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Genetic and phenotypic characterisation of an ABC transporter system implicated in narasin resistance of *Enterococcus faecium*

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Abstract

Antibiotic resistant strains of bacteria are increasingly prevalent all over the world. Better understanding of the resistance occurrence and spread is needed to stop this development. A narasin (Nar) resistance gene was recently discovered on the same plasmid as a vancomycin (Van) resistant gene (Nar/*vanA* plasmid). This study sought to examine the relationship between the ionophore coccidiostat Nar used in rearing of broiler chicken and the clinically important antibiotic vancomycin and initiate the characterization of the molecular mechanism of Nar resistance.

Strains of *Enterococcus faecium (E. faecium)* isolated from Norwegian poultry production had previously been examined for the presence of the Nar resistance genes (encoding an ATPase and ABC transporter) of the Nar operon). The Nar operons from a subset of these strains were sequenced and analysed for differences in nucleotide and amino acid sequences. Strains with varying Nar resistance were also screened for resistance of four ionophores currently authorised for use in Norway. Nine different Nar operons were also introduced into separate cells of *Escherichia coli (E. coli)* DH5α and the drug-hypersensitive *E. coli* DH5α *ΔacrAB* and cross-resistance were determined by screening a panel of antibiotics. Lastly, an experiment was designed to determine if a selective pressure of Nar could enhance conjugational transfer of the Nar/*vanA* plasmid between strains of *E. faecium*.

Translated ATPase and ABC transporter differed only at three and one amino acid positions, respectively, when compared to the consensus sequence. These differences did not seem to explain the variation in Nar resistance. No cross-resistance was found between the antimicrobials for *E. coli* DH5α nor the *E. coli* DH5α *ΔacrAB* carrying their individual Nar operons. Results indicate that there is a correlation between resistance to Nar, and the ionophores Salinomycin and Maduramicin, but not Monensin and Lasolacid. Findings also suggest that a reduced Nar susceptibility can be co-transferred with the *vanA* resistance gene without the selective pressure of Nar.

Indeed, use of Nar may have taken part, and may still take part in the persistence of Van resistance in enterococci if introduced back into rearing practices. Yet, further research is necessary.

Sammendrag

Antibiotika resistente bakterier er i økende antall over hele verden, og det er ansett for å være et økende samfunnsmedisinsk problem. Bedre forståelse av spredningen og forekomsten er nødvendig for å kunne stoppe denne utviklingen. Et narasin (Nar) resistensgen ble nylig funnet på et plasmid sammen med et vancomycin (Van) resistensgen (Nar/*vanA* plasmid). Denne studien ønsket å undersøke forholdet mellom den ionofore koksidiostaten Nar, brukt i produksjonen av slaktekyllling, og det klinisk viktige antibiotikumet vancomycin, og samtidig initiere karakteriseringen av de molekylære mekanismene bak Nar resistens.

Bakteriestammer av *Enterococcus faecium (E. faecium)*, isolert fra norsk kylling produksjon hadde tidligere blitt undersøkt for tilstedeværelsen av Nar resistensgenene (som koder for en ATPase og ABC transporter) i Nar operonet. Nar operonet fra en undergruppe av stammene ble sekvensert og analysert for forskjeller i nukleotid og aminosyre sekvensene. Stammer med varierende Nar resistens ble i tillegg undersøkt for resistens mot fire andre ionoforer tillatt for bruk i Norge. Ni forskjellige Nar operon ble også klonet og introdusert inn i individuelle celler av *Escherichia coli (E. coli)* DH5α og hypersensitiv *E. coli* DH5α *ΔacrAB*, og kryss-resistens ble undersøkt mot et panel av antibiotika. Et eksperiment ble designet for å finne ut om et selektivt press fra Nar kan øke overføringen av Nar/*vanA* plasmidet mellom to stammer av *E. faecium.*

Når sammenlignet med konsensussekvensen varierte proteinene fra ABC transporteren og ATPasen på henholdsvis én og tre posisjoner i aminosyrekjeden. Disse forskjellene så ikke ut til å kunne forklare variasjonene i stammenes Nar resistens. Ingen kryss-resistens ble funnet mellom panelet av antibiotika for *E. coli* DH5α eller *E. coli* DH5α *ΔacrAB* med deres individuelle Nar operon. Resultatene indikerte også at det er en sammenheng mellom resistens for Nar, og ionoforene Salinomycin og Maduramicin, men ikke for Monensin og Lasolacid. Funn tyder også på at redusert Nar mottakelighet kan overføres med *vanA* resistensgenet uten selektivt press fra Nar.

Bevis tyder på at Nar kan ha på påvirket, og kan fortsatt påvirke forekomsten av resistens mot Van i enterococci hvis antibiotikumet er reintrodusert i fôret til kylling. Men, videre forskning er nødvendig.

Abbreviations

ABC:	ATP (adenosine triphosphate)-binding cassette
AMP:	Ampicillin
AR:	Antibiotic Resistance
AZI:	Azithromycin
BAC:	Bacitracin
CIP:	Ciprofloxacin
CHL:	Chloramphenicol
COL:	Colistin
DAP:	Daptomycin
DNA:	Deoxyribonucleic acid
dNTPs:	Deoxynucleotides
ECOFF:	Epidemiological cut-off
ERY:	Erythromycin
FOT:	Cefotaxime
GM:	Gentamicin
HGT:	Horizontal Gene Transfer
LAS:	Lasolacid
LZ:	Linezolid
MATE:	Multidrug and Toxic Compound Extrusion
MAD:	Maduramicin
MCS:	Multiple Cloning Site
MDR:	Multidrug resistance
MERO:	Meropenem
MFS:	Major Facilitator Superfamily
MH:	Mueller-Hinton
MIC:	Minimal Inhibitory Concentration
MON:	Monensin
NAL:	Nalidixic acid
NAR:	Narasin
NVI:	Norwegian Veterinary Institute
ORF:	Open Reading Frame
PCR:	Polymerase Chain Reaction
RND:	Resistance-Nodulation-Division
SAL:	Salinomycin
SMR:	Small Multidrug Resistance
SMX:	Sulfamethoxazole
STR:	Streptomycin
SVA:	National Veterinary Institute of Sweden
SYN:	Quinuprostin/Dalfopristin
T4SS:	Type IV secretion system
TAZ:	Ceftazidime
TET:	Tetracycline
TGC:	Tigecycline
TRI:	Trimethoprim
VAN:	Vancomycin
VI:	Virginiamycin
VRE:	Vancomycin Resistant Enterococci

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1. Introduction

An increasing number of antibiotic resistant bacteria occur all over the world. This important problem can have devastating consequences, and the World Health Organization (WHO) has even stated that antibiotic resistance (AR) is one of the most important threats to human health. In order to stop this development, it is essential that we gain better understanding regarding the occurrence of antibiotic resistant bacteria and the reason for development and spread of AR. This thesis will study the relationship between resistance to the coccidiostat narasin (Nar), used in rearing of broiler chicken, and the clinically important antibiotic vancomycin (Van). Further, initiating the characterisation of the molecular mechanism of Nar resistance.

1.1 Antibiotics

Antibiotic drugs are, per definition, antimicrobial agents able to kill or inhibit growth of microorganisms (Cantón & Ruiz-Garbajosa 2011). It is a compound generally assumed to be produced by one microorganism to kill a competing microbe in its environment. Yet, some scientist believe that antibiotics may in fact be signalling molecules, which happens to kill the cell if amounts are high (Clardy et al. 2009; Linares et al. 2006; Yim et al. 2007).

Nevertheless, antibiotics are commonly found in nature, and are believed to have existed for billions of years. Traces of tetracycline (Tet) has even been found in human skeletal remains in Sudan dating back to 350-550 CE (Aminov 2010; Bassett et al. 1980). Indicating that these ancient people were exposed to Tet via foods. Since its discovery, antibiotics have led to significant drop of child mortality and death from diseases caused by pathogenic microorganisms (Blair et al. 2015). It has greatly increased life expectancy (Bérdy 2012; Van Hoek et al. 2011) and is considered crucial for invasive surgeries. It also revolutionised animal medicine for the same reasons (VKM 2015). However, in recent decades these advances have been threatened due to the emergence and spread of antibiotic resistant bacterial strains; microbes resistant to the effect of antibiotics (Fernández & Hancock 2012). In the beginning of our ‘antibiotic era’ it was assumed that an evolution of AR was unlikely. Yet, time proved otherwise. Just a few years after the introduction of medically used antibiotics, a significant increase in resistant bacteria was found. Nobody had initially expected that the exposed bacteria could develop such a wide variety of resistance mechanism when exposed to these new-found chemicals. It was highly surprising. More surprisingly so was however, the microbe’s ability to inter-change genes, known today as horizontal gene-transfer (HGT). Though, it was later found

that bacterial resistance was not a new mechanism after all. Resistance had existed before the discovery and medicinal use of antibiotics. In fact, AR is a natural phenomenon prevalent in nature (D'Costa et al. 2011). The development is considered an expected result due to the vast interactions of various microbes in the environment (Munita & Arias 2016). Yet, the excessive and inappropriate use of antibiotics for humans and animals, together with poor infection control practices have led AR to become a serious threat to global public health. Numerous ecological studies have discovered that the worlds increased antibiotic consumption has contributed, and is still contributing to AR development and spread in various strains and species. And of most concern, is the development and spread of resistance genes in pathogenic bacteria. Not only does resistant bacteria directly impact human and animal health, but it also present great economic costs due to increased healthcare costs, treatment failures and prolonged hospital stays (WHO 2014).

Antibiotics inhibit or kill bacteria by a variety of mechanisms. They are usually classified based on the cellular component or system they affect, as well as if they induce cell death (bactericidal) or inhibit cell growth (bacteriostatic) (Kohanski et al. 2010). The bactericidal effect of antibiotics is a complex process by which physical interactions occur between the drug molecule and the bacterial-specific target. It involves modulations and alterations at a molecular level. Common mechanisms of antibiotics can be the disruption of cell wall biosynthesis or the membrane structure itself. Other mechanisms can affect nucleotide metabolism and repair, or the protein synthesis through regulation or inhibition (see figure 1.2.2).

As the role of antibiotics was discovered, it was not only applied in the treatment of human diseases, but also in the maintenance of animal health in the rearing of food animals (Schwarz et al. 2001). It is, for example, used in the treatment of a disease or as a prophylactic agent (acting to prevent a disease) in poultry. Yet, also in food animal rearing, increasing amounts of antibiotic resistant bacteria have been found over the past decades, highlighting the observed association between antibiotic use and prevalence of resistant bacteria.

1.2 Antibiotic resistance

To understand the problem of AR it is useful to understand some relevant concepts. Resistance to antimicrobials may either be intrinsic or acquired. Intrinsic resistance is the natural resistance to a given antibiotic in all members of a microbial species due to an inherent characteristic of

the organism (Gang & Jie 2016). It is a characteristic expressed by an intrinsic resistance gene in the bacterial chromosome. The gene's presence is also independent of previous antibiotic exposure.

Acquired resistance in bacteria occur through either mutations in existing genes or the acquisition of additional genes (Rice 2016). It is the capability of a species or strain of microorganism to survive exposure to a drug that was formerly effective against it. Mutations occur when the microbes replicate themselves erroneously, but mutational resistance develops when a spontaneous mutation occurs at a locus in the microbial chromosome that results in decreased susceptibility to a given antibiotic (Capita & Alonso-Calleja 2013). These spontaneous mutations usually result in changes in an antimicrobial target, and may be transferred vertically. If mutations occur in regulatory regions or regulators, antimicrobial resistance may be promoted by the overproduction of an intrinsic resistance determinant, such as efflux pumps (Beinlich et al. 2001), or the target gene.

Acquired resistance can also occur through HGT. HGT refers to the process by which genetic material, such as plasmids, may be transferred to other bacterial cells within the same strain or species, or between two different bacterial species (VKM 2015). There are at least three different processes by which HGT may occur - conjugation, transduction and transformation (Soucy et al. 2015) (Figure 1.2.1).

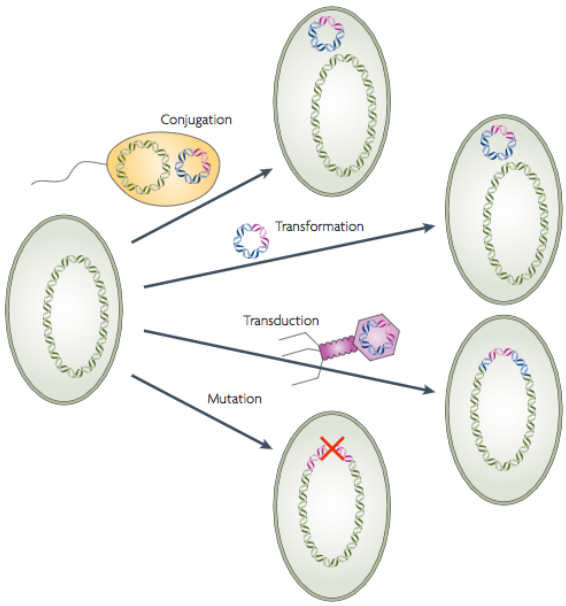

Figure 1.2.1: Mechanisms of resistance acquisition. DNA resistance gene (pink) could be transferred to another cell by three mechanisms; conjugation: transfer from one cell to another, transformation: transfer of naked DNA from environment into new cell and transduction: phage-mediated transfer into a new cell. DNA resistance could also occur by mutation of an existing gene. (Reprinted with permission from (Andersson & Hughes 2010)

Conjugation is the transfer of genetic material directly from one donor to a recipient cell (Ilangovan et al. 2015). It is a process mediated by a type IV secretion system (T4SSs), a large macromolecular complex involved in pilus biogenesis that is able to transport not only DNA, but also toxins and effector proteins (Cabezón et al. 2015). Bacterial conjugation is considered as one of the main mechanisms of HGT, and a key element in the potential dissemination of AR to human pathogens.

Transduction is the transfer of genetic material from one bacterium to another via species specific bacteriophages. The bacteriophage picks up foreign DNA from one bacteria, packs the foreign DNA inside its shell, transports it and inserts it along with its own genome into a bacterial cell. Transformation is the inclusion of exogenous DNA from the environment into bacteria or archaea. The genetic material then becomes integrated into the bacterial genome from where it can be expressed.

Any microbe resistant to an antimicrobial will have a proliferative advantage when exposed to it. After time, a new resistant population will have outcompeted the original susceptible population. This combined effect of being able to transfer genes, fast growth rates and the genetic process of mutations, can explain the extraordinary rates at which bacteria can adapt, and it may also explain why exposure to an antibiotic environment seems to induce spread of bacterial resistance.

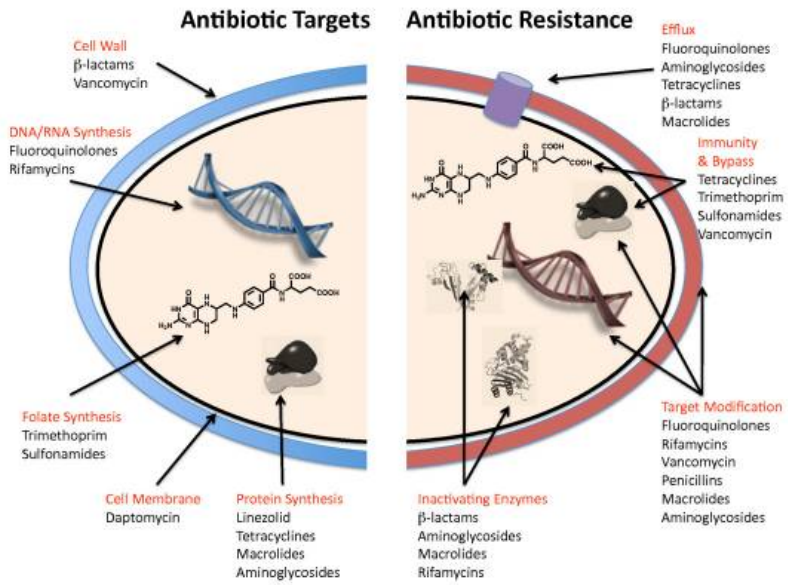

Figure 1.2.2: Schematic illustration of antibiotic targets and antibiotic resistance mechanisms. (Reprinted with permission under the Creative Commons Attribute License from Wright (2010)

1.2.1 Molecular mechanisms of antibiotic resistance

The molecular mechanisms behind AR have been extensively studied (Davies & Davies 2010), and there are several mechanisms (Figure 1.2.1) by which a bacterial cell can become resistant.

1. Changes in the cell envelop, therefore limiting antimicrobials access to target sites (Van Hoek et al. 2011),
2. Active efflux of the antibiotic compound from the microbial cell (e.g. membrane inserted ATP-dependent efflux system),
3. Enzymatic alterations of the antibiotic (Mc Dermott et al. 2003)
4. Degradation of the antimicrobial compound (Blair et al. 2015),
5. Acquisition of alternative metabolic pathways to those inhibited by the drug,
6. Modification of antibiotic targets (e.g. methylation),
7. Overproduction of the target enzyme.

Although all mechanisms are relevant and important in the discussion about AR, the current study will focus on efflux pumps (2.). It will also consider the effect potential mutations of the pump encoding gene may have on bacterial tolerance.

One of the first description of the efflux pump systems as a resistance mechanism was to Tet in *Escherichia coli* (*E. coli)* (Ball et al. 1980; McMurry et al. 1980). They are now, however, recognised as ubiquitous resistance mechanisms present in all organisms (Blanco et al. 2016). It was first assumed that these pumps arose as a way of surviving exposure to a hostile environment (antimicrobials), yet these pumps are also found in the microorganism that produces the antibiotic substance, indicating that they must also have another purpose. Indeed, they are today considered as transport proteins with an important role in the extrusion of toxic substrates and metabolites produced by the cell itself (Webber & Piddock 2003). These toxic substrates also include nearly all classes of clinically used antibiotics. Additionally, it is widely accepted that the efflux system activity is part responsible for the 'intrinsic resistance' of bacteria to some antibiotics.

There are in general five familial classes of bacterial efflux systems capable of transporting antimicrobials out of the cell (Figure 1.2.1.1):

1. The major facilitator superfamily (MFS)
2. The ATP (adenosine triphosphate)-binding cassette (ABC) family
3. The resistance-nodulation-division (RND) family
4. The small multidrug resistance (SMR) family
5. The multidrug and toxic compound extrusion (MATE) family

These families have been classified based on their sequence similarities, substrate specificity, number of constituents (single or multiple), energy source and number of transmembrane-spanning regions (Blanco et al. 2016). The MFS, SMR, MATE and RND families utilise the proton/ion motive force (energy that is generated by the transfer of electrons or protons over a semipermeable membrane) as their energy source, while the ABC family utilises ATP hydrolysis to extrude compounds (Tillotson & Tillotson 2010). Although much can be said about the different classes of efflux pumps, the focus of the current study will be upon the ABC transporters.

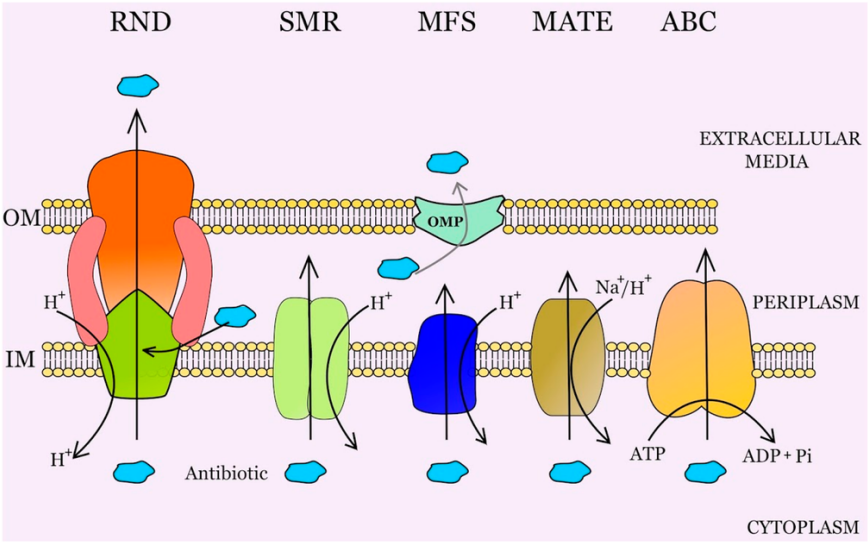

Figure 1.2.1.1: Schematic illustration of the five major families of bacterial efflux systems: the resistance- nodulation-division (RND) family, the small multidrug resistance (SMR) family, the major facilitator superfamily (MFS), the multidrug and toxic compound extrusion (MATE) family and the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily. IM: Inner membrane. OM: Outer membrane. OMP: Outer membrane protein. (Reprinted with permission under the Creative Commons Attribute License from Blanco et al. (2016)

The ABC transporter efflux pumps are found in all organisms (Pohl et al. 2011). It is one of the largest families of efflux pumps consisting of multiple subunits. The prototypical ABC transporter consists of four domains, of which two are transmembrane proteins and two are cytoplasmically localised nucleotide-binding domains - ATPase subunits (Hernando-Amado et

al. 2016; Tillotson & Tillotson 2010). It is located only in the inner membrane of the cell wall (Figure 1.2.1.1).

The different efflux pump systems may be specific for one substance, or they may be able to transport several substances. In the latter case, they are called multidrug resistance (MDR) pumps. A MDR transporter is not a class of its own, but it may belong to any of the five superfamilies, and they are normally found in most living organisms. Normal cellular activity results in various waste products, which commonly need to be exported out of the cell. Efflux pumps can serve as monitors for chemical concentration in the cell, extruding substances that may endanger the cells wellbeing. However, cells may also deploy pumps to adapt and defend the cell against unwanted, cell threatening substances in their environment. When adding to the fact that exposure to antibiotics contribute to the development of more MDR pumps (Chuanchuen et al. 2001), these pumps are considered to play a major role in AR. In fact, studies have found that many pathogenic bacteria have developed resistance to several antibiotics due to the acquisition of MDR transporters.

While all living organisms contain genes for efflux pumps in their chromosome, some are present in mobile elements such as plasmids. Plasmids are small mobile elements of DNA that exist separately from the main bacterial chromosome (Bennett 2008). They do not carry any of the core genes needed for normal cell function, instead they carry genes that enable the cell to exploit certain environmental situations, such as the survival in the presence of potentially lethal antimicrobials. They are also more easily transferred between bacteria, which can explain the increased prevalence of AR seen in response to the global, increased use of antibiotics.

1.3 Cross and co-resistance

Cross-resistance occurs when the same or similar mechanism(s) of resistance applies to more than one type of antimicrobial (VKM 2015), e.g. a MDR pump that extrudes several antimicrobial substances. One example is the cross-resistance that has been reported between the biocide triclosan and antibiotics in *Pseudomonas*, mediated by MDR pumps (Braoudaki & Hilton 2004; Chuanchuen et al. 2001).

Co-resistance occurs when resistance genes encoding different resistance mechanism are located on a mobile genetic element such as a plasmid, transposon or integron (Baker-Austin et

al. 2006). The mobile element can then be transferred into a bacterial isolate making it resistant to more than one antimicrobial. This type of co-resistance and co-selection increases the risk of resistant bacteria surviving and further disseminating its resistance genes (Cantón & Ruiz-Garbajosa 2011).

1.4 Defining resistance

Although knowledge on AR and relevant resistance mechanisms are increasing, there is still discussion about how resistance should be classified; where the cut-off values are and what these values are based on. There is not always a clear definition of the difference between a 'resistant' and a 'non-resistant' bacteria. The term 'reduced susceptibility' or 'increased tolerance' to an antibiotic may therefore be more appropriate, although this is not the consensus. Two terms could be used to describe the level of resistance in bacteria; epidemiological and clinical resistance. By gathering the Minimal Inhibitory Concentration (MIC) of a large quantity of bacterial isolates, epidemiological breakpoints can be determined (Martínez et al. 2015). Isolates with a certain MIC are plotted to get a distribution curve, and the epidemiological cut-off (ECOFF) values are determined as the upper MIC value of the part of the curve representing the majority of the population (figure 1.4.1). Isolates above these values are considered resistant, yet, not clinically resistant. The clinical cut-off values are usually found above the epidemiological breakpoints, and it refers to bacterial resistance associated with therapeutic failure - clinical resistance (MacGowan 2008).

The widespread human use of compounds with antimicrobial activity, heavy metals and disinfectants could exert a selective pressure on bacterial populations in our surroundings (Baquero 2001). Bacteria with low-level resistance may evolve high-level resistance under this pressure. Therefore, prevalence of low-level resistance should be considered a warning sign of future evolutionary development of high-level, clinical resistance. It also underscores the importance of epidemiological resistance monitoring.

In this study, epidemiological resistance has been explored among bacterial strains, and the more appropriate terms of 'reduced susceptibility' and 'increased tolerance' are used.

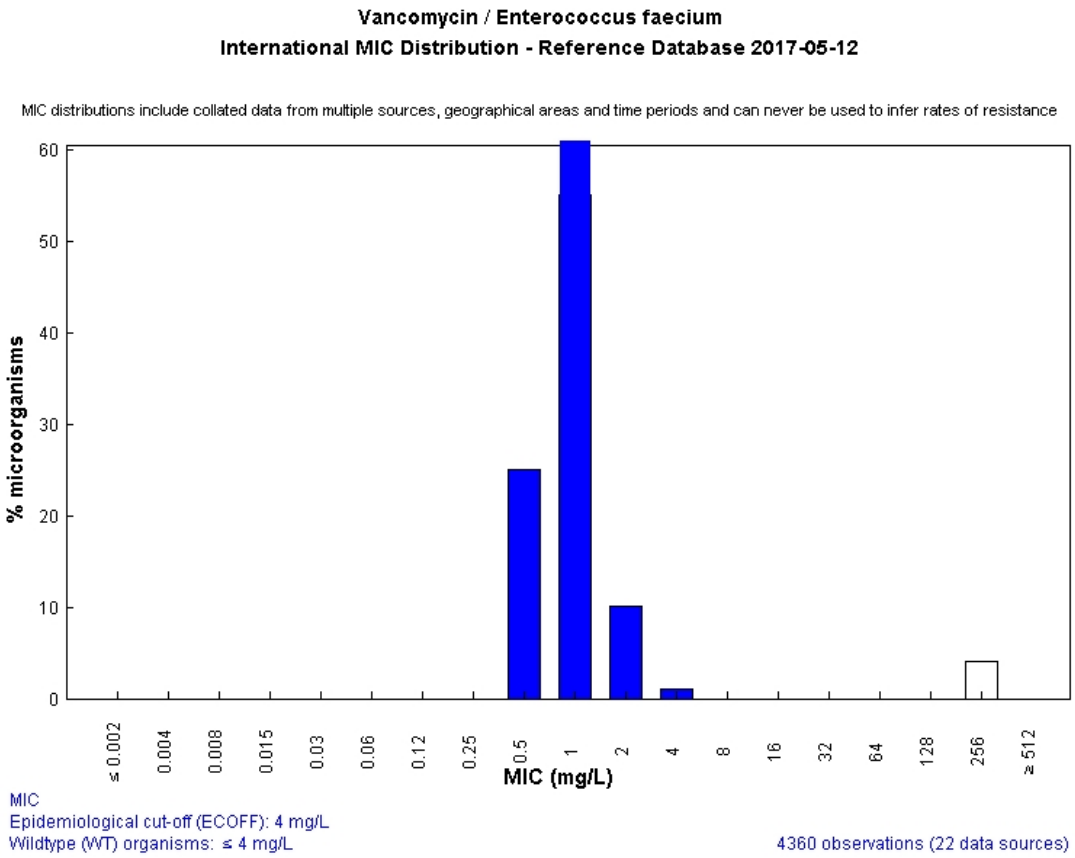

Figure 1.4.1: Epidemiological cut-off (ECOFF) for vancomycin in E. faecium (EUCAST 2017)

1.5 Possible transmission routes of bacterial resistance genes

The development and spread of AR has led to the discussion about appropriate antibiotic use, especially in nutritional and veterinary settings. As previously mentioned, antibiotics are being used extensively in veterinary settings in addition to treatment of humans, and AR genes are increasingly found.

As bacteria can preserve and harbour AR genes even though the selective pressure has seized, a larger reservoir of resistance genes is generated. Of particular concern, is the potential reservoir in commensal bacteria of food-producing animals (van den Bogaard & Stobberingh 2000). It is believed that the reservoirs in production animals, and other reservoirs contribute to the dissemination of AR genes and antibiotic resistant bacteria to clinically relevant antibiotics.

1.6 Diseases in poultry

Coccidiosis is an intestinal disease in animals, especially poultry, caused by protozoan parasites called coccidia (Sloper et al. 1982). It spreads between animals by contact with infected faeces, and typically does so even before symptoms of the disease are seen (VKM 2015). Therefore, preventative measures are usually preferred, e.g. coccidiostats. Coccidiostats are drugs used to treat or prevent infections caused by coccidia. They are often used as animal feed additives and two major groups of coccidiostats are available – ionophores and non-ionophores. Ionophores are antiprotozoal chemicals that also inhibit or kill some bacterial strains (Kevin Ii et al. 2009), whereas non-ionophores do not (VKM 2015). Therefore, by administering ionophores in animal feed, some bacterial infections may also be controlled. This has been found to be true for enterococci, staphylococci and *Clostridium perfringens* (*C. perfringens*), where the latter is considered the predominant cause of necrotic enteritis (Al-Sheikhly & Al-Saieg 1980), a disease characterised by severe necrosis of the intestinal mucosa (Dahiya et al. 2006).

C. perfringens is normally found in caecal contents of poultry, but can proliferate and start producing toxins under certain conditions (Dahiya et al. 2006; VKM 2015). Similar to coccidiosis, necrotic enteritis shows no clear symptoms until the disease is widespread. The clinical form of necrotic enteritis causes decreased appetite or anorexia and diarrhoea, among other symptoms. The resulting impaired growth and feed utilisation in chicken production is a concerning consequence of both diseases. As is the risk of poor litter bedding quality, and in general, the detrimental effect on chicken welfare. Preventative measures, such as the ionophore antibiotics have therefore been considered very important in poultry rearing.

In 2015, eleven coccidiostats, both ionophores and non-ionophores, were authorised for use as feed additive in the EU (VKM 2015). But as Norway is exempted from this field due to the EEA agreement, only five has been approved; all ionophores. These include Nar, Monensin (Mon), Lasolacid (Las), Salinomycin (Sal) and Maduramicin (Mad). Yet, only two are currently being used; Nar for broilers and Mon for turkeys (VKM 2015).

1.7 Narasin

Nar is a polyether ionophore with prophylactic activity, meaning it has the ability to prevent diseases such as coccidiosis (Wang & Sporns 2000). It belongs to the family of the structurally similar polyether ionophores Mon, Las, Mad and Sal (Figure 1.3.1). Its biological activity is

based on the molecule's ability to form lipid soluble and reversible complexes with metal ions, and by working as carriers by mediating electrically neutral exchange-diffusion type of cation transport across membranes (Kevin Ii et al. 2009).

Until recently, Nar has been used to prevent coccidiosis in poultry in the Norwegian chicken industry. However, over the past years the use of antibiotics, especially Nar have been under severe scrutiny. It came to the point at which chicken filet sales dropped significantly, fostered by news reports that antibiotics had been found in store-bought chicken filets. Filets that were from chickens reared with Nar-supplemented feed. Many chicken breeders abandoned the use of Nar, and the Norwegian government started the process of phasing it out of Norwegian poultry rearing (Ministries 2015).

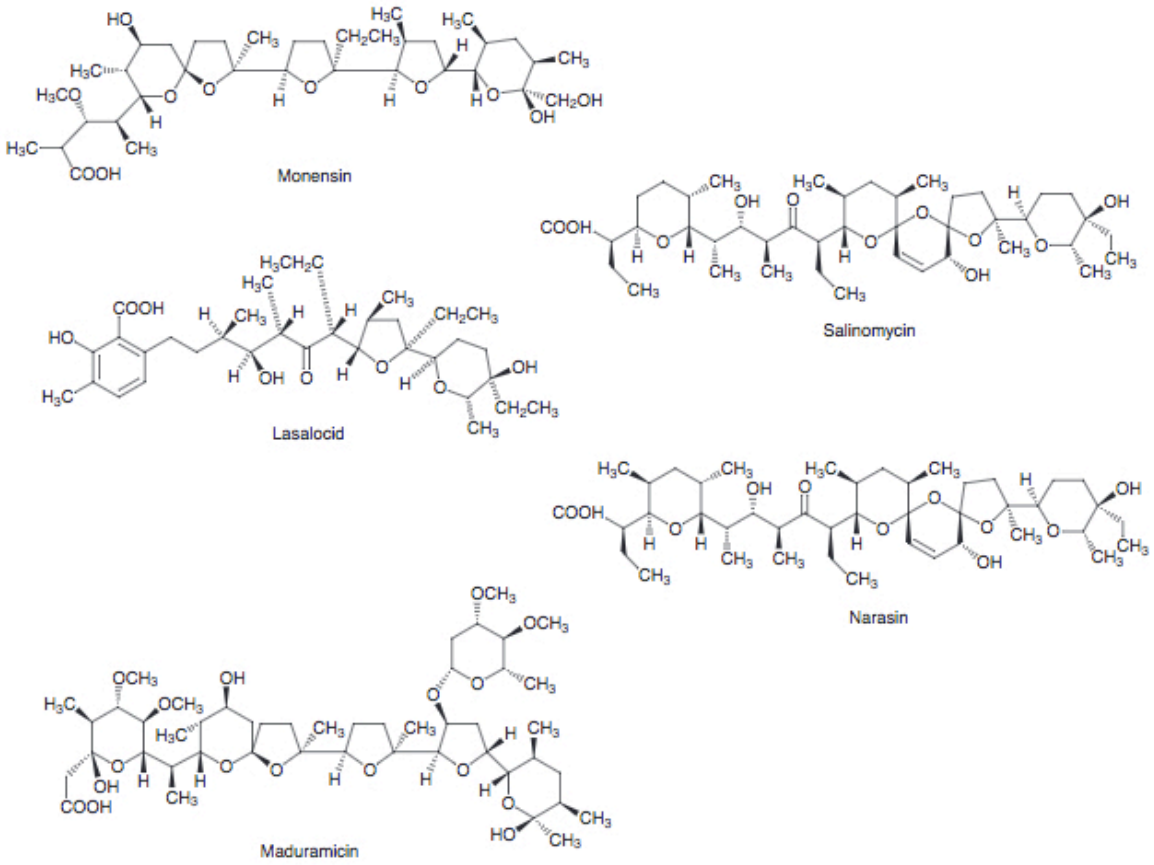

Figure 1.7.1: Molecular structure of Lasolacid, Monensin, Maduramicin, Salinomycin and Narasin (Adapted with permission from Elliott et al. (1998)

1.8 Concerns regarding narasin

Although, some people could be allergic or react to Nar, it is not the main reason for the concern. It is the possible occurrence of Nar resistant bacteria able to mediate cross- or co-resistance.

So, the main threat is not necessarily that the bacteria are resistant to the ionophore Nar, but that these bacteria could in fact, also be resistant to clinically, very important antibiotics, such as Van. Van is today, considered one of the last resort antibiotics to treat several diseases caused by bacteria already resistant to most clinically used antibiotics, e.g. septicaemia and skin and bone infections. A study by Nilsson et al. (2012) found that the presence of genes potentially encoding an ABC transporter system correlated with elevated MIC for Nar in *Enterococcus faecium* (*E. faecium*). These genes were situated on the same plasmid as a Van resistance gene (*vanA*). This presents a possible serious issue of co-resistance between the feed additive Nar and the clinically important antibiotic Van. Adding to this issue, is evidence of a non-negligible prevalence of bacteria with resistance to ionophores. In fact, the Norwegian surveillance program, NormVet, found that between 2002 – 2013, 50 to 80% of their tested poultry had Nar resistant enterococci (VKM 2015). Bacteria that are a part of their normal intestinal flora (Lu et al. 2003).

While the use of Nar has been mostly discontinued in the Norwegian chicken industry today, Mon is still being used in feed for turkey rearing. Due to these ionophores structural similarities, it is possible that the Nar efflux system, of which we still have little knowledge, may be able to transport Mon across a bacterial cell membrane (cross-resistance). If so, even though Nar is discontinued, a selective pressure may still be present due to the use of Mon. Indeed, other substances may also induce such a selective pressure if the Nar resistance genes encodes a MDR pump. If the bacterial cells, harbouring the Nar/*vanA* plasmid, are exposed to this kind of selective pressure, they will have a proliferative advantage. Thus, creating a population of cells harbouring the *vanA* gene, presenting an increased risk of dissemination among a bacterial population, potentially including human pathogens.

1.9 Aim of study

The specific aim of the study was to characterise the Nar operon, consisting of the two Nar resistance genes (ATPase and ABC transporter), that has been correlated with transferrable increased Nar resistance and co-resistance to Van. The aim is further divided into 3 sub-aims as follows.

Sub-aim 1: sequence and compare the Nar resistance operons from isolates with varying Nar susceptibility.

Sub-aim 2: find out if Nar resistance is accompanied with resistance to clinically relevant antibiotics by cloning the Nar operon and determine the AR profile of a hypersusceptible *E. coli* strain expressing the Nar resistance operon

Sub-aim 3: find out if Nar promotes horizontal gene transfer of the Nar operon between strains of *E. faecium*

2. Materials and methods

2.1 Materials

2.1.1 Bacterial strains

Samples were collected from meat, caecal content or boot swabs used to sample the environment of the animal houses. These were collected for Normvet's routine surveillance program of Van resistant *E. faecium* (VRE) prevalence in Norwegian poultry production. Samples were further inoculated onto either selective plates supplemented with Van, or non-selective plates. Strains for the current study were chosen based on their Nar resistance which had been routinely tested on SVA-microtiter plates in connection with the isolation of the strains. Additionally, one genomic DNA sample from a VRE isolate received from the National Veterinary Institute of Sweden (SVA) was included in the study.

Moreover, fourteen isolates collected in 2014 that had not been tested on the same antibiotic panel as the previous isolates were included in the study and screened on VetMIC microtiter plates against a panel of antibiotics including Van and Nar (see table 2.1.3 for isolates).

2.1.2 DNA extracts

All isolates were screened by Jannice Schau Slettemås at the Norwegian Veterinary Institute (NVI) for the presence of the *vanA* and the Nar genes by Polymerase Chain Reaction (PCR) (see section 2.2.1) and the DNA was made available for the current study.

2.1.3 Vector (pBAD30)

For the current study an *E. coli* plasmid vector pBAD30 was used (Figure 2.1.3). It is a small plasmid expression vector of around 4.9kb and has been genetically engineered to include

restriction sites, and a promoter for gene expression. It also contains the important multiple cloning site (MCS). This is a stretch of different restriction sites located downstream of the promoter. If a gene is introduced in the MCS, the expression of the gene should be under control of the promoter, making it possible to regulate the gene expression.

The promoter of pBAD30 is a P*ara*BAD or P_{BAD} promoter. It keeps the vector under tight control when working with its associate regulator AraC (Guzman et al. 1995). In *E.coli* the P_{BAD}-promoter is strongly induced by presence of L(+) arabinose and in the absence of glucose (Newman & Fuqua 1999).

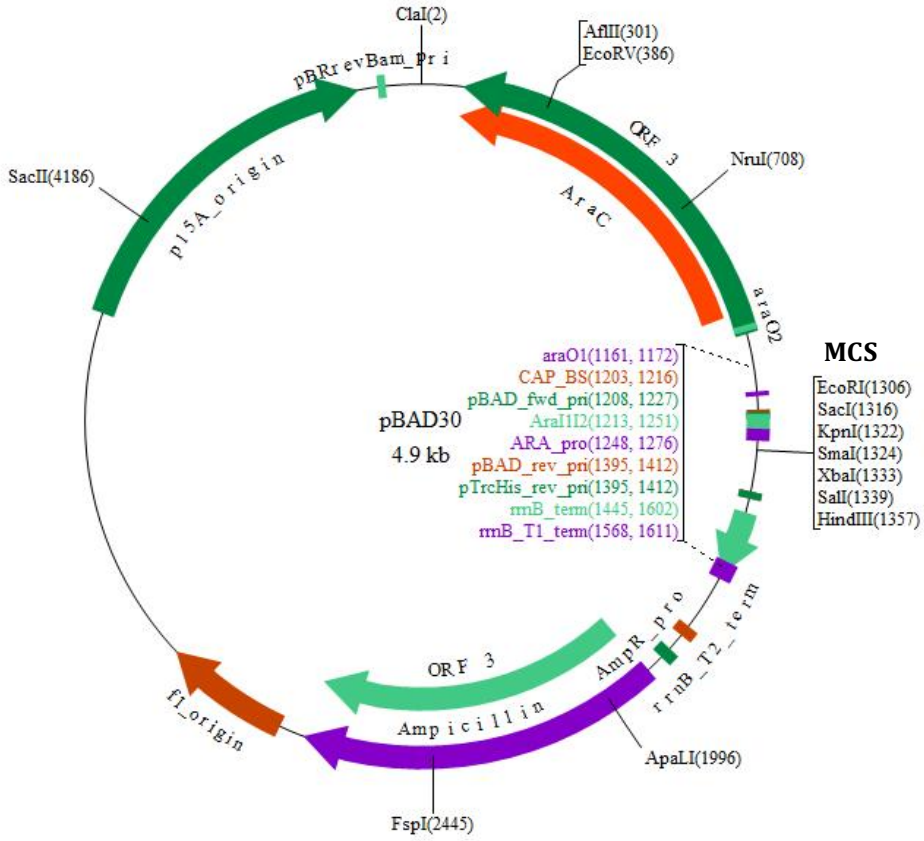

Figure 2.1.3: Schematic representation of the pBAD30 vector. The plasmid diagram was generated using BVTech Plasmid software (BV Tech Inc., Bellevue, WA).

pBAD30 was chosen to express the ATPase and ABC transporter in *E. coli* DH5α and an *E. coli* DH5α Δ*acrAB* mutant. All strains and plasmids used in this study are presented in table 2.1.3.

Table 2.1.3: Strains and plasmids used in this study

Strains or plasmids	Description
Enterococcus faecium	
2004-01-850-1	(Fe), Nar resistant, Van susceptible
2006-01-1700-1	(Fe), Nar and Van susceptible
2006-01-1145-1	(Fe), Nar resistant, Van susceptible
2006-01-1190-1	(Fe), Nar and Van susceptible
2006-01-2608-1	(Fe), Nar and Van susceptible
2006-01-1115-1	(Fe), Nar resistant, Van susceptible
2006-01-1111-1	(Fe), Nar resistant, Van susceptible
2006-01-1117-1	(Fe), Nar resistant, Van susceptible
2006-01-1151	(Fe), Nar resistant, Van susceptible
2006-01-1402	(Fe), Nar resistant, Van susceptible
2013-01-3934	Nar and Van resistant (selective)
2013-01-4826	Nar susceptible, Van resistant
2004-01-1251-1	(Fe), Nar and Van susceptible
2004-01-1301-1	(Fe), Nar resistant, Van susceptible
2004-01-1343-1	(Fe), Nar resistant, Van susceptible
2006-01-3433	Nar and Van resistant (selective)
2009-01-1808-4	Nar and Van resistant (selective)
2006-01-1131-1	(Fe), Nar resistant, Van susceptible
2006-01-1148-1	(Fe), Nar resistant, Van susceptible
2006-01-1154-1	(Fe), Nar resistant, Van susceptible
2006-01-1152-1	(Fe), Nar resistant, Van susceptible
2013-01-5191	Nar and Van resistant (selective)
2014-01-7513	
2014-01-7512	
2014-01-7483	
2014-01-7479	
2014-01-7377	
2014-01-7207	
2014-01-7050	
2014-01-6934	
2014-01-4539	
2014-01-2995	
2014-01-1914	
2014-01-1741	
2014-01-7394	
2014-01-7584	
2012-70-76-8	
Escherichia coli	
DH5α	
DH5α Δ*acrAB*	Drug-hypersensitive
Plasmid vector	
pBAD30	*E. coli* expression vector

(Fe): Isolated from faeces (selective): isolated on agar supplemented with vancomycin

2.1.4 DH5α Δ*acrAB*

The *E. coli* DH5α Δ*acrAB* mutant is drug-hypersensitive compared to DH5α (Simm et al. 2012). This is due to a knock-out mutation of its *acrA* gene encoding a membrane fusion protein and *acrB* encoding a multidrug efflux system. It causes inactivation of the bacterial RND

transporter complex. The cell is consequently sensitised to many drugs due to its reduced ability to transport toxic compounds out of the cell.

2.1.5 Kits used in this study

Kits used in this study includes; QIAquick® PCR Purification Kit (QIAGEN, Germany), QIAquick® Gel Extraction Kit (QIAGEN, Germany), Rapid DNA Ligation kit (Thermo Scientific, USA), HindIII and KpnI restriction enzymes (Thermo Fisher Scientific, USA) and DreamTaq and Phusion polymerases (Thermo Fisher Scientific, USA). All procedures were carried out according to the manufacturer's instructions. Yet, the following changes were made for some methods:

Rapid DNA Ligation kit (Thermo Scientific)

Incubation time was increased from five minutes to fifteen minutes.

2.2 Methods

2.2.1 Polymerase chain reaction

PCR is a well-known method of DNA amplification widely used in lab-settings. This method was used in the current study to amplify the Nar operon carried by the *E. faecium* strains (Table 2.1.3).

A PCR reaction mixture contains all the components needed to synthesise new and exact copies of target DNA molecules. The basic reaction mix contains water (sterile), a buffer for optimal activity of the DNA polymerase, dNTPs, which are the building blocks of the DNA, in addition to primers with sequences complementary to sequences of the target DNA. The Taq polymerase and template DNA is subsequently added to the mixture, before reactions are loaded into a Thermal Cycler (Agilent technologies SureCycler 8800). Primers for PCR in the current study were custom-designed to anneal at the beginning and end of the Nar operon, with an annealing temperature of 56°C. When heating the solution to 98°C the double-stranded DNA will separate. The temperature is then decreased to optimal annealing temperature and increased again to allow elongation by the chosen DNA polymerase. There are different polymerases that may be used for PCR. For the methods presented here a DreamTaq and a High-Fidelity Phusion

polymerase were used. DreamTaq, as the name indicates is a Taq polymerase. It was isolated from the thermophilic bacterium of *Thermus aquaticus* back in 1976 (Chien et al. 1976) and is frequently used in PCR due to its thermostable properties. While DreamTaq is a reliable enzyme for use in PCR, the Phusion Taq polymerase also provides high fidelity (accuracy in DNA replication) due to a processivity-enhancing domain. As such it makes it more eligible for use during cloning, as the method relies on accuracy of the cloned gene sequence. Standard PCR mixes and programs used were designed according to the recommendations of the manufacturers of DreamTaq and Phusion polymerase. PCR mixes and programs are presented in table 2.2.1.1 and 2.2.1.2, respectively. Primers used in this study (sequencing and cloning) are described in table 2.2.1.3.

Table 2.2.1.1: PCR mixes for DreamTaq and Phusion polymerase

Taq polymerase	PCR mix	Volume (50µl)
DreamTaq polymerase	Water	32.75
	10X DreamTaq Buffer	5
	dNTP Mix (10mM each)	1
	Forward primer (5 µM)	5
	Reverse primer (5 µM)	5
	DreamTaq (5 U/µl)	0.25
	Template DNA	1
Phusion polymerase	Water	27.5
	5X Phusion High-Fidelity Buffer	10
	dNTP Mix (10mM each)	1
	Forward primer (5 μM)	5
	Reverse primer (5 µ*M)*	5
	Phusion (2 U/µl)	0.5
	Template DNA	1

Table 2.2.1.2: PCR program for DreamTaq and Phusion polymerase

Taq polymerase	Operation	Temperature	Time
DreamTaq polymerase			
	Denaturation	95°C	3 min
35 cycles	Denaturation	95°C	30 seconds
	Annealing	56°C	30 seconds
	Elongation	72°C	1 min/kb
	Elongation	72°C	10 min
Phusion polymerase			
	Denaturation	98°C	3 min
35 cycles	Denaturation	98°C	15 seconds
	Annealing	56°C	15 seconds
	Elongation	72°C	15 seconds/kb
	Elongation	72°C	5 min

Table 2.2.1.3: Primers used in PCR for sequencing and cloning

Experiment	Primer	Sequence (5'-3')
Sequencing		
	Forward 1	TCTGTTCTTCGTTCAAAACC
	Forward 2	CGGAAAAGATGTTTGGAAAG
	Forward 3	GACGCTCTCTGGCGTGCATG
	Forward 4	TCGAGTAGGACGACAAGC
	Forward 5	TTAATAAAGGAGTAATGATTGG
	Forward 6	AGATTTGCCGGATTGGTTC
	Reverse 1	ATTTTTTAATGAATCGTTGCAC
	Nar rev	CTTTCCAAACATCTTTTCCG
	pBAD_for	ACACTTTGCTATGCCATAGC
	pBAD_rev	TCAGGTGGGACCACCGC
Cloning		
	ABC_C-His-For	GATCAAGCTTTTAAGCGCCTTCTACCATATC
	ATPase_N-His-Rev	GATCGGTACCTAAGGAGGTTTCTAAA

2.2.2 Gel electrophoresis

Gel-electrophoresis was performed to determine the quality and purity of the PCR product. A 1% agarose gel was made with Gel-red™ (10 000x concentrate, Biotium) as fluorescent DNA stain, diluted to working concentration. When the gel had set, 5 µl of each PCR product was mixed with 1.5 µl loading dye (6X LD, Thermo Fisher Scientific, USA) and loaded onto the gel. GeneRuler 1kb (Thermo Fisher Scientific, USA) was used as reference DNA ladder. The gel was run under constant voltage set to 80V, and the gel was run for 1 hour before being scanned by a Molecular Imager® (Chemi Doc™ CRS+ Imaging System, Bio-Rad). Results were documented and analysed using the Image Lab™ software (Bio-Rad).

2.2.3 Sequencing

A subset of 21 isolates with varying MIC to Nar were chosen for amplification by PCR and further sequencing (Table 2.1.3). PCR procedure, reaction mixtures and programs used for the experiment are described in section 2.2.1. Primers for sequencing had been designed to anneal to the Nar operon at appropriate sites in the gene to promote sequence overlapping (Figure 2.2.3.1). As PCR did not provide satisfactory products for all the isolates, PCR was also run with High-Fidelity Phusion to get the products necessary for further sequencing.

Figure 2.2.3.1: Nucleotide sequence of Nar operon from isolate strain 2006-01-1700-1.

Nar operon

AGGAAATAATACGAACTAACAATGGAATCATAGGTTTAAATTTAAAATTATTTTTATTGACTGTTAAA
ATAAAAAAGAATATTATTGGAGCTGTAAAAGAACTTTGGTTTGAAACAAAGTATAAAGGGGTATTTTA
TGAACGATACGTATTGTATTGGTTCATAAAAAAGGTGCGTTTTGTTCAGACTGGAAAGCTCGTTATAA
AATCATGTCAAAAGAAAAGGGAGTTATGTACCATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAA
AAATTTGGTAAATTCCAGGCGTTGAAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTT
TATCGGACCAAATGGAGCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACG
AAGGAGATGTCCAAATATTCGGAAAAGATGTTTCGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTATTTATCAA
ACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTTGAACTTGATCCAAAGA
AAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGTTTGATTGCTGCACTTTCAGTTGAA
TCTGATCTGTATATTTTAGATGAACCGACTTCAGGACTAGATCCATTGATGGAAGCAGTATTCCAAGA
AGAAGTAGAAAAAATCAAAAATGATGGCAAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTG
AACGATTAGCAGATAAAGTAGCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAA
TTGCGTCATTTGACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCT
CTCTGGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCGATGA
ATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCGCCAACGCTCGAA
GATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAAAGAAAATGAATGAAAAATTT
GCGCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCGATTGGAAAAAAATAATTGTTTGGGTTTT
AGGTTTGGGTTTGTTCTCAGGAGCATATGTACCAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTT
TAGGGATGTTTGAAACGATGCAAAATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATA
GGTACGGATTATACTTTAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAAT
GATTATCTCAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGTTGATCAAT
CTTTTATTAGGTCTTTTAATCGGCGGACTCATGATGAGTTTTGGTGTAAAAACGATTGATGCCGAAGG
AGCTTTCTTGTTCGGAGGATCAATTGCATTGGCGGGAATTATCGGTGGTGTATTGGCACTTGTGATGT
CGCAGATTATGGCGACTTCTACTGGAGCAACCGGCTCGACATTAAGTCTTATAGGACTTTTGTATATC
GTGCGCGCTGGAACAGATGTGTCTAATCTTGATCTATCAATGTTCAATCCAATGGGATGGATTTACTT
GACCTATCCTTTCACAAAAAATAACTGGCTACCATTATTATTTGCTTTGATTTTTAGTCTTGTTTTTA
CCGTACTTGCGTTTGTGTTGGAAGAACATCGCGACATGGGCGCAGGTTATCTTCCTGAACGAGAAGGA
CGTGCGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAGTAAT
GATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATGGAGACATGCAAG
TCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTGGCGTTTCCATTGAAGAATCC
TTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCACAATCTTGCCAATCGCGGTGGTCAATAA
ATTATTTGCAGAAGAAACAAGACTGCATCTGAGTCAACTGTATGTAACGAAGATTACGCGAGGCCAAT
TATATTGGACAACGATATTTTTAGCTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGA
TTAGGTGGAACGGCGATTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGC
TGGATACAATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCGGCGGAATC
TTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCACGCTTACCGATGGAAGA
ATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCGTCTTCTTATTTGTCGGCTATTTAG
GATACAAACGCCGTGATATGGTAGAAGGCGCTTAAAAATTGAATTTCTAAAAAACCGTTCAGATTTAC
GAAAGTCGATTTGA

Coloured fields mark where primers anneal to operon; red: forward 2, yellow: forward 3, blue: forward 4: green: forward 5, orange: forward 6. Green nucleotides indicate ATPase protein, while purple nucleotides indicate ABC transporter protein.

Sequence alignment

Sequences were assembled for each operon and the complete operon sequences were compared using the Clustal omega multiple sequence alignment software (version 1.2.4, EMBL-EBI, Hinxton, UK).

2.2.4 Cloning

Molecular cloning can be referred to as the creation of recombinant DNA molecules (DNA formed from new combinations of genetic material) (Alberts et al. 2002). By isolating target DNA fragments, hereafter referred to as inserts, one can ligate and introduce the fragments into a cloning vector and as such create recombinant molecules. These may be further transformed into bacteria or other suitable hosts for propagation, analysis of sequence and/or expression of the resulting protein. In this study, the aim is to execute a successful cloning of the Nar operon into chemically competent cells of *E. coli* DH5α and the hypersensitive mutant *E. coli* DH5α Δ*acrAB* to test the function of the transporter protein.

Chemically competent cells

Competent cells refer to bacterial cells able to take up foreign DNA (Sigma-Aldrich 2017b). It is a prerequisite for the bacteria to undergo transformation. Transformation, as discussed in the introduction, is the process of which foreign DNA is introduced into a cell. Bacteria can be naturally competent, yet in the laboratory, more often either chemically or electro competent cells are made as it is a relatively easy and cheap method. While electro competent cells are washed free of salts and permeabilised by electroshock treatment, chemically competent cells are treated with TSS buffer (recipe in appendix 6.3) and a short heat shock. For the current study, cells were made competent through the latter process. Heat treating the cells at an appropriate temperature is thought to open transient pores in the membrane, allowing the plasmid to enter.

Bacterial cultures of *E. coli* DH5α and *E. coli* DH5α Δ*acrAB* in LB-broth were grown overnight at 37°C in a shaking incubator. The next morning 200 µl of each culture was suspended in two separate sterile Falcon tubes (totalling four tubes), with 20 ml LB-broth, and incubated in a shaking incubator at 37°C until OD_{600} reached 0.4 - 0.6. The tubes were then put on ice for 10 minutes to slow down the growth, before being centrifuged for 10 minutes at 3000*g* at 4°C. While tubes were being centrifuged, TSS buffer and sterile Eppendorf tubes were chilled on ice. After centrifugation, while still being kept on ice, the supernatant was removed and the remaining pellet suspended in chilled TSS buffer. Volume of TSS buffer equalled 10% of original culture amount. As original amount was 20 ml, 2 ml of chilled TSS buffer was added to each tube and the pellet was resuspended. The bacterial suspension was divided into aliquots in the chilled Eppendorf tubes and marked by which bacterial culture it contained (DH5α or

DH5α Δ*acrAB*). As transformation of the competent cells were to be done the following day, aliquots were frozen at -70°C.

PCR with primers for cloning

The Nar operon from 8 isolates (of the 21 isolates for sequencing, table 2.1.3) was amplified from genomic DNA by PCR using High-Fidelity Phusion polymerase and primers designed for cloning (see section 2.2.1 for full procedure). These primers were custom-made, and designed to amplify the entire Nar operon from the start codon of the first gene to the stop codon of the second gene and introduced important features for subsequent protein expression (table 2.2.4.1). When cloning primers anneal and flank the target gene, the nucleotide sequence sections, essential for the cloning process, are added and amplified along with the gene.

Table 2.2.4.1: Primers for cloning

Primers	Sequence (5'-3')
Cloning	
ABC_C-His-For	GATCGGTACCTAAGGAGGTTTCTAAATGACAGAAATTGTAAAAGTAC
ATPase_N-His-Rev	GATCAAGCTTTTAAGCGCCTTCTACCATATC

Coloured fields mark the following; yellow: leading sequence, green: restriction site KpnI, purple: restriction site HindIII, blue: Shine-Dalgarno sequence, orange: start codon, red: stop codon

The primer annealing at the start of the first gene include (from 5' to 3' direction) a leader sequence of four nucleotides (GATC), followed by six nucleotides defining the restriction site of KpnI and fifteen nucleotides introducing an optimized Shine-Dalgarno sequence in front of the cloned gene. This is followed by the ATG-start codon and a sequence that anneals appropriately to the start of the target sequence. Shine-Dalgarno sequence refers to a short section of the DNA that in mRNA helps recruit the ribosome for translation and protein synthesis in prokaryotes. The consensus sequence is AGGAGG, however it may vary some depending on the bacteria. In *E. coli* for example, the consensus sequence is AGGAGGU. The middle of the consensus sequence is generally located around 10 base pairs upstream of the start codon.

The primer annealing at the end of the target sequence is reverse complemented and contains (in 5' – 3' direction) a leader sequence of four nucleotides (GATC), followed by six nucleotides

defining the restriction site HindIII, a TAA stop-codon and a sequence that anneals properly to the end of the second gene of the Nar operon.

As successful molecular cloning depends on the correct DNA sequence being transferred, PCR for cloning was run with the High-Fidelity Phusion DNA polymerase in accordance with the protocol described in section 2.2.1. The amount and purity of the PCR products were analysed by gel-electrophoresis as described in section 2.2.2.

Digestion

Digestion refers to the process of cleaving the inserts and plasmid DNA by restriction enzymes. To remove residues of the PCR process before digestion, the products were purified using a QIAquick® PCR Purification Kit, (QIAGEN, Germany) and the amplicon concentration was evaluated using a NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific, USA).

A digestion reaction of 50 µl was prepared for both restriction enzymes for all the purified PCR products and the vector DNA (pBAD30). In addition, two separate control reactions were prepared for KpnI and HindIII, with the vector to estimate the digestion efficiency of the respective restriction enzymes. Full recipe of each digestion reaction is shown in table 2.2.4.2. As a rule of thumb, the mass (m) of DNA per reaction should be 500ng. Therefore, volume (V) of DNA necessary for the digestion reaction was adjusted accordingly by the formula $V = \frac{m}{\rho}$, where ρ is the mass concentration of the DNA sample in ng/µl.

Table 2.2.4.2: Digestion reaction mix

	Volume (µl)		
	Digestion reaction	**Control 1**	**Control 2**
FastDigest buffer 10x	5	5	5
Water	43 – x	44 – x	44 - x
DNA	x	x	x
KpnI	1	1	-
HindIII	1	-	1

When prepared, all reactions were incubated at 37°C for 1 hour, before their whole amount was run on a 1% agarose gel. The band pattern on the gel was analysed and compared to the 1kB GeneRuler DNA ladder to make sure the DNA had been properly digested (only one band should appear in each lane of the gel). Digestion products were extracted from the gel using the

QIAquick® Gel Extraction Kit (QIAGEN, Germany) according to the manufacturers' instructions.

Ligation

While the digestion enzymes cleave the DNA, a ligase is needed to combine the nucleotide sequences of the insert with the vector. A T4 ligase from the Rapid DNA ligation Kit is used. It creates a phosphodiester bond between the cleaved 5' and 3' ends of the insert and vector, thus, circularising the DNA. The concentration of DNA in a ligation reaction mix may affect the rate of ligation. As a rule of thumb, a molar ratio of 3:1 (insert:vector) is recommended. Amount of vector and insert for this reaction was calculated accordingly. The rest of the ligation process was performed as recommended by the manufacturer of the ligation kit used (Rapid DNA Ligation kit, Thermo Scientific, USA).

Transformation

First, chemically competent cells were thawed on ice. Five µl of ligation reaction was subsequently added to the cells. Two transformations were prepared for each of the inserts in addition to the control of only the purified vector pBAD30 which had been digested with both enzymes and put through a ligation reaction. The tubes with competent cells mixed with ligation reaction were incubated on ice for 10 minutes, then moved to 42^{o}C for a 40 second incubation before they were put on ice again for 2 minutes. To spur cell growth 1 ml LB medium was added and the tubes were incubated for an hour at 37^{o}C in a shaking incubator. Tubes were then centrifuged for 5 minutes at 6000*g*. Eight hundred µl of the supernatant was discarded and the pellet was resuspended in the remaining LB-medium. The suspension was then distributed on LB-agar plates supplemented with 50 µg/ml ampicillin (LB-Amp plates) and incubated overnight at 37^{o}C. LB plates have been made with ampicillin (Amp) because of the assumed, unstable nature of the vector pBAD30 within the cloned cell. In the absence of Amp, the vector carrying the Amp resistance is not necessary for the survival of the cell, and it would not be replicated with cell division. Cells without the vector would then outnumber the cells carrying the vector. Therefore, Ampi is added to the plates to present a selective pressure to ensure that cells do not survive unless they carry the vector that encodes Amp resistance. However, for the experiment of transformation, it was also specifically added to be able to select the bacteria that had taken up the plasmid from the majority that had not. If the bacterial suspension had been

plated on a normal LB-plate without Amp, the plate would have been overgrown with bacteria and it would not have been possible to separate the plasmid containing bacteria (transformants).

Colonies formed were subsequently tested by Colony PCR to identify transformants positive for introduction of the Nar operon in the correct position of the vector.

Colony PCR

After incubation, five colonies from each of the LB-amp plates were picked and separately suspended in 20 µl Milliq water in sterile Eppendorf tubes. The same loop used to suspend the colonies were used to inoculate new LB-amp plates for cultivation of the transformant. The plates were incubated at 37^{o}C overnight.

Inoculated Eppendorf tubes were thereafter boiled for 5 minutes to release DNA into the water and the supernatant was used as DNA template in the following PCR. As there would be a great deal of reactions run by Colony PCR, only 12.5 µl PCR reactions were prepared for each template. They were run with DreamTaq and analysed by Gel electrophoresis.

2.2.5 Cross-resistance

Cross-resistance is the ability of a resistance mechanism against a certain antibiotic to infer resistance to other related or unrelated compounds. Resistance to antibiotics can be determined in different ways, but in this study, the minimal inhibitory concentration (MIC) was used. As the name describes, MIC is determined by the lowest concentration of a chemical (antibiotic) that prevents visible growth of a bacterium. Here we want to test for cross-resistance in a subset of VRE isolates and *E. faecium* with varying Nar MIC. Additionally, the strains of *E. coli* DH5α and *E. coli* DH5α Δ*acrAB* containing the different recombinant Nar operons or empty vector were also tested. Antibiotics have been chosen for each of the tests as shown below (table 2.2.5.1).

Table 2.2.5.1: Antibiotic compounds used in this study

Method	Antibiotic compound	
MIC testing for ionophores		
	Narasin	Maduramicin
	Monensin	Lasolacid
	Salinomycin	
MIC testing of isolates collected in 2014		
	Narasin	Vancomycin
	Ampicillin	Erythromycin
	Virginiamycin	Gentamicin
	Streptomycin	Kanamycin
	Tetracycline	Chloramphenicol
	Bacitracin	Linezolid
MIC testing on EUVSEC plates		
	Sulfamethoxazole	Nalidixic acid
	Trimethoprim	Cefotaxime
	Ciprofloxacin	Chloramphenicol
	Tetracycline	Colistin
	Meropenem	Ampicillin
	Azithromycin	Gentamicin
Manual MIC testing		
	Ciprofloxacin	Colistin
	Nalidixic acid	Bacitracin
	Streptomycin	Trimethoprim
Horizontal gene transfer on EUVENC and VetMIC plates		
	Ampicillin	Vancomycin
	Erythromycin	Gentamicin
	Tetracycline	Narasin
	Chloramphenicol	Bacitracin
	Virginiamycin	Linezolid
	Teicoplanin	Quinuprostin/Dalfopristin
	Daptomycin	Ciprofloxacin
	Tigecycline	

MIC testing of isolates

A subset of 21 isolates (table 2.1.3) were tested for cross-resistance to the following ionophore antibiotics: Nar, Mon, Sal, Mad and Las. These antibiotics were in 2015 accepted for use as coccidiostats in Norwegian broiler chicken production (VKM 2015). Yet, two of them are used in Norway (per 2015). Nar for rearing of broiler chickens and Mon for rearing of turkeys. As such, they were tested to see if resistance to Nar could be related to resistance of the other available ionophores.

For MIC-testing, a broth microdilution method was performed. The test is performed using a 96 U bottom well plate as seen in figure 2.2.5.1 with columns (1-12) and rows (A-H) indicating each well (Nunc™ MicroWell™ Plates with Nunclon™ Delta Surface). Each of the five antibiotics was added to their individual row and in a decreasing concentration along the rows (Table 2.2.5.2).

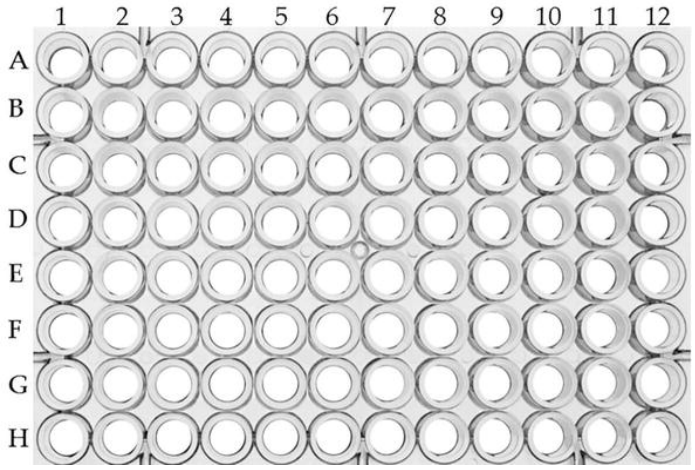

Figure 2.2.5.1: An example of the 96U well plate used for manual MIC testing

First, isolates were streaked onto plates of blood-agar for incubation overnight at 37°C. Then, bacterial suspensions of the desired strains were prepared by suspending a small number of colonies from the plates in physiologic salt water to a McFarland value of 0.5. From these tubes, 30 µl was transferred to 10 ml Mueller-Hinton (MH) broth, tubes were vortexed and the bacterial suspensions were distributed to their individual well plate in 100 µl aliquots.

Secondly, two-fold dilution series of the five antibiotics were prepared from concentrated stocks. Appropriate MIC values were decided based on observations from earlier papers, but they were also adjusted, appropriately during the experiments. All substances were dissolved in Methanol; thus, they were all kept on ice during preparation and distribution to prevent evaporation. Stocks of antibiotics were as follows: Nar 1.6 mg/ml, Mon 1.6 mg/ml, Sal 1.6 mg/ml, Mad 1.6mg/ml and Las 100 µg/ml. Two µl of every antibiotic dilution was added to their specific well to achieve MIC values descending from 32 to 0.125 µg/ml. For Las, however, the MIC values descended from 4 to 0.125 µg/ml. Wells in column 10 for Nar, Mon, Sal, and Mad and column 7 for Las were used as broth control (without the addition of antibiotic) (table 2.2.5.2).

Table 2.2.5.2: Antibiotic concentration (µg/ml) in wells on the VetMIC plate

Well no.	Antibiotic concentration (µg/ml)	
	Nar (A), Mon (B), Sal (C), Mad (D)	Las (E)
1	32	4
2	16	2
3	8	1
4	4	0.5
5	2	0.25
6	1	0.125
7	0.5	0
8	0.25	-
9	0.125	-
10	0	-

Plates were then stacked (three by three) in lidded plastic boxes with moist paper (a 'humidity chamber') to inhibit evaporation and incubated at 35°C for 20-22 hours.

MIC testing of isolates collected in 2014

Fourteen isolates collected in 2014 were tested for a susceptibility against a panel of antibiotics that included Nar and Van. Two sets of plates were used for this experiment; 96U well plates and VetMIC plates. MIC testing on 96U well plates was performed as described previously under 'MIC testing of isolates', screened with a two-fold dilution of Nar.

Both Nar and Van were, however, tested on VetMIC plates for enterococci (ver. 3, Art.nr. E395100, SVA). A bacterial suspension was prepared as described previously under 'MIC testing of isolates'. It was further distributed into the plates in 50 µl aliquots by a Sensititre AIM^{TM} Automated Inoculation Delivery System (ThermoFisher Scientific, USA). Plates were placed in a 'humidity chamber' and incubated at 35°C for 18-20 hours.

MIC testing on EUVSEC plates

The *E. coli* DH5α and *E. coli* DH5α Δ*acrAB* strains containing the different recombinant Nar operon plasmids as well as the empty vector control pBAD30 were tested on Sensititre® Trek EUVSEC plates. These plates are dry microdilution plates standardised for *E. coli* strains containing freeze-dried compounds of the antibiotic as describe in table 2.2.5.1. Concentrations have been prepared and quality controlled by the manufacturer.

Bacterial suspensions were prepared basically as previously described for the isolates collected in 2014 and *E. faecium*. However, 20 µl of bacterial suspension in physiological salt water was suspended in two separate tubes of 11ml MH-cation adjusted broth (Sensititre™ Cation Adjusted MH-broth, Thermo Scientific, USA). Both tubes were added 0.01% carbenicillin (50 µg/ml) and one was additionally added 0.01% L (+) arabinose. As previously described, arabinose works as an inducer of the P_{BAD} promoter. Therefore, it is introduced here to promote expression of the Nar operon to test if that would have any effect on resistance and thereby the MIC. The un-induced culture was also used for the experiment as it, ideally, works as a negative control if the promoter is 'tight' (i.e. it does not generate expression of the gene without an inducer, and in turn will not influence resistance level).

All suspensions were distributed into Sensititre® Trek EUVSEC plates in 50 µl aliquots using a Sensititre AIM™ Automated Inoculation Delivery System (ThermoFisher Scientific, USA). Subsequently, plates were covered by plastic film to prevent evaporation and incubated for 18-22 hours at 35°C.

Manual MIC testing

To expand this resistance evaluation of the constructs, more antibiotic compounds were included in MIC testing. However, only the hypersensitive strain of DH5α Δ*acrAB* containing the constructs were tested at this point. The bacterial suspension was prepared as describe above in 'MIC testing on EUVSEC plates' with carbenicillin and L (+) arabinose, yet the rest of the method was executed as in 'MIC testing of isolates' with manual preparation of the antibiotic dilution series and 100 µl distribution into 96 U well plates. The following antibiotics were used: Cip, Nal, Tet, streptomycin (Str) Col, bacitracin (Bac) and Tri.

As construct MIC for Str, Col and Bac were unknown the dilution series had to be tried out and adjusted accordingly. Bac was replaced with Tri after testing four of the constructs. The two plates of each cloned construct were placed in 'humidity chambers' and incubated at 35°C for 18-22 hours.

2.2.5 Horizontal gene transfer

As described in the introduction of this study, horizontal gene transfer (HGT), refers to the transmission of genetic material between bacterial strains or species. The aim of the current

method will be to test for possible transfer of the Nar resistance gene from a donor strain to a recipient strain. Additionally, the method will seek to determine if presence of Nar in the environment may induce HGT.

Donor strains and a recipient strain were chosen for the attempt of transferring the Nar resistant gene based on their resistance profile. The strains were screened against a panel of antibiotics to have a resistance profile for each of them. This panel could then be used to determine if a conjugational transfer had succeeded. Tet and Van was chosen for selection and counter selection. Two VRE strains with the Nar resistance gene and susceptibility to Tet, were chosen as the donor strains. While a Nar sensitive *E. faecium* strain (2012-70-76-8), with high (MIC >128) resistance to Tet, was chosen as the recipient strain.

The donor and recipient strains were inoculated on Blood agar plates and incubated overnight at 37°C. The following day single colonies were picked from each strain and suspended in 5 ml buffered MH- broth. All tubes with bacterial suspension were further incubated in a shaking incubator at 37°C overnight. Cultures were then diluted 1:100 in 5 ml buffered MH-broth and growth was continued until OD_{600} of 0.5. Forty µl of a donor culture was mixed with 360 µl of recipient culture. So, two cultures were prepared, one for each donor mixed with the recipient. Additionally, three controls were made. Two control mixtures were made with 40 µl of each donor culture in separate tubes and 360 µl of buffered MH-broth. The third control was prepared with 40 µl of buffered MH-broth, and 360 µl of the recipient culture. From each of the five cultures, 20 µl was spotted on MH-agar plates, with and without supplementation of Nar (4 µg/ml). Spots were left to dry, then plates were incubated overnight at 37°C. The following day, each of the spots were transferred to 10 ml MH-broth, and a ten-fold dilution series was prepared in MH-broth; 1x; 10x; 100x; 1000x; 10,000x; 100,000x; 1000,000x. Five µl of every dilution was spotted in duplicates on pre-prepared MH-agar plates, supplemented with either Tet 8 µg/ml or Tet 8 µg/ml and Van 4 µg/ml. Spots were left to dry and incubated at 37°C overnight.

A subset of the conjugants (bacterial strains with the transferred plasmid) were further tested for antibiotic susceptibility on VetMIC and EUVENC plates. Testing on VetMIC plates was performed as described in 'MIC testing of isolates collected in 2014'. Test on EUVENC plates was performed as described in 'MIC testing on EUVSEC plates', but the EUVSEC plates

(designed for *E. coli)* were substituted by EUVENC plates designed for enterococci. As for *E. coli* and EUVSEC plates, evidence have shown that enterococci are able to acquire resistance to the antimicrobials in these plates (Hammerum et al, 2010).

3. Results

During an 11-year period between the years 2003-2013, 988 isolates of *E. faecium* were collected by NVI under the Norm-Vet program. These isolates were sampled mostly from broiler chickens and turkeys, and collected either from the environment of the animal houses, or from meat or caecal content of the animals. These isolates were routinely screened for antibiotic resistance against a panel of at least 12 antimicrobial compounds in microbroth dilution assays and the results were reported as minimum inhibitory concentration (MIC) of the relevant antibiotics. In this screen, the MIC for the ionophore Nar varied greatly between isolates (0.12-32 µg/ml). Four isolates of the 988 had a MIC for Van of 256 µg/ml, and rest of the isolates had a MIC for Van of 4 µg/ml or below. A smaller set of isolates (86) were also isolated on selective plates supplemented with Van. These were screened for antibiotic resistance against 12 antimicrobial compounds. All 86 isolates had a MIC for Van of 128 µg/ml, while MIC for Nar varied between 0.5-16 µg/ml. However, most (98%) presented a MIC of either 4 or 8 µg/ml.

The Van resistant isolates were screened for the presence of the *vanA* gene (providing resistance to Van) and the genes encoding the ATPase and the ABC transporter of the Nar operon. In addition, a subset of van susceptible isolates, with varying Nar MIC (0.12-32 µg/ml) were screened for the presence of the Nar genes. The *vanA* gene was detected in all van resistant isolates. The genes of the Nar resistance operon were detected in isolates with MIC for Nar ranging from 0.5-32 µg/ml.

3.1 Sequencing

In order to understand the diverse MIC values associated with different isolates of *E. faecium* a subset of the isolates was chosen for sequencing to determine if differences in the nucleotide or amino acid sequences of the Nar resistance genes could explain the different MIC values. The subset of isolates that was sequenced and the alignment of the resulting nucleotide sequences is shown in appendix 6.2. Alignments of the translated amino acid sequences are

shown in figure 3.1.1 (ATPase) and 3.1.2 (ABC transporter). Results from sequencing of the amplified Nar operon from genomic DNA were translated into amino acid sequences using ExPASy translate tool (Swiss Institute of Bioinformatics, SIB (2017)).

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A)
2004-01-1343-1      MTEIVKVQGLQKKFGKFQALKDVSFTVNAGEVVGFIGPNGAGKSTTIRTLLGIINRDEGD
2006-01-1154-1      MTEIVKVQGLQKKFGKFQALKDVSFTVNAGEVVGFIGPNGAGKSTTIRTLLGIINRDEGD
2004-01-850-1       MTEIVKVQGLQKKFGKFQALKDVSFTVNAGEVVGFIGPNGAGKSTTIRTLLGIINRDEGD
2006-01-1700-1      MTEIVKVQGLQKKFGKFQALKDVSFTVNAGEVVGFIGPNGAGKSTTIRTLLGIINRDEGD
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2006-01-1152-1      MTEIVKVQGLQKKFGKFQALKDVSFTVNAGEVVGFIGPNGAGKSTTIRTLLGIINRDEGD
2004-01-1301-1      MTEIVKVEGLQKKFGKFQALKDVSFTVNAGEVVGFIGPNGAGKSTTIRTLLGIINRDEGD
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2006-01-1700-1      VQIFGKDVWKDSLEIHKRISYVPGDVALWGSLTGGEIIDLFIKLHGGGSKAKRDYLIKRF
2006-01-1145-1      VQIFGKDVWKDSLEIHKRISYVPGDVALWGSLTGGEIIDLFIKLHGGGSKAKRDYLIKRF
2006-01-1190-1      VQIFGKDVWKDSLEIHKRISYVPGDVALWGSLTGGEIIDLFIKLHGGGSKAKRDYLIKRF
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2006-01-1152-1      VQIFGKDVWKDSLEIHKRISYVPGDVALWGSLTGGEIIDLFIKLHGGGSKAKRDYLIKRF
2004-01-1301-1      VQIFGKDVWKDSLEIHKRISYVPGDVALWGSLTGGEIIDLFIKLHGGGSKAKRDYLIKRF
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2004-01-1343-1      ELDPKKKAKGYSKGNRQKVGLIAALSVESDLYILDEPTSGLDPLMEAVFQEEVEKIKNDG
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2004-01-850-1       ELDPKKKAKGYSKGNRQKVGLIAALSVESDLYILDEPTSGLDPLMEAVFQEEVEKIKNDG
2006-01-1700-1      ELDPKKKAKGYSKGNRQKVGLIAALSVESDLYILDEPTSGLDPLMEAVFQEEVEKIKNDG
2006-01-1145-1      ELDPKKKAKGYSKGNRQKVGLIAALSVESDLYILDEPTSGLDPLMEAVFQEEVEKIKNDG
2006-01-1190-1      ELDPKKKAKGYSKGNRQKVGLIAALSVESDLYILDEPTSGLDPLMEAVFQEEVEKIKNDG
2006-01-2608-1      ELDPKKKAKGYSKGNRQKVGLIAALSVESDLYILDEPTSGLDPLMEAVFQEEVEKIKNDG
2006-01-1111-1      ELDPKKKAKGYSKGNRQKVGLIAALSVESDLYILDEPTSGLDPLMEAVFQEEVEKIKNDG
2006-01-1151        ELDPKKKAKGYSKGNRQKVGLIAALSVESDLYILDEPTSGLDPLMEAVFQEEVEKIKNDG
2006-01-1402        ELDPKKKAKGYSKGNRQKVGLIAALSVESDLYILDEPTSGLDPLMEAVFQEEVEKIKNDG
2013-01-3934        ELDPKKKAKGYSKGNRQKVGLIAALSVESDLYILDEPTSGLDPLMEAVFQEEVEKIKNDG
2004-01-1251-1      ELDPKKKAKGYSKGNRQKVGLIAALSVESDLYILDEPTSGLDPLMEAVFQEEVEKIKNDG
2006-01-3433        ELDPKKKAKGYSKGNRQKVGLIAALSVESDLYILDEPTSGLDPLMEAVFQEEVEKIKNDG
2006-01-1131-1      ELDPKKKAKGYSKGNRQKVGLIAALSVESDLYILDEPTSGLDPLMEAVFQEEVEKIKNDG
2006-01-1152-1      ELDPKKKAKGYSKGNRQKVGLIAALSVESDLYILDEPTSGLDPLMEAVFQEEVEKIKNDG
2004-01-1301-1      ELDPKKKAKGYSKGNRQKVGLIAALSVESDLYILDEPTSGLDPLMEAVFQEEVEKIKNDG
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2004-01-1343-1      KAILLSSHILSEVERLADKVAIIRRGEVVETGTLDELRHLTRSTVTLVTKGDIEKLATLS
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2004-01-850-1       KAILLSSHILSEVERLADKVAIIRRGEVVETGTLDELRHLTRSTVTLVTKGDIEKLATLS
2006-01-1700-1      KAILLSSHILSEVERLADKVAIIRRGEVVETGTLDELRHLTRSTVTLVTKGDIEKLATLS
2006-01-1145-1      KAILLSSHILSEVERLADKVAIIRRGEVVETGTLDELRHLTRSTVTLVTKGDIEKLATLS
2006-01-1190-1      KAILLSSHILSEVERLADKVAIIRRGEVVETGTLDELRHLTRSTVTLVTKGDIEKLATLS
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2006-01-1111-1      KAILLSSHILSEVERLADKVAIIRRGEVVETGTLDELRHLTRSTVTLVTKGDIEKLATLS
2006-01-1151        KAILLSSHILSEVERLADKVAIIRRGEVVETGTLDELRHLTRSTVTLVTKGDIEKLATLS
2006-01-1402        KAILLSSHILSEVERLADKVAIIRRGEVVETGTLDELRHLTRSTVTLVTKGDIEKLATLS
2013-01-3934        KAILLSSHILSEVERLADKVAIIRRGEVVETGTLDELRHLTRSTVTLVTKGDIEKLATLS
2004-01-1251-1      KAILLSSHILSEVERLADKVAIIRRGEVVETGTLDELRHLTRSTVTLVTKGDIEKLATLS
2006-01-3433        KAILLSSHILSEVERLADKVAIIRRGEVVETGTLDELRHLTRSTVTLVTKGDIEKLATLS
2006-01-1131-1      KAILLSSHILSEVERLADKVAIIRRGEVVETGTLDELRHLTRSTVTLVTKGDIEKLATLS
2006-01-1152-1      KAILLSSHILSEVERLADKVAIIRRGEVVETGTLDELRHLTRSTVTLVTKGDIEKLATLS
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2006-01-1700-1      GVHDFVQKDGKATFSADNEAMNTILTEATKLGVIKIESVPPTLEDLFMRHYEG
2006-01-1145-1      GVHDFVQKDGKATFSADNEAMNTILTEATKLGVIKIESVPPTLEDLFMRHYEG
2006-01-1190-1      GVHDFVQKDGKATFSADNEAMNTILTEATKLGVIKIESVPPTLEDLFMRHYEG
2006-01-2608-1      GVHDFVQKDGKATFSADNEAMNTILTEATKLGVIKIESVPPTLEDLFMRHYEG
2006-01-1111-1      GVHDFVQKDGKATFSADNEAMNTILTEATKLGVIKIESVPPTLEDLFMRHYEG
2006-01-1151        GVHDFVQKDGKATFSADNEAMNTILTEATKLGVIKIESVPPTLEDLFMRHYEG
2006-01-1402        GVHDFVQKDGKATFSADNEAMNTILTEATKLGVIKIESVPPTLEDLFMRHYEG
2013-01-3934        GVHDFVQKDGKATFSADNEAMNTILTEATKLGVIKIESVPPTLEDLFMRHYEG
2004-01-1251-1      GVHDFVQKDGKATFSADNEAMNTILTEATKLGVIKIESVPPTLEDLFMRHYEG
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2006-01-1131-1      GVHDFVQKDGKATFSADNEAMNTILTEATKLGVIKIESVPPTLEDLFMRHYEG
2006-01-1152-1      GVHDFVQKDGKATFSADNEAMNTILTEATKLGVIKIESVPPTLEDLFMRHYEG
2004-01-1301-1      GVHDFVQKDGKATFSADNEAMNTILTEATKLGVIKIESVPPTLEDLFMRHYEG
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B)

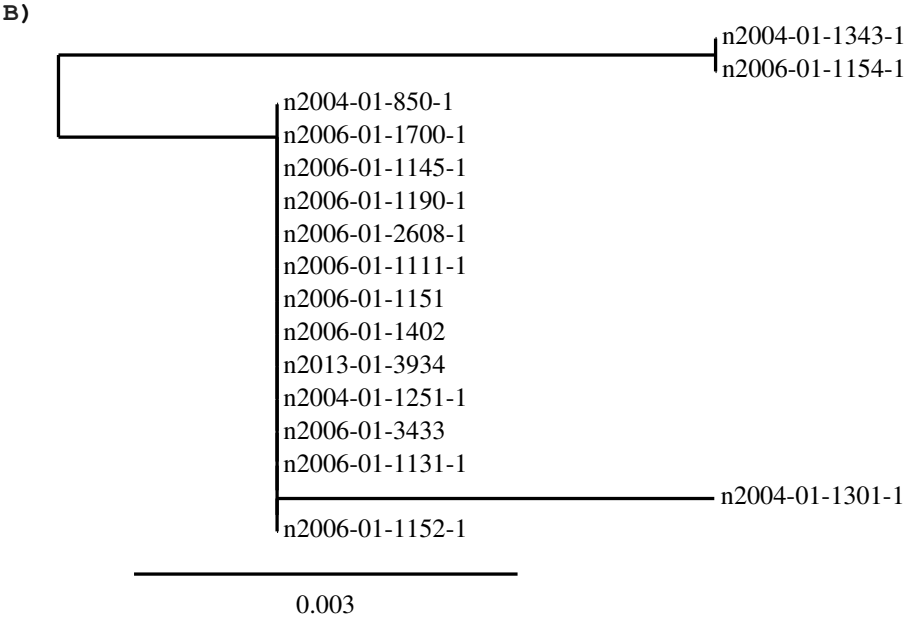

*Figure 3.1.1: A) Alignment of the amino acid sequence of the ATPase encoded by the Nar operon from 16 bacterial strains B) Phylogenetic tree of aligned ATPase protein sequences from 16 bacterial strains *(asterisk) indicates positions which have a single, fully conserved residue. : (colon) indicates conservation between groups of similar properties. The same colour in a column indicates identical amino acids*

Results from sequencing and alignment show that there are few differences between the strains of varying Nar susceptibility. Most residues are fully conserved, both for the ATPase protein and the ABC transporter protein. While one difference could be seen in the ABC transporter,

three differences were found in the ATPase protein sequence (all indicated in colour). The phylogenetic trees presented in figure 3.1.1B and 3.1.2B provide an illustration of the evolutionary relationship between the ATPase and ABC transporter genes, respectively, of the different strains.

A)

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2004-01-850-1       MNEKFARWNVLFIQYVKRDWKKIIVWVLGLGLFSGAYVPAFEEIAKGQGLLGMFETMQNP
2006-01-1700-1      MNEKFARWNVLFIQYVKRDWKKIIVWVLGLGLFSGAYVPAFEEIAKGQGLLGMFETMQNP
2006-01-1145-1      MNEKFARWNVLFIQYVKRDWKKIIVWVLGLGLFSGAYVPAFEEIAKGQGLLGMFETMQNP
2006-01-2608-1      MNEKFARWNVLFIQYVKRDWKKIIVWVLGLGLFSGAYVPAFEEIAKGQGLLGMFETMQNP
2006-01-1151        MNEKFARWNVLFIQYVKRDWKKIIVWVLGLGLFSGAYVPAFEEIAKGQGLLGMFETMQNP
2006-01-1402        MNEKFARWNVLFIQYVKRDWKKIIVWVLGLGLFSGAYVPAFEEIAKGQGLLGMFETMQNP
2013-01-3934        MNEKFARWNVLFIQYVKRDWKKIIVWVLGLGLFSGAYVPAFEEIAKGQGLLGMFETMQNP
2006-01-1154-1      MNEKFARWNVLFIQYVKRDWKKIIVWVLGLGLFSGAYVPAFEEIAKGQGLLGMFETMQNP
2006-01-1190-1      MNEKFARWNVLFIQYVKRDWKKIIVWVLGLGLFSGAFVPAFEEIAKGQGLLGMFETMQNP
2006-01-1111-1      MNEKFARWNVLFIQYVKRDWKKIIVWVLGLGLFSGAFVPAFEEIAKGQGLLGMFETMQNP
2004-01-1251-1      MNEKFARWNVLFIQYVKRDWKKIIVWVLGLGLFSGAFVPAFEEIAKGQGLLGMFETMQNP
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2006-01-1152-1      MNEKFARWNVLFIQYVKRDWKKIIVWVLGLGLFSGAFVPAFEEIAKGQGLLGMFETMQNP
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2006-01-2608-1      AMISMVGPTPIKIGTDYTLGAMYAQEMLLFCGLFAMIISALHVVSHTRKEEELGLTELVR
2006-01-1151        AMISMVGPTPIKIGTDYTLGAMYAQEMLLFCGLFAMIISALHVVSHTRKEEELGLTELVR
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2006-01-1152-1      AMISMVGPTPIKIGTDYTLGAMYAQEMLLFCGLFAMIISALHVVSHTRKEEELGLTELVR
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2006-01-1700-1      SFRVGRQANSLAVISEMLLINLLLGLLIGGLMMSFGVKTIDAEGAFLFGGSIALAGIIGG
2006-01-1145-1      SFRVGRQANSLAVISEMLLINLLLGLLIGGLMMSFGVKTIDAEGAFLFGGSIALAGIIGG
2006-01-2608-1      SFRVGRQANSLAVISEMLLINLLLGLLIGGLMMSFGVKTIDAEGAFLFGGSIALAGIIGG
2006-01-1151        SFRVGRQANSLAVISEMLLINLLLGLLIGGLMMSFGVKTIDAEGAFLFGGSIALAGIIGG
2006-01-1402        SFRVGRQANSLAVISEMLLINLLLGLLIGGLMMSFGVKTIDAEGAFLFGGSIALAGIIGG
2013-01-3934        SFRVGRQANSLAVISEMLLINLLLGLLIGGLMMSFGVKTIDAEGAFLFGGSIALAGIIGG
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2006-01-1190-1      SFRVGRQANSLAVISEMLLINLLLGLLIGGLMMSFGVKTIDAEGAFLFGGSIALAGIIGG
2006-01-1111-1      SFRVGRQANSLAVISEMLLINLLLGLLIGGLMMSFGVKTIDAEGAFLFGGSIALAGIIGG
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2004-01-1343-1      SFRVGRQANSLAVISEMLLINLLLGLLIGGLMMSFGVKTIDAEGAFLFGGSIALAGIIGG
2006-01-3433        SFRVGRQANSLAVISEMLLINLLLGLLIGGLMMSFGVKTIDAEGAFLFGGSIALAGIIGG
2006-01-1131-1      SFRVGRQANSLAVISEMLLINLLLGLLIGGLMMSFGVKTIDAEGAFLFGGSIALAGIIGG
2006-01-1152-1      SFRVGRQANSLAVISEMLLINLLLGLLIGGLMMSFGVKTIDAEGAFLFGGSIALAGIIGG
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2004-01-850-1       VLALVMSQIMATSTGATGSTLSLIGLLYIVRAGTDVSNLDLSMFNPMGWIYLTYPFTKNN
2006-01-1700-1      VLALVMSQIMATSTGATGSTLSLIGLLYIVRAGTDVSNLDLSMFNPMGWIYLTYPFTKNN
2006-01-1145-1      VLALVMSQIMATSTGATGSTLSLIGLLYIVRAGTDVSNLDLSMFNPMGWIYLTYPFTKNN
2006-01-2608-1      VLALVMSQIMATSTGATGSTLSLIGLLYIVRAGTDVSNLDLSMFNPMGWIYLTYPFTKNN
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2006-01-1151        VLALVMSQIMATSTGATGSTLSLIGLLYIVRAGTDVSNLDLSMFNPMGWIYLTYPFTKNN
2006-01-1402        VLALVMSQIMATSTGATGSTLSLIGLLYIVRAGTDVSNLDLSMFNPMGWIYLTYPFTKNN
2013-01-3934        VLALVMSQIMATSTGATGSTLSLIGLLYIVRAGTDVSNLDLSMFNPMGWIYLTYPFTKNN
2006-01-1154-1      VLALVMSQIMATSTGATGSTLSLIGLLYIVRAGTDVSNLDLSMFNPMGWIYLTYPFTKNN
2006-01-1190-1      VLALVMSQIMATSTGATGSTLSLIGLLYIVRAGTDVSNLDLSMFNPMGWIYLTYPFTKNN
2006-01-1111-1      VLALVMSQIMATSTGATGSTLSLIGLLYIVRAGTDVSNLDLSMFNPMGWIYLTYPFTKNN
2004-01-1251-1      VLALVMSQIMATSTGATGSTLSLIGLLYIVRAGTDVSNLDLSMFNPMGWIYLTYPFTKNN
2004-01-1301-1      VLALVMSQIMATSTGATGSTLSLIGLLYIVRAGTDVSNLDLSMFNPMGWIYLTYPFTKNN
2004-01-1343-1      VLALVMSQIMATSTGATGSTLSLIGLLYIVRAGTDVSNLDLSMFNPMGWIYLTYPFTKNN
2006-01-3433        VLALVMSQIMATSTGATGSTLSLIGLLYIVRAGTDVSNLDLSMFNPMGWIYLTYPFTKNN
2006-01-1131-1      VLALVMSQIMATSTGATGSTLSLIGLLYIVRAGTDVSNLDLSMFNPMGWIYLTYPFTKNN
2006-01-1152-1      VLALVMSQIMATSTGATGSTLSLIGLLYIVRAGTDVSNLDLSMFNPMGWIYLTYPFTKNN
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2004-01-850-1       WLPLLFALIFSLVFTVLAFVLEEHRDMGAGYLPEREGRATAKKSLLSVPGLFFKINKGVM
2006-01-1700-1      WLPLLFALIFSLVFTVLAFVLEEHRDMGAGYLPEREGRATAKKSLLSVPGLFFKINKGVM
2006-01-1145-1      WLPLLFALIFSLVFTVLAFVLEEHRDMGAGYLPEREGRATAKKSLLSVPGLFFKINKGVM
2006-01-2608-1      WLPLLFALIFSLVFTVLAFVLEEHRDMGAGYLPEREGRATAKKSLLSVPGLFFKINKGVM
2006-01-1151        WLPLLFALIFSLVFTVLAFVLEEHRDMGAGYLPEREGRATAKKSLLSVPGLFFKINKGVM
2006-01-1402        WLPLLFALIFSLVFTVLAFVLEEHRDMGAGYLPEREGRATAKKSLLSVPGLFFKINKGVM
2013-01-3934        WLPLLFALIFSLVFTVLAFVLEEHRDMGAGYLPEREGRATAKKSLLSVPGLFFKINKGVM
2006-01-1154-1      WLPLLFALIFSLVFTVLAFVLEEHRDMGAGYLPEREGRATAKKSLLSVPGLFFKINKGVM
2006-01-1190-1      WLPLLFALIFSLVFTVLAFVLEEHRDMGAGYLPEREGRATAKKSLLSVPGLFFKINKGVM
2006-01-1111-1      WLPLLFALIFSLVFTVLAFVLEEHRDMGAGYLPEREGRATAKKSLLSVPGLFFKINKGVM
2004-01-1251-1      WLPLLFALIFSLVFTVLAFVLEEHRDMGAGYLPEREGRATAKKSLLSVPGLFFKINKGVM
2004-01-1301-1      WLPLLFALIFSLVFTVLAFVLEEHRDMGAGYLPEREGRATAKKSLLSVPGLFFKINKGVM
2004-01-1343-1      WLPLLFALIFSLVFTVLAFVLEEHRDMGAGYLPEREGRATAKKSLLSVPGLFFKINKGVM
2006-01-3433        WLPLLFALIFSLVFTVLAFVLEEHRDMGAGYLPEREGRATAKKSLLSVPGLFFKINKGVM
2006-01-1131-1      WLPLLFALIFSLVFTVLAFVLEEHRDMGAGYLPEREGRATAKKSLLSVPGLFFKINKGVM
2006-01-1152-1      WLPLLFALIFSLVFTVLAFVLEEHRDMGAGYLPEREGRATAKKSLLSVPGLFFKINKGVM
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2004-01-850-1       IGWLIAFVVMGAAYGSIYGDMQVFLGGNELMKQMFTQSGVSIEESFTATIMMVMIGLVTI
2006-01-1700-1      IGWLIAFVVMGAAYGSIYGDMQVFLGGNELMKQMFTQSGVSIEESFTATIMMVMIGLVTI
2006-01-1145-1      IGWLIAFVVMGAAYGSIYGDMQVFLGGNELMKQMFTQSGVSIEESFTATIMMVMIGLVTI
2006-01-2608-1      IGWLIAFVVMGAAYGSIYGDMQVFLGGNELMKQMFTQSGVSIEESFTATIMMVMIGLVTI
2006-01-1151        IGWLIAFVVMGAAYGSIYGDMQVFLGGNELMKQMFTQSGVSIEESFTATIMMVMIGLVTI
2006-01-1402        IGWLIAFVVMGAAYGSIYGDMQVFLGGNELMKQMFTQSGVSIEESFTATIMMVMIGLVTI
2013-01-3934        IGWLIAFVVMGAAYGSIYGDMQVFLGGNELMKQMFTQSGVSIEESFTATIMMVMIGLVTI
2006-01-1154-1      IGWLIAFVVMGAAYGSIYGDMQVFLGGNELMKQMFTQSGVSIEESFTATIMMVMIGLVTI
2006-01-1190-1      IGWLIAFVVMGAAYGSIYGDMQVFLGGNELMKQMFTQSGVSIEESFTATIMMVMIGLVTI
2006-01-1111-1      IGWLIAFVVMGAAYGSIYGDMQVFLGGNELMKQMFTQSGVSIEESFTATIMMVMIGLVTI
2004-01-1251-1      IGWLIAFVVMGAAYGSIYGDMQVFLGGNELMKQMFTQSGVSIEESFTATIMMVMIGLVTI
2004-01-1301-1      IGWLIAFVVMGAAYGSIYGDMQVFLGGNELMKQMFTQSGVSIEESFTATIMMVMIGLVTI
2004-01-1343-1      IGWLIAFVVMGAAYGSIYGDMQVFLGGNELMKQMFTQSGVSIEESFTATIMMVMIGLVTI
2006-01-3433        IGWLIAFVVMGAAYGSIYGDMQVFLGGNELMKQMFTQSGVSIEESFTATIMMVMIGLVTI
2006-01-1131-1      IGWLIAFVVMGAAYGSIYGDMQVFLGGNELMKQMFTQSGVSIEESFTATIMMVMIGLVTI
2006-01-1152-1      IGWLIAFVVMGAAYGSIYGDMQVFLGGNELMKQMFTQSGVSIEESFTATIMMVMIGLVTI
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2004-01-850-1       LPIAVVNKLFAEETRLHLSQLYVTKITRGQLYWTTIFLAIFAGVVGIGLASAGLGGTAIS
2006-01-1700-1      LPIAVVNKLFAEETRLHLSQLYVTKITRGQLYWTTIFLAIFAGVVGIGLASAGLGGTAIS
2006-01-1145-1      LPIAVVNKLFAEETRLHLSQLYVTKITRGQLYWTTIFLAIFAGVVGIGLASAGLGGTAIS
2006-01-2608-1      LPIAVVNKLFAEETRLHLSQLYVTKITRGQLYWTTIFLAIFAGVVGIGLASAGLGGTAIS
2006-01-1151        LPIAVVNKLFAEETRLHLSQLYVTKITRGQLYWTTIFLAIFAGVVGIGLASAGLGGTAIS
2006-01-1402        LPIAVVNKLFAEETRLHLSQLYVTKITRGQLYWTTIFLAIFAGVVGIGLASAGLGGTAIS
2013-01-3934        LPIAVVNKLFAEETRLHLSQLYVTKITRGQLYWTTIFLAIFAGVVGIGLASAGLGGTAIS
2006-01-1154-1      LPIAVVNKLFAEETRLHLSQLYVTKITRGQLYWTTIFLAIFAGVVGIGLASAGLGGTAIS
2006-01-1190-1      LPIAVVNKLFAEETRLHLSQLYVTKITRGQLYWTTIFLAIFAGVVGIGLASAGLGGTAIS
2006-01-1111-1      LPIAVVNKLFAEETRLHLSQLYVTKITRGQLYWTTIFLAIFAGVVGIGLASAGLGGTAIS
2004-01-1251-1      LPIAVVNKLFAEETRLHLSQLYVTKITRGQLYWTTIFLAIFAGVVGIGLASAGLGGTAIS
2004-01-1301-1      LPIAVVNKLFAEETRLHLSQLYVTKITRGQLYWTTIFLAIFAGVVGIGLASAGLGGTAIS
2004-01-1343-1      LPIAVVNKLFAEETRLHLSQLYVTKITRGQLYWTTIFLAIFAGVVGIGLASAGLGGTAIS
2006-01-3433        LPIAVVNKLFAEETRLHLSQLYVTKITRGQLYWTTIFLAIFAGVVGIGLASAGLGGTAIS
2006-01-1131-1      LPIAVVNKLFAEETRLHLSQLYVTKITRGQLYWTTIFLAIFAGVVGIGLASAGLGGTAIS
2006-01-1152-1      LPIAVVNKLFAEETRLHLSQLYVTKITRGQLYWTTIFLAIFAGVVGIGLASAGLGGTAIS
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2004-01-850-1       AMKNESTMDLTDFLAAGYNFLPSILFYIGLAALALGWLPKFGKVIYAYLGYSFALNYFGG
2006-01-1700-1      AMKNESTMDLTDFLAAGYNFLPSILFYIGLAALALGWLPKFGKVIYAYLGYSFALNYFGG
2006-01-1145-1      AMKNESTMDLTDFLAAGYNFLPSILFYIGLAALALGWLPKFGKVIYAYLGYSFALNYFGG
2006-01-2608-1      AMKNESTMDLTDFLAAGYNFLPSILFYIGLAALALGWLPKFGKVIYAYLGYSFALNYFGG
2006-01-1151        AMKNESTMDLTDFLAAGYNFLPSILFYIGLAALALGWLPKFGKVIYAYLGYSFALNYFGG
2006-01-1402        AMKNESTMDLTDFLAAGYNFLPSILFYIGLAALALGWLPKFGKVIYAYLGYSFALNYFGG
2013-01-3934        AMKNESTMDLTDFLAAGYNFLPSILFYIGLAALALGWLPKFGKVIYAYLGYSFALNYFGG
2006-01-1154-1      AMKNESTMDLTDFLAAGYNFLPSILFYIGLAALALGWLPKFGKVIYAYLGYSFALNYFGG
2006-01-1190-1      AMKNESTMDLTDFLAAGYNFLPSILFYIGLAALALGWLPKFGKVIYAYLGYSFALNYFGG
2006-01-1111-1      AMKNESTMDLTDFLAAGYNFLPSILFYIGLAALALGWLPKFGKVIYAYLGYSFALNYFGG
2004-01-1251-1      AMKNESTMDLTDFLAAGYNFLPSILFYIGLAALALGWLPKFGKVIYAYLGYSFALNYFGG
2004-01-1301-1      AMKNESTMDLTDFLAAGYNFLPSILFYIGLAALALGWLPKFGKVIYAYLGYSFALNYFGG
2004-01-1343-1      AMKNESTMDLTDFLAAGYNFLPSILFYIGLAALALGWLPKFGKVIYAYLGYSFALNYFGG
2006-01-3433        AMKNESTMDLTDFLAAGYNFLPSILFYIGLAALALGWLPKFGKVIYAYLGYSFALNYFGG
2006-01-1131-1      AMKNESTMDLTDFLAAGYNFLPSILFYIGLAALALGWLPKFGKVIYAYLGYSFALNYFGG
2006-01-1152-1      AMKNESTMDLTDFLAAGYNFLPSILFYIGLAALALGWLPKFGKVIYAYLGYSFALNYFGG
                    ************************************************************

2004-01-850-1       ILDLPDWFSKTAIQSWIPRLPMEEFDGTIFAVITVISIVFLFVGYLGYKRRDMVEGA
2006-01-1700-1      ILDLPDWFSKTAIQSWIPRLPMEEFDGTIFAVITVISIVFLFVGYLGYKRRDMVEGA
2006-01-1145-1      ILDLPDWFSKTAIQSWIPRLPMEEFDGTIFAVITVISIVFLFVGYLGYKRRDMVEGA
2006-01-2608-1      ILDLPDWFSKTAIQSWIPRLPMEEFDGTIFAVITVISIVFLFVGYLGYKRRDMVEGA
2006-01-1151        ILDLPDWFSKTAIQSWIPRLPMEEFDGTIFAVITVISIVFLFVGYLGYKRRDMVEGA
2006-01-1402        ILDLPDWFSKTAIQSWIPRLPMEEFDGTIFAVITVISIVFLFVGYLGYKRRDMVEGA
2013-01-3934        ILDLPDWFSKTAIQSWIPRLPMEEFDGTIFAVITVISIVFLFVGYLGYKRRDMVEGA
2006-01-1154-1      ILDLPDWFSKTAIQSWIPRLPMEEFDGTIFAVITVISIVFLFVGYLGYKRRDMVEGA
2006-01-1190-1      ILDLPDWFSKTAIQSWIPRLPMEEFDGTIFAVITVISIVFLFVGYLGYKRRDMVEGA
2006-01-1111-1      ILDLPDWFSKTAIQSWIPRLPMEEFDGTIFAVITVISIVFLFVGYLGYKRRDMVEGA
2004-01-1251-1      ILDLPDWFSKTAIQSWIPRLPMEEFDGTIFAVITVISIVFLFVGYLGYKRRDMVEGA
2004-01-1301-1      ILDLPDWFSKTAIQSWIPRLPMEEFDGTIFAVITVISIVFLFVGYLGYKRRDMVEGA
2004-01-1343-1      ILDLPDWFSKTAIQSWIPRLPMEEFDGTIFAVITVISIVFLFVGYLGYKRRDMVEGA
2006-01-3433        ILDLPDWFSKTAIQSWIPRLPMEEFDGTIFAVITVISIVFLFVGYLGYKRRDMVEGA
2006-01-1131-1      ILDLPDWFSKTAIQSWIPRLPMEEFDGTIFAVITVISIVFLFVGYLGYKRRDMVEGA
2006-01-1152-1      ILDLPDWFSKTAIQSWIPRLPMEEFDGTIFAVITVISIVFLFVGYLGYKRRDMVEGA
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B)

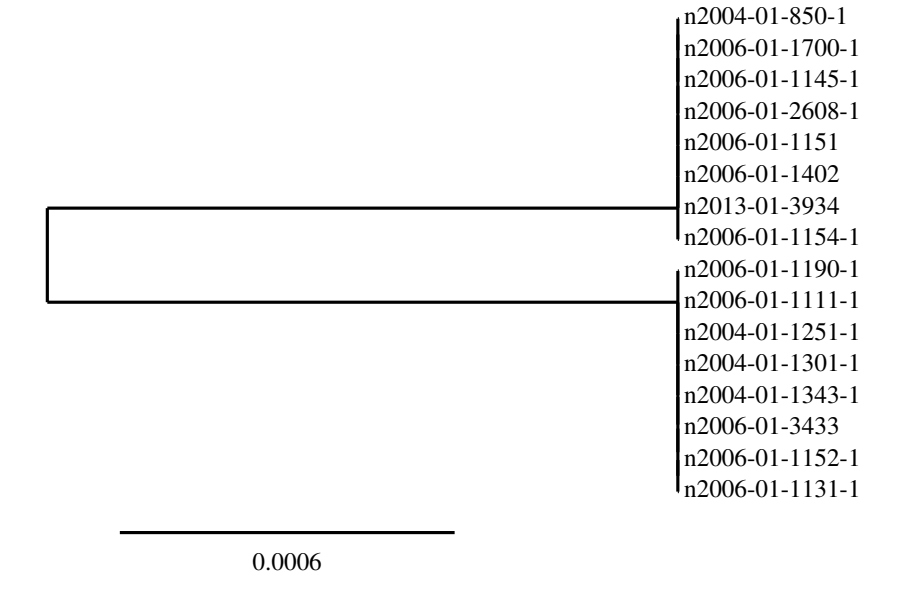

*Figure 3.1.2: A) Alignment of ABC transporter protein sequences from 16 bacterial strains B) Phylogenetic tree of aligned ABC transporter protein sequences from 16 bacterial strains *(asterisk) indicates positions which have a single, fully conserved residue. : (colon) indicates conservation between groups of strongly similar properties. The same colour in a column indicates identical amino acids*

Additionally, figure 3.1.3 presents an alignment of the upstream region of the Nar operons from three different isolates. The start codon of the ATPase protein is marked in purple. The Nar promoter region from the isolate 2013-01-4826 seem to stand out from the other two. Five differences in the nucleotide sequence can be seen in the figure below.

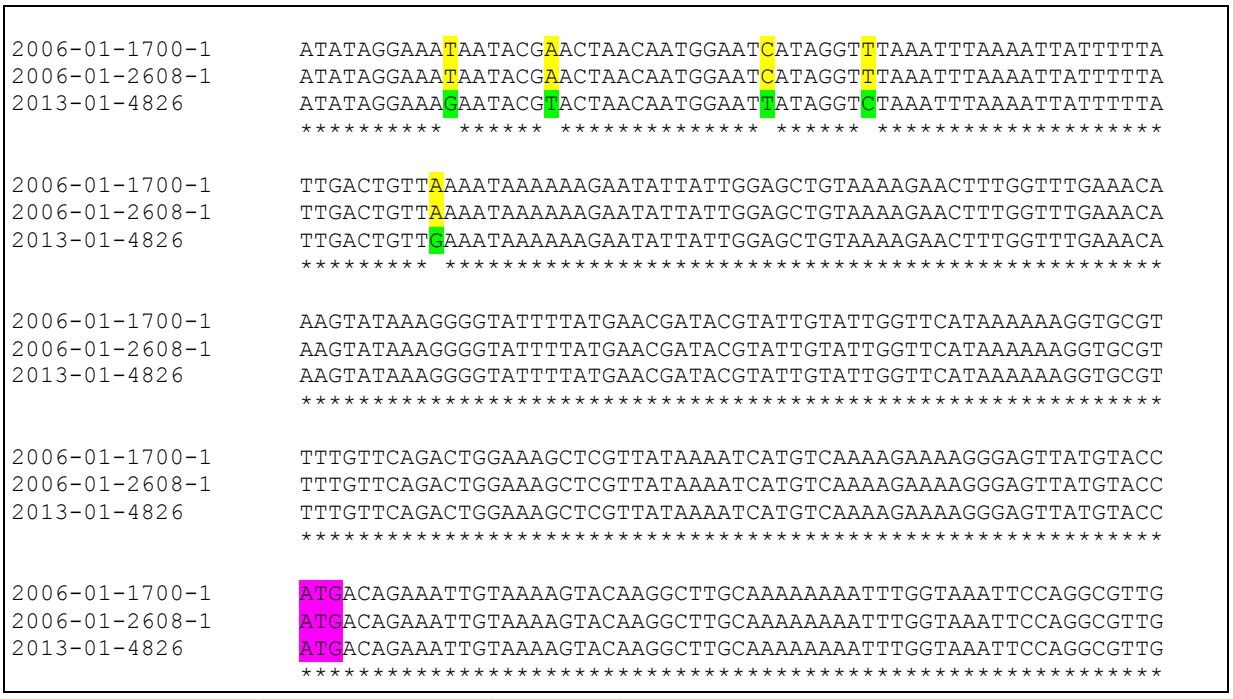

Figure 3.1.3: Alignment of the upstream region of Nar operon from three isolates

3.2 Cloning

A total of 9 operons were cloned. Alignments show that 7 operons were cloned correctly since the alignments between the sequence of the cloned gene and the template (the genomic copy in *E. faecium*) were identical. However, one cloned operon, SVA-01-233, contained two difference from the template sequence, and another cloned operon (2013-01-4826) could not be aligned due to a short template sequence. See appendix 6.3 for alignments.

3.3 Cross-resistance

3.3.1 MIC testing

As some Nar resistant isolates were collected from turkeys, which are not reared with a diet supplemented with Nar, but with Mon, it is possible that the Nar operon can provide resistance against Mon and other ionophores in addition to Nar. Alternatively, it is possible that mutations

in the proteins of the efflux pump can change its substrate preference and switch from being a Nar transporter to being a Mon transporter. As such, a subset of *E. faecium* strains were tested against all ionophores permitted for use in Norway. Results from MIC test of 21 bacterial isolates are presented in the table below (table 3.3.1). These isolates had previously been tested for Nar and Van susceptibility, and these test results were included here for comparison.

Table 3.3.1: Cross-resistance testing of bacterial isolates

Isolate no.	MIC susceptibility						
	Previous test		Current				
	Nar	Van	Nar	Mon	Sal	Mad	Las
2004-01-850-1	32	2	4	2	4	16	2
2006-01-1700-1	1	1	8	2	8	32	2
2006-01-1145-1	2	2	8	8	8	32	4
2006-01-1190-1	0,5	1	0.5	8	2	4	8
2006-01-2608-1	0,5	4	1	>32	1	4	1
2006-01-1115-1	32	1	4	4	4	16	2
2006-01-1111-1	32	1	8	8	8	32	2
2006-01-1117-1	32	1	8	8	8	32	2
2006-01-1151	8	128	8	4	8	16	2
2006-01-1402	16	128	8	8	8	32	2
2013-01-3934	4	128	8	4	8	16	2
2013-01-4826	0,5	128	4	8	8	32	2
2004-01-1251-1	0,5	2	8	>32	8	32	2
2004-01-1301-1	4	1	8	4	8	32	2
2004-01-1343-1	4	1	8	4	8	32	4
2006-01-3433	8	128	8	8	8	32	2
2009-01-1808-4	8	128	8	4	8	16	2
2006-01-1131-1	16	1	8	8	8	32	2
2006-01-1148-1	16	1	4	4	8	16	2
2006-01-1154-1	32	1	4	8	4	16	2
2006-01-1152-1	32	1	8	8	8	32	2

Narasin (Nar), vancomycin (Van), monensin (Mon), salinomycin (Sal), maduramicin (Mad), lasolacid (Las).

Results show that, for most of the isolates, Nar susceptibility found in the previous test does not correspond with the Nar susceptibility found in experiments of the current study. The Nar susceptibility in this study varies between 0.5 to 8, while tests performed during the initial screening of the isolates found a MIC variation for the same isolates between 0.5 and 32. All isolates presented high MIC values (32) towards Mad, except for two isolates (2006-01-1190-1 and 2006-01-2608) that presented a lower MIC of 4. Additionally, two isolates (2006-01-2608-1 and 2004-01-1251-1) presented high MIC (>32) towards Mon, while other isolates had MIC of ≤8. All isolates presented a Las MIC between 1 and 8, however most presented a MIC

of 2 (17 of 21). Similar MIC could be seen with Sal; between 1 and 8. Yet for Sal, most isolates presented a MIC of 8 (16 of 21).

When comparing the MIC values of the different ionophores for the same strains, it is possible to see two isolates standing out, 2006-01-1190-1 and 2006-01-2608-1, as highlighted in table 3.3.1. As mentioned, both have a Nar MIC of ≤1, and at the same time these are also the only two isolates with a Sal MIC ≤2. Both isolates also present a Mad MIC of 4, while all other isolates had a Mad MIC of ≥16. This indicates that a low Nar MIC is associated with low MICs for Sal and Mad and that there can be cross-resistance between these ionophores.

Table 3.3.2: MIC testing of isolates collected in 2014

Strains	Previously		Current		
	Nar	Van	Nar		Van
			Manually	*VetMIC*	*VetMIC*
Isolates					
2014-01-7513	Nd	Nd	8	8	128
2014-01-7512	Nd	Nd	8	8	128
2014-01-7483	Nd	Nd	8	8	128
2014-01-7479	Nd	Nd	8	8	128
2014-01-7377	Nd	Nd	8	8	128
2014-01-7207	Nd	Nd	8	2	128
2014-01-7050	Nd	Nd	8	4	128
2014-01-6934	Nd	Nd	8	4	128
2014-01-4539	Nd	Nd	8	8	128
2014-01-2995	Nd	Nd	8	4	128
2014-01-1914	Nd	Nd	8	8	128
2014-01-1741	Nd	Nd	8	4	128
2014-01-7394	Nd	Nd	8	4	128
2014-01-7584	Nd	Nd	8	4	128
Controls					
2013-01-5191	4	128	8	8	128
2013-01-4826	0,5	128	4	1	64

Nd: Not detected. Fourteen isolates from 2014 in addition to two controls were tested for Nar and Van resistance on VetMIC plates (Nar and Van) and on manual administered plates with Nar.

Fourteen isolates collected in 2014, and two control strains were screened for susceptibility of a panel of antibiotics that included Nar and Van. Two sets of plates were used. Screening on VetMIC plates included both Nar and Van, while 96U well plates contained only Nar (manually). When tested manually, all isolates presented a Nar MIC of 8. When tested on VetMIC plates 50% of strains presented the same Nar MIC as for manual test, while one strain

(2014-01-7207) had a Nar MIC of 2 compared to 8 in the manual test. One control (2013-01-4826) had a Nar MIC of 1 compared to 4 (manually) and 0.5 (previous test). The other control (2013-01-5191) presented a Nar MIC of 8 by VetMIC test, compared to 8 (manually) and 4 (previous test). All strains (100%) presented a Van MIC of 128 when tested on VetMIC plates. Controls, 2013-01-5191 and 2013-01-4826, had Van MIC 128 and 64, respectively. Table 3.3.3 presents the labelling of the constructs throughout the rest of this study.

Table 3.3.3: Labelling of constructs

***E. faecium* containing the Nar operon**	*Vector (pBAD30) containing inserts amplified from E. faecium*
SVA-01-233	pBAD-SVA
2006-01-1190-1	pBAD-1190
2006-01-2608-1	pBAD-2608
2006-01-1151	pBAD-1151
2013-01-4826	pBAD-4826
2004-01-1251-1	pBAD-1251
2006-01-1131-1	pBAD-1131
2006-01-1154-1	pBAD-1154
2006-01-1152-1	pBAD-1152
Control containing empty vector	
pBAD30	pBAD30

Table 3.3.4: MIC testing of DH5α containing a plasmid with the Nar operon under control of an arabinose inducible promoter or the empty vector control pBAD30

DH5α transformants	MIC susceptibility (µg/mL)													
	Smx	Tri	Cip	Tet	Mero	Azi	Nal	Fot	Chl	Tgc	Taz	Col	Amp	Gm
pBAD30														
No inducer	≤8	≤0.25	0.03	≤2	≤0.03	8	32	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.03	≤2	≤0.03	8	32	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
pBAD-SVA														
No inducer	≤8	≤0.25	0.03	≤2	≤0.03	8	32	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.03	≤2	≤0.03	8	32	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
pBAD-1190														
No inducer	≤8	≤0.25	0.03	≤2	≤0.03	4	32	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.03	≤2	≤0.03	4	32	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
pBAD-2608														
No inducer	≤8	≤0.25	0.03	≤2	≤0.03	8	32	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.03	≤2	≤0.03	8	32	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
pBAD-1151														
No inducer	≤8	≤0.25	0.03	≤2	≤0.03	8	32	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.03	≤2	≤0.03	8	32	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
pBAD-4826														
No inducer	≤8	≤0.25	0.03	≤2	≤0.03	4	32	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.03	≤2	≤0.03	4	32	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
pBAD-1251														
No inducer	≤8	≤0.25	0.03	≤2	≤0.03	8	32	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.03	≤2	≤0.03	8	32	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
pBAD-1131														
No inducer	≤8	≤0.25	0.03	≤2	≤0.03	8	32	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.06	≤2	≤0.03	8	32	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
pBAD-1154														
No inducer	≤8	≤0.25	0.06	≤2	≤0.03	8	64	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.03	≤2	≤0.03	8	32	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
pBAD-1152														
No inducer	≤8	≤0.25	0.06	≤2	≤0.03	8	64	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.03	≤2	≤0.03	8	64	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5

Tested on Sensititre® Ttrek EUVSEC. The inducer L (+) arabinose (0.01%) was added to one suspension of each isolate Sulfamethoxazole (Smx), Trimethoprim (Tri), Ciprofloxacin (Cip), Tetracyclin (Tet), Meropenem (Mero), Azithromycin (Azi), Nalidixic acid (Nal), Cefotaxime (Fot), Chloramphenicol (Chl), Tigecycline (Tgc), Ceftazidime (Taz), Colistin (Col), Ampicillin (Amp), Gentamicin (Gm).

Each of the Nar operons (in plasmid) from the nine different strains, in addition to the empty vector (pBAD30), were introduced into separate competent cells of DH5α and the hypersensitive DH5α Δ*acrAB*. All strains were tested on EUVSEC plates, and results of the screening are presented in table 3.3.4 (DH5α) and 3.3.5 (DH5α Δ*acrAB*). Some differences were found between induced and un-induced DH5α clones containing Nar operon from pBAD-1131, pBAD-1154, and pBAD-1152 for Cip. Without inducer, pBAD-1154 and pBAD-1152 presented Cip MIC of 0.06, while it was 0.03 with inducer. The opposite was observed for pBAD-1131. With inducer Cip MIC was 0.06, and without inducer it was 0.03. Additionally, pBAD-1154 presented differences between induced and un-induced clones for Nal. A higher Nal MIC was seen for the un-induced (64), than the induced (32). The pBAD-1152 transformant

also presented a higher Nal MIC (64), than the rest of the transformants (32). Results show no difference between transformants, with or without the addition of the inducer (L (+) arabinose) for the following antibiotic compounds; Smx, Tmr, Tet, Mero, Fot, Chl, Tgc, Taz, Col, Amp and Gm.

Table 3.3.5: Manual MIC testing of DH5α ΔacrAB containing plasmid carrying Nar operon or the empty vector pBAD30

DH5α Δ*acrAB* transformants	MIC susceptibility (µg/mL													
	Smx	**Tri**	**Cip**	**Tet**	**Mero**	**Azi**	**Nal**	**Fot**	**Chl**	**Tgc**	**Taz**	**Col**	**Amp**	**Gm**
pBAD30														
No inducer	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
pBAD-SVA														
No inducer	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
pBAD-1190														
No inducer	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
pBAD-2608														
No inducer	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
pBAD-1151														
No inducer	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
pBAD-4826														
No inducer	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
pBAD-1251														
No inducer *	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
L (+) arabinose	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
pBAD-1131														
No inducer	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
pBAD-1154														
No inducer	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
pBAD-1152														
No inducer	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5
L (+) arabinose	≤8	≤0.25	0.015	≤2	≤0.03	≤2	16	≤0.25	≤8	≤0.25	≤0.5	≤1	>64	≤0.5

*Tested on Sensititre® Ttrek EUVSEC. The inducer L (+) arabinose (0.01%) was added to one suspension of each isolate Sulfamethoxazole (Smx), Trimethoprim (Tri), Ciprofloxacin (Cip), Tetracyclin (Tet), Meropenem (Mero), Azithromycin (Azi), Nalidixic acid (Nal), Cefotaxime (Fot), Chloramphenicol (Chl), Tigecycline (Tgc), Ceftazidime (Taz) Colistin (Col), Ampicillin (Amp), Gentamicin (Gm). * Suspension contaminated*

Similarly, to the DH5α transformants cross-resistance test for hypersensitive DH5α Δ*acrAB* transformants found no differences between the induced and un-induced. No growth was observed in the wells containing the following antibiotics; Smx, Tmr, Tet, Mero, Azi, Fot, Chl, Tgc, Taz, Col and Gm. There was only growth of all transformants, including control in three of the fourteen antibiotics; Cip, Nal and Amp, all transformants presenting MIC of 0.015, 16 and >64, respectively.

Table 3.3.6: DH5α ΔacrAB containing Nar operon were tested for cross-resistance of selected antibiotics

MIC susceptibility (µg/mL)							
DH5α Δ*acrAB* transformants	**Cip**	**Nal**	**Tet**	**Str**	**Col**	**Bac**	**Tri**
pBAD30							
No inducer	0.01	12.5	3.13	15.63	0.78	>200	0.08
L (+) arabinose	0.01	12.5	3.13	15.63	0.78	>200	0.08
pBAD-SVA							
No inducer	0.01	12.5	3.13	Nd	0.78	>200	Nd
L (+) arabinose	0.01	12.5	3.13	Nd	0.78	>200	Nd
pBAD-1190							
No inducer	0.01	12.5	3.13	15.63	0.78	Nd	0.08
L (+) arabinose	0.01	12.5	3.13	15.63	0.78	Nd	0.08
pBAD-2608							
No inducer	0.02	12.5	3.13	15.63	0.78	Nd	0.08
L (+) arabinose	0.01	12.5	3.13	7.81	0.78	Nd	0.08
pBAD-1151							
No inducer	0.01	12.5	3.13	7.81	0.78	Nd	0.08
L (+) arabinose	0.01	12.5	3.13	15.63	0.78	Nd	0.08
pBAD-4826							
No inducer	0.01	12.5	3.13	15.63	0.78	Nd	0.08
L (+) arabinose	0.01	12.5	3.13	31.25	0.78	Nd	0.08
pBAD-1251							
No inducer	0.01	12.5	3.13	7.81	0.78	Nd	0.08
L (+) arabinose	0.01	12.5	3.13	7.81	0.78	Nd	0.08
pBAD-1131							
No inducer	0.01	12.5	3.13	15.63	0.78	Nd	0.08
L (+) arabinose	0.01	12.5	3.13	15.63	0.78	Nd	0.08
pBAD-1154							
No inducer	0.01	12.5	3.13	Nd	0.78	>200	Nd
L (+) arabinose	0.01	12.5	3.13	Nd	0.78	>200	Nd
pBAD-1152							
No inducer	0.01	12.5	3.13	Nd	0.78	>200	Nd
L (+) arabinose	0.01	12.5	3.13	Nd	0.78	>200	Nd

Nd: Not detected. Ciprofloxacin (Cip), Nalidixic acid (Nal), Tetracycline (Tet), Streptomycin (Str), Colistin (Col), Bacitracin (Bac), Trimethoprim (Tri)

Results from cross-resistance testing of hypersensitive DH5α Δ*acrAB* to Cip, Nal, Tet, Str, Col, Bac and Tri are presented in table 3.3.5. No difference in susceptibility was found between the transformants, with or without inducer, for Nal, Tet, Col or Tri. All strains were highly susceptible to Cip (0.01), yet one strain (pBAD-2608) had susceptibility of (0.02). Susceptibility to Str varied between the strains, from 7.81 to 31.25. Yet, 9 out of 14 tested had a MIC of 15.63, while only one strain (pBAD-4826) had a MIC of 31.25 when induced. Four strains, three of which contained the vector carrying the Nar operon, while one contained empty vector (pBAD30), were screened for Bac susceptibility. All four strains, both un-induced and induced, had a high-level of resistance to Bac (>200).

3.4 Horizontal gene transfer (conjugation)

Bacteria were grown, and the culture was split up onto two different MH-agar plates. One of which was supplemented with Nar, and one with no supplementation. This was done to determine if the Nar resistance could be transferred between two strains of *E. faecium* and if Nar-pressure could enhance conjugation-frequency. Tet was used to select for the recipient strain whereas Van was used to select for the plasmid. Therefore, if the experiment is successful one would expect to find growth of only the recipient and potential conjugants on the plates with Tet (as recipient strain is resistant to Tet, while donors are not), and on the plates with Tet and Van one would expect to find only strains resistant to both agents (i.e. conjugants carrying both the Tet and Van resistance gene).

Table 3.4.1 presents results from HGT experiment. No growth was found for the controls on MH-agar with Tet and Van (i.e. the recipient culture with MH broth, and the two donor cultures with MH broth). Yet, one of the controls (2014-01-7512) had some growth (5 cfu) on the plate supplemented with Tet. No growth was found on plates supplemented with Tet, or Tet and Van for all conjugants grown previously on MH-agar with Nar. Growth was found, however, on plates supplemented with Tet and Van for conjugants prepared with VRE strain 2014-01-7513 (11 cfu) and 2014-01-7512 (1 cfu) grown previously on MH-agar plate without Nar.

Table 3.4.1: Colony forming units (cfu) on MH agar supplemented with tetracycline (Tet) or vancomycin (Van)) and tetracycline (Tet), inoculated with conjugation cultures grown on duplicate plates of MH agar with or without Nar.

Suppl.	Strains	MH-agar (cfu)		MH-agar with Nar (4 µg/ml) (cfu)	
		1	2	1	2
Tet	**Recipient**				
	E. faecium 2012-70-76-8	1.5×10^7	1.5×10^7	<1	<1
	Control (VRE donor strain)				
	2014-01-7512	4	1	<1	<1
	2014-01-7513	<1	<1	<1	<1
	Conjugant				
	Recipient + 2014-01-7512	8.0×10^6	2.9×10^6	<1	<1
	Recipient + 2014-01-7513	4.8×10^6	1.1×10^7	<1	<1
Tet + van	**Conjugant**				
	Recipient + 2014-01-7512	1	<1	<1	<1
	Recipient + 2014-01-7513	6	5	<1	<1

Suppl.: Supplement

Results from antibiotic susceptibility testing of a subset of conjugants are presented in table 3.4.2. The tests show that recipient strain of *E. faecium* was resistant to Tet, Erythromycin (Ery), Cm and Syn, while VRE donor strains presented only resistance to Van and Nar. In comparison, all conjugants presented resistance to Van, Tet, Ery, Cm, Tei and Syn. Interestingly, not all conjugants were resistant to Nar even though they were resistant to Van.

Table 3.4.2: Recipient strain (E. faecium), both donor strains of VRE (A: 2014-01-7513, B: 2014-01-7512) and 5 colonies from Tet + Van plate were inoculated in EUVENC plates (E. faecium and conjugate strains) and VetMIC plates (conjugants and VRE donor strains)

		MIC values														
		Van	Tet	Nar	Ery	Gm	Amp	Cip	Chl	Lz	Tei	Syn	Dap	Tgc	Bac	Vi
Pre-conjugation	**Recipient strain**															
	E. faecium 2012-70-76-8	2	64	0.5	>128	≤8	4	0.5	8	2	≤0.5	8	1	0.06	2U	16
	Donor strain															
	2014-01-7513	128	0.5	8	0.5	4	4	Nd	4	1	Nd	Nd	Nd	Nd	2U	0.5
	2014-01-7512	128	0.5	8	0.5	4	4	Nd	4	1	Nd	Nd	Nd	Nd	2U	2
Post- conjugation	**Conjugant**															
	2014-01-7513															
	cfu 1	>128	128	4	>128	≤8	4	0.5	8	2	64	8	1	0.12	4U	8
	cfu 2	>128	128	0.5	>128	≤8	4	0.5	8	2	>64	8	1	0.12	4U	8
	cfu 3	>128	64	Nd	128	≤8	4	0.5	8	2	64	8	1	0.12	Nd	Nd
	cfu 4	>128	128	Nd	>128	16	4	0.5	8	2	64	8	1	0.12	Nd	Nd
	2014-01-7512															
	cfu 1	>128	128	0.5	>128	16	4	0.5	8	2	>64	8	1	0.12	2U	8

Vancomycin (Van), Tetracycline (Tet), Narasin (Nar), Erythromycin (Ery), Gentamicin (Gm), Ampicillin (Amp), Ciprofloxacin (Cip), Chloramphenicol (Chl), Linezolid (Lz), Teicoplanin (Tei), Quinupristin/dalfopristin (Syn), Daptomycin (Dap), Tigecycline (Tgc), Bacitracin (Bac), Virginiamycin (Vi). *Nd: Not detected. Yellow fields indicate strain resistance to antibiotic*

4. DISCUSSION

Occurrence of mutations in nucleotide and amino acid sequence of Nar operon

Sixteen isolates were examined for differences in nucleotide and amino acid sequences of the Nar operon to determine if this could explain their observed variation in Nar resistance. Results showed a total of twelve nucleotide position differences in the Nar operon when compared to the wild-type, mediating three amino acid substitutions in the ATPase protein and one in the ABC transporter protein.

Mutations in nucleotide sequences do not necessarily confer alterations in the coded protein, yet synonymous mutations can influence gene expression (Kudla et al. 2009). A synonymous mutation refers to the substitution of one nucleotide with another in a gene that do not alter the encoded amino acid sequence. This is possible because of codon degeneracy - amino acids in nucleotide sequences encoded by several codons (triplet of nucleotides). Hence, a synonymous mutation would not lead to an alteration in the amino acid chain and protein. An alteration in gene expression, however, may occur.

The ABC gene encoding the transporter porin only differed in one position of the amino acid sequence (position 37). Fifty percent of the genes encoded a Phenylalanine, whereas the other half encoded a Tyrosine. These amino acids are quite similar as they both carry a hydrophobic side chain (Sigma-Aldrich 2017a), consequently, an alteration of the protein is not certain.

At the start of the ATPase protein, the amino acid glutamine at position 8 had been replaced with a proline (Q8P). Glutamine is a relatively long polar amino acid, whilst proline is a highly rigid amino acid. Such a substitution often introduces sterical constraints in the folding of the protein. Indeed, this mutation could theoretically alter the function of the encoded efflux pump system and the cells antimicrobial susceptibility. In two other strains, the amino acid methionine had been replaced by isoleucine M261I. Isoleucine is a somewhat bulkier amino acid than methionine. Yet, as both are aliphatic amino acids (Addgene 2017), they present similar properties in the protein and are therefore unlikely to cause a significant change in the protein. The mutation of isoleucine to threonine (I274T) however, could mediate an effect on antimicrobial susceptibility, as they are quite different from each other (i.e. isoleucine is aliphatic and threonine has a polar uncharged side group (Addgene 2017).

The two genes of the Nar operon encode different parts of the efflux pump. While the ABC transporter is the porin structure (i.e. the tunnel through the membrane which transports the compounds), the ATPase converts the energy needed to transport the compounds through the porin (Ames & Joshi 1990). A mutation in the ATPase could alter binding of ATP and induce a loss-of-function of the efflux pump (Jones & George 1999). Thereby, reducing its ability to transport compounds through the membrane. If a mutation occurs in the porin itself, it might lead to a structural change of the protein, making the transport channel tighter/looser and/or affecting the mobility of the transmembrane segments (Srinivasan et al. 2014). Thus, making it more difficult, or easier for the compounds to be exported through the porin.

Notwithstanding the mutations of the amino acid chains, all strains displayed a high degree of similarity throughout the Nar operon. Yet, all strains presenting mutational substitutions were compared with their observed Nar susceptibility. Two strains (2006-01-1190-1 and 2006-01-2608-1) presented lower MICs for Nar and Sal then the rest. They also presented different amino acids from each other and the wild-type in position 37 of the porin. As they have a similar MIC to each other, yet contain different amino acids, it is likely that the mutational substitution in this position is not important for the function of the protein. Rest of findings showed that no other strains had a notable difference in MIC from the rest, either in the previous tests or the current. However, susceptibility to Nar did not vary in this study as it had in the previous test. None presented high levels of resistance (16 μg/ml or 32 μg/ml) as was previously found. Though, this correlates with the lack of amino acid differences in the Nar operon.

As results came in, additional alignment of the upstream promoter region was performed to further examine possible explanations. The promoter region of the Nar operon has not yet been characterised. It is possible that variations in susceptibility could also be explained by mutations in a repressor or promoter region. A previous study by Suzuki et al. (2014) found that when a mutation occurred in the gene of a local repressor (AcrR) of an *acrAB* operon in *E. coli*, it exhibited significantly higher expression levels of *acrAB*. They concluded that the occurring mutation disrupted the expression of the repressor, allowing increased expression of *acrAB* and thus, increased efflux of antimicrobials from the cell and an overall increased tolerance to antimicrobials. It also showed that mutations occurring in the open reading frame (ORF) of the gene would create less changes in susceptibility than the changes in expression level caused by mutations in the repressor gene. However, as the upstream sequence of the Nar operon is not determined, evidence for this remains unclear. Nonetheless, during PCR, it proved very difficult for the designed primer (Forward 1) to anneal to the upstream region. This indicates that the upstream region differs greatly between isolates. In the end, PCR products would only be attained from three different strains. One of the strains conferred five nucleotide position differences from the wild-type. Yet, the sequences were too short to make a proper analysis of the upstream region, and too few to appropriately analyse the effect of the mutations on antimicrobial susceptibility. This could have been improved by redesigning primers and including more strains for testing, yet as the upstream region seems to differ greatly between strains that could also have been problematic. Another possible solution could be to do a stepwise sequencing of the upstream region directly from the genomic DNA from the different

E. faecium strains. A reverse primer could be designed that binds in the ATPase gene then designing new primers as sequences arrive from sequencing for the previous primer.

Cross-resistance to other antimicrobials when Nar operon is introduced in E. coli

The Nar operon was successfully introduced into cells of *E. coli* DH5α and hypersensitive *E. coli* DH5α *ΔacrAB*. Where they were further screened for cross-resistance to 14 different antimicrobials, either automatically or manually, or both. Results showed that no cross-resistance was found between the antimicrobials for *E. coli* DH5α nor the hypersensitive *E. coli* DH5α *ΔacrAB* carrying the Nar operon.

Antimicrobial susceptibility in transformants tested automatically, did not differ from the control carrying the empty vector. These results suggest that the ABC transporter as encoded by the Nar operon, did not transport any of the tested drugs, and therefore did not mediate any effect on antimicrobial resistance for *E. coli* DH5α, or the hypersensitive *E. coli* DH5α *ΔacrAB*.

As *E. coli* are Gram negative bacteria they have an extra layer (the outer membrane) in the cell envelop. enterococci, however, are Gram positive bacteria without the extra layer. This difference could explain the problem of detecting an effect of the ABC transporter in *E. coli,* since this bacterium is intrinsically resistant to many of the drugs putatively transported by the proteins encoded by the Nar-operon. This is mainly because the outer membrane of *E. coli* can prevent certain drugs from entering the cell. An effect of the Nar resistance genes could have been seen if some of these compounds had been able to enter the cells of *E. coli*, before being subsequently transported out of the cell by the ABC efflux pump. Yet, this did not seem to have occurred. The regulatory systems between *E. coli* and enterococci could also be different. But, as the regulatory system for gene expression was circumvented by expressing the genes from the arabinose inducible plasmid, it did not apply here. Although some studies show that ABC transporter could in fact, be MDR pumps (Lubelski et al. 2007), results from the current study indicate that the ABC transporter as encoded by the Nar operon is not, at least in *E. coli.*

Some differences in susceptibility could also be seen between the *E. coli* and the hypersensitive *E. coli*. Results showed that tolerance to Ciprofloxacin and Nalidixic acid were reduced in the hypersensitive strain, when compared to the wild type *E. coli.* This is presumably due to the mutant's lack of the very important AcrAB efflux pump system, which reduced the mutant's

ability to extrude compounds from the cell. Manual testing of Cip and Nal also support these findings, as MIC values are similar to each other. These similarities also validate the manual procedure.

Both *E. coli* strains, mutant and non-mutant, presented high level of resistance to Amp, as was anticipated. The vector (pBAD30) carrying the Nar operon, also contain a gene encoding a beta-lactamase (Guzman et al. 1995). This enzyme cuts the beta-lactam ring of Amp, therefore making the transformants resistant to it (Addgene 2017). In addition to high level of Amp resistance, mutant *E. coli* also presented high level of resistance to Bac. Bac present antimicrobial mechanisms through inhibiting cell wall synthesis of mainly Gram-positive bacteria. In fact, Bac has been suggested as a treatment for infection with VRE (Manson et al. 2004). However, there has been some evidence of an association between Bac resistance and resistance to Nar. Therefore, Bac was included in this study to further examine this possibility. Although E. coli are generally resistant to Bac, we hypothesized that absence of the AcrAB system might have made the bacteria susceptible to the antibiotic. Yet, results presented a high-level of resistance for the hypersensitive strain of *E. coli*, indicating that Bac was not able to inhibit cell wall synthesis even in the absence of the very important drug transporter AcrAB.

Cross-resistance to other ionophores

When tested for cross-resistance of other ionophores, two strains, presented high-level resistance to Mon (32 µg/ml), but their Nar MIC varied (1 and 8 µg/ml). In comparison, the rest of the strains conferred a higher susceptibility to Mon (2 to 8 µg/ml), whilst their tolerance to Nar varied (0.5 to 8 µg/ml). When comparing Mon MIC to the resistance of Nar it did not seem to relate to a cross-resistance effect, e.g. an increase in Nar MIC would also be seen as an increase in Mon MIC, and the other way around. A possible correlation could, however, be seen between two of the other ionophores and Nar. Two strains with reduced Nar MIC, also had a reduced MIC for both Sal and Mad. Yet, no other strains presented a correlation between an increased Nar MIC and increased resistance to other antimicrobials. If this observation is correct it may indicate that the Nar efflux pump could be transporting not only Nar, but also Sal and Mad. Nar and Sal are highly similar, therefore, cross-resistance was likely to occur. These results are also supported by the findings of Butaye et al. (2000). In their study, a correlation was found between resistance to Nar and Sal in isolates of enterococci collected from (mainly) poultry. However, they did not find a correlation between Nar resistance and tolerance to the two ionophores, Mon and Las. Mad on the other hand is more dissimilar to the other compounds

and the indicated cross-resistance found in this study was more unexpected. These findings should be confirmed by future experiments. If the cross-resistance effect is mediated by the efflux pump, use of Mad and Sal in rearing practices may present a selective pressure, even though the use of Nar has been discontinued. Neither of these antimicrobials are used in Norway today, though, they are both approved for use. Nevertheless, strains for this study had been chosen based on Nar MIC from previous tests to create a scale of susceptibility (0.5 – 32 µg/ml) that we hoped would serve as a good basis for comparison of cross-resistance. Results indicate that there is a correlation between resistance to Nar, Sal and Mad, but not Mon and Las. However, as the "old" MIC-values did not correlate with the ones found in the current study, the selection of isolates did not make it possible to draw a conclusion regarding cross resistance between Nar and other ionophores.

Transfer of Van and Nar resistance to another E. faecium strain

Dissemination of resistance genes through HGT is a process found to play an important role in the creation of resistant human pathogens. It is supported by the evidence of increased resistance to all antibiotics introduced into health care practices throughout the years (Bennett 2008). A Nar resistance gene was recently discovered on the same plasmid as a *vanA* resistance gene (Nilsson et al. 2016), in addition to evidence of a possible Nar and Van co-resistance transfer between *E. faecium* strains (Nilsson et al. 2012). An experiment was designed to determine if Nar pressure could enhance conjugational transfer of the Nar/*vanA* plasmid between strains of *E. faecium*.

Antibiotic screening of donor strains showed resistance to Van and Nar, while presenting susceptibility to Tet and Ery. In contrast, the recipient strain was susceptible to both Van and Nar, and resistant to Tet, Ery, Chl and Syn. We hypothesized that these opposite resistance profiles would make it possible to determine a conjugational transfer of the plasmid. While plates with Tet would select for recipient strain, Van would select for the plasmid (containing the resistance gene to Van).

Post-conjugation, all five conjugants presented resistance to the following antimicrobials: Van, Tet, Ery, Chl, Tei and Syn. One of the screened conjugants (cfu 1) also presented resistance to Nar. In addition to its own original resistance profile, conjugants had acquired resistance to Van and Nar (one strain). This suggests a successful conjugational transfer of the resistance genes carried by the Nar/*vanA* plasmid from the donor strain. However, interestingly, no conjugation

occurred between the strains under the selective pressure of Nar. This may be due to several factors, one of which could be an incorrect Nar concentration to stimulate transfer. It is possible that strains with low level resistance to Nar could have been inhibited by an incorrect, high concentration. By optimising plates with a lower concentration of Nar, this could perhaps, be avoided. Moreover, results showed that Nar resistance was not transferred for most of the strain (4 of 5). This might be explained by the existence of two different plasmids, where one carries the *vanA* gene, while the other carries the Nar operon. Another possibility is that something happened during the MIC test that created a false result.

Nevertheless, findings of this study agree with the study of Nilsson et al. (Nilsson et al. 2012), that a reduced Nar susceptibility can be co-transferred with the *vanA* resistance gene. It occurred in this study for one out of 5 strains. Indeed, use of Nar may have taken part, and may still take part in the persistence of Van resistance in enterococci if introduced back into rearing practices. Also, as VRE have been shown to transfer resistance genes to other enterococci even without a selective pressure, such a persistence should be taken seriously.

Limitations of experimental design

Some limitations of the experimental design must be taken into consideration. Although, sequences of both genes were obtained from 16 different strains, variations in Nar MIC were limited, presenting only low to medium resistance. A wider variation in MIC which also includes a higher level of resistance to Nar would create an improved basis for evaluation between mutational occurrences in the genes and Nar MIC.

Cross-resistance testing also presents some difficulties. Susceptibility screening relies on visual evaluation of bacterial growth, presenting possibilities of human errors.

The Nar operon was cloned into an *E. coli* expression vector (pBAD30) and introduced into cells of *E. coli* DH5α and hypersensitive *E. coli* DH5α *ΔacrAB*. However, no cross-resistance was observed. Since it has been suggested that there may be cross resistance between Nar and Bac, Sal and Mad and not even the *ΔacrAB*-mutant of DH5α was susceptible to these compounds it is likely that cloning of the Nar operon into an *E. faecium* expression vector, and introduction and testing of the constructs in *E. faecium* would have been more informative. The HGT experiment presented some insight into possible antimicrobial cross-resistance when *E.*

faecium carries a Nar resistance gene. But, as the HGT experiment was not repeated more than once due to limited time, random results cannot be disregarded.

Conclusion and prospective studies

Although results from this study did not see a co-resistance between Nar and Mon, a correlation cannot be disregarded. Indeed, several of the isolates showing resistance to Nar were collected from turkeys bred with Mon supplemented feed, yet it is not certain that Mon is the causative agent. Additionally, although no increased resistance could be seen for *E. coli* when carrying the Nar operon, it does not exclude the possibility of cross-resistance if carried by another bacterium or if other antibiotics would have been tested.

By including several *E. faecium* strains with varied Nar MIC, further studies could better examine a possible correlation between mutational occurrence in the Nar operon and susceptibility to Nar. In addition, it is necessary to characterise the upstream region of the Nar operon in order to compare the sequences of the promoter region and correlate these findings with differences in Nar resistance. Furthermore, there is evidence of possible cross-resistance between certain ionophores, however, the transport capability of the proteins encoded by the Nar operon has not yet been fully characterised. More studies are needed to do this. Prospective studies are suggested to further examine the possibility of cross-resistance of the Nar operon in strains of *E. faecium*.

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6. Appendices

Appendix 6.1 - Recipe TSS buffer

Preparation

To make 50 mL:

- 5g PEG 8000
- 1.5 mL 1M $MgCl_2$ (or 0.30g $MgCl_2*6H_20$)
- 2.5 mL DMSO
- Add LB to 50 mL

Filter sterilize (0.22 µm filter)

Store at 4˚C or -20˚C

Appendix 6.2 – Alignment of DNA sequences from Nar operon from 16 strains

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2004-01-1301-1      ATGACAGAAATTGTAAAAGTACCAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
2004-01-850-1       ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
2006-01-1700-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
2006-01-1145-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
2006-01-2608-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
2006-01-1151        ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
2006-01-1402        ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
2013-01-3934        ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
2006-01-1190-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
2006-01-1111-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
2004-01-1251-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
2006-01-3433        ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
2006-01-1131-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
2006-01-1152-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
2004-01-1343-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
2006-01-1154-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
                    ********************* ************************************

2004-01-1301-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
2004-01-850-1       AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
2006-01-1700-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
2006-01-1145-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
2006-01-2608-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
2006-01-1151        AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
2006-01-1402        AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
2013-01-3934        AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
2006-01-1190-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
2006-01-1111-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
2004-01-1251-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
2006-01-3433        AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
2006-01-1131-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
2006-01-1152-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
2004-01-1343-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
2006-01-1154-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
                    ************************************************************

2004-01-1301-1      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
2004-01-850-1       GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
2006-01-1700-1      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
2006-01-1145-1      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
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2006-01-1402        GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
2013-01-3934        GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
2006-01-1190-1      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
2006-01-1111-1      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
2004-01-1251-1      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
2006-01-3433        GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
2006-01-1131-1      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
2006-01-1152-1      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
2004-01-1343-1      GCAGGAAAGTCAACGACGATCCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
2006-01-1154-1      GCAGGAAAGTCAACGACGATCCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
                    ******************* ****************************************

2004-01-1301-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
2004-01-850-1       GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
2006-01-1700-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
2006-01-1145-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
2006-01-2608-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
2006-01-1151        GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
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2013-01-3934        GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
2006-01-1190-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
2006-01-1111-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
2004-01-1251-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
2006-01-3433        GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
2006-01-1131-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
2006-01-1152-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
2004-01-1343-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
2006-01-1154-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
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2004-01-1301-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
2004-01-850-1       TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
2006-01-1700-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
2006-01-1145-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
2006-01-2608-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
2006-01-1151        TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
2006-01-1402        TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
2013-01-3934        TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
2006-01-1190-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
2006-01-1111-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
2004-01-1251-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
2006-01-3433        TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
2006-01-1131-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
2006-01-1152-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
2004-01-1343-1      TATGTTCCTGGAGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
2006-01-1154-1      TATGTTCCTGGAGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
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2004-01-850-1       TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
2006-01-1700-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
2006-01-1145-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
2006-01-2608-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
2006-01-1151        TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
2006-01-1402        TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
2013-01-3934        TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
2006-01-1190-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
2006-01-1111-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
2004-01-1251-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
2006-01-3433        TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
2006-01-1131-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
2006-01-1152-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
2004-01-1343-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGCGATTATTTAATCAAACGATTT
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2006-01-2608-1      GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
2006-01-1151        GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
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2013-01-3934        GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
2006-01-1190-1      GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
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2004-01-1251-1      GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
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2006-01-1154-1      GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
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2006-01-2608-1      TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
2006-01-1151        TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
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2013-01-3934        TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
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2006-01-1152-1      TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
2004-01-1343-1      TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
2006-01-1154-1      TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
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2006-01-1154-1      CTAGATCCATTGATGGAAGCAGTATTCCAAGAAGAAGTAGAAAAAATCAAAAATGATGGC
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2006-01-1402        AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
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2006-01-1131-1      AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
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2006-01-1154-1      AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
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2006-01-1145-1      GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
2006-01-2608-1      GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
2006-01-1151        GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
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2006-01-1131-1      GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
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2006-01-1131-1      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
2006-01-1152-1      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
2004-01-1343-1      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
2006-01-1154-1      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
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2004-01-1301-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
2004-01-850-1       GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
2006-01-1700-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
2006-01-1145-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
2006-01-2608-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
2006-01-1151        GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
2006-01-1402        GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
2013-01-3934        GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
2006-01-1190-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
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2004-01-1251-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
2006-01-3433        GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
2006-01-1131-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
2006-01-1152-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
2004-01-1343-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCGACTTTTTCTGCTGACAATGAAGCG
2006-01-1154-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCGACTTTTTCTGCTGACAATGAAGCG
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2004-01-1301-1      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
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2006-01-1145-1      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
2006-01-2608-1      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
2006-01-1151        ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
2006-01-1402        ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
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2006-01-1190-1      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
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2006-01-1131-1      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
2006-01-1152-1      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
2004-01-1343-1      ATAAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGACAAAAATCGAATCTGTACCG
2006-01-1154-1      ATAAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGACAAAAATCGAATCTGTACCG
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2004-01-850-1       CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
2006-01-1700-1      CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
2006-01-1145-1      CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
2006-01-2608-1      CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
2006-01-1151        CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
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2006-01-1190-1      CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
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2006-01-1111-1      AGAAAATGAATGAAAAATTTGCGCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCG
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2006-01-1154-1      AGAAAATGAATGAAAAATTTGCCCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCG
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2006-01-2608-1      ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATATGTAC
2006-01-1151        ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATATGTAC
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2004-01-1251-1      ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATTTGTAC
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2004-01-1343-1      ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATTTGTAC
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2006-01-1145-1      CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
2006-01-2608-1      CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
2006-01-1151        CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
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2006-01-1700-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
2006-01-1145-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
2006-01-2608-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
2006-01-1151        ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
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2013-01-3934        ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
2006-01-1190-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
2006-01-1111-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
2004-01-1251-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
2006-01-3433        ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
2006-01-1131-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
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2004-01-1343-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
2006-01-1154-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
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2004-01-1301-1      TAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAATGATTATCT
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2006-01-1154-1      TAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAATGATTATCT
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2004-01-1251-1      CAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
2006-01-3433        CAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
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2004-01-850-1       TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
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2006-01-1151        TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
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2006-01-1190-1      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
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2004-01-1251-1      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
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2006-01-1131-1      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
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2006-01-1154-1      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
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2004-01-1343-1      CGATTGATGCCGAAGGAGCTTTCTTGTTCGGAGGATCAATTGCATTGGCGGGAATTATCG
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2006-01-1190-1      GTGGTGTATTGGCACTTGTGATGTCGCAGATTATGGCGACTTCTACTGGAGCAACCGGCT
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2004-01-850-1       CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
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2006-01-1145-1      CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
2006-01-2608-1      CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
2006-01-1151        CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
2006-01-1402        CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
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2006-01-1152-1      CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
2004-01-1343-1      CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
2006-01-1154-1      CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
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2004-01-1251-1      TTGTGTTGGAAGAACATCGCGACATGGGCGCAGGTTATCTTCCTGAACGAGAAGGACGTG
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2006-01-1111-1      CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
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2006-01-1131-1      CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
2006-01-1152-1      CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
2004-01-1343-1      CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
2006-01-1154-1      CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
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2006-01-1151        TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
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2004-01-1251-1      TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
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2006-01-1152-1      TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
2004-01-1343-1      TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
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2006-01-1700-1      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
2006-01-1145-1      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
2006-01-2608-1      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
2006-01-1151        GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
2006-01-1402        GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
2013-01-3934        GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
2006-01-1190-1      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
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2006-01-1152-1      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
2004-01-1343-1      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
2006-01-1154-1      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
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2004-01-850-1       GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
2006-01-1700-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
2006-01-1145-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
2006-01-2608-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
2006-01-1151        GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
2006-01-1402        GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
2013-01-3934        GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
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2006-01-1190-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
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2004-01-1251-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
2006-01-3433        GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
2006-01-1131-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
2006-01-1152-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
2004-01-1343-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
2006-01-1154-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
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2004-01-1301-1      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
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2006-01-1700-1      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
2006-01-1145-1      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
2006-01-2608-1      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
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2006-01-1152-1      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
2004-01-1343-1      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
2006-01-1154-1      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
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2004-01-1301-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
2004-01-850-1       GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
2006-01-1700-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
2006-01-1145-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
2006-01-2608-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
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2006-01-1402        GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
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2006-01-1190-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
2006-01-1111-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
2004-01-1251-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
2006-01-3433        GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
2006-01-1131-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
2006-01-1152-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
2004-01-1343-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
2006-01-1154-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
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2004-01-1301-1      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
2004-01-850-1       CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
2006-01-1700-1      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
2006-01-1145-1      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
2006-01-2608-1      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
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2006-01-1111-1      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
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2006-01-1152-1      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
2004-01-1343-1      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGCTTAGGTGGAACGGCGA
2006-01-1154-1      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGCTTAGGTGGAACGGCGA
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2004-01-850-1       TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
2006-01-1700-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
2006-01-1145-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
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2006-01-1402        TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
2013-01-3934        TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
2006-01-1190-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
2006-01-1111-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
2004-01-1251-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
2006-01-3433        TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
2006-01-1131-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
2006-01-1152-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
2004-01-1343-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
2006-01-1154-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
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2004-01-1301-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
2004-01-850-1       ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
2006-01-1700-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
2006-01-1145-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
2006-01-2608-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
2006-01-1151        ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
2006-01-1402        ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
2013-01-3934        ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
2006-01-1190-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
2006-01-1111-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
2004-01-1251-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
2006-01-3433        ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
2006-01-1131-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
2006-01-1152-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
2004-01-1343-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
2006-01-1154-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
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2006-01-1145-1      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
2006-01-2608-1      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
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2006-01-1402        CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
2013-01-3934        CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
2006-01-1190-1      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
2006-01-1111-1      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
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2006-01-1152-1      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
2004-01-1343-1      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
2006-01-1154-1      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
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2004-01-1301-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
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2006-01-1700-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
2006-01-1145-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
2006-01-2608-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
2006-01-1151        GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
2006-01-1402        GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
2013-01-3934        GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
2006-01-1190-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
2006-01-1111-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
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2006-01-3433        GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
2006-01-1131-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
2006-01-1152-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
2004-01-1343-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGCTGGATTCCAC
2006-01-1154-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGCTGGATTCCAC
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2006-01-1700-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
2006-01-1145-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
2006-01-2608-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
2006-01-1151        GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
2006-01-1402        GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
2013-01-3934        GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
2006-01-1190-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
2006-01-1111-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
2004-01-1251-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
2006-01-3433        GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
2006-01-1131-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
2006-01-1152-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
2004-01-1343-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
2006-01-1154-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
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2006-01-1145-1      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
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2013-01-3934        TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
2006-01-1190-1      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
2006-01-1111-1      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
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2006-01-3433        TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
2006-01-1131-1      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
2006-01-1152-1      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
2004-01-1343-1      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
2006-01-1154-1      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
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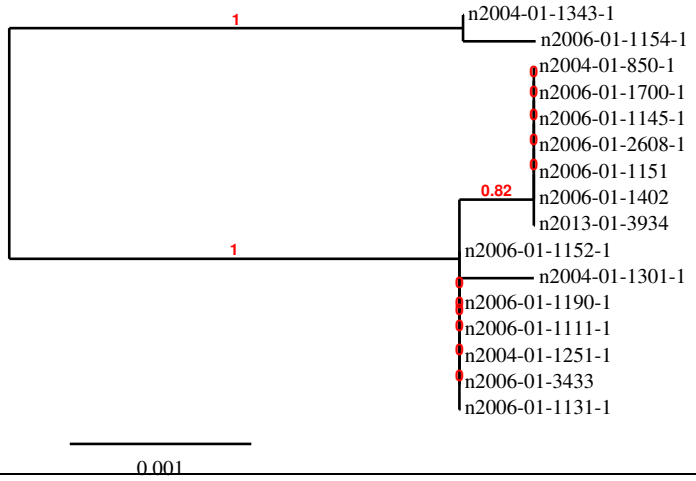

Figure 6.2.1: A) *Alignment of amplified narasin operon from 16 isolates B)* Phylogenetic tree of aligned narasin operon genomic sequence

Appendix 6.3 - alignment of chromosomal DNA and cloned DNA

Tables in appendix 6.3 present the following eight alignments; figure 6.3.1: 2006-01-2608-1, figure 6.3.2: 2006-01-1151, figure 6.3.3: 2006-01-1251-1, figure 6.3.4: 2006-01-1131-1, figure 6.3.5: 2006-01-1154-1, figure 6.3.6: 2006-01-1152-1, figure 6.3.7: 2006-01-1190-1 and figure 6.3.8: SVA-01-233. Alignments show no differences for first 7 alignments between the chromosomal DNA and the cloned DNA. Alignment of cloned SVA-01-233 and template sequences show two differences.

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Clone_2006-01-2608-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAAATTTGGTAAATTCCAGGCGTTG
                          ************************************************************

Chrom_2006-01-2608-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
Clone_2006-01-2608-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
                          ************************************************************

Chrom_2006-01-2608-1      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
Clone_2006-01-2608-1      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
                          ************************************************************

Chrom_2006-01-2608-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
Clone_2006-01-2608-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
                          ************************************************************

Chrom_2006-01-2608-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
Clone_2006-01-2608-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
                          ************************************************************

Chrom_2006-01-2608-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
Clone_2006-01-2608-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
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Chrom_2006-01-2608-1      GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
Clone_2006-01-2608-1      GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
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Clone_2006-01-2608-1      TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
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Chrom_2006-01-2608-1      CTAGATCCATTGATGGAAGCAGTATTCCAAGAAGAAGTAGAAAAAATCAAAAATGATGGC
Clone_2006-01-2608-1      CTAGATCCATTGATGGAAGCAGTATTCCAAGAAGAAGTAGAAAAAATCAAAAATGATGGC
                          ************************************************************

Chrom_2006-01-2608-1      AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
Clone_2006-01-2608-1      AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
                          ************************************************************

Chrom_2006-01-2608-1      GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
Clone_2006-01-2608-1      GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
                          ************************************************************

Chrom_2006-01-2608-1      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
Clone_2006-01-2608-1      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
                          ************************************************************

Chrom_2006-01-2608-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
Clone_2006-01-2608-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
                          ************************************************************

Chrom_2006-01-2608-1      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
Clone_2006-01-2608-1      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
                          ************************************************************
```

```
Chrom_2006-01-2608-1      CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
Clone_2006-01-2608-1      CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
                          ************************************************************

Chrom_2006-01-2608-1      AGAAAATGAATGAAAAATTTGCGCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCG
Clone_2006-01-2608-1      AGAAAATGAATGAAAAATTTGCGCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCG
                          ************************************************************

Chrom_2006-01-2608-1      ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATATGTAC
Clone_2006-01-2608-1      ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATATGTAC
                          ************************************************************

Chrom_2006-01-2608-1      CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
Clone_2006-01-2608-1      CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
                          ************************************************************

Chrom_2006-01-2608-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
Clone_2006-01-2608-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
                          ************************************************************

Chrom_2006-01-2608-1      TAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAATGATTATCT
Clone_2006-01-2608-1      TAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAATGATTATCT
                          ************************************************************

Chrom_2006-01-2608-1      CAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
Clone_2006-01-2608-1      CAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
                          ************************************************************

Chrom_2006-01-2608-1      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
Clone_2006-01-2608-1      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
                          ************************************************************

Chrom_2006-01-2608-1      TGATCAATCTTTTATTAGGTCTTTTAATCGGCGGACTCATGATGAGTTTTGGTGTAAAAA
Clone_2006-01-2608-1      TGATCAATCTTTTATTAGGTCTTTTAATCGGCGGACTCATGATGAGTTTTGGTGTAAAAA
                          ************************************************************

Chrom_2006-01-2608-1      CGATTGATGCCGAAGGAGCTTTCTTGTTCGGAGGATCAATTGCATTGGCGGGAATTATCG
Clone_2006-01-2608-1      CGATTGATGCCGAAGGAGCTTTCTTGTTCGGAGGATCAATTGCATTGGCGGGAATTATCG
                          ************************************************************

Chrom_2006-01-2608-1      GTGGTGTATTGGCACTTGTGATGTCGCAGATTATGGCGACTTCTACTGGAGCAACCGGCT
Clone_2006-01-2608-1      GTGGTGTATTGGCACTTGTGATGTCGCAGATTATGGCGACTTCTACTGGAGCAACCGGCT
                          ************************************************************

Chrom_2006-01-2608-1      CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
Clone_2006-01-2608-1      CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
                          ************************************************************

Chrom_2006-01-2608-1      TTGATCTATCAATGTTCAATCCAATGGGATGGATTTACTTGACCTATCCTTTCACAAAAA
Clone_2006-01-2608-1      TTGATCTATCAATGTTCAATCCAATGGGATGGATTTACTTGACCTATCCTTTCACAAAAA
                          ************************************************************

Chrom_2006-01-2608-1      ATAACTGGCTACCATTATTATTTGCTTTGATTTTTAGTCTTGTTTTTACCGTACTTGCGT
Clone_2006-01-2608-1      ATAACTGGCTACCATTATTATTTGCTTTGATTTTTAGTCTTGTTTTTACCGTACTTGCGT
                          ************************************************************

Chrom_2006-01-2608-1      TTGTGTTGGAAGAACATCGCGACATGGGCGCAGGTTATCTTCCTGAACGAGAAGGACGTG
Clone_2006-01-2608-1      TTGTGTTGGAAGAACATCGCGACATGGGCGCAGGTTATCTTCCTGAACGAGAAGGACGTG
                          ************************************************************

Chrom_2006-01-2608-1      CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
Clone_2006-01-2608-1      CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
                          ************************************************************

Chrom_2006-01-2608-1      TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
Clone_2006-01-2608-1      TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
                          ************************************************************

Chrom_2006-01-2608-1      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
Clone_2006-01-2608-1      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
                          ************************************************************

Chrom_2006-01-2608-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
Clone_2006-01-2608-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
                          ************************************************************

Chrom_2006-01-2608-1      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
```

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Clone_2006-01-2608-1      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
                          ************************************************************

Chrom_2006-01-2608-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
Clone_2006-01-2608-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
                          ************************************************************

Chrom_2006-01-2608-1      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
Clone_2006-01-2608-1      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
                          ************************************************************

Chrom_2006-01-2608-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
Clone_2006-01-2608-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
                          ************************************************************

Chrom_2006-01-2608-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
Clone_2006-01-2608-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
                          ************************************************************

Chrom_2006-01-2608-1      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
Clone_2006-01-2608-1      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
                          ************************************************************

Chrom_2006-01-2608-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
Clone_2006-01-2608-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
                          ************************************************************

Chrom_2006-01-2608-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
Clone_2006-01-2608-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
                          ************************************************************

Chrom_2006-01-2608-1      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
Clone_2006-01-2608-1      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
                          ************************************************************
```

Figure 6.3.1: Alignment of chromosomal and cloned Nar operon DNA sequence from bacterial strain 2006-01-2608-1

```
Chrom_2006-01-1151        ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAAATTTGGTAAATTCCAGGCGTTG
Clone_2006-01-1151        ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAAATTTGGTAAATTCCAGGCGTTG
                          ************************************************************

Chrom_2006-01-1151        AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
Clone_2006-01-1151        AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
                          ************************************************************

Chrom_2006-01-1151        GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
Clone_2006-01-1151        GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
                          ************************************************************

Chrom_2006-01-1151        GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
Clone_2006-01-1151        GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
                          ************************************************************

Chrom_2006-01-1151        TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
Clone_2006-01-1151        TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
                          ************************************************************

Chrom_2006-01-1151        TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
Clone_2006-01-1151        TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
                          ************************************************************

Chrom_2006-01-1151        GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
Clone_2006-01-1151        GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
                          ************************************************************

Chrom_2006-01-1151        TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
Clone_2006-01-1151        TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
                          ************************************************************

Chrom_2006-01-1151        CTAGATCCATTGATGGAAGCAGTATTCCAAGAAGAAGTAGAAAAAATCAAAAATGATGGC
Clone_2006-01-1151        CTAGATCCATTGATGGAAGCAGTATTCCAAGAAGAAGTAGAAAAAATCAAAAATGATGGC
                          ************************************************************

Chrom_2006-01-1151        AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
```

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Clone_2006-01-1151      AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
                        ************************************************************

Chrom_2006-01-1151      GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
Clone_2006-01-1151      GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
                        ************************************************************

Chrom_2006-01-1151      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
Clone_2006-01-1151      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
                        ************************************************************

Chrom_2006-01-1151      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
Clone_2006-01-1151      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
                        ************************************************************

Chrom_2006-01-1151      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
Clone_2006-01-1151      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
                        ************************************************************

Chrom_2006-01-1151      CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
Clone_2006-01-1151      CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
                        ************************************************************

Chrom_2006-01-1151      AGAAAATGAATGAAAAATTTGCGCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCG
Clone_2006-01-1151      AGAAAATGAATGAAAAATTTGCGCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCG
                        ************************************************************

Chrom_2006-01-1151      ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATATGTAC
Clone_2006-01-1151      ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATATGTAC
                        ************************************************************

Chrom_2006-01-1151      CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
Clone_2006-01-1151      CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
                        ************************************************************

Chrom_2006-01-1151      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
Clone_2006-01-1151      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
                        ************************************************************

Chrom_2006-01-1151      TAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAATGATTATCT
Clone_2006-01-1151      TAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAATGATTATCT
                        ************************************************************

Chrom_2006-01-1151      CAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
Clone_2006-01-1151      CAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
                        ************************************************************

Chrom_2006-01-1151      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
Clone_2006-01-1151      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
                        ************************************************************

Chrom_2006-01-1151      TGATCAATCTTTTATTAGGTCTTTTAATCGGCGGACTCATGATGAGTTTTGGTGTAAAAA
Clone_2006-01-1151      TGATCAATCTTTTATTAGGTCTTTTAATCGGCGGACTCATGATGAGTTTTGGTGTAAAAA
                        ************************************************************

Chrom_2006-01-1151      CGATTGATGCCGAAGGAGCTTTCTTGTTCGGAGGATCAATTGCATTGGCGGGAATTATCG
Clone_2006-01-1151      CGATTGATGCCGAAGGAGCTTTCTTGTTCGGAGGATCAATTGCATTGGCGGGAATTATCG
                        ************************************************************

Chrom_2006-01-1151      GTGGTGTATTGGCACTTGTGATGTCGCAGATTATGGCGACTTCTACTGGAGCAACCGGCT
Clone_2006-01-1151      GTGGTGTATTGGCACTTGTGATGTCGCAGATTATGGCGACTTCTACTGGAGCAACCGGCT
                        ************************************************************

Chrom_2006-01-1151      CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
Clone_2006-01-1151      CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
                        ************************************************************

Chrom_2006-01-1151      TTGATCTATCAATGTTCAATCCAATGGGATGGATTTACTTGACCTATCCTTTCACAAAAA
Clone_2006-01-1151      TTGATCTATCAATGTTCAATCCAATGGGATGGATTTACTTGACCTATCCTTTCACAAAAA
                        ************************************************************

Chrom_2006-01-1151      ATAACTGGCTACCATTATTATTTGCTTTGATTTTTAGTCTTGTTTTTACCGTACTTGCGT
Clone_2006-01-1151      ATAACTGGCTACCATTATTATTTGCTTTGATTTTTAGTCTTGTTTTTACCGTACTTGCGT
                        ************************************************************

Chrom_2006-01-1151      TTGTGTTGGAAGAACATCGCGACATGGGCGCAGGTTATCTTCCTGAACGAGAAGGACGTG
Clone_2006-01-1151      TTGTGTTGGAAGAACATCGCGACATGGGCGCAGGTTATCTTCCTGAACGAGAAGGACGTG
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                    ************************************************************

Chrom_2006-01-1151      CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
Clone_2006-01-1151      CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
                        ************************************************************

Chrom_2006-01-1151      TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
Clone_2006-01-1151      TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
                        ************************************************************

Chrom_2006-01-1151      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
Clone_2006-01-1151      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
                        ************************************************************

Chrom_2006-01-1151      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
Clone_2006-01-1151      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
                        ************************************************************

Chrom_2006-01-1151      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
Clone_2006-01-1151      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
                        ************************************************************

Chrom_2006-01-1151      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
Clone_2006-01-1151      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
                        ************************************************************

Chrom_2006-01-1151      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
Clone_2006-01-1151      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
                        ************************************************************

Chrom_2006-01-1151      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
Clone_2006-01-1151      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
                        ************************************************************

Chrom_2006-01-1151      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
Clone_2006-01-1151      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
                        ************************************************************

Chrom_2006-01-1151      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
Clone_2006-01-1151      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
                        ************************************************************

Chrom_2006-01-1151      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
Clone_2006-01-1151      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
                        ************************************************************

Chrom_2006-01-1151      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
Clone_2006-01-1151      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
                        ************************************************************

Chrom_2006-01-1151      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
Clone_2006-01-1151      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
                        ************************************************************
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Figure 6.3.2: Alignment of chromosomal and cloned Nar operon DNA sequence for bacterial strain 2006-01-1151

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Chrom_2004-01-1251-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAAATTTGGTAAATTCCAGGCGTTG
Clone_2004-01-1251-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAAATTTGGTAAATTCCAGGCGTTG
                          ************************************************************

Chrom_2004-01-1251-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
Clone_2004-01-1251-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
                          ************************************************************

Chrom_2004-01-1251-1      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
Clone_2004-01-1251-1      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
                          ************************************************************

Chrom_2004-01-1251-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
Clone_2004-01-1251-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
                          ************************************************************

Chrom_2004-01-1251-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
Clone_2004-01-1251-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
                          ************************************************************
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Chrom_2004-01-1251-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
Clone_2004-01-1251-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
                          ************************************************************

Chrom_2004-01-1251-1      GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
Clone_2004-01-1251-1      GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
                          ************************************************************

Chrom_2004-01-1251-1      TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
Clone_2004-01-1251-1      TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
                          ************************************************************

Chrom_2004-01-1251-1      CTAGATCCATTGATGGAAGCAGTATTCCAAGAAGAAGTAGAAAAAATCAAAAATGATGGC
Clone_2004-01-1251-1      CTAGATCCATTGATGGAAGCAGTATTCCAAGAAGAAGTAGAAAAAATCAAAAATGATGGC
                          ************************************************************

Chrom_2004-01-1251-1      AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
Clone_2004-01-1251-1      AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
                          ************************************************************

Chrom_2004-01-1251-1      GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
Clone_2004-01-1251-1      GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
                          ************************************************************

Chrom_2004-01-1251-1      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
Clone_2004-01-1251-1      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
                          ************************************************************

Chrom_2004-01-1251-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
Clone_2004-01-1251-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
                          ************************************************************

Chrom_2004-01-1251-1      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
Clone_2004-01-1251-1      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
                          ************************************************************

Chrom_2004-01-1251-1      CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
Clone_2004-01-1251-1      CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
                          ************************************************************

Chrom_2004-01-1251-1      AGAAAATGAATGAAAAATTTGCGCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCG
Clone_2004-01-1251-1      AGAAAATGAATGAAAAATTTGCGCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCG
                          ************************************************************

Chrom_2004-01-1251-1      ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATTTGTAC
Clone_2004-01-1251-1      ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATTTGTAC
                          ************************************************************

Chrom_2004-01-1251-1      CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
Clone_2004-01-1251-1      CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
                          ************************************************************

Chrom_2004-01-1251-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
Clone_2004-01-1251-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
                          ************************************************************

Chrom_2004-01-1251-1      TAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAATGATTATCT
Clone_2004-01-1251-1      TAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAATGATTATCT
                          ************************************************************

Chrom_2004-01-1251-1      CAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
Clone_2004-01-1251-1      CAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
                          ************************************************************

Chrom_2004-01-1251-1      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
Clone_2004-01-1251-1      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
                          ************************************************************

Chrom_2004-01-1251-1      TGATCAATCTTTTATTAGGTCTTTTAATCGGCGGACTCATGATGAGTTTTGGTGTAAAAA
Clone_2004-01-1251-1      TGATCAATCTTTTATTAGGTCTTTTAATCGGCGGACTCATGATGAGTTTTGGTGTAAAAA
                          ************************************************************

Chrom_2004-01-1251-1      CGATTGATGCCGAAGGAGCTTTCTTGTTCGGAGGATCAATTGCATTGGCGGGAATTATCG
Clone_2004-01-1251-1      CGATTGATGCCGAAGGAGCTTTCTTGTTCGGAGGATCAATTGCATTGGCGGGAATTATCG
                          ************************************************************
```

```
Chrom_2004-01-1251-1      GTGGTGTATTGGCACTTGTGATGTCGCAGATTATGGCGACTTCTACTGGAGCAACCGGCT
Clone_2004-01-1251-1      GTGGTGTATTGGCACTTGTGATGTCGCAGATTATGGCGACTTCTACTGGAGCAACCGGCT
                          ************************************************************

Chrom_2004-01-1251-1      CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
Clone_2004-01-1251-1      CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
                          ************************************************************

Chrom_2004-01-1251-1      TTGATCTATCAATGTTCAATCCAATGGGATGGATTTACTTGACCTATCCTTTCACAAAAA
Clone_2004-01-1251-1      TTGATCTATCAATGTTCAATCCAATGGGATGGATTTACTTGACCTATCCTTTCACAAAAA
                          ************************************************************

Chrom_2004-01-1251-1      ATAACTGGCTACCATTATTATTTGCTTTGATTTTTAGTCTTGTTTTTACCGTACTTGCGT
Clone_2004-01-1251-1      ATAACTGGCTACCATTATTATTTGCTTTGATTTTTAGTCTTGTTTTTACCGTACTTGCGT
                          ************************************************************

Chrom_2004-01-1251-1      TTGTGTTGGAAGAACATCGCGACATGGGCGCAGGTTATCTTCCTGAACGAGAAGGACGTG
Clone_2004-01-1251-1      TTGTGTTGGAAGAACATCGCGACATGGGCGCAGGTTATCTTCCTGAACGAGAAGGACGTG
                          ************************************************************

Chrom_2004-01-1251-1      CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
Clone_2004-01-1251-1      CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
                          ************************************************************

Chrom_2004-01-1251-1      TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
Clone_2004-01-1251-1      TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
                          ************************************************************

Chrom_2004-01-1251-1      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
Clone_2004-01-1251-1      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
                          ************************************************************

Chrom_2004-01-1251-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
Clone_2004-01-1251-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
                          ************************************************************

Chrom_2004-01-1251-1      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
Clone_2004-01-1251-1      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
                          ************************************************************

Chrom_2004-01-1251-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
Clone_2004-01-1251-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
                          ************************************************************

Chrom_2004-01-1251-1      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
Clone_2004-01-1251-1      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
                          ************************************************************

Chrom_2004-01-1251-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
Clone_2004-01-1251-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
                          ************************************************************

Chrom_2004-01-1251-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
Clone_2004-01-1251-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
                          ************************************************************

Chrom_2004-01-1251-1      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
Clone_2004-01-1251-1      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
                          ************************************************************

Chrom_2004-01-1251-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
Clone_2004-01-1251-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
                          ************************************************************

Chrom_2004-01-1251-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
Clone_2004-01-1251-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
                          ************************************************************

Chrom_2004-01-1251-1      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
Clone_2004-01-1251-1      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
                          ************************************************************
```

Figure 6.3.3: Alignment of chromosomal and cloned Nar operon DNA sequence for bacterial strain 2006-01-1251-1

```
Chrom_2006-01-1131-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
Clone_2006-01-1131-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
                          ************************************************************

Chrom_2006-01-1131-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
Clone_2006-01-1131-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
                          ************************************************************

Chrom_2006-01-1131-1      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
Clone_2006-01-1131-1      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
                          ************************************************************

Chrom_2006-01-1131-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
Clone_2006-01-1131-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
                          ************************************************************

Chrom_2006-01-1131-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
Clone_2006-01-1131-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
                          ************************************************************

Chrom_2006-01-1131-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
Clone_2006-01-1131-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
                          ************************************************************

Chrom_2006-01-1131-1      GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
Clone_2006-01-1131-1      GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
                          ************************************************************

Chrom_2006-01-1131-1      TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
Clone_2006-01-1131-1      TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
                          ************************************************************

Chrom_2006-01-1131-1      CTAGATCCATTGATGGAAGCAGTATTCCAAGAAGAAGTAGAAAAAATCAAAAATGATGGC
Clone_2006-01-1131-1      CTAGATCCATTGATGGAAGCAGTATTCCAAGAAGAAGTAGAAAAAATCAAAAATGATGGC
                          ************************************************************

Chrom_2006-01-1131-1      AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
Clone_2006-01-1131-1      AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
                          ************************************************************

Chrom_2006-01-1131-1      GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
Clone_2006-01-1131-1      GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
                          ************************************************************

Chrom_2006-01-1131-1      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
Clone_2006-01-1131-1      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
                          ************************************************************

Chrom_2006-01-1131-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
Clone_2006-01-1131-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
                          ************************************************************

Chrom_2006-01-1131-1      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
Clone_2006-01-1131-1      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
                          ************************************************************

Chrom_2006-01-1131-1      CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
Clone_2006-01-1131-1      CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
                          ************************************************************

Chrom_2006-01-1131-1      AGAAAATGAATGAAAAATTTGCGCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCG
Clone_2006-01-1131-1      AGAAAATGAATGAAAAATTTGCGCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCG
                          ************************************************************

Chrom_2006-01-1131-1      ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATTTGTAC
Clone_2006-01-1131-1      ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATTTGTAC
                          ************************************************************

Chrom_2006-01-1131-1      CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
Clone_2006-01-1131-1      CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
                          ************************************************************

Chrom_2006-01-1131-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
Clone_2006-01-1131-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
                          ************************************************************
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Chrom_2006-01-1131-1      TAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAATGATTATCT
Clone_2006-01-1131-1      TAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAATGATTATCT
                          ************************************************************

Chrom_2006-01-1131-1      CAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
Clone_2006-01-1131-1      CAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
                          ************************************************************

Chrom_2006-01-1131-1      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
Clone_2006-01-1131-1      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
                          ************************************************************

Chrom_2006-01-1131-1      TGATCAATCTTTTATTAGGTCTTTTAATCGGCGGACTCATGATGAGTTTTGGTGTAAAAA
Clone_2006-01-1131-1      TGATCAATCTTTTATTAGGTCTTTTAATCGGCGGACTCATGATGAGTTTTGGTGTAAAAA
                          ************************************************************

Chrom_2006-01-1131-1      CGATTGATGCCGAAGGAGCTTTCTTGTTCGGAGGATCAATTGCATTGGCGGGAATTATCG
Clone_2006-01-1131-1      CGATTGATGCCGAAGGAGCTTTCTTGTTCGGAGGATCAATTGCATTGGCGGGAATTATCG
                          ************************************************************

Chrom_2006-01-1131-1      GTGGTGTATTGGCACTTGTGATGTCGCAGATTATGGCGACTTCTACTGGAGCAACCGGCT
Clone_2006-01-1131-1      GTGGTGTATTGGCACTTGTGATGTCGCAGATTATGGCGACTTCTACTGGAGCAACCGGCT
                          ************************************************************

Chrom_2006-01-1131-1      CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
Clone_2006-01-1131-1      CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
                          ************************************************************

Chrom_2006-01-1131-1      TTGATCTATCAATGTTCAATCCAATGGGATGGATTTACTTGACCTATCCTTTCACAAAAA
Clone_2006-01-1131-1      TTGATCTATCAATGTTCAATCCAATGGGATGGATTTACTTGACCTATCCTTTCACAAAAA
                          ************************************************************

Chrom_2006-01-1131-1      ATAACTGGCTACCATTATTATTTGCTTTGATTTTTAGTCTTGTTTTTACCGTACTTGCGT
Clone_2006-01-1131-1      ATAACTGGCTACCATTATTATTTGCTTTGATTTTTAGTCTTGTTTTTACCGTACTTGCGT
                          ************************************************************

Chrom_2006-01-1131-1      TTGTGTTGGAAGAACATCGCGACATGGGCGCAGGTTATCTTCCTGAACGAGAAGGACGTG
Clone_2006-01-1131-1      TTGTGTTGGAAGAACATCGCGACATGGGCGCAGGTTATCTTCCTGAACGAGAAGGACGTG
                          ************************************************************

Chrom_2006-01-1131-1      CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
Clone_2006-01-1131-1      CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
                          ************************************************************

Chrom_2006-01-1131-1      TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
Clone_2006-01-1131-1      TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
                          ************************************************************

Chrom_2006-01-1131-1      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
Clone_2006-01-1131-1      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
                          ************************************************************

Chrom_2006-01-1131-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
Clone_2006-01-1131-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
                          ************************************************************

Chrom_2006-01-1131-1      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
Clone_2006-01-1131-1      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
                          ************************************************************

Chrom_2006-01-1131-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
Clone_2006-01-1131-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
                          ************************************************************

Chrom_2006-01-1131-1      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
Clone_2006-01-1131-1      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
                          ************************************************************

Chrom_2006-01-1131-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
Clone_2006-01-1131-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
                          ************************************************************

Chrom_2006-01-1131-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
Clone_2006-01-1131-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
                          ************************************************************

Chrom_2006-01-1131-1      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
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Clone_2006-01-1131-1      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
                          ************************************************************

Chrom_2006-01-1131-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
Clone_2006-01-1131-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
                          ************************************************************

Chrom_2006-01-1131-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
Clone_2006-01-1131-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
                          ************************************************************

Chrom_2006-01-1131-1      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
Clone_2006-01-1131-1      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
                          ************************************************************
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Figure 6.3.4: Alignment of chromosomal and cloned Nar operon DNA sequence for bacterial strain 2006-01-1131-1

```
Chrom_2006-01-1154-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAAATTTGGTAAATTCCAGGCGTTG
Clone_2006-01-1154-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAAATTTGGTAAATTCCAGGCGTTG
                          ************************************************************

Chrom_2006-01-1154-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
Clone_2006-01-1154-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
                          ************************************************************

Chrom_2006-01-1154-1      GCAGGAAAGTCAACGACGATCCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
Clone_2006-01-1154-1      GCAGGAAAGTCAACGACGATCCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
                          ************************************************************

Chrom_2006-01-1154-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
Clone_2006-01-1154-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
                          ************************************************************

Chrom_2006-01-1154-1      TATGTTCCTGGAGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
Clone_2006-01-1154-1      TATGTTCCTGGAGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
                          ************************************************************

Chrom_2006-01-1154-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGCGATTATTTAATCAAACGATTT
Clone_2006-01-1154-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGCGATTATTTAATCAAACGATTT
                          ************************************************************

Chrom_2006-01-1154-1      GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
Clone_2006-01-1154-1      GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
                          ************************************************************

Chrom_2006-01-1154-1      TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
Clone_2006-01-1154-1      TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
                          ************************************************************

Chrom_2006-01-1154-1      CTAGATCCATTGATGGAAGCAGTATTCCAAGAAGAAGTAGAAAAAATCAAAAATGATGGC
Clone_2006-01-1154-1      CTAGATCCATTGATGGAAGCAGTATTCCAAGAAGAAGTAGAAAAAATCAAAAATGATGGC
                          ************************************************************

Chrom_2006-01-1154-1      AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
Clone_2006-01-1154-1      AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
                          ************************************************************

Chrom_2006-01-1154-1      GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
Clone_2006-01-1154-1      GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
                          ************************************************************

Chrom_2006-01-1154-1      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
Clone_2006-01-1154-1      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
                          ************************************************************

Chrom_2006-01-1154-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCGACTTTTTCTGCTGACAATGAAGCG
Clone_2006-01-1154-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCGACTTTTTCTGCTGACAATGAAGCG
                          ************************************************************

Chrom_2006-01-1154-1      ATAAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGACAAAAATCGAATCTGTACCG
Clone_2006-01-1154-1      ATAAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGACAAAAATCGAATCTGTACCG
                          ************************************************************

Chrom_2006-01-1154-1      CCAACGCTTGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
Clone_2006-01-1154-1      CCAACGCTTGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
                          ************************************************************
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Chrom_2006-01-1154-1      AGAAAATGAATGAAAAATTTGCCCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCG
Clone_2006-01-1154-1      AGAAAATGAATGAAAAATTTGCCCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCG
                          ************************************************************

Chrom_2006-01-1154-1      ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATATGTAC
Clone_2006-01-1154-1      ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATATGTAC
                          ************************************************************

Chrom_2006-01-1154-1      CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
Clone_2006-01-1154-1      CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
                          ************************************************************

Chrom_2006-01-1154-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
Clone_2006-01-1154-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
                          ************************************************************

Chrom_2006-01-1154-1      TAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAATGATTATCT
Clone_2006-01-1154-1      TAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAATGATTATCT
                          ************************************************************

Chrom_2006-01-1154-1      CAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
Clone_2006-01-1154-1      CAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
                          ************************************************************

Chrom_2006-01-1154-1      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
Clone_2006-01-1154-1      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
                          ************************************************************

Chrom_2006-01-1154-1      TGATCAATCTTTTATTAGGTCTTTTAATCGGCGGACTCATGATGAGTTTTGGTGTAAAAA
Clone_2006-01-1154-1      TGATCAATCTTTTATTAGGTCTTTTAATCGGCGGACTCATGATGAGTTTTGGTGTAAAAA
                          ************************************************************

Chrom_2006-01-1154-1      CGATTGATGCCGAAGGAGCTTTCTTGTTCGGAGGATCAATTGCATTGGCGGGAATTATCG
Clone_2006-01-1154-1      CGATTGATGCCGAAGGAGCTTTCTTGTTCGGAGGATCAATTGCATTGGCGGGAATTATCG
                          ************************************************************

Chrom_2006-01-1154-1      GTGGTGTATTGGCACTTGTGATGTCGCAGATTATGGCGACTTCTACTGGAGCAACCGGCT
Clone_2006-01-1154-1      GTGGTGTATTGGCACTTGTGATGTCGCAGATTATGGCGACTTCTACTGGAGCAACCGGCT
                          ************************************************************

Chrom_2006-01-1154-1      CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
Clone_2006-01-1154-1      CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
                          ************************************************************

Chrom_2006-01-1154-1      TTGATCTATCAATGTTCAATCCAATGGGATGGATTTACTTGACCTATCCTTTCACAAAAA
Clone_2006-01-1154-1      TTGATCTATCAATGTTCAATCCAATGGGATGGATTTACTTGACCTATCCTTTCACAAAAA
                          ************************************************************

Chrom_2006-01-1154-1      ATAACTGGCTACCATTATTATTTGCTTTGATTTTTAGTCTTGTTTTTACCGTACTTGCGT
Clone_2006-01-1154-1      ATAACTGGCTACCATTATTATTTGCTTTGATTTTTAGTCTTGTTTTTACCGTACTTGCGT
                          ************************************************************

Chrom_2006-01-1154-1      TTGTGTTGGAAGAACATCGCGACATGGGCGCAGGTTATCTTCCTGAACGAGAAGGACGTG
Clone_2006-01-1154-1      TTGTGTTGGAAGAACATCGCGACATGGGCGCAGGTTATCTTCCTGAACGAGAAGGACGTG
                          ************************************************************

Chrom_2006-01-1154-1      CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
Clone_2006-01-1154-1      CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
                          ************************************************************

Chrom_2006-01-1154-1      TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
Clone_2006-01-1154-1      TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
                          ************************************************************

Chrom_2006-01-1154-1      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
Clone_2006-01-1154-1      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
                          ************************************************************

Chrom_2006-01-1154-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
Clone_2006-01-1154-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
                          ************************************************************

Chrom_2006-01-1154-1      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
Clone_2006-01-1154-1      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
                          ************************************************************
```

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Chrom_2006-01-1154-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
Clone_2006-01-1154-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
                          ************************************************************

Chrom_2006-01-1154-1      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGCTTAGGTGGAACGGCGA
Clone_2006-01-1154-1      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGCTTAGGTGGAACGGCGA
                          ************************************************************

Chrom_2006-01-1154-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
Clone_2006-01-1154-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
                          ************************************************************

Chrom_2006-01-1154-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
Clone_2006-01-1154-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
                          ************************************************************

Chrom_2006-01-1154-1      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
Clone_2006-01-1154-1      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
                          ************************************************************

Chrom_2006-01-1154-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGCTGGATTCCAC
Clone_2006-01-1154-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGCTGGATTCCAC
                          ************************************************************

Chrom_2006-01-1154-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
Clone_2006-01-1154-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
                          ************************************************************

Chrom_2006-01-1154-1      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
Clone_2006-01-1154-1      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
                          ************************************************************
```

Figure 6.3.5: Alignment of chromosomal and cloned Nar operon DNA sequence for bacterial strain 2006-01-1154-1

```
Chrom_2006-01-1152-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAAATTTGGTAAATTCCAGGCGTTG
Clone_2006-01-1152-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAAATTTGGTAAATTCCAGGCGTTG
                          ************************************************************

Chrom_2006-01-1152-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
Clone_2006-01-1152-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
                          ************************************************************

Chrom_2006-01-1152-1      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
Clone_2006-01-1152-1      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
                          ************************************************************

Chrom_2006-01-1152-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
Clone_2006-01-1152-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
                          ************************************************************

Chrom_2006-01-1152-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
Clone_2006-01-1152-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
                          ************************************************************

Chrom_2006-01-1152-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
Clone_2006-01-1152-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
                          ************************************************************

Chrom_2006-01-1152-1      GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
Clone_2006-01-1152-1      GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
                          ************************************************************

Chrom_2006-01-1152-1      TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
Clone_2006-01-1152-1      TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
                          ************************************************************

Chrom_2006-01-1152-1      CTAGATCCATTGATGGAAGCAGTATTCCAAGAAGAAGTAGAAAAAATCAAAAATGATGGC
Clone_2006-01-1152-1      CTAGATCCATTGATGGAAGCAGTATTCCAAGAAGAAGTAGAAAAAATCAAAAATGATGGC
                          ************************************************************

Chrom_2006-01-1152-1      AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
Clone_2006-01-1152-1      AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
                          ************************************************************
```

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Chrom_2006-01-1152-1      GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
Clone_2006-01-1152-1      GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
                          ************************************************************

Chrom_2006-01-1152-1      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
Clone_2006-01-1152-1      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
                          ************************************************************

Chrom_2006-01-1152-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
Clone_2006-01-1152-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
                          ************************************************************

Chrom_2006-01-1152-1      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
Clone_2006-01-1152-1      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
                          ************************************************************

Chrom_2006-01-1152-1      CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
Clone_2006-01-1152-1      CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
                          ************************************************************

Chrom_2006-01-1152-1      AGAAAATGAATGAAAAATTTGCGCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCG
Clone_2006-01-1152-1      AGAAAATGAATGAAAAATTTGCGCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCG
                          ************************************************************

Chrom_2006-01-1152-1      ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATTTGTAC
Clone_2006-01-1152-1      ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATTTGTAC
                          ************************************************************

Chrom_2006-01-1152-1      CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
Clone_2006-01-1152-1      CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
                          ************************************************************

Chrom_2006-01-1152-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
Clone_2006-01-1152-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
                          ************************************************************

Chrom_2006-01-1152-1      TAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAATGATTATCT
Clone_2006-01-1152-1      TAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAATGATTATCT
                          ************************************************************

Chrom_2006-01-1152-1      CAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
Clone_2006-01-1152-1      CAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
                          ************************************************************

Chrom_2006-01-1152-1      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
Clone_2006-01-1152-1      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
                          ************************************************************

Chrom_2006-01-1152-1      TGATCAATCTTTTATTAGGTCTTTTAATCGGCGGACTCATGATGAGTTTTGGTGTAAAAA
Clone_2006-01-1152-1      TGATCAATCTTTTATTAGGTCTTTTAATCGGCGGACTCATGATGAGTTTTGGTGTAAAAA
                          ************************************************************

Chrom_2006-01-1152-1      CGATTGATGCCGAAGGAGCTTTCTTGTTCGGAGGATCAATTGCATTGGCGGGAATTATCG
Clone_2006-01-1152-1      CGATTGATGCCGAAGGAGCTTTCTTGTTCGGAGGATCAATTGCATTGGCGGGAATTATCG
                          ************************************************************

Chrom_2006-01-1152-1      GTGGTGTATTGGCACTTGTGATGTCGCAGATTATGGCGACTTCTACTGGAGCAACCGGCT
Clone_2006-01-1152-1      GTGGTGTATTGGCACTTGTGATGTCGCAGATTATGGCGACTTCTACTGGAGCAACCGGCT
                          ************************************************************

Chrom_2006-01-1152-1      CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
Clone_2006-01-1152-1      CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
                          ************************************************************

Chrom_2006-01-1152-1      TTGATCTATCAATGTTCAATCCAATGGGATGGATTTACTTGACCTATCCTTTCACAAAAA
Clone_2006-01-1152-1      TTGATCTATCAATGTTCAATCCAATGGGATGGATTTACTTGACCTATCCTTTCACAAAAA
                          ************************************************************

Chrom_2006-01-1152-1      ATAACTGGCTACCATTATTATTTGCTTTGATTTTTAGTCTTGTTTTTACCGTACTTGCGT
Clone_2006-01-1152-1      ATAACTGGCTACCATTATTATTTGCTTTGATTTTTAGTCTTGTTTTTACCGTACTTGCGT
                          ************************************************************

Chrom_2006-01-1152-1      TTGTGTTGGAAGAACATCGCGACATGGGCGCAGGTTATCTTCCTGAACGAGAAGGACGTG
Clone_2006-01-1152-1      TTGTGTTGGAAGAACATCGCGACATGGGCGCAGGTTATCTTCCTGAACGAGAAGGACGTG
                          ************************************************************
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Chrom_2006-01-1152-1      CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
Clone_2006-01-1152-1      CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
                          ************************************************************

Chrom_2006-01-1152-1      TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
Clone_2006-01-1152-1      TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
                          ************************************************************

Chrom_2006-01-1152-1      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
Clone_2006-01-1152-1      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
                          ************************************************************

Chrom_2006-01-1152-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
Clone_2006-01-1152-1      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
                          ************************************************************

Chrom_2006-01-1152-1      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
Clone_2006-01-1152-1      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
                          ************************************************************

Chrom_2006-01-1152-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
Clone_2006-01-1152-1      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
                          ************************************************************

Chrom_2006-01-1152-1      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
Clone_2006-01-1152-1      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
                          ************************************************************

Chrom_2006-01-1152-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
Clone_2006-01-1152-1      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
                          ************************************************************

Chrom_2006-01-1152-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
Clone_2006-01-1152-1      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
                          ************************************************************

Chrom_2006-01-1152-1      CAAAATTATGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
Clone_2006-01-1152-1      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
                          ************************************************************

Chrom_2006-01-1152-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
Clone_2006-01-1152-1      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
                          ************************************************************

Chrom_2006-01-1152-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
Clone_2006-01-1152-1      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
                          ************************************************************

Chrom_2006-01-1152-1      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
Clone_2006-01-1152-1      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
                          ************************************************************
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Figure 6.3.6: Alignment of chromosomal and cloned Nar operon DNA sequence for bacterial strain 2006-01-1152-1

```
Chrom_2006-01-1190-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAAATTTGGTAAATTCCAGGCGTTG
Clone_2006-01-1190-1      ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAAATTTGGTAAATTCCAGGCGTTG
                          ************************************************************

Chrom_2006-01-1190-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
Clone_2006-01-1190-1      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
                          ************************************************************

Chrom_2006-01-1190-1      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
Clone_2006-01-1190-1      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
                          ************************************************************

Chrom_2006-01-1190-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
Clone_2006-01-1190-1      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
                          ************************************************************

Chrom_2006-01-1190-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
Clone_2006-01-1190-1      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
                          ************************************************************

Chrom_2006-01-1190-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
```

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Clone_2006-01-1190-1      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
                          ************************************************************

Chrom_2006-01-1190-1      GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
Clone_2006-01-1190-1      GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
                          ************************************************************

Chrom_2006-01-1190-1      TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
Clone_2006-01-1190-1      TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
                          ************************************************************

Chrom_2006-01-1190-1      CTAGATCCATTGATGGAAGCAGTATTCCAAGAAGAAGTAGAAAAAATCAAAAATGATGGC
Clone_2006-01-1190-1      CTAGATCCATTGATGGAAGCAGTATTCCAAGAAGAAGTAGAAAAAATCAAAAATGATGGC
                          ************************************************************

Chrom_2006-01-1190-1      AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
Clone_2006-01-1190-1      AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
                          ************************************************************

Chrom_2006-01-1190-1      GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
Clone_2006-01-1190-1      GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
                          ************************************************************

Chrom_2006-01-1190-1      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
Clone_2006-01-1190-1      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
                          ************************************************************

Chrom_2006-01-1190-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
Clone_2006-01-1190-1      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
                          ************************************************************

Chrom_2006-01-1190-1      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
Clone_2006-01-1190-1      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
                          ************************************************************

Chrom_2006-01-1190-1      CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
Clone_2006-01-1190-1      CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
                          ************************************************************

Chrom_2006-01-1190-1      AGAAAATGAATGAAAAATTTGCGCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCG
Clone_2006-01-1190-1      AGAAAATGAATGAAAAATTTGCGCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCG
                          ************************************************************

Chrom_2006-01-1190-1      ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATTTGTAC
Clone_2006-01-1190-1      ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATTTGTAC
                          ************************************************************

Chrom_2006-01-1190-1      CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
Clone_2006-01-1190-1      CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
                          ************************************************************

Chrom_2006-01-1190-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
Clone_2006-01-1190-1      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
                          ************************************************************

Chrom_2006-01-1190-1      TAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAATGATTATCT
Clone_2006-01-1190-1      TAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAATGATTATCT
                          ************************************************************

Chrom_2006-01-1190-1      CAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
Clone_2006-01-1190-1      CAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
                          ************************************************************

Chrom_2006-01-1190-1      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
Clone_2006-01-1190-1      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
                          ************************************************************

Chrom_2006-01-1190-1      TGATCAATCTTTTATTAGGTCTTTTAATCGGCGGACTCATGATGAGTTTTGGTGTAAAAA
Clone_2006-01-1190-1      TGATCAATCTTTTATTAGGTCTTTTAATCGGCGGACTCATGATGAGTTTTGGTGTAAAAA
                          ************************************************************

Chrom_2006-01-1190-1      CGATTGATGCCGAAGGAGCTTTCTTGTTCGGAGGATCAATTGCATTGGCGGGAATTATCG
Clone_2006-01-1190-1      CGATTGATGCCGAAGGAGCTTTCTTGTTCGGAGGATCAATTGCATTGGCGGGAATTATCG
                          ************************************************************

Chrom_2006-01-1190-1      GTGGTGTATTGGCACTTGTGATGTCGCAGATTATGGCGACTTCTACTGGAGCAACCGGCT
Clone_2006-01-1190-1      GTGGTGTATTGGCACTTGTGATGTCGCAGATTATGGCGACTTCTACTGGAGCAACCGGCT
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                         ************************************************************

Chrom_2006-01-1190-1     CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
Clone_2006-01-1190-1     CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
                         ************************************************************

Chrom_2006-01-1190-1     TTGATCTATCAATGTTCAATCCAATGGGATGGATTTACTTGACCTATCCTTTCACAAAAA
Clone_2006-01-1190-1     TTGATCTATCAATGTTCAATCCAATGGGATGGATTTACTTGACCTATCCTTTCACAAAAA
                         ************************************************************

Chrom_2006-01-1190-1     ATAACTGGCTACCATTATTATTTGCTTTGATTTTTAGTCTTGTTTTTACCGTACTTGCGT
Clone_2006-01-1190-1     ATAACTGGCTACCATTATTATTTGCTTTGATTTTTAGTCTTGTTTTTACCGTACTTGCGT
                         ************************************************************

Chrom_2006-01-1190-1     TTGTGTTGGAAGAACATCGCGACATGGGCGCAGGTTATCTTCCTGAACGAGAAGGACGTG
Clone_2006-01-1190-1     TTGTGTTGGAAGAACATCGCGACATGGGCGCAGGTTATCTTCCTGAACGAGAAGGACGTG
                         ************************************************************

Chrom_2006-01-1190-1     CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
Clone_2006-01-1190-1     CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
                         ************************************************************

Chrom_2006-01-1190-1     TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
Clone_2006-01-1190-1     TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
                         ************************************************************

Chrom_2006-01-1190-1     GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
Clone_2006-01-1190-1     GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
                         ************************************************************

Chrom_2006-01-1190-1     GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
Clone_2006-01-1190-1     GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
                         ************************************************************

Chrom_2006-01-1190-1     CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
Clone_2006-01-1190-1     CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
                         ************************************************************

Chrom_2006-01-1190-1     GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
Clone_2006-01-1190-1     GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
                         ************************************************************

Chrom_2006-01-1190-1     CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
Clone_2006-01-1190-1     CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
                         ************************************************************

Chrom_2006-01-1190-1     TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
Clone_2006-01-1190-1     TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
                         ************************************************************

Chrom_2006-01-1190-1     ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
Clone_2006-01-1190-1     ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
                         ************************************************************

Chrom_2006-01-1190-1     CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
Clone_2006-01-1190-1     CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
                         ************************************************************

Chrom_2006-01-1190-1     GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
Clone_2006-01-1190-1     GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
                         ************************************************************

Chrom_2006-01-1190-1     GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
Clone_2006-01-1190-1     GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
                         ************************************************************

Chrom_2006-01-1190-1     TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
Clone_2006-01-1190-1     TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
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Figure 6.3.7: Alignment of chromosomal and cloned Nar operon DNA sequence for bacterial strain 2006-01-1190-1

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CHROME_SVA       ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
CLONE_SVA        ATGACAGAAATTGTAAAAGTACAAGGCTTGCAAAAAAAATTTGGTAAATTCCAGGCGTTG
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                ************************************************************

CHROME_SVA      AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
CLONE_SVA       AAAGATGTCTCATTCACAGTAAACGCCGGTGAAGTTGTTGGTTTTATCGGACCAAATGGA
                ************************************************************

CHROME_SVA      GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
CLONE_SVA       GCAGGAAAGTCAACGACGATTCGTACACTGCTAGGAATCATCAACCGAGACGAAGGAGAT
                ************************************************************

CHROME_SVA      GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
CLONE_SVA       GTCCAAATATTCGGAAAAGATGTTTGGAAAGATAGTCTAGAAATCCATAAACGAATTTCG
                ************************************************************

CHROME_SVA      TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
CLONE_SVA       TATGTTCCTGGGGATGTTGCTCTTTGGGGCAGCCTGACTGGTGGAGAGATCATTGATCTA
                ************************************************************

CHROME_SVA      TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
CLONE_SVA       TTTATCAAACTTCATGGCGGCGGGAGCAAAGCAAAGCGTGATTATTTAATCAAACGATTT
                ************************************************************

CHROME_SVA      GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
CLONE_SVA       GAACTTGATCCAAAGAAAAAAGCCAAAGGTTACTCTAAAGGAAATCGTCAAAAAGTCGGT
                ************************************************************

CHROME_SVA      TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
CLONE_SVA       TTGATTGCTGCACTTTCAGTTGAATCTGATCTGTATATTTTAGATGAACCGACTTCAGGA
                ************************************************************

CHROME_SVA      CTAGATCCATTGATGGAAGCAGTATTCCAAGAAGAAGTAGAAAAAATCAAAAATGATGGC
CLONE_SVA       CTAGATCCATTGATGGAAGCAGTATTCCAAGAAGAAGTAGAAAAAATCAAAAATGATGGC
                ************************************************************

CHROME_SVA      AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
CLONE_SVA       AAAGCGATTCTATTATCTTCACATATTTTAAGTGAAGTTGAACGATTAGCAGATAAAGTA
                ************************************************************

CHROME_SVA      GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
CLONE_SVA       GCAATCATTCGACGTGGAGAAGTAGTTGAAACAGGTACATTAGATGAATTGCGTCATTTG
                ************************************************************

CHROME_SVA      ACTCGCTCAACAGTTACATTGGTGACAAAAGGCGATATTGAGAAACTTGCGACGCTCTCT
CLONE_SVA       ACTCGCTCAACAGTTACATTGGTGACAAAAGGTGATATTGAGAAACTTGCGACGCTCTCT
                ******************************** ************************

CHROME_SVA      GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
CLONE_SVA       GGCGTGCATGATTTTGTTCAAAAAGACGGCAAAGCAACTTTTTCTGCTGACAATGAAGCG
                ************************************************************

CHROME_SVA      ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
CLONE_SVA       ATGAATACGATTCTGACCGAGGCAACCAAATTAGGTGTGATAAAAATCGAATCTGTACCG
                ************************************************************

CHROME_SVA      CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
CLONE_SVA       CCAACGCTCGAAGATTTATTCATGCGTCACTACGAAGGCTGATTGTCGGAACGGAGGAAA
                ************************************************************

CHROME_SVA      AGAAAATGAATGAAAAATTTGCGCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCG
CLONE_SVA       AGAAAATGAATGAAAAATTTGCGCGTTGGAACGTATTGTTCATTCAATACGTGAAACGCG
                ************************************************************

CHROME_SVA      ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATTTGTAC
CLONE_SVA       ATTGGAAAAAAATAATTGTTTGGGTTTTAGGTTTGGGTTTGTTCTCAGGAGCATATGTAC
                ********************************************************* *****

CHROME_SVA      CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
CLONE_SVA       CAGCATTTGAAGAGATTGCTAAAGGACAAGGTCTTTTAGGGATGTTTGAAACGATGCAAA
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                ************************************************************

CHROME_SVA      ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
CLONE_SVA       ATCCAGCGATGATCTCGATGGTTGGACCTACACCAATCAAAATAGGTACGGATTATACTT
                ************************************************************

CHROME_SVA      TAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAATGATTATCT
CLONE_SVA       TAGGAGCGATGTATGCTCAAGAGATGTTGCTGTTTTGCGGATTGTTCGCAATGATTATCT
                ************************************************************

CHROME_SVA      CAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
CLONE_SVA       CAGCACTTCATGTGGTGAGCCACACGCGAAAAGAAGAAGAATTAGGTTTGACTGAATTGG
                ************************************************************

CHROME_SVA      TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
CLONE_SVA       TTCGCTCATTTCGAGTAGGACGACAAGCCAATTCATTAGCTGTTATCAGTGAGATGCTGT
                ************************************************************

CHROME_SVA      TGATCAATCTTTTATTAGGTCTTTTAATCGGCGGACTCATGATGAGTTTTGGTGTAAAAA
CLONE_SVA       TGATCAATCTTTTATTAGGTCTTTTAATCGGCGGACTCATGATGAGTTTTGGTGTAAAAA
                ************************************************************

CHROME_SVA      CGATTGATGCCGAAGGAGCTTTCTTGTTCGGAGGATCAATTGCATTGGCGGGAATTATCG
CLONE_SVA       CGATTGATGCCGAAGGAGCTTTCTTGTTCGGAGGATCAATTGCATTGGCGGGAATTATCG
                ************************************************************

CHROME_SVA      GTGGTGTATTGGCACTTGTGATGTCGCAGATTATGGCGACTTCTACTGGAGCAACCGGCT
CLONE_SVA       GTGGTGTATTGGCACTTGTGATGTCGCAGATTATGGCGACTTCTACTGGAGCAACCGGCT
                ************************************************************

CHROME_SVA      CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
CLONE_SVA       CGACATTAAGTCTTATAGGACTTTTGTATATCGTGCGCGCTGGAACAGATGTGTCTAATC
                ************************************************************

CHROME_SVA      TTGATCTATCAATGTTCAATCCAATGGGATGGATTTACTTGACCTATCCTTTCACAAAAA
CLONE_SVA       TTGATCTATCAATGTTCAATCCAATGGGATGGATTTACTTGACCTATCCTTTCACAAAAA
                ************************************************************

CHROME_SVA      ATAACTGGCTACCATTATTATTTGCTTTGATTTTTAGTCTTGTTTTTACCGTACTTGCGT
CLONE_SVA       ATAACTGGCTACCATTATTATTTGCTTTGATTTTTAGTCTTGTTTTTACCGTACTTGCGT
                ************************************************************

CHROME_SVA      TTGTGTTGGAAGAACATCGCGACATGGGCGCAGGTTATCTTCCTGAACGAGAAGGACGTG
CLONE_SVA       TTGTGTTGGAAGAACATCGCGACATGGGCGCAGGTTATCTTCCTGAACGAGAAGGACGTG
                ************************************************************

CHROME_SVA      CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
CLONE_SVA       CGACGGCGAAGAAATCACTACTTTCTGTACCTGGTTTGTTTTTCAAGATTAATAAAGGAG
                ************************************************************

CHROME_SVA      TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
CLONE_SVA       TAATGATTGGTTGGCTGATCGCATTTGTGGTTATGGGAGCTGCGTATGGCTCCATTTATG
                ************************************************************

CHROME_SVA      GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
CLONE_SVA       GAGACATGCAAGTCTTTCTTGGCGGAAATGAACTGATGAAACAAATGTTCACTCAATCTG
                ************************************************************

CHROME_SVA      GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
CLONE_SVA       GCGTTTCCATTGAAGAATCCTTTACGGCAACGATCATGATGGTAATGATTGGATTAGTCA
                ************************************************************

CHROME_SVA      CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
CLONE_SVA       CAATCTTGCCAATCGCGGTGGTCAATAAATTATTTGCAGAAGAAACAAGACTGCATCTGA
                ************************************************************

CHROME_SVA      GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
CLONE_SVA       GTCAACTGTATGTAACGAAGATTACGCGAGGCCAATTATATTGGACAACGATATTTTTAG
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                ************************************************************

CHROME_SVA      CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
CLONE_SVA       CTATTTTTGCTGGAGTCGTAGGCATTGGCTTAGCATCAGCGGGATTAGGTGGAACGGCGA
                ************************************************************

CHROME_SVA      TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
CLONE_SVA       TTTCTGCGATGAAAAATGAATCGACTATGGATCTGACCGATTTCTTAGCTGCTGGATACA
                ************************************************************

CHROME_SVA      ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
CLONE_SVA       ATTTTCTCCCTTCCATCTTATTTTATATTGGTTTGGCTGCTTTAGCGTTAGGCTGGTTGC
                ************************************************************

CHROME_SVA      CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
CLONE_SVA       CAAAATTTGGAAAAGTAATCTATGCTTATCTAGGCTATTCCTTTGCTTTGAATTATTTCG
                ************************************************************

CHROME_SVA      GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
CLONE_SVA       GCGGAATCTTAGATTTGCCGGATTGGTTCTCAAAAACGGCGATTCAAAGTTGGATTCCAC
                ************************************************************

CHROME_SVA      GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
CLONE_SVA       GCTTACCGATGGAAGAATTTGATGGAACGATTTTTGCAGTAATTACTGTTATCAGTATCG
                ************************************************************

CHROME_SVA      TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
CLONE_SVA       TCTTCTTATTTGTCGGCTATTTAGGATACAAACGCCGTGATATGGTAGAAGGCGCTTAA
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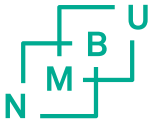
Figure 6.3.8: Alignment of chromosomal and cloned Nar operon DNA sequence for bacterial strain SVA-01-233



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