Escherichia coli Ribosomal RNA deletion strain

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Running title: *E. coli rrn* deletion strain

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1 Introduction

Ribosome function in bacteria constitutes the most energetically expensive process in the cell. As such production of rRNA presents an important target for regulating the energy flux and coordinating other physiological processes. Multiple mechanisms for regulating rRNA production have been described (reviewed in (41)). We previously constructed a strain *E. coli* in which all chromosomal rRNA gene function was eliminated from the chromosome and replaced instead with a single plasmid based rRNA operon (TA series) (1, 2). With the wealth of ribosomal crystal data now available this deletion series has been particularly useful for ribosome structure function studies (34, 36, 46, 54).

While the TA deletion series has been useful for functional studies of *E. coli* ribosomes, there are limitations to using the strains for more physiological studies of rRNA transcription. The progenitor to the TA series, strain TX has a number of known chromosomal mutations (22). It is a derivative of *E. coli* strain TX135, a lysogen with a temperature-sensitive inducible Mu phage. The provenance of the strain is unclear and whether it has an additional chromomosomal mutations is unknown.

Additionally, before the introduction of the phage λ red method of allelic exchange in *E. coli* methods for generating precise chromosomal deletions were cumbersome (14, 60). Ribosomal RNA operons in the TA series were inactivated by replacing a portion of the 16S and 23S genes with an antibiotic or *lacZ* marker, leaving the rRNA promoters and 5S genes intact. In addition, these markers precluded their subsequent use in the deletion strain, further limiting cloning options. The TA deletion series retains rRNA promoters in the inactivated rRNA operons. Since transcription from these operons usually represent eighty to ninety percent of total RNA synthesis in rich media at 37°C, this non-productive transcription incurs a substantial metabolic burden on the cell and titrates out other components such as initiation factor 3, further confounding physiological interpretation of data obtained with the strains (13).

At fast growth rates in rich media at 37°C as much as 70% of *E. coli*'s resources are devoted to the translation machinery and protein synthesis (45). The ribosome forms the core of the translation machinery and its function is critical in ensuring cells grow at a maximal rate (Bremer: growth rate ∝ number of ribosome × peptide chain elongation rate 17).

Growth rate is determined by the concentration of ribosomes and the peptide chain elongation rate (17). Ribosome synthesis is rate-limited by rRNA transcription and thus growth rate itself is limited by the rRNA transcription level(16, 47).

Feedback regulation is one mechanism of rRNA regulation in *E. coli* (reviewed in 40). Electron microscopy of rRNA operons reveal that in *E. coli* cells with *rrn* operon deletions, a feedback mechanism ensures a sufficient amount of rRNA is made by increasing the initiation frequency at the *rrn* promoters and possibly also increasing RNA polymerase elongation rates (12, 56). Likewise in cells with increased rRNA genes dosage there is no apparent increase in rRNA transcription (3, 31). Thus feedback regulation can compensate for changes in gene dosage.

E. coli has 7 *rrn* operons but with multifork DNA replication this number may increase to as many as 38 *rrn* operons. At high growth rates *rrn* operons are not fully saturated with RNAPs suggesting that the capacity for rRNA transcription has not yet been reached (8). Reducing the rRNA gene dosage by eliminating rRNA genes results in an increased number of RNAPs per rRNA gene from enhanced promoter initiation frequency and RNAP elongation rate (53 RNAPs/rRNA gene in WT to 71 RNAPs/rRNA gene in Δ4 12). Since fewer rRNA genes leads to increased initiation from the *rrn* promoters, the question of whether promoter saturation eventually limits the number of RNAPs transcribing the remaining rRNA operons can be directly addressed by examining additional rRNA gene deletions. If saturation of the existing rRNA genes has been reached then additional rRNA genes should increase rRNA levels and thereby enhancing growth rate.

2 Materia and Methods

2.1 Growth conditions

Strains plasmids and oligonucleotides used in this work are described in Table 1. Luria-Bertani (Lennox) media was used for growth in rich media. M9 minimal media was used for growth in a defined media. This was supplemented variously with uracil (20 µg/ml), casamino acids (Difco) (0.1%) and serine (0.5 mg/ml). Glucose (0.2%) was used as the carbon source. Antibiotics were used at the following concentrations: ampicillin

100 µg/ml, kanamycin 30 µg/ml, spectinomycin 30 µg/ml, chloramphenicol 30 µg/ml.

2.2 Plasmid construction

The pSC101 based ribosomal RNA plasmids pK4-15 and p19cr used the minimal pSC101 plasmids described in ref 26. Plasmid pK4-16 is derived from plasmid pTH18kr and plasmid p19cr is derived from pTH19cr (26). The *rrn*B operon was amplified by PCR with primers BF-Bam and BR-Bam (Table 2). The pSC101 backbone plasmid was amplified with primers THF-Bam and THR-Bam eliminating the Plac promoter and multiple cloning site in the original plasmid. The integrity of *rrn*B was confirmed by sequencing and in cases where a mutation was located, fragment exchange with wildtype sequence was performed by restriction enzyme cloning (Figure 1).

2.3 Southern blots

Genomic DNA was digested with restriction enzymes BamHI and PstI (NEB). Digested DNA was run on a 0.6% agarose gel. Gels were briefly treated with 0.25 N HCl and transferred overnight onto a nylon membrane (Millipore, Ny+) via capillary transfer with alkaline transfer buffer (0.4 N NaOH, 1 M NaCl). DNA was crosslinked onto the membrane with UV light (Stratalinker, Stratagene).

Membranes were prehybridized in modified Church hybridization buffer (0.5 M sodium phosphate pH 7.1, 2 mM EDTA, 7% SDS, 0.1% sodium pyrophosphate) for 2 hours at 68°C (11). Ribosomal RNA 16S probes were DIG-labelled dUTP (Roche diagnostics) in a PCR reaction with primers TA227 and TA236 (Table 2). Hybridization, washing and detection were performed as recommended by the manufacturer (Roche Diagnostics) for chemiluminescent detection.

2.4 Sucrose gradients

Ribosomes were prepared from cells grown in LB media at 37°C as previously described (25). Sucrose gradients were prepared either by the

freeze-thaw method as described in ref 37 or by using the Gradient Master (BioComp Instruments). Ribosome subunits were separated on a 10 - 40% sucrose gradients by centrifugation in either a Sorvall SW-28 or SW-41 rotor. Samples were centrifuged in a SW-41 rotor at 35000 rpm for 2.5 hours or in the SW-28 rotor at 20000 rpm for 15 hours. Samples were fractionated with a BioComp Piston gradient fractionator (BioComp Instruments) attached to BioRad FPLC for fraction collection and inline UV detection.

2.5 Microscopy

Cells were grown with shaking in LB to an O.D.600 between 0.1 and 0.2 for at least three generations. Nucleoids were stained with DAPI (0.1 µg/ml) (Probes, InVitrogen) for 5 minutes at room temperature. A small aliquot of cells (5 µl) was placed onto an agarose pad (1% in M9 minimal media) prepared on a glass slide. Samples were covered with a coverslip and then viewed with a Leica DM4000 B microscope. Simultaneous phase-contrast and fluorescence was used to image the cells through a 1.3 numerical aperture 100× objective lens.

3 Results

3.1 Strain construction

E. coli MG1655 a completely sequenced and well characterized derivative of the wildtype *E. coli* K-12 strain was chosen for making the rrn deletion strains (6, 27, 50). Variations from the published genome sequence for MG1655 strains have been described 50. This strain is a derivative of ??? ().

Each of the seven rRNA operons was completely deleted using the PCR allelic exchange method described in ref 14 to give seven kanamycin marked *rrn* reletion strains (Table 1). Deletions spanned as much of the control elements around each of the rRNA operons including upstream promoter elements, FIS binding sites and downstream terminators as possible without interfering with upstream or downstream genes (Figure 3).

Successive P1 transduction of a kanamycin marked rrn deletion and resolution of this resistance marker was used to combine individual *rrn* deletions culminating in a strain with all chromosomal *rrn* operons removed. Rrn deletions were ordered to minimize impact from loss of spacer tRNAs.

Resolution of the antibiotic marker with FLP resolvase leaves an 85 bp scar site. Deletions of the *rrn* operons were confirmed by both PCR (data not shown) and Southern blots (Figure 2). Supplemental tRNA and rRNA genes were provided by the tRNA plasmid ptRNA67 (p15A ori, SpcR) (1) and rRNA plasmid pKK3535 (pBR322 ori, AmpR) (9) at the delta five and six stages respectively (Figure 2).

3.2 Maximal growth rates

The question of ribosome and indirectly rRNA levels required to sustain cells at a given growth rate can partially be addressed with the *rrn* deletion strains. Reducing rRNA copy number will force cells to transcribe more rRNA from fewer rRNA operons to maintain the same rRNA level 12. Growth rate of a *rrn* deletion strain compared to the growth rate of the deletion strain suggests that either rRNA, tRNA or both are growth limiting. This implies cells are unable to cope with the increased demand for rRNA through their usual mechanism of increasing *rrn* transcription probably due to some physiological limitation. Whether this limitation represents a combination of *rrn* promoter initiation or transcription rate or some other reason will give interesting insight the mechanism of *rrn* transcription regulation.

E. coli responds to fewer rrn copies by increasing *rrn* initiation 12. Growth rates of the *rrn* deletion strains were compared under fast growth (rich media at 37°C) and slow growth conditions (minimal M9 media at 37°C) (Figure 5).

[what is the influence of rrn copy number on growth rate and does how does this explain either a growth rate dependent or feedback control? Note Bremer argues against feedback control! Does feedback regulation not imply a preset homeostatic level and then why copy number variation - ie. can we increase growth rate of a strain with a single rrn operon by increasing copy number or conversely can we reduce growth rate of a strain by eliminating rRNA operons?]

A reproducible but statistically insignificant growth rate lag compared to the wildtype was observed with each additional *rrn* deletion upto Δ3. At the deletion of fourth *rrn* operon the relative growth rate decreased substantially by 72% with respect to the wildtype. Deletion of the fifth operon required the addition of tRNA genes on a plasmid since the deletion would remove all copies of the unique rRNA spacer tRNA ile and ala isoacceptors. The doubling time for the Δ5 ptRNA67 strain was significantly faster than the Δ4 strain suggesting that the presence of the tRNA plasmid was responsible for faster growth. This tRNA limitation would partially also explain the dramatic doubling time increase from the Δ3 to the Δ4 strain. Indeed, addition of a tRNA plasmid to the Δ4 strain reduced the doubling time to a rate comparable to Δ5 ptRNA67 but not the Δ3 strain. Addition of a rRNA plasmid to the Δ4 and Δ5 ptRNA67 strain had no significant effect on growth rates suggesting that these strains, despite their slower doubling times, are tRNA but not rRNA limited. The most significant growth rate change from one *rrn* operon deletion to the next was the Δ5 to Δ6 deletion. Only the Δ6 deletion strain show a substantially faster growth rate after transformation with an rRNA plasmid suggesting that rRNA gene copy number is limited in this background.

Issues with plasmid maintenance in the deletion series became apparent after differences in plasmid transformation was noted (Fig 4). For example it was shown that there was a significant reduction in the transformation efficiency of plasmid pNKwt in SQ53. This was independent of the *rrn* operon since pK4-16 a pSC101 rrnB plasmid transformed SQ53 without a reduction in efficiency. It was also independent of the replicon since a pBR322 plasmid also does not show this reduction in transformation efficiency. What is curious is that after transforming ptRNA67 plasmid into the cell the transformation efficiency is restored. It has previously been reported that uncharged tRNA interact with RNA I which regulates ColE1 plasmid replication (58).

Furthermore in the course of measuring growth rates of the deletion series we noticed a significant growth rate difference between deletions strains transformed with an *rrn* plasmid derived from a pBR322 backbone such as pKK3535 and pSTL102 to those transformed with a pSC101 derived backbone like pHKrrnC or pK4-16. Strains carrying the pKK3535 plasmid grew substantially slower. Comparison of the growth rates of wildtype *E. coli* MG1655 transformed with different *rrn* plasmids suggested that even in a wildtype background, slower growth rate correlated

with the presence of pMB1 *rrn* plasmids but not with a pSC101 *rrn* plasmids (Table 4). In subsequent experiments pSC101 based *rrn* plasmids were used.

Growth rates were also measured under more defined conditions that required less intensive rRNA transcription (M9 minimal media with glucose). As expected, the growth rate differences were attenuated in minimal media but the overall trend remained the same as seen with growth in rich media (Table 3).

Growth rate and tRNA/rRNA complementation data surprisingly suggested that the *rrn* deletion strains are mostly tRNA-limited. Only the Δ6 strain showed any evidence of rRNA limitation.

3.3 Nucleoid structure

The contribution of transcription to chromosome supercoiling, spatial localization and chromosome segregation in prokaryotes is currently a topic of much interest (7). The deletion series enabled the study of effects of successive removal of highly transcribed domains from the chromosome and their effects on supercoiling and chromosomal domain localization.

Loss of transcription induced supercoiling might be a cause of chromosomal instability possibly through loss of supercoiling domain structure generated by active transcription. Pulsed-field gel electrophoresis analysis of the chromosome after digestion with the restriction enzyme Not I suggested that chromosomal rearrangements had occurred.

Nucleoids of the deletion strains were examined under early log phase growth after staining with DAPI and examining cells under fluorescent microscopy. Since nucleoids are subject to photo- or autolytic degradation which result in diffuse nucleoids (63) we were careful to minimize exposure to light. Results show that during early log phase growth wildtype cells, as expected, have a compact nucleoid (Figure 6). The Δ6 strain nucleoid however is decondensed but after transformation with an *rrn* plasmid, appeared more structured.

3.4 Hydroxyurea sensitivity

Hydroxyurea inhibits DNA replication by targeting ribonucleotide reductase the enzyme responsible for de novo synthesis of deoxyribonucleotides (44). It has been shown to induce the SOS response (4). We were interested in investigating the *rrn* deletion series to see if there was a correlation with the observed nucleoid abnormalities and sensitivity to hydroxyurea. Also since we predicted more intensive *rrn* transcription with each successive *rrn* operon deletion, we wanted to establish whether there was a correlation between the number of deleted *rrn* operons and the strain's HU sensitivity. This would support an RNA polymerase stalling model which would predict more stalling of DNA replication because of the larger array of RNA polymerases transcribing the rRNA operon. The RNAP roadblock would be relieved by increasing rRNA gene copy number.

The deletion strains showed an increasing sensitivity to HU as more *rrn* operons were deleted (Figure 7). Dependence of HU sensitivity on *rrn* copy number was confirmed by complementation with an *rrn* plasmid. Strains transformed with an *rrn* plasmid in most cases showed at least a 100-fold greater survival rate on LB plates at 37°C (Figure 7). The effect was more dramatic in strains with a greater number of *rrn* deletions. Transformation of such strains with a tRNA plasmid had no such obvious effect on HU resistance suggesting that rRNA rather than tRNA gene dosage was responsible for increased HU resistance.

Additional plasmid copies of the *rrn* genes restored HU resistance in the deletion strains presumably by preventing stalled RNAP complexes. We wanted to see which components of the traditional replication fork repair pathway *recB*, *recA* and *ruvABC* were involved. Increased HU resistance from an *rrn* plasmid was *recB* dependent but *recA* independent.

3.5 Nalidixic acid resistance

DNA gyrase (GyrA) is one of the topoisomerases responsible for maintaining chromosomal topology. The quinolone antibiotic nalidixic acid specifically targets DNA gyrase forming a ternary complex that blocks DNA replication (10). Further pleiotropic responses include inhibition of initiation and elongation of DNA replication, blocking of RNAP, DNA damage and SOS induction (30, 42, 59). Since chromosome condensation is

affected in the deletion strains we examined the consequences of agents that interfere with chromosome topology.

Deletion of rRNA operons clearly offered strains some protective effect to nalidixic acid compared to the wildtype strain (Figure 8). While most noticeable with the Δ6 strain which only showed a 10-fold reduction of efficiency of plating (EOP) at 5 mM Nal, wildtype and other deletion strains show a more substantial 4-5 log EOP reduction. Transformation of the Δ6 strain with a rRNA plasmid restored sensitivity of the strain to nalidixic acid at 37°C again showing that this effect is rRNA specific. Curiously this result was not seen at 42°C.

Resistance to nalidixic acid also showed a temperature dependence. A slight increase in naldixic acid resistance was observed in the wildtype strain but a more noticeable increase in resistance was seen in strains with 4 or more deletions after incubation at 42°C compared to incubation at 37°C (Figure 8). Transformation with either a tRNA or a rRNA plasmid had no mitigating effect on nalidixic acid resistance at higher temperatures suggesting that unlike HU resistance, the effects are not directly related to either rRNA or tRNA expression but rather an indirect consequence of deleting *rrn* genes from the chromosome.

3.6 Flow cytometry

Fluorescent microscopy and altered sensitivity to agents that affect DNA replication like hydroxyurea and nalidixic acid suggested a problem with a DNA replication. For more quantitatively analysis of DNA replication problems we performed flow cytometry analysis of the deletion strains. Cell size was measured relative to DNA content in exponentially growing cells and in cells treated with cephelexin and rifampicin to inhibit division but also to allow DNA replication that had already initiated to finish. DNA replication defects like asynchrony, origin overinitiation, DNA fragmentation and mis-segregation of chromosome are manifest as altered peaks which are readily apparent after rifampin-cephelexin runout. Runout experiments suggested no apparent defect in DNA replication with most strains showing a normal four and eight chromosome configuration after drug treatment. The only exception being Δ6 (SQ110) strain confirming the earlier fluorescent microscopy observation of decondensed nucleoids. The data showed less defined peaks after run out hinting at DNA

replication problems in this strain (missegregation, asynchrony or DNA fragmentation).

SQ88 showed a small peak between 4 and 8 chromosome peaks possibly indicating pairwise segregation and defective segregation ((33)).

A further observation was that size as determined by forward scatter seem to correlate to gene dosage. The fewer *rrn* operons the smaller the cell size. The Δ6 strain was smallest in size corresponding to a single chromosomal *rrn* operon. The Δ6 and Δ7 strains with an *rrn* plasmid were correspondingly much larger in size.

4 Discussion

4.1 Growth rates

Growth rate is proportional to ribosome concentration which is itself determined by levels of rRNA transcription. Since protein synthesis and rRNA synthesis are a major energy sink in cellular metabolism, regulation of rRNA transcription is tightly controlled to avoid unnecessary energetic cost. Regulation of rRNA transcription is the critical rate-limiting control point at which a variety of internal chemical signals such as carbon source and amino acid availability are combined with external signals such as media status and integrated and relayed to the polymerases involved in transcription. The complexity of rRNA transcription regulation is reflected in the number of controversial viewpoints regarding mechanisms of rRNA transcription regulation.

- Growth rate dependent control is gene dosage independent (3, 57) - Growth rate dependent control independent of feedback response (deletion strains and rrnP1 mutation analysis) (57) - Loss of dksA increases rrnBP1 transcription in presence of absence of ppGpp (43) - greA no effect - protein elongation rate in modulated as a function of growth rate by the tuning of intracellular concentrations o fall tRNA isoacceptors

Reasons for the decreased growth rate with *rrn* plasmid pKK3535 are not known but have previously been described (3). It is known though that sucrose gradient profiles of cells with pKK3535 plasmid have a higher proportion of ribosome subunits suggesting that there are ribosome assembly problems with pKK3535. In addition mutations within the 23S rRNA of pKK3535 increase the growth rate over the wildtype supporting

the idea that the folding dynamics of 23S rRNA may be altered to allow more efficient ribosome assembly (Mankin, personal communication)

Growth rate dependent control or feedback control of rRNA transcription is gene dosage independent ((3, 57). Although the mechanism by which growth rate dependent control or ribosome feedback control is achieved is not fully understood both predict that increasing rrn gene dosage in the rrn deletion series by transforming rrn deletion strains with an rrn plasmid should not alter the level of rRNA per cell since transcription from existing chromomsomal rrn copies is modulated to adjust either for an rrn gene dosage increase or decrease (3, 12, 31, 57. Thus cells producing a similar level of rRNA, everything else being equal, should have comparable growth rates.

17 calculate a Vmax for *rrn* promoters at 110 initiations per minute.

4.2 Hydroxyurea sensitivity

Although hydroxyurea targets ribonucleotide reductase, mutations sensitizing the cells to hydroxyurea but not directly linked to DNA replication have also been described including obgE and seqA (23, 52). Mutations in cydA obtained as suppressors of temperature lethality in a dnaEts strain independently conferred resistance to hydroxyurea (51).

The idea that DNA replication inhibition by hydroxyurea arises solely from dNTP starvation is also challenged by genetic evidence that strains with mutant DNA pol V encoded by umuCD are resistant to HU (24). Lethality was also shown to proceed through toxin/antitoxin pair relBE and mazEF.

Increased ATP production has been reported in strain with a rRNA antitermination factor mutation which reduces the amount on rRNA available ((49)). Inhibition of protein synthesis with chloramphenicol or spectinomycin increases level of ATP in the cell((48))

4.3 Nalidixic acid resistance

The role of central metabolism components isocitrate dehydrogenase in contributing to nalidixic acid resistance has been described (29, 35). Ensuing work though reveal that the acrA and tolC mutants also confer re-

sistance to Nal suggesting that this is due to NalR is due to efflux rather than any direct effect (28).

Mutations in RNA polymerase have also been described (5). Altering ppGpp concentrations also influence nalR.

Protein synthesis is necessary for the lethality of nalidixic acid since chloramphenicol protects from cell death (15, 38). Similarities to the previously described cell death at non-permissive temperatures in a dnaEts strain are striking (51). Suppressor of cell death were isolated in a cydA gene coding cytochrome bd electron transport chain.

Like thymineless death, DNA replication is neither necessary nor sufficient for cell death though protein synthesis is apparently required (15, 20, 38, 39, 62).

A possible mechanism of cell death by quinolones is the breakdown of iron regulation which results in the production of reactive oxygen species (21, 32). Gyrase inhibition results in hydroxyl radical formation which if treated with an iron chelator results in reduced cell death. Deletion of iscS cysteine desulfurase and atpC a subunit of ATP synthase also results in dramatic survival in the presence of norfloxacin.

4.4 Applications

Recent interest in *E. coli* systems biology and the potential use of the SQ deletion strains for looking at RNAP regulation at rRNA operons and also translation power (8, 18, 19, 53).

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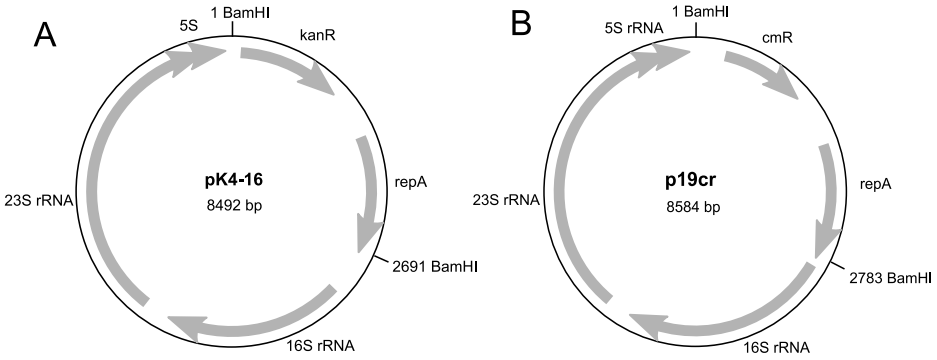


Figure 1: pSC101 rrnB plasmid maps

Table 1: Strains and plasmids used in this work

Strain	Genotype	Source/Reference
MG1655	*ilvG rfb-50 rph-1*	6
SQ11	Δ*rrn*E::KmR	This work
SQ16	Δ*rrn*B::KmR	This work
SQ20	Δ*rrn*G::KmR	This work
SQ22	Δ*rrn*A::KmR	This work
SQ24	Δ*rrn*D::KmR	This work
SQ26	Δ*rrn*H::KmR	This work
SQ34	Δ*rrn*C::KmR	This work
SQ37	Δ*rrn*E	This work
SQ40	Δ*rrn*EG	This work
SQ49	Δ*rrn*GBA	This work
SQ53	Δ*rrn*GBAD	This work
SQ78	Δ*rrn*GADE	This work
SQ88	Δ*rrn*GADEH(ptRNA67)	This work
SQ141	Δ*rrn*GADEHB(pKK3535,ptRNA67)	This work
SQ2203	Δ*rrn*GADEHB(ptRNA67)	This work
SQ2158	Δ*rrn*GADEHBC(pK4-16, ptRNA67)	This work
SQ171	Δ*rrn*GADEHBC(pKK3535,ptRNA67)	This work
SQ110	Δ*rrn*GADBHC(ptRNA67)	This work
SQ351	Δ*rrn*GADEHBCΔlacZYA(pKK3535,ptRNA67)	This work
SQ2062	SQ53(ptRNA67)	This work
SQ2066	SQ53(pK4-16)	This work
SQ2068	SQ78(pK4-16)	This work
SQ2199	SQ78(ptRNA67)	This work
SQ2197	SQ78(pK4-16, ptRNA67)	This work
SQ2196	SQ88(pK4-16, ptRNA67)	This work
SQ2194	SQ110(pK4-16, ptRNA67)	This work
SQZ10	Δ*rrn*GADEHBC(pCsacB,ptRNA67)	This work
SZ7	Δ*rrn*GADEHBCΔrecA(pKK3535,ptRNA67)	This work
Plasmid		
pKK3535		9
pKK45		9
pSTL102		55
pHKrrnCsacB		61
pTH19kr		26
pK4-16		This work
p19cr		This work
pBADrrnB		This work
ptRNA67		61
pKD46		14
pCP20		14
pKD13		14

Table 2: Oligo used in this work

Oligo name	Sequence (5' - 3')
rnaAF	atttaaccgacaaaccgacgctgaaataagcataaagaatgtgtaggctggagctgcttc
rnaAR	tgcgccaatgcaaaaaggccatccgtcaggatggccttctattccggggatccgtcgacc
rnaBF	cgctcgaaaaactggcagttttaggctgatttggttgaatgtgtaggctggagctgcttc
rnaBR	gtagatatgacgacaggaagagtttgtagaaacgcaaaaaattccggggatccgtcgacc
rnaCF	ttttattcctccttagtatgccaccaggaagtgtgattacgtgtaggctggagctgcttc
rnaCR	gccggtagaaggatttacttcggagagggttatttcagatattccggggatccgtcgacc
rnaDF	cgcaggtaatccattaattgaatgttagttcgaaaagcaagtgtaggctggagctgcttc
rnaDR	gactttgggggcattattggccttgtgcaagtcttttagtattccggggatccgtcgacc
rnaEF	acgtttgcgcaacgctcgcgaatttttctctttcaatggtggtgtaggctggagctgcttc
rnaER	cagaactgacatgagattcccttcatcatgcaaataattgacatatgaatatcctccttag
rnaGF	tgttgcatatcattatgcaaccttaaccatgaatttagttgtgtaggctggagctgcttc
rnaGR	ataaaacgagcccttcggggctcgtttttgtctataagtattccggggatccgtcgacc
rnaHF	gcaaaaaccggcacaatgattaaaagatgagcggttgaaagtgtaggctggagctgcttc
rnaHR	atgcagaggatttttgcgattctggcaataatagatatacattccggggatccgtcgacc
AdelF	aagatgtcaggcggtgaaac
AdelR	ggctgattttgtggtggagt
BdelF	tgccttttgtatggcaatga
BdelR	caatgccaaatgtgttccag
rrnCP1	gggcaaaatggtgccgggttcata
TA243	gcctgcataccgttgtcgatag
TA242	cgagggcatttttatcgcaggt
DdelR	attacgcgctgaccgattt
TA304	attcgacgataccggctttg
EdelR	ttactgaaggcagcgtctcc
GdelF	gccatgccattatgtctcct
GdelR	cgctggcacagcaaatact
HdelF	ggtgcgtacgggtaaaccta
HdelR	caaatgcagggatagccataa
THF-Bam	tcggatcccagcctgaatggcgaatg
THR-Bam	tcggatcctggggtgcctaatgagtgag
BF-Bam	tcggatccagcgttacggcttcgaaa???
BR-Bam	tcggatccctcatctctgaaaacttccg???
TA227	ggcctaacacatgcaagtcgaa
TA236	ctctacgcatttcaccgcta

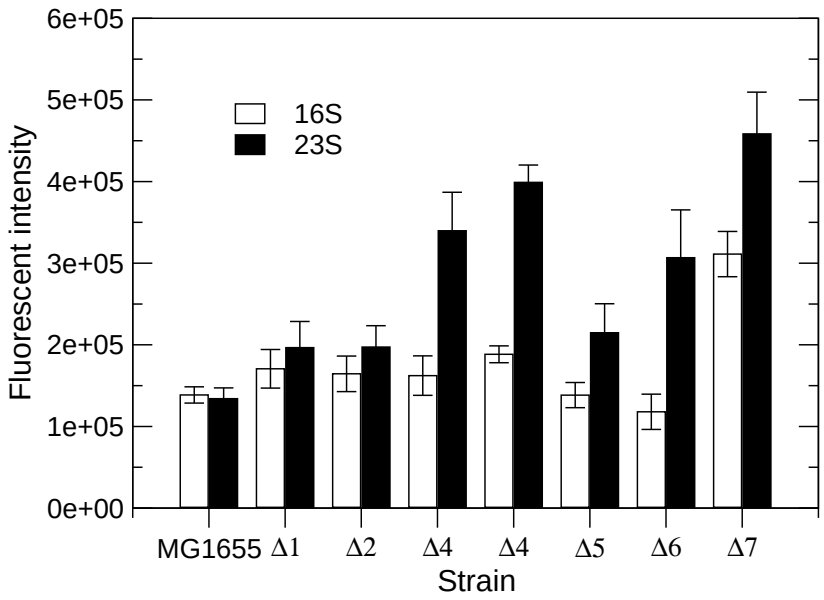
Table 3: Growth of deletion series in LB and M9 minimal media

Strain	LB	M9
MG1655	26.6 ± 1.4	39
SQ37	26.2 ± 2.4	37
SQ40	25.9 ± 2.1	40
SQ42	25.7 ± 1.8	38
SQ49	28.2 ± 2.2	39
SQ53	36.8 ± 1.5	42
SQ78	35.3 ± 0.4	42
SQ88	34.4 ± 0.8	39
SQ141	43.2 ± 4.2	58
SQ170	40.8 ± 2.4	57
SQZ1	35.5 ± 2.9	ND
SQZ10	33	ND
SQ110	ND	74

Table 4: *rrn* plasmid growth retardation

Strain	Doubling time (min)
MG1655	28.6
MG1655(pBR322)	29.1
MG1655(pKK3535)	40.8
MG1655(pKK45)	37.8
MG1655(pSTL102)	39.2
MG1655(pHKrrnc)	27.5

Strain		pK4-16	ptRNA67	pK4-16, ptRNA67
MG1655	31.7 ± 1.3 (0.95)			
Δ1 (SQ37)	32.3 ± 1.5 (1.13)			
Δ2 (SQ40)	33.3 ± 2.3 (1.20)			
Δ3 (SQ49)	33.2 ± 1.0			
Δ4 (SQ53)	43.7 ± 2.8 (2.08)	43.7 ± 1.2	35.8 ± 0.4	37.6 ± 5.3
Δ4 (SQ78)	43.1 ± 0.7 (2.18)	48.0 ± 3.4	36.9 ± 1.3	37.5 ± 1.0
Δ5 (SQ88)			38.2 ± 1.2 (1.48)	37.1 ± 1.3
Δ6 (SQ110)			59.5 ± 0.6 (2.64)	41.8 ± 2.3
Δ7 (SQ171)				38.4 ± 1.4 (1.46)



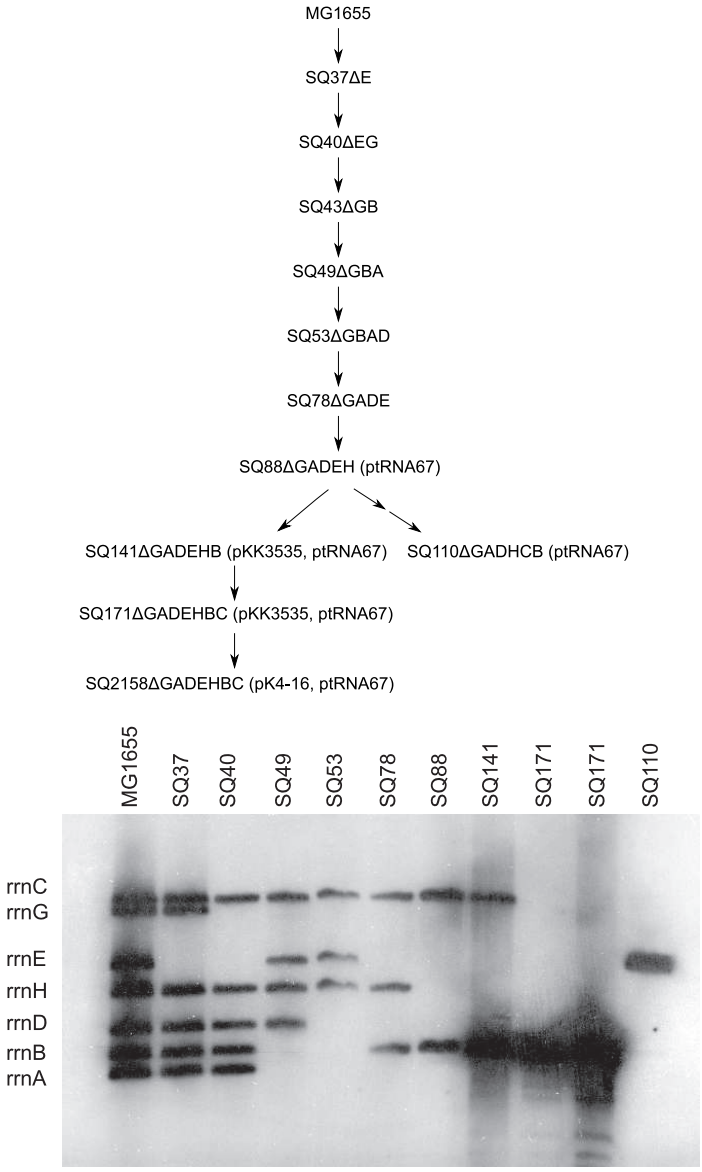


Figure 2: (A) Order of ribosomal RNA deletions to generate an *E. coli* strain with no chromosomal rRNA operons. (B) Confirmation of the rrn deletions by Southern blot using TA227-TA236 16S as a probe

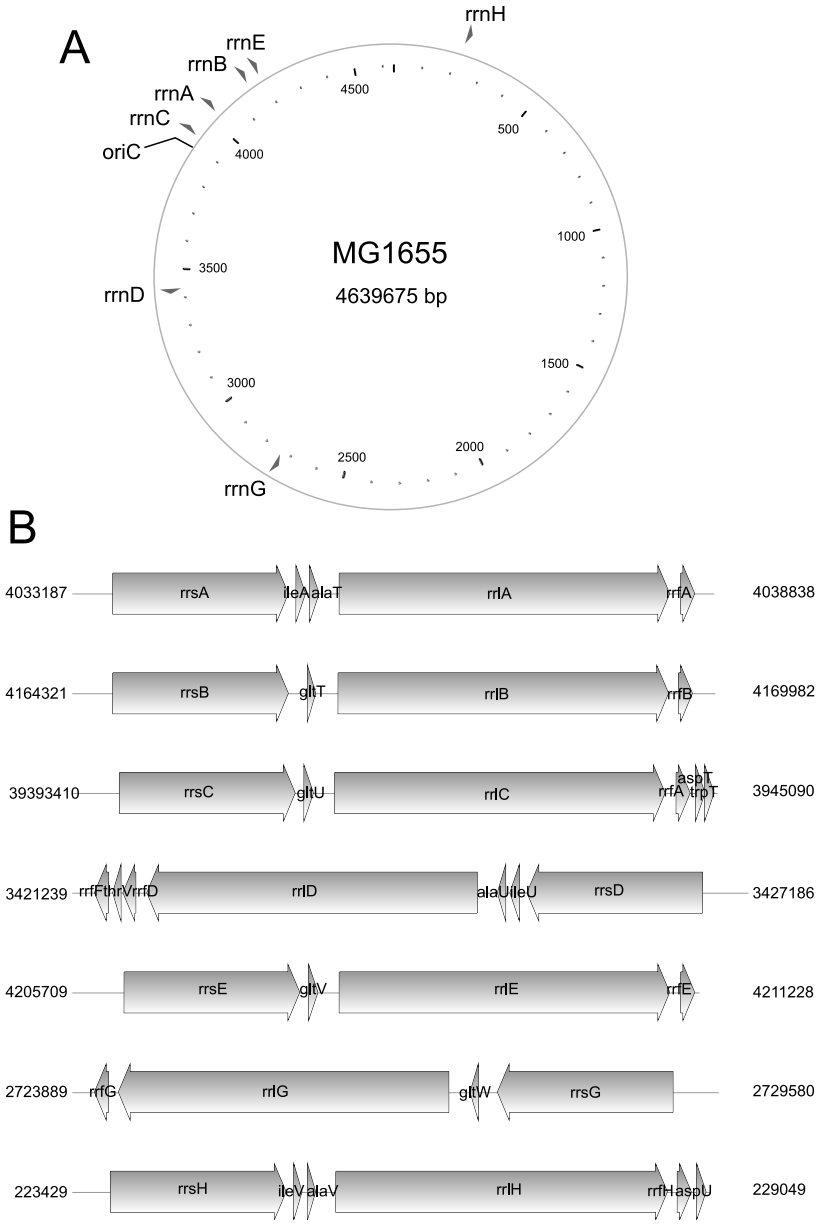


Figure 3: Extent of deletions of the *E. coli* ribosomal RNA operon mapped with respect to Genbank version U00096.2 of the *E. coli* genomic sequence. Absolute chromosome coordinates indicated on either side of each operon mark the 3' end of the deletion oligonucleotide homology with genomic DNA.

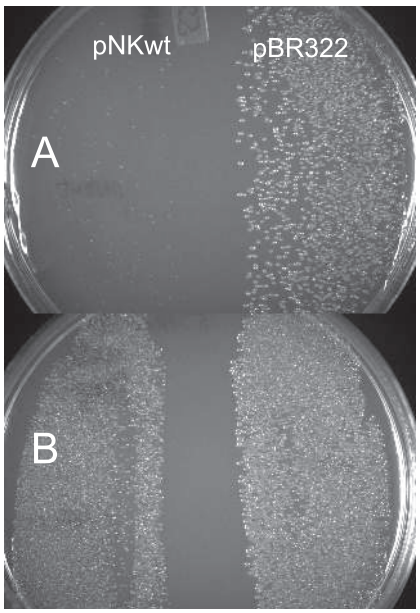


Figure 4: Transformation efficiency of strains (A) SQ53 and (B) SQ53(ptRNA76) with *rrn* plasmid pNKwt and a pBR322 control plasmid

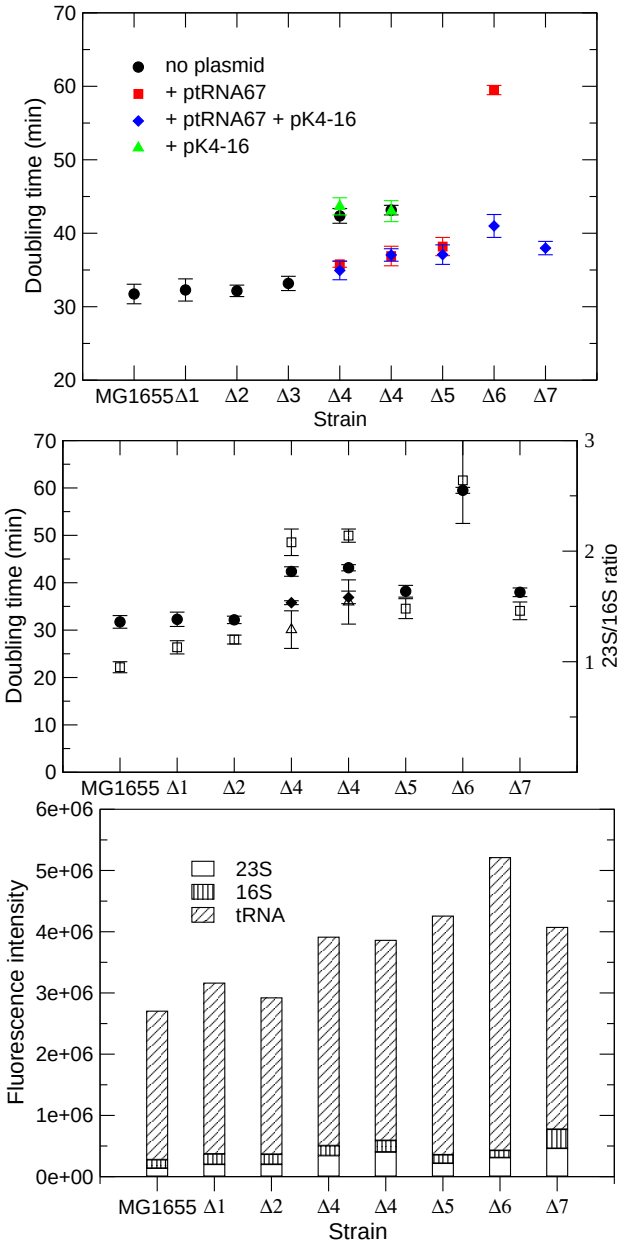


Figure 5: Growth rates of *E. coli rrn* deletion strains grown in LB media at 37°C with and without the tRNA and rRNA plasmids. Error bars represent the standard deviation of at least three independent experiments.

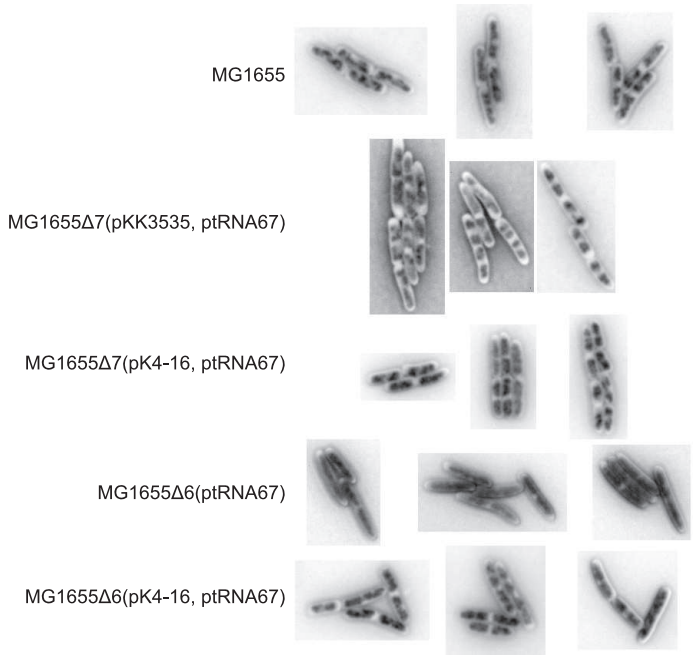


Figure 6: Nucleoid structure of *rrn* deletion strains.

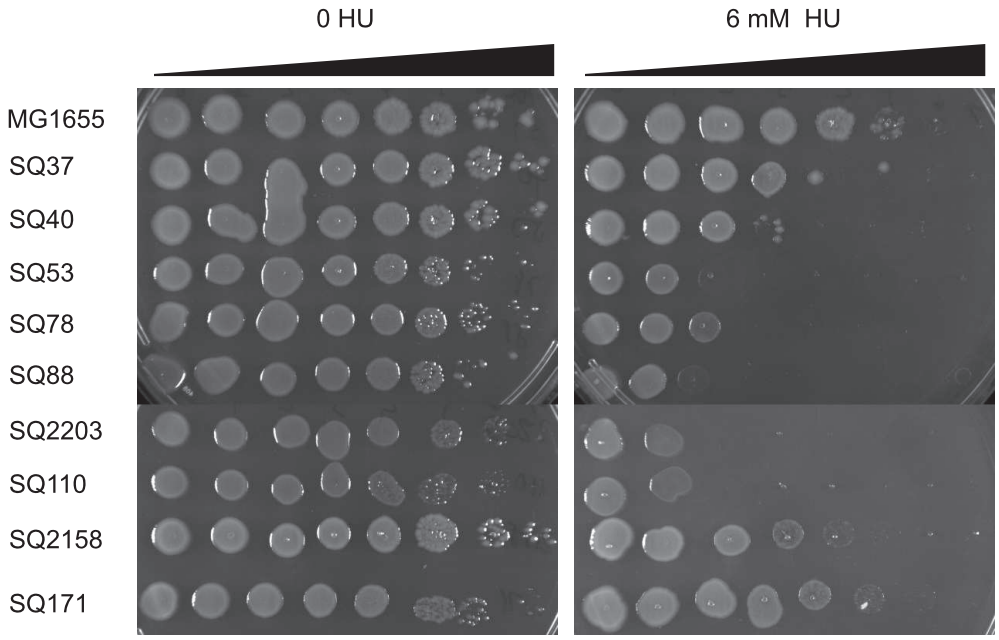


Figure 7: Hydroxyurea sensitivity of *rrn* deletions strains

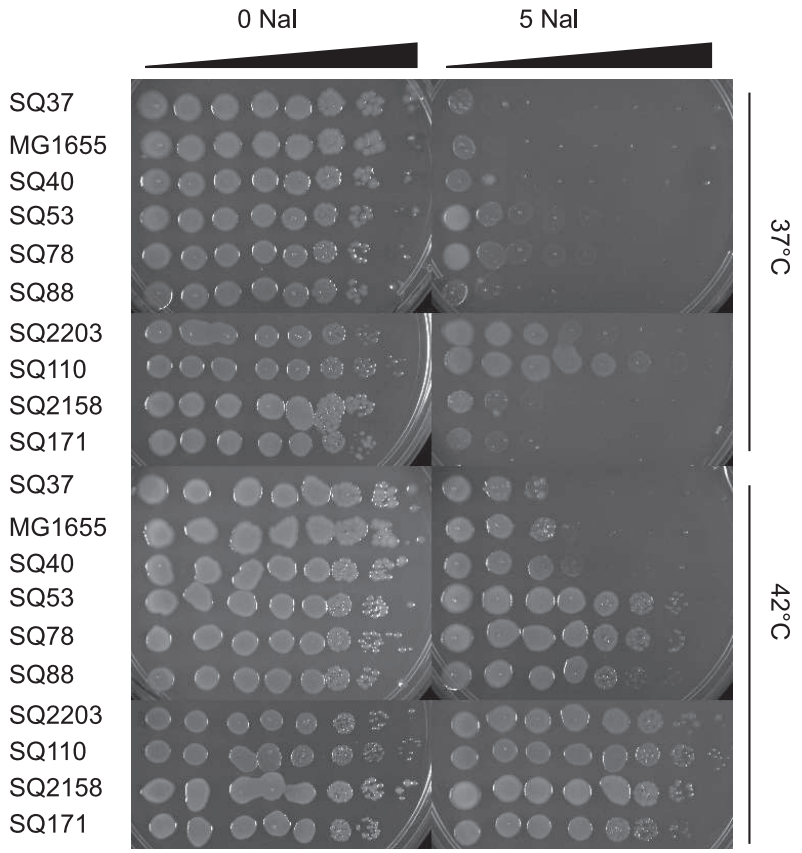


Figure 8: Nalidixic acid resistance of *rrn* deletions strains