Team
Uppsala
University

Downregulation of Antibiotic Resistance using Engineered small RNA

UPPSALA UNIVERSITET

Abstract

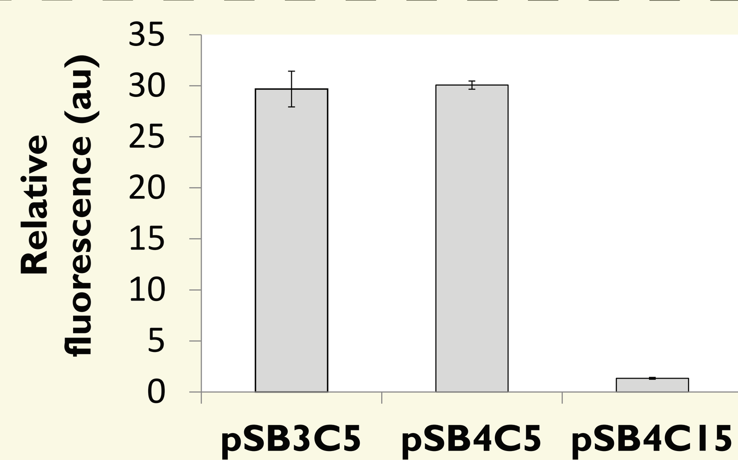
Infections caused by antibiotic resistant bacteria is a serious global healthcare problem causing 25 000 deaths in EU alone every year. We have developed new methods for targeting the resistance itself - making resistant bacteria sensitive to old antibiotics once again. Working with genes from multi-resistant bacteria isolated at Uppsala University Hospital, we have developed an anti-resistance system using small RNAs to silence the resistance genes and we are currently working on transcriptional super-repressors to repress resistance genes and native defense mechanisms.

Achievements

Downregulated Antibiotic Resistance by 90%!

- Constructed a universal screening system
- Constructed REAL low-copy backbones
- Constructed thermosensitive backbones
- Constructed lacIq backbones
- Improved an existing part in the registry
- Successfully isolated sRNA from a randomized library
- Had a totally awesome iGEM-summer

Low-copy Backbones



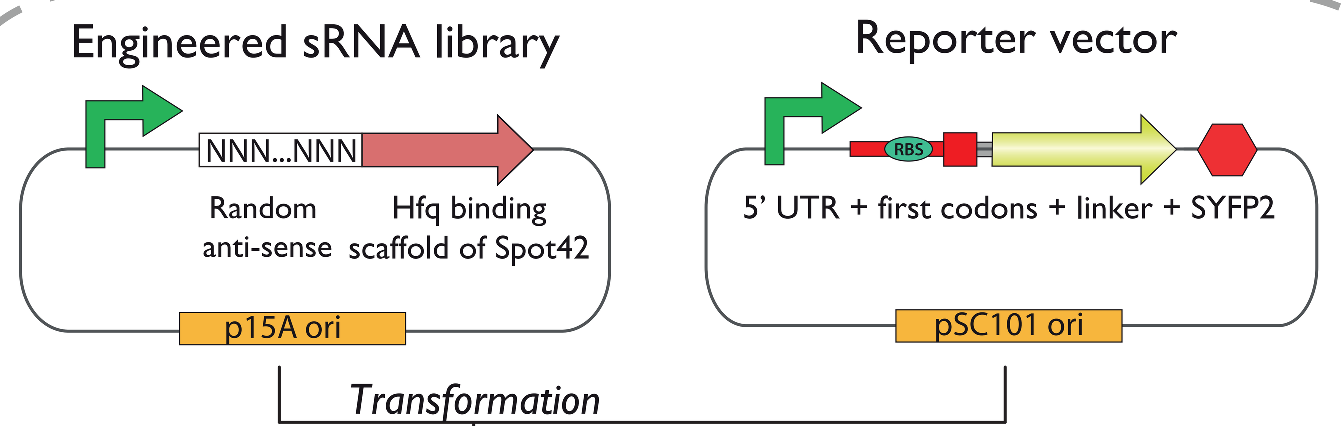
The classic pSB4C5 plasmid, and most likely the whole pSB4x5 series, are not low copy backbones as specified in the registry. Our expression measurements done with flow cytometry strongly indicate that they have a much higher copy number, similar to that of p15A plasmids such as pSB3C5.

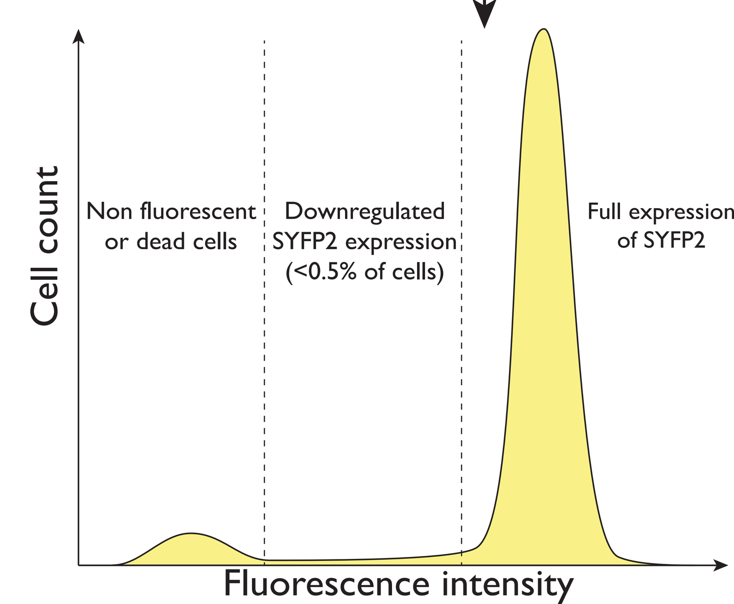
We have provided a new series of real low copy backbones, the pSB4x15 series. These backbones also come in lacIq as well as thermosensitive versions.

References

[1] Sharma, V., Yamamura, A. and Yokobayashi, Y. (2011) *Engineering Artifical Small RNA for Conditional Gene Silencing*. ACS Synth. Biol. 2012, 1, 6–13

[2] Lu, T.K., Collin, J.J. (2009) *Engineered Bacteriophage Targeting Genenetworks as Adjuvant for Antibiotic Theraphy*. PNAS vol.106 no.12 4629-4634

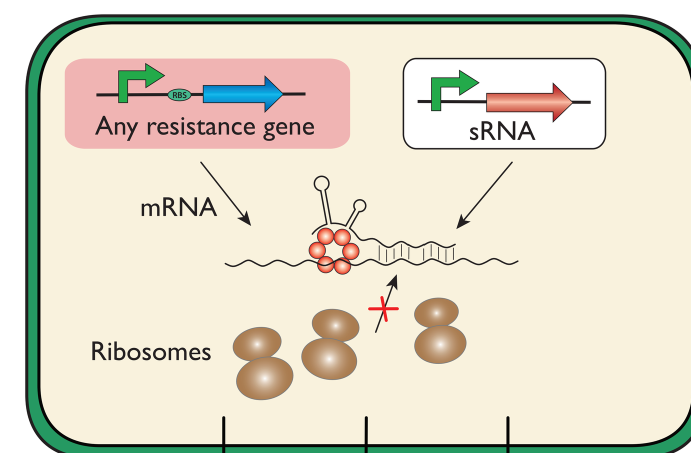




Using primers with randomized nucleotides, we obtained a huge library (10^{18} different theoretical combinations) of randomized anti-sense small RNAs. A reporter vector was constructed by translationally fusing the 5'UTR and the first codons of the aac(6') resistance gene to the reporter gene SYFP2. Starting with 10^{7} cells, we could select 20 000 cells with lower expression of SYFP2 using a Fluorescence Activated Cell Sorter (FACS), plate them to verify loss of fluorescence and then characterize them further.

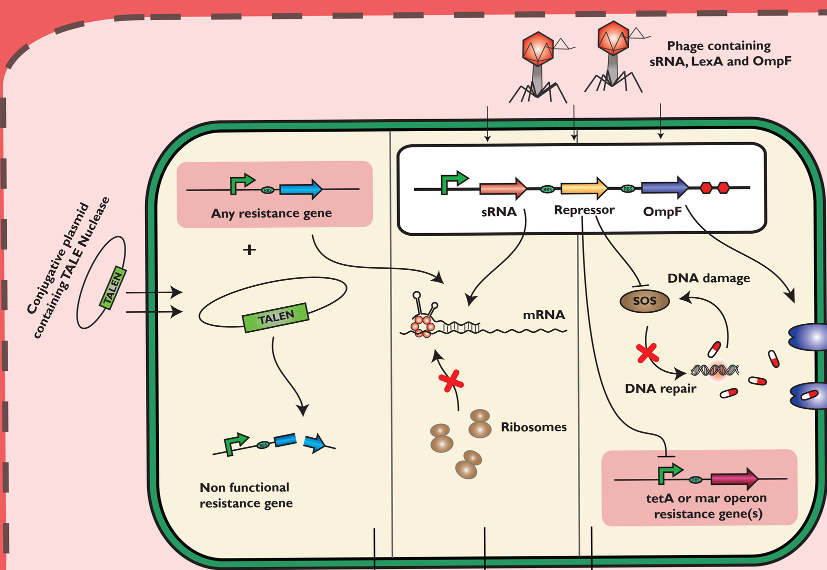
Visual screening - Loss of fluorescence

- *Characterization*
- *Sequencing*
- *Modelling*
- *Testing*



Lower Resistance

Resistance is Futile

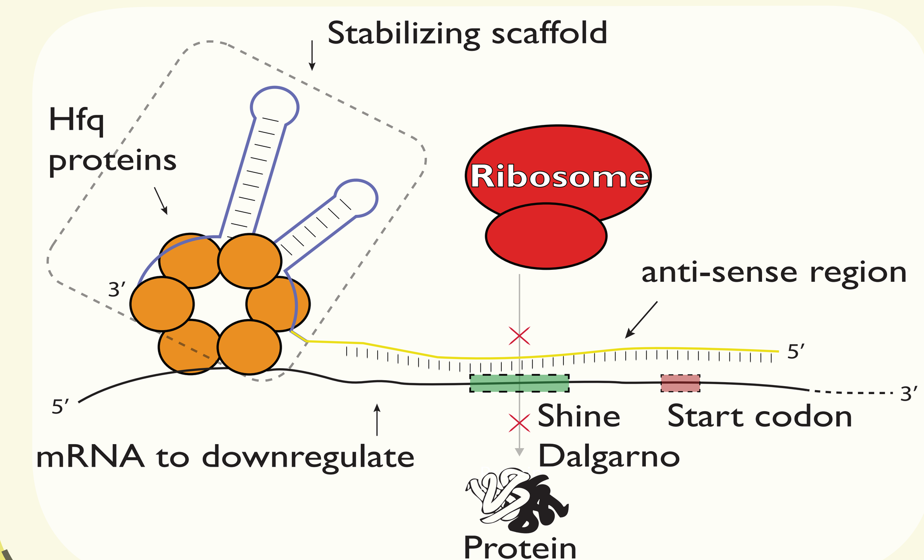


Combine working small RNAs with transcriptional superrepressors and include designed DNA cutting enzymes such as TALENs. Deliver this into a patient infected with resistant bacteria using engineered phages [2] or conjugative plasmids. Then, we belive that resistance is futile.

RESISTANCE IS FUTILE

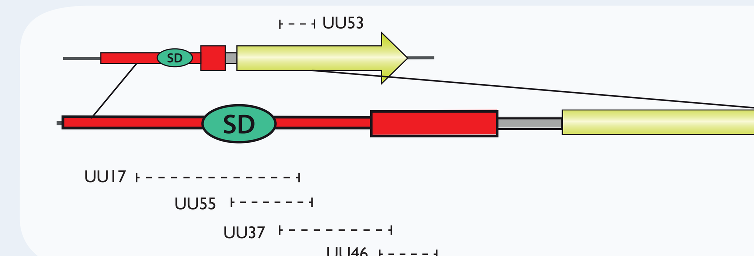
Native small RNA

sRNA are small regulatory RNAs that can be found in bacteria. In our project we chose to work with Spot42, a trans-acting hfq-dependent small RNA [1]. The trans-acting sRNAs consist of one hfq-binding scaffold and an anti-sense region, the latter responsible for binding to the target mRNA, often inhibiting ribosome binding and translation.



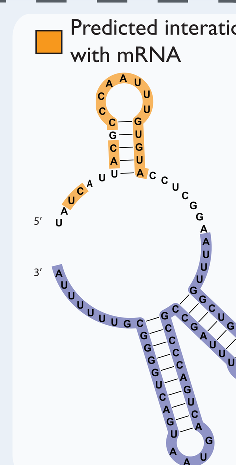
Modelling

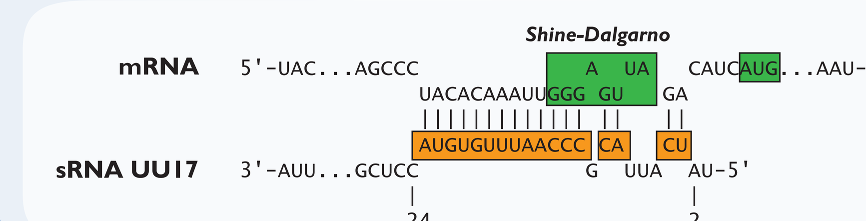
Before testing our sRNAs on the native resistance gene and not only a translational fusion, we made some modelling to predict the sRNA binding to the target mRNA. In the figure below, the predicted binding region of five different sRNAs is shown. Those sRNA that according to our model interacts with the 5'UTR or the first codons of the gene of interest were further analyzed by secondary structure modelling and testing against the full length resistance gene.



sRNA clone UU17

One of the best isolated sRNAs was predicted to bind with 17 hybridizing bp of which 13 bp with perfect complementary match to the target mRNA. Our model of the secondary structure shows a stem-loop structure in the anti-sense region.

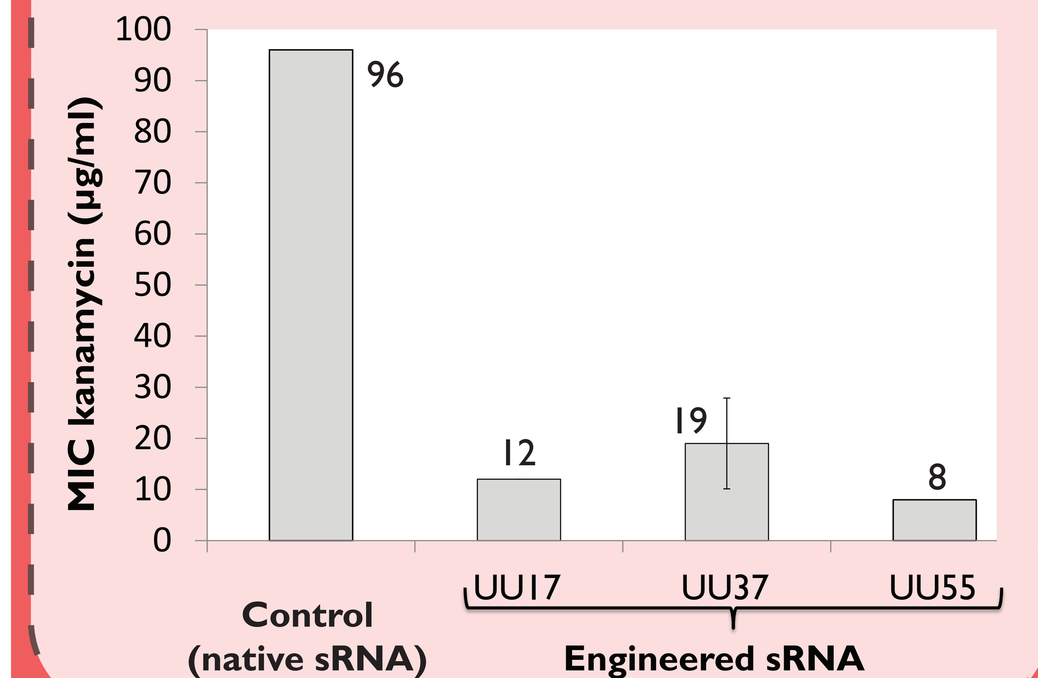




Results

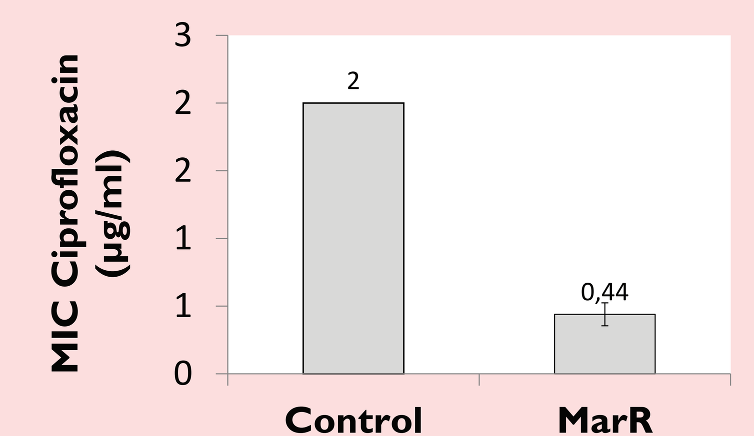
Kanamycin resistance

Using our small RNAs, we were able to downregulate kanamycin resistance conferred by the clinical multi-resistance plasmid pUUH239.2 in *E coli* with up to 90%.



Ciprofloxacin resistance

Expression of the MarR transcriptional super-repressor in *E. coli* resistant to ciprofloxacin lowered the resistance with 80%.



Conclusion

From a large randomized library, we screened a total of 10^{7} cells and identified three small RNAs that were shown to downregulate kanamycin resistance with up to 90% on a translational level. Modelling of the sRNA-mRNA interaction suggest that these sRNAs bind close to the Shine-Dalgarno (SD) sequence. Our modelling of the secondary structure suggest that it might be favourable with a stem-loop structure in the anti-sense region of the sRNA. We also showed that we can downregulate resistance with 80% on a transcriptional level using the MarR transcriptional super-repressor. We hypothesize that combining a small RNA on the translational level and a super-repressor on a transcriptional level could lower the resistance even further. A combined silencing of resistance on multiple levels might also make it harder for the bacteria to develop resistance to our system. By delivering these constructs into the bacteria using a phage or a conjugative plasmid, they could be used as adjuvants to be taken together with antibiotics.

TEKNAT TEKNIK OCH NATURVETENSKAP
Uppsala studentkår
SciLifeLab
Uppsala Genome Center
SIGMA-ALDRICH
VINNOVA
analytikjena
CLC bio Accelerating Scientific Research