DEVELOPMENT OF PHAGE-ASSISTED EVOLUTION AND RIBOREGULATION STRATEGIES

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Riboswitches are short RNA sequences that change conformation in the presence of a specific molecule, regulating gene expression in the process. Present in many species of bacteria and some eukaryotes, these molecules are of great interest for their regulatory properties, which could be used in metabolic studies, or in medical, industrial and environmental contexts.
However, they are very substrate-specific, creating a problem for their use with novel compounds; and current development procedures suffer from issues like being too laborious and not using *in vivo* conditions. By using T7 phage along with a double selection system, we developed a way of obtaining riboswitches that are either improved or have a different specificity; and could even be used to develop novel riboswitches not present in nature.

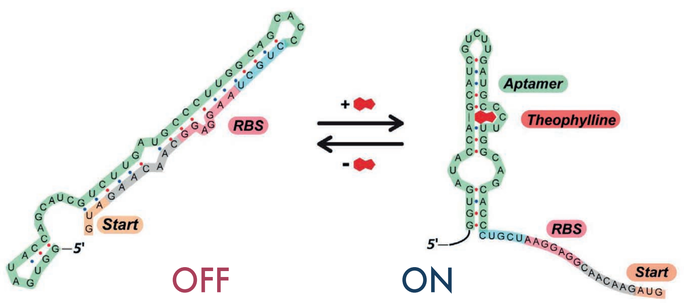
Once established, it could be used for other types of sensors, such as protein or RNA receptors.

Alternative strategies based on phage transduction were also tested, along with inducible RNA systems based on the phage Qβ.

These processes have yielded different results, in the form of a randomised library of the theophylline riboswitch in phages, a working transduction system, and an inducible RNA plasmid.

Elements

Theophylline Riboswitch

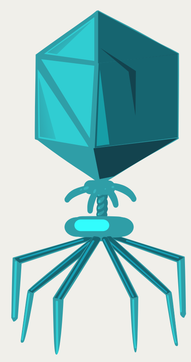


- In absence of ligand, it is closed (OFF) and blocks ribosome binding
- In presence of theophylline it activates (ON) and allows ribosome binding

Selection Strains

POSITIVE	NEGATIVE
T7 essential gene *cmk*	T7 exclusion gene *pifA*
Teophylline present	Teophylline absent
Riboswitch State selected ON / Open	Riboswitch State selected OFF / Closed

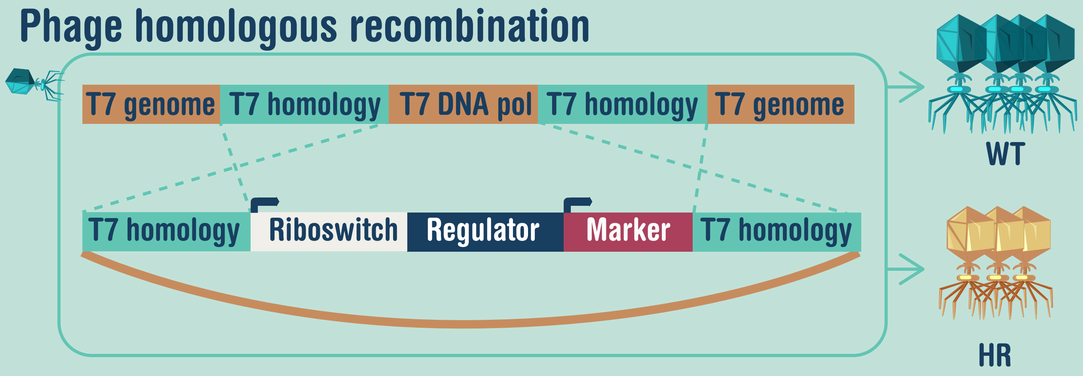
T7 Bacteriophage



- Infects *Escherichia coli*
- Model system for molecular evolution and genetics
- 40 kb genome encoding 55 proteins
- Injects DNA in 10 minutes
- Replicates in 17 minutes at 37°C (High-fitness strains ~11 min)
- Produces an average of 100 offspring per replication

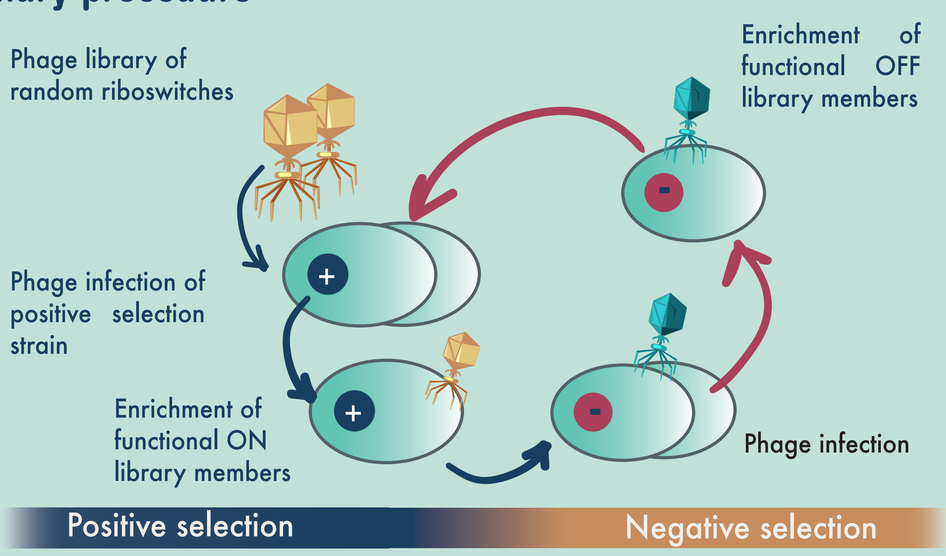
PHAGE-BASED EVOLUTION (Project 1)

Phage homologous recombination

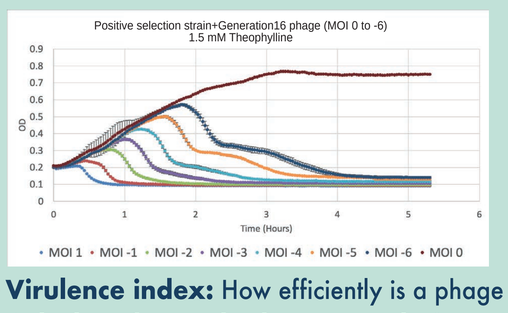


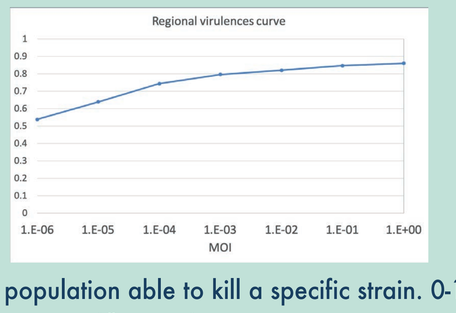
- **Randomized Riboswitch** sequence with **8-n** fragment after the stem
- **Riboswitch plasmid library**, regulating expression of the cI repressor from phage λ
- T7 **infection**
- **"Homologous recombination"**, exchanging its DNA polymerase for our construct
- **Riboswitch library of homologous recombined (HR) phages**

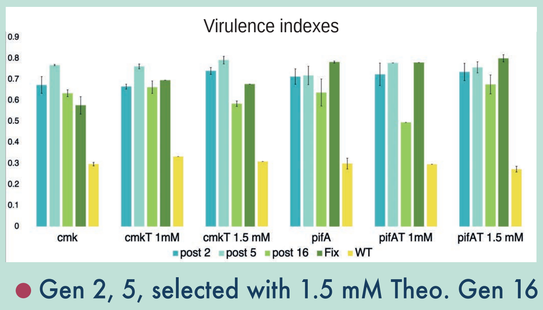
Evolutionary procedure

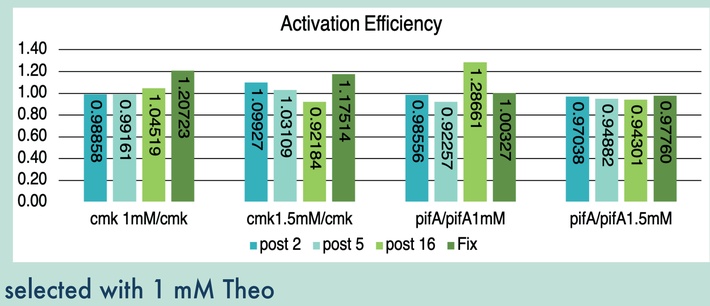


Results





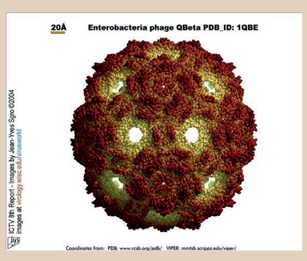




- **Virulence index:** How efficiently is a phage population able to kill a specific strain. 0-1 values
- **Calculate for each phage population on a specific strain**
- **Regional virulence**: $1-(A_i/A_0)$, with A_0= AUC of MOI 0, and A_i = AUC of MOIs 1-6
- Gen 2, 5, selected with 1.5 mM Theo. Gen 16 selected with 1 mM Theo
- Virulence indexes for 5 different phage strains in positive and negative selections
- **"Activation efficiency"**: Difference between switch being ON/OFF in each case

Qβ INDUCIBLE RNA SYSTEM (Project 2)

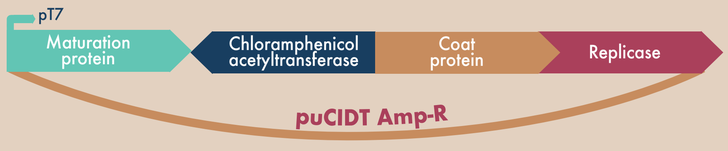
Qβ phage



- Leviviridae family
- Smallest known phage (28 nm)
- (+) ssRNA: 3 ORFs, 4 proteins
- CRISPR resistant

Strategy

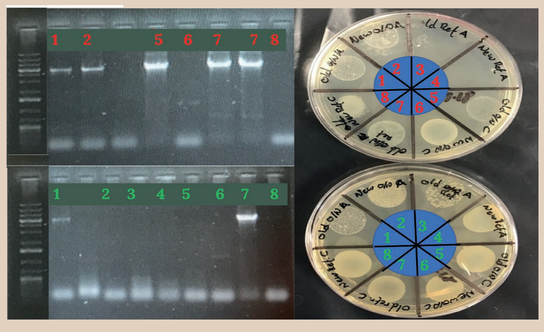
- Introduce gene of interest inside phage's genome
- Introduce phage genome as part of plasmid
- Set up in inducible cell system, allowing for regulated expression



- Cell line with inducible T7 DNA pol
- Plasmid will give Amp resistance

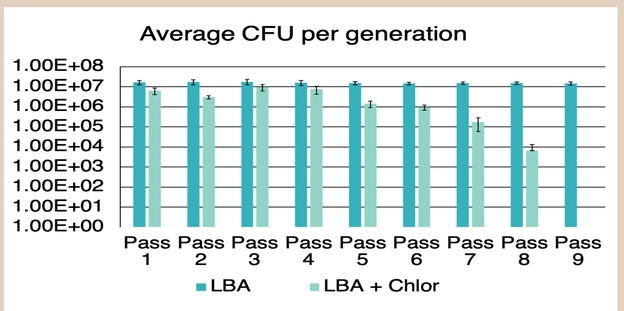
Results

Elimination of original resistance



- Sparser growth in Amp after several passages
- PCR of plasmid gives band in AMP but not in Cm
- Possible loss of plasmid but retain viral RNA

Confirmation of temporary resistance



- Serial passage to eliminate resistance
- Lower CFU over time for Cm cultures
- Confirmation of transient expression