1. Mutagenesis Assay iGEM Heidelberg

In the directed evolution approaches PREDCEL and PACE, Heidelberg selects for beneficial mutations in a protein of interest encoded on a M13 phage genome. In order to generate a pool of protein mutants to select from in the first place, the efficient introduction of mutations during phage genome replication is essential. Mutagenesis-inducing plasmids that enhance the mutation rate in *E. coli* by inhibiting DNA repair mechanisms have been described (Development of potent in vivo mutagenesis plasmids with broad mutational spectra, Badran et al., 2015). When transformed into *E. coli* hosts, these plasmids should cause mutation rates several orders of magnitude higher than usually expected under laboratory conditions. The exact mutation rate induced by different mutagenesis plasmids could, however, vary between laboratories, as *E. coli* growth conditions and hence expression strength of the mutagenic proteins (e.g. error-prone polymerase subunits) could differ. However, robust induction of high mutation rates are critical for the success of PREDCEL and PACE and hence of major importance for other teams to reproduce their directed evolution methods. Therefore, the Heidelberg iGEM Team performed a small inter-lab study to evaluate the performance of different mutagenesis inducing plasmids and find the construct setup most robust and hence suitable for future iGEM teams to use for in vivo directed evolution. **(Results on Heidelberg WIKI)**

Mutagenesis Assay – Spontaneous Resistance Acquisition

Due to exceptionally high mutagenesis levels of *E. coli* cells transformed with one of the mutagenesis plasmids, these cells can more quickly adopt to environmental changes. Hence, one way to measure the mutagenesis levels is simply to quantify the level of spontaneous antibiotic resistance acquisition. Therefore, *E. coli* were transformed with mutagenesis plasmids or remain untransformed (as control) followed by incubation in presence or absence of different antibiotics on agar plates. After 18-21 hours of incubation at 37 °C, colonies are counted. A higher number of colonies (i.e. clones with spontaneous resistance acquired) thereby indicates a higher mutation rate in the corresponding *E. coli* population due to the presence of the mutagenesis plasmid.

Mutagenesis Assay - Protocol

1. Preparation of the agar stabs
    - plate cells from each agar stab on an agar plate containing 100 mM Glucose (to inhibit the mutagenesis plasmids) and 1000-fold diluted 34 mg/ml chloramphenicol stock.
    - After 8-12 hours of incubation at 37 °C pick a single colony to inoculate the overnight culture (step 2)

2. Inoculate an overnight culture of 4 ml 2xYT medium with 200 mM arabinose (to induce the mutagenesis plasmids) and 4 µl of 34 mg/ml chloramphenicol stock (resistance of all mutagenesis plasmids)
    - negativ control: for each mutagenesis inducing plasmid an overnight culture is prepared but instead of arabinose 200 mM glucose is added to inhibit the mutagenesis plasmids
3. Incubate the overnight cultures at 37 °C for 18-21 hours
4. 4. Prepare 2xYT agar plates with 100 mM glucose + antibiotic (see table #1 below). Label all plates properly: name of antibiotic + dilution
5. Each plate is divided into three sections. +undiluted overnight culture +10-fold diluted overnight culture +100-fold diluted overnight culture
6. Plate 50 µl directly on the appropriate plate for undiluted samples (without centrifugation) and mix 10-fold or 100-fold diluted samples with 2xYT medium and then plate them.

Table #1

culture#	antibiotic	working concentration [µg/ml]
1	Streptomycin	50
2	Streptomycin	50
3	Streptomycin	50
4	Carbenicillin	50
5	Carbenicillin	50

culture#	antibiotic	working concentration [µg/ml]
6	Carbenicillin	50
7	Kanamycin	30
8	Kanamycin	30
9	Kanamycin	30
10	Tetracyclin	10
11	Tetracyclin	10
12	Tetracyclin	10
13	negativ control #1	0
14	negativ control #2	0

culture#	antibiotic	working concentration [µg/ml]
15	negativ control #3	0

7. Incubate agar plates at 37 °C for 18-21 hours
8. Count the number of colonies on each plate and fill in your results in table #2.

Table #2

#	antibiotic	MP #	# of colonies undiluted	# of colonies 10-fold diluted	# of colonies 100-fold diluted
1	Streptomycin	1			
2	Streptomycin	2			
3	Streptomycin	3			
4	Carbenicillin	1			
5	Carbenicillin	2			

#	antibiotic	MP #	# of colonies undiluted	# of colonies 10-fold diluted	# of colonies 100-fold diluted
6	Carbenicillin	3			
7	Kanamycin	1			
8	Kanamycin	2			
9	Kanamycin	3			
10	Tetracyclin	1			
11	Tetracyclin	2			
12	Tetracyclin	3			
13	negativ control	1			

#	antibiotic	MP #	# of colonies undiluted	# of colonies 10-fold diluted	# of colonies 100-fold diluted
14	negativ control	2			
15	negativ control	3			