Subcloning into yeast vectors and transformations

Subcloning of EFE BioBricks into yeast vector pRS406

The BioBricks used:

- pADH1 EFE tEno2
- pTEF2 EFEopt tCYC1
- pADH1 EFEopt tCYC1

They, along with the vector pRS406, were cut with XbaI and SpeI using the following protocol:

DNA	500 ng
SpeI	2 µl
XbaI	2 µl
Buffer	4 µl
Water	to 40 µl

Incubated for 1 hour at 37 ºC. Afterwards, the plasmid was dephosphorylated by adding 2 µl FastAP and incubated at 37 ºC for another 20 minutes. Electrophoresis gel was run for 20 minutes. The corresponding bands were cut from the gel, purified, and quantified. The ligation was done using the following protocol:

	for 1:3 (vector:insert)	for 1:5 (vector:insert)
Plasmid	1 µl	1 µl
DNA	3 µl	5 µl
T4 ligase	1 µl	1 µl

T4 Buffer	2 µl	2 µl
Water	13 µl	11 µl

Incubated overnight at 15 ºC.

The BioBricks were transformed into DH5α cells. Colony validation PCR was done according to the protocol:

DreamTaq	8.5 µl
Primer 1	1.5 µl
Primer 2	1.5 µl
DNA	half a colony
Water	12.5 µl

Program

Step	Time	Temperature (ºC)	# of cycles
Initial step	3 min	95	
Denaturaturation	30 s	95	35
Anneling	30 s	48	
Extension	2 min	72	
Final extension	10 min	72	
Hold	∞	15	

The correct candidates were inoculated and left at 37 ºC and 220 rpm for 15-17 hours. Afterwards the mini preps were prepared, quantified, and sent for sequencing. The sequences were later analyzed and the best and the most accurate match was chosen.

Subcloning of SUC2 BioBricks into yeast vector pRS305

The BioBricks:

pFCY2 SUC2 tFba1

pTEF2 SUC2 tCYC1

They, along with the vector pRS406, were cut with XbaI and SpeI using the following protocol:

DNA	500 ng
SpeI	2 µl
XbaI	2 µl
Buffer	4 µl
Water	to 40 µl

Incubate for 1 hour at 37 ºC. Afterwards, the plasmid was dephosphorylated by adding 2 µl FastAP and incubated at 37 ºC for another 20 minutes. Electrophoresis gel was run for 20 minutes. The corresponding bands were cut from the gel, purified, and quantified. The ligation was done using the following protocol:

	for 1:3	for 1:5
Plasmid	1 µl	1 µl
DNA	3 µl	5 µl
T4 ligase	1 µl	1 µl

T4 Buffer	2 µl	2 µl
Water	13 µl	11 µl

Incubated overnight at 15 ºC.

The BioBricks were transformed into DH5α cells. Colony validation PCR was done according to the protocol:

DreamTaq	8.5 µl
Primer 1	1.5 µl
Primer 2	1.5 µl
DNA	half a colony
Water	12.5 µl

Program

Step	Time	Temperature (ºC)	# of cycles
Initial step	3 min	95	
Denaturaturation	30 s	95	35
Anneling	30 s	48	
Extension	2 min	72	
Final extension	10 min	72	
Hold	∞	15	

The correct candidates were inoculated and left at 37 ºC and 220 rpm for 15-17 hours. Afterwards the mini preps were prepared, quantified, and sent for sequencing. The sequences were later analyzed and the best and the most accurate match was chosen.

Yeast transformation and screening for positive candidates

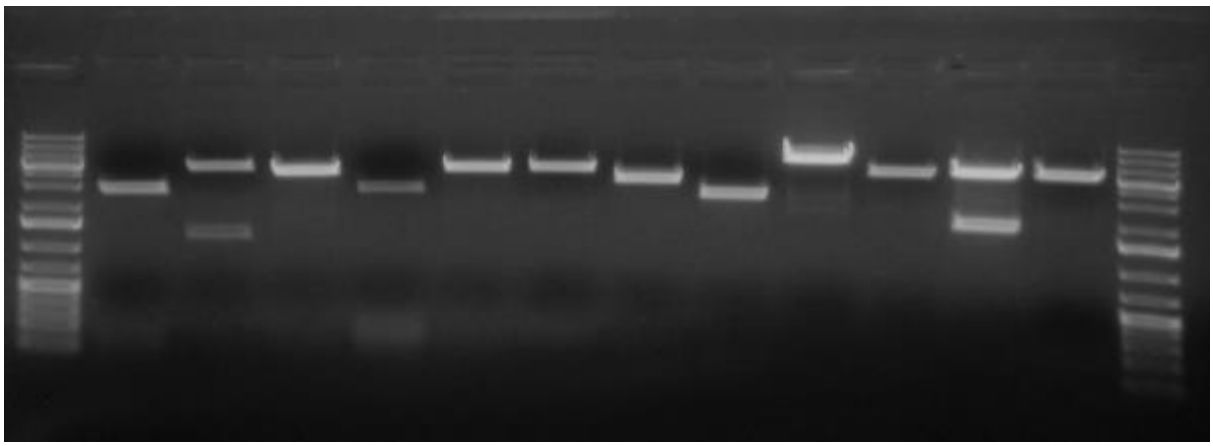
Yeast strain CEN-PK 2.1C was first transformed with the EFE BioBrick. Afterwards the colony validation was done using the following protocol:

DreamTaq	8.5 µl
Primer 1	1.5 µl
Primer 2	1.5 µl
DNA	half a colony
Water	12.5 µl

with the DNA being heated to 100 ºC for 10 minutes in a 30 µl solution of 20 mM NaOH.

Program

Step	Time	Temperature (ºC)	# of cycles
Initial step	3 min	95	
Denaturaturation	30 s	95	35
Anneling	30 s	48	
Extension	2 min	72	
Final extension	10 min	72	
Hold	∞	15	



The candidates had double the expected size which means that there were probably two copies of each BioBrick.

The correct candidates were inoculated and left at 30 ºC and 220 rpm for 15-17 hours. Afterwards the minipreps were prepared, quantified, and sent for sequencing. The sequences were later analyzed and the best and the most accurate match was chosen, which was transformed with the SUC2 BioBrick.

The screening was done through the same process. In the end, there was a functioning yeast strain with EFE and SUC2 BioBricks.