Cloning: Restriction digestion and Ligation

Restriction digestion

Restriction digestion was used for BioBrick cloning. All DNA fragments (tRNA-fragments and ptRNA_backbone) were previously amplified via PCR and purified using the PCR & DNA Cleanup Kit from New England BioLabs.

1. Set up reaction as follows:

Table 1: Representation of the composition of a digestion reaction

Component	50 µL reaction
DNA	1 µg
10x NEBuffer 2.1	5 µL
EcoRI	1.0 µL (10 units)
PstI	1.0 µL (10 units)
Nuclease-free water	To 50 µL

2. Incubate at 37°C for 5-15 minutes as both enzymes are Time-Saver qualified.
3. Purify DNA by agarose gel electrophoresis followed by the Zymoclean™ Gel DNA Recovery Kit.

Ligation

Ligation was performed using a molar ratio of 1:3 for vector to insert.

Vector DNA mass: 50 ng
Insert DNA mass (single tRNA fragments): 15 ng
Insert DNA mass (all tRNA fragments): 75 ng

1. Set up reaction as follows:

Table 2: Representation of the composition of a ligation reaction

Component	20 µL reaction
T4 DNA Ligase Buffer (10x)	2 µL
Vector DNA	50 ng
Insert DNA	15 ng / 75 ng
T4 DNA Ligase	1.0 µL
Nuclease-free water	To 20 µL

2. Gently mix the reaction by pipetting up and down.
3. Incubate at room temperature for 10 minutes.
4. Heat inactivate at 65°C for 10 minutes.
5. Chill on ice and transform 1-5 µL of the reaction into 50 µL competent cells.