Restriction & Ligation

Digesting the plasmid and inserting a new part in the plasmid.

Estimated bench time: 30 min
Estimated total time: 17 hours

MATERIALS

- Thermocycler
- Centrifuge
- Sterile 0.2 mL PCR tubes
- Inserts
- Plamsid
- Restriction enzymes
- rCutSmart buffer
- MilliQ
- 6x Purple gel loading dye
- 1X TAE buffer
- Agarose
- Sybr Safe nucleic acid dye
- 10 kb DNA ladder
- T4 DNA ligase
- T4 DNA ligase reaction buffer
- Ice bucket
- Shrimp Alkaline dephosphatase

SETUP & PROTOCOL

Restriction

1. Thaw all components -except the restriction enzymes- on ice.
2. Dephosphorylate the plasmids with 1 µL shrimp alkaline dephosphatase for 15 minutes at 37 °C.
3. Calculate the amount of DNA insert needed for the reaction using the NEBioCalculator ligation with 1200 ng plasmid:

   https://nebiocalculator.neb.com/#!/ligation
4. Wear gloves and pipette 5 µL 10X CutSmart buffer, 1 µL for each restriction enzyme and 1200 ng DNA (the DNA amount calculated in step 3) for the insert into separate 0.2 mL clean DNAse-free Eppendorf tubes. Calculate the amount of MilliQ needed to have a volume of up to 50 microliters and add to the tubes. Add the restriction enzymes last.
5. Program the thermocycler with the following sequences of temperatures and place the restriction mixtures in the thermocycler. Start the program.
   a. 60 minutes at 37 °C
   b. 20 minutes at 80 °C
   c. Forever at 4 °C
6. Run gel electrophoresis and do a gel extraction following the protocol Gel Electrophoresis & Gel Extraction.

The protocol continues on the next page

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SETUP & PROTOCOL

Ligation

1. Thaw all components except the T4 DNA ligase and place on ice.
2. Mix the plasmid and insert in the molar ratio of 1:5 according to the NEBioCalculator ligation in a sterile 0.2 mL PCR tube. Make up the volume to 16 µL with sterile water.
3. Add 2 µL 10X T4 DNA Ligase buffer and cool the mixture on ice.
4. Add 2 µL T4 DNA Ligase and mix well.
5. Tap the tube and shortly centrifuge to collect everything in the bottom of the tube.
6. Incubate the reaction for 30 minutes at room temperature or overnight at 16 ºC in a programmed thermocycler.
7. Store the ligation products at -20ºC in the freezer.