# MIX PREPARATION

ADAPTED FROM THE BONNET TEAM PROTOCOL REPOSITORY

## STEPS:

1. Primers design
2. PCR with Q5 polymerase
3. Gel Extraction
4. Gibson Assembly
5. Transformation
6. Sequence verification
7. Storage

## MATERIALS:

- Q5® Hot Start High-Fidelity DNA polymerase (NEB)
- Primers 20µM
- Template adjusted to 1ng/µL
- Ultrapure Water
- PCR tubes
- PCR machine
- Agarose gel material

# PROTOCOL:

- Depending on the final quantity of fragment needed and PCR yield, you can perform either a PCR with 20µL, 40µL or several times 40µL. Mix 8µL Water + 10µL Q5 + 1µL Template + 0.5µL each Primer.
- Prepare the cycles' times and temperatures according to the user's manual and primers' Tm. Use of the NEB $T_m$ Calculator is highly recommended.
- Prepare a 0.8% agarose gel for PCR products larger than 1kb and 1.5% for products smaller than 1kb.
- Complement the PCR reaction with loading dye to a 1X final concentration in the gel and a 1kb ladder or 100bp ladder according to the size of your expected fragment.
- Image the gel and cut the band at the right size.

## Gel Extraction:

- Extract the DNA from the band at the right size with a gel extraction kit (Ex. The Macherey-Nagel™ NucleoSpin™ Gel and PCR Clean-up Kit). Follow the protocol from the user's manual.
- Measure the DNA concentration with Nanodrop.

## Gibson Assembly:

### Materials

- 2X Gibson Assembly Mix (see Gibson Assembly Mix protocol)
- Insert fragment(s) from PCR or ordered
- Vector fragment (after PCR, DpnI and clean-up)
- Ultrapure water
- Thermo-cycler at 50°C

### Protocol:

- Calculate the volume of insert and vector to mix in the Gibson Assembly reaction. For 2 fragments assembly (one insert and one vector), the optimum is to mix 100ng of vector with a 3-fold molar ratio of insert; for more than 2 fragments assembly, mix 100ng of vector with a 1-fold molar ratio of insert. The total volume of insert(s) and vector has to be 10µL or less, if the calculated total volume is higher, the quantity of vector can be reduced down to 50ng and the volume of insert calculated accordingly.
- Mix the vector and insert(s) fragments according to previously calculated proportions with 10µL of the Gibson Assembly Mix and adjust the total volume of the reaction to 20µL with water. As negative control of assembly, mix the vector alone in the same proportion than previously with 10µL of Gibson

Assembly Mix and adjust the total volume of the reaction to 20µL with water.

- Place the reactions at 50°C for one hour

**Note : For a better conservation of your Gibson Assembly reaction at room temperature you can incubate your reaction 10min at 80°C.**

## Transformation

See transformation protocol.