COLONY PCR

ADAPTED FROM THE BONNET TEAM PROTOCOL REPOSITORY

MATERIALS:

- 2X One-taq quick load master mix (NEB)
- Primers at 20µM
- Colonies
- PCR tubes
- PCR machine
- Agar gel material

PROTOCOL:

- For each cloning, perform **two colony PCR** from two different colonies.
- For each colony PCR, pick one colony and re-suspend it in 10µL of sterile water (in PCR tube).
- Pre-mix the One-Taq master mix, primers and water for the corresponding number of reactions, such as for one reaction : 5µL of master mix, 0.25µL of each primer, 3.5µL of water.
- Keep the colony re-suspended in water at 4°C, to use afterward to inoculate the culture for plasmid extraction.
- Mix 9µL of the pre-mix with 1µL of the re-suspended colony.
- Place the tubes in the PCR machine with the following PCR cycle :

1. 95°C 5min
2. 95°C 20s
3. Temperature dependent on primers – 30s
4. 68°C – 1min/kb
5. Cycle 30 Times the 3 last steps
6. 68°C 5 min

7. Hold at 12°C

- Prepare a 0.8% agarose gel for PCR products larger than 1kb and 1.5% for products smaller than 1kb.
- Load directly 5µL of the PCR reaction in the gel and a 1kb ladder or 100bp ladder according to the size of your expected fragment.
- Image the gel