

SEQUENCING AND GLYCEROL STOCK

ADAPTED FROM THE BONNET TEAM PROTOCOL REPOSITORY

MATERIALS

- GATC barcodes
- Tubes 1.5mL
- Primers
- dd water
- 50% glycerol

PROTOCOL

- Send the extracted plasmid DNA for sequencing with the appropriate primers to verify the sequence spanning the entire cloned construct, follow GATC procedure (5 μ L DNA at 30 to 100ng/ μ L + 5 μ L of one Primer at 5 μ M).
- For each construct, choose one streak from a clone with the correct sequence. Inoculate 2mL of LB with appropriate antibiotic with a colony picked from the streak.
- Place the culture at 37°C with agitation during 6 hours, until the culture is turbid.
- Mix 1.2mL of culture with 400 μ L of 50% glycerol (15% glycerol final).
- Annotate the tube (Name of construct / Strain / Antibiotic / Book ref / Date / Your name).
- Place the glycerol stock at -80°C.