Colony PCR

To check for the correct construct after assembly.

Materials

- Transformed plates
- LB medium/ nutritionally rich medium
- Primers
- PCR master mix (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0012702_DreamTaq_K1071_UG.pdf)
- Nuclease-free water

Procedure

1. Pick one colony and dissolve in 100 µL media
2. Take 1 µL as DNA sample for PCR
3. Here's a sample reaction mix that can be followed

PCR Volumes (25 ul/sample):

Reagents	Volume(ul)
Dream Taq PCR Master Mix(2X)	12.5
Forward Primer	0.1-1.uM
Reverse primer	0.1-1.uM
Template DNA	~ 1ul
Nuclease free water	Make up to 25ul
Total Volume	25ul

4. Here's a sample PCR run protocol for Dream Taq 2X master mix.

Step	Temperature °C	Time	Number of cycles
Initial denaturation	95	10 min	1
Denaturation	95	30s	25
Annealing	Tm-5	30s	
Extension	72	1 min	
Final Extension	72	10min	1

5. Final hold temperature at4 °C.

6. Run agarose gel electrophoresis (1-2% based on the size of the insert) on the PCR samples.

7. Select the colonies with the right construct size, prepare overnight liquid cultures of these samples.

8. Perform a mini plasmid prep on these overnight cultures. (QIAGEN: QIAprep Spin Miniprep Kit (50))

9. Send the plasmid preps with its primers for sequencing.