Colony PCR

Introduction

By David Faurdal, adapted in part from NEB's one-taq protocol.

The purpose of this protocol is to confirm correct insertion of fragments after assemblies, such as 3A, Gibson, or Golden Gate. As the fragments run on the gel won't be used for cloning purposes, there is no reason to use high-fidelity polymerases on this, just use one-taq.

Materials

- Transformants from whatever assembly method you fashion
- Eppendorf tubes
- Steril toothpicks/inoculation lops/pipette tips for transferring colonies
- Sterile water
- LB media with apropriate antibiotics

Procedure

Preparing the template DNA from the transformants:

1. Pick a number of transformants, typically 3-10, from each plate of interest and mark them on the back of the plate.
2. Set up 2 eppendorf tubes for each colony and mark them accordingly:
3. Fill the first one (1) with 15 µl MQ water.
4. The other one (2) remains empty for now.
5. Transfer each colony to the eppendorf containing 15 µl water using a sterile toothpick, inoculation loop or autoclaved pipette tip.
6. Transfer 5 µl of the water from (1) to the empty (2) tube. This tube (2) is now for safekeeping in case the colony PCR shows that the transformant in question contains the correct insertion.
7. Boil the (1) tubes for 10 minutes at 98 °C. Prepare the PCR mastermix, while the colonies are boiling.

Setting up the PCR itself:

8. Set up a 25 µl reaction for each colony to be screened, as per NEB'S one-taq PCR protocol (see the protocol for troubleshooting).

Table1

	A	B
1	Component	25 µl reaction
2	5x OneTaq Standard Reaction Buffer	5 µl
3	10 mM dNTP	0,5 µl
4	10 µM Forward Primer	0,5 µl
5	10 µM Reverse Primer	0,5 µl
6	OneTaq DNA Polymerase	0.125 µl
7	Template	1 µl from the (1) tube
8	Nuclease-Free Water	up to 25 µl

9. Run the PCR in the thermocycler (use this website to calculate temperatures used based upon the primers and polymerase used).

10. Run the products on a gel to check for correct insertion.

11. Prepare an O/N culture from the (2) tubes that have the correct insertions by adding 1 ml LB media to the tube, mixing it and transfer a W-tube containing 4 ml LB media.