2016 Protocols

PCR Cleanup and Sequencing Preparation

Adapted from
https://www.nucleics.com/DNA_sequencing_support/exonucleaseI-SAP-PCR-protocol.html

Purpose:

To clean-up colony PCR reaction from 16S Colony PCRr and prepare it for sanger sequencing at GeneWiz.

Materials:

- Quantitative Gel Scanner
- ExoSapIt
- ddH_2O
- 1% agarose gel
- 0.5X TBE
- Loading buffer 1kb DNA ladder

Procedure:

Quantification and ExoSap It protocol: Adapt from Nucleics Protocol found at: https://www.nucleics.com/DNA_sequencing_support/exonucleaseI-SAP-PCR-protocol.html

1. Make a 1% 0.5x TBE agarose gel
2. Combine 5 µl PCR product to 1 µl loading buffer, add to the agarose gel
3. Load a 1 kb DNA ladder along-side samples as a standard with known molecular weight
4. Run gel electrophoresis until the ladder bands have separated
5. Using a Quantitative Gel Scanner, record the peaks of each band, including the band with known concentration from the ladder.
6. Use these peaks to extrapolate the concentration of each PCR product sample. See example calculation below.

Lane	Name	peak	calc ng	µL loaded	ng/µL
1	1636bp band	34300	30	1	30
2	PCR #1	32000	32.156	5	6.43

7. Calculate the amount of PCR product need to achieve 100 ng. Transfer that amount to a PCR tube.
8. For every 5 µl PCR product, add 2µL ExoSap It. Mix and quickly spin to collect.
9. Place in Thermocycler and heat to 37°C for 15 min and 80°C for 15 min. Place immediately on ice.

Sequencing Set Up (GeneWhiz)

1. Add ddH_2O
2. Transfer 10 µl of each sample into two separate tube. Label well!
3. Add 5 µl or 5 µM appropriate DNA sequencing primer to the sample, forward and reverse in separate tubes.

Wrap tube in parafilm and send to GeneWhiz along with the seqeuncing document