[iGEM 2017] DpnI Digestion

Introduction

DpnI digests methylated DNA-- this serves to remove the template DNA from a tube containing PCR product.

This protocol describes the DpnI Digestion of 24 uL of PCR Product. This is the standard amount for a 25 uL PCR that has had 1 uL removed for running on a gel. If you are working with a different volume, scale the CutSmart and DpnI volumes accordingly so the ratios of each solution component to the total volume are all preserved.

DPNI reactions can be performed more DPNI (up to 10% total reaction volume), or for longer times (up to 8 hours at 37C) if template contamination is a concern. (i.e. you've previously had a lot of template bleed through, or you are using this PCR to clone something that is only a few BP different).

The typical cloning pipeline is:
PCR -> Gel -> **DpnI** -> PCR Purification -> Gibson Assembly -> Transformation -> Colony PCR -> Inoculation -> Miniprep

Materials

- PCR Product
- CutSmart Buffer
- DpnI

Procedure

DpnI Digestion

1. Into 24 uL PCR Product in a 0.2mL Tube, add:

   2.7 uL CutSmart Buffer (~10% of total volume)
   0.5 uL DpnI (~2% of total volume)
   This can be scaled to larger reaction volumes. However be sure to take into account that you will not be removing 2µl from a 50µl PCR to run on a gel. So you can't simply double the components.

2. Flick the tube and spin down

3. Run the "DpnI" procedure on the Thermal Cycler, for 27 uL Volume

   The procedure is 1 hour of 37C, then 20 minutes of 85C, then hold at 4C.
   The 37C step can be performed for up to 8 hours before heat killing, if additional template removal is required.
   The 80C heat kill step can be omitted, provided you are immediately moving to PCR purification. This is useful if you need to save time.

4. Proceed to PCR Purification.