DpnI Digestion

Overview

DpnI digests methylated DNA[i]-- this serves to remove the template DNA from a tube containing PCR product. This reduces background transformation of template plasmid. Note that this process is not recommended or needed for purification of restriction enzyme digests/Gel purification products.

Materials

- Product to be digested (typically PCR product 24µl in volume)
    - Tubes should be labeled with primer name and 10µM, dates and initials should be included on the side
- DpnI
    - As with all restriction enzymes, keep in the freezer (-20C)until immediately before use. Keep in Stratacooler when not in freezer. Enzyme is stored in glycerol (50%) so it should not freeze.
- Cutsmart 10x Buffer
    - Comprised of (1x): 50mM Potassium Acetate, 20mM Tris-acetate, 10mM Magnesium Acetate, 100µg.mL BSA, pH 7.9
    - This buffer is stored at -20C but an aliquot is usually stored at 4C.

Procedure

1. Determine the correct volume of 10x Cutsmart to add to PCR product tube so that the final concentration is 1x. (Typically 2.7µl 10x Cutsmart to a reaction with 0.5µL of DpnI and 24µL of PCR product [25µL PCR with 1µL used for gel])
    - 0.5µL of DpnI is more than enough[ii] for a 25µL PCR[iii].
2. Add correct amount of 10x Cutsmart to each PCR tube.
    - Typically 2.7µL for .5µL DpnI and 24µL PCR product
    - No need to change to a new PCR tube
3. Add correct amount of DpnI to each tube (typically 0.5µL)
    - Due to glycerol in storage buffer, enzyme should not make up more than 10% the total volume of reaction[iv] (e.g. maximum of 2.5µl DpnI in 25µl reaction).
4. Flick and spin down. Place in thermocycler[v]. Heat at 37C for 1 hour, heat inactivate by heating at 80C for 20 minutes, hold at 4C.

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[i] Specifically GA(methyl)T(opposite base methylated)C

[ii] Smaller volumes would just as well, but are too difficult to pipette.

[iii] Consider the input of plasmid DNA into your PCR (likely 1nM/a few ngs)

[iv] Technically, no more than 5% of final reaction volume should be glycerol. Presented as such for presentability.

[v] Hypothetically this could be done in a heat block with beads (or in a 1.7mL centrifuge tube)

- Reactions can be incubated at 37C for up to 8 hours, for digestion of larger amounts of DNA. Alternatively, larger volumes of DpnI can be used.
- Heat inactivation step can be skipped if reaction is immediately PCR purified, as this removes the enzyme anyway. This can be useful to save time.