PCR Purification

Overview

This protocol covers column based purification of PCR products[i] using NEB reagents (NEB Monarch PCR & DNA Cleanup Kit).

Materials

- PCR/DpnI product
  - Depending on the volume, temporary tubes may be needed.
- Labeled 1.7mL centrifuge tubes (1 per sample)
  - Tubes should be labeled with a key, initials and date. Unless PCR product is to be preserved in which case it should be fully labeled with primers and template used.
- Keyed spin columns[ii] (1 per sample)
  - Make sure you are using the correct columns. Not the ones for minipreps
- DNA Binding buffer
  - Guanidine and Isopropanol-based binding buffer
- DNA Wash buffer
  - Ethanol-based wash buffer
- DNA Elution buffer
  - Comprised of: 10mM Tris, 0.1mM EDTA, pH 8.5

Procedure

1. Dilute samples with DNA Binding Buffer. Add buffer in a **2:1 ratio** of binding buffer to sample for fragments **larger than 2kb** (e.g. add 54µl Binding buffer to 27µl DNA). For fragments **smaller than 2kb** add buffer in a **5:1 ratio** (e.g. add 135µl Binding buffer to 27µl DNA).
   - PCR tubes can hold 200µl of total volume. If the total volume after adding buffer will exceed 200µl, transfer DNA to a keyed temporary 1.7µl tube then add buffer.
2. Mix either by pipetting up and down or by flicking and spinning.
3. Apply each sample to appropriate spin column. Close cap and centrifuge at 6,000 rpm[iii] for 1 minute.
4. Discard flow through (dump out fluid from lower collection tube, then replace the upper column).

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[i] This kit can also be used to clean up ssDNA (e.g. cDNA, oligonucleotides, phage)

[ii] 5µg total binding capacity

[iii] Anecdotal evidence suggests that this might increase DNA binding (by giving more time) and thus lead to higher yields

- Note that collection tubes can safely hold 600µl without contact to column. Optionally, flow through can only be discarded at the final stage for small enough samples (<200µl).

5. Add 200µl of DNA Wash buffer. Centrifuge at 13,300 rpm for 1 minute
6. Add 200µl of DNA wash buffer. Let stand 1 minute.[iv]
7. Centrifuge at 13,300 rpm for 1 minute. Discard flow through.
8. Perform dry spin by centrifuging again at 13,300 rpm for 1 minute.
9. Remove top column and place into appropriate final 1.7mL tube. Throw away collection tube
    - Do not allow column to make contact with any liquid in collection tube. If contact is made, repeat dry spin.
10. Elute by adding between 6-20µl elution buffer to column (typically 12-15µl used). For greatest yield ensure that elution buffer is applied to membrane, not sides of column.
    - Greater volumes lead to greater yields of DNA, but lower concentration.
11. Let stand 4 minutes. Spin at 13,300 RPM for 1 minute.
12. *(Optional)*: Repeat step 10 and 11, using eluate (liquid that just came out of column), in place of elution buffer. Let stand for 1 minute instead of 2.
    - This step increases yield and should be performed if yields have been low, or if a large amount of DNA is needed (e.g. same backbone being used for a variety of Gibson reactions).
13. Discard column. Measure and record concentration using nanodrop. Store at 4C short term (>1 week), or at -20C long term.

[iv] Addition made based on positive results with Qiagen buffer PE. Might not be needed (due to two wash steps). Given that this step is of unknown efficacy and that purity is usually fine, this step may be skipped if time is really short (though I imagine this would be fairly rare).