Qiagen PCR Purification

Overview

This protocol covers column based purification of PCR products, using Qiagen reagents and Epoch Min-Elute columns.

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 Min-Elute spin columns[i] (1 per sample)
  - Make sure you are using the correct columns. Not the ones for minipreps
- Buffer PB
  - Guanidine and Isopropanol-based binding buffer
  - Composed of: 5 M Gu-HCl, 30% Isopropanol
- Buffer PE (Wash buffer)
  - Comprised of:[ii] 10 mM Tris-HCl pH 7.5, 80% ethanol. Used to wash away proteins, salts, and various other contaminants.
- Buffer EB (Elution Buffer)
  - Comprised of: 10 mM Tric-cl pH 8.5
  - Used to elute DNA from column

Procedure

1. Add Buffer PB to DNA in a **5:1 ratio** of PB to sample (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 6000 rpm for 1 minute[iii].
4. Discard flow through (dump out fluid from lower collection tube, then replace the upper column).

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[i] ~6 µg total binding capacity (via Epoch Life Sciences [email])

[ii] Qiagen has not revealed the exact recipe, this is a close approximation.
Source: https://openwetware.org/wiki/Qiagen_Buffers

[iii] Anecdotal evidence is given on openwetware that this reduction in speed increases yield (due to longer DNA binding time)

- 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 **750µL of Buffer PE**. Let stand for 1 minute.
6. Centrifuge at 13,300 rpm for 1 minute. Discard flow through.
    - It is important to **discard flow through <u>before</u> performing dry spin**.
7. Perform **dry spin** by centrifuging again at 13,300 rpm for 1 minute.
8. *(Optional)*: Preheat Buffer EB to **55C**[iv]
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 Buffer EB to the column membrane (typically 12-15µl used).
    - Ensure that Buffer EB is **applied to membrane, <u>not</u> sides of column**.
    - Greater volumes lead to greater yields of DNA, but lower concentration.
11. Let stand 4 minutes[v]. 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] Supposedly increases yield, especially for large >5kb DNA.
[v] Adaption from original 1 minute elution, which supposedly increases yield.