[iGEM 2017] PCR Purification (NEB)

Introduction

Purify out extraneous reagents, enzymes, etc. from the PCR and DpnI process. Results in pure, linear dsDNA fragments that can be stored at -20C for several weeks.

This protocol also describe Nanodrop quantification of results.

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

Notes:
- This protocol follows the Monarch NEB kit protocol. See the documentation for details and troubleshooting.

Materials

- Monarch PCR & DNA Cleanup Kit
  - Make sure DNA Wash Buffer has had ethanol added. If not, add it yourself according to instructions on the bottle.
- Spin column from the Monarch Kit
  - A spin column consists of a column portion and a collection tube portion. Make sure you have both!
- 1.7ml Microcentrifuge Tubes
  - A final tube labeled with the primers, template and date of the PCR.
  - A temp tube for PCRs larger than 50µls.
- DNA samples
  - These will typically be in 0.2 mL tubes, as they have just completed DpnI digestion.

Procedure

Purify

1. Dilute sample with DNA Cleanup Binding Buffer. *(PCR tubes hold 200µl. If sample volume + buffer exceeds 200µl, first spin down the sample in the PCR tube, then transfer the sample to a 1.7ml microcentrifuge tube prior to adding buffer.)*

   For double stranded DNA of length 2kb or greater, add a 2:1 ratio of DNA Cleanup Binding Buffer to sample. *The typical DpnI volume is 27 uL, so then you'd add 54 uL buffer.*
   For double stranded DNA of length less 2kb add a 5:1 ratio of DNA Cleanup Binding Buffer to sample. *The typical DpnI volume is 27 uL, so then you'd add 135 uL buffer.*

2. Spin down sample, and transfer sample into spin column. Close cap, and then centrifuge at 13,000 rpm for 1 minute.

3. Discard flow-through. Add 200µl of DNA Wash Buffer to column and centrifuge at 13,000 rpm for 1 minute.

4. Repeat wash by adding 200µl of DNA Wash Buffer to column and centrifuging at 13,000 rpm for 1 minute.

5. Discard flow-through. Centrifuge at 13,000 rpm for 1 minute.

6. Transfer column to labeled final 1.7ml microcentrifuge tube, being careful not to allow tip of column to come into contact with any flow-through.

   CRITICAL If any doubt, simply discard flow-through and spin at 13,000 rpm for 1 minute. Liquid remaining on column will lead to ethanol contamination of final product, which lowers yield, quality, and potentially inhibits downstream reactions.

7. Add 6 or more µl (generally we use 12 ul) of DNA Elution Buffer to silica column, making sure that elution buffer is absorbed by silica membrane, and is not left on the side of the column.

   Higher volumes of DNA Elution Buffer lead to greater total yields, but reduce concentration. Concentration is more important than total yield in most molecular cloning reactions.
   However, if you are PCRing a fragment which will be used in many gibson reactions (this is often the case with backbone PCRs), you may want to use 20 uL Elution Buffer so you do not run out of the fragment.
   DNA Elution Buffer can be warmed to 50C to increase yields, especially with large fragments (≥10kb)

01:00 8. Incubate at room temperature for minimum of 1 minute

9. Centrifuge at 13,000 rpm for 1 minute.

10. (Optional) Re-elute using the already spun through elution by performing steps 7-9, using the already spun through elution instead of elution buffer.

Quantification

11. Select double stranded DNA on nanodrop.

12. Blank with 1.7µl of DNA Elution Buffer.

13. Measure 1.7µl samples, making sure to clean pedestal between each sample.

    CRITICAL When nanodropping, only pipette to the first stop. Putting air bubbles in the sample will lead to inaccurate (lower) purity values.

14. Measure 1.7µl samples, making sure to clean top and bottom parts of pedestal with a kimwipe between each sample.

    Make sure to check for bubbles detected if the flashing warning sign comes up. Sometimes spinning down the tube in the benchtop centrifuge can help remove micro-bubbles.

15. Record ng/µl, 260/230 and 260/280

    260/230 and 260/280 should both be above 1.8 for a reasonably pure sample.
    Roughly, 260/280 measures the amount of nucleic acid / the amount of protein in the sample, while 260/230 measures the amount of nucleic acid / the amount of salt contaminants in the sample. For more details, use Google or check out Thermo-Fisher's documentation.
    ng/µl will depend on length of DNA purified. (Longer length = greater ng/µl)

16. Before turning off nanodrop, wipe with a kimwipe and DI water. Place nanodrop lever in down position.

Storage

17. Purified DNA can be stored for 2+ weeks at 4C or at -20 indefinitely. After use in Gibson assembly, store at -20C in the PCR purifications box. Make sure to log **ALL** (Template mp number, primers used, date performed, nanodrop values, etc) information on the drive spreadsheet associated with said box.