DNA Column Purification

Linear or circular non-genomic DNA, such as plasmid or PCR-generated DNA, may need to be purified following a reaction to remove buffer and enzyme components in preparation of downstream reactions or transformation into a host. Thermostable PCR DNA polymerases, DNA primers, and dNTPs from a PCR, or certain non-heat-inactivable restriction endonucleases and reaction buffers often need to be purified away from DNA products. Column purification broadly purifies products in a 50 bp – 23 kb range, unlike size-selective gel-purification, but column purification is also quicker, has lower DNA loss, and is easier to produce DNA without salt contamination.

We essentially use the Qiagen QIAquick DNA cleanup protocol, but instead of buying the smaller kits, we buy 500 mL bottles of bulk Qiagen buffers for common stocks, except homemade Buffer PE, and buy cheaper EconoSpin columns in bulk (Epoch Life Science 3010-250). The basic procedure below is adapted from Qiagen and experience. Written by Shyam Bhakta.

Materials

- 1.5 mL microcentrifuge tube, for each DNA sample.
- Spin column with collection tube:
  Micro column: 5 µg DNA capacity, 5 µL elution (*EconoSpin 3010-250; Zymo 11-501*)
  Mini column: 30–40 µg DNA capacity, 30–50 µL elution (*Econo Spin 1920-250*)
    - *Micro columns are typically used for gel purifications and reaction cleanups, as less DNA binding capacity with smaller silica bed volume is needed, which allows smaller elution volume and higher DNA concentration.*
    - *Using a vacuum manifold, collection tubes are only used for drying columns (step 10), and thus may be reused, with fresh ones saved if needed.*
    - *Zymo has purification kits for 5, 25, 100, and 500 µg capacities.*
    - *Zymo Spin-I columns claim a range of 50 bp – 23 kb (lower end verified); Quiagen QIAquick claims 100 bp – 10 kb. EconoSpin: unknown.*
- Buffer PB, DNA-binding buffer
    - *Same as the Buffer PB used as an optional miniprep wash buffer.*
- Buffer PE, wash buffer
    - *For the original buffer bottle, make sure "Ethanol added" is checked on the lid.*
    - *Labmade PE is made by mixing: 20 mL filter-sterilized 0.5 M Tris-HCl pH 7.8, 180 g or mL sterile MilliQ water, and 800 mL 96–100% ethanol, made in detergent-free glassware.*
- Eluent: elution buffer EB / DNA-grade Tris buffer / TE, or nuclease-free deionized water.
    - *Elution buffer ensures optimal pH for DNA elution from silica membrane, solvates DNA better than water, and maintains an alkaline pH that protects DNA from hydrolysis and degradation from local pH extremes during freeze-thaw cycles.*
    - *Qiagen elution buffer is 10 mM Tris-Cl pH 8.5; TE buffer (Tris-Cl + EDTA) pH 8–9 is also a common elution buffer, adding EDTA, whose purpose is to protect DNA from contaminating nucleases by chelating the necessary Mg^{2+} cofactor. Prefer TE with low 0.1 mM EDTA over the common 1 mM, as 1 mM may be enough to inhibit downstream Mg^{2+}-dependent enzymatic reactions. Buffer must be DNA-grade.*
    - *For electrotransformation, DNA in elution buffer does not seem to contribute enough salt to cause arcing or reduce . Still, using large DNA volumes in library transformation efficiency might still benefit from elution in water.*
    - *Heat the eluent only before use to preserve elution buffer and plastic integrity.*

Summary

1. **Mix** DNA with 5 volumes PB.
2. **Bind** DNA to micro column; spin/vacuum.
3. **Wash** column with 500 µL PE; spin/vacuum.
4. **Dry** column with a 2 min spin.
5. **Elute** DNA in 5–15 µL EB or water; spin.

Procedure

1. **Mix** DNA sample with **5 volumes Buffer PB**. Triturate or vortex to mix.
    - *You can pipette-mix it in the DNA tube and move the mixture directly to the column.*
2. **DNA–matrix binding**: Pipette or decant DNA mixture into the labelled spin column set inside a collection tube or upon a vacuum manifold.
   Centrifuge 15–30 s, 18000×*g*, and discard the flow-through by decanting or aspiration.
   For vacuum manifold, apply vacuum until all columns are completely drained.
    - *Aspiration can result in less buffer contamination of the DNA, as decanting dirties the collection tube wall and lip, which can then transfer to the spin column and elution tube.*
3. **Wash** the spin column by adding **500 µL Buffer PE**.
   Centrifuge and drain, or vacuum, as in step 7.
4. **Dry** the column by centrifuging 2 min to remove residual wash buffer. (*cannot be vacuumed*)
   Then *quickly* separate and discard collection tube and place the column in the remaining fresh, labeled 1.5 mL tube.
    - *Residual ethanol in matrix inhibits elution. Allowing columns to rest in elution tubes for a few min, even heated in the same block as your eluent, may slightly improve elution by allowing residual ethanol to evaporate.*
5. **Elute** the DNA by adding **5–20 L elution buffer EB** or water, opt. heated, to the center of the column matrix. Let stand for 1–4 min.
   Centrifuge 30 s, 15000×*g* with open tube caps braced for rotation.
   Check column and tube labels match, and discard column.
    - *Warming the eluent, up to 60°C, can improve elution yield, especially of larger DNAs.*
    - *Prefer an elution buffer to water; read notes in Materials.*
    - *Since the rotor turns counterclockwise, face elution tube caps clockwise over adjacent holes if there is space, or against the bottom of the tube adjacent to the right, stacking the cap over the adjacent tube's cap if tightly packed. The caps must be braced for the rotation, not able to move, or else they will break off.*
    - *Centrifuging at a lower speed than step 10 may prevent tube caps from breaking.*

- DNA concentration and purity can be estimated using a NanoDrop™ spectrophotometer's microvolume pedestal using only 1–1.5 µL sample or TECAN NanoQuant Plate. It can show presence of high levels of typical salt contaminants (especially guanidinium) via the 260/230 nm absorbance ratio. See Microvolume Spectrophotometry.

- Store purified DNA at 4° for short-term use and 20°C for longer-term. Thaw briefly at room temp and (for best practice) keep on ice or 1°C block during usage. Linear DNA is more prone to degradation at ends than circular plasmid DNA. Cohesive (recessed, "sticky") ends are rather unstable and should be used in assemblies soon after generating and purifying, as the ends are not very stable for long periods even when frozen.