**Minipreps & Plasmid Purification**

A ***miniprep*** is the isolation and purification of plasmid DNA from a small (~1-3ml) sample of cells, usually bacterial cells. Plasmid DNA is a small circular piece of DNA that is foreign with respect to the cell type (usually bacterial cells); this is different from the cell’s own chromosomal DNA. Thus, the plasmid DNA needs to be purified separately from the chromosomal DNA. While there are commercially available kits for prepping plasmid DNA, it’s also very possible & easy to do using homemade buffers.

The following protocol is an ***alkaline lysis method*** used for purifying DNA from a ~3ml liquid culture of bacterial cells. The method relies on the fact that, under alkaline (pH = 11) conditions, both plasmid and chromosomal DNA are denatured; subsequent neutralization with the high salt buffer potassium acetate in the presence of SDS causes the cell’s chromosomal DNA and proteins to form an insoluble aggregate, while the plasmid DNA stays in solution. Spinning the sample in a microcentrifuge separates these insoluble aggregates from the soluble plasmid in solution. Alternatively, addition of pure chloroform and subsequent centrifugation will cause the plasmid DNA to separate into a different phase from the rest of the components of the cell. The plasmid DNA is then itself precipitated into a pellet using isopropanol, washed in ethanol, and resuspended in pure water.

The DNA purified here can be used for restriction enzyme digestion or amplification by PCR.

**Miniprep (Alkaline Lysis Method)**

1. Pellet cells by spinning in a microcentrifuge at 12,000rpm for 2min. at room temp. (~25°C)
   * If using a 3ml cell suspension, keep cells in the same microtube by first transferring 1.5ml of cells to a 1.7ml microtube, spin, remove supernatant, then transfer the remaining 1.5ml cells to the tube and spin again; remove supernatant as much as possible without disturbing pellet
2. Resuspend cell pellet in 100ul **TEG buffer**: vortex until there are no more clumps
3. Chill on ice for about a minute
4. Set vortex to lowest setting and carefully hold tube, uncapped, on the vortex with one hand, making sure the buffer inside is actually spinning around without going out of the tube; with the other hand, add 200ul **NaOH/SDS buffer** one drop at a time while vortexing gently
5. Inc. on ice 5min.
6. Add 150ul **KOAc buffer** and invert 6X to mix gently
7. Inc. on ice 5min.
   * Optional: for very pure DNA (to be used in sensitive downstream applications, like sequencing or transfection in mammalian cells), add 500ul **pure chloroform** and invert 4X to mix gently; spin in microcentrifuge at 12,000rpm for 5min. at room temp.; transfer ~350ul of the upper phase to a fresh tube and skip to step #10
8. Spin in microcentrifuge at 12,000rpm for 5min. at room temp.
9. Carefully transfer supernatant to a fresh microtube; don’t transfer the flaky white stuff!
10. Add 1ml **100% isopropanol** and invert 6X to mix gently
11. Inc. at room temp 2min.
12. Spin in microcentrifuge at 12,000rpm for 10min. at room temp.
13. Remove supernatant carefully without disturbing the DNA pellet
14. Wash the DNA pellet by adding 1ml **70% ethanol**
15. Spin in microcentrifuge at 12,000rpm for 10min. at room temp.
16. Remove as much supernatant as possible without disturbing the pellet
17. Allow DNA pellet to dry completely, about 15-30min.
    * Leave the tube open on your bench; pellet should change color/turn translucent
18. Resuspend pellet in 50ul **DW/RNase** and mix by pipetting up and down a few times, without introducing any bubbles
19. Let the reconstituted DNA sit at room temp. for at least 10min. before moving on

**Reagents**

* TEG buffer
* NaOH/SDS buffer
* KOAc buffer
* Pure chloroform (optional)
* 100% isopropanol
* 70% ethanol
* DW/RNase

**Recipes**

**TEG Buffer, 50ml**

* 1.25ml 1M Tris, pH 8.0 (25mM final)
* 146.1mg EDTA (10mM final)
* 450.4mg glucose (50mM final)
* Add ddH2O up to 50ml final volume

**NaOH/SDS buffer, 20ml (make fresh each time)**

* 400ul 10N NaOH
* 1ml 20% SDS
* 18.6ml ddH2O

**KOAc Buffer, 100ml**

* 29.442g potassium acetate
* Add ddH2O up to 60ml
* Add 11.5ml glacial acetic acid
* Add 28.5ml ddH2O to get final buffer

**70% ethanol**

* 70ml 100% ethanol
* 30ml ddH2O

**DW/RNase, 1ml (store at -20°C)**

* 40ul 1mg/ml RNase A stock
* 960ul ddH2O
* Make 20 x 50ul single-use aliquots before freezing, and thaw just what you need right before you use it (repeatedly thawing and re-freezing one big stock is not advised)

**DNA Quantification**

DNA is classically quantified by spectrophotometric absorption at wavelengths of 260nm and 280nm. The ratio of absorption at 260/280 gives a reading of the purity of the DNA; pure DNA should have a 260/280 ratio of 1.8, while pure RNA will have a 260/280 ratio of 2.0. Contamination with chloroform or proteins will cause this ratio to deviate from these values significantly. The more modern nanodrop can also give DNA concentration and purity measurements.

Whichever you choose, it should be able to do it for you; but generally,

[concentration of DNA in ug/ml] = [Absorbance at 260nm] x [dilution factor] x 50ug/ml

Say you diluted your sample 1:50 by adding 1ul of undiluted sample + 49ul of buffer for 50ul total, and you read the absorbance of this 1:50 diluted sample at 260nm and 280nm wavelengths. The A260 = 0.2108 and the A280 = 0.1146. The concentration of the undiluted sample is then: (0.2108)x(50)x(50ug/ml) = 527ug/ml. The A260/A280 = 1.84, indicating fairly pure DNA.

If you don’t have access to a spectrophotometer, you can also run a sample of your DNA on an agarose gel and compare with a DNA standard of known concentration. For example, if you load 10ul of your DNA on a gel and notice that the band intensity is roughly equivalent to the DNA standard, which you know is 100ng, then you can assume that 10ul of your sample contains 100ng; this works out to be 10ng/ul, or 10ug/ml.

**Gel Visualization & Purification**

Following plasmid DNA purification, the minipreps can be analyzed by various methods. Two main methods in classical molecular cloning are ***restriction enzyme digestion*** and ***PCR amplification***. Both methods produce fragments that need to be purified. The fragments can be subjected to agarose gel electrophoresis, and the band at the correct/expected size can be excised from the gel and purified. While purification can be performed with a commercially available kit with >80% recovery (QIAquick Gel Extraction kit, Qiagen), a simple method utilizing cotton filtration with ~30-60% recovery may be sufficient for most downstream cloning applications.

**Agarose Gel Purification**

The following protocol is adapted from Sun et al. (2012).

*\*If using ethidium bromide to visualize DNA bands on agarose gels, do not dispose of anything containing ethidium bromide in normal trash! Separate the ethidium bromide waste into a special waste receptacle; and contact a specialized chemical waste management company to remove the waste and dispose of it safely\**

1. Pierce a 0.5ml microtube from the inside with a 22-gauge needle
2. Insert absorbent cotton (for example, from cotton swabs) into the bottom of the 0.5ml microtube, up to the 200ul mark, loosely
3. Run the digested DNA or PCR reaction on an agarose gel (see How to Run an Agarose Gel guide)
4. Safely visualize the bands
5. Protecting your eyes and skin, cut the desired band(s) with a clean, sharp razor and place gel slice into the 0.5ml microtube, on top of the cotton cushion (slice should ideally include only the DNA band, ~2mm x 10mm max)
   * If using UV light for visualization, don’t expose the bands to UV for too long - it will damage the DNA
6. Cap the 0.5ml microtube and place into a 1.7ml microtube adaptor tube without a lid; spin in a microcentrifuge at room temp. according to the size of the fragment:
   * For a fragment less than 1kb, spin at 5,000rpm for 5min.
   * For a fragment of 1-2kb, spin at 10,000rpm for 5min.
   * For a fragment of 2-3kb, spin at 10,000rpm for 10min.
7. Remove the 0.5ml microtube; there should be ~15-30ul of recovered DNA in the gel-making buffer
   * To remove the gel-making buffer, which may not be optimal for subsequent use, isopropanol precipitation and resuspension in DW/RNase is performed as listed above in steps #10-19 of the miniprep protocol

**Citations**

Sun, Y., Sriramajayam, K., Luo, D., Liao, D.J. (2012) A quick, cost-free method of purification of DNA fragments from agarose gel. J Cancer 3:93-95. doi: 10.7150/jca.4163