Gel Extraction

Overview

This protocol covers gel extraction and purification using a Qiagen MinElute kit. Gel extraction is used to purify DNA of a specific length. All centrifugation steps should be carried out for 1 minute at max speed (13,300 RPM).

Background

Occasionally, DNA of a specific length will need to be purified from a sample containing multiple different lengths/pieces of DNA. Frequently, gel extraction is used during restriction enzyme based cloning, in order to remove isolate sticky ended insert DNA without any of the digested or undigested vector[i]. Less commonly, gel extraction is used to isolate a correct length band from a polymerase chain reaction with off target or non-specific amplification.

The fundamental principle of gel extraction is that using electrophoresis, different size bands can be separated on agarose gels. Then the gel can be visualized and the correct size band can be excised, the agarose dissolved, and the DNA purified.[ii] Importantly, gel extraction has a reputation in labs as being "voodoo", i.e. everybody has a different protocol and nobody is sure what works the best or what works decently. On the upside, most protocols that need gel purified inserts (Gibson Assembly, ligations), are quite robust to purity concerns as well as to low concentrations.

Materials

- Agarose
    - Ideally of high quality. Do not use low melt agarose.
- Gel Box and Lid
- 6x Purple Gel Loading Dye (NEB)
- TAE Buffer
- Buffer EB
- ETBR or Syber Green (or equivalent)
- DNA Sample
- Blue blocking (orange) glasses.
- Promega Gel x-tracta
- Buffer QG
    - Composed of: 5.5 M guanidine thiocyanate, 20mM Tris-HCl, pH 6.6
    - Note: **Do not add bleach to any waste containing buffer QG**. Toxic gases will be released.
- Buffer PE
- Isopropanol (100%)

[i] Digested vector has sticky ends which can re-ligate to the insert to form the original plasmid, and undigested vector already is just the original plasmid.

[ii] This also has the side effect of removing protein contaminants as well.

- Blue EconoSpin spin column.
- 1.7mL Microcentrifuge Tubes
    - One temporary tube, one final tube.

Procedure

1. Prepare a 1%[iii] agarose gel with the thick side of a 6 well comb.
    - Note that you will be able to purify 2 samples per gel.
    - Take extra care here to make a good gel (dissolve agarose completely) and to use a gel box that works well.
    - Either ETBR or Syber Safe can be used (Syber Safe is preferred due to increased brightness).
    - For larger fragments (>3kb) 0.8% Agarose works well.
2. Add 6x loading dye to sample tube (e.g. 10µL 6x loading dye to 50µL of product).
    - **Note** that when using Syber Safe the **no SDS** loading dye should be used.
3. Load 12µL of ladder in lane 1 of gel.
4. Load entire sample + dye into the gel.
    - It is recommended that you put one lane of space between each well with DNA. This prevents cross contamination
    - It is also recommended that the gel is loaded in the backroom to prevent the contents of the well from spilling out, I.e movement of the gel once loaded should be minimal.
5. Run gel for long enough to produce quality separation.
    - 30 minutes typically works well
6. Bring Gel(s) to ChemiDoc MP imager. Insert blue tray. Set gel imager to Syber Green for the blue tray (regardless of whether or not you are using Syber Green). Image gel.
7. Determine which bands are the correct ones (to be isolated).
8. Label a 1.7mL temporary tube for each band to be isolated.
9. Preheat heat block to 50C
10. Put on orange glasses and pull tray out. Turn on the transilluminator. Locate the correct size bands.
    - In some cases (especially with ETBR or faint bands) lab lights will need to be turned off.

[iii] Lower gel percentages can lead to better yields/higher purity, however band separation might not be optimal. Going above 1% is not recommended unless needed.

11. Either zero the balance with the empty temp tube that will hold the gel, or zero the balance with an empty gel x-tracta tool. (This is done so that the mass of the gel punch may be measured).
12. Use the gel x-tracta[iv] to obtain each band. Note that you will have to perform two punches with for each band (use one x-tracta and do both punches before squeezing).
    - To use gel x-tracta, place x-tracta on one edge desired band, gently push straight down (wiggling if necessary), pull out at slight angle (20-40 degrees), perform another punch on the remaining part of the band (getting as little extra agarose as possible), place gel x-tracta in tube and squeeze hard and fast.
    - Remember to get the least amount of agarose without DNA as possible.
    - When expelling agarose plug, make sure to squeeze hard and fast.
13. With the extracted gel either in the x-tracta tool or in the temp tube (depending on what the balance was zeroed with), measure the mass of the gel punch.
14. Based on the mass of the gel punch, add an amount of buffer QG, in uL, equal to 3 times the mass of the gel extract in milligrams. (IE a gel that weighs .2g/200mg will receive 600uL of QG).
15. Vortex briefly.
16. Incubate at 50C[v] for 10 minutes. Vortexing every 2-3 minutes.
17. While sample(s) incubate, label a min-elute column (white).
18. Ensure that the agarose is completely dissolved. The tube should be entirely liquid like and not look thick or ripple when inverted
19. After dissolution, check that the color of the solution is correct. It should be yellow. If it orange or violet, add 10µL 3M sodium acetate (pH 5.0)[vi].
20. Add a volume of Isopropanol (in uL) equivalent to the mass of the gel punch(in mg) pipetting to mix. (For example, a gel punch that weighs .2g/200mg will get 200uL of isopropanol).
    - Once Isopropanol has been added, do not centrifuge sample.
21. Apply 700µL of sample(s) to column. Spin through at 13,300 RPM for 1 minute. Discard flow through
    - Some have said that results are better if flow through is pipetted out rather than dumped.
22. Apply remaining portion of sample to column. Spin through. Discard flow through.

[iv] Alternatively, use a razorblade. If using a razor blade, be sure to trim the gel as much as possible.
[v] The temperature given is 42-50C. Anecdotally some people swear that melting by hand (i.e. 37C is better).
[vi] Some people swear that performing this step every time improves yields.

23. Add 750µL buffer PE to column. Remove column from collection tube. Invert column a few times to wash Buffer QG from the walls of the tube.
24. Incubate 5 minutes[vii]. Spin through. Discard flow through.
25. Add 750µL buffer PE. Remove column from collection tube. Invert column a few times to wash Buffer QG from the walls of the tube. Spin through. Discard flow through.
26. Add 750µL buffer PE. Remove column from collection tube. Invert column a few times to wash Buffer QG from the walls of the tube. Spin through. Discard flow through.[viii]
27. Pre-warm aliquot of buffer EB to 50C in heat block.
28. Spin through again to dry sample(s). If significant flow through, discard flow through and dry again.
29. Transfer column(s) to final tubes. Apply 15µL of pre-warmed elution buffer to the column(s). Incubate at room temperature for 4 minutes. Spin through.
30. (Optional) for increased (~15%) yield, run eluate through column again.
31. Nanodrop to quantify.[ix]
32. Store at 4C for use on the same day, and -20C (gel extractions box) for longer term storage.

[vii] This step (and the inversion steps) [allegedly] greatly reduce QG buffer carry over in the purified product.
[viii] Adaption from original Qiagen protocol (via ADH), this additional wash has been found to increase purity.
[ix] If DNA is present but impure, a PCR purification may clean up the sample.