SPRI BEAD CLEANUP AND AGAROSE GEL ELECTROPHORESIS

OBJECTIVES

- Clean up Illumina 16S amplicon libraries using SPRI beads
- Check library size using agarose gel electrophoresis

PART 1. PCR cleanup using SPRI beads

Principle: Solid-phase reversible immobilization (SPRI) beads are an effective, convenient, and reasonably priced way to purify dsDNA. DNA binds to magnetic SPRI beads under certain chemical conditions (e.g. ~10% PEG and 1M NaCl) and can be washed and eluted in water or other low salt buffer.

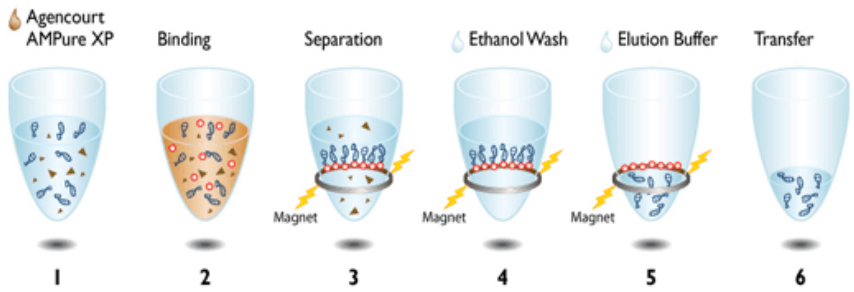


Image: Beckman Coulter

Longer DNA molecules bind preferentially to SPRI beads and the lower cutoff of binding can be varied by varying the ratio of bead buffer to sample. This is useful here because it enables us to selectively purify larger DNA molecules, which avoids excess primers from PCR and primer or adapter-dimers that were generated during PCR. The gel image below shows a DNA ladder that has been "cleaned up" using SPRI beads at the indicated ratio of beads:ladder [e.g.: ratio of 2.5x = 2.5 volumes beads per volume ladder].

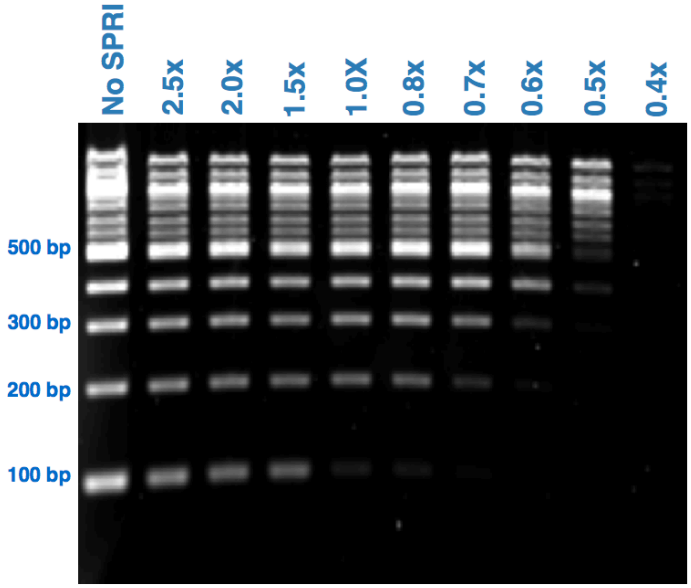


Image: http://core-genomics.blogspot.com/

Beckman Coulter Ampure beads are one of several commercially available SPRI bead products. These are relatively expensive, but it is not difficult to prepare your own SPRI beads. For a home brew recipe see: https://ethanomics.files.wordpress.com/2012/08/serapure_v2-2.pdf

The magnetic stands we will be using were designed with OpenSCAD and 3-D printed by Mark Stenglein: https://github.com/stenglein-lab/3D_parts/tree/master/mag_rack

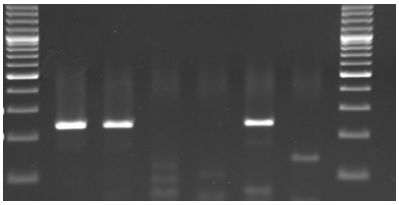
Materials and Equipment

- 16S Illumina PCR libraries prepared previously
- SPRI beads in appropriate buffer
- 1.5 ml Eppendorf tubes
- Magnets
- Freshly prepared 70% ethanol
- 10 mM Tris-HCl pH 8.0
- Pipets (20, 200 µl) and tips

Protocol

1. Vortex or shake bottle of SPRI beads to ensure the beads are evenly dispersed.
2. Allow SPRI beads to come to room temperature
3. Add 20 µl of your Illumina 16S PCR product to a 1.5 ml Eppendorf tube.
4. Add 1x volume (20 µl) of well-mixed beads to each sample.
5. Mix beads and PCR by vortexing or pipetting.
6. If necessary, pulse down tubes to remove liquid from caps. Don't pellet the beads.
7. Incubate for 2 minutes at room temperature then put tubes on magnetic stand. Leave the tubes on the magnetic stand until step 14.
8. Allow the beads to separate for 2-4 minutes.
9. Remove and discard the supernatant using P200 pipet.
10. Add 200 µl freshly prepared 70% ethanol to each tube
11. Incubate beads in ethanol for 1 minute then remove and discard ethanol wash.
12. Repeat ethanol wash step. As much as possible, remove all ethanol.
13. Allow beads to air dry. Wait until visible EtOH has evaporated off and beads are no longer wet/shiny. But do not wait until they appear dry and cracked at that will reduce yield.
14. Remove the tube from the magnetic rack and add 20 µl of 10 mM Tris pH 8.
15. Resuspend the beads by vortexing or pipetting well.
16. If necessary, pulse down tubes. Don't pellet the beads.
17. Incubate the beads for 2 minutes then transfer back to magnetic stand.
18. Allow the beads to separate for ~1 minute.
19. Keeping the tube on magnetic stand, remove DNA-containing supernatant. Avoid transferring any beads.
20. Transfer DNA to new, labeled 1.5 ml Eppendorf tube.

PART 2: Characterization of 16S libraries by agarose gel



Here, we are looking for our 16 PCRs to have produced single bands of DNA the expected size (our 16S primers amplify an insert of 254 bp plus the primer/adapter lengths). We can also compare the intensities of the bands from the various samples.

Materials and Equipment

- Gel electrophoresis rig, gel casts, and comb (MiniPCR mini 16)
- Microwave
- Agarose
- 100 ml of 1x TBE buffer
- Loading buffer and DNA stain (Green Gel Loading Buffer with DNA Stain)
- Cleaned Illumina 16S libraries
- DNA ladder
- Erlenmeyer flask
- Pipet (20 µl) and tips

Protocol

1. We will prepare two gels for the group by melting 0.2 g of agarose in 20 ml of 1x TBE for each gel in a microwave. After tempering, molten gel will be poured into a cast with a comb to create wells and left at room temperature until solidified. After removing gel combs, we will submerge each gel in a buffer tank with 30 ml of 1x TBE solution.

2. Add 4 µl of 6x loading buffer with DNA stain to 20 ul volume of cleaned Illumina library. [Typically, you would only add loading buffer to a small portion of your library and save the rest for sequencing, but we will not be sequencing these libraries].

3. Add 1 µl of 6x loading buffer with DNA stain to the ~5 ul remaining volume of your pre-cleaned Illumina library.

4. Mix each sample by vortexing or pipetting.

5. Pipet 4 µl of your cleaned and pre-cleaned samples into open wells on the gel. We will instruct you. We will provide instructions for the sample loading order on the gels. We will also add a ladder and some control samples for the group.

6. Run electrophoresis for ~30 min and visualize bands with the built in transilluminator.