B. Faircloth & T. Glenn
November 19, 2011
Ecol. and Evol. Biology
Univ. of California – Los Angeles

The goal here is to create a substitute for AMPure XP that is of equal effectiveness in comparison to the commercial product but far more cost-effective ($19/mL versus $0.46/mL).

Suggested Reading

Rohland N, Reich D. **Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture.** Genome Research. Early Online Access. Doi: 10.1101/gr.128124.111

DeAngelis MM, Wang DG, Hawkins TL: **Solid-phase reversible immobilization for the isolation of PCR products**. *Nucleic Acids Res* 1995, **23**:4742–4743.

Fisher S, *et al.*: **A scalable, fully automated process for construction of sequence-ready human exome targeted capture libraries.** *Genome Biol* 2011, **12**:R1.

Lundin S, Stranneheim H, Pettersson E, Klevebring D, Lundeberg J: **Increased throughput by parallelization of library preparation for massive sequencing.** *PLoS One* 2010, **5**:e10029.

Contributing Authors

This protocol is derived from the referenced protocol created by Nadin Rohland. Please do not cite this protocol as being originally produced by me.

Materials

Here, I list stock solutions that are purchased pre-mixed and sterilized. This is in an attempt to minimize variation to the degree possible. You can certainly prepare your own stock solutions at appropriate pH.

- Sera-mag SpeedBeads (Fisher # 09-981-123)[1]
- PEG-8000 (Amresco 0159)
- 0.5 M EDTA, pH 8.0 (Amresco E177)
- 1.0 M Tris, pH 8.0 (Amresco E199)
- Tween 20 (Amresco 0777)
- 5 M NaCL
- Fermentas ladder(s) (Ultra-low range: Fisher # FERSM1211, 50 bp: FERSM0371)
- Rare-earth magnet stand (Ambion AM10055 or NEB S1506S)

Optional

- Agencourt SPRIPlate Super Magnet Plate (Beckman Coulter A32782)

[1] These are the same beads used by Beckman, per Orapure product sheet and http://bit.ly/vmiDzU

Changes

- Minor wording changes
- v2.2 – added exact Fisher mix for with-bead library prep

Steps

1. In a 50 mL conical using sterile stock solutions, prepare TE (10 mM Tris-HCl, 1 mM EDTA = 500 µL 1 M Tris pH8 + 100 µL 0.5 M EDTA, fill conical to 50 mL mark with dH_20).
2. Mix Sera-mag SpeedBeads and transfer 1 mL to a 1.5 mL microtube.
3. Place SpeedBeads on magnet stand until beads are drawn to magnet.
4. Remove supernatant with P200 or P1000 pipetter.
5. Add 1 mL TE to beads, remove from magnet, mix, return to magnet.
6. Remove supernatant with P200 or P1000 pipetter.
7. Add 1 mL TE to beads, remove from magnet, mix, return to magnet.
8. Remove supernatant with P200 or P1000 pipetter.
9. Add 1 mL TE to beads and remove from magnet. Fully resuspend and set microtube in rack (i.e. not on magnet stand).
10. Add 9 g PEG-8000 to a new 50 mL, sterile conical.
11. Add 10 mL 5 M NaCL (or 2.92 g) to conical.
12. Add 500 µL 1 M Tris-HCL to conical.
13. Add 100 µL 0.5 M EDTA to conical.
14. Fill conical to ~ 49 mL using sterile dH_20. You can do this by eye, just go slowly.
15. Mix conical for about 3-5 minutes until PEG goes into solution (solution, upon sitting, should be clear).
16. Add 27.5 uL Tween 20 to conical and mix gently.
17. Mix 1 mL SpeedBead + TE solution and transfer to 50 mL conical.
18. Fill conical to 50 mL mark with dH_20 (if not already there) and gently mix 50 mL conical until brown.
19. Test against AMPure XP using aliquots of ladder (Fermentas GeneRuler). I recommend the 50 bp ladder in place of the ultra-low range ladder.
20. Wrap in tinfoil (or place in dark container) and store at 4°C.
21. Test monthly – see Testing, next page.

You may also wish to prep an extra 50 mL of PEG solution that lacks Sera-mag SpeedBeads so that you can use it in a bead-inclusive library preparation protocol, derived from Fisher (2011). In that case, just:

1. Add 10 g PEG-8000 to a new 50 mL, sterile conical.
2. Add 25 mL 5 M NaCL (or 7.3 g) to conical.
3. Fill conical to ~ 49 mL using sterile dH_20. You can do this by eye, just go slowly.
4. Mix conical for about 3-5 minutes until PEG goes into solution (solution, upon sitting, should be clear).

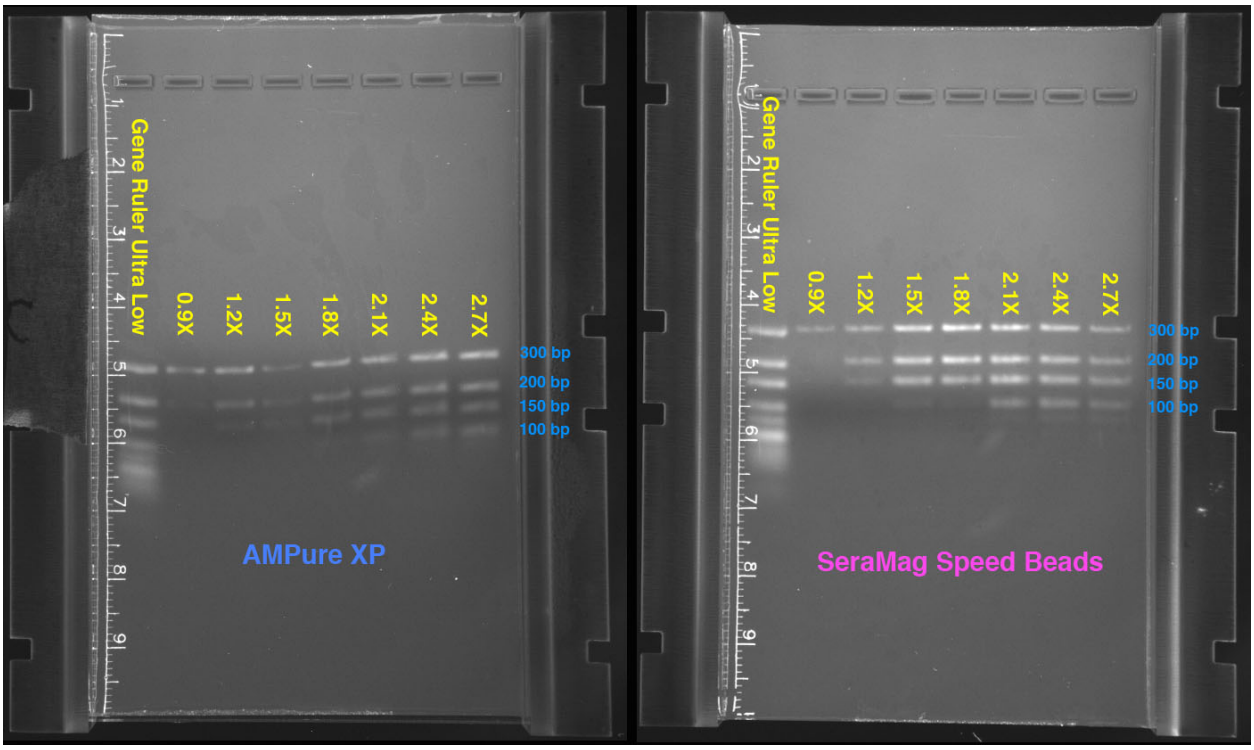
Testing

You should test the Serapure mixture to ensure that it is working as expected. You can do this using DNA ladder (Fermentas GeneRuler – NEB ladders may cause problems):

1. Prep fresh aliquots of 70% EtOH.
2. Mix 2 µL GeneRuler with 18 µL dH_20.
3. Add 20 µL GeneRuler mixture to a volume of Serapure and/or AMPure (the specific volume depends on whether you are trying exclude small fragments or not; see the figure on the next page).
4. Incubate mixture 5 min. at room temperature.
5. Place on magnet stand.
6. Remove supernatant.
7. Add 500 µL 70 % EtOH.
8. Incubate on stand for 1 min.
9. Remove supernatant.
10. Add 500 µL 70% EtOH.
11. Incubate on stand for 1 min.
12. Remove supernatant.
13. Place beads on 37°C heat block for 3-4 min. until dry.
14. Rehydrate with 20 µL dH_20.
15. Place on magnet stand.
16. Transfer supernatant to new tube.

17. Mix supernatant with 1 µL loading dye.
18. Electrophorese in 1.5 % agarose for 60 min. at 100 V.

The following image compares the results of "purifying" a mix of 2 µL Fermentas Ultra Low Range Ladder + 18 µL dH_20 using several different amounts of AMPure or Serapure solution to DNA solution. AMPure is on the left, "Serapure" is on the right. After preparing 20 µL of ladder + water mix, we combined that with the volumes of AMPure or Serapure listed below and then purified using the standard protocol:



As you can see, the volume of AMPure or SeraPure controls the size of fragments recovered. More specifically, it is the ratio of PEG solution used to the volume of the DNA in solution which makes the difference, not the count of beads in solution (provided they are above the minimum level). This is what makes it possible to do "double-SPRI" size selection.