Amplicon Library Purification for 454 Sequencing (from Amplicon Library Preparation Method Manual for the GS Junior Titanium Series from Roche, with modifications)

Materials:

70% ethanol (200 µL per sample)

Molecular grade water

PCR products

AMPure beads (33.75 µL per sample for 300 bp product; amount will vary depending on desired product size, level of purity required, and lot calibration. Check with Mark Dasenko to find out best ratio of beads to DNA)

Magnetic ring stand

1x TE Buffer (20 µL per sample)

Procedure:

1. Add 22.5 µL water and 22.5µL PCR product to a PCR tube.
2. Vortex AMPure beads until completely resuspended, then add proper amount to the PCR tube (33.75 µL for a 75% ratio of bead suspension to DNA solution) and mix well by pipetting.
3. Incubate for 10 minutes at room temperature.
4. Place in magnetic ring stand and incubate for 5 minutes at room temperature.
5. While still in the magnetic stand, remove and discard supernatant without disturbing beads on the side of the tube.
6. Remove from magnetic stand and add 100 µL 70% ethanol, and mix well.
7. Place in magnetic stand and incubate for 1 minute.
8. While still in the magnetic stand, remove and discard supernatant without disturbing beads on the side of the tube.
9. Repeat steps 6-8 and remove as much supernatant as possible.
10. While in magnetic stand, let sit for 15 minutes with caps off to allow remaining EtOH to evaporate.
11. Add 22.5 µL 1x TE Buffer and mix by pipetting. Make sure all beads are resuspended.
12. Place in magnetic stand and incubate for 2 minutes.
13. While still in magnetic stand, transfer 20 µL supernatant into new tube without disturbing the beads on the side of the tube. This is the purified product.