Ampure XP bead-based library purification		
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Manual AMPure® XP Bead-Based MPS Library Purification

1 Purpose

1.1 To purify the amplified libraries of nonspecific amplification product, and unincorporated primers and nucleotides.

2 Preparation

2.1 Retrieve the amplified library plate and, if necessary, allow to thaw and equilibrate to room temperature.

2.2 Retrieve the following reagents. Where necessary, allow to thaw and/or equilibrate to room temperature for at least 30 minutes.

AMPure® XP bead solution
20 mg/mL Proteinase K
50mM Tris-HCl (pH 8.0), 10mM $CaCl_2$
10mM Tris-HCl (pH 8.5)
100% EtOH
Distilled water

2.3 Record the reagent lot numbers in LIMS.

2.4 Retrieve a new 96-well, Eppendorf semi-skirted PCR plate and label it with the run name followed by “purified libraries”.

2.5 Proteinase K working solution:

2.5.1 Proteinase K working solution must be made fresh for each purification run. 20 mg/mL Proteinase K is available in single use aliquots and any remaining solution should be discarded.

2.5.2 Prepare 360 µg/mL Proteinase K (Pro-K) working solution

2.5.2.1 Retrieve and label a new 1.5 mL microcentrifuge tube.

2.5.2.2 In the microcentrifuge tube, create the working solution as presented below in Table 1:

Table 1: 360µg/mL Pro-K Working Solution

20mg/mL Pro-K (stock)	50mM Tris-HCl (pH 8.0), 10mM $CaCl_2$	360µg/mL Pro-K (Total Volume)
4µL	216µL	220µL

2.6 Prepare 80% Ethanol working solution:

2.6.1 Pour 40mL of 100% ethanol into a new, 50mL conical tube.

2.6.2 Add 10mL distilled water to bring the volume up to 50mL.

2.7 Prepare two (2) reagent reservoirs:

2.7.1 Retrieve and label one reservoir for the ethanol transfer.

2.7.2 Retrieve and label a second reservoir for the waste.

2.8 **WITNESS STEP:** Have a witness confirm the plate names written on the input (amplified libraries) and output (purified libraries) plates.

3 Procedure

3.1 A multi-channel pipette should be used for the following purification procedure. For ease of transfer, reagents should be placed in an 8-tube strip prior to addition to the samples.

3.2 If, at any point in the procedure, the bead pellet is unintentionally disturbed, return the supernatant to its well and allow the beads to pellet and the sample to clear before continuing.

3.3 Vortex the amplified library plate on a plate mixer at 1000 rpm for 1 minute, then centrifuge at 1000 rpm for 1 minute.

3.4 Remove the seal from the amplified library plate.

3.5 Add 25µL of the Proteinase K working solution to each well of an 8-tube strip.

3.6 From the 8-tube strip, transfer 5µL of the Proteinase K working solution to each amplified library.

3.7 Perform 3 washes following the instructions listed under step 3.8 below.

3.8 Wash:

3.8.1 Add the appropriate amount of AMPure® XP beads to each library according to Table 2 by following steps 3.8.1.1-3.8.1.3 below:

3.8.1.1 Prepare an 8-tube strip by adding well mixed, room-temperature AMPure® XP beads to each well of the strip in the volumes indicated in Table 2 below. The amount of AMPure® XP beads aliquoted to each well of the 8-tube strip depends on the number of columns in use and which wash step is being performed. If all wells in a column are not in use, proceed as if all wells were in use.

Table 2: AMPure® XP beads per well of 8-tube strip

	1 Column (8 samples)	2 Column (16 samples)	3 Column (24 samples)	4 Column (32 samples)
1st Wash	40µL	70µL	100µL	130µL
2nd Wash	35µL	60µL	85µL	110µL
3rd Wash	35µL	60µL	85µL	110µL

3.8.1.2 From the 8-tube strip, transfer the AMPure® XP beads to each amplified library in the volume indicated in Table 3 below to form a 1:1 mixture of beads to sample. A multi-channel pipette may be used.

Table 3: AMPure® XP beads per library

1st Wash	30µL
2nd Wash	25µL
3rd Wash	25µL

3.8.1.3 Mix thoroughly by pipetting up and down.

3.8.2 Incubate at room temperature for 5 minutes.

3.8.3 Place the samples on a magnetic stand at room temperature for an additional 5 minutes.

3.8.4 Leaving the plate on the magnetic stand, remove and discard the supernatant. Take care not to disturb the bead pellet.

3.8.5 Pour approximately 15mL of 80% ethanol working solution into the ethanol reservoir.

3.8.6 From the ethanol reservoir, use a multi-channel pipette to add 200µL of 80% ethanol to each well and incubate the plate at room temperature for approximately 30 seconds.

3.8.7 Remove and discard the supernatant from each sample into the waste reservoir.

3.8.8 Repeat steps 3.8.6-3.8.7 for a second ethanol rinse.

3.8.9 With the plate on the magnetic stand, allow the beads to air-dry for 5 minutes.

3.8.10 To elute the DNA, remove the plate from the magnetic stand and add 28µl of 10mM Tris-HCl (pH 8.5) to each library following steps 3.8.10.1-3.8.10.3 below:

3.8.10.1 Prepare an 8-tube strip by adding 10mM Tris-HCl (pH 8.5) in the appropriate volumes as indicated in Table 4 below.

Table 4: 10mM Tris-HCl (pH 8.5) per well of 8-tube strip

	1 Column (8 samples)	2 Column (16 samples)	3 Column (24 samples)	4 Column (32 samples)
10mM Tris-HCl (pH 8.5)	30µL	60µL	90µL	120µL

3.8.10.2 From the 8-tube strip, transfer 28µL of 10mM Tris-HCl (pH 8.5) to each amplified library.

3.8.10.3 Resuspend the beads by pipetting up and down.

3.8.11 Incubate at room temperature for 2 minutes.

3.8.12 Place the plate on the magnetic stand until the beads pellet and the sample appears clear.

3.8.13 Being careful not to disturb the bead pellet, transfer 25µl of the supernatant to a new well as indicated below.

Wash 1&2: Transfer the elutant to an available well in an unused column. Additional 96-well plates of any kind may be used, if needed.

Wash 3: The final elutant should be transferred to a new well in the previously labeled purified library 96-well Eppendorf semi-skirted PCR plate. This plate should be loaded starting with column 2. The layout of samples on the purified library plate should match the layout of the original amplified library plate.

3.8.14 Repeat steps 3.8.1-3.8.13 twice for a total of 3 washes.

3.9 Seal the purified library plate and store at -20° C.