Manual AMPure® XP Bead-Based MPS Library Purification

1 Purpose

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

2 LIMS Processing

2.1 Refer to the LIMS Process Manual for the general worksheet processing protocols.

2.2 The index primer combinations used are indicated by the index primer identifier suffix at the end of each sample name (e.g., sample_A1, sample_B1).

3 Preparation

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

3.2 Retrieve the following reagents. Allow the reagents to thaw and/or equilibrate to room temperature. It is recommended for the bead solution to equilibrate to room temperature for at least 30 minutes.

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

3.3 Record the reagent lot numbers in LIMS.

3.4 Retrieve a new 96-well plate and label it with the run name and date, followed by "purified libraries."

3.5 Proteinase K working solution:

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

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

3.5.2.1 Retrieve and label a new 1.5 mL microcentrifuge tube.

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

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

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

3.6 Prepare 80% Ethanol working solution:

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

3.6.2 Add 10 mL laboratory-grade water to bring the volume up to 50 mL.

3.7 Prepare two (2) reagent reservoirs:

3.7.1 Retrieve and label one reservoir for the ethanol transfer.

3.7.2 Retrieve and label a second reservoir for the waste.

4 Procedure

4.1 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.

4.2 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.

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

4.4 Remove the seal from the amplified library plate.

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

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

4.7 Perform 3 washes following the instructions listed under step 4.8.

4.8 Wash:

4.8.1 Add the appropriate amount of AMPure® XP beads to each library according to Table 2 by following steps 4.8.1.1 - 4.8.1.3 below:

4.8.1.1 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	40 µL	70 µL	100 µL	130 µL
2nd Wash	35 µL	60 µL	85 µL	110 µL
3rd Wash	35 µL	60 µL	85 µL	110 µL

4.8.1.2 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. If needed, mix AMPure® XP beads by pipetting prior to transfer. A multi-channel pipette may be used.

Table 3: AMPure® XP beads per library

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

4.8.1.3 With new pipette tips, mix thoroughly by pipetting up and down.

4.8.2 Incubate at room temperature for 5 minutes.

4.8.3 Centrifuge the plate for 1 minute at 1000 rpm. Then place the samples on a magnetic stand at room temperature for an additional 5 minutes.

4.8.4 During the incubation steps in 4.8.2 and 4.8.3, prepare aliquots of the elution buffer as described below in 4.8.11.1.

4.8.5 Leaving the plate on the magnetic stand, remove and discard the supernatant. Take care not to disturb the bead pellet. Recommendation: use a multi-channel L-20 pipette set at 20 µL for 3 passes, changing pipette tips for each pass.

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

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

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

4.8.9 Repeat steps 4.8.7-4.8.8 for a second ethanol rinse. Following the second ethanol rinse and removal of ethanol with a multichannel L-200 pipette, use a multichannel L-20 pipette set at 20 µL to remove any residual ethanol.

4.8.10 Remove the plate from the magnetic stand and allow the beads to air-dry for 2-3 minutes.

4.8.11 To elute the DNA, add 28 µl of 10 mM Tris-HCl (pH 8.5) to each library following steps 4.8.11.1-4.8.11.3 below.

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

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)
10 mM Tris-HCl (pH 8.5)	30 µL	60 µL	90 µL	120 µL

4.8.11.2 From the 8-tube strip, transfer 2 8µL of 10 mM Tris-HCl (pH 8.5) to each amplified library.

4.8.11.3 Resuspend the beads by pipetting them up and down. Recommendation: First, add 28 µL of the elution buffer to all the wells. Then mix with a multichannel L-20 pipette set at 20 µL.

4.8.12 Centrifuge the plate for 1 minute at 1000 rpm, the incubate at room temperature for 2 minutes.

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

4.8.14 Being careful not to disturb the bead pellet, transfer 25 µL of the supernatant to a new well as indicated below. Recommendation: Use an L-20 pipette and transfer in two steps. First transfer 15 µL then transfer an additional 10 µl.

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 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.

4.8.15 Repeat steps 4.8.1-4.8.14 twice for a total of 3 washes.

4.9 Seal the purified library plate using a type B seal and store at -20 °C.

Note: Prior to finalizing your worksheet in LIMS, ensure that all sample suffixes are present and correct.