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 test batch processing protocol.

2.2 The index primers used will be indicated with the addition of the sample well as a suffix to each sample name. This suffix will always be added last, e.g., sample_A1, sample_B1.

2.3 As you fill performed by/date, suffixes may be removed for some quality control samples.

2.3.1 Prior to the completion of the test batch, you must ensure that the appropriate suffixes are present for every output sample.

2.3.2 To correct incorrect/missing suffixes if the test batch has not been finalized:

2.3.2.1 Sample names can be edited in batches directly in the test batch by selecting the sample(s) and clicking on the *Edit* button located below the Output Samples.

2.3.3 To correct incorrect/missing suffixes if the test batch has already been finalized:

2.3.3.1 Navigate to the manage sample tram stop in LIMS.

2.3.3.2 Search by test batch output samples using the test batch ID for your batch.

2.3.3.3 Click the checkbox for samples with an incorrect/missing suffix and click the *Edit* button at the top of the page. This must be done individually for each sample that needs correction.

2.3.3.4 In the OCME ID field, add the appropriate suffix to the end of the sample name.

2.3.3.5 Click *Save* and enter your E-signature.

2.3.3.6 Repeat these steps for each sample that needs correction.

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
50mM Tris-HCl (pH 8.0), 10mM $CaCl_2$
10mM Tris-HCl (pH 8.5)
100% EtOH
Distilled 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 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 and 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: 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

3.6 Prepare 80% Ethanol working solution:

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

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

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

4.6 From the 8-tube strip, transfer 5µL 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	40µL	70µL	100µL	130µL
2nd Wash	35µL	60µL	85µL	110µL
3rd Wash	35µL	60µL	85µL	110µL

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. A multi-channel pipette may be used.

Table 3: AMPure® XP beads per library	
1st Wash	30µL
2nd Wash	25µL
3rd Wash	25µL

4.8.1.3 Mix thoroughly by pipetting up and down.

4.8.2 Incubate at room temperature for 5 minutes.

4.8.3 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 and4.8.3, 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 20µl for 3 passes, changing pipette tips for each pass.

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

4.8.7 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.

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 20µl to remove any residual ethanol.

4.8.10 With the plate on the magnetic stand, allow the beads to air-dry for 2-3 minutes.

4.8.11 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 4.8.11.1-4.8.11.3 below:

4.8.11.1 Prepare an 8-tube strip by adding 10mM 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)
10mM Tris-HCl (pH 8.5)	30µL	60µL	90µL	120µL

4.8.11.2 From the 8-tube strip, transfer 28µL of 10mM 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 28µl of the elution buffer to all the wells. Then mix with a multichannel L-20 pipette set at 20µl.

4.8.12 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 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 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 test batch in LIMS, ensure that all sample suffixes are present and correct.