Normalization of MPS Libraries

1 Purpose

1.1 To normalize purified libraries to approximately 4 nM in preparation for sequencing.

1.2 Libraries whose concentrations fall within the range of 2.0 nM and 8.0 nM need not be normalized, and may proceed directly to pooling.

1.2.1 For hair-sample libraries whose concentrations are less than or equal to 2.0 nM, consult a supervisor or technical lead for guidance on whether to proceed with pooling.

2 LIMS Processing

2.1 Refer to the LIMS process Manual for general test batch processing protocol.

2.2 LIMS is unable to perform normalization calculations for purified libraries with concentrations greater than 200 nM.

2.2.1 For libraries with concentrations greater than 200 nM, calculate the appropriate volumes of purified library and 10mM Tris-HCl + 0.1% Tween 20 (pH 8.5) needed to achieve a normalized library concentration of 4 nM. A dilution factor of 0.1 should be used to achieve this. Record calculations in the LIMS data entry tab.

3 Normalization

3.1 Retrieve and thaw the purified library plate. Once thawed, vortex on a plate mixer at 1000 rpm for 1 minute, then centrifuge at 1000 rpm for 1 minute.

3.2 Retrieve 10 mM Tris-HCl + 0.1% Tween 20 (pH 8.5) and log the reagent lot number in LIMS.

3.3 Retrieve a new 96-well plate and label it with the run name and “normalized libraries”.

3.4 Consult the MiSeq Normalization sheet for your run in LIMS.

3.5 If dilutions are needed, retrieve and label the appropriate number of 1.5 mL microcentrifuge tubes.

3.6 Perform the sample dilutions by adding purified libraries and 10 mM Tris-HCl + 0.1% Tween 20 (pH 8.5) to the appropriate 1.5 mL microcentrifuge tubes in the volumes indicated in Table 1.

Table 1: Dilutions		
Dilution	**Amount of Purified Library (uL)**	**Amount of 10 mM Tris-HCl + 0.1% Tween 20 (pH 8.5) (uL)**
0.25	3 or (2)	9 or (6)
0.2	2	8
0.1	2	18

3.7 Vortex your dilutions, then centrifuge briefly.

3.8 The normalized library plate should be loaded starting with column 2. The layout of samples on the normalized library plate should match the layout of the original amplified library plate.

3.9 Referring to the MiSeq Normalization sheet for your run, pipette the appropriate volume of the purified library into its corresponding well of the normalized library plate.

3.10 Add the indicated volume of 10 mM Tris-HCl + 0.1% Tween 20 (pH 8.5) to each library to complete the normalization.

3.11 Seal the plate and vortex on a plate mixer at 1000 rpm for 1 minute, then centrifuge at 1000 rpm for 1 minute.

3.12 4nM libraries may be quantified before proceeding to pooling. Refer to the Library Quantification with PowerSeq® Quant MS kit protocol to quantify normalized libraries.

4 Re-Normalization of libraries

4.1 If quantified, normalized libraries that have concentrations of less than 2.0 nM or greater than 8.0 nM should be re-normalized and quantified before pooling.

4.2 If re-normalization is required, follow the normalization procedure above.

4.3 Re-normalized libraries should be placed in the next available well on the original normalized library plate. Note the location of the re-normalized libraries within the plate load tab of the associated LIMS test batch.

4.4 Re-normalized libraries may be quantified before proceeding to pooling. Refer to the Library Quantification with PowerSeq® Quant MS kit protocol to quantify normalized libraries.

4.5 Re-normalized libraries with concentrations between 2.0 nM to 8.0 nM may proceed to pooling.

4.6 If re-normalized libraries continue to yield values outside the range of 2.0 nM to 8.0 nM, the corresponding purified libraries should be requantified and normalized. These normalized libraries should be quantified before pooling. If newly normalized libraries continue to yield

concentrations outside the accepted range, consult with a supervisor or the technical lead for guidance on proceeding to pooling.

4.6.1 Further troubleshooting may be performed on those samples, including re-amplification, re-extraction and/or consultation with the vendor.