Normalization and Pooling of MPS Libraries		
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Manual Normalization and Pooling of MPS Libraries

1 Purpose

1.1 To normalize the purified libraries to approximately 4nM and pool the normalized libraries in preparation for sequencing.

1.2 Libraries whose quantitation values are less than or equal to 4.0nM but greater than 2.0nM will not need be normalized, and may proceed directly to step 4 (Pooling).

1.2.1 For non-hair-sample libraries whose quantitation values are less than or equal to 2.0nM, please consult a supervisor or technical lead for guidance on whether or not to proceed with pooling.

2 Normalization

2.1 Preparation

2.1.1 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.

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

2.1.3 Retrieve and label a new Eppendorf semi-skirted 96-well plate with the run name and "4nM normalized libraries".

2.1.4 Consult the "Normalization Tab" of the Library Dilution spreadsheet for your run and/or LIMS.

2.1.4.1 If additional dilutions are indicated on the Library Dilution spreadsheet, retrieve and label the appropriate number of 1.5 mL microcentrifuge tubes.

2.2 Procedure

2.2.1 Perform any necessary additional dilutions as indicated on the Library Dilution spreadsheet:

2.2.1.1 Steps 2.2.1.2-2.2.1.3 below create a 1:10 (0.1) dilution. If a dilution greater than 0.1 is indicated, perform additional 1:10 serial dilutions.

2.2.1.2 To the previously prepared 1.5mL microcentrifuge tubes, aliquot 2µL of the appropriate purified libraries.

2.2.1.3 Add 18µL 10mM Tris-HCl + 0.1% Tween 20 (pH 8.5), mix thoroughly and centrifuge briefly.

2.2.2 Perform normalization to 4nM

2.2.2.1 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.

2.2.2.2 Referring to the Library Dilution spreadsheet and/or LIMS, pipette the appropriate volume of the purified library into its corresponding well of the normalized Library plate.

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

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

2.3 4nM libraries must be quantified before proceeding to pooling (step 4). Refer to the Manual MPS Library Quantitation with PowerSeq® Quant MS protocol to quantify normalized libraries.

3 Re-Normalization of libraries

3.1 Normalized libraries that have a quantitation value of 2.0 nM or less should be re-normalized and quantified before pooling.

3.2 If re-normalization is required, follow the normalization procedure above (step 2).

3.3 Re-normalized libraries should be placed in the next available well on the original normalized plate, for quantitation. Note the location of the re-normalized libraries within the associated LIMS test batch.

3.4 Re-normalized libraries must be quantified before proceeding to pooling (step 4). Refer to the Manual MPS Library Quantitation with PowerSeq® Quant MS protocol to quantify normalized libraries.

3.5 If re-normalized libraries continue to yield values of 2.0 nM or less, the corresponding source (non-normalized) libraries should be requantified, and then normalized for quantification again. If newly normalized libraries continue to yield values of 2.0nM or less, please consult with a supervisor or the technical lead for guidance on proceeding to pooling. Normalized libraries with quantitation values greater than 2.0 nM shall proceed to pooling (Step 4).

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

4 Creating the Library pool

4.1 Retrieve a single 1.5 mL microcentrifuge tube and label with the run name followed by the word "pool."

4.2 Add 5 µL of each library due to be included in the pool to the microcentrifuge tube, including positive and negative controls. Libraries pooled may include both normalized/re-normalized libraries and purified libraries with concentrations less than 4 nM.

4.3 The tube containing the pooled libraries stored at -20 °C freezer. Seal the plate containing the normalized libraries and store at -20 °C freezer.