Sep 12, 2022

Final QC, Pooling and Sequencing

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dx.doi.org/10.17504/protocols.io.yxmvmnw29g3p/v1

CGORD Palmer Wet-Lab Protocols

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ABSTRACT

This protocol is conducted after a set of libraries are completed and ready to quantify and pool. This protocol outlines the final steps before submitting for sequencing.

DOI

dx.doi.org/10.17504/protocols.io.yxmvmnw29g3p/v1

PROTOCOL CITATION

Katarina A Cohen, Khai-Minh H Nguyen, Oksana Polesskaya, Abraham Palmer 2022. Final QC, Pooling and Sequencing . **protocols.io** https://protocols.io/view/final-qc-pooling-and-sequencing-b8i8ruhw

CREATED

May 01, 2022

LAST MODIFIED

Sep 12, 2022

PROTOCOL INTEGER ID

61760

MATERIALS TEXT

Equipment

- PC Running Excel
- Pipette 1-10uL
- Pipette 20-200uL
- Bioanalyzer
- Qubit Flourometer

Reagents

- Ultrapure Distilled, Nuclease Free Water **Contributed by users** In 2 steps

Consumables

- Pipette Tips
- 1.5mL Eppendorf Tubes
- Qubit Assay
- Tapestation D1000

BEFORE STARTING

Ensure all libraries that will be pooled are uniquely indexed.

Library QC

1
- Quantify purity and concentration of library with Nanodrop and a Qubit Assay
- Obtain average fragment size of library with Tapestation (D1000 Assay)

1.1
- Libraries should have an average fragment size between 420bp - 650bp.
- 260/280 should be around 1.80 - 2
- 260/230 should be around 2-2.2.
- We have been able to get good data from libraries with relatively poor nanodrop purities.
- Qubit concentrations can widely range. We get a range from 10ng/ul - 60ng/ul

Pooling

2 Download **Pooling Template.xlsx**

3
- Increase "Target Vol (uL) per Sample" if any Sample Vol is lower than 1ul

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- Use Ultrapure Distilled, Nuclease Free Water **Contributed by users** when adding water to final pool

4 Enter library name in Column A "Sample Name"

5 Enter qubit concentrations in column B

6 Enter average fragment size of library in column C for each library.

7 Increase "Target Vol (uL) per Sample" if any Sample Vol is lower than 1ul

8 Label new tube as Riptide Pool ## , and Date. Add calculated volume of water (shown in cell I6). Add calculated volumes of sample shown in column E.

- Use Ultrapure Distilled, Nuclease Free Water **Contributed by users** when adding water to final pool

Checking Pooling

9 **RECOMMENDED OPTIONAL STEP**
Check pooling with an illumina MiSeq run

- % Reads Identified for each library shouldn't vary more than 30% from each other.

Sample-Barcode List

10 **YYYY-MM-DD-Flowcell Sample-Barcode list.xlsx**

- Download the file above
- Check Twist Bioscience site for updates to the sample barcodes used for the Twist 96-Plex Kit.

11 Open the library file created in the "EPMotion - Normalization and Randomization".

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- Go to the "Sample_Randomization" Tab

12 When the "Sample_Randomization" tab is opened, copy and paste the randomized Transponder ID's into Column A of the Flowcell Sample-Barcode list.

- NOTE: If less than 96 samples are processed in a library, delete the unfilled rows within that 96 library set. (Essentially, you want to delete the sample barcodes that are not associated with a sample).

13 Transfer any comments from the library file into Column G of the Sample barcode list
- Ensure that the comment matches with the correct sample ID.

14 Enter the PCR index barcode used for that particular library (ex. 1,2,3,4,5...) in Column D

15 Enter Library name in Column E

Submitting for Sequencing

16 Submit 30ul of Pool
- Platform: NovaSeq S4
- Run Type: PE150

You can find the updated inline i7 and i5 index sequences on Twist Bioscience Site

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