Jun 06, 2022

Pooling

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protocol .

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The throughput of modern sequencers grows higher and higher, allowing hundreds of millions of reads in a single microfluidic chamber. For many purposes this is actually far more than a single library requires. To keep sequencing cost-effective, researchers often pool together multiple libraries before sequencing, each with a unique molecular barcode (or unique combination of multiple barcodes). The sequencer then reads each library molecule's biological base sequence as well as the barcode sequence; these barcodes are matched back to the sequences expected from the libraries, and thus each molecule can be attributed to its library of origin even though the libraries were mixed. The pool of libraries needs to be prepared at a specific molarity. Thus when you combine libraries into a pool to sequence together, you must take care to match not only their relative molarities but also the absolute total molarity of the pool.

Allyson Hirsch, George Testo 2022. Pooling. **protocols.io**
https://protocols.io/view/pooling-carzsd76

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Supplies

- Table of Volumes
- Sterile 1.5 mL tube
- Green temporary seal(s)
- Foil seal(s)
- Sharpie

Equipment

- 10uL pipette & tips
- 20uL pipette & tips

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Preparations

1 Print plate table with volume of each amplicon to be pooled together (equal molar).

2 DNA Away and Ethanol the workstation.

3 Remove PCR plate(s) from the **-20 °C** freezer and thaw plates to be pooled while finishing workstation prep.

4

Spin thawed plates down in centrifuge.

Pooling Sample(s)

5 

Pipette the correct volume from the PCR plate according to the table into the labeled 1.5 mL tube

5.1 Use a temporary seal to help keep wells uncontaminated and to keep track of samples being added.

5.2 Clearly mark off when a sample has been added to the pooling tube.

6 Once an entire plate's samples have been added, place a freezer seal on the plate and seal well.

7 When all samples have been added to the pooling 1.5 mL tube, clean the workstation and place the PCR plates and pooling tube in the - **20 °C** freezer for temporary storage.