**LIBRARY QC Template** Initials: Date:

*Diagram

Description automatically generated*LIBRARY NAME:

1. Determine concentration of library using Qubit HS DNA assay and record **concentration in ng/µl**.

*Note: Make sure you click “Calculate Stock” on the Qubit, specify the number of µl you added, and change the units to ng/µl*

Concentration (ng/µl) = \_\_\_\_\_\_\_\_\_\_\_\_\_\_

\* Dilute library to ~1ng/µl in a minimum of 20µl. This 1ng/µl dilution is the library you will need for tapestation, qPCR, and loading the sequencing instrument!

1. Determine the average size of your library using the HS D1000 Tapestation assay and set a region around your library. Record the **average size** and **estimated molarity** here.

*Note: If there are primer dimers/adapter dimers, you should stop, perform a bead clean up and repeat the QC.*

Average Size (bp) = \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Estimated Molarity (nM) = \_\_\_\_\_\_\_\_\_\_\_\_\_

1. qPCR: Kapa Quant kit

*Note 1: There are single use aliquots of the standards and PhiX 10e-3 run-to-run control in the -20˚C*

*Note 2: This is strictly a template, you should read the Kapa Library Quantification Protocol before using this.*

*Note 3: If you’re using the NGS Core’s Kapa Library Quant kit, there is a $50 fee for each use.*

1. Calculate the number of reactions to prepare master mix for: **B**) Make Master Mix:

|  |  |  |
| --- | --- | --- |
| **Reaction** | **Replicates** | **Subtotal** |
| Standards 1-5 | 3 | 15 |
| PhiX 10e-3 | 3 | 3 |
| Library (1 library) | 3 |  |
| Total (Sum of Subtotal + 2) | -- |  |

|  |  |  |
| --- | --- | --- |
|  | 1X | \_\_\_ X |
| 2X KAPA MM | 6µl |  |
| dH2O | 2µl |  |
| Total | 8µl/rxn |  |

1. Make serial dilutions of libraries according to Figure 2

***Note: Perform two serial dilutions each in duplicate. The first dilution is 1:100. Using the first dilution, making a second 1:100 dilution for a final dilution of 1:10,000.***

Dilutions:

1. 1:100 dilution 🡪 2µl Library + 198µl 1X Dilution Buffer
2. 1:10,000 dilution 🡪 2µl of diluted library from (1) + 198µl 1X Dilution Buffer

***Load the 1:10,000 dilution!***

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A |  |  |  |  |  |  |  |  |  |  |  |  |
| B |  | STD 1 |  |  | Library rep 1 |  |  |  |  |  |  |  |
| C |  | STD 2 |  |  | Library rep 2 |  |  |  |  |  |  |  |
| D |  | STD 3 |  |  |  |  |  |  |  |  |  |  |
| E |  | STD 4 |  |  |  |  |  |  |  |  |  |  |
| F |  | STD 5 |  |  |  |  |  |  |  |  |  |  |
| G |  | PhiX |  |  |  |  |  |  |  |  |  |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |

1. Set up qPCR plate according to plate map

- Add 8µl of Master Mix to each well changing pipet tips each time.

- Then add 2µl of template, changing pipet tips each time.

\* Do not change pipet dial as you go! Even if the volume changes over time.

1. Analyze the Ct values using the ‘kapa\_quant\_libqc\_data\_analysis\_template.xls’ and record your PhiX and Library molarities here:

PhiX 10e-3 (nM) = \_\_\_\_\_\_\_\_\_\_

Library (nM) = \_\_\_\_\_\_\_\_\_\_\_\_\_

***Diagram

Description automatically generated***