

# NGS Library QC

## **General Information**

| Order Number                     | 2007UNHS-0288 |      | Name of Customer |  | Gregory Randolph |   | Date of Order |      | 2020-07-16 |      |
|----------------------------------|---------------|------|------------------|--|------------------|---|---------------|------|------------|------|
|                                  |               |      |                  |  |                  |   |               |      |            |      |
| Final QC Result of DNA sample(s) |               |      |                  |  |                  |   |               |      |            |      |
| Arrival Dat                      | te            | Exp  | eriment Date     |  | Sample count     | ] | Pass          | Fail |            | Hold |
| 2020-07-20                       |               | 2020 | 0-08-18 17:13    |  | 1                |   | 0             | 0    |            | 1    |

| Final QC Result of RNA sample(s) |                 |              |      |      |      |  |  |  |
|----------------------------------|-----------------|--------------|------|------|------|--|--|--|
| Arrival Date                     | Experiment Date | Sample count | Pass | Fail | Hold |  |  |  |
| N/A                              | N/A             | N/A          | N/A  | N/A  | N/A  |  |  |  |

The QC criteria refer to the specification requirements of a single run. In any case, we may encounter the shortage of sample volume or amount due to various reasons such as a library construction failure. In these cases a request of an additional sample will be inevitable.

Therefore, we recommend double the amount to be supplied at first place to minimize any delay of the whole procedure.

\* Pass : Samples automatically move forward to the next steps.

\* Hold : A specific instruction should be given by the client for further processing. PSOMAGEN, INC. does not proceed to the next step until we have received the client's confirmation.

\* Fail : Samples have failed to meet all the criteria set and cannot proceed to the next step. Sample(s) will be put on hold until further written notice from the client.

# Library QC Result of DNA

| Arrival Date | 2020-07-20   | Experiment Date | 2020-08-18 17:13 | Tested by | SL |  |  |  |
|--------------|--|-----------------|------------------|-----------|----|--|--|--|
| Comment      | The sample has been placed on hold due to broad and multiple peaks. Please confirm how to proceed furthermore. |                 |                  |           |    |  |  |  |

| # | Library Name  | Library Type            | Conc.<br>(ng/ul) | Conc.<br>(ng/ul)Conc.<br>(nM)Size<br>(bp)vol.<br> |     | Result* |      |      |
|---|---------------|-------------------------|------------------|---|-----|---------|------|------|
| 1 | NovaSeq2-Full | Custom pre-made library |                  | 6.95  | 613 | 91      | Hold | Size |

Experiment Condition

D5000 Screen Tape



### Library QC Method

#### 1. Library Size Check

To verify the size of PCR enriched fragments, we check the template size distribution by running on a Agilent TapeStation 2200 or 4200.

#### 2. Library Quanity Check

In order to achieve the higher quality of data on Illumina sequencing platforms, it is important to create optimum cluster densities across every lane of every flow cell. This requires accurate quantitation of DNA library templates. Therefore, PSOMAGEN, INC. quantify prepared libraries using qPCR method for PCR free libraries and Picogreen method for PCR plus libraries according to each protocol guidelines.

## Library QC Criteria

|                         |                                      | QC Criteria        |           |         |  |
|-------------------------|--------------------------------------|--------------------|-----------|---------|--|
| Requested lib           | Platform                             | Concentration (nM) | Size (bp) |         |  |
|                         | TruSeg Nano library                  | HiSeq              | 2         | 450 800 |  |
|                         | Trused Natio library                 | HiSeq X***         | 3         | 430-800 |  |
| Shotgun librany         | Trused DCP Free library*             | HiSeq              | 2         | 450-800 |  |
| Shotgun horary          | hused PCK Free library               | HiSeq X***         | 2.5       |         |  |
|                         | KADA library propagation (DCD Free)* | HiSeq              | 2         | 450-800 |  |
|                         | KAPA library preparation(PCK Free)   | HiSeq X***         | 2.5       |         |  |
| Even library            | SuraSalast past                      | HiSeq              | 2         | 250,400 |  |
| Exoni library           | Sureselect_post                      | NovaSeq***         | 2         | 250-400 |  |
| BNA library             | Truson Strandod BNA library          | HiSeq              | 2         | 200-400 |  |
| RNA library             | Trused stranded KNA library          | NovaSeq***         | 2         |         |  |
| Pre-made library        |                                      | HiSeq              | 2         | 200 000 |  |
| (Illumina compatible)** |                                      | HiSeq X***         | 3         | 200-800 |  |

\*TruSeq PCR Free library sizes measured on the Bioanalyzer or TapeStation are substantially larger than would be predicted or derived from sequencing data due to structure of sequencing adapter. Library sizes being reported are calculated from sizes measured after fragmentation.

\*\*The minimum requirement for pre-made libraries is 2nM. However, we recommend sending 5nM at 10ul volume for our evaluation of sample quality. In many cases, measurement at the time of sample preparation shows substantial discrepancies from measurement at the time of sample arrival due to sample disruption during transition or different measurement procedures. Please take precautions and send enough concentration and volume to avoid any possible delays.

\*\*\*Criteria for the HiSeqX and NovaSeq can be changed.

# Library QC Fail Reason (Results)

Low Yield : Samples that failed/on hold due to not meeting QC criteria depending on library type.

No Yield: Samples that failed exome capture and resulted in no library yield.

Size : Samples that failed due to Library size above or below QC criteria depending on library. Compared to fragmentation QC results, library size is out of expected range.

ETC.: Only for special circumstances which does not fall under the above three categories. Separate comment will be left regarding this circumstance.