



## RNA Sequencing Methods

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Samples were DNase treated with Invitrogen DNase (RNase free). Library preparation was performed using Illumina's Stranded Total RNA Prep Ligation with Ribo-Zero Plus kit and 10bp unique dual indices (UDI). Sequencing was done on a NovaSeq X Plus, producing paired end 150bp reads. Demultiplexing, quality control, and adapter trimming was performed with bcl-convert (v4.2.4)<sup>1</sup>. Sequencing statistics are included in the '*RNA Sequencing Stats.xlsx*' file.

No additional trimming or filtering was performed on reads before they are uploaded to Box. If you received analysis on your RNA data post-sequencing you will have received a separate text document with your analysis files describing the methods and version numbers used for your samples.

What is an md5sum?

The md5sum functions as a file's compact digital fingerprint. md5sums are used to verify the integrity of files between two servers. If you calculate the md5sum of the file on your server, it should match that of the file listed in the '*DNA Sequencing Stats.xlsx*' file. If it does not match, the file was corrupted either being uploaded or downloaded to box, please let SeqCenter know if this is the case.

Calculating the md5sum:

- Windows users, please see [this tutorial](#).
- MacOS users, please see [this tutorial](#).
- Linux users, please see [this tutorial](#).

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<sup>1</sup> [bcl-convert](#): A proprietary Illumina software for the conversion of bcl files to basecalls.