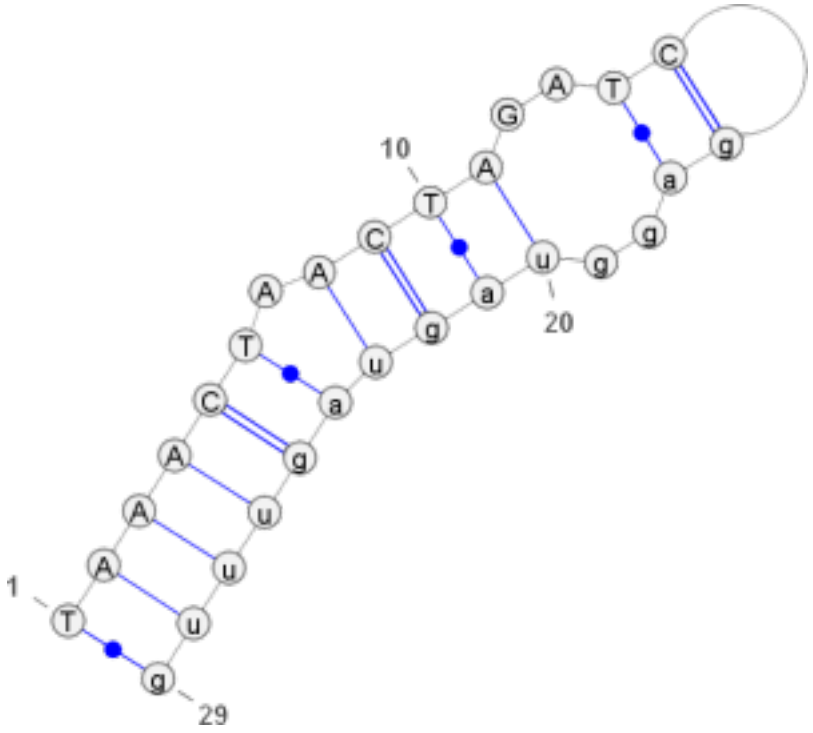
miRNA Sequencing with RNAstable® and SeqMatic miRNA kit

SeqMatic

Sample Information

- <10ng of human miRNA (small ~16nt RNA) was extracted from samples using Qiagen miRNeasy® extraction kit.
- 12 samples were stored using RNAstable® with standard protocol
- Samples were shipped from South America to the US via a standard package envelope



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Sample Processing

- Samples were reconstituted with nuclease free water.
- Samples sent directly to library generation without cleanup.
- Sequencing libraries were generated using the SeqMatic miRNA library preparation kit.

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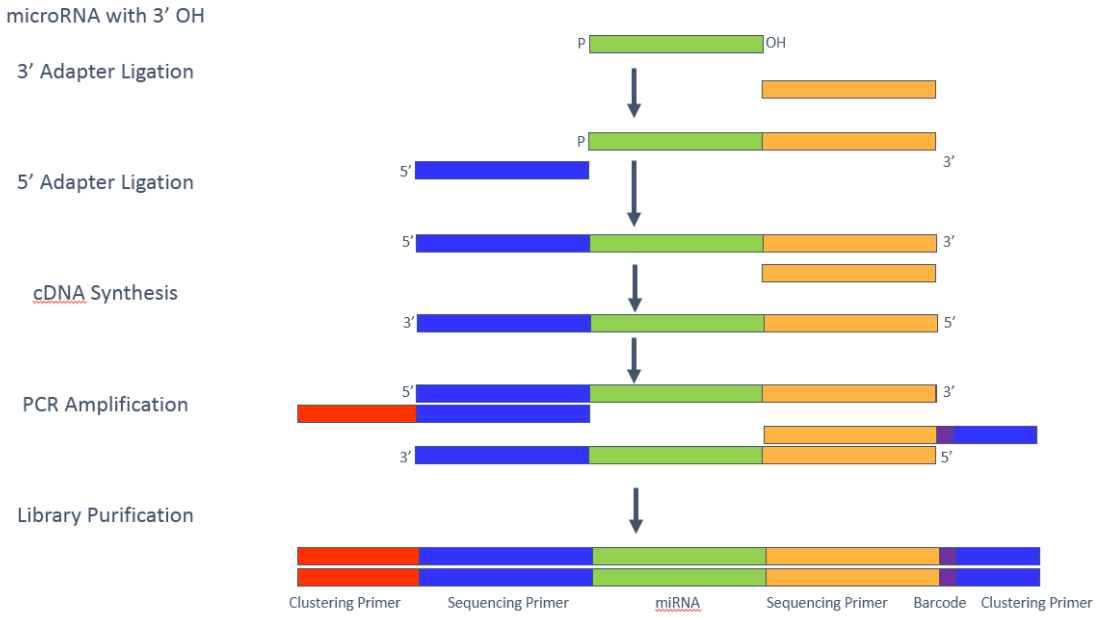
Library Generation Overview

- Unique 3’ and 5’ adapter oligos were ligated to the ends of each miRNA.
- cDNA was generated, followed by PCR.
- A unique barcode was added for multiplexed sequencing.



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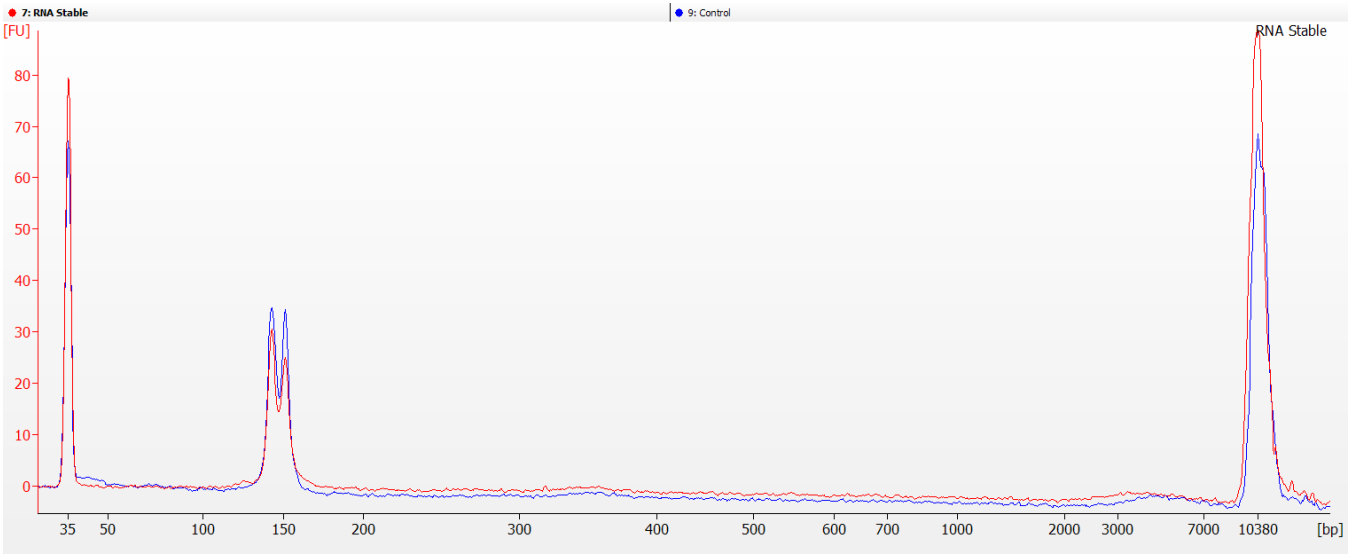
Library Quality

- RNAstable samples look identical to control RNA.
- High yield: samples generated > 2nM of library necessary for sequencing.
- High purity: clean separation of adapter dimer bands after gel electrophoresis.



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Sequencing

- Sequencing was performed on the Illumina® MiSeq® instrument.
- A single read of 50bp is sufficient for miRNA profiling and expression analyses.
- Instrument provides a comprehensive sequencing platform with onboard clustering, sequencing, and data analysis.

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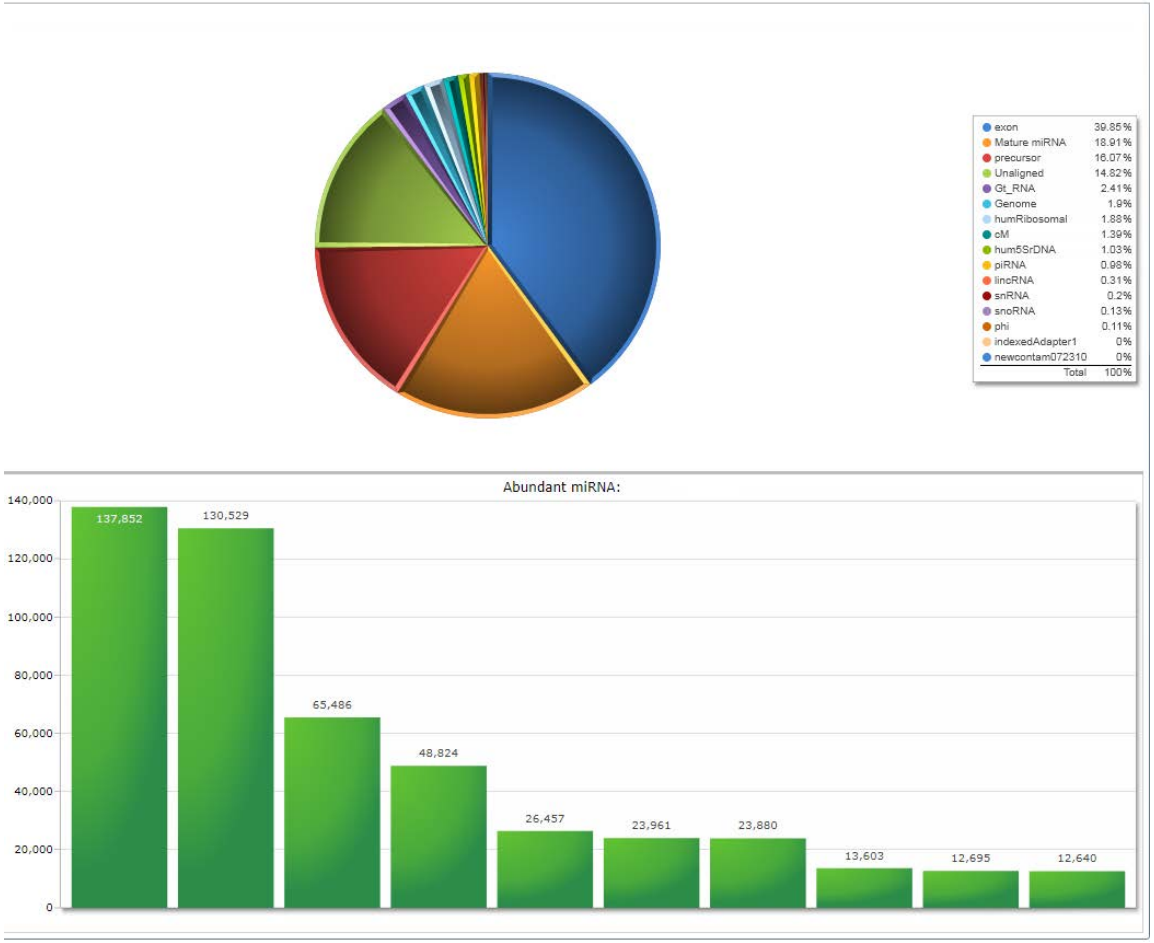
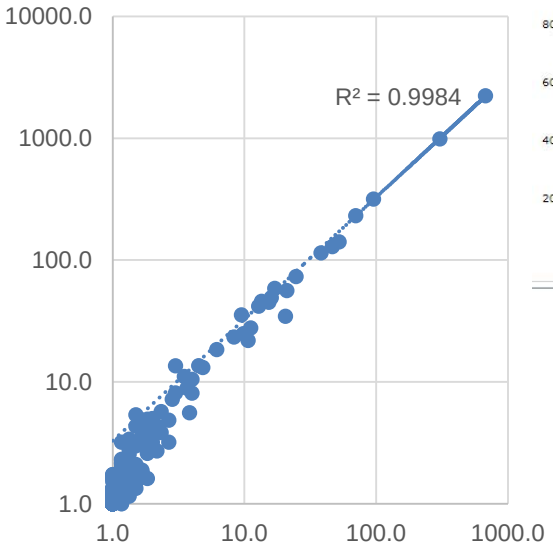
Data Analysis

- All libraries aligned to human miRbase.
- Adapter dimer contamination rate was very low.
- Replicate samples generated similar expression profiles with high R^2 value.





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Summary

- RNAstable provides a good method of shipping low amounts of miRNA across the world.
- RNAstable does not interfere with enzymatic reactions during library generation.
- SeqMatic miRNA sample preparation kit provides a robust method of generating Illumina sequencing libraries with very low sample input.
- Libraries generated showed no loss of yield or any biases.

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