QIAseq miRNA Library Kit

The “next-generation” in miRNA sequencing products
What challenges are there with miRNA Sequencing?

- **Sample input is one of many challenges facing miRNA sequencing reactions.**
  - Kits may require up to 1 microgram of input, lower input level more than 1ng
  - May not be compatible or optimized for bio-fluids

- **Data quality may be low**
  - On Target miRNA NGS Reads account for only 20-30% of total reads
  - Adapter Dimers eat at your read budget
  - Gel band excision is not an exact science (not 100% miRNA)
  - Biofluid samples may be largely contaminated with other RNA types similar in size

- **Workflow can be tedious and unreliable**
  - Gel workflows take multiple days, unlikely to get on the sequencer in days
  - Large sample numbers are tedious with gels
  - Bead based methods still have high background as dimers and contaminants are still in high percentage
  - Optimization from sample isolation not available
QIAseq miRNA Library Kit: miRNA specificity that outpaces the competition

PAGE gel after standard library prep protocol

Illumina

NEB

AD = Adapter Dimer

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QIAseq miRNA overview

What is the kit? miRNA-focused next-generation sequencing library prep kit and integrated bioinformatics/data analysis solution

What is the product used for? Preparation of mature miRNome libraries from any species

What sequencers are the libraries compatible with? Illumina sequencers

What can be done with the sequencing data?
• Differential expression calculations of miRNA from highly multiplexed samples
• Novel miRNA discovery
• Identification of IsomiRs

What are distinguishing features of the prep kit?
• “Gel-free” prep kit for miRNA sequencing
• Broad RNA input: 500 ng to 1 ng
• Library prep from cells and tissues of any species
• Library prep from serum, plasma and other biofluids
• Integrated Unique Molecular Index (UMI) technology
• Rapid workflow
• Highly optimized chemistry
• “All-in-one-box” solution

QIAseq miRNA Library Kit: Unparalleled miRNA-focused sequencing for accurate digital quantification
QIAsseq miRNA mapping rates and specs

**miRNA mapping rates routinely observed**
- **Cell lines:** 50-60% or greater
- **Tissues:** 75% or greater
- **Serum/plasma:** 15-30% or greater

**Specs**
- **Sample type:** Cells, fresh/frozen tissue, FFPE tissue, serum/plasma, biofluids
  - Animal and plant samples
  - Any species
- **Total RNA input range (cells/tissues):** 500 ng to 1 ng
- **Total RNA input recommendation (serum/plasma):** 5 µl when RNA has been isolated from 200 µl of sample
- **What RNAs are included in library prep?** Highly optimized for miRNA
  - piRNAs will also be efficiently sequenced
- **Multiplex capability:** 48 samples
- **Sequencer compatibility:** Illumina
- **Total library construction time:** 8 hours
Overview of products

**QIAseq miRNA Library Kit**
- **12 rxn:** 331502
- **96 rxn:** 331505
- **What's included?** 3' ligation, 5' ligation, reverse-transcription, cDNA cleanup, library amplification and library cleanup reagents; quality control primers

**QIAseq miRNA NGS 12 Index IL**
- **12 rxn:** 331592
- **What’s included?** Sequencing adapters, primers and indexes compatible with Illumina platforms. **12 indexes for 12 samples.**

**QIAseq miRNA NGS 48 Index IL**
- **96 rxn:** 331595
- **What's included?** Sequencing adapters, primers and indexes compatible with Illumina platforms. **Two 48 indexes for 96 samples.**
QIAseq miRNA Library Kit

Other kits

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<th>P5 Capture</th>
<th>5’ adaptor</th>
<th>miRNA</th>
<th>3’ adaptor</th>
<th>Sample Index</th>
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- 50 bp read
- 48 possible sample indexes

QIAseq miRNA Library Kit

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<tr>
<th>P5 Capture</th>
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- 75 bp read to make use of the UMI
  - Note: 50 bp read is possible, but the UMI will not be sequenced
- 48 possible sample indexes

UMI enables precision quantification of miRNA molecules following next-generation sequencing
QIAseq miRNA: Save a Day of Workflow-8 Hours

Step 1: 3’ ligation

Step 2: 5’ ligation

Step 3: Reverse-transcription with Unique Molecular Index (UMI) assignment

Step 4: QMN Bead prep

Step 5: cDNA cleanup

Step 6: Library amplification and Sample Index assignment

Step 7: Library cleanup

Steps 8-11: Library Pre-Seq QC, Determining Library Conc, Prep for Seq, Data Analysis

Elimination of adapter dimer from sequencing library
Unique Molecular Index (UMI) principle

Original Sample

miRNA 1  
RT with UMI  
Amplify & Sequence

miRNA 2

Raw Reads

• Original sample \(\rightarrow\) (3:2 ratio of “miRNA 1” to “miRNA 2”)
  • miRNA 1: 3 molecules
  • miRNA 2: 2 molecules
• Interpretation of “raw reads” \(\rightarrow\) (2:1 of “miRNA 1” to “miRNA 2”)
  • miRNA 1: 12 reads
  • miRNA 2: 6 reads
• Interpretation of “UMIs” (3:2 ratio of “miRNA 1” to “miRNA 2”)
  • Reads are collapsed based on “molecule counts”
  • miRNA 1: 12 reads BUT 3 molecules are identified due to UMIs
  • miRNA 2: 6 reads BUT 2 molecules are identified due to UMIs

Instead of number of reads, the number of unique UMIs are counted, which accurately reflects the original status of the transcript
Value of UMIs

- **Assessment of raw miRNA reads:** Sequencing of the same miRNA molecule over and over, resulting in an overestimation of miRNA expression
  - *The lower the RNA input, the worse this effect is*
- **Assessment of miRNA UMIs:** Individual miRNA molecules are being counted, resulting in a true assessment of miRNA expression
  - *The lower the RNA input, the more powerful the UMIs are*

UMIs give a true readout of miRNA expression
QIAseq miRNA Library Kit: Straightforward workflow = Highly reproducible results

- **QIAseq miRNA Library Kit workflow on kidney total RNA**
  - **RNA Amounts:** 500 ng, 100 ng, 10 ng, 1 ng
  - **Sample QC:** Bioanalyzer (BA)
  - **Sequencing:** NextSeq, 75 bp Single Read

The QIAseq miRNA workflow enables robust, reproducible results from 500 ng to 1 ng
QIAseq miRNA Library Kit: miRNA specificity that outpaces the competition

- QIAseq miRNA, Illumina TruSeq® Small RNA, and NEB NEBNext® on HCT 116 total RNA
- **RNA Amounts:** 100 ng (QIAseq miRNA), 1 µg (Illumina), 100 ng (NEB)

**PAGE gel after standard library prep protocol**

With the QIAseq miRNA standard protocol, a robust, specific miRNA library is generated with negligible background. Other commercial options are fraught with side-products, including adapter dimers.
QIAseq miRNA Library Kit: Outsequencing the competition

- **Next generation sequencing:** QIAseq miRNA and Illumina TruSeq
  - **RNA input amounts:** 100 ng HCT 116 total RNA
  - *For Illumina TruSeq, prior to sequencing, miRNA library was excised/purified from a PAGE gel*
  - **MiSeq:** 75 bp Single-Read (QIAseq miRNA) and 50 bp Single-Read (Illumina)

**Illumina TruSeq vs. QIAseq miRNA**

- **2X+ Reads with QIAseq miRNA compared to Illumina Small RNA Kit!**

With QIAseq miRNA, increase your mapped miRNA reads (result of reduced bias and improved sensitivity) while reducing your workflow time
Data Analysis

- **Primary Analysis:** http://ngsdataanalysis.sabiosciences.com/QIAseqmiRNA/
  - **Well-characterized species:**
    - UMI (Molecular Tag: MT) counting and mapping (species-specific miRBase, genome)
  - **Poorly-characterized species:**
    - UMI (MT) counting and mapping ("all of miRBase")

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- **Secondary Analysis:** http://qiagen.com/GeneGlobe
  - Differential expression analysis
  - Multiple normalization methods offered that are routinely used for miRNA analysis
    - geNorm
    - Total Molecular Tag Count
    - DESeq2
    - Trimmed Mean of M (edgeR)
Summary

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