TECHNICAL NOTE

Ion Torrent

Ion RNA-Seq Solution

SUMMARY

Comprehensive, quantitative and accurate data for RNA studies

Single day workflow

Simple sample preparation and data analysis

Affordable system and low cost per sample

Ion RNA-Seq solution removes the barriers to NGS for microarray users

Introduction

Next-generation sequencing opened a new frontier in RNA expression studies. Unlike microarrays and other genomic techniques that rely on previous knowledge and gene-specific consumables, RNA-Seq generates a precise digital output of specific individual transcriptomes. However, most researchers have been unable to access this technique due to the high cost of equipment and reagents, long and cumbersome workflows, and complicated data analysis tools.

The Ion Personal Genome Machine™ (PGM™) Sequencer enables every researcher to use RNA-Seq for their RNA profiling studies without any significant changes to their current pipelines or personnel. The Ion PGM™ Sequencer, in combination with the Ion Total RNA-Seq Kit developed by Ambion®, generates comprehensive and quantitative data for RNA studies, offering a fast, cost-effective solution without the need to pool hundreds of samples, along with a simple workflow and intuitive data analysis solutions. The Ion semiconductor sequencing technology removes the barriers to entry for next-generation sequencing and empowers microarray and qPCR users to take their research to a higher level today.

Single day workflow

Ion Torrent offers a complete solution for performing RNA-Seq studies, from fast library preparation and simple, rapid sequencing workflows, to intuitive data analysis tools (Figure 1).

The Ion Total RNA-Seq Kit provides flexible workflows to generate whole transcriptome or small RNA libraries from human and non-human samples. Starting with just 200ng of total RNA or 5ng of miRNA, the library construction can take as little as 6 hours. Unlike methods that ligate adapters to double-stranded cDNA, the Ion Total RNA-Seq Kit utilizes proprietary Ambion® technology to attach the adapters in a directional manner that preserves strand information in the resulting libraries (patent pending). In addition, both the 3' and 5' adapters are attached simultaneously, reducing ligation and clean-up steps. The library is then clonally amplified and the resulting templates are ready to be sequenced.

The Ion OneTouch™ automated workflow allows preparation of templates in about 3 hours with just minutes of hands on time. The sequencing run takes less than 1 hour. Sequence reads are processed in the Torrent Server and a simple file is exported to common genomics data analysis software tools such as Partek® Genomics Suite™.

Partek® Genomics Suite™, a primary software tool for microarray gene expression analysis, has partnered with Ion Torrent and offers an intuitive user interface and comprehensive workflows for complete RNA-Seq data analysis.

The Chip is the Machine™: multiple chips for multiple applications

Depending on the throughput requirements of the experiment, customers will be able to choose between the Ion 314™, Ion 316™ or Ion 318™ sequencing chips. The only difference between the chips is the number of interrogating wells, ranging from 1M for the Ion 314™ sequencing chip, 6M for the Ion 316™ and 12M for the Ion 318™. These sequencing chips can produce from hundreds of thousands to up to 8M reads. Applications that require a small number of reads, such as the sequencing of small RNAs, low complexity transcriptomes (e.g. viruses, bacteria) or targeted gene expression, can be performed on the Ion 314™ or Ion 316™ sequencing chips. We anticipate that the Ion 318™ chip will be optimal for human whole-transcriptome experiments, surpassing the sensitivity and dynamic range levels achieved with microarrays and bringing all the added benefits of RNA-Seq. All the chips are compatible with the Ion PGM™ Sequencer. No additional instrumentation, ancillary equipment or software is required for the larger capacity chips.

Figure 1. Ion RNA-Seq workflow

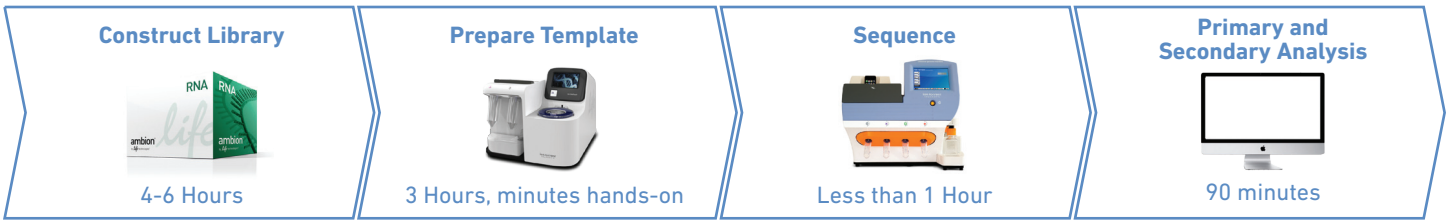
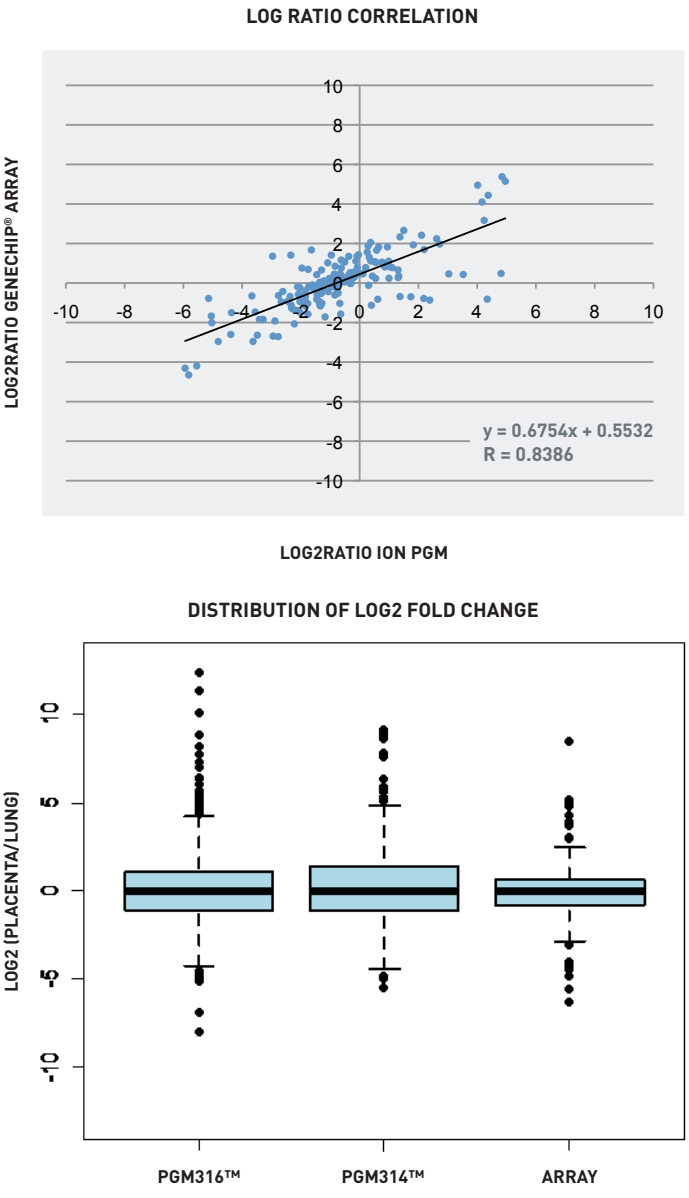


Table 1.
The first column shows the total number of miRNAs that can theoretically be detected by each platform based on their respective content. PGM™ detection threshold is not content dependent and the number represents the total number of miRNAs included in the miRBase up to date. Detection by TaqMan® is calculated at Ct<40. The number of RNAs that all 3 platforms can theoretically detect is shown in the second column and the actual number of miRNAs detected by each platform is indicated on the last column.

	Total Assays	Intersection Assays	Intersection Detected
TaqMan®	748	708	308
GeneChip® Array	847	708	216
Ion 314™	**904**	**708**	**230**
Ion 316™*	**904**	**708**	**358**

Figure 2.
(A) Correlation between Ion PGM RNA-Seq data and miRNA microarrays.
(B) Dynamic range of LogRatio values obtained with the Ion 316™ and Ion 314™ chips, and with GeneChip® miRNA arrays.



The PGM™ Sequencer produces better expression data than microarrays

We sequenced miRNAs from human lung and placenta samples on the Ion 314™ and Ion 316™ sequencing chips (3 replicates of lung for both chips, 3 replicates of placenta for Ion 314™ and 2 for Ion 316™) and compared the results with expression profiling experiments conducted on GeneChip® miRNA microarrays (3 replicates per tissue type) and TaqMan® miRNA TLDA cards (2 replicates per tissue type). The number of different miRNA molecules detected with the Ion PGM™ Sequencer is superior to the different molecules detected with either TaqMan® or microarrays (Table 1). This demonstrates that the sensitivity with the Ion 316™ sequencing chip surpasses the sensitivity of microarrays for detecting miRNA species.

The correlation between the LogRatio values obtained with the Ion 316™ sequencing chip and those obtained with the GeneChip® miRNA arrays is high (Figure 2A). LogRatio values obtained with TaqMan® also show very high correlation with the Ion PGM™ values (R=0.86).

LogRatios from the microarray experiments are compressed compared with the values obtained with the Ion PGM™ Sequencer. The digital nature of the Ion PGM™ sequencing data allows for wider dynamic range of LogRatio values compared with the limited dynamic range obtained by microarrays (Figure 2B). While the dynamic range of microarray data is predetermined by the signal intensity thresholds of the systems, the Ion PGM™ sequencing dynamic range is theoretically unlimited and is only a function of the number of reads obtained for that experiment. Compression can also be due to cross-hybridization of isomirs and closely related miRNA species, and the inability of microarrays to discriminate between precursor and mature miRNA molecules.

The Ion PGM™ Sequencer has the ability to detect both mature and star(*) forms for annotated miRNAs (Figure 3). A representative example is illustrated with the analysis of PGM™ sequence data for hsa-mir-126* and hsa-mir-126 sequences. These reads from placenta libraries also show detection of both 5' and 3' isomirs (data not shown). Accurate isomir detection is very difficult using other gene expression technologies.

Figure 3.
Ability of the Ion PGM™ Sequencer to distinguish mature vs. star (*) miRNA forms. The table shows the Log2 value of the Ct for TaqMan®, hybridization signal for microarrays and normalized Log2 values for mapped PGM™ reads.

Platform	hsa-miR-126	hsa-miR-126*
TaqMan®	21.4	26.5
Microarray	15.1	Not Detected
Ion PGM™	**13.2**	**10.9**

The Ion Torrent PGM™ with the Ion Total RNA-Seq Kit developed by Ambion® bring the power of next generation sequencing for RNA expression to every researcher.

- It provides better, more accurate and more comprehensive data than microarrays—the LogRatios are more accurate, closely related transcript species can be distinguished, strand information is preserved, and quantitative digital output is provided.
- It is affordable—the equipment cost is less than most microarray scanners. Unlike other NGS technologies, the Ion PGM™ solution does not require pooling hundreds of samples to achieve cost-effective pricing. The price of sequencing a single sample per chip is affordable and comparable to that of microarrays.
- It is fast—a single day workflow with a sequencing run under 1 hour.
- It is simple—the workflow is easy to perform with limited hands-on-time and the data analysis tools are intuitive and optimized for RNA expression analysis.

The Ion PGM™ Sequencer puts RNA-Seq within the reach of any lab and empowers them with a complete, precise and digital output of individual transcriptomes.

Ordering Infomation

Description	Part No.
Personal Genome Machine	
Ion PGM™ System Includes PGM™ Sequencer (4462917) and Torrent Server (4462918)	4462921
Service Contracts Rapid Exchange Program AB Assurance Plan	 ZGEXSCIONPGMSYS ZG11SCIONPGMSYS
Semiconductor Sequencing Chips	
Ion 314™ Chip Kit (8 Chips)	4462923
Ion 316™ Chip Kit (8 Chips)*	4466616
Reagent Kits	
Ion Total RNA-Seq Kit (12 reactions)	4466666
Ion Xpress™ Template Kit (10 reactions)	4466457
Ion Sequencing Kit (8 reactions)	4466456
Ion Control Materials Kit (3 reactions each per control)	4466465

* The content provided herein may relate to products that have not been officially released and is subject to change without notice.

Contact

In North America
1-87-SEQUENCE (1-877-378-3623)

Outside North America
+1-203-458-8552
www.iontorrent.com

Ion Torrent by Life Technologies | 7000 Shoreline Court | Suite 201 |South San Francisco, CA 94080 USA
Phone +1-203-458-8552 | Toll Free in North America 1-87-SEQUENCE (1-877-378-3623)

www.lifetechnologies.com | www.iontorrent.com | http://ioncommunity.iontorrent.com CO23050 0611

ion torrent

by life technologies™