Whole Genome Analysis of Small RNA

Innovations in Small RNA Analysis — a streamlined workflow for whole-genome discovery, profiling and validation analysis of expression patterns.

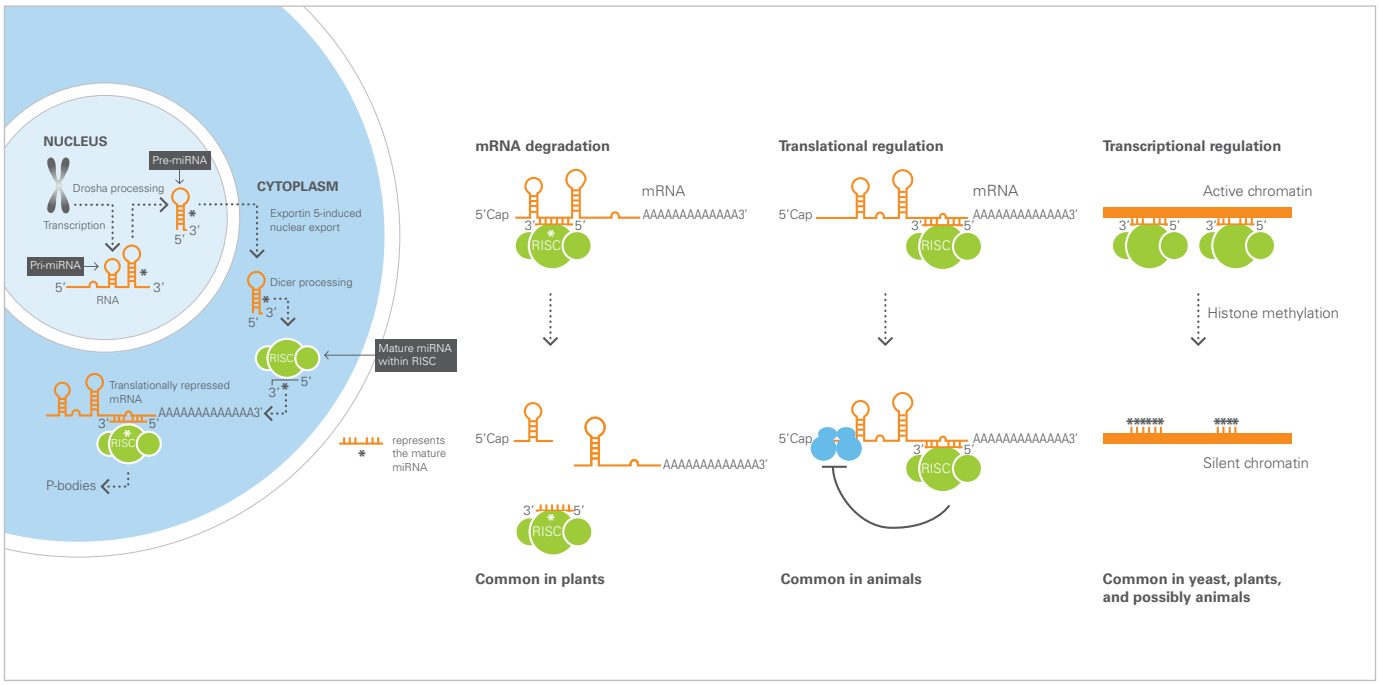

Figure 1. Small RNA — critical regulators of gene regulation. Small RNA regulates gene expression through several mechanisms including; mRNA degradation, transcriptional inhibition and histone modification.

Small Non-coding RNA

Small non-coding RNAs are rapidly gaining recognition as significant effectors of gene regulation in organisms spanning the evolutionary spectrum. Animals, plants, and fungi contain several distinct classes of small RNA, including microRNA (miRNA), short interfering RNA (siRNA), piwi-interacting RNA (piRNA), and repeat associated siRNA RNA (rasiRNA). These molecules are typically only ~18–40 nucleotides in length, however their effect on cellular processes is profound.

Small RNA has been shown to play critical roles in developmental timing, cell fate, tumor progression, neurogenesis, transposon silencing, viral defense, and many other cellular processes. Small RNAs function in gene regulation by binding to their targets and negatively affecting gene expression using mechanisms such as heterochromatin modification, translational inhibition, mRNA decay and even nascent peptide turnover mechanisms.

Analysis of Small Non-coding RNA

Previously, discovery and analysis of small RNA expression was limited by laborious sample preparation methods and targeted detection technology. Array technologies held great promise for global analysis of expression patterns but are biased toward known targets and limited in their sensitivity to detect novel small RNA molecules expressed in low abundance.

Applied Biosystems' Solutions for Small RNA Analysis

Applied Biosystems provides a broad portfolio of products for the discovery, profiling and validation of small RNA. The SOLiD™ RNA Expression Solution is a robust method for hypothesis-neutral, whole-genome discovery analysis of expression patterns without the limitations of microarrays.

Applied Biosystems TaqMan® Assays are the gold standard for quantitation of expression levels and provide a rapid

Small RNA Discovery Workflow

1. RNA Isolation

Description

RNA is isolated from various sample types; including blood, cultured cells, and tissue (fresh/frozen and formalin-fixed paraffin-embedded [FFPE]).

Technical Considerations

- RNA quality is critical for library generation
- Amount of input RNA is reduced for clinical samples

Applied Biosystems' Solutions

MagMAX™-96 for Microarrays Total RNA Isolation Kit → RNA isolation from standard samples

***mir*Vana™ miRNA Isolation Kit** → micro RNA isolation from standard samples

RNAqueous Micro-Kit → Small sample / LCM samples

RecoverAll™ Total Nucleic Acid Isolation Kit (AM1975) → FFPE samples

Leukolock™ Kit Total RNA Isolation → Human blood

Benefits of Applied Biosystems' Solutions

- Applied Biosystems provides multiple kits for RNA purification that support isolation from a variety of sample types including whole blood, FFPE and LCM
- RNA isolation kits enable the generation of quality RNA from various sources

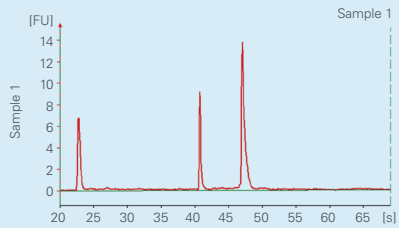

Figure 4: High quality RNA isolated with MagMAX™-96 for Microarrays Total RNA Isolation Kit: K652 cells were harvested and re-suspended in RNALater™. RNA was then isolated using the MagMAX-96 Total RNA protocol. 28s/18s ratios of >1.8 and RIN (RNA integrity number) of >9.9 were obtained for all samples.

2. Library Prep

cDNA is generated by reverse transcription from adaptors ligated to ends of the small RNA molecule. cDNA is then amplified, using primers complementary to adaptors, and purified.

- Current protocols are labor intensive — 4–5 days with multiple purification steps
- Current protocols are technically challenging — requiring highly trained technicians — and give highly variable results
- High input RNA amounts of 10ug limit ability to analyze clinical samples

SOLiD™ Small RNA Expression Kit

- Streamlined protocol — one day library prep
- Simplified workflow — 5 easy steps with a single purification
- 1-500ng input RNA

TABLE 1: Comparison of standard protocols for small RNA library preparation.

	Bartel Method	SOLiD™ Small RNA Kit
# Days	5	1
Input RNA	10ug	1-500ng
Purification Steps Bartel	4–5	1

3. Discovery

cDNA is amplified and sequenced. Sequence is then mapped back to reference genome sequences. Relative expression levels are calculated based on the number of tags.

- Clonal and stochastic amplification
- Ability to discover novel small RNA
- Sensitivity to detect small RNA present at levels less than 1 copy per cell

SOLiD™ System

SOLiD™ ePCR Kit

Thermal Cyclers

- The SOLiD™ System provides a global method for the discovery of small RNA without the limitations of microarrays
- The SOLiD™ System generates greater than 120M tags per slide or 240M tags per run, providing the sensitivity to detect RNA present in low abundance

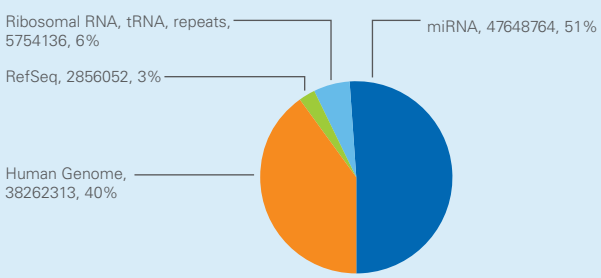

Figure 5: Distribution of 94M reads from a single slide on the SOLiD Analyzer. 40% sequence reads mapped to the human genome likely contain novel, uncharacterized small RNAs.

4. Expression Profiling

Quantitative analysis of differential expression patterns to identify specific profiles which correlate to a particular phenotype.

- Sensitivity to detect 1 copy per cell
- Dynamic range of 5 logs or greater
- Number of RNA species analyzed and number of samples

TaqMan® MicroRNA Arrays

SOLiD™ System

Applied Biosystems microRNA primer pool products

- TaqMan® Arrays provide a **rapid, simplified workflow** for **profiling known miRNA** species
- The SOLiD™ System provides a **highly sensitive method for profiling known and unknown** small RNA

Arrays	SOLiD	TaqMan
10^2-10^3 fold	10^5-10^6 fold	10^7-10^8 fold

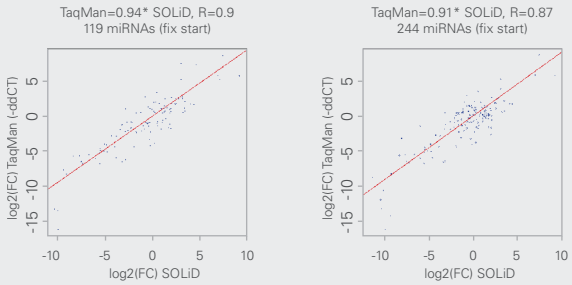

Figure 6: SOLiD™ System and TaqMan data compared across 244 miRNA. Correlation increased further by restricting analysis to microRNA with significant differential expression.

5. Validation

Validation of the presence of a particular small RNA molecule in system.

- Sensitivity to detect 1 copy per cell
- Dynamic range of up to 7 logs
- Number of RNA species analyzed and number of samples

TaqMan® MicroRNA Assays

Custom TaqMan® Small RNA Assays (Early Access)

Real-Time PCR Instrumentation

- Gold standard validation of SOLiD results
- Highly specific — quantitate only the biologically active mature miRNAs
- Fast, simple and scalable

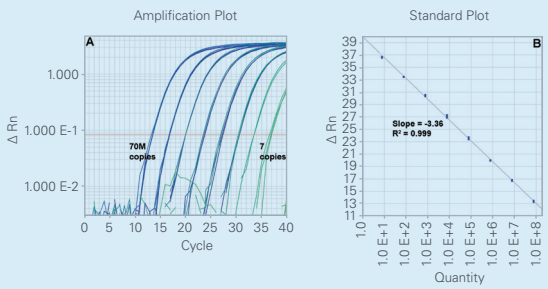

Figure 7: Dynamic range and sensitivity of the TaqMan lin-4 miRNA assay. (A) Amplification plot over seven orders of magnitude. Synthetic RNA input ranged from 7 to 70,000,000 copies in PCR; (B) Standard curve.

method of profiling or validating known sets of small RNA. Applied Biosystems also provides a comprehensive line of products for RNAi which will not be discussed in the context of this workflow. For more information about these products, contact your local representative, or visit **www.ambion.com/rnai**

The SOLiD™ Small RNA Expression Kit provides a streamlined workflow that greatly reduces the time, cost and experimental variability associated with library preparation. Researchers may now generate small RNA libraries in a single day with a simple, easy to use protocol (Figure 3). The SOLiD™ Small RNA Expression Kit converts RNA into a library suitable for emulsion PCR in 5 easy steps with a single purification. This simplified protocol results in a reduction in experimental variability and improves the detection of biologically relevant changes. The SOLiD™ System generates greater than 100M mappable reads per slide to deliver the dynamic range required to detect changes in molecules present at less than 1 copy per cell. Together with the SOLiD™ Small RNA Expression Kit, Applied Biosystems provides an optimized solution for small RNA analysis that expands the boundaries of conventional research.

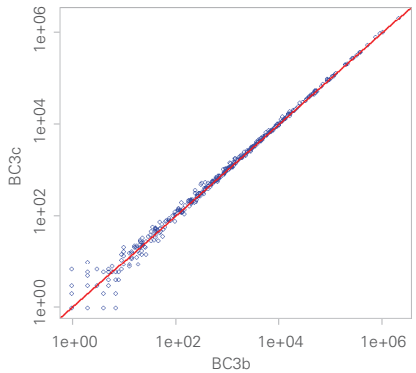

Figure 2. The SOLiD™ System provides a highly reproducible method for gene expression analysis with a dynamic range of 5-6 logs. A single small RNA library was amplified by ePCR and deposited on to 2 separate slide segments and sequenced in a single run on the SOLiD™ Analyzer.

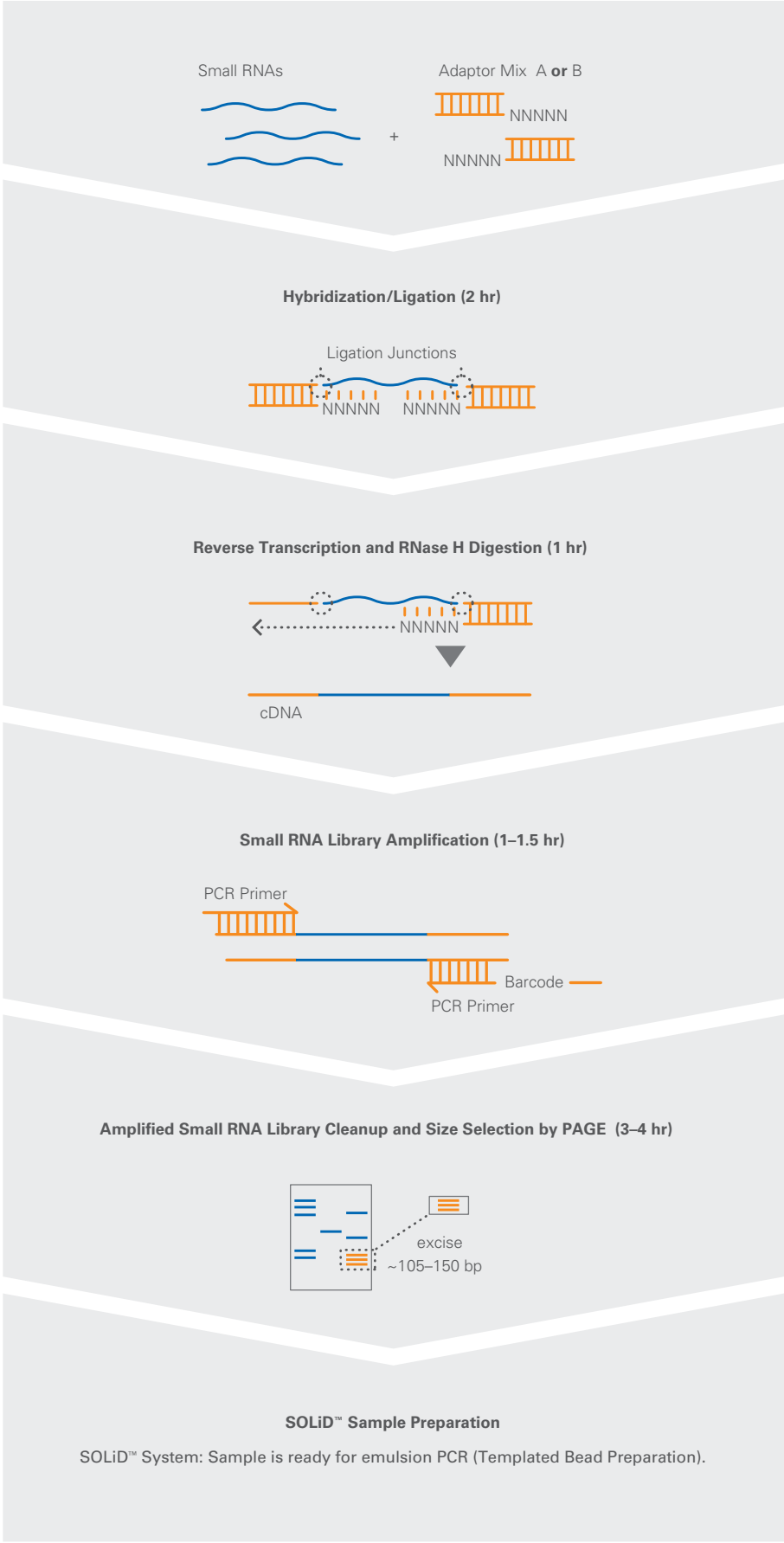

Figure 3. Overview of SOLiD™ Small RNA Expression Kit Procedure.

PRODUCT ORDERING INFORMATION

Description	P/N
Sample Prep	
MELT™ Total Nucleic Acid Isolation System	AM1983
RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE	AM1975
*mir*VANA™ miRNA Isolation Kit	AM1560
RNAqueous Micro Kit (LCM sample)	AM1931
Leukolock™ Kit Total RNA Isolation (Human blood)	AM1933
Library Prep	
SOLiD™ Small RNA Expression Kit	Inquire
Discovery/ Profiling	
SOLiD™ System	4387830
SOLiD™ ePCR Kit V2	4400834
SOLiD™ Bead Enrichment Kit	4387894
SOLiD™ Bead Deposition Kit	4387895
SOLiD™ Slide Kit	4391888
SOLiD™ Buffer Kit	4387918
SOLiD™ Instrument Buffer Kit	4387919
SOLiD™ Sequencing Probes Kit V2	4400412
SOLiD™ Fragment Library Sequencing Kit V2	4400467
Profiling and Validation	
TaqMan® MicroRNA Assays	mirna.appliedbiosystems.com
Custom TaqMan® Small RNA Assays (Early Access)	Contact your local sales representative

Applied Biosystems

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