NuGEN's RNA-Seq Portfolio

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Workflow Solutions for NextGen Sequencing

Library Construction

Ovation Ultralow Methyl-Seq Library System
Ovation RRBS Methyl-Seq System

Ovation Ultralow Library System V2

Ovation Rapid Library Systems (PCR Free)

Ovation Low Complexity System (Amplicon-seq)

Custom Target Enrichment including library preparation

Ovation Custom Target Enrichment System

Ovation Cancer Panel 2.0

Ovation human Mitochondrial Target Enrichment System

Ovation Fusion Panel

cDNA Generation

Ovation RNA-Seq System V2

Ovation FFPE RNA-Seq System

Strand Specific RNA-Seq Complete Workflow

Ovation Universal RNA-seq MultiplexSystem
Ovation Human Blood RNA-Seq MultiplexSystems
Ovation Human FFPE RNA-Seq MultiplexSystems
Ovation Mouse RNA-Seq System
Ovation Drosophila RNA-Seq System
Ovation Rat RNA-Seq System
Ovation Arabidopsis RNA-Seq System
Ovation Complete Prokaryotic RNA-Seq Systems

New! Ovation SoLo RNA-Seq System (single cell and ultralow RNA input)

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RNA-Seq Portfolio Consists of 4 Major Product

	RNA-Seq v2	Universal RNA-Seq	SoLo RNA-Seq	Fusion (Targeted RNA-Seq)
Technology	- SPIA	- InDA-C	- InDA-C	- Target Enrichment
Product	- Sample to cDNA - Whole transcriptome or poly (A) selected RNA	- Sample to library - Whole transcriptome, stranded - Customizable for targeted depletion	- Sample to library - Whole transcriptome, stranded - Customizable for targeted depletion	- Sample to library - Targeted RNA-Seq - Customizable for target enrichment
Applications	- Low to medium input samples, FFPE	- Medium to high input samples	- Ultralow input samples, single cells, liquid biopsy, FFPE	- Biomarker discovery and diagnostics
Competition	- Many	- Roche (Kapa), Illumina, Clontech	- Clontech, homebrew DropSeq	- Archer, Illumina, Nanostring

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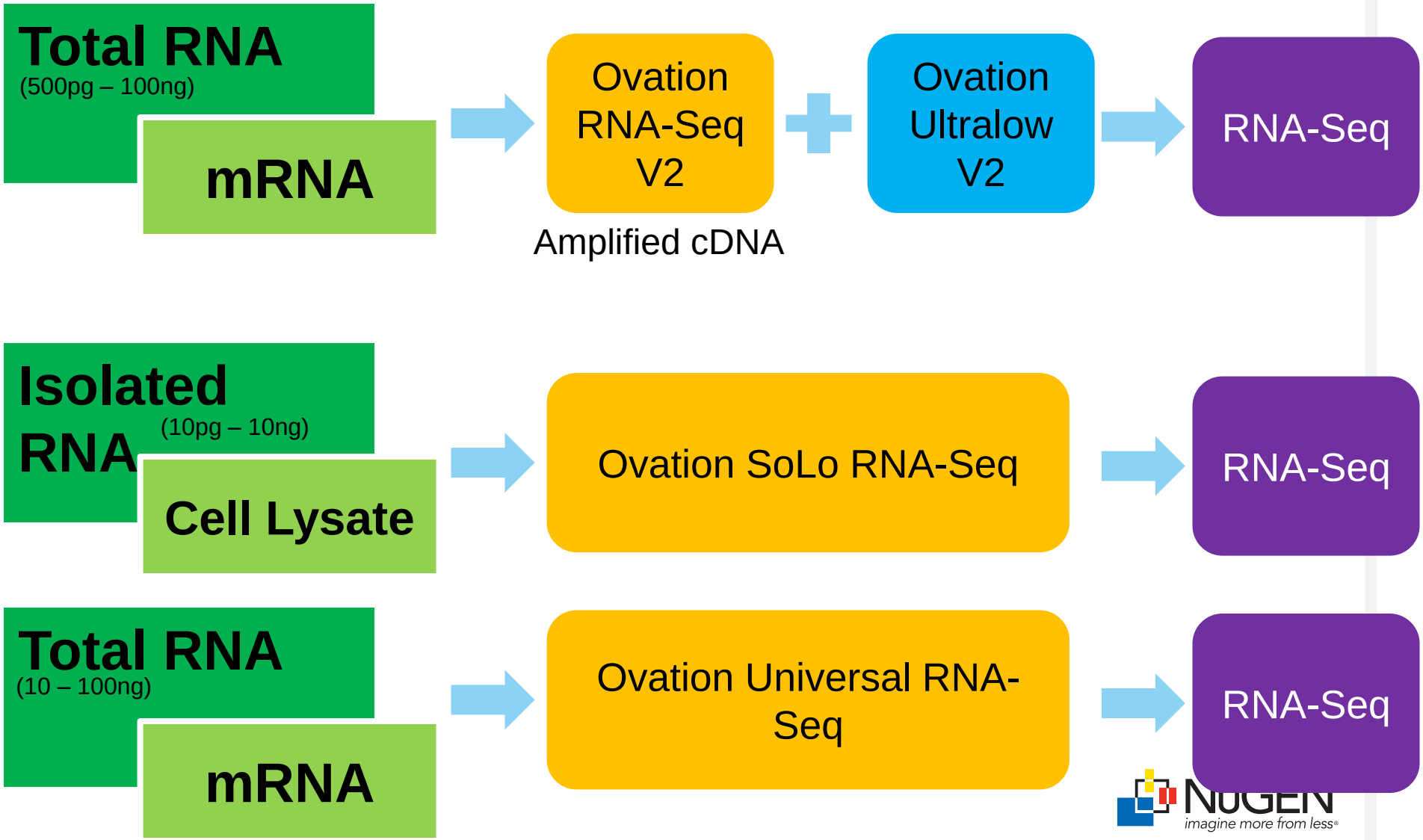
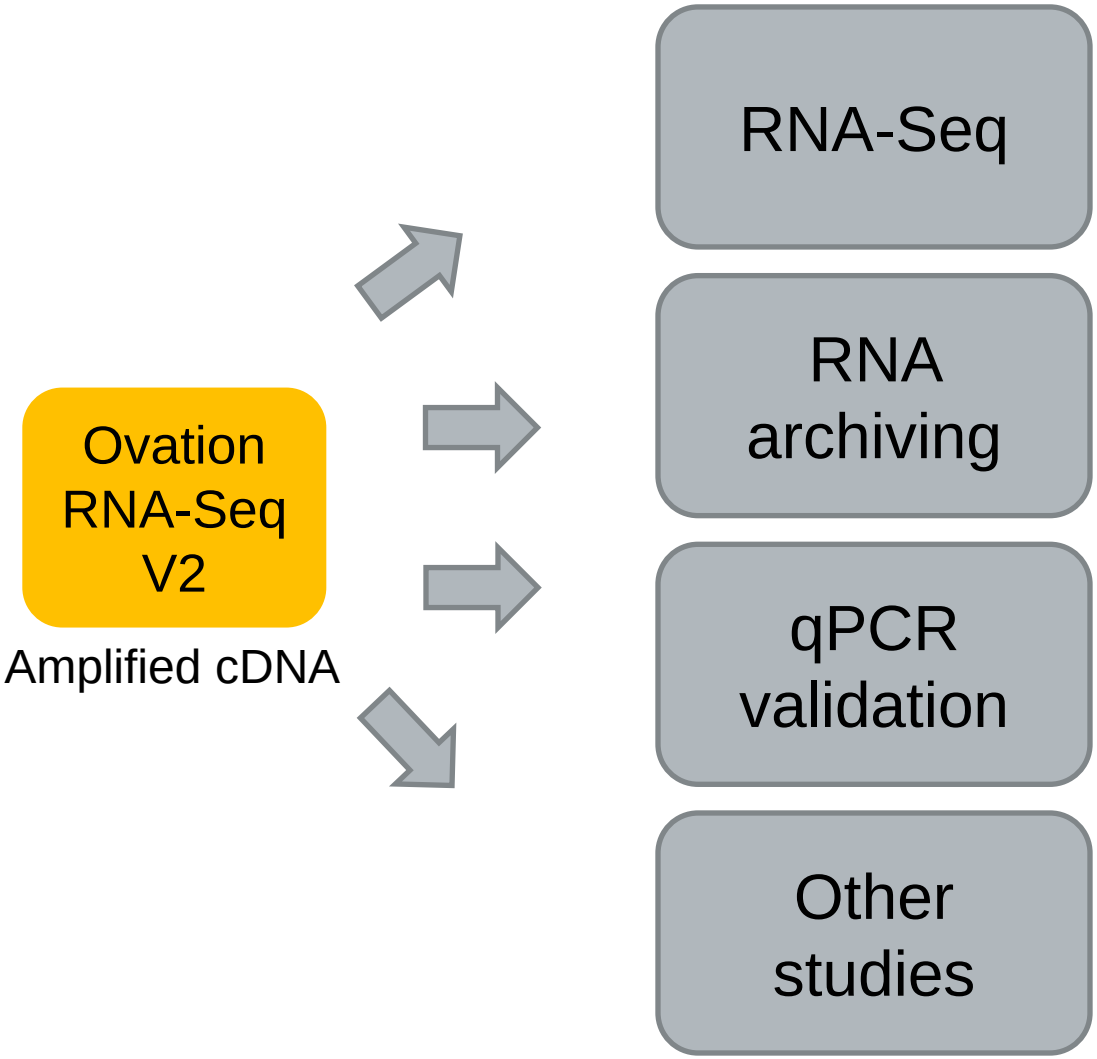
Comparison RNA-Seq V2 vs SoLo vs Universal



Ovation RNA-Seq V2

- The gold standard in RNA amplification with hundreds of peer-reviewed publications
  - **Low RNA input requirements:** Reproducible amplification from 500pg – 100ng of RNA
  - **Complete transcriptome representation**
  - **Versatile:** Amplified cDNA compatible with a range of library preparation systems, including Ovation Ultralow V2

Ovation RNA-Seq V2



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Ovation RNA-Seq V2

Customer Bottleneck	Key Features	Customer Benefits
Limited amount of material for studies	Low RNA input (500 pg – 100 ng)	Allow interrogation of a wide range of sample types including degraded samples.
Require whole transcript or transcriptome analysis	Random and Oligo(T) priming	Complete biology from total RNA or mRNA
Inability to perform multiple experiments on limited sample	Micrograms of amplified cDNA	Sample available for multiple applications (qPCR, RNA archiving, orthogonal experiments)
Long, complicated workflows with high failure rates	Automatable, robust workflow with hundreds of publications	It works!; Amenable to high throughput on a variety of platforms (Agilent Bravo, Perkin Elmer Sciclone, Beckman BiomekFx, Hamilton Starlet)

Remember:

- No rRNA depletion!
- No poly(A) selection module included!

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Case Studies – Viral Pathogen Detection

Problem: Encephalitis is a severe neurological syndrome where the causative agent remains unidentified in the majority of cases due to the limited sensitivity and specificity of currently used diagnostic assays.

Method: Use Ovation RNA-Seq V2 for RNA amplification followed by NGS to detect putative viral pathogens. Libraries prepared with the Nextera XT system.

Results: The use of RNA amplification (Ovation RNA-Seq V2) lead to the identification of HHV-1 in CSF from a patient with acute encephalitis at a time when specific antibodies could not yet be detected.

Perlejewski, et. al., Next-generation sequencing (NGS) in the identification of encephalitis-causing viruses: Unexpected detection of human herpesvirus 1 while searching for RNA pathogens . *Journal of Virological Methods*. 2015

Case Studies – Viral Pathogen Sequencing

Problem: The Seoul virus (genus Hantavirus, family Bunyaviridae) is an emerging pathogen associated with hemorrhagic fever and acute kidney injury. The full genomic sequence is not known.

Method: Use Ovation RNA-Seq V2 for RNA amplification followed by NGS to determine the viral genomic sequence. Libraries constructed with the Nextera XT system.

Results: The sequencing results provided 99.9% genome coverage allowing production of a consensus genome sequence.

Miles, et. al., Complete Genome Sequence of Seoul Virus Strain Tchoupitoulas. *Genome Announcements*. 2016

Case Studies – Astrocyte-specific Contributions to Alzheimer's Disease

Problem: Alzheimer's disease is characterized by deficits in cerebral metabolic rates of glucose in the posterior cingulate. Astrocytes have a role in energy storage and brain immunity. Characterization of astrocytes from the posterior cingulate of Alzheimer's disease can help understand this disease.

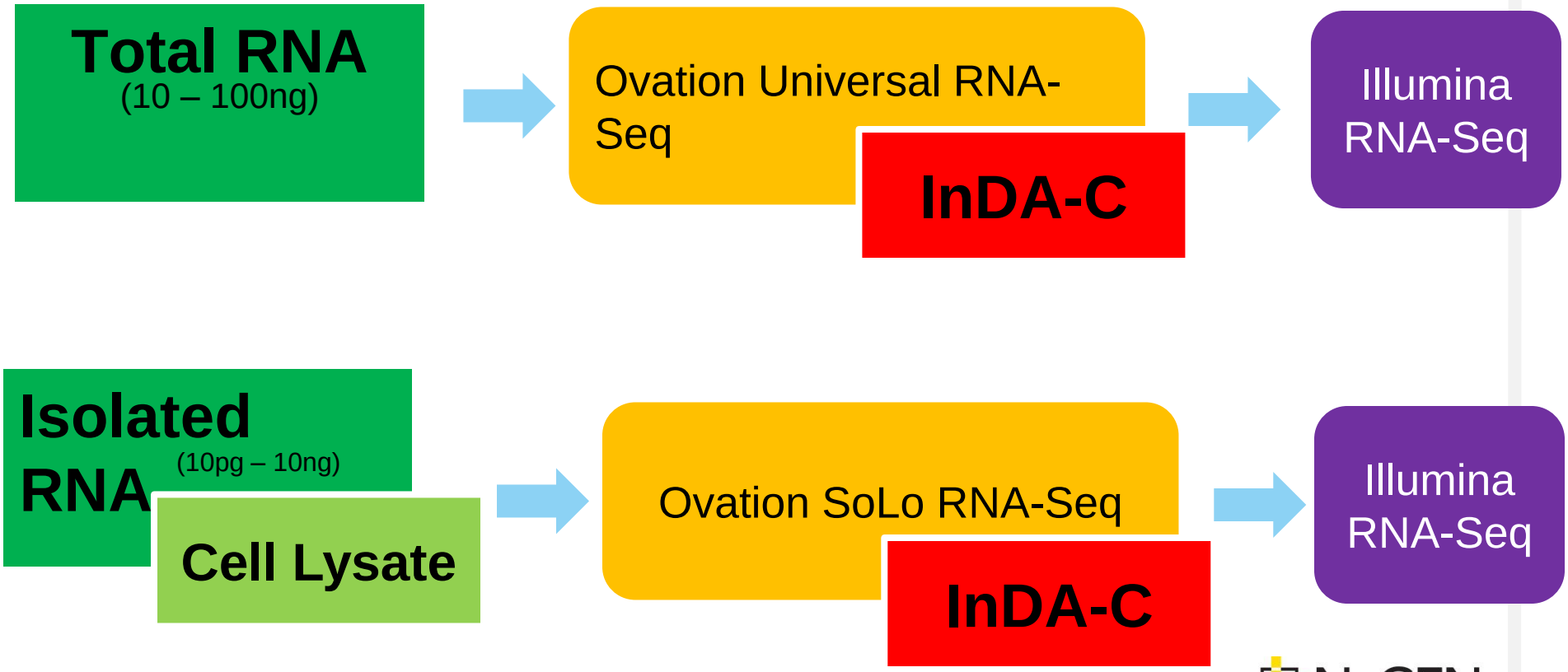
Method: Astrocytes were extracted by laser capture microdissection (LCM) from brain samples. RNA was isolated with the Arcturus PicoPure RNA Isolation Kit and amplified with the Ovation RNA-Seq V2 system. Libraries were constructed with the Illumina TruSeq v2 system.

Results: Identified differentially expressed immune response genes, a number of which have been implicated in beta amyloid generation or clearance.

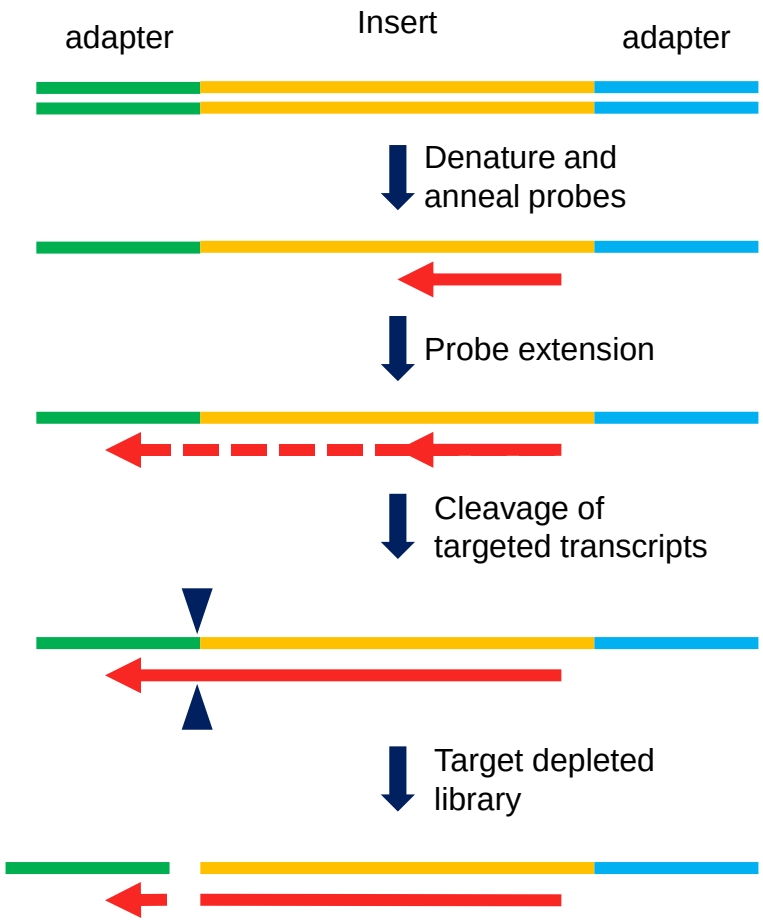
Sekar, et. al., Alzheimer's disease is associated with altered expression of genes involved in immune response and mitochondrial processes in astrocytes. *Neurobiology of Aging*. 2015

The "InDA-C" Product Line

RNA-Seq workflows with targeted depletion of <u>any</u> transcript from <u>any</u> organism



Benefits of the InDA-C approach



- Enriches for desired transcripts by targeted elimination of unwanted sequences *following library construction*
- Original total RNA population is not perturbed as with hybridization capture methods
- InDA-C primer design is specific to targeted transcript
- Custom designs to target virtually any class of unwanted transcripts from any species

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Ovation Universal RNA-Seq System

- Standard to low RNA input
- Whole transcriptome representation
- Strand Specificity
- Reduced Sequencing Costs:
  - 96 unique barcode adaptors for multiplex sequencing
  - Transcript Depletion: Targeted depletion of unwanted transcripts
- Customizable transcript depletion

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Ovation Universal RNA-Seq System Benefits

Customer Bottleneck	Key Features	Customer Benefits
Multiple projects with different input amounts, quality and organism	Range of sample inputs with customized transcript depletion	Single workflow for variable samples reducing experiment-to-experiment variability
High cost of sequencing	Reduced sequencing costs	Remove uninformative reads to reduce sequencing costs and simplify data analysis
Long, complicated workflow	Simple one day workflow	Get results faster while minimizing potential errors
Need multiple kits for a complete solution	Complete system with no additional reagents needed; modular transcript depletion (coming soon)	No additional kits to purchase and maintain

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Case Studies – Multi-Organism Study

Problem: Chemokines and their receptors are involved in oncogenesis and in tumor progression, invasion, and metastasis. The role of these chemokines in alcoholic hepatitis is not well understood.

Method: Use Ovation Human FFPE RNA-Seq System to study this disease in human samples and mouse model. Allowed use of a single workflow for different organism samples with a simple change in the InDA-C probes.

Results: Gained insight into the mechanism of alcoholic hepatitis mediated by chemokine signaling.

Liu, et. al., IL-8 signaling is up-regulated in alcoholic hepatitis and DDC fed mice with Mallory Denk Bodies (MDBs) present. *Experimental and Molecular Pathology*. 2015

Case Study – Genome Transcription

Problem: Transcription of eukaryotic genomes produces protein-coding mRNAs and diverse noncoding RNAs (ncRNAs), including enhancer RNAs (eRNAs) that are rapidly degraded, difficult to detect, and thus far have not been mappable. Mapping of transient RNAs is required, however, for analysis of RNA sequence, function, and fate.

Method: Use Ovation Human Blood RNA-Seq System to develop a new method to study transcription of transient transcripts. Utilize ability of the kit to access low input and fragmented RNA along with rRNA depletion.

Results: Gained novel insight into transient transcripts generated during human genome transcription.

Schwalb, et. al., TT-seq maps the human transient transcriptome. *Science*. 2016

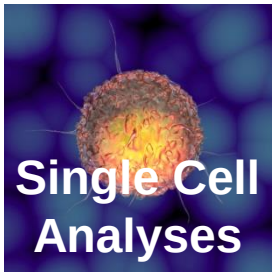
Ovation SoLo RNA-Seq System

- Ultralow RNA input
- Whole transcriptome representation
- Strand Specificity
- Reduced Sequencing Costs:
  - 96 unique barcode adaptors for multiplex sequencing (192 available BTO)
  - Transcript Depletion: Targeted depletion of unwanted transcripts
- Customizable transcript depletion
- N8 molecular tag to identify unique molecules

Ovation SoLo RNA-Seq System Benefits

Customer Bottleneck	Key Features	Customer Benefits
Inconsistent performance at ultra low input range or single cell level	High efficiency library construction	Excellent gene representation from 1-500 cells or 10pg – 10 ng of purified RNA
Input limited to isolated RNA	Direct integration with cell lysis protocol in addition to purified RNA	Supports multiple sample types
High signal-to-noise ratio at the ultra-low input ranges	Improved depletion method and primer design for ribosomal depletion of 98%; can be applied to other transcripts	Superior signal-to-noise ratio for better sensitivity and data quality. Ability to customize for any gene, any genome, any organism
Accurate representation of biological data	Analyses of whole transcriptome with option of data normalization with N8	More accurate and true representation of biological data

Where is Ovation SoLo RNA-Seq System Applicable?

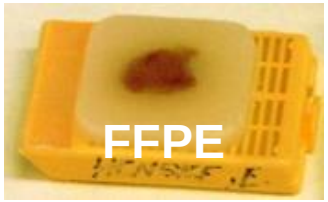


Analyses of

- Cellular heterogeneity (oncology, immunology, stem cells, neuroscience)
- Single cell transcriptomics

Analyses of

- Circulating tumor cells
- Cell-free nucleic acids
- Exosomes



Analyses of

- Compromised and/or archived samples
- Fragmented nucleic acids

Case Study – Nuclear RNA Profiling in Neurons

Customer: An academic group – name confidential

Problem: It is difficult to isolate whole neuron cells for transcriptome profiling, so researchers in the field are attempting to study neurons by profiling nuclear RNA instead. This customer previously tested out Clontech kits but was unsuccessful.

Method: Isolate RNA from mouse neuronal nuclei, which have about 1/10 the RNA of the whole cell. Tested 2.5 and 5 ng inputs.

Current progress: Customer has done one experiment and is analyzing the data. Interestingly, they found an unusually high number of transcripts in their analysis. Initially there was concern that the intron content of the data is somewhat high while intergenic sequence is also a bit higher than expected and rRNA content is very low. However, the strandedness of the data is very high (97-99%), indicating that the data is transcript derived. They are exploring new ways to analyze this kind of data. Is planning to repeat experiments with lower input.

Case Study – Urine RNA Profiling for Biomarker Detection

Customer: An academic group – name confidential

Problem: Customer is looking for new diagnostic markers in urine samples. However, it is a challenge to obtain enough RNA at a high enough quality for most available kits. Poly (A) based low input kits are not a good option since they don't capture all of the different extracellular RNA in the sample.

Method: Isolate RNA using the Norgen Biotek urine RNA kit.

Current progress: Customer has completed one round of sequencing and is analyzing data. Had some concerns with slightly low exon content and low alignment, but early analysis indicates these sequences are derived from extracellular and ncRNA as well as (expected) pathogens present in the samples.

Case Study – Exosome Sequencing

Customer: An academic group – name confidential

Problem: Liquid biopsy (exosomes) for cancer marker detection

Method: Isolated exosomes using SBI ExoQuick. Total RNA from exosomes used in workflow. Human InDA-C module selected for depletion.

Current progress: Customer has done first pass sequencing and strandedness and rRNA depletion is good. They had issues with alignment (caused by contaminating sequences). Sent in house universal RNA control for them to test out.

Determining the Best Solution for a Customer

	RNA-Seq V2	Universal	SoLo
Input	**Low input** (500 pg – 100 ng)	**Standard input** (10 – 100 ng)	**Ultralow input** (10 pg – 10 ng; cell lysate)
Degraded samples	✓	✓	✓
Strandedness	—	✓	✓
Transcript depletion	—	✓	✓
Automation	✓	✓	—
Alternative library construction	✓	—	—

Questions?

THANK YOU!