LEXOGEN

- Founded in 2007
- HQ in Vienna, Austria
- Lexogen, Inc., NH, the USA
- Multinational team
- Products & Services

Developing **innovative technologies** that will allow to **resolve** the **complexity** of the **transcriptome**

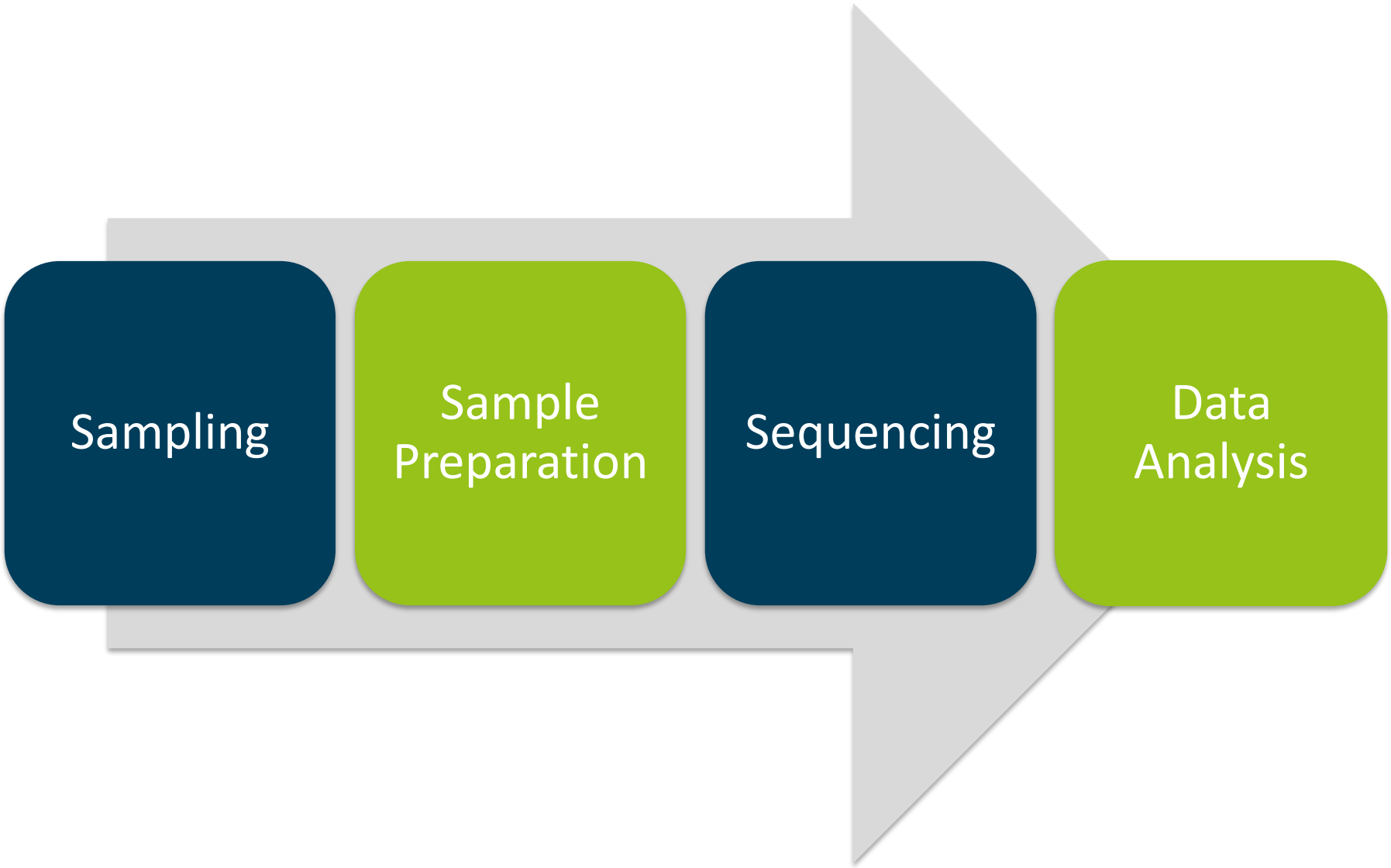
100%

RNA Sequencing Tools

www.lexogen.com

Enabling Complete Transcriptome Sequencing

RNA-Seq project pipeline



Challenges in RNA-Seq library prep

- Amount of input material

- RNA quality
  - High Quality
  - Low Quality (FFPE)

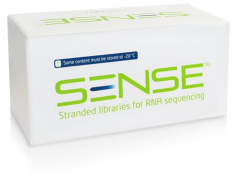
- Ribosomal RNA elimination

- Strand-specificity

Challenges in RNA-Seq library prep

- Reproducibility 

All Lexogen Kits
- Time of the library prep 

- Library complexity 

- Bioinformatics 

PRODUCT PORTFOLIO IN THE RNA-SEQ WORKFLOW

Sampling	RNA Preparation	NGS Library Prep	NGS Data Analysis
SLAMseq Metabolic RNA Labeling Kit » Explorer Modules » Kinetics Modules	**SPLIT RNA Extraction Kit**	**Expression Profiling** » QuantSeq 3′ mRNA-Seq Library Prep Kits » QuantSeq-Flex Targeted RNA-Seq Library Prep Kit	**QuantSeq Data Analysis** » QuantSeq Pipeline on Blue-bee® Genomics Platform » SLAMdunk Pipeline for QuantSeq-SLAMseq Data on Bluebee® Genomics Platform » QuantSeq Pipeline in Partek® Flow®
	RiboCop rRNA Depletion Kit	**Whole Transcriptome** » SENSE mRNA-Seq Library Prep Kit » CORALL Total RNA-Seq Library Prep Kit	**Mix² RNA-Seq Data Analysis Software**
	Poly(A) RNA Selection Kit	**Small RNA-Seq Library Prep Kit**	
	cDNA Preparation		
	TeloPrime Full-Length cDNA Amplification Kit V2		
	NGS Controls		
	Spike-in RNA Controls » SIRV-Set 1 (Iso Mix E0, E1, E2) » SIRV-Set 2 (Iso Mix E0) » SIRV-Set 3 (Iso Mix E0 / ERCC)		**SIRV Suite Spike-In Analysis Software**
Services			
Library Preparation » QuantSeq 3′ mRNA-Seq » QuantSeq on SLAMseq RNA » QuantSeq on Blood-RNA (Globin Block) » Small RNA-Seq	**Automation and Sequencing**		**Data Analysis and Reporting**

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Lexogen's solution for gene expression profiling

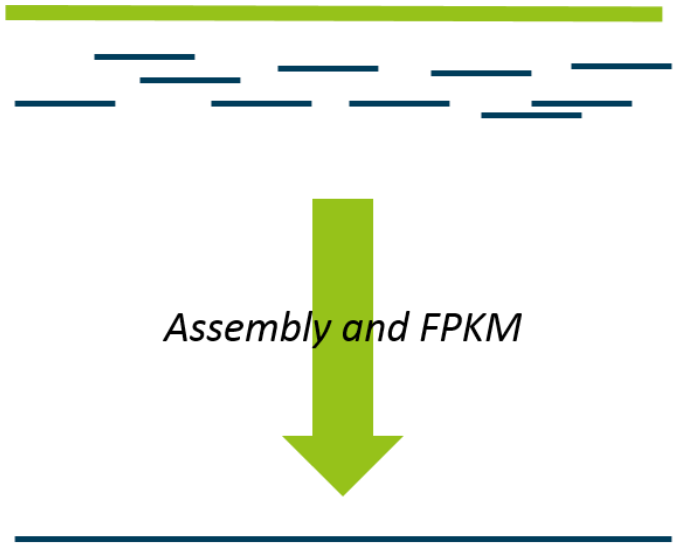
QUANT™ SEQ
Sequencing that counts

High-throughput version
(384 samples, 4x96 barcodes)

Two major types of RNA-Seq – which strategy?

Whole Transcriptome Sequencing

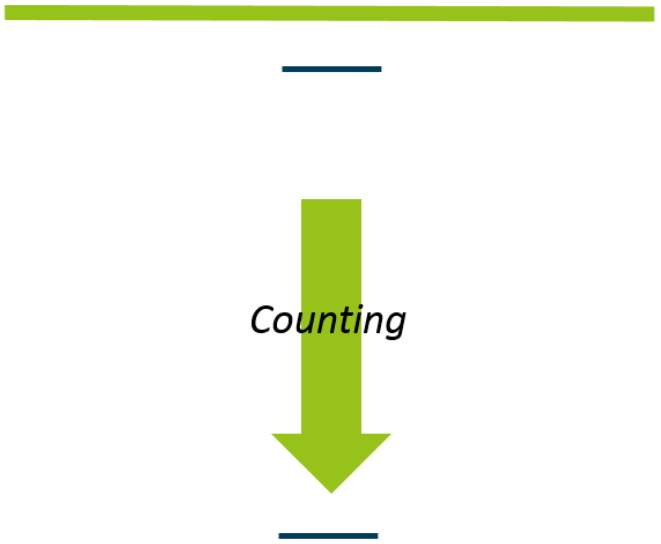
- mRNA or total RNA-Seq
- *de novo* assembly of transcripts
- Isoform detection
- Expression profiling (RPKM needed)



Multiple fragments per transcript

Expression Profiling Sequencing

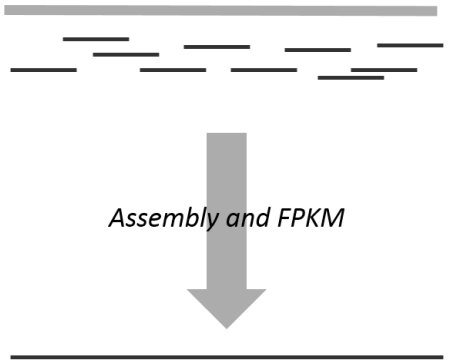
- mRNA
- Counting the number of sequences
- Sequencing one fragment per transcript



One fragment per transcript

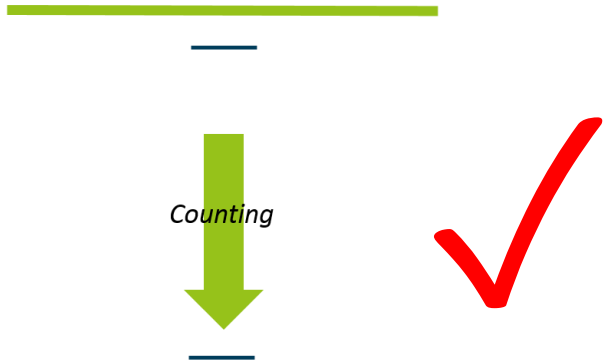
Which RNA-Seq Experiment Strategy?

Whole Transcriptome Sequencing



Multiple fragments per transcript

Expression Profiling Sequencing

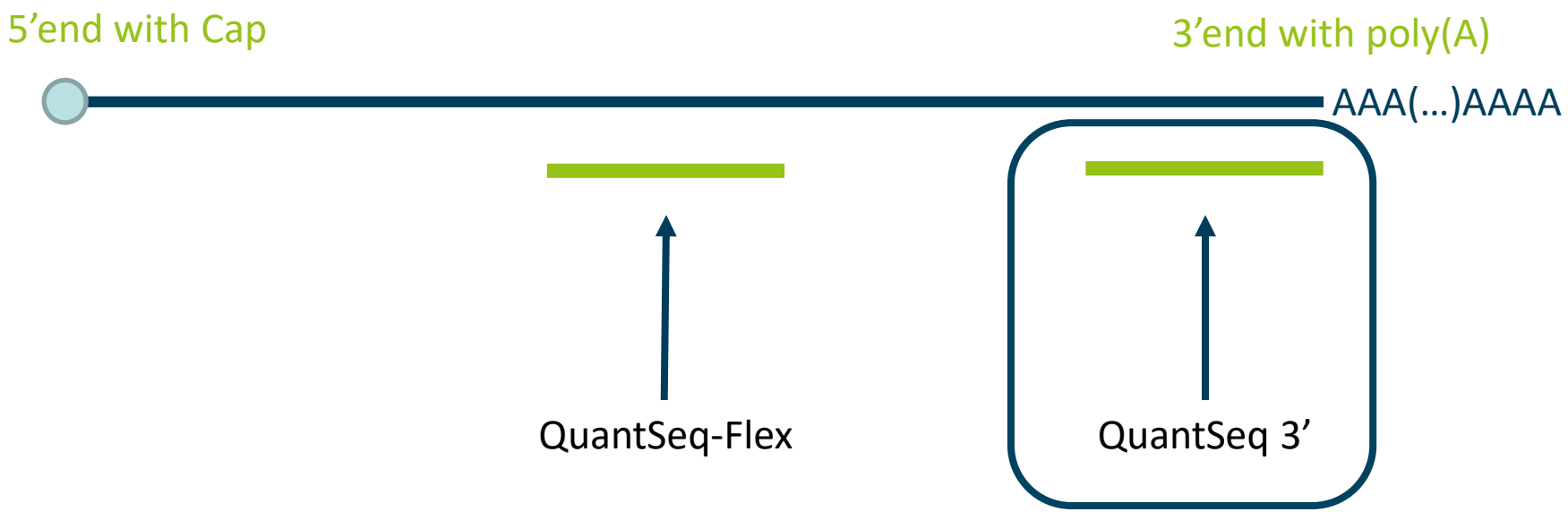


One fragment per transcript

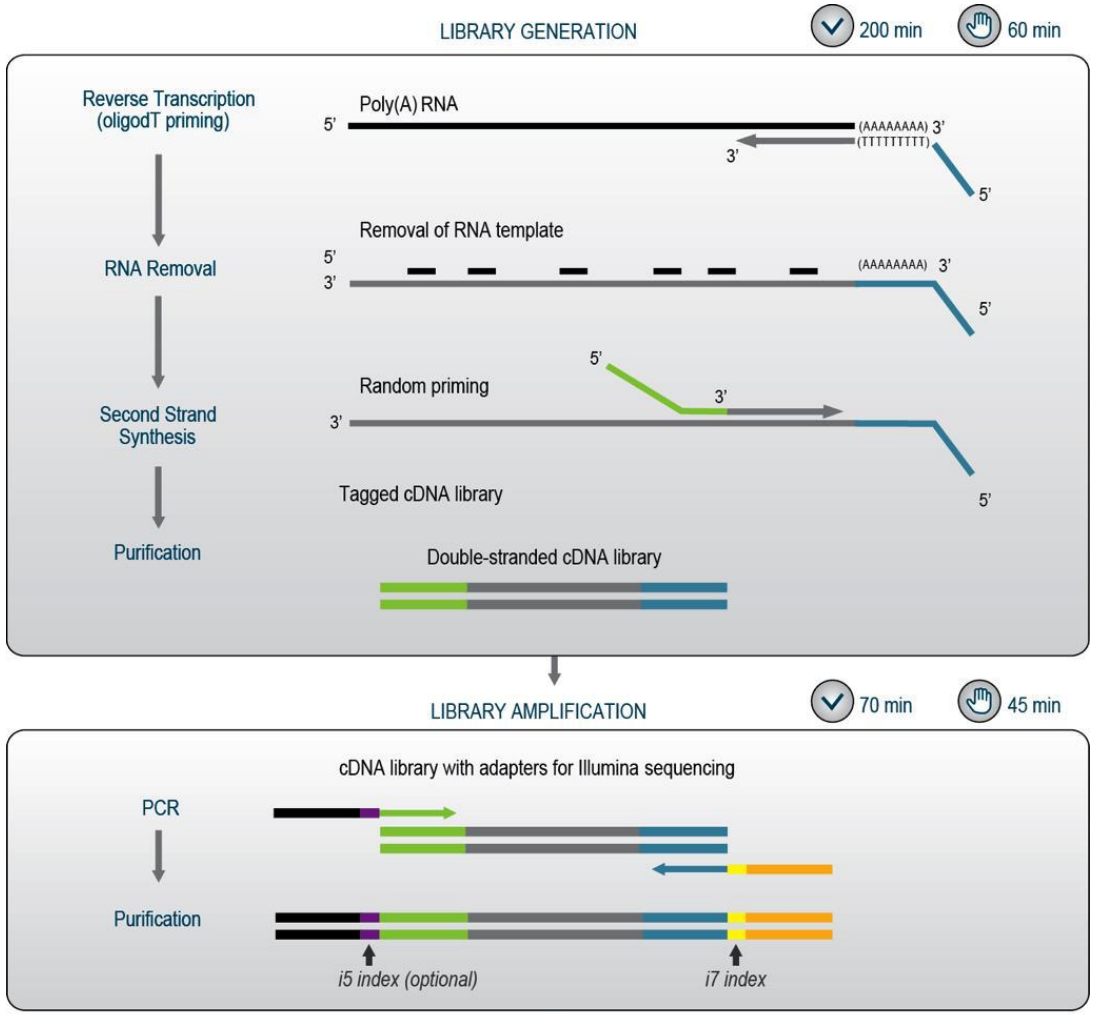
Only 1 read for 1 transcript is enough → higher multiplexing is possible, less computational effort → cheaper and faster

2 types of QuantSeq

- QuantSeq 3’mRNA-Seq Library Prep Kit
- QuantSeq-Flex Targeted mRNA-Seq Library Prep Kit



QuantSeq Workflow



From 100 pg total RNA input
4.5 hrs library prep time, 2 hrs hands-on time
barcodes available: 96x96 (all unique)

QuantSeq 3’ FWD and REV

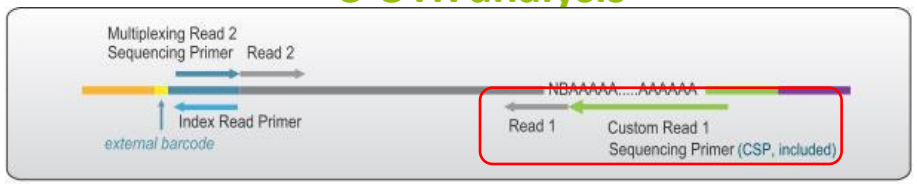
Cat.No. 015 (FWD):

Gene expression analysis

- NGS reads will be generated **towards** the **poly(A) tail**
- Read 1 sequencing linker is located in **5´part of the second strand synthesis primer**

Cat.No. 016 (REV):

3’UTR analysis



- NGS reads start **directly at the 3’end** of transcripts
- Read 1 sequencing linker is located on the **5’end of the oligodT primer**
- **Custom Sequencing Primer** (CSP) covers poly(T) stretch and replaces Multiplexing Read 1 Sequencing Primer - Information must be provided to sequencing facility along with the CSP (see UG for more information)!
- Recommended for **PE sequencing**

Strand-specific mapping of 3’ ends

- ✓ QuantSeq covers the very 3’ end and saves more than 90 % sequencing depth while still determining gene expression accurately.

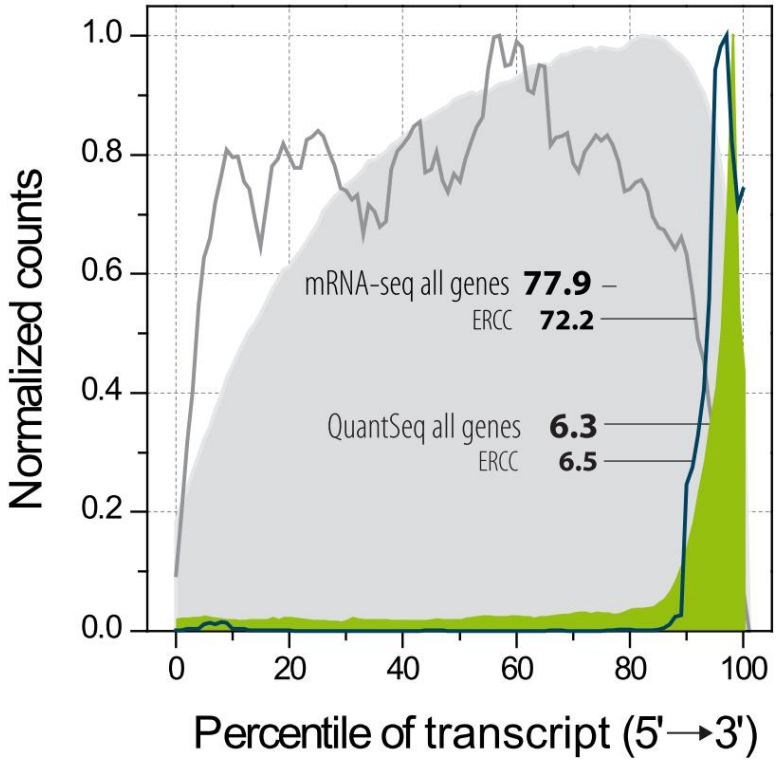
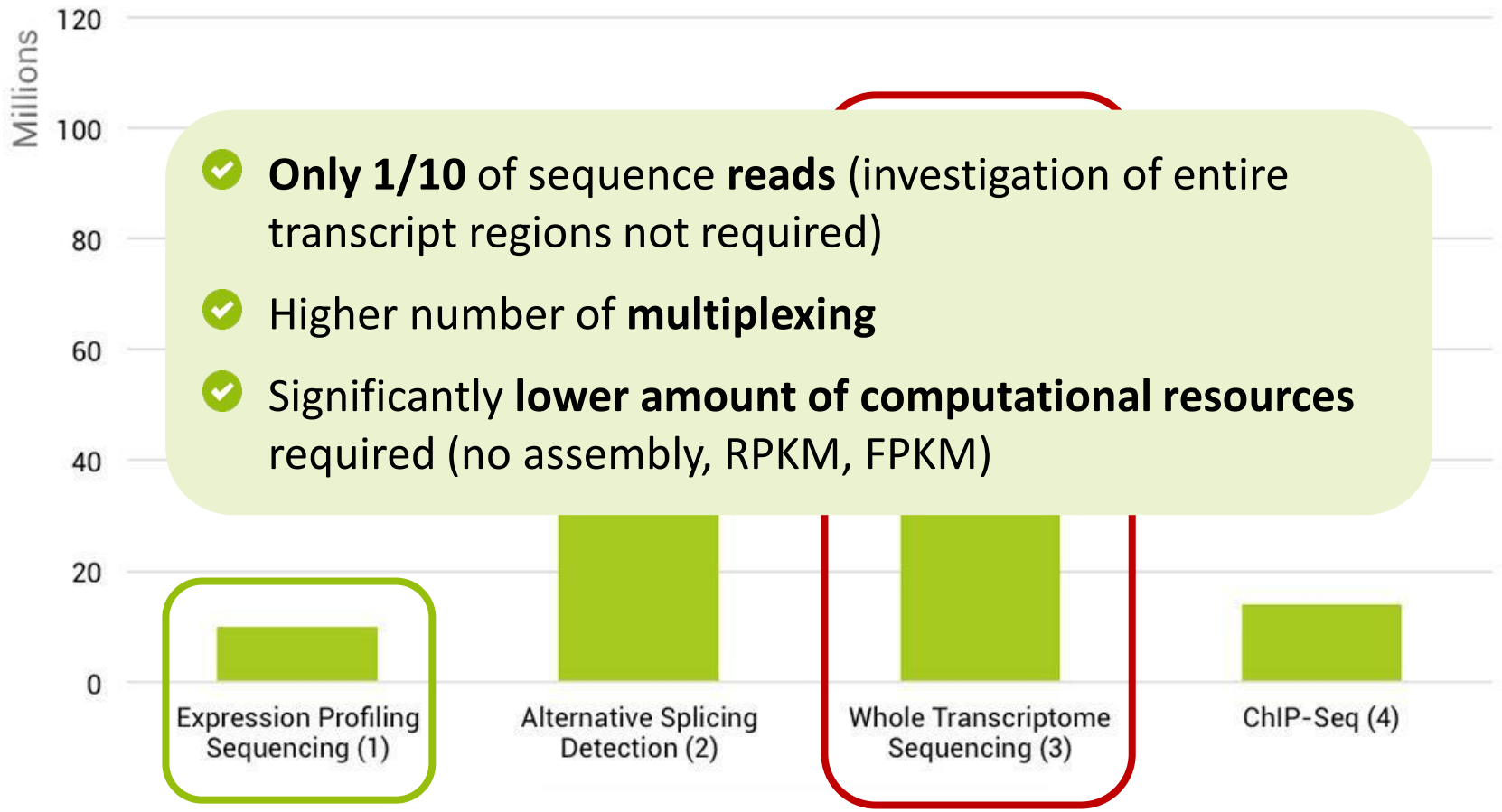


Figure | Coverage versus normalized transcript length in QuantSeq and standard mRNA-Seq. RSeQC-derived coverage is plotted for all transcript (areas) and the ERCC mix only (lines), for QuantSeq (green) and mRNA-Seq (grey). Numbers give the area under the curve (AUC) values as a measure for sequence coverage.

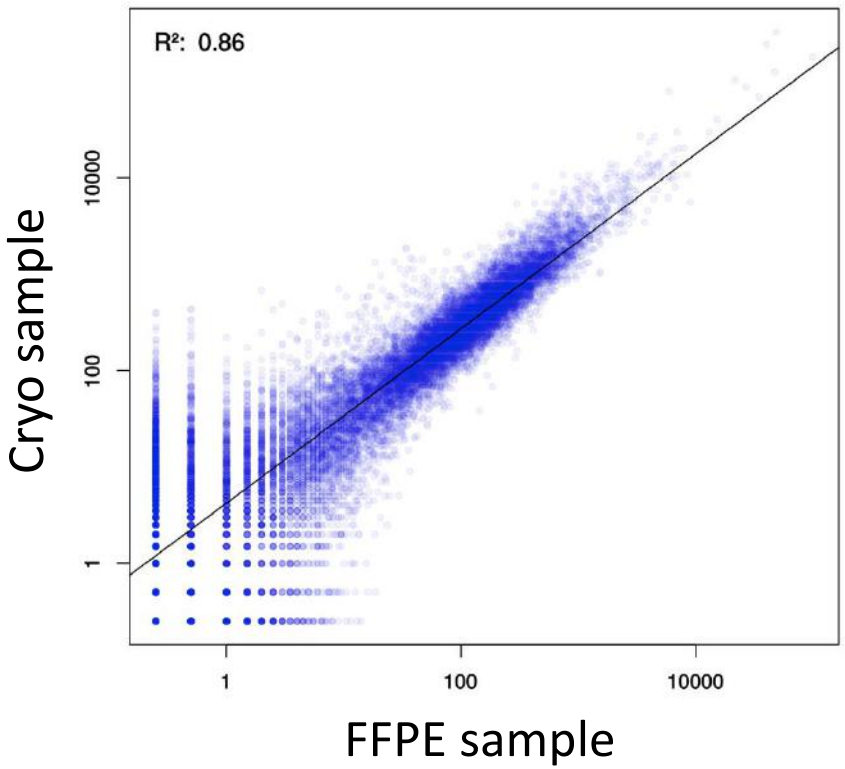
Required number of sequencing reads

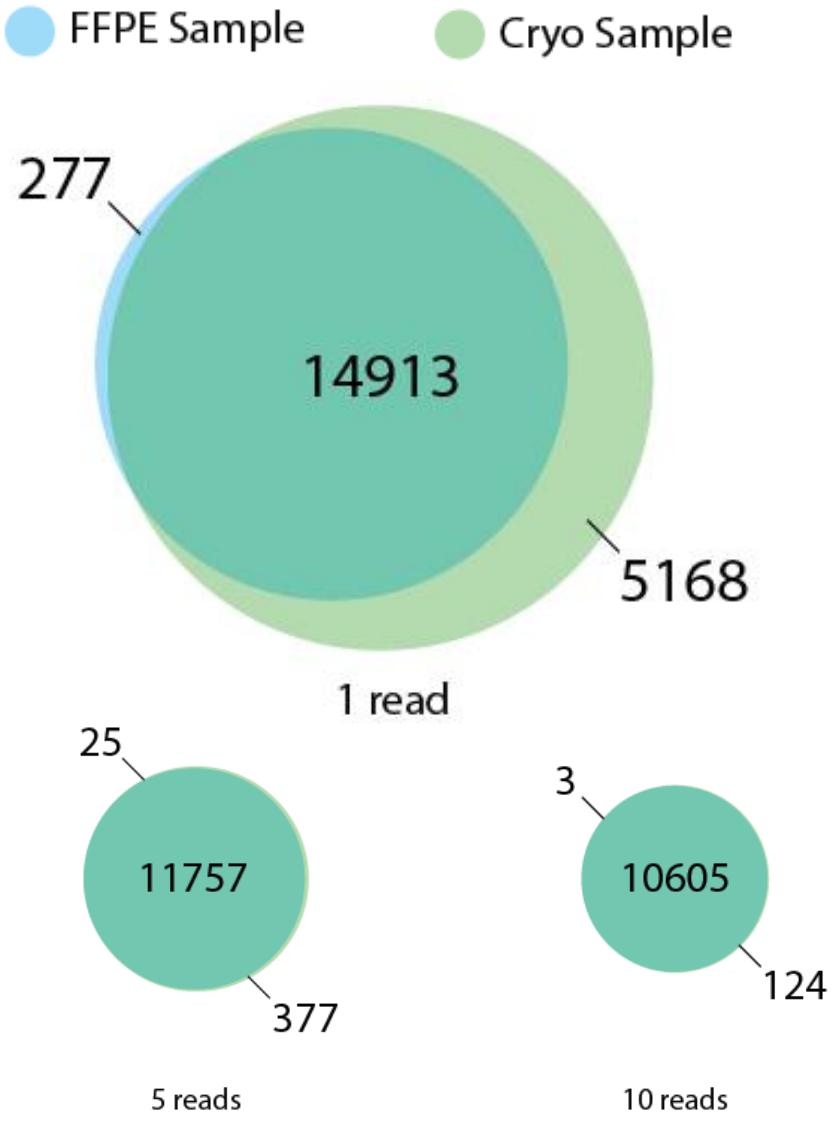


- **Only 1/10** of sequence **reads** (investigation of entire transcript regions not required)
- Higher number of **multiplexing**
- Significantly **lower amount of computational resources** required (no assembly, RPKM, FPKM)

1) Liu Y., et al., RNA-seq differential expression studies: more sequence or more replication? Bioinformatics 30(3):301-304 (2014) 2) Liu Y., et al., Evaluating the impact of sequencing depth on transcriptome profiling in human adipose. Plos One 8(6):e66883 (2013) 3) Bentley, D. R. et al. Accurate whole human genome sequencing using reversible terminator chemistry. Nature 456, 53–59 (2008) 4) Rozowsky, J.et al., PeakSeq enables systematic scoring of ChIP-seq experiments relative to controls. Nature Biotech. 27, 65-75 (2009).

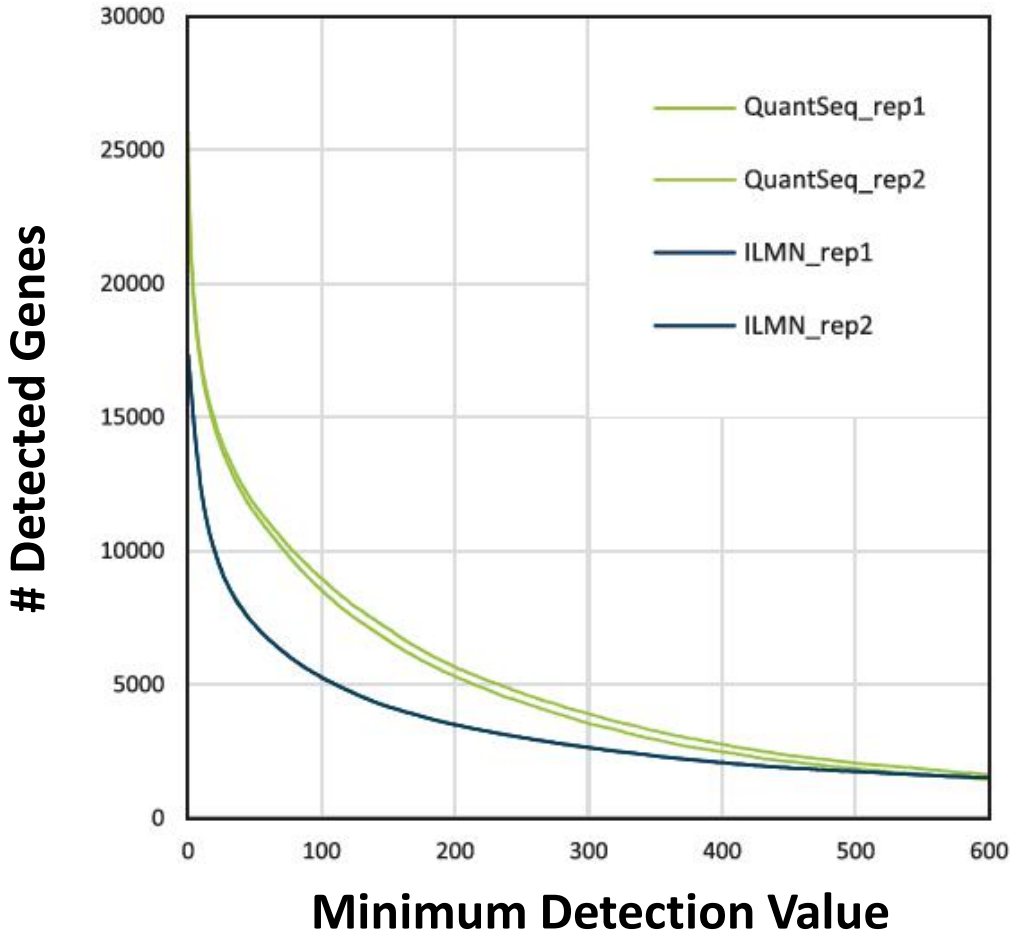
QuantSeq quantifications of FFPE and fresh frozen (cryo) samples correlate well





Xenograft of MOLP-8 human tumor cell line, split and processed either as fresh frozen cryo-block (RIN 8.3) or FFPE material (RIN 2.8, DV_{200} of 87 %).

QuantSeq outperforms Microarrays in the detection of low and medium abundant transcripts



Minimum detection value

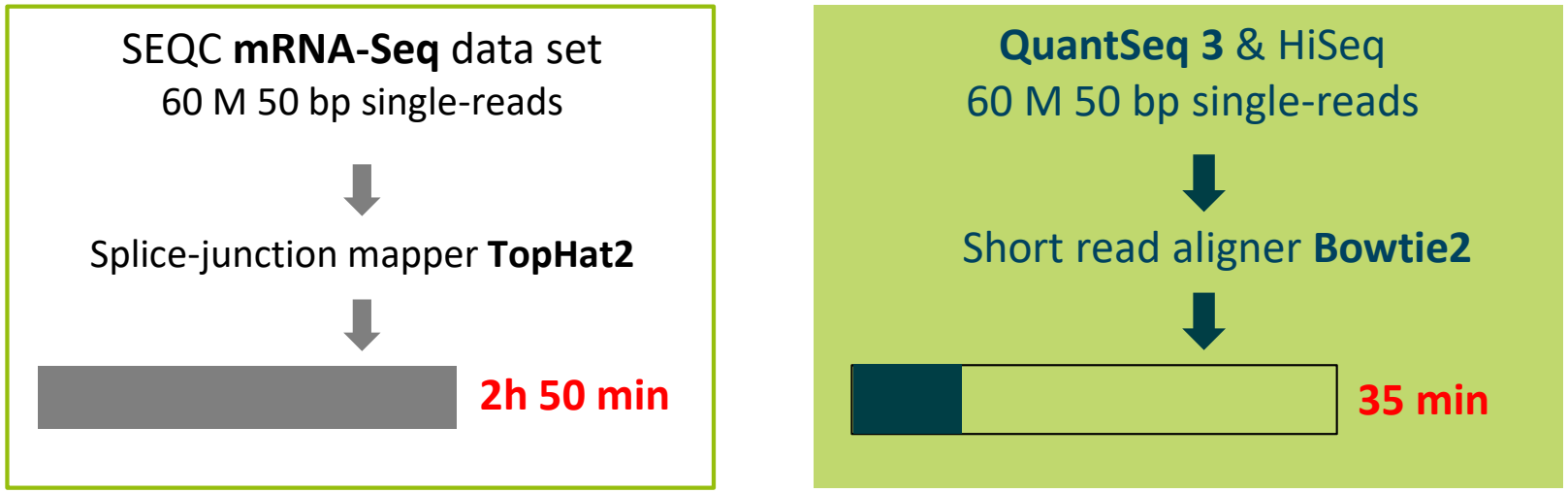
- **Illumina HT12 array:** quantile normalized signal levels (Yu et al.)
- **QuantSeq:** counts based on total gene reads

Yu, J. et al. (2015). *BMC Genomics.* 16:710

Simple bioinformatics analysis

Alignment:

- Read mapping is simplified by skipping the junction detection.



Concentration calculation:

- QuantSeq is streamlined for gene-read counting. No calculation of FPKM/RPKM values are needed.
- High strand-specificity reduces ambiguous counting.

Streamlined data analysis pipeline – FREE for QuantSeq users on Bluebee platform

www.bluebee.com/quantseq/

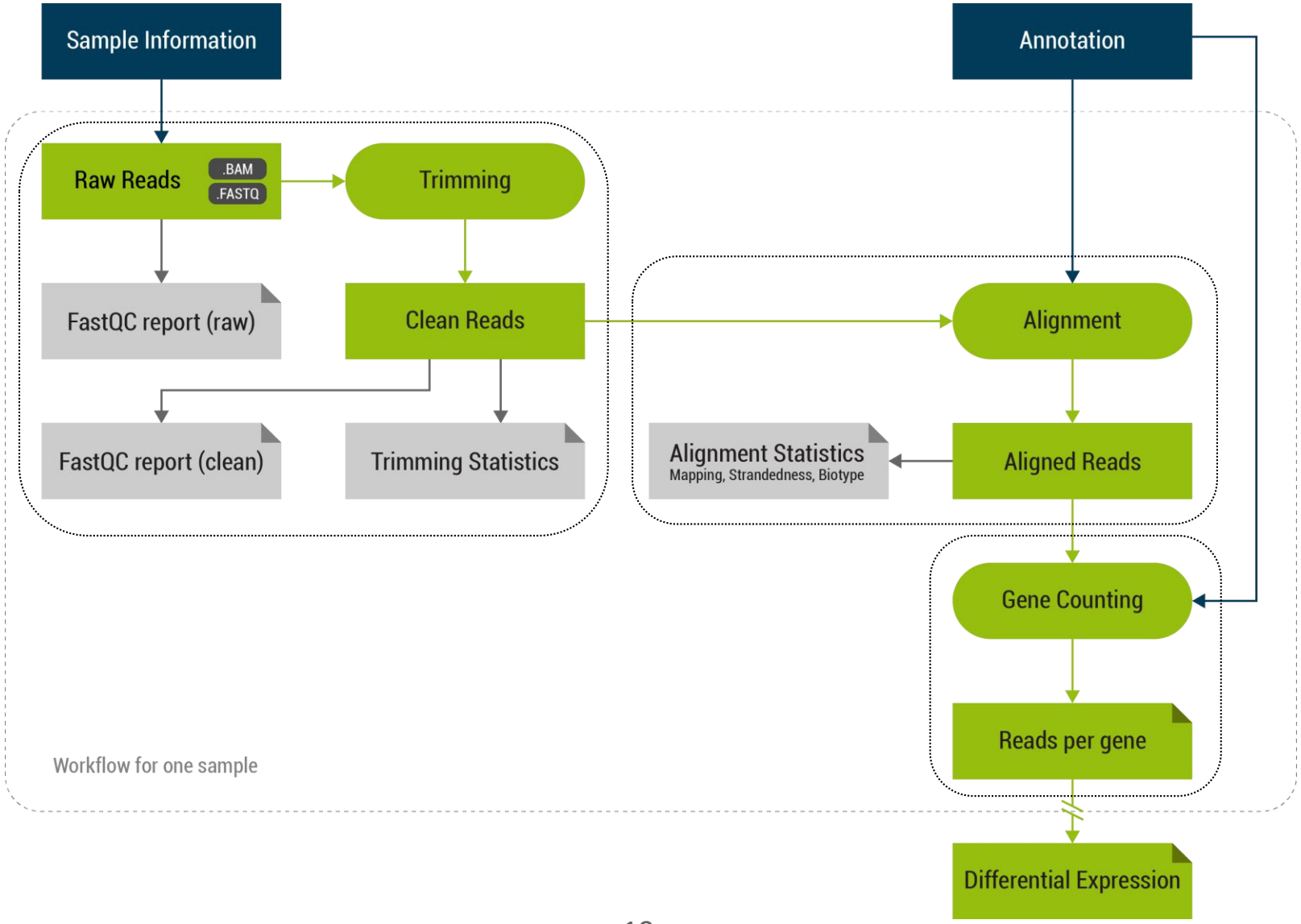
- **User-friendly data analysis** on the **Bluebee** genomics analysis platform
- For any user, also **without bioinformatics background**
- **Free for QuantSeq customers** – code is included in the kit
- **Data analysis only takes 10 minutes** once set up

QuantSeq sequencing data (zipped fastq)

BLUEBEE High Performance Genomics

mapping statistics
read counts
differential expression
PCA plot and MA plot

Data analysis workflow



Five simple steps to have your data analyzed

Register

on the Bluebee platform using the kit code provided with the QuantSeq kit (FWD or REV for Illumina, single read runs only)

Get connected

Connect your sequencer with the Bluebee platform (using the Bluebee Service Connector)

Upload your data

zipped fastq file = sequencing data file

Run your pipeline

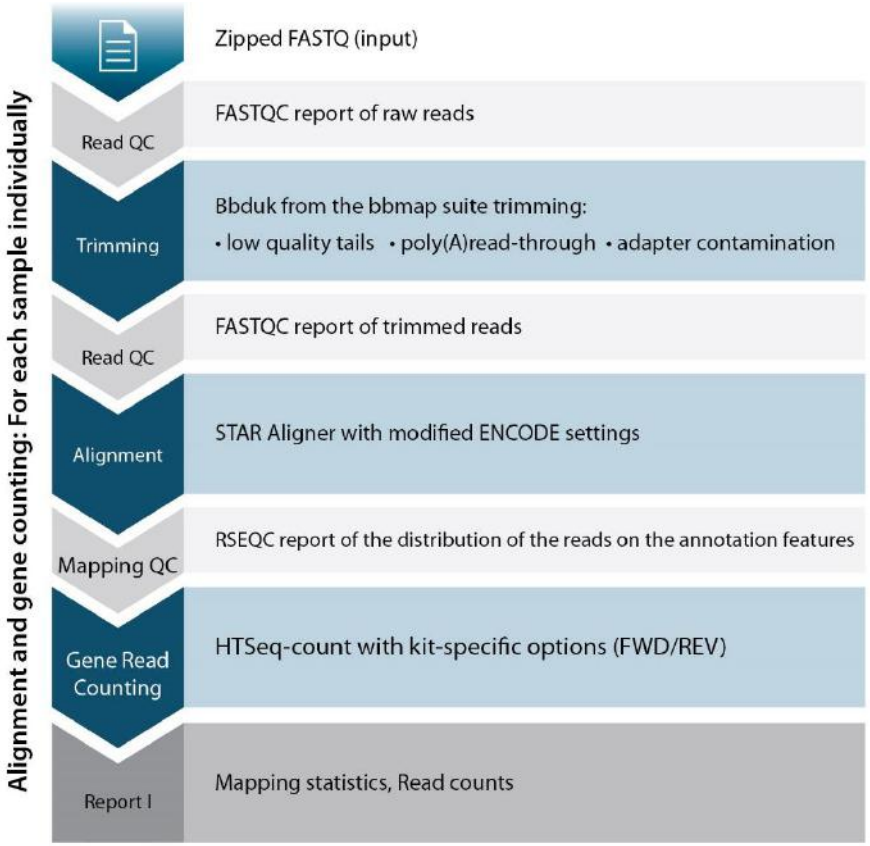
Choose QuantSeq FWD or REV for Illumina pipeline

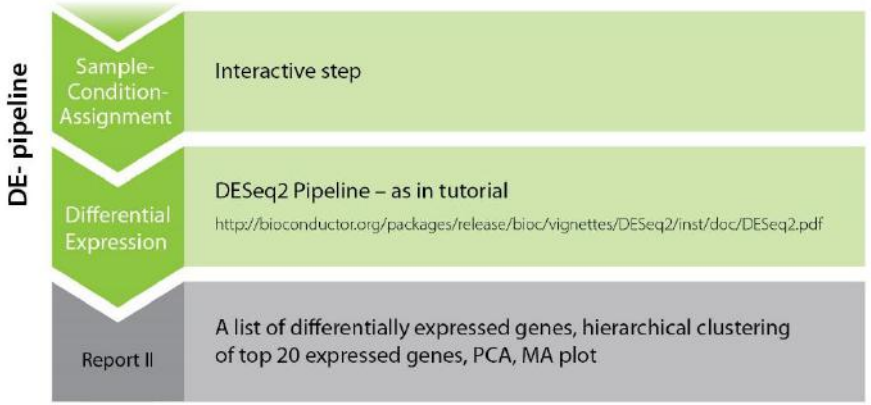
Get your results

Mapping statistics and read counts
DE analysis (list of DE genes, heat map, PCA plot and MA plot)
FastQC, RSeQC

Remark: For a detailed walk through of each of these steps, please have closer look at the **video-tutorials.**

Bluebee data analysis – overview





For **more information and support** please contact us at bioinfo@lexogen.com

QuantSeq: Cost Calculations

		QuantSeq	mRNA-Seq
Libraries / lane		96 (3M reads)	15 (20M reads)
Cost / rxn		USD 19.80	USD 51.00
Total library prep costs		USD 1900.80	USD 765.00
1 seq. lane (300M SR100 reads)		USD 1500.00	USD 1500.00
Data evaluation		**included @ BlueBee**	significant
RNA to data	total	USD 3400.80	USD 2265
	per rxn	**USD 35.43**	**USD 151.00**

RNA-seq based expression profiling including sequencing and data evaluation

~36 USD per sample

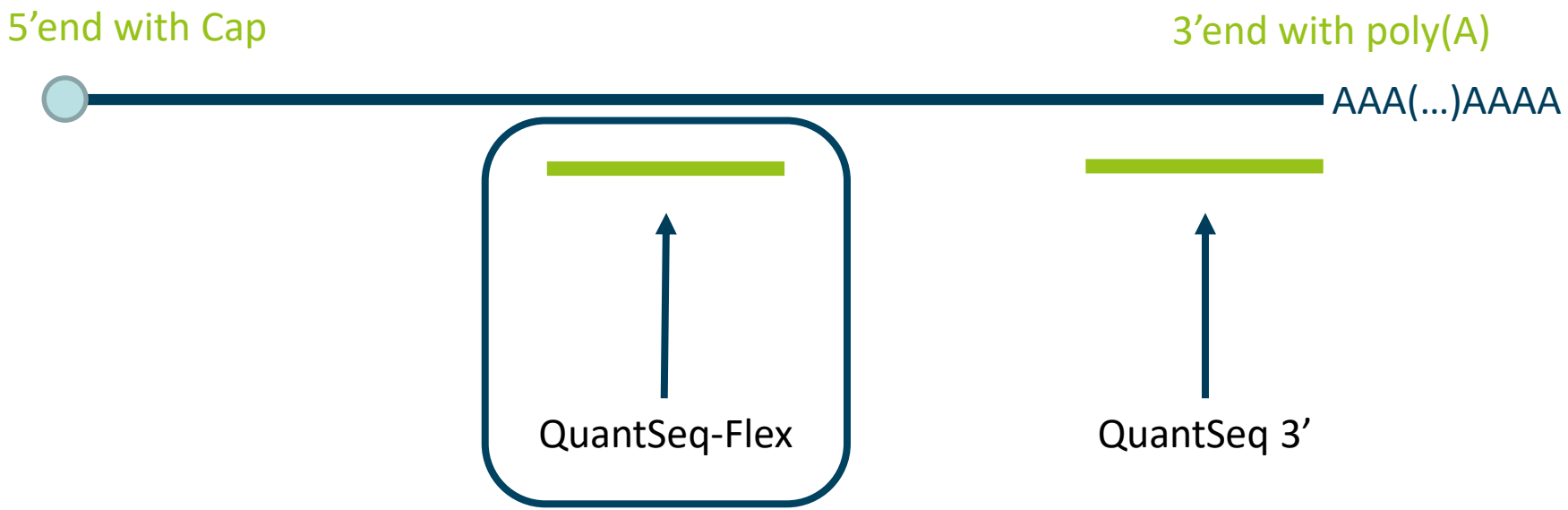
QuantSeq versus standard mRNA-Seq

	QuantSeq	mRNA-Seq [1]
Overall protocol time	4 h 30’	12 h
Hands-on time	1h 45’	4h 30’
Computing Time [2]	35’	2h 50’
Total RNA input	0.1 – 2000 ng	100 – 1000 ng
Input-output correlation (R^2) [3]	0.97	0.82
Strandedness [3]	> 99.9 %	93.4 % - 97.8 %
Differential expression (AUC) [4]	0.84 – 0.90	0.74 – 0.78

1 TruSeq mRNA stranded library preparation (Illumina)
2 mRNA-Seq needs a splice-junction mapper such as TopHat2, QuantSeq only a short-read aligner such as Bowtie2
3 Calculated on ERCC-mapping reads
4 Area under curve (see slide “accurate gene quantification even at low read depths”)

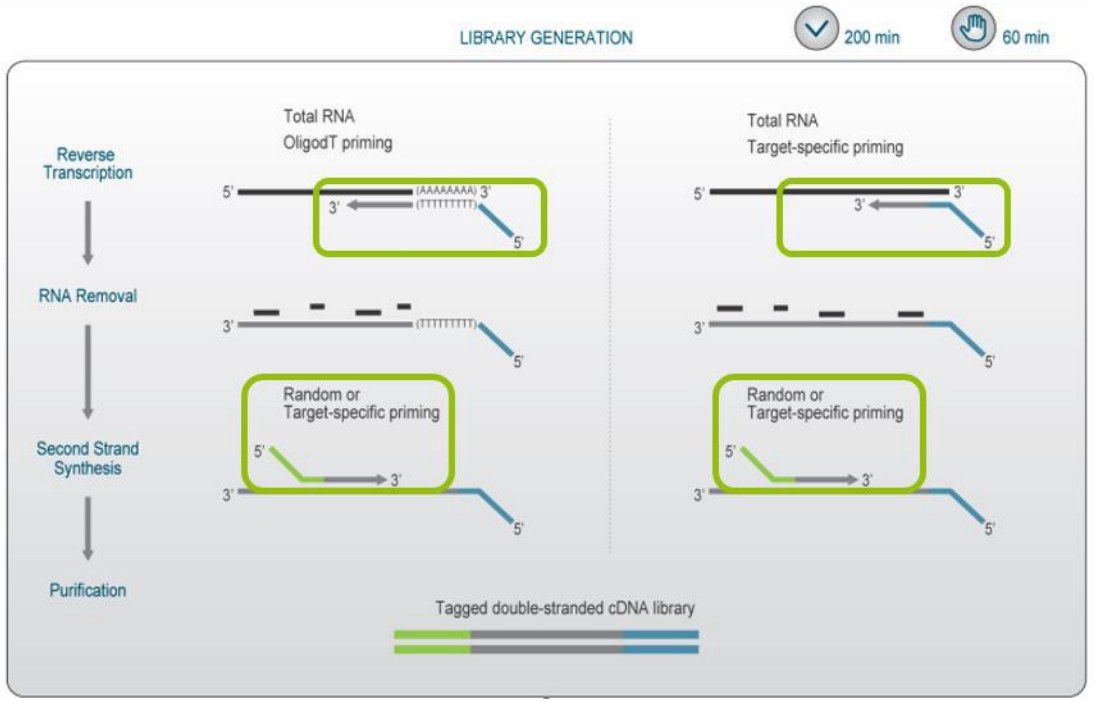
2 types of QuantSeq

- QuantSeq 3’mRNA-Seq Library prep kit
- QuantSeq-Flex Targeted mRNA-Seq Library prep kit



RNA-Seq experiments tailored to your needs

Application	RT	SSS
QuantSeq 3’ mRNA-Seq	Oligo-dT	Random
Targeted 3’ mRNA-Seq	Oligo-dT	Targeted
Targeted RNA-Seq, novel fusions	Targeted	Random
Targeted RNA-Seq, known targets only	Targeted	Targeted



QuantSeq - Summary

- Highly efficient and very cost-efficient solution for expression profiling (Alternative to microarrays, qPCR and standard RNA-Seq)
- Fast (only 4.5 hours) and simple all-in one protocol (fewer steps = less chance of error)
- Suitable for degraded RNA samples (e.g., FFPE)
- Wide input range:
  - For Illumina: 100 pg – 2 µg of total RNA
  - For Ion Torrent: 5 ng – 2 µg of total RNA
- High level of multiplexing (up to 384 samples/lane)
- > 99.9 % strand-specificity
- Optimized for shorter reads (SR50, 100). Library Sizes around 335-456 bp with insert sizes of 203-324 bp
- Usage of custom primers for targeted RNA-Seq (QuantSeq-FLEX)
- Automation-friendly (autoQuantSeq for Illumina)
  - For Sciclone NGS and Zephyr liquid handlers of PerkinElmer and the Hamilton Microlab STAR Workstations, Agilent Bravo, Beckman.

Customer publications

The effector AWR5 from the plant pathogen *Ralstonia solanacearum* is an inhibitor of the TOR signalling pathway

Crina Popa, Liang Li, Sergio Gil, Laura Tatjer, Keisuke Hashii, Mitsuaki Tabuchi, Núria S. Coll, Joaquín Ariño & Marc Valls

Sci. Rep. 6, 27058; doi: 10.1038/srep27058 (2016)

Transcriptional profiling and muscle cross-section analysis reveal signs of ischemia reperfusion injury following total knee arthroplasty with tourniquet

Jonathan B. Muyskens, Austin D. Hocker, Douglas W. Turnbull, Steven N. Shah, Brick A. Lantz, Brian A. Jewett, Hans C. Dreyer

Physiological Reports Published 5 January 2016 Vol. 4 no. e12671 DOI: 10.14814/phy2.12671

Find the latest QuantSeq publications at https://www.lexogen.com/publications/#quantseqpub

Quote from paper in Nature: Dysregulation of expression correlates with rare-allele burden and fitness loss in maize
Karl A. G. Kremling1, Shu-Yun Chen2,3, Mei-hsiu Su2, nicholas K. Lepak4, M. Cinta Romay2, Kelly L. Swarts1,5, Fei Lu2,6, Anne Lorant7, Peter J. Bradbury4 & Edward S. Buckler1,2,4

We automated a 3‘ mRNA sequencing method (**QuantSeq**, Lexogen GmBH), which **is more efficient and accurate than mRNA sequencing** and deals well with paralogues.“

Watch our QuantSeq webinars!

Dr. Jernej Ule (Francis Crick Institute)
Dr. Gregor Rot (Institute of Molecular Life Sciences University of Zurich)
https://www.lexogen.com/webinar-expressrna/

Oscar Harari (Department of Psychiatry at Washington University in St Louis)
https://www.lexogen.com/webinar-analysis-of-the-transcriptome/

Free QuantSeq data analysis pipeline on the Bluebee platform

Jekaterina Aleksejeva (Lexogen)
https://www.lexogen.com/webinar-free-quantseq-data-analysis/

Kevin Roy (Standford University School of Medicine)
https://www.lexogen.com/webinar-mapping-nuclear-exosome-targeted-polya-tails-with-3-rna-seq/

Behnam Abasht (University of Delaware)
https://www.lexogen.com/webinar-gene-expression-analysis-using-3-rna-sequencing/

QUANT™ SEQ

Sequencing that counts

Automation

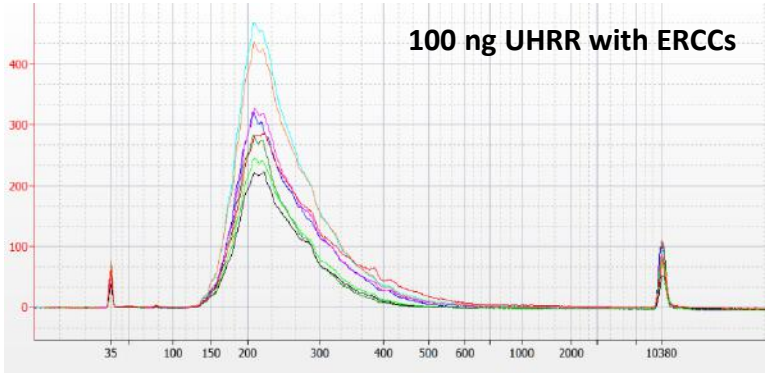
QuantSeq has been automated on various liquid handling instruments

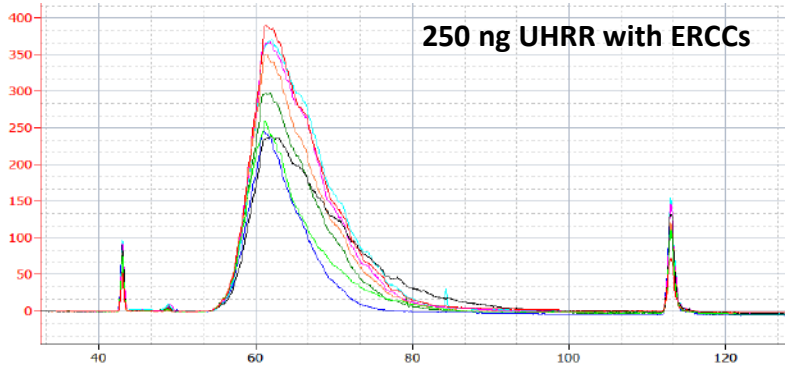
- ✓ **Perkin Elmer Sciclone / Zephyr**
- ✓ **Hamilton Microlab STAR**
- ✓ **Beckmann Biomek FxP**
- ✓ **Agilent Bravo NGS Workstation (B)** – Application Note available
- ✓ **Eppendorf EpMotion 5075**

- Script files are available at **www.lexogen.com**
- **Dummy Reagents** for QuantSeq Automation (Cat. No. 019.384) – **free of charge**
  - Set of 3 **mock solutions** for **volume checks** and **consistency**
- **Purification Module** with Magnetic Beads (Cat. No. 022.24)

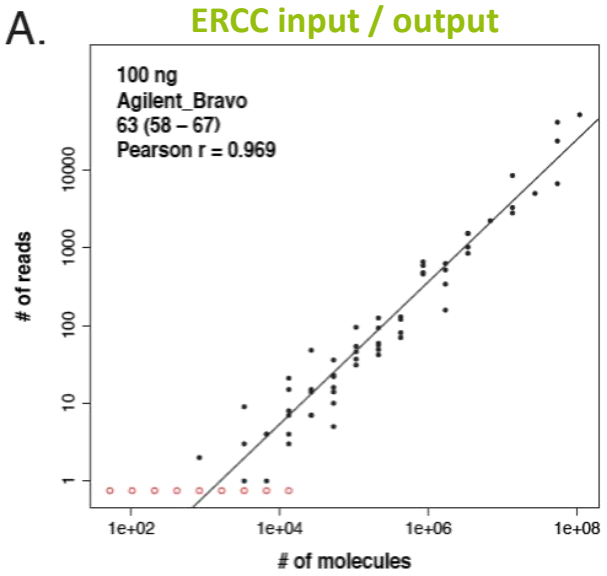
Efficient, **robust**, and **reproducible** library preparation in a **high-throughput format**, with minimal drop out.

QuantSeq Automation on Agilent Bravo shows high consistency for technical replicates

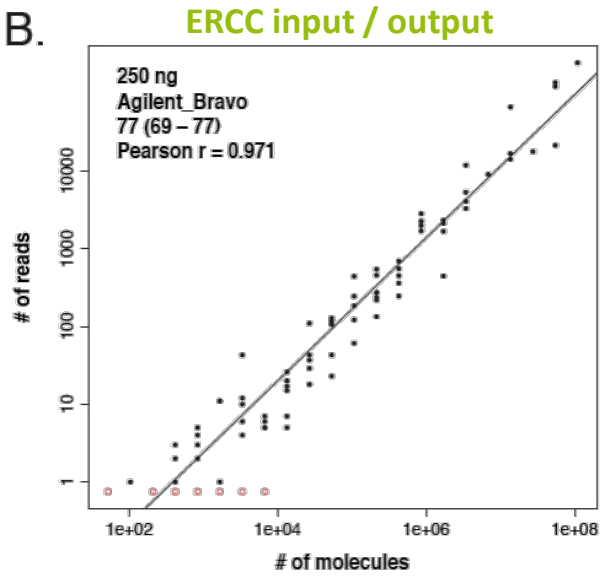




A.



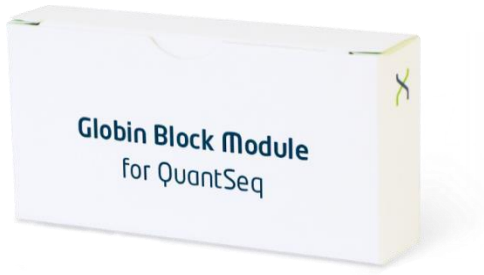
B.



QUANT™ SEQ

Sequencing that counts

Globin Block Modules



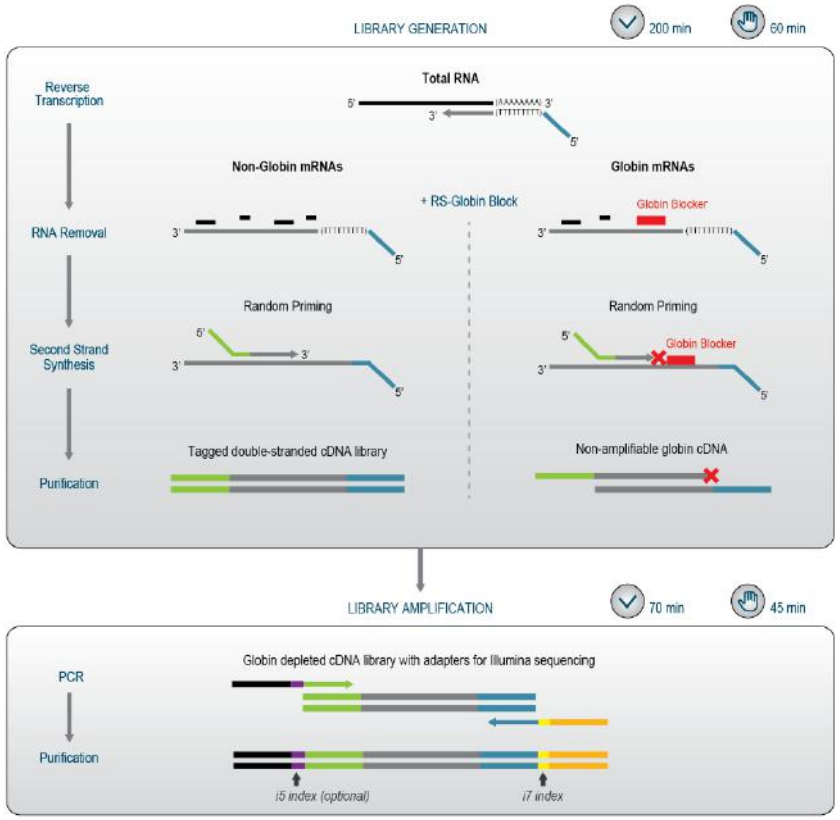
Blood as sample type

- **Informative**
- **low cost**
- **minimally invasive**
- **highly accessible**
- **biomarker discovery** and **monitoring**

- **high abundance** of **globin mRNA** (HBA, HBB)
- **50-80 %** of **sequencing reads**
- **limiting gene detection** and **quantification sensitivity**

Seamless gene expression profiling of blood samples

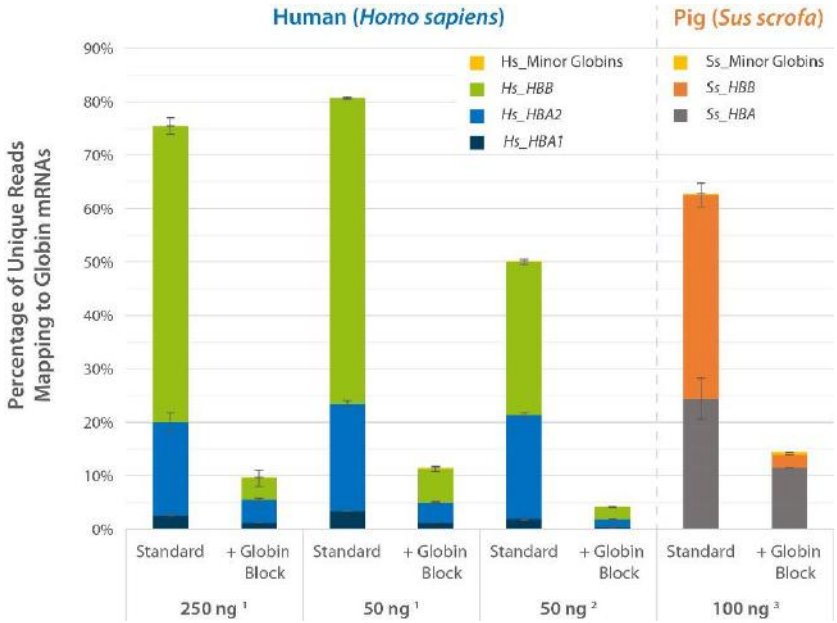
- For **human** (*Homo sapiens*, Cat. No. 070.96) and **pig** (*Sus scrofa*, Cat. No. 071.96) **blood samples**
- Efficient **globin depletion** for **less than USD 4.98** per sample
- Block globin mRNA **during QuantSeq** library prep – **no additional steps** required, simple exchange of RNA Removal Solutions (RS vs. RS-GBs)
- **Input** starting **from 50 ng** of **total RNA**
- **Reduce globin mapping** reads by **up to 91 %**
- **Detect** and **quantify thousands** of **additional genes**



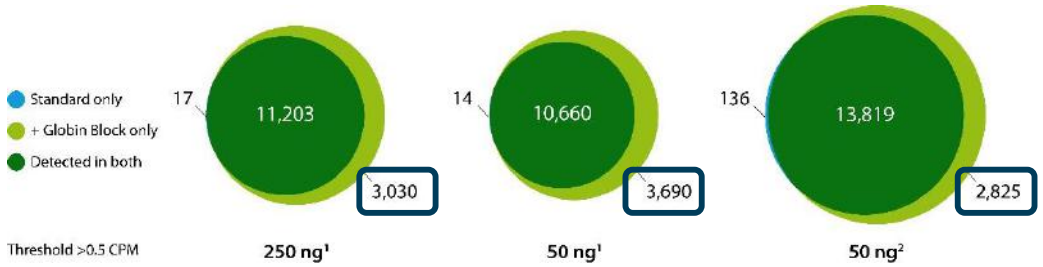
Efficiently deplete globin mRNA for less than $ 4.98 per sample!

Efficiently deplete globin mRNA and detect and quantify thousands of additional genes

Globin mRNAs **reads reduced** from **50 – 80 %, to as low as 5 %**



Gene detection is **increased** by **thousands** of **additional genes**



NEW

i5 Unique Dual Indexing Kits

QUANT SEQ™ Sequencing that counts

SENSE™ Making sense of RNA sequencing

i5 Unique Dual Indexing Add-on Kit – Your benefits

96 i5
x
96 i7

Enhanced multiplexing capacity

Benefit from up to 9,216 different i5/i7 index combinations, or up to 96 unique dual indexing barcoding options for all QuantSeq and SENSE libraries

Minimize index mis-assignment

Up to 96 unique dual indexing barcode options enable you to detect and quantify index hopping

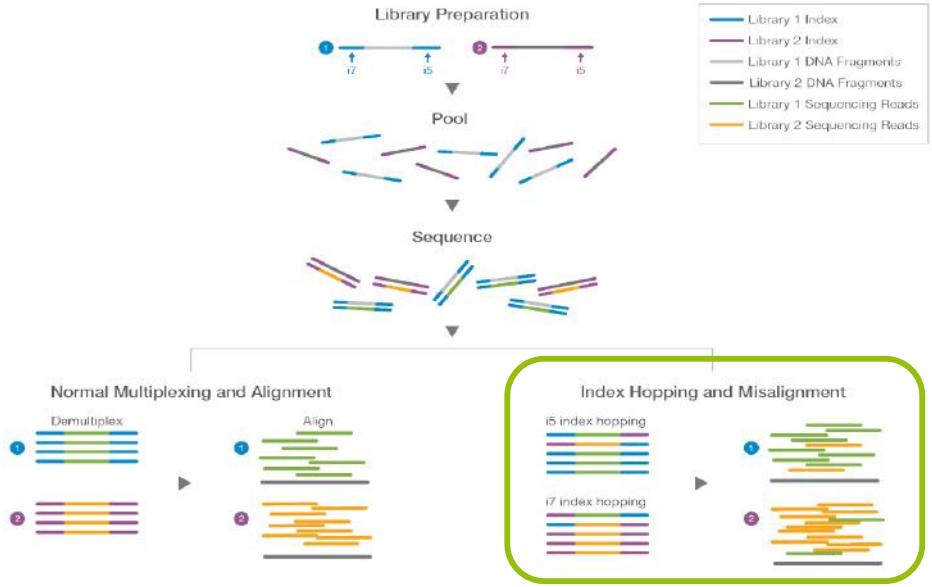
Index Balance Checker

Use our online help tool to choose the optimal index combination for your RNA-Seq experiment

Dual indexing rationale – index hopping

Problem: Index hopping/switching

= **mis-assignment** that results in the incorrect assignment of libraries from the expected index to a different index (in the multiplexed tool)



Resource: Illumina Whitepaper 770-2017-004-D (2017) *Effects of Index Misassignment on Multiplexing and Downstream Analysis. Learn why it happens and best practices to reduce the impact of index hopping.*

Reason: Index hopping

- **Left-over primers** causing issues with the ExAmp (exclusion amplification chemistry)
- **Patterned flow cells:** HiSeq3000/4000, NovaSeq
- **Slightly elevated levels**: **0.1-2 %** depending on type, quality, and handling library

Solution

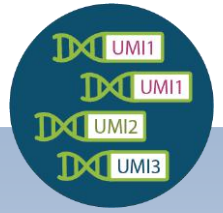
- **Thorough purification protocols** to remove free adapter (always **two rounds** of post PCR, optional repurification of lane mix)
- **96 i5 x 96 i7 unique dual indices** NEW

NEW

Unique Molecular Identifiers

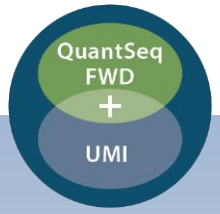
QUANT™ SEQ
Sequencing that counts

UMI Second Strand Synthesis Module – Your benefits



De-duplicate your RNA-Seq data

Tag individual transcripts to detect and eliminate amplification bias for unbiased gene expression profiling



Fully integrated within QuantSeq

Save your precious time and add UMIs during QuantSeq library prep with a simple solution exchange, no additional steps required

Data analysis tool available

De-duplicate your QuantSeq data using the *collapse_UMI_bam* command-line analysis tool, available from Lexogen

UMI working principle

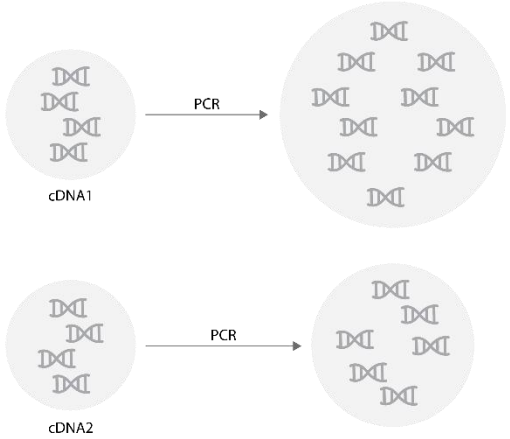
UMI = Unique Molecular Identifier, also known as Molecular Barcode

Library prep without UMI

Problem: Sequence specific PCR bias:

- Pre-PCR: identical cDNA fragment pools (cDNA1 and cDNA2) with the same size
- Post-PCR: cDNA1 and cDNA2 sizes differ

Inaccurate gene and **isoform quantification**

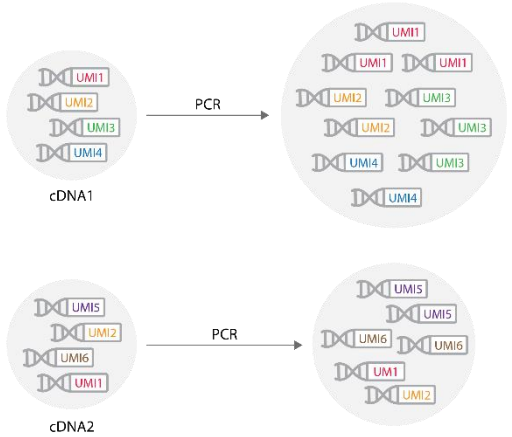


Library prep with UMI

Solution to sequence specific PCR bias:

- Ligation of random oligonucleotides (UMIs) to DNA fragments before PCR
- Distinguish pre-PCR fragments with identical sequence
- Number of distinct UMIs after PCR is the same as the number of fragment copies before PCR

Unbiased gene expression profiling



UMI Second Strand Synthesis Module for QuantSeq FWD (Illumina, Read 1) (Cat. No. 081.96)

- **Kit components:** UMI Second Strand Synthesis Mix (USS)
- **Compatibility:**
  - only for QuantSeq FWD (Cat. No. 015)
  - **NOT** compatible with QuantSeq REV (Cat. No. 016) or Ion Torrent (Cat. No. 012)
- **Design:** random primers including 6 bp UMIs. UMI sequence is located between the partial P5 adapter and the random priming sequence.
- **Usage:**
  - simple solution exchange
  - UMIs added during the second strand synthesis step of QuantSeq
  - USS replaces Second Strand Synthesis Mix 1 (SS1) from standard QuantSeq FWD kit at step 7

Kit Components and Storage Conditions

NEW

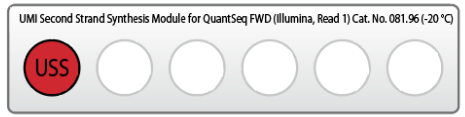


Figure 2. Location of kit component.

UMI Second Strand Synthesis Module for QuantSeq FWD (Illumina, Read 1) (Cat. No. 081.96)	Tube Label	Volume* 96 rxn	Storage
UMI Second Strand Synthesis Mix	**USS** ●	1056 µl	-20 °C

*including 10 % surplus

Second Strand Synthesis

NOTE: This protocol replaces steps 7 and 8 of the detailed protocol from the QuantSeq 3′ mRNA-Seq Library Prep Kit for Illumina User Guide (015UG009, 015UG110). Step 8 has not been changed for UMI libraries and is included here for ease of reference.

Follow steps 1 - 6 as indicated in the QuantSeq 3′ mRNA-Seq Library Prep Kit for Illumina User Guides (see above).

7 Add 10 µl of UMI Second Strand Synthesis Mix (**USS** ●) to the reaction. Mix well by pipetting, and seal the plate. **REMARK:** Use a pipette set to 30 µl for efficient mixing.

8 Incubate the plate for 1 minute at 98 °C in a thermocycler, and slowly cool down to 25 °C by setting the ramp speed to 10 % (0.5 °C/second). Incubate the reaction for 30 minutes at 25 °C. Quickly spin down the plate at room temperature before removing the sealing foil.

Proceed to step 9 of the detailed protocol in the QuantSeq 3′ mRNA-Seq Library Prep Kit for Illumina User Guides (see above).

User Information will be added in the **QuantSeq User Guide.**

UMI Second Strand Synthesis Module for QuantSeq – key features

- **Tag individual transcripts during QuantSeq** library preparation by a simple solution exchange
- **Identify PCR amplification bias** and **de-duplicate** your **sequencing data** for unbiased gene expression profiling
- **6 bp UMIs** added **during second strand synthesis** – no additional steps, same streamlined QuantSeq workflow
- **Single-read sequencing** (SR 75 or higher) enough – UMI read-out at the start of Read 1
- Compatible with **QuantSeq 3‘ mRNA-Seq (FWD) for Illumina**
- Compatible with **Globin Block Module** and **Unique Dual Indexing Module** – use for single and dual-indexed libraries
- **Data Analysis Tool *collapse_UMI_bam*** for collapsing reads by UMIs available from Lexogen

QUANT™ SEQ

Sequencing that counts

Full Service

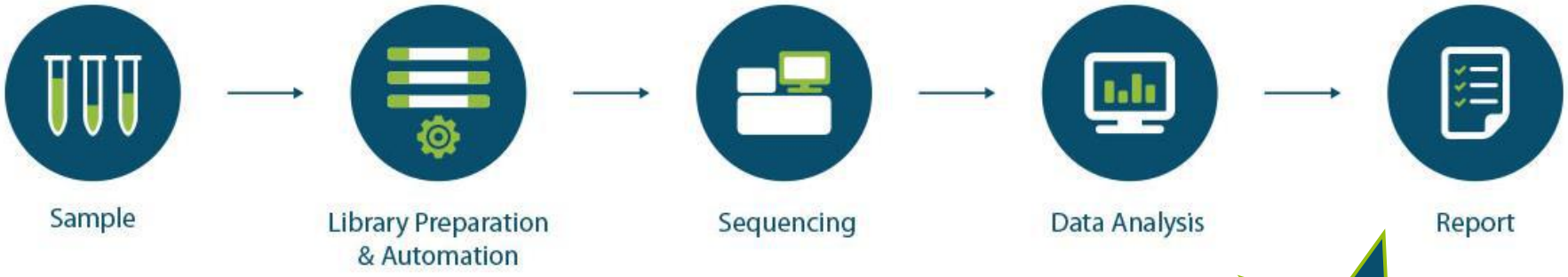
Fully integrated service-workflow: from RNA to evaluated data

Sample	Library Preparation & Automation	Sequencing	Data Analysis	Report
Your **RNA** samples (on dry ice)	Our **QuantSeq FWD** library prep	**Illumina HiSeq 2500**, **SR75**, minimum 190 M reads / lane **Illumina NextSeq 500**, **SR75**, 400 M reads per run (Illumina – specified)	**Report:** Demultiplexing, read quality control, trimming and filtering procedures, mapping, and data analysis down to differential gene expression (DGE), further analyses available on request.	

- Fully integrated package: **economical** and **convenient**
- **Includes RNA QC, library preparations, NGS run, and data analysis**
  NGS data QC, mapping, counting, (differential) gene expression analysis
- **Project management and support** by the experts in the field

Pricing depends on the number of samples to be processed, the desired degree of multiplexing, and the sequencing mode. For a quote or an example report, please inquire at services@lexogen.com

Now available: **QuantSeq SERVICE**



NEW

- **From RNA to evaluated data**
- Fully integrated package: economical, and convenient

- **Includes RNA QC, library preparations, NGS run (SR75), and data analysis**
- NGS data QC, mapping, counting, (differential) gene expression analysis

- **Pricing - depending on number of samples**

- **Project management and support** by the experts in the field

Product portfolio in the RNA-Seq workflow

Sampling	RNA Preparation	NGS Library Prep	NGS Data Analysis
SLAMseq Metabolic RNA Labeling Kit » Explorer Modules » Kinetics Modules	**SPLIT RNA Extraction Kit**	**Expression Profiling** » QuantSeq 3′ mRNA-Seq Library Prep Kits » QuantSeq-Flex Targeted RNA-Seq Library Prep Kit	**QuantSeq Data Analysis** » QuantSeq Pipeline on Blue- bee® Genomics Platform » SLAMdunk Pipeline for QuantSeq-SLAMseq Data on Bluebee® Genomics Platform » QuantSeq Pipeline in Partek® Flow®
	RiboCop rRNA Depletion Kit	**Whole Transcriptome** » SENSE mRNA-Seq Library Prep Kit » CORALL Total RNA-Seq Library Prep Kit	**Mix² RNA-Seq Data Analysis Software**
	Poly(A) RNA Selection Kit	**Small RNA-Seq Library Prep Kit**	
	cDNA Preparation		
	TeloPrime Full-Length cDNA Amplification Kit		
	NGS Controls		
	Spike-in RNA Controls » SIRV-Set 1 (Iso Mix E0, E1, E2) » SIRV-Set 2 (Iso Mix E0) » SIRV-Set 3 (Iso Mix E0 / ERCC)		**SIRV Suite Spike-In Analysis Software**
Services			
Library Preparation » QuantSeq 3′ mRNA-Seq » QuantSeq on SLAMseq RNA » QuantSeq on Blood-RNA (Globin Block) » Small RNA-Seq	**Automation and Sequencing**		**Data Analysis and Reporting**

SLAMseq

NEW

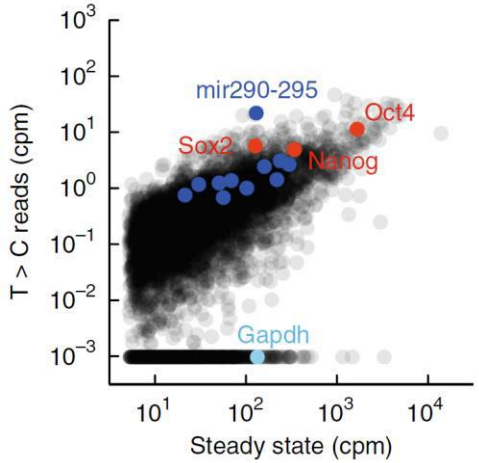
Metabolic RNA-Seq

The transcriptome is not completely described by measuring RNA steady state-levels

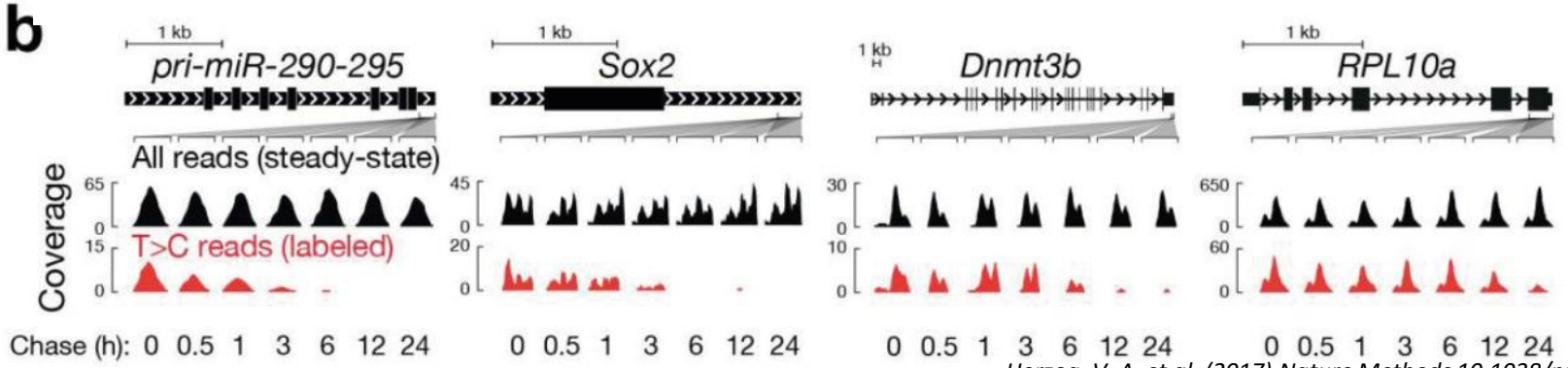
- **Conventional RNA-Seq** experiments measure **RNA steady-state levels**
- **Metabolic RNA-Seq** can measure **RNA synthesis** and **degradation separately**

Gain novel insights in gene expression and its controls!

RNA synthesis



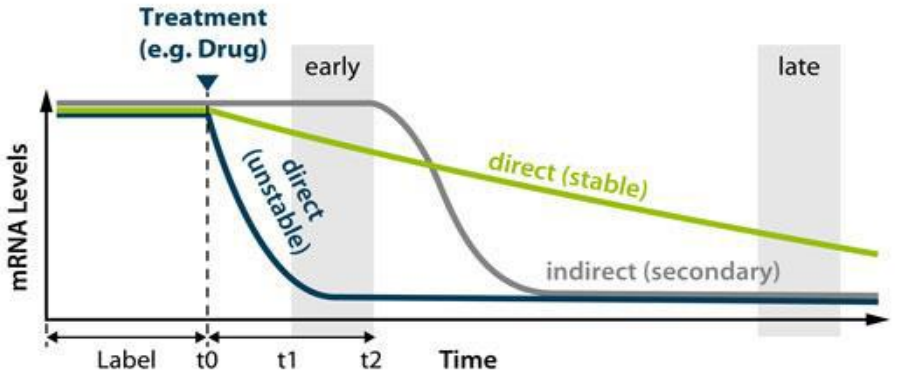
RNA degradation

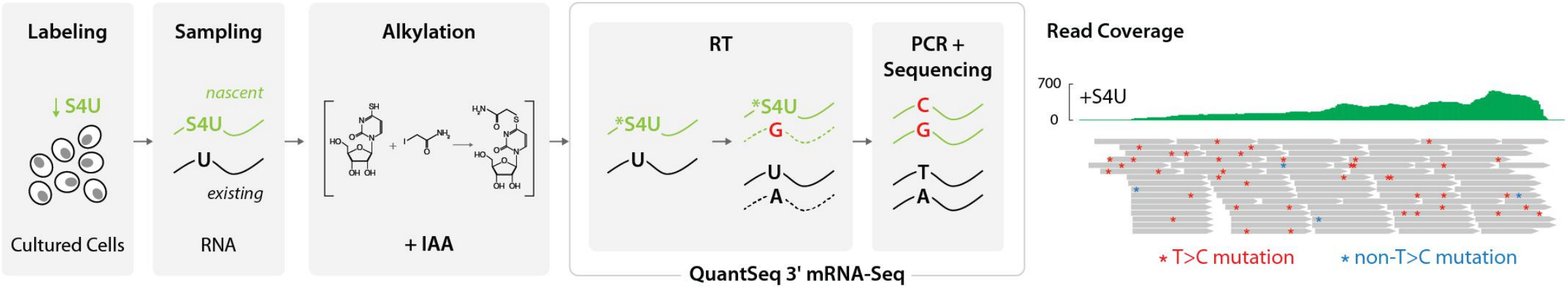


Herzog, V. A. et al. (2017) Nature Methods 10.1038/nmeth.4435

SLAMseq METABOLIC RNA LABELING KITS

Identify direct transcriptional targets of any gene





Lexogen's SLAMseq Explorer and Kinetics Kits

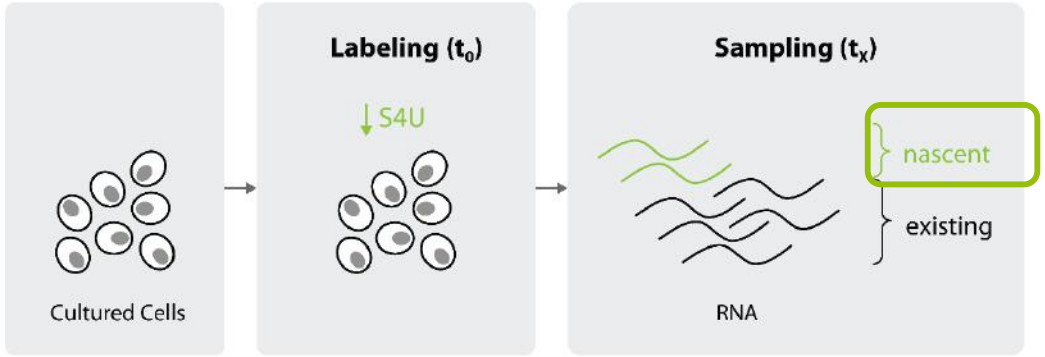
Kit Type	Module	Application
SLAMseq Explorer Kit	**Cell Viability Titration Module** (Cat. No. 059.24)	•Assess S4U toxicity in target cell lines •Optimize S4U labeling concentrations
	S4U Incorporation Module (Cat. No. 060.24)	•Measure S4U uptake and incorporation rates using HPLC analysis
SLAMseq Kinetics Kit	**Anabolic Kinetics Module** (Cat. No. 061.24)	•Label newly transcribed RNA with S4U •Measure nascent RNA expression •Analyze RNA synthesis kinetics
	Catabolic Kinetics Module (Cat. No. 062.24)	•Label existing RNA with S4U •Assess transcript stability •Analyze RNA degradation kinetics

- **Kit size** and **format: 24-well cell culture plate** format using **24× 0.5 - 1 ml** growth medium, all **examples** in the **User Guide** are based on 24-well format. **Up-** and **down-scaling** to suit different culture vessel sizes is **possible**.
- Interested in bigger kits sizes? Please contact info@lexogen.com

SLAMseq Kinetics Kits – measure nascent RNA expression and transcript stability

Anabolic Kinetics Module – RNA syns

= S4U-labeling of newly synthesized (nascent) RNA



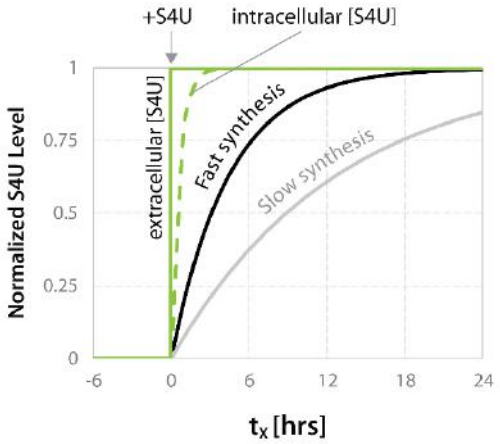
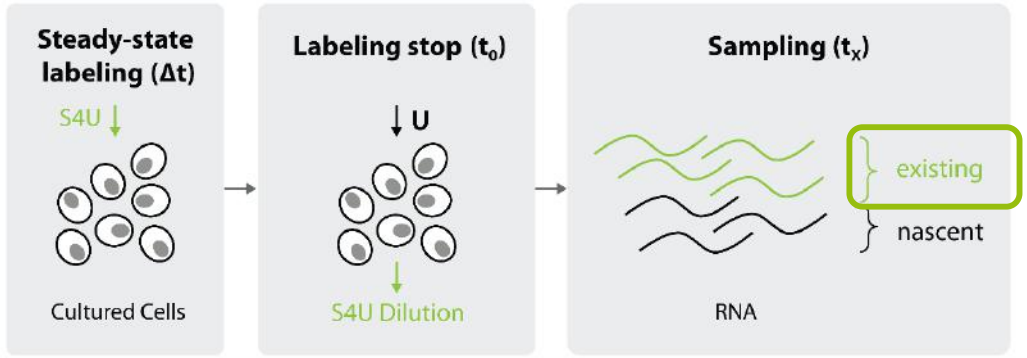


Figure | Anabolic kinetics labeling experiment time course.

Catabolic Kinetics Module – RNA degradation rates

= S4U-labeling of existing RNA



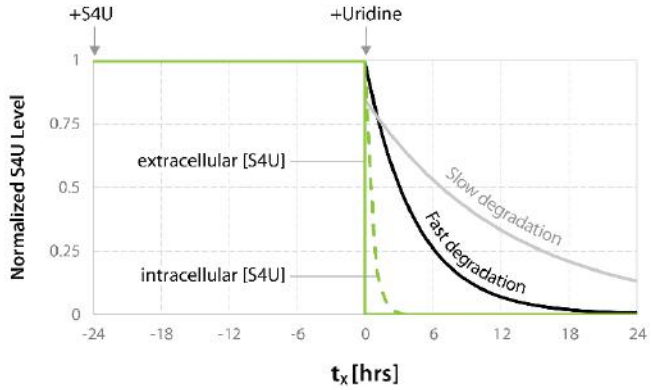
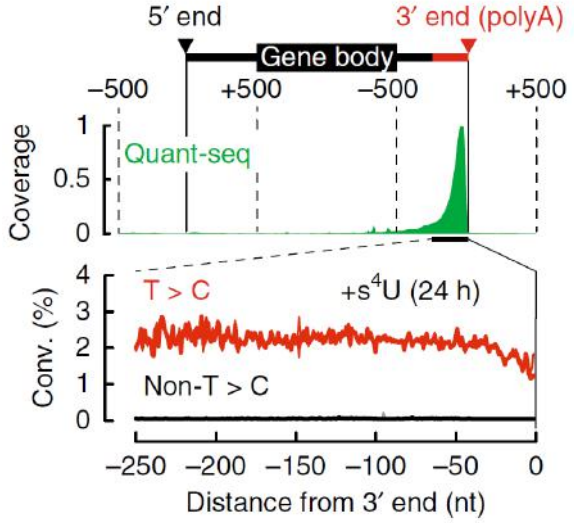
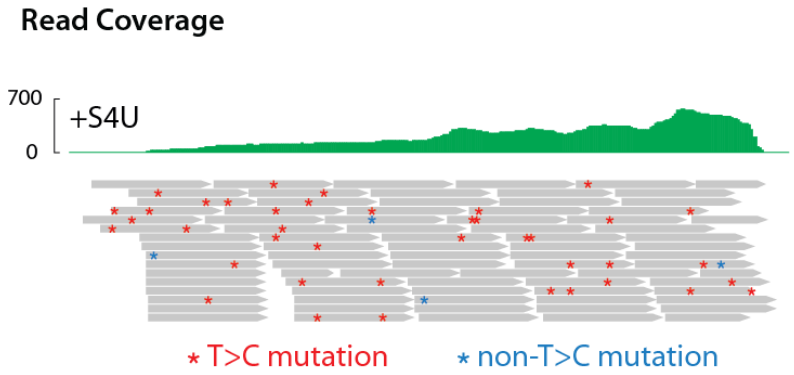


Figure | Catabolic kinetics labeling experiment time course.

QuantSeq 3‘ mRNA-Seq library preparation is ideal for SLAMseq samples

- **Cost-effective** library preparation
- **Low read depth:** 3 M reads for gene expression, 20-30 M reads for SLAMseq
- **Poly(A)-selective:** functional, fully processed RNA pol II transcript, no requirement for rRNA depletion
- **Facilitated data analysis:** highest strandedness, no length normalization
- **Sensitive:** from 100 pg total RNA input; standard input for SLAMseq is 1 µg
- **Robust quantification of T > C conversions**, covers inherently U-rich 3’ UTRs





Herzog, V. A. et al. (2017) Nature Methods 10.1038/nmeth.4435

Product portfolio in the RNA-Seq workflow

Sampling	RNA Preparation	NGS Library Prep	NGS Data Analysis
SLAMseq Metabolic RNA Labeling Kit » Explorer Modules » Kinetics Modules	**SPLIT RNA Extraction Kit**	**Expression Profiling** » QuantSeq 3′ mRNA-Seq Library Prep Kits » QuantSeq-Flex Targeted RNA-Seq Library Prep Kit	**QuantSeq Data Analysis** » QuantSeq Pipeline on Blue- bee® Genomics Platform » SLAMdunk Pipeline for QuantSeq-SLAMseq Data on Bluebee® Genomics Platform » QuantSeq Pipeline in Partek® Flow®
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Services			
Library Preparation » QuantSeq 3′ mRNA-Seq » QuantSeq on SLAMseq RNA » QuantSeq on Blood-RNA (Globin Block) » Small RNA-Seq	**Automation and Sequencing**		**Data Analysis and Reporting**

SLAMdunk – Streamlined data analysis pipeline

- **Streamlined and user-friendly SLAMseq-QuantSeq data analysis** integrated on the **Bluebee** genomics analysis platform
  https://www.bluebee.com/lexogen

- **DUNK: D**igital **U**nmasking of **N**ucleotide conversion-containing **k**-mers
- **Modified alignment algorithm** to report **read counts** for **T>C containing reads**
- For any user – **no specialized bioinformatics knowledge required**
- **Highly secure** cloud-based solution
  - **Five simple steps** to have your **SLAMseq-QuantSeq data analyzed: Register, get connected, upload** compressed fastq files, **select** species and **run** pipeline.
  - **Output: statistics** about **T->C conversion rates** and **alignments** falling into **unique 3' UTR** regions (CPM, T coverage, read counts, multimapping, etc). In addition, SLAMdunk produces further statistical and diagnostic information. Results can be downloaded in batch or individually.
- **Complete metabolic RNA-Seq solution** – from **RNA labeling** to **NGS library preparation** and **data analysis**

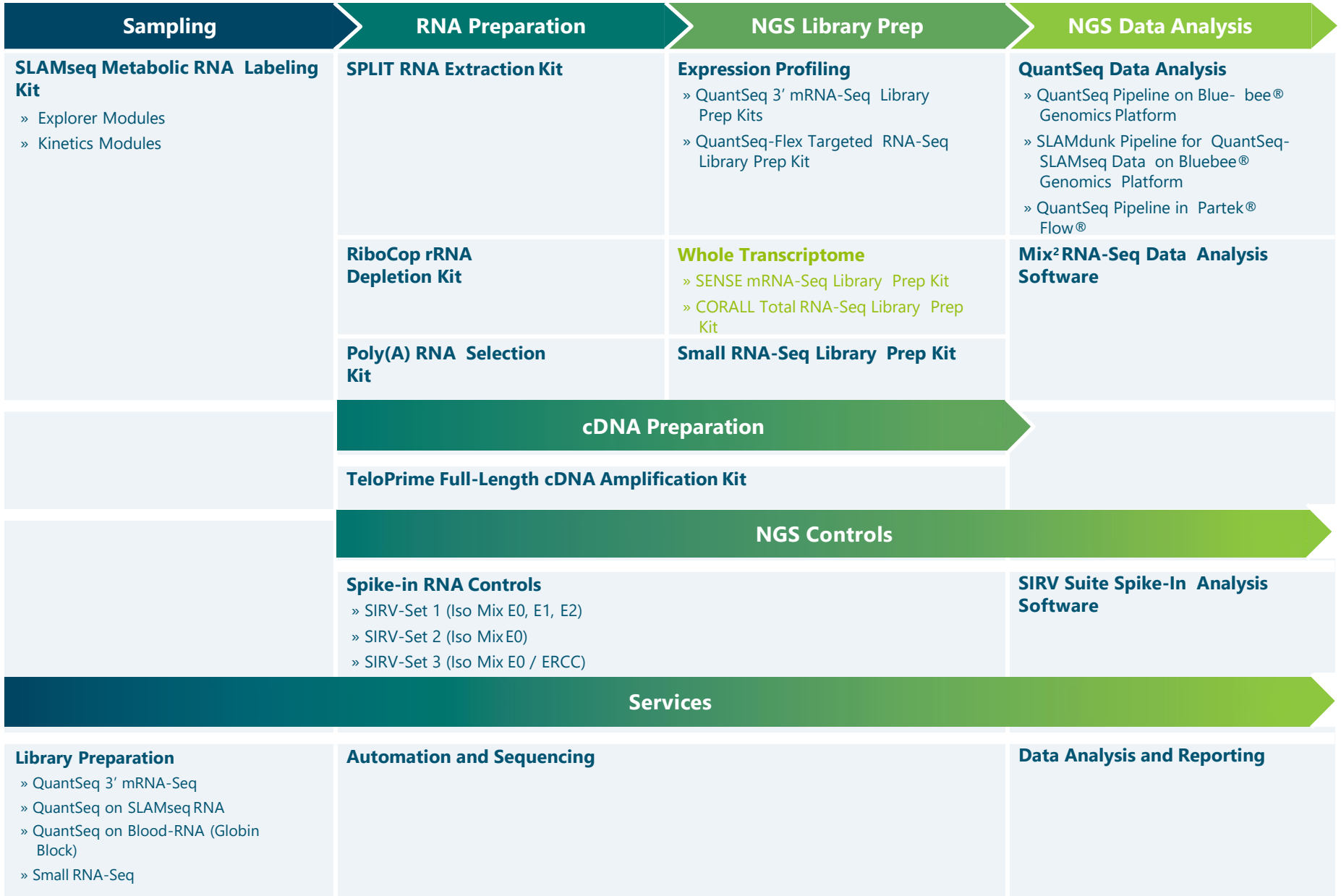
Developed by the Zuber (IMP), the von Haesler (MFPL) and the Ameres (IMBA) groups

Herzog, V. A. et al. (2017) Nature Methods 10.1038/nmeth.4435
Neumann, Tobias, Herzog, V.A., Muhar, M., von Haeseler, A., Zuber, J., Ameres, S.L. & Rescheneder, P., unpublished data

SLAMseq Kit – summary

- Analyze **transcriptome-wide kinetics** of **RNA synthesis** and **turnover**
- **Measure newly synthesized RNA expression** and **transcript stability**
- Gain **novel insights** into the **control** of **gene expression**
- Assess **toxicity** and **incorporation** of **4-Thiouridine (S4U)** in **cultured cells**
- **Only two extra steps** added to a standard RNA-Seq workflow:
  - **Labeling** of **RNA** by **adding S4U** to the culture medium
  - **Pre-processing** of the **total RNA** with **iodoacetamide** to alkylate 4-thiol group
- **Combine** with **QuantSeq 3' mRNA-Seq** or **QuantSeq Flex Targeted RNA-Seq Kits** for **time-** and **cost-efficient**, **high-throughput metabolic sequencing** from **multiple timepoints** and in **replicates**
- **SLAMdunk:** available as **user-friendly data analysis pipeline** on Bluebee for **analyzing SLAMseq-QuantSeq sequencing data**
  - **Modified alignment algorithm** to report **read counts** for **T>C containing reads**
  - Herzog et al., *Thiol-linked alkylation of RNA to assess expression dynamics* (Nature Methods, 2017: DOI:10.1038/nmeth.4435)

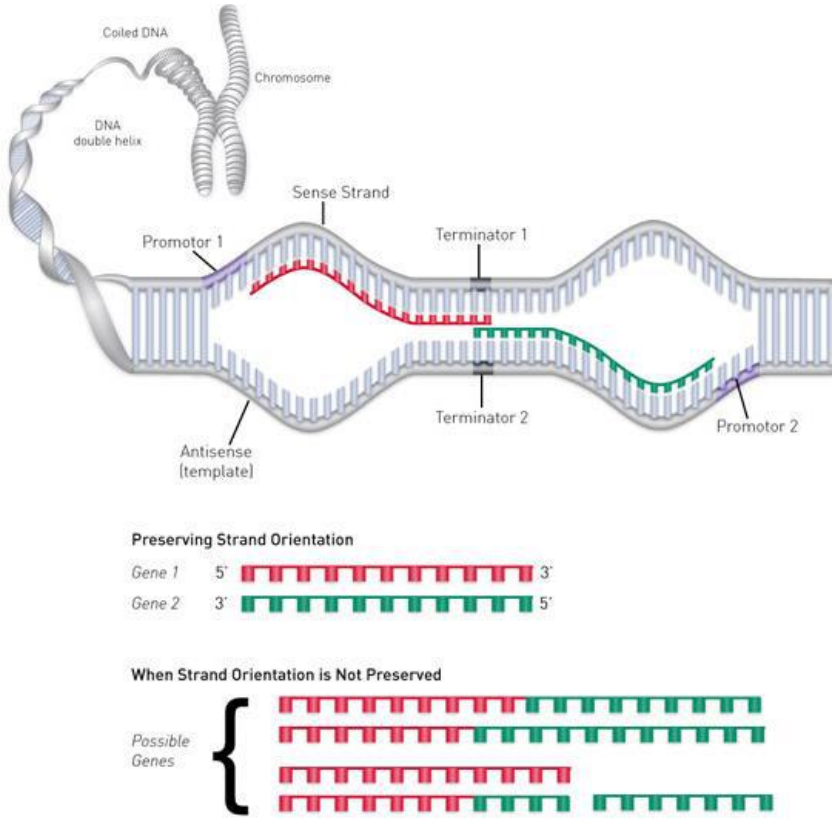
Product portfolio in the RNA-Seq workflow



SENSE™

Making sense of RNA sequencing

Strand-specific RNA sequencing is a crucial factor



About 25% of human genes share expressed mRNA regions and up to 10% of genes in the human genome participate in bi-directional promoters*.

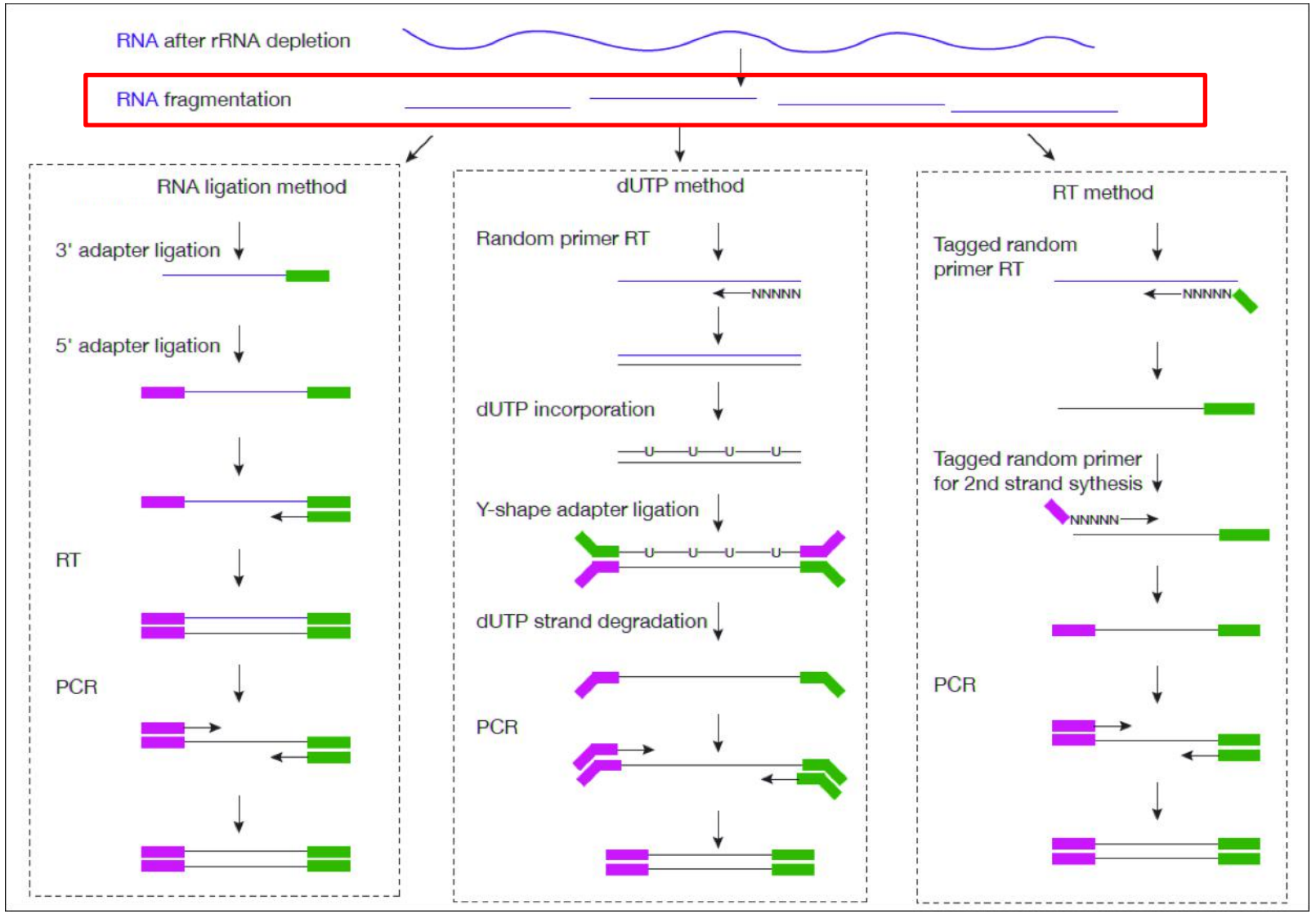
Stranded libraries **preserve information** about transcribed strand

- Detect **antisense** transcripts
- **Overlapping** genes
- **Fusion** genes

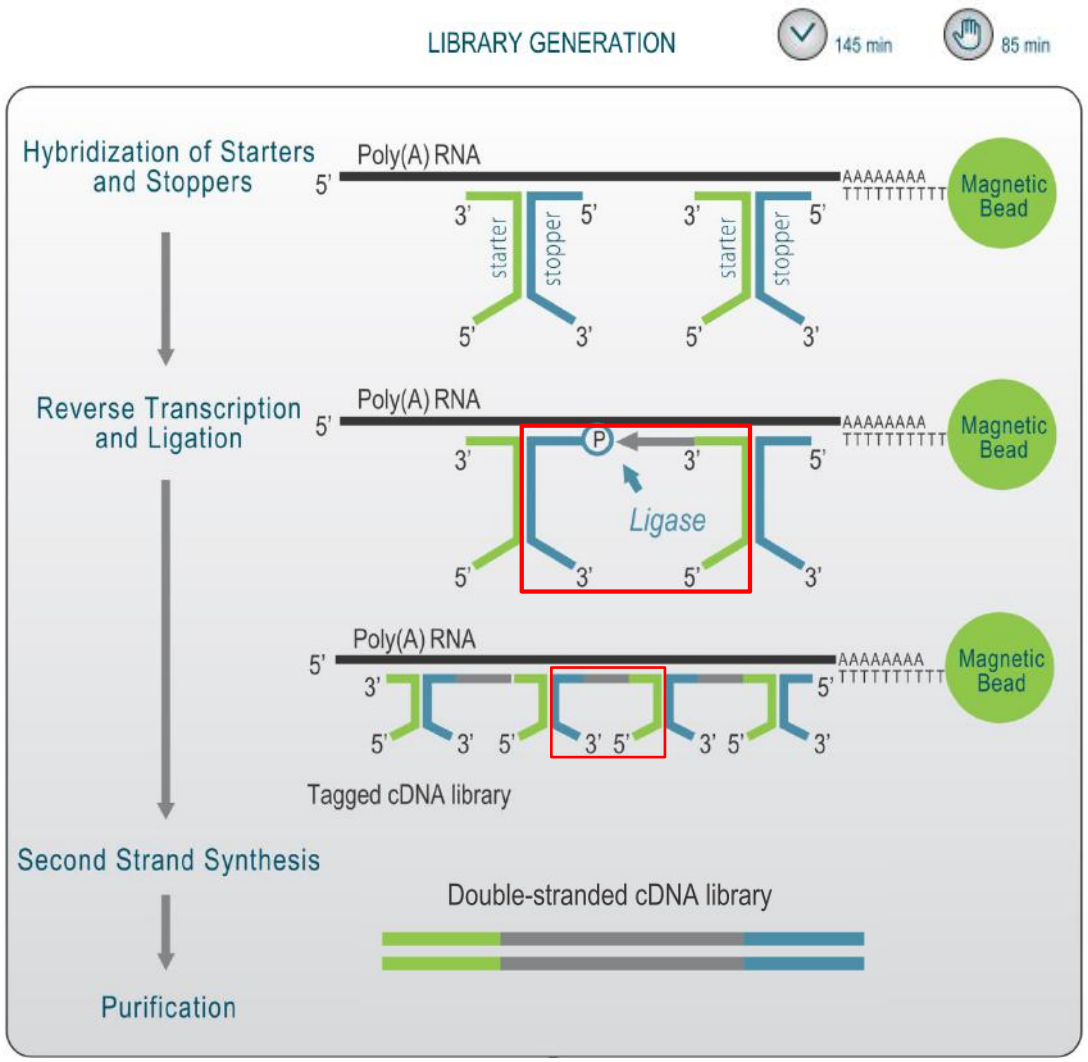
- Measure **gene expression**
- **Annotate** the structures of all transcribed genes
- Measure the extent of **alternative splicing**

* Engström, P.G. *et al.,* 2006, **Complex Loci in Human and Mouse Genomes**, *PLoS Genetics* V.2, p.0564-0577.

RNA-Seq library prep methods

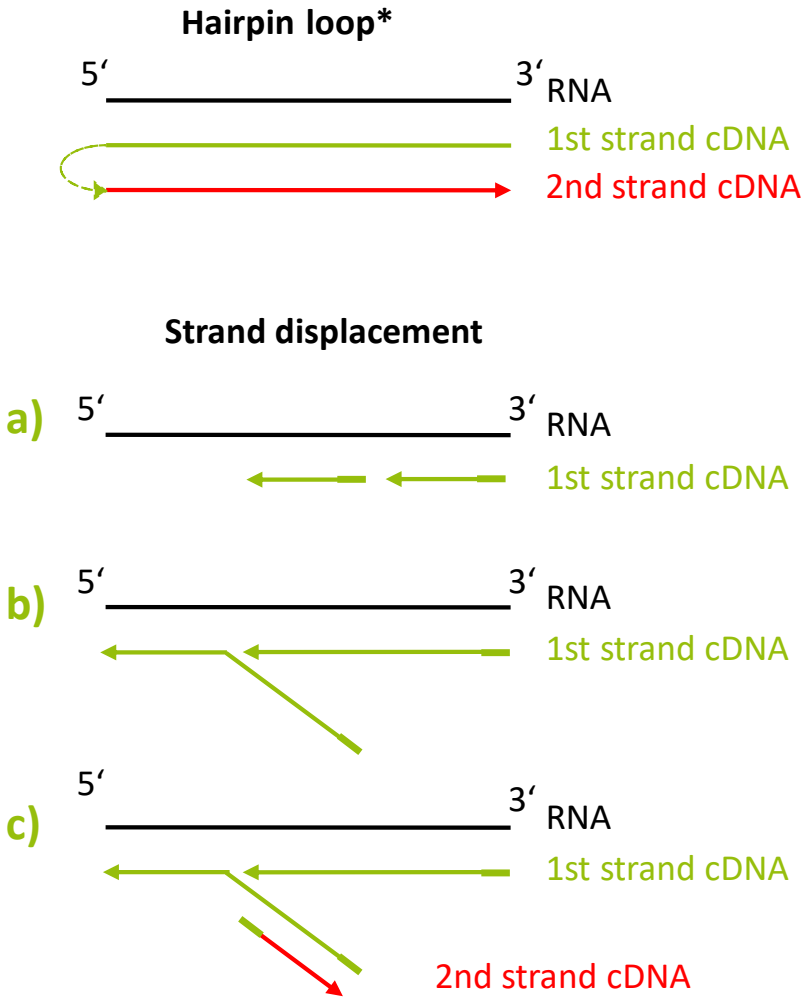


Lexogen's proprietary strand displacement stop/ligation technology



1. **Random hybridization** of **starter/stopper** heterodimers including sequencing platform-compatible linker sequences, to the RNA
2. **Extension** of the starter to the next hybridized heterodimer by reverse transcription
3. **Ligation** of the newly synthesized cDNA insert to the stopper
4. Hydrolysis of the RNA during **second strand synthesis** and conversion of the library to **double-stranded DNA**

Avoiding reverse transcription artifacts affecting strand-specificity



SENSE

- **No fragmentation**
- **No new 5’ ends**
- **No hairpin loop artifacts**

SENSE

- **Efficient stop of extension** (of the Starter at the next Stopper)
- **No strand displacement**

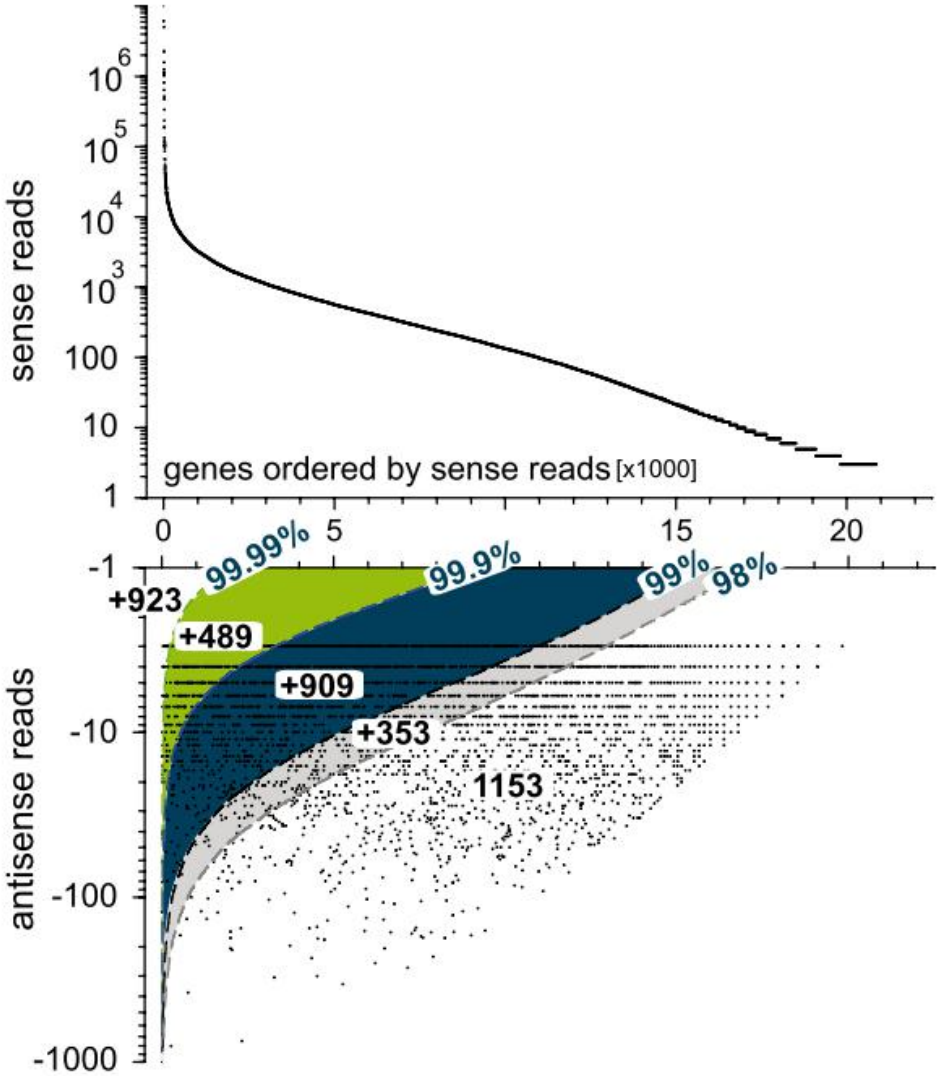
*Perocchi, F., et al., 2007, **Antisense artifacts in transcriptome microarray experiments are resolved by actinomycin D,** Nucleic Acids Res. 35(19): e128

Highly reproducible strand-specificity

- ✓ Mean strand-specificity: 99.922% (ERCCs)

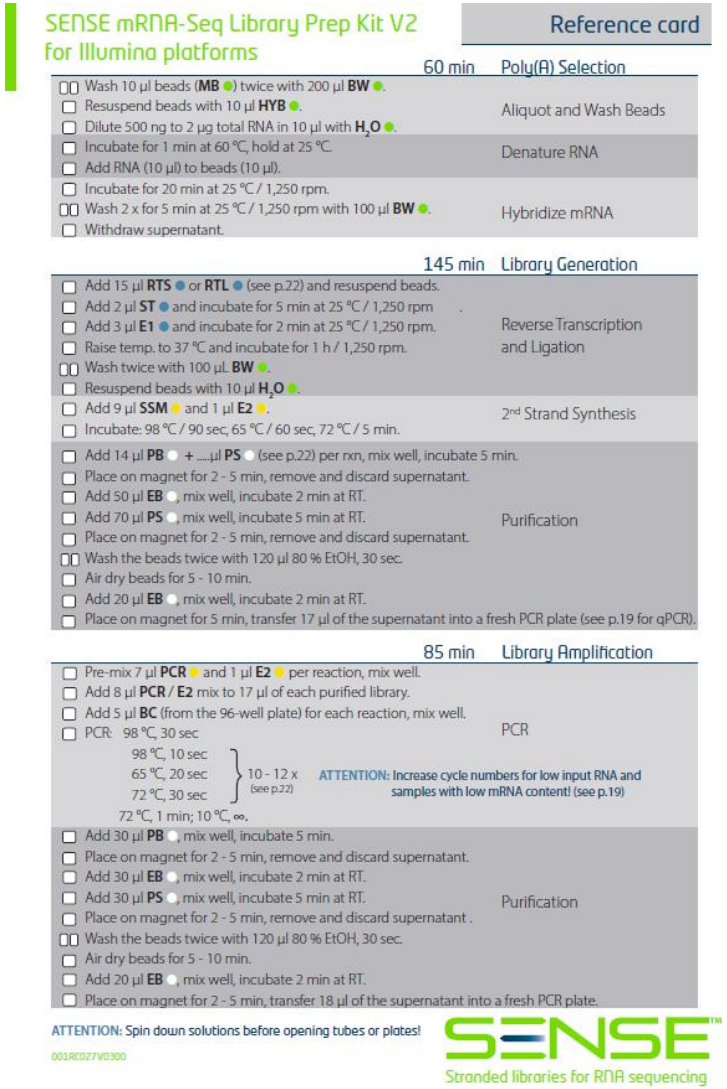
Experimenter 1		Experimenter 2		Experimenter 3	
Sample	Strandedness, %	Sample	Strandedness, %	Sample	Strandedness, %
12782 ACATTA	**99.927**	12790 AATGAA	**99.981**	12798 GATCAC	**99.975**
12783 GGTGAG	**99.975**	12791 GATTGT	**99.968**	12799 CGCGGA	**99.948**
12784 CGAAGG	**99.911**	12792 ATAAGA	**99.947**	12800 CCTAAG	**99.925**
12785 AAGACA	**99.944**	12793 GCCACA	**99.958**	12801 GGCTGC	**99.910**
12786 TAATCG	**99.943**	12794 GAACCT	**99.893**	12802 ACCAGT	**99.957**
12787 CGCAAC	**99.841**	12795 CGGTTA	**99.933**	12803 GTGCCA	**99.810**
12788 AATAGC	**99.693** min	12796 AACGCC	**99.886**	12804 AGATAG	**99.993** max
12789 TTAACT	**99.949**	12797 CAGATG	**99.864**	12805 TCGAGG	**99.987**

>99.9% strand-specificity ensures confident antisense transcript detection



Strand specificity	Antisense transcripts detected
99.99%	2904 (100%)
99.90%	2415 (83%)
99.00%	1506 (52%)
98.00%	1153 (40%)

SENSE



SENSE mRNA-Seq Library Prep Kit V2
for Illumina platforms

Reference card

60 min — Poly(A) Selection

- ☐☐ Wash 10 µl beads (**MB** ●) twice with 200 µl **BW** ●.
- ☐ Resuspend beads with 10 µl **HYB** ●.
- ☐ Dilute 500 ng to 2 µg total RNA in 10 µl with H_2O ●.
- ☐ Incubate for 1 min at 60 °C, hold at 25 °C.
- ☐ Add RNA (10 µl) to beads (10 µl).
- ☐ Incubate for 20 min at 25 °C / 1,250 rpm.
- ☐☐ Wash 2 x for 5 min at 25 °C / 1,250 rpm with 100 µl **BW** ●.
- ☐ Withdraw supernatant.

Aliquot and Wash Beads

Denature RNA

Hybridize mRNA

145 min — Library Generation

- ☐ Add 15 µl **RTS** ● or **RTL** ● (see p.22) and resuspend beads.
- ☐ Add 2 µl **ST** ● and incubate for 5 min at 25 °C / 1,250 rpm.
- ☐ Add 3 µl **E1** ● and incubate for 2 min at 25 °C / 1,250 rpm.
- ☐ Raise temp. to 37 °C and incubate for 1 h / 1,250 rpm.
- ☐☐ Wash twice with 100 µl. **BW** ●.
- ☐ Resuspend beads with 10 µl H_2O ●.

Reverse Transcription and Ligation

- ☐ Add 9 µl **SSM** ● and 1 µl **E2** ●.
- ☐ Incubate: 98 °C / 90 sec, 65 °C / 60 sec, 72 °C / 5 min.

2nd Strand Synthesis

- ☐ Add 14 µl **PB** ○ + ...µl **PS** ○ (see p.22) per rxn, mix well, incubate 5 min.
- ☐ Place on magnet for 2 - 5 min, remove and discard supernatant.
- ☐ Add 50 µl **EB** ○, mix well, incubate 2 min at RT.
- ☐ Add 70 µl **PS** ○, mix well, incubate 5 min at RT.
- ☐ Place on magnet for 2 - 5 min, remove and discard supernatant.
- ☐☐ Wash the beads twice with 120 µl 80 % EtOH, 30 sec.
- ☐ Air dry beads for 5 - 10 min.
- ☐ Add 20 µl **EB** ○, mix well, incubate 2 min at RT.
- ☐ Place on magnet for 5 min, transfer 17 µl of the supernatant into a fresh PCR plate (see p.19 for qPCR).

Purification

85 min — Library Amplification

- ☐ Pre-mix 7 µl **PCR** ● and 1 µl **E2** ● per reaction, mix well.
- ☐ Add 8 µl **PCR / E2** mix to 17 µl of each purified library.
- ☐ Add 5 µl **BC** (from the 96-well plate) for each reaction, mix well.
- ☐ PCR: 98 °C, 30 sec
  98 °C, 10 sec
  65 °C, 20 sec
  72 °C, 30 sec
  } 10 - 12 x (see p.22)
  72 °C, 1 min; 10 °C, ∞.

PCR

ATTENTION: Increase cycle numbers for low input RNA and samples with low mRNA content! (see p.19)

- ☐ Add 30 µl **PB** ○, mix well, incubate 5 min.
- ☐ Place on magnet for 2 - 5 min, remove and discard supernatant.
- ☐ Add 30 µl **EB** ○, mix well, incubate 2 min at RT.
- ☐ Add 30 µl **PS** ○, mix well, incubate 5 min at RT.
- ☐ Place on magnet for 2 - 5 min, remove and discard supernatant .
- ☐☐ Wash the beads twice with 120 µl 80 % EtOH, 30 sec.
- ☐ Air dry beads for 5 - 10 min.
- ☐ Add 20 µl **EB** ○, mix well, incubate 2 min at RT.
- ☐ Place on magnet for 2 - 5 min, transfer 18 µl of the supernatant into a fresh PCR plate.

Purification

ATTENTION: Spin down solutions before opening tubes or plates!

001RC027V0300

SENSE™
Stranded libraries for RNA sequencing

TruSeq

Ribo-Zero™ Deplete and Fragment RNA

Make RRP

1 Dilute the total RNA with nuclease-free ultra pure water to a final volume of 10 µl in the new 96-well 0.3 ml PCR plate labeled with the RRP barcode.
2 Add 5 µl of rRNA Binding Buffer to each well of the RRP plate.
3 Add 5 µl of one of the following reagents to each well of the RRP plate, depending on the kit you are using:
• Globin Removal Mix
• rRNA Removal Mix
• rRNA Removal Mix - Gold
• rRNA Removal Mix - Plant
4 Gently pipette the entire volume of each well of the RRP plate up and down 6 times to mix thoroughly.
5 Seal the RRP plate with a Microseal 'B' adhesive seal.
6 Return the following to -15°C to -25°C storage:
• rRNA Binding Buffer
• One of the following, depending on the kit you are using:
— Globin Removal Mix
— rRNA Removal Mix
— rRNA Removal Mix - Gold
— rRNA Removal Mix - Plant

Incubate 1 RRP

7 Place the sealed RRP plate on the pre-programmed thermal cycler. Close the lid, then select and run the RNA Denaturation program.
a Choose the pre-heat lid option and set to 100°C
b 68°C for 5 minutes
8 After the 5 minute incubation, place the RRP plate on the bench and incubate at room temperature for 1 minute

Make RRP

9 Vortex the room temperature RNA Removal Bead tube vigorously to resuspend the beads.
10 Add 95 µl of RNA Removal Beads to each well of the new 96-well 0.3 ml PCR plate labeled with the RRP barcode.
11 Remove the adhesive seal from the RRP plate.
12 Transfer the entire contents from each well of the RRP plate to the corresponding well of the RRP plate containing rRNA Removal Beads.
13 Adjust the pipette to 45 µl, then with the tip of the pipette at the bottom of the well, pipette quickly up and down 20 times to mix thoroughly.
14 Incubate the RRP plate at room temperature for 1 minute.
15 Place the RRP plate on the magnetic stand at room temperature for 1 minute.
16 Transfer all of the supernatant from each well of the RRP plate to the corresponding well of the new 96-well 0.3 ml PCR plate labeled with the RCP barcode.
17 Place the RCP plate on the magnetic stand at room temperature for 1 minute.
18 Return the rRNA Removal Beads to 2°C to 8°C storage.

Clean Up RCP

19 Vortex the RNAClean XP beads until they are well dispersed, then add 90 µl of well-mixed RNAClean XP beads to each well of the RCP plate containing ribosomal depleted RNA. Gently pipette the entire volume up and down 10 times to mix thoroughly.
NOTE
If starting with degraded total RNA, add 153 µl of well-mixed RNAClean XP beads to each well of the RCP plate containing ribosomal depleted RNA.
20 Incubate the RCP plate at room temperature for 15 minutes.
Start time: _____ Stop time: _____
21 Place the RCP plate on the magnetic stand at room temperature, for 5 minutes to make sure that all of the beads are bound to the side of the wells.
Start time: _____ Stop time: _____
22 Remove and discard all of the supernatant from each well of the RCP plate.
23 With the RCP plate on the magnetic stand, add 200 µl freshly prepared 70% EtOH to each well without disturbing the beads.
24 Incubate the RCP plate at room temperature for 30 seconds, and then remove and discard all of the supernatant from each well.
25 Let the RCP plate stand at room temperature for 15 minutes to dry, and then remove the plate from the magnetic stand.
Start time: _____ Stop time: _____
26 Centrifuge the thawed, room temperature Elution Buffer to 600 x g for 5 seconds.
27 Add 11 µl Elution Buffer to each well of the RCP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.
28 Incubate the RCP plate at room temperature for 2 minutes.
Start time: _____ Stop time: _____
29 Place the RCP plate on the magnetic stand at room temperature for 5 minutes.
Start time: _____ Stop time: _____
30 Return the Elution Buffer to 2°C to 8°C storage.
31 Transfer 8.5 µl supernatant from the RCP plate to the new 96-well 0.3 ml PCR plate labeled with the DFP barcode.
32 Add 8.5 µl Elute, Prime, Fragment High Mix to each well of the DFP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.
33 Seal the DFP plate with a Microseal 'B' adhesive seal.
34 Return the Elute, Prime, Fragment High Mix to -15°C to -25°C storage and the RNAClean XP Beads tube to 2°C to 8°C storage.

Incubate 1 DFP

35 Place the sealed DFP plate on the pre-programmed thermal cycler. Close the lid, then select and run the Elution 2 - Frag - Prime program.
a Choose the pre-heat lid option and set to 100°C
b 94°C for 8 minutes
c Hold at 4°C
36 Remove the DFP plate from the thermal cycler when it reaches 4°C and centrifuge briefly.
Proceed immediately to Synthesize First Strand cDNA on page 8.

Synthesize First Strand cDNA

Add FSA

38 Remove the adhesive seal from the DFP plate.
39 Centrifuge the thawed First Strand Synthesis Mix Act D tube to 600 x g for 5 seconds.
40 Add 50 µl SuperScript II to the First Strand Synthesis Act D Mix tube. If you are not using the entire contents of the First Strand Synthesis Act D Mix tube, add SuperScript II at a ratio of 1 µl SuperScript II for each 9 µl First Strand Synthesis Act D Mix. Mix gently, but thoroughly, and centrifuge briefly.
Label the First Strand Synthesis Mix Act D tube to indicate that the SuperScript II has been added.
41 Add 8 µl of First Strand Synthesis Mix Act D and SuperScript II mix to each well of the DFP plate. Gently pipette the entire volume up and down 6 times to mix thoroughly.
42 Seal the DFP plate with a Microseal 'B' adhesive seal and centrifuge briefly.
43 Return the First Strand Synthesis Mix Act D tube to -15°C to -25°C storage immediately after use.

Incubate 2 DFP

44 Place the sealed DFP plate on the pre-programmed thermal cycler. Close the lid, then select and run the Synthesize 1st Strand program.
a Choose the pre-heat lid option and set to 100°C
b 25°C for 10 minutes
c 42°C for 15 minutes
d 70°C for 15 minutes
e Hold at 4°C
45 When the thermal cycler reaches 4°C, remove the DFP plate from the thermal cycler and proceed immediately to Synthesize Second Strand cDNA on page 11.

Synthesize Second Strand cDNA

Add SMM

46 Remove the adhesive seal from the DFP plate.
47 Do one of the following:
• If using the in-line control reagent:
— Centrifuge the thawed End Repair Control tube to 600 x g for 5 seconds.
— Dilute the End Repair Control to 1/50 in Resuspension Buffer (For example, 2 µl End Repair Control + 98 µl Resuspension Buffer) before use.
— Add 5 µl of diluted End Repair Control to each well of the DFP plate.
• If not using the in-line control reagent, add 5 µl of Resuspension Buffer to each well of the DFP plate.
48 Centrifuge the thawed Second Strand Marking Master Mix to 600 x g for 5 seconds.
49 Add 20 µl of thawed Second Strand Marking Master Mix to each well of the DFP plate. Gently pipette the entire volume up and down 6 times to mix thoroughly.
50 Seal the DFP plate with a Microseal 'B' adhesive seal.
51 Return the Second Strand Marking Master Mix tube to -15°C to -25°C storage after use.

Incubate 3 DFP

52 Place the sealed DFP plate on the pre-heated thermal cycler. Close the lid and incubate at 16°C for 1 hour.
Start time: _____ Stop time: _____
53 Remove the DFP plate from the thermal cycler and place it on the bench.
54 Remove the adhesive seal from the DFP plate.
55 Let the DFP plate stand to bring it to room temperature.

Clean Up DFP

56 Vortex the AMPure XP beads until they are well dispersed.
57 Add 90 µl of well-mixed AMPure XP beads to each well of the DFP plate containing 50 µl of ds cDNA. Gently pipette the entire volume up and down 10 times to mix thoroughly.
58 Incubate the DFP plate at room temperature for 15 minutes.
Start time: _____ Stop time: _____
59 Place the DFP plate on the magnetic stand at room temperature, for 5 minutes to make sure that all of the beads are bound to the side of the wells.
Start time: _____ Stop time: _____
60 Remove and discard 155 µl supernatant from each well of the DFP plate.
61 With the DFP plate on the magnetic stand, add 200 µl freshly prepared 80% EtOH to each well without disturbing the beads.
62 Incubate the DFP plate at room temperature for 30 seconds, and then remove and discard all of the supernatant from each well.
63 Repeat steps 6 and 7 one time for a total of two 80% EtOH washes.
64 Let the DFP plate stand at room temperature for 15 minutes to dry, and then remove the plate from the magnetic stand.
Start time: _____ Stop time: _____
65 Centrifuge the thawed, room temperature Resuspension Buffer to 600 x g for 5 seconds.
66 Add 17.5 µl Resuspension Buffer to each well of the DFP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.
Start time: _____ Stop time: _____
67 Incubate the DFP plate at room temperature for 2 minutes.
Start time: _____ Stop time: _____
68 Place the DFP plate on the magnetic stand at room temperature for 5 minutes.
69 Transfer 15 µl supernatant (ds cDNA) from the DFP plate to the new 96-well 0.3 ml PCR plate labeled with the ALP barcode.

Adenylate 3' Ends

Add ATL

70 Do one of the following:
• If using the in-line control reagent:
— Centrifuge the thawed A-Tailing Control tube to 600 x g for 5 seconds.
— Dilute the A-Tailing Control to 1/100 in Resuspension Buffer (For example, 1 µl A-Tailing Control + 99 µl Resuspension Buffer) before use. Discard the diluted A-Tailing Control after use.
— Add 2.5 µl of diluted A-Tailing Control to each well of the ALP plate.
• If not using the in-line control reagent, add 2.5 µl of Resuspension Buffer to each well of the ALP plate.
80 Add 12.5 µl of thawed A-Tailing Mix to each well of the ALP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.
81 Seal the ALP plate with a Microseal 'B' adhesive seal.

Incubate 1 ALP

82 1 Place the sealed ALP plate on the pre-programmed thermal cycler. Close the lid, then select and run the ATAIL30 program.
a Choose the pre-heat lid option and set to 100°C
b 37°C for 30 minutes
c 70°C for 5 minutes
d Hold at 4°C
83 When the thermal cycler temperature is 4°C, remove the ALP plate from the thermal cycler, then proceed immediately to Ligate Adapters on page 17.

Ligate Adapters

Add LIG

84 Do one of the following:
• If using RNA Adapter tubes, centrifuge the thawed tubes to 600 x g for 5 seconds.
• If using a RAP:
— Thaw the plate for 10 minutes at room temperature on the benchtop. Visually inspect the wells to make sure that they all are thawed.
Start time: _____ Stop time: _____
— Remove the adapter plate tape seal.
— Centrifuge the plate to 280 x g for 1 minute to collect all of the adapter to the bottom of the well.
— Remove the plastic cover. Save the cover if you are not processing the entire plate at one time.
— If it is the first time using this RAP, apply the RAP barcode label to the plate.
NOTE
• The RAP is single-use for each well.
• Illumina recommends that the RAP does not undergo more than 4 freeze-thaw cycles.
85 Centrifuge the Ligation Control (if using Ligation Control) and Stop Ligation Buffer tubes to 600 x g for 5 seconds.
86 Immediately before use, remove the Ligation Mix tube from -15°C to -25°C storage.
87 Remove the adhesive seal from the ALP plate.
88 Do one of the following:
• If using the in-line control reagent:
— Dilute the Ligation Control to 1/100 in Resuspension Buffer (For example, 1 µl Ligation Control + 99 µl Resuspension Buffer) before use. Discard the diluted Ligation Control after use.
— Add 2.5 µl of diluted Ligation Control to each well of the ALP plate.
• If not using the in-line control reagent, add 2.5 µl of Resuspension Buffer to each well of the ALP plate.
89 Add 2.5 µl of Ligation Mix to each well of the ALP plate.
90 Return the Ligation Mix tube to -15°C to -25°C storage immediately after use.
91 Do one of the following:
• If using RNA Adapter tubes, add 2.5 µl of the thawed RNA Adapter Index to each well of the ALP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.
• If using a RAP:
— Place the RAP on the benchtop so that the part number barcode, on the long side of the plate, is facing you and the clipped corner is on the lower left.
— Do one of the following to pierce the foil seal:
— If using the entire plate at one time, use the bottom of a clean 96-well semi-skirted PCR plate to pierce a hole in all of the well seals simultaneously. Gently, but firmly, press the clean plate over the foil seal.
— If using only part of the plate, use the bottom of a clean eight-tube strip, with caps attached, to pierce holes in the seals of the wells that will be used for ligation. Repeat with a new, clean eight-tube strip, with caps attached, for each row or column of adapters that will be used for ligation.
— Using an eight-tip multichannel pipette, transfer 2.5 µl of the thawed RNA Adapter from the RAP well to each well of the ALP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.
92 Seal the ALP plate with a Microseal 'B' adhesive seal.
93 Centrifuge the ALP plate to 280 x g for 1 minute.

Incubate 2 ALP

94 Place the sealed ALP plate on the pre-heated thermal cycler. Close the lid and incubate at 30°C for 10 minutes.
Start time: _____ Stop time: _____
95 Remove the ALP plate from the thermal cycler.

Add STL

96 Remove the adhesive seal from the ALP plate.
97 Add 5 µl of Stop Ligation Buffer to each well of the ALP plate to inactivate the ligation. Gently pipette the entire volume up and down 10 times to mix thoroughly.

Clean Up ALP

98 Vortex the AMPure XP Beads for at least 1 minute or until they are well dispersed.
99 Add 42 µl of mixed AM Pure XP Beads to each well of the ALP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.
100 Incubate the ALP plate at room temperature for 15 minutes.
Start time: _____ Stop time: _____
101 Place the ALP plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.
Start time: _____ Stop time: _____
102 Remove and discard 79.5 µl supernatant from each well of the ALP plate.
103 With the ALP plate on the magnetic stand, add 200 µl freshly prepared 80% EtOH to each well without disturbing the beads.
104 Incubate the ALP plate at room temperature for 30 seconds, and then remove and discard all of the supernatant from each well.
105 Repeat steps 6 and 7 one time for a total of two 80% EtOH washes.
106 With the ALP plate on the magnetic stand, let the samples air-dry at room temperature for 15 minutes.
Start time: _____ Stop time: _____
107 Remove the ALP plate from the magnetic stand.
108 Add 52.5 µl Resuspension Buffer to each well of the ALP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly or until the beads are fully resuspended.
109 Incubate the ALP plate at room temperature for 2 minutes.
Start time: _____ Stop time: _____
110 Place the ALP plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.
Start time: _____ Stop time: _____
111 Transfer 50 µl supernatant from each well of the ALP plate to the corresponding well of the new 0.3 ml PCR plate labeled with the CAP barcode.
112 Vortex the AMPure XP Beads until they are well dispersed.
113 Add 50 µl of mixed AM Pure XP Beads to each well of the CAP plate for a second cleanup. Gently pipette the entire volume up and down 10 times to mix thoroughly.
114 Incubate the CAP plate at room temperature for 15 minutes.
Start time: _____ Stop time: _____
115 Place the CAP plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.
Start time: _____ Stop time: _____
116 Remove and discard 95 µl supernatant from each well of the CAP plate.
117 With the CAP plate on the magnetic stand, add 200 µl freshly prepared 80% EtOH to each well.
118 Incubate the CAP plate at room temperature for 30 seconds, and then remove and discard all of the supernatant from each well.
119 Repeat steps 20 and 21 one time for a total of two 80% EtOH washes.
120 With the CAP plate on the magnetic stand, let the samples air-dry at room temperature for 15 minutes, and then remove the plate from the magnetic stand.
Start time: _____ Stop time: _____
121 Add 22.5 µl Resuspension Buffer to each well of the CAP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly or until the beads are fully resuspended.
122 Incubate the CAP plate at room temperature for 2 minutes.
Start time: _____ Stop time: _____
123 Place the CAP plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.
Start time: _____ Stop time: _____
124 Transfer 20 µl supernatant from each well of the CAP plate to the corresponding well of the new 0.3 ml PCR plate labeled with the PCR barcode.

Enrich DNA Fragments

Make PCR

125 Add 5 µl of thawed PCR Primer Cocktail to each well of the PCR plate.
126 Add 25 µl of thawed PCR Master Mix to each well of the PCR plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.
127 Seal the PCR plate with a Microseal 'B' adhesive seal.

Amp PCR

128 1 Place the sealed PCR plate on the pre-programmed thermal cycler. Close the lid, then select and run PCR to amplify the plate.
a Choose the pre-heat lid option and set to 100°C
b 98°C for 30 seconds
c 11 cycles of:
— 98°C for 10 seconds
— 60°C for 30 seconds
— 72°C for 30 seconds
d 72°C for 5 minutes
e Hold at 4°C

Clean Up PCR

Remove the adhesive seal from the PCR plate.
Vortex the AMPure XP Beads until they are well dispersed.
Do one of the following, depending on the adapter type used:
• If using the RNA Adapter tubes, add 50 µl of the mixed AM Pure XP Beads to each well of the PCR plate containing 50 µl of the PCR amplified library. Gently pipette the entire volume up and down 10 times to mix thoroughly.
• If using the RAP, add 47.5 µl of the mixed AMPure XP Beads to each well of the PCR plate containing 50 µl of the PCR amplified library. Gently pipette the entire volume up and down 10 times to mix thoroughly.
Incubate the PCR plate at room temperature for 15 minutes.
Start time: _____ Stop time: _____
Place the PCR plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.
Start time: _____ Stop time: _____
Remove and discard 95 µl supernatant from each well of the PCR plate.
With the PCR plate on the magnetic stand, add 200 µl freshly prepared 80% EtOH to each well without disturbing the beads.
Incubate the PCR plate at room temperature for 30 seconds, and then remove and discard all of the supernatant from each well.
Repeat steps 7 and 8 one time for a total of two 80% EtOH washes.
With the PCR plate on the magnetic stand, let the samples air-dry at room temperature for 15 minutes, and then remove the plate from the magnetic stand.
Start time: _____ Stop time: _____
Add 32.5 µl Resuspension Buffer to each well of the PCR plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.
Incubate the PCR plate at room temperature for 2 minutes.
Start time: _____ Stop time: _____
Place the PCR plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.
Start time: _____ Stop time: _____
142 Transfer 30 µl supernatant from each well of the PCR plate to the corresponding well of the new 0.3 ml PCR plate labeled with the TSPS barcode.
SAFE STOPPING POINT
If you do not plan to proceed immediately to Validate Library on page 27, you can safely stop the protocol here. If you are stopping, seal the TSPS plate with a Microseal 'A' adhesive seal and store at -15°C to -25°C for up to 7 days.

NEW

CORALL™

Cover all bases

CORALL TOTAL RNA-SEQ LIBRARY PREP KIT

NEW

Excellent 5’ representation

Get full transcript coverage starting at the very 5’ end

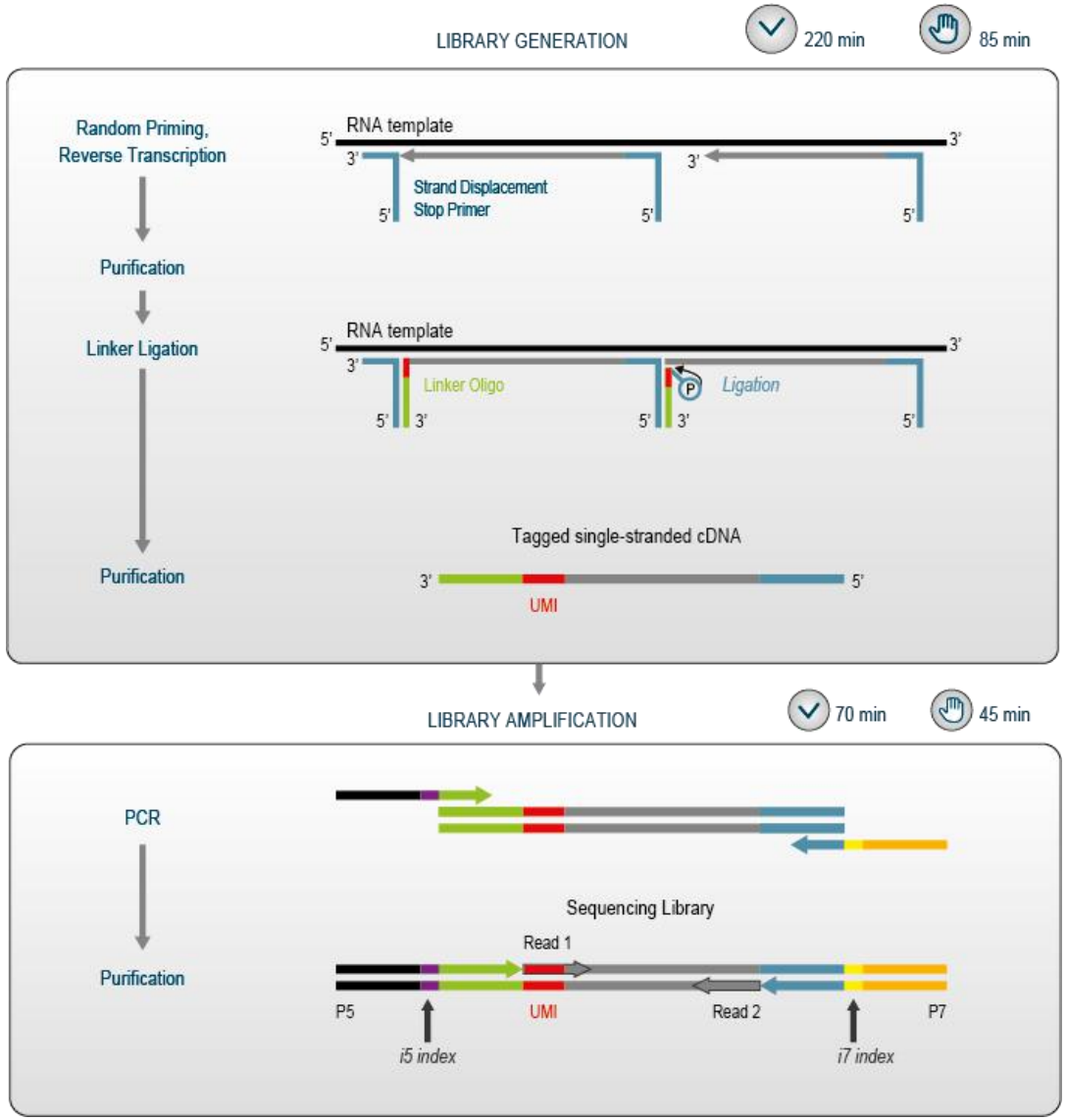
Fast and easy library prep

Prepare libraries in only 5 hours and save research time

Unique Molecular Identifiers included

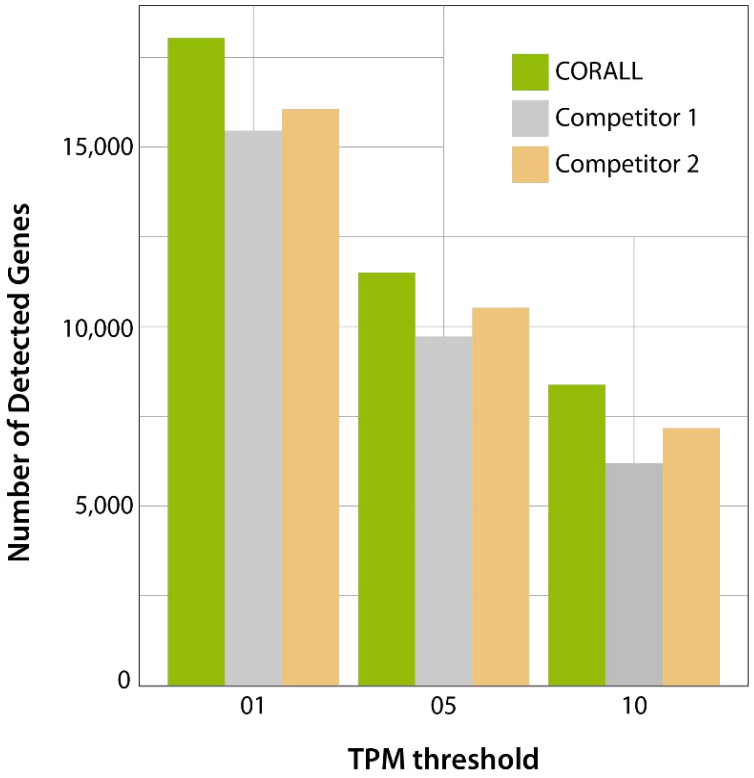
Tag individual transcripts to detect and eliminate amplification bias

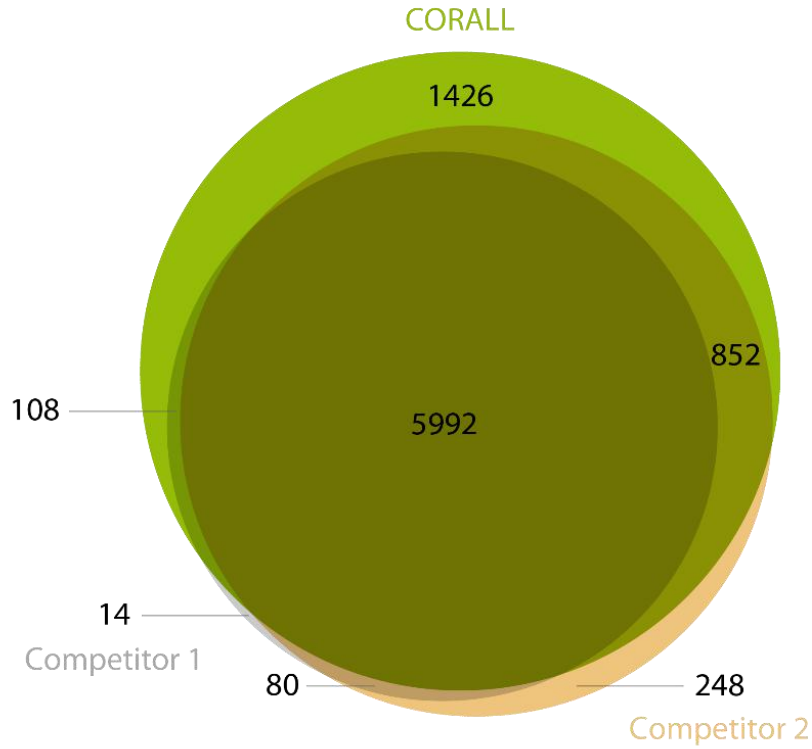
CORALL WORKFLOW



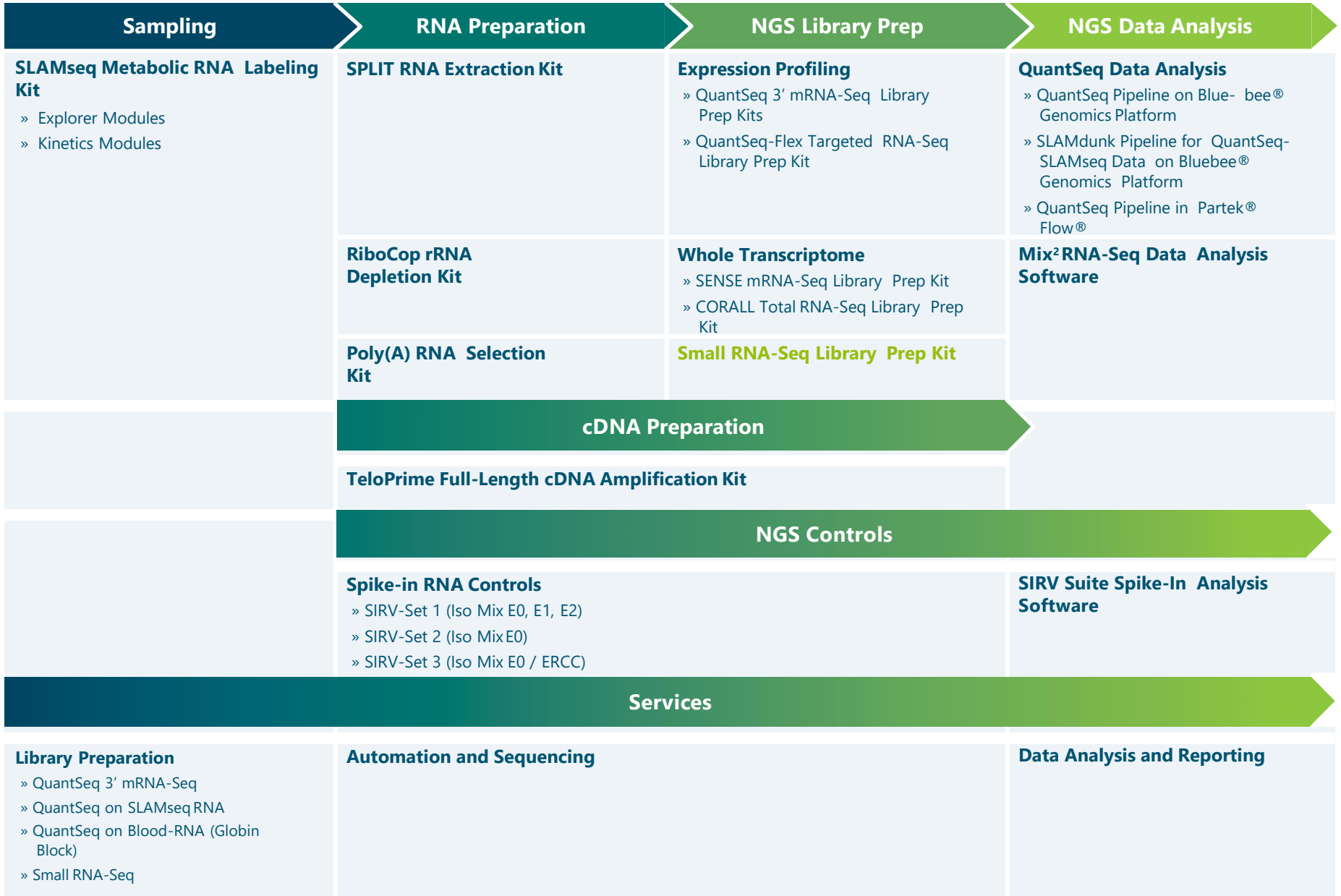
CORALL - ENHANCED GENE DETECTION

- ✓ Surpassing gene detection levels, even at high TPM thresholds
- ✓ 96% of all genes detected by two other methods covered and additional 20% detected (TPM>10)
- ✓ 76% of exclusively detected genes are protein-coding genes



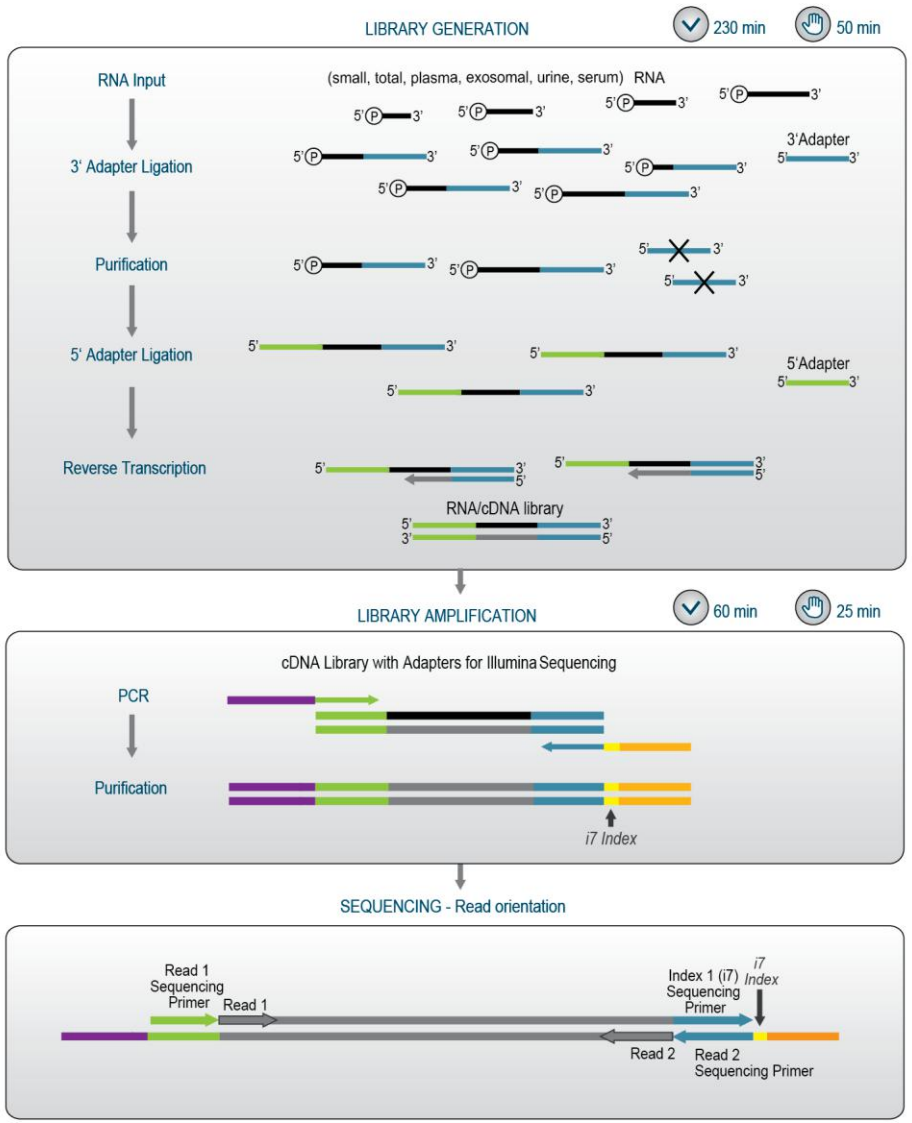


Product portfolio in the RNA-Seq workflow



Small RNA-Seq Kit

Lexogen's Small RNA-Seq Kit for Illumina



Exceptional miRNA discovery and high reproducibility

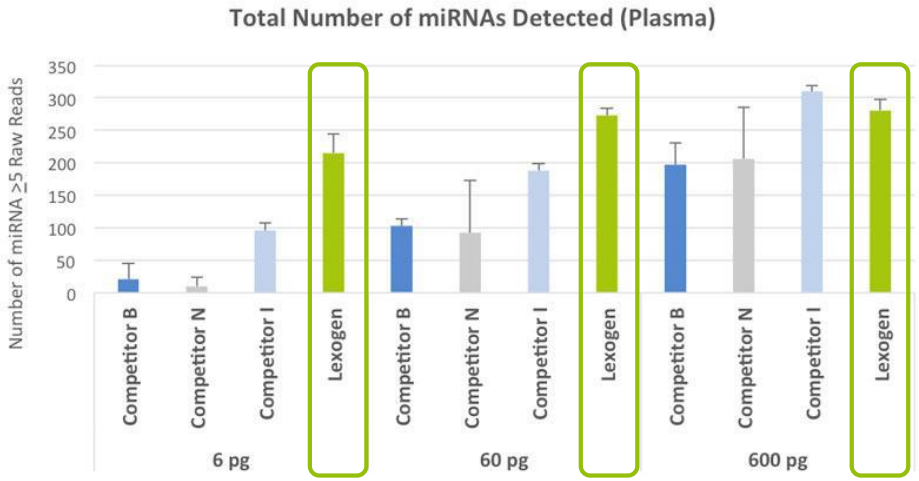


Figure | Total number of miRNAs detected across 4 different protocols. Dilution series (6 pg, 60 pg, and 600 pg) of purified plasma RNA used for library prep from different vendors were performed. The obtained libraries were sequenced at equal molarity at ~ 1.5 – 2M Total Raw Reads per sample. Lexogen's Small RNA-Seq Kit showed much higher numbers of detected miRNAs at ≥ 5 Raw Reads across all concentrations tested.

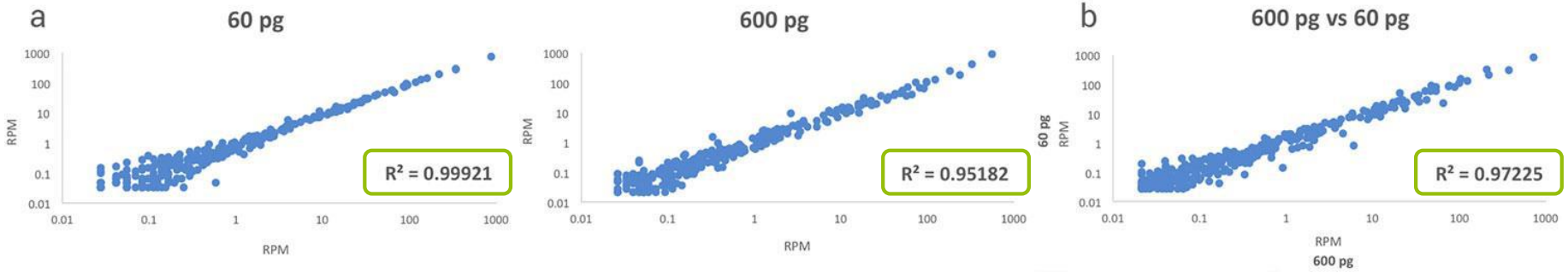


Figure | a) Dilution series of purified plasma RNA in Small RNA-Seq shows high inter-replicate correlation Reads per Million (RPM) across concentrations tested (60 pg and 600 pg). **b)** Dilution series of purified plasma RNA in Small RNA-Seq shows high correlation RPM across concentrations tested (60 pg and 600 pg). As an example, only data for 60 pg and 600 pg are shown.

sRNA-Seq Kit – key features

- **Discovery and profiling of small RNA (**≤ 200 nt), including miRNA, piRNA, siRNA, snoRNA, tsRNA, srRNA, U-RNA
- **Gel-free user-friendly workflow** (bundle version with Purification Module with Magnetic Beads available (Cat. No. 058))
- **Wide input range** from **50 pg to 1 µg of RNA**
  (1 ng – 1,000 ng of cellular total RNA or 50 pg – 1,000 ng enriched small RNA including plasma, serum, and urine)
- **Optimized** for **low RNA content samples** such as **liquid biopsies** (e.g., plasma, serum, and urine) including **exosomes**
- **All-in-one** protocol – only **75 minutes hands-on** time
- **High reproducibility** for inter-replicates and across concentrations
  (R^2 0.999 to 0.952)
- **Exceptional miRNA discovery** compared to other protocols, **in particular** for **low RNA inputs**
- **Combination** with **SPLIT RNA Extraction Kit** offers the complete solution for small RNA analysis

Product portfolio in the RNA-Seq workflow

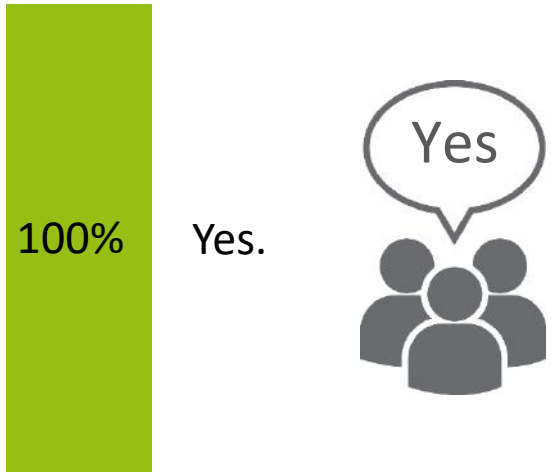
Sampling	RNA Preparation	NGS Library Prep	NGS Data Analysis
SLAMseq Metabolic RNA Labeling Kit » Explorer Modules » Kinetics Modules	**SPLIT RNA Extraction Kit**	**Expression Profiling** » QuantSeq 3′ mRNA-Seq Library Prep Kits » QuantSeq-Flex Targeted RNA-Seq Library Prep Kit	**QuantSeq Data Analysis** » QuantSeq Pipeline on Blue- bee® Genomics Platform » SLAMdunk Pipeline for QuantSeq-SLAMseq Data on Bluebee® Genomics Platform » QuantSeq Pipeline in Partek® Flow®
	RiboCop rRNA Depletion Kit	**Whole Transcriptome** » SENSE mRNA-Seq Library Prep Kit » CORALL Total RNA-Seq Library Prep Kit	**Mix² RNA-Seq Data Analysis Software**
	Poly(A) RNA Selection Kit	**Small RNA-Seq Library Prep Kit**	
	cDNA Preparation		
	TeloPrime Full-Length cDNA Amplification Kit		
	NGS Controls		
	Spike-in RNA Controls » SIRV-Set 1 (Iso Mix E0, E1, E2) » SIRV-Set 2 (Iso Mix E0) » SIRV-Set 3 (Iso Mix E0 / ERCC)		**SIRV Suite Spike-In Analysis Software**
Services			
Library Preparation » QuantSeq 3′ mRNA-Seq » QuantSeq on SLAMseq RNA » QuantSeq on Blood-RNA (Globin Block) » Small RNA-Seq	**Automation and Sequencing**		**Data Analysis and Reporting**

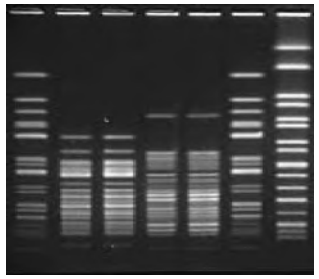
SiRVs™

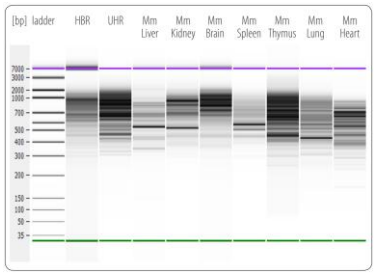
Spike-in RNA Variant Control Mixes

Do you use controls in your experiments?

Do you use spike in controls
in your **RNA** experiments?

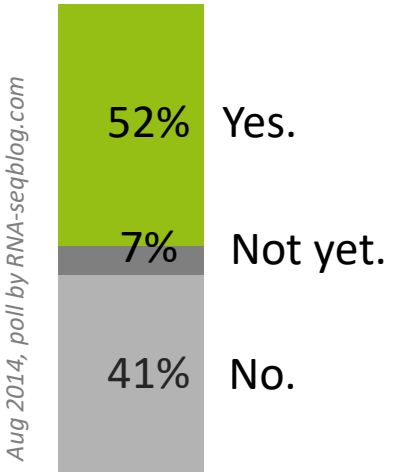






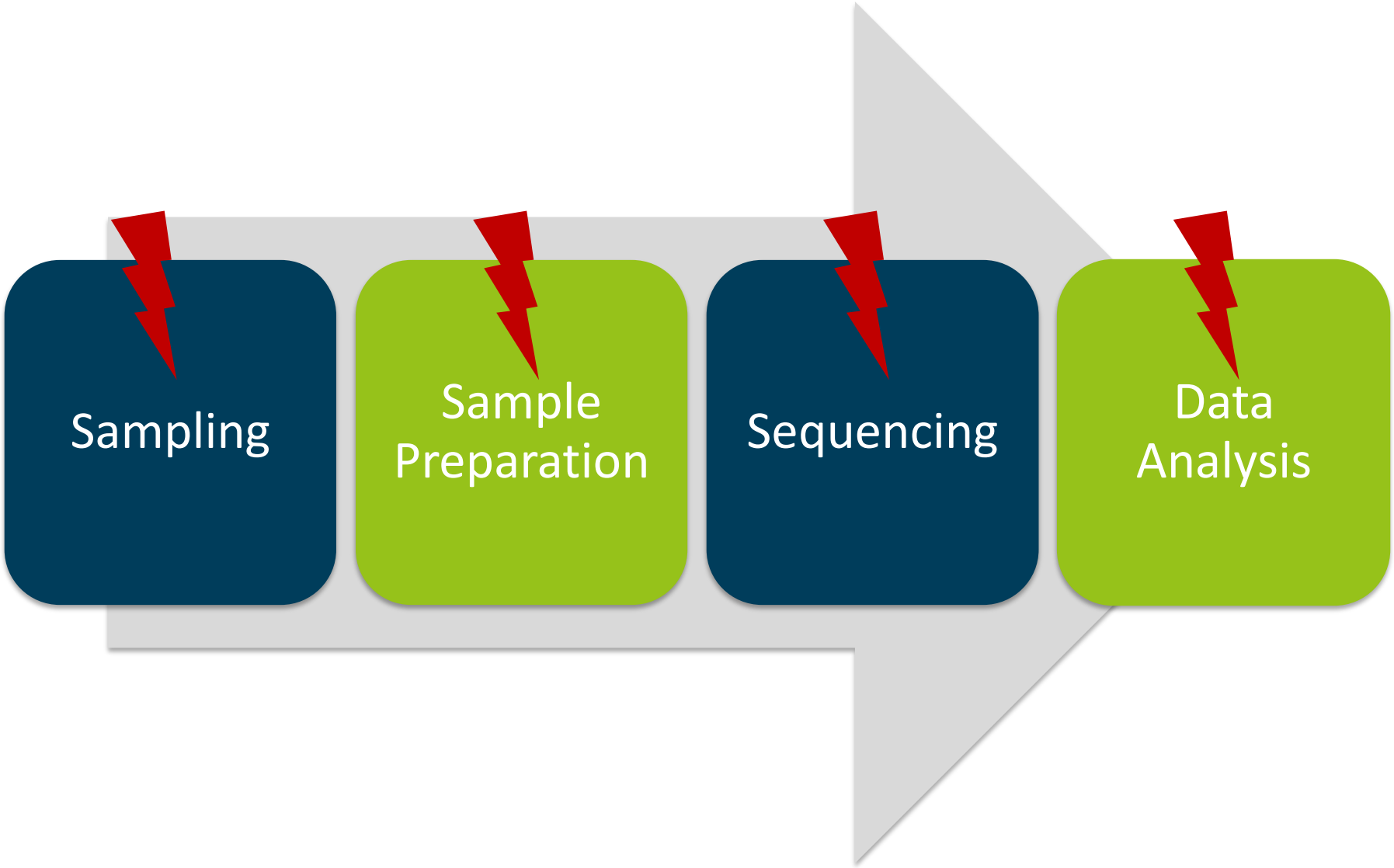
~ 100 USD

Do you use spike in controls
in your **RNA-Seq** experiments?



48 samples	your samples
Library preparation	950 -1˙630 USD
125 PE, 300 Mreads	2˙500 USD
Bioinformatics	substantial
	> 3˙450 USD

Biases in RNA-Seq



Spike-in controls are essential in RNA-Seq

Validation and **Monitoring** of the RNA-seq workflow assesses

- **technical parameters** such as biases and sensitivity
- **functional aspects** like isoform detection and quantification

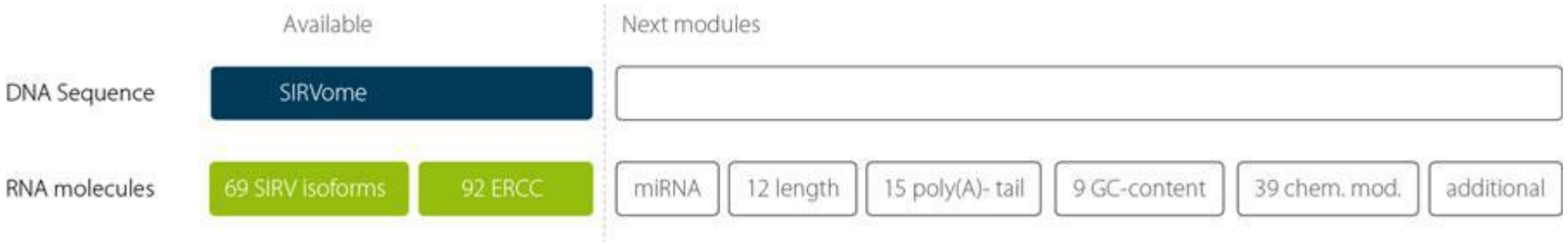
Know the **performance** and the **limits** of your RNA-seq workflow and data evaluation pipeline.

Concordance determines whether samples are comparable

- **in experiments** and
- **between experiments**

Know the **technical variability** between **samples** and **experiments** before investigating **biological variance**.

SIRV modules address transcriptome complexity in a defined and condensed manner



- **Transcriptome properties:** high complexity and large concentration differences, several RNA classes with specific properties

- **SIRVs as a family of modules:** each module probes a specific aspect
  - **SIRV isoform module:** transcription and splicing variants -> isoforms
  - **ERCC single isoform module** -> concentrations

The SIRVome = SIRV genome

Current SIRV genome: **7 SIRV isoform** genes and **92 ERCC genes**

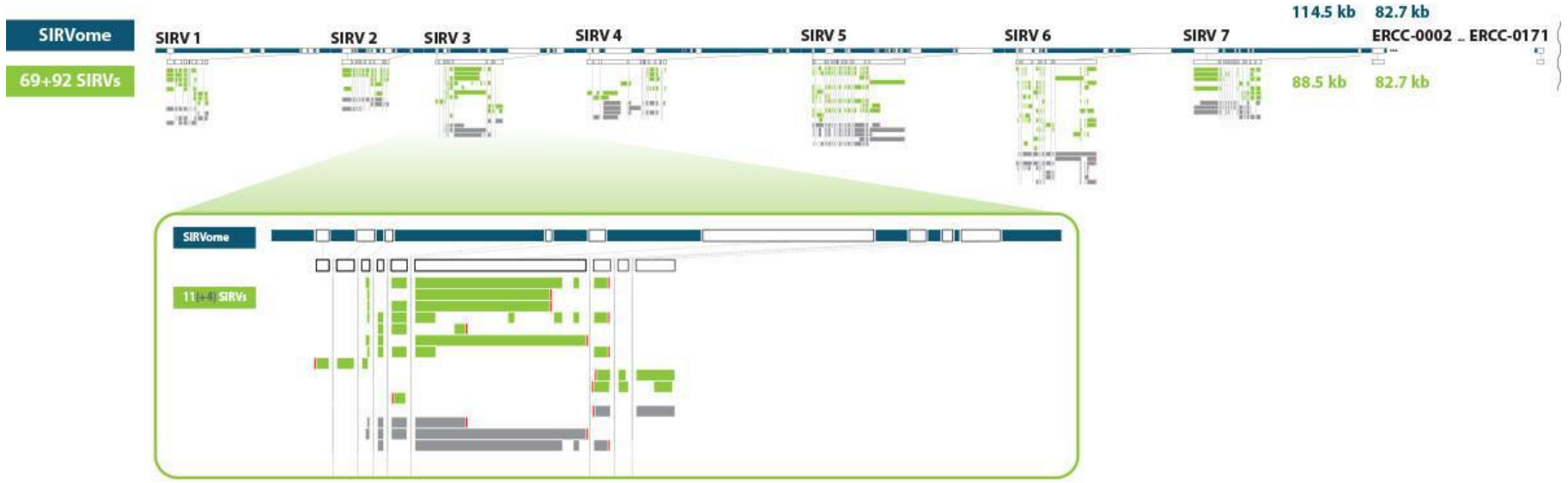


Figure | SIRVome and SIRV transcripts. The SIRV isoform and ERCC genes are lined up on a SIRVome. Pullout, the Compact Coverage Visualization (CCV) shows intron regions common to all transcripts as short standardized gaps irrespective of the original sequence length, which provides a more comparable overview of the actual SIRV transcripts.

- **Sequences** and **gene definitions:** multi-FASTA and GTF files for the individual SIRV genes (isoforms and ERCCs) and the entire SIRVome are freely accessible at https://www.lexogen.com/sirvs
- **GenBank**: SIRV isoform sequences can be accessed using No KX147759 to KX147765 for SIRV1 to SIRV7

SIRV isoform module

- **7 artificial genes** (derived from human model genes) with 6-18 transcript variants each, **69 *in-vitro* transcript variants** in total
- **Length 191 - 2528 nt, poly(A_{30})-tail**, **GC**-content **29.5 – 51.2 %**
- **Canonical GT-AG exon-intron junctions** with known exceptions
- **Intron sequences:** random, maintain GC content of upstream and downstream exons
- **No significant similarity** to any **known sequences**
- Three mixes **E0, E1, and E2** with molar ratios of transcripts at **magnitudes of 0, 1, and 2,** respectively
- Also available in **conjunction with ERCCs**

SIRVs mimic the complexity of the transcriptome

- **Alternative splicing**
- **Promoter** and **poly(A) site usage**
- **Overlapping genes**
- **Antisense transcription**

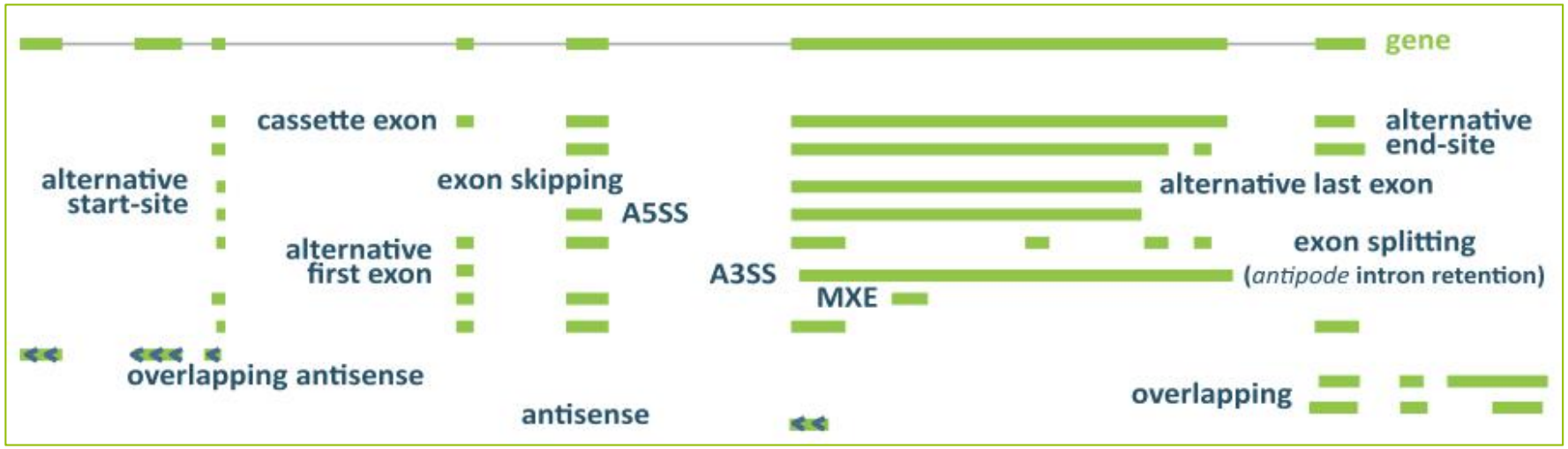
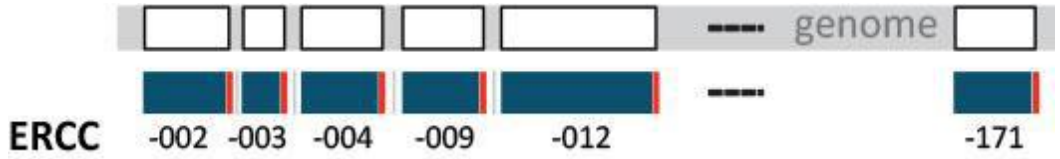


Figure | SIRVs design overview. SIRVs genes mimic human model genes to represent in their entity all main aspects of alternative transcription in numerous repeats and variations. A5SS and A3SS, alternative 5'/3' splice sites, MXE, mutually exclusive exons.

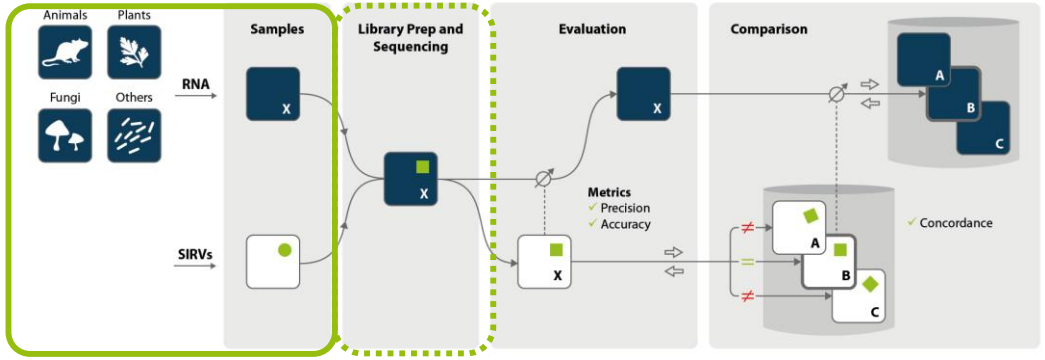
ERCC module



- Developed by the External RNA Controls Consortium (ERCC)
- **Exemplary studies** by **the FDA Sequencing Quality Control** (**SEQC)** Consortium and the **Association of Biomolecular Resource Facilities (ABRF**)
- **92 artificial transcripts** with **non-overlapping sequences following the 1 gene, 1 exon, and 1 transcript layout**
- Unique sequence identities makes them well suited for measuring **technical parameters irrespective of isoforms**
- **Standardized assessment** of **gene expression platforms** such as quantitative **RT-PCR, microarrays, and NGS** technologies
  - **Dynamic range**
  - **Dose response**
  - **Lower limit** of **detection** and **efficiency**
  - **Fold change response** of RNA sequencing pipelines,

within the complexity boundaries of monoexonic, non-overlapping RNA sequences.

Spiking of samples



SIRV SUITE

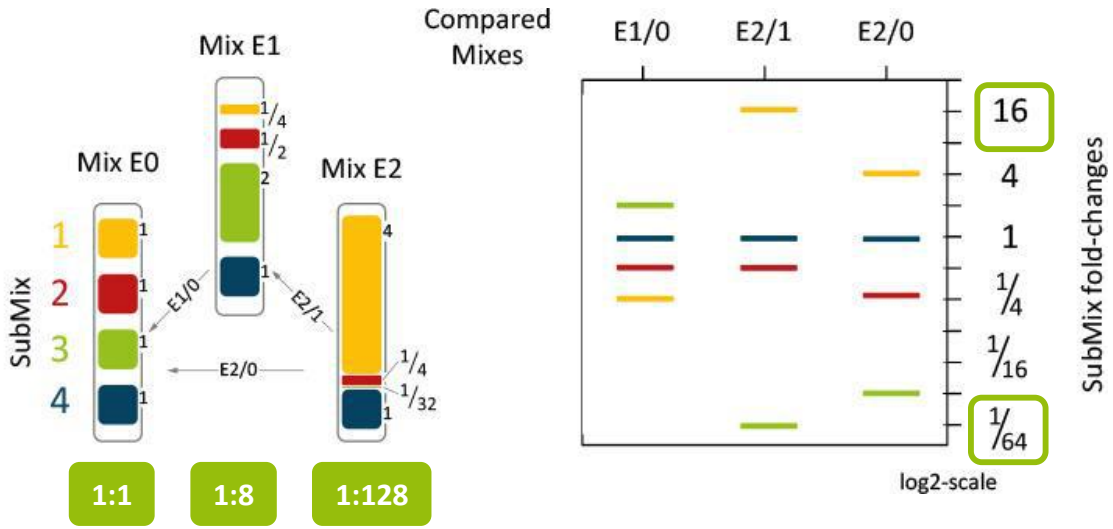
Experiment Designer*

- Spike SIRVs into sample: **during or after RNA extraction**
- SIRVs have **exclusive sequences** – spike into **any sample or organism**
- SIRVs are **polyadenylated** – library prep can start from poly(A) selected fractions as well as from total RNA, depleted RNA, etc.
- **Spike-in RNA amount**: only a **small fraction** (usually 1 %) **of all NGS reads** mapping to the SIRV genome, the “SIRVome”.

* For SIRV isoforms

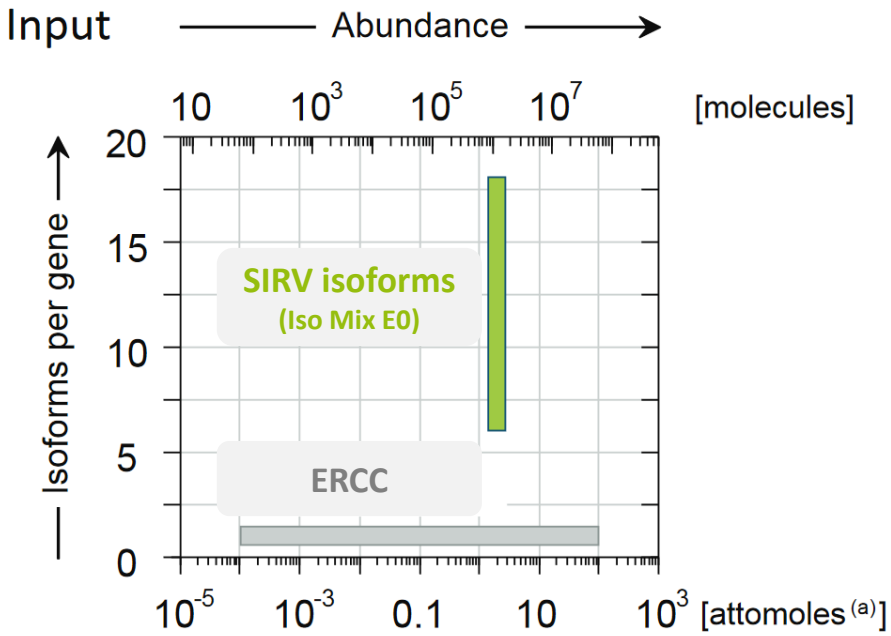
Spike-in RNA Variant Control Mixes (SIRV set 1)

- **3 predefined SIRV mixes**: E0, E1, and E2
- Each mix contains **all 69 SIRVs** in **different concentration** ratios

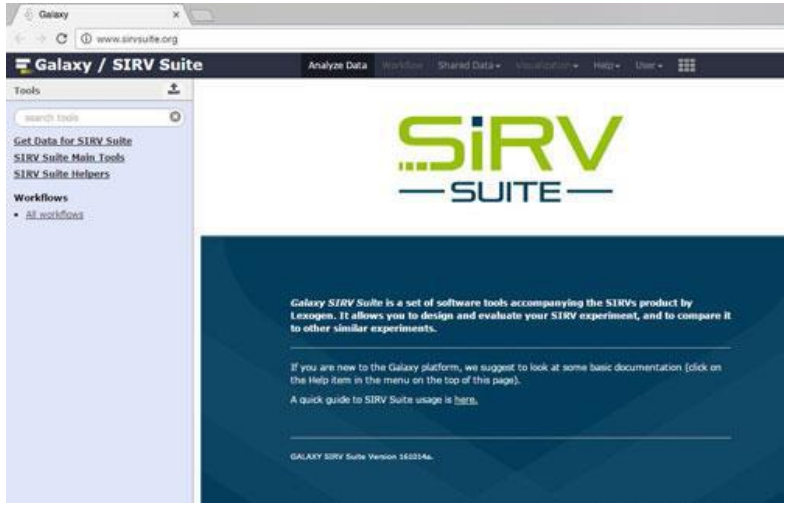


SIRV-Set 2 and SIRV-Set 3 contain the isoform mix E0

- **SIRV-Set 2** (Cat. No. 050.xx) contains mix **E0 only**
- **SIRV-Set 3** (Cat. No. 051.xx) contains mix **E0** in mixture **with** the **ERCC** mix
- These sets may come in **dried format.**



- **A set of freely available software tools** accompanying SIRV isoforms
- **Streamlined** and **unified data evaluation process** embedded in the **Galaxy environment** (galaxyproject.org)
- **Evaluator report (pdf)** as **standardized documentation** of SIRV isoforms evaluation
  - **Modules:**
    - **Designer:** plan experiments
    - **Specifier**: enter experiment information
    - **Evaluator:** evaluate SIRV isoforms data and obtain a standardized but customizable report and data files for downstream applications
    - **Comparator:** Cross-sample comparisons and referencing
    - **Data Base:** experiment metadata, SIRV isoform reads, quality metrics



Webinar: https://www.lexogen.com/controlling-rna-seq-experiments-using-spike-in-rna-variants/

SIRVs – key features

- **Spike-ins** to RNA-Seq experiments with **exclusive sequences** (platform and sample independent)
- **SIRV isoforms** provide **isoform complexity** through **mimicking transcription** and **alternative splicing** variations
- **ERCCs** provide **abundance complexity** through **high dynamic range** (10^6)
- **Assess** your pipeline for **transcript isoform detection** and **quantification performance**
- **Determine accuracy** and **precision** in concentration and differential expression measurements
- **Validation** of **workflows** and **experiments** under authentic conditions
- **Identify** the sources of **errors** and help **improving experimental workflows** from library preparation, over sequencing up to data evaluations
- Provide an universal marker for **comparing** RNA-Seq **experiments**
- **SIRV Suite: Streamlined** and **unified data evaluation process** embedded in **Galaxy**
- **SIRV-Set 2 and 3** as **dried format – easy delivery, storage** and **handling**

Watch our SIRVs webinar

Lukas Paul, PhD
Head of Services, Lexogen

https://www.lexogen.com/controlling-rna-seq-experiments-using-spike-in-rna-variants/

- The **concept of complex RNA spike-ins** covering **transcription** and **splicing events**
- **Design of SIRV mixes** to allow for **RNA-Seq** pipeline **quality control** and **validation**
- Assessment of **differential gene expression** at the transcript level
- The **SIRV Suite**, a Galaxy-based platform for easy and complete spike-in experiment design, data evaluation and comparison
- The **concept of the quality matrix** (coverage CoD, accuracy, precision) and **concordance**
- The SIRV Suite data base to find and research **comparable RNA-Seq experiments** - on the transcript level
- **Upcoming RNA spike-in modules**: from transcript length, poly(A) variants to base modifications

Product portfolio in the RNA-Seq workflow

Sampling	RNA Preparation	NGS Library Prep	NGS Data Analysis
SLAMseq Metabolic RNA Labeling Kit » Explorer Modules » Kinetics Modules	**SPLIT RNA Extraction Kit**	**Expression Profiling** » QuantSeq 3′ mRNA-Seq Library Prep Kits » QuantSeq-Flex Targeted RNA-Seq Library Prep Kit	**QuantSeq Data Analysis** » QuantSeq Pipeline on Blue- bee® Genomics Platform » SLAMdunk Pipeline for QuantSeq-SLAMseq Data on Bluebee® Genomics Platform » QuantSeq Pipeline in Partek® Flow®
	RiboCop rRNA Depletion Kit	**Whole Transcriptome** » SENSE mRNA-Seq Library Prep Kit »SENSE Total RNA-Seq Library Prep Kit	**Mix² RNA-Seq Data Analysis Software**
	Poly(A) RNA Selection Kit	**Small RNA-Seq Library Prep Kit**	

cDNA Preparation
TeloPrime Full-Length cDNA Amplification Kit

NGS Controls	
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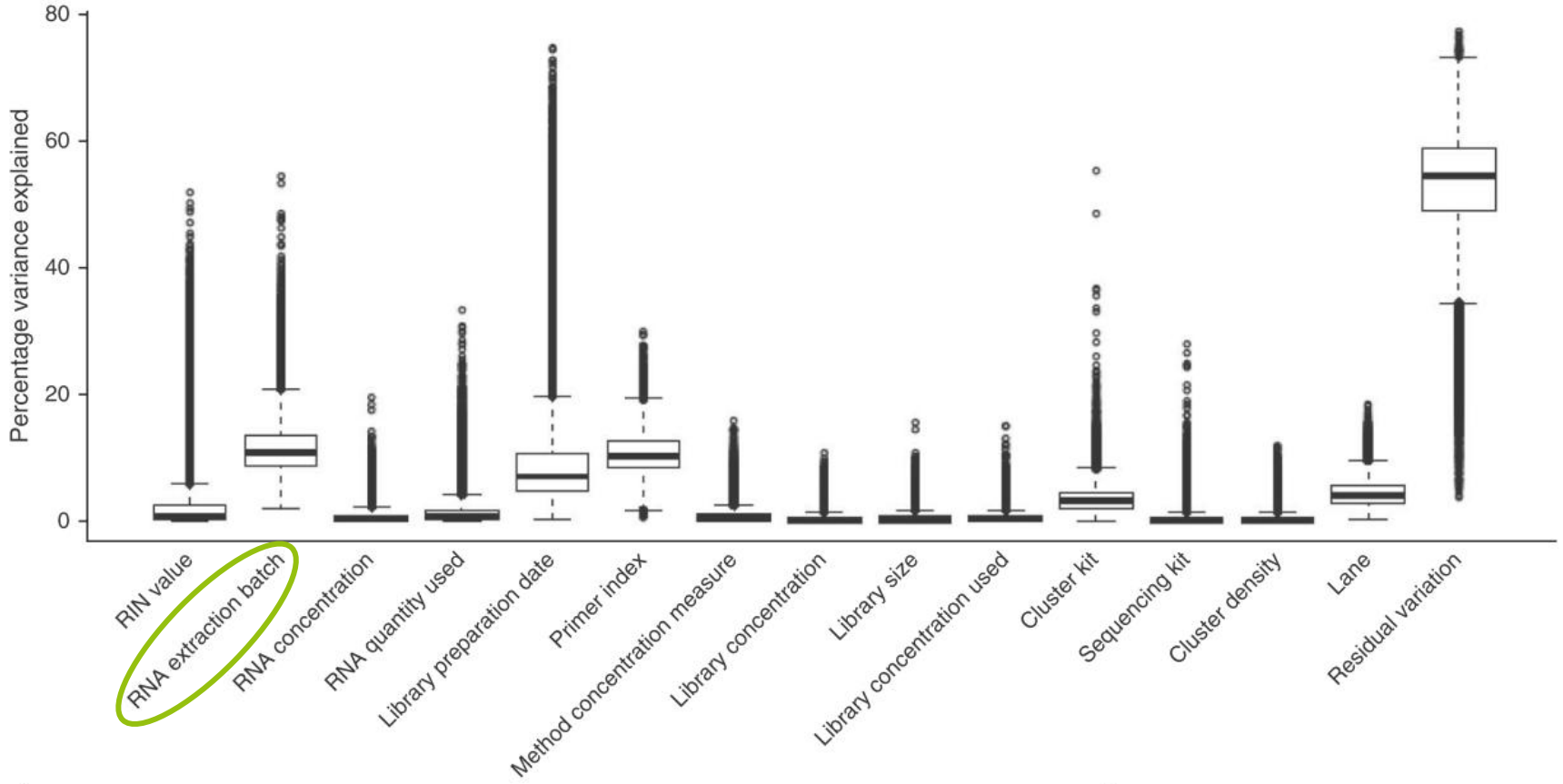
Services		
Library Preparation » QuantSeq 3′ mRNA-Seq » QuantSeq on SLAMseq RNA » QuantSeq on Blood-RNA (Globin Block) » Small RNA-Seq	**Automation and Sequencing**	**Data Analysis and Reporting**

SPLIT

Fractions for pure RNA sequencing

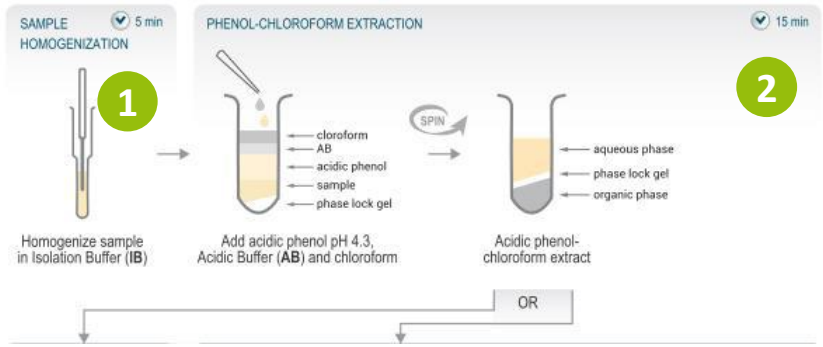
Sources of variation in RNA sequencing data*

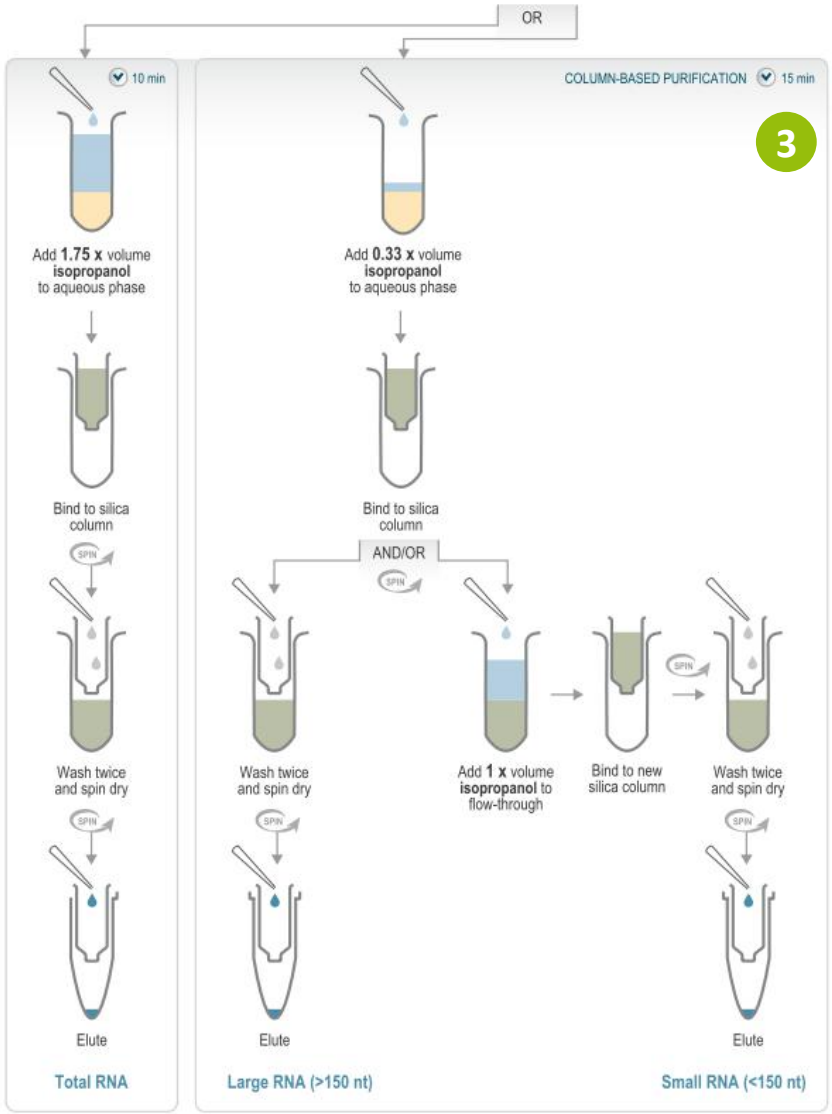
Sample | RNA Extraction | RNA QC | RNA Library Prep | Library QC | Sequencing | Data Analysis | Conclusion



*Hoen, P. A. C 't, *et al.*, 2013, **Reproducibility of high-throughput mRNA and small RNA sequencing across laboratories**, *Nature Biotechnology*

SPLIT RNA Extraction Kit





1. **Sample homogenization** in a highly chaotropic isolation buffer (guanidinium)

2. **Acidic phenol-chloroform** extraction aided by phase lock gel tubes

3. **Silica-column purification** and **fractioning** of RNA in
   - **total RNA** or
   - **small** and **large RNA** fractions

   by using different volumes of isopropanol

User supplied materials:
Phenol solution pH 4.3, Chloroform, Isopropanol, Ethanol

*Free trial kit (3 extractions including splitting option)

Extraction of high quality RNA

- **No genomic DNA** contamination (no slot-retained band on agarose gel)
- Extraction of **RNA > 10,000 nt**
- **No size bias** and **loss of long RNAs** due to gDNA removal columns
- **No RNA degradation** induced by DNase heat inactivation

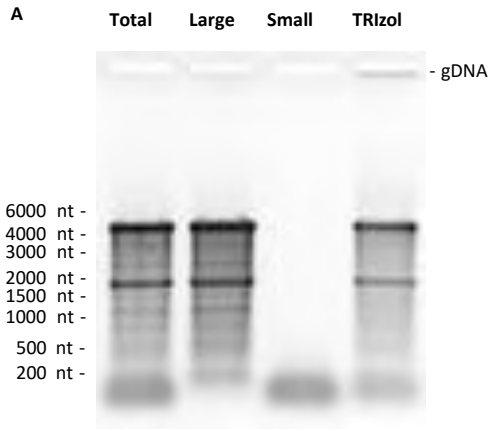


Figure A | The SPLIT protocol extracts RNA free of gDNA contamination. In comparison gDNA contamination is present after TRIzol extraction and becomes visible as a slot-retained band on a denaturing agarose gel. Importantly, gDNA contamination cannot be detected with Agilent's Bioanalyzer RNA chip (see Figure B).

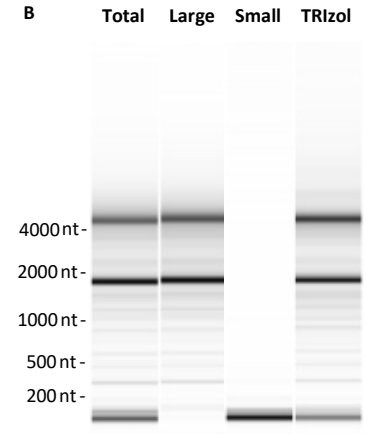


Figure B | The SPLIT protocol enables the extraction of high quality RNA. Importantly, no major difference between SPLIT extracted and gDNA contaminated RNA extracted with Trizol / isopropanol precipitation can be detected on Agilent's Bioanalyzer RNA chip. All samples have a RIN of 8.2 - 8.3.

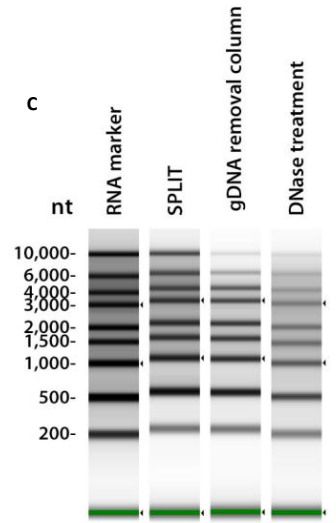


Figure C | The SPLIT protocol preserves RNA integrity and recovers the complete RNA size range. RNA integrity of an RNA marker (200 – 10,000 nt) after employing different gDNA removal methods was assessed on Agilent's TapeStation.

SPLIT RNA Extraction Kit – Summary

- RNA for **any downstream application**
- Total RNA from **< 17 nt** to **> 10,000 nt**
- Efficient recovery of **sRNA**
- **Splitting option** into large and small RNA fractions (cut-off ~150 nt)
- **Highest** RNA **integrity** and **purity** (RIN > 8; up to RIN 10 for cell culture)
- RNA **free of genomic DNA**
  - without RNA degradation induced by DNase heat inactivation
  - without size bias due to gDNA removal columns
- **High yield** (> 100 µg RNA / column) and **extraction efficiency** (down to 0.5 mg tissue)
- **Species-independent** RNA extraction (e.g., mammals, plants, insects, and others)
- User Guide protocols for **animal / plant tissue**, **cell** culture and **fluid** samples
- **Convenient** and **fast** protocol (only 30 - 35 min)

Tested organisms

- **Mammals** e.g., human, mouse, rat, pig
- **Plants** e.g., Arabidopsis thaliana, Crocus sativus, tomato, Norway spruce (Picea abies), Craterostigma plantagineum, ryegrass (Lolium perenne), Pak choi (Brassica rapa ssp. Chinensis), wallflower (Erysimum Brassicaceae)
- **Insects** e.g., Drosophila
- **Bacteria** e.g. E.coli, Bacillus subtilis
- **Others** e.g., Sea anemone (Nematostella), Jellyfish (Aurelia), Frog (Xenopus laevis), fungi (Fusarium), P. infestans sporangia, zoospores

Customer publications

InFusion: Advancing Discovery of Fusion Genes and Chimeric Transcripts from Deep RNA-Sequencing Data

Konstantin Okonechnikov, Aki Imai-Matsushima, Lukas Paul, Alexander Seitz, Thomas F. Meyer, Fernando Garcia-Alcalde

PLoS One. 2016 Dec 1, doi:10.1371/journal.pone.0167417

Differences in DNA Repair Capacity, Cell Death and Transcriptional Response after Irradiation between a Radiosensitive and a Radioresistant Cell Line

Mireia Borràs-Fresneda, Joan-Francesc Barquinero, Maria Gomolka, Sabine Hornhardt, Ute Rössler, Gemma Armengol & Leonardo Barrios

Sci. Rep. 6, 27043; doi:10.1038/srep27043 (2016)

Alternative Splice Forms Influence Functions of Whirlin in Mechanosensory Hair Cell Stereocilia

Seham Ebrahim, Neil J. Ingham, Morag A. Lewis, Michael J.C. Rogers, Runjia Cui, Bechara Kachar, Johanna C. Pass, Karen P. Steel

doi:10.1016/j.celrep.2016.03.081

Excessive Osteocytic Fgf23 Secretion Contributes to Pyrophosphate Accumulation and Mineralization Defect in Hyp Mice

Sathish K. Murali, Olena Andrukhova, Erica L. Clinkenbeard, Kenneth E. White, Reinhold G. Erben

PLoS Biol 14(4): e1002427. doi: 10.1371/journal.pbio.1002427

Customer publications

Amphiregulin lacks an essential role for the bone anabolic action of parathyroid hormone.
Freya F. Jay, Mithila Vaidya, Sabrina M. Porada, Olena Andrukhova, Marlon R. Schneider, Reinhold G. Erben
Mol Cell Endocrinol. 2015 Sep 28. pii: S0303-7207(15)30097-6. doi: 10.1016/j.mce.2015.09.031.

Nuclear accumulation of CDH1 mRNA in hepatocellular carcinoma cells.
Ghafoory S, Mehrabi A, Hafezi M, Cheng X, Breitkopf-Heinlein K, Hick M, Huichalaf M, Herbel V, Saffari A, Wölfl S.
Oncogenesis. 2015 Jun 1;4:e152. doi: 10.1038/oncsis.2015.11.

Find the latest SPLIT publications at https://www.lexogen.com/publications/#splitpub

Product portfolio in the RNA-Seq workflow

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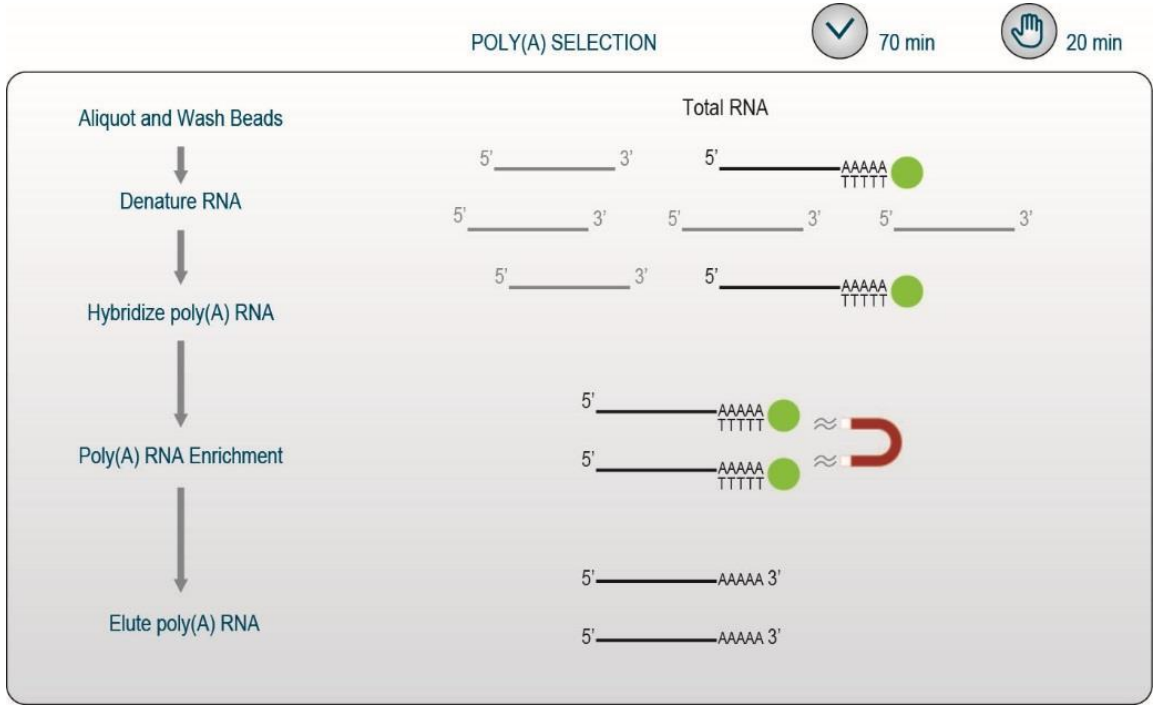
Poly(A) RNA
Selection Kit

Poly(A) RNA Selection Kit



1. **Highly specific binding** of **poly(A) RNA** to oligo(dT)30 beads

2. **Enrichment of poly(A) RNA** through magnetic bead-based purification

Poly(A) RNA Selection Kit - Summary

- **Highly specific poly(A) enrichment** from total RNA (ct rRNA mapping of only 0.0003% of all reads)
- **Straightforward** protocol - only **one binding step** required due to high specificity
- **Recovery of 1-3%** poly(A) RNA of total RNA (depending on the input material)
- **Up- and down-scaling** of the protocol (500 ng - 100 µg of total RNA)
- **Rapid** turnaround – only 20 minutes hands-on time
- **Automation**-friendly
- Poly(A) RNA as **pure eluate** or for **direct insertion into downstream applications**
- **Various downstream applications** such as RNA-Seq library preparation (SENSE Total RNA-Seq, microarrays, SAGE, RACE, cDNA synthesis, cDNA library construction, RT-PCR and others)

Product portfolio in the RNA-Seq workflow

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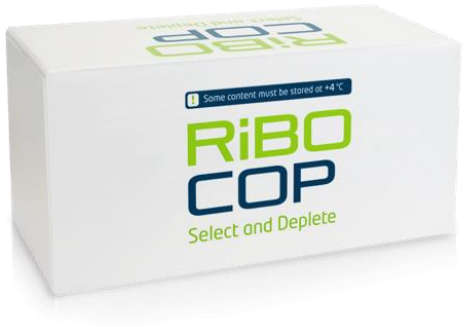
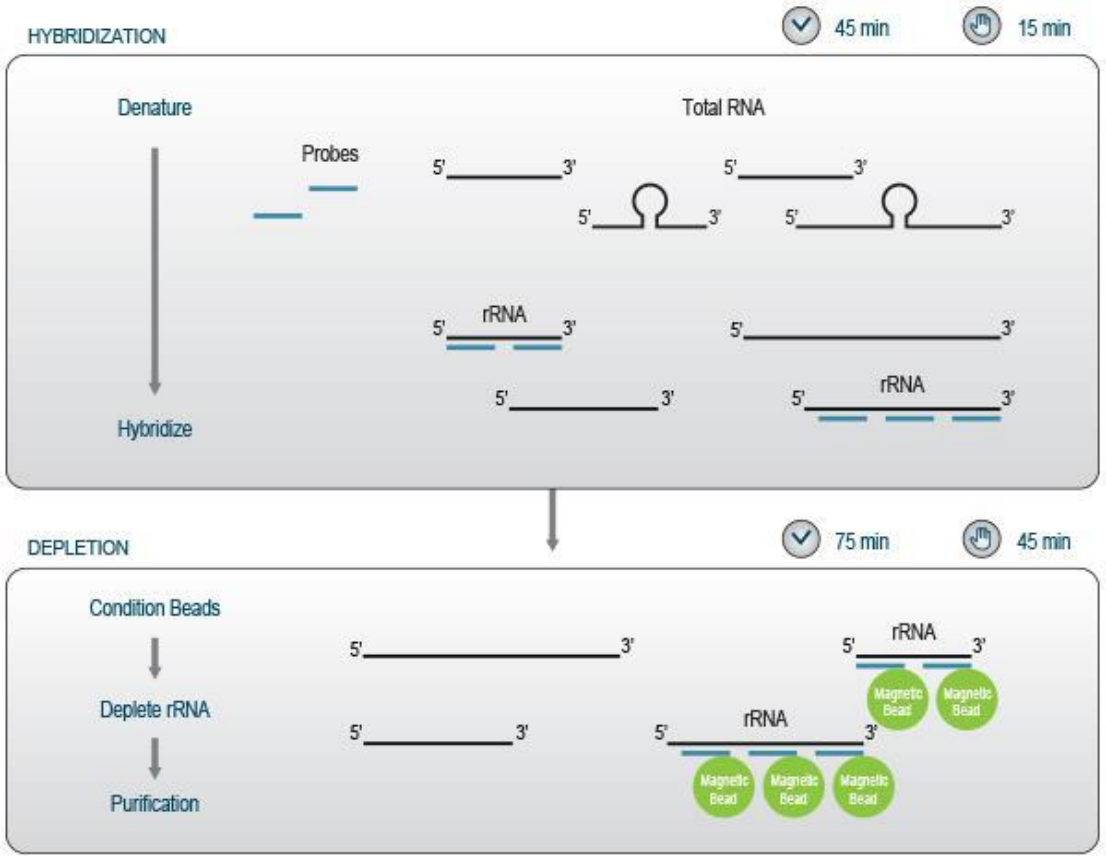
Library Preparation » QuantSeq 3′ mRNA-Seq » QuantSeq on SLAMseq RNA » QuantSeq on Blood-RNA (Globin Block) » Small RNA-Seq	**Automation and Sequencing**		**Data Analysis and Reporting**

RiBO COP™

Select and Deplete



RiboCop rRNA Depletion Kit V1.2



1. Specific depletion of **rRNA** sequences through **hybridization** to **affinity probes**

2. Magnetic bead-based purification

RiboCop rRNA Depletion Kit – Summary

- **Highly specific elimination (> 98%)** of **cytoplasmic** and **mitochondrial rRNA**
- For **human**, **mouse,** and **rat** samples
- Suitable for any quality RNA, **intact** and **degraded RNA** (e.g., FFPE samples)
- Standard recovery from **1 - 3% of input RNA** (depending on the input material)
- **Up- and down-scaling** of the protocol (1 ng - 1 µg of total RNA / prep)
- **Convenient** and **fast** protocol – rRNA depleted RNA in only 2 hours
- **Automation**-friendly protocol
- **Simple** and **robust** workflow retaining **full-length transcripts** – no enzymatic reactions or mechanical shearing steps
- **All-in one** solution – no additional purification modules required
- **Seamless integration** in any downstream application (e.g., RNA-Seq library preps including Lexogen's SENSE Total RNA-Seq; Cat. No. 042.04*/08/24/96)

Product portfolio in the RNA-Seq workflow

Sampling	RNA Preparation	NGS Library Prep	NGS Data Analysis
SLAMseq Metabolic RNA Labeling Kit » Explorer Modules » Kinetics Modules	**SPLIT RNA Extraction Kit**	**Expression Profiling** » QuantSeq 3′ mRNA-Seq Library Prep Kits » QuantSeq-Flex Targeted RNA-Seq Library Prep Kit	**QuantSeq Data Analysis** » QuantSeq Pipeline on Blue- bee® Genomics Platform » SLAMdunk Pipeline for QuantSeq-SLAMseq Data on Bluebee® Genomics Platform » QuantSeq Pipeline in Partek® Flow®
	RiboCop rRNA Depletion Kit	**Whole Transcriptome** » SENSE mRNA-Seq Library Prep Kit »SENSE Total RNA-Seq Library Prep Kit	**Mix² RNA-Seq Data Analysis Software**
	Poly(A) RNA Selection Kit	**Small RNA-Seq Library Prep Kit**	

cDNA Preparation
TeloPrime Full-Length cDNA Amplification Kit

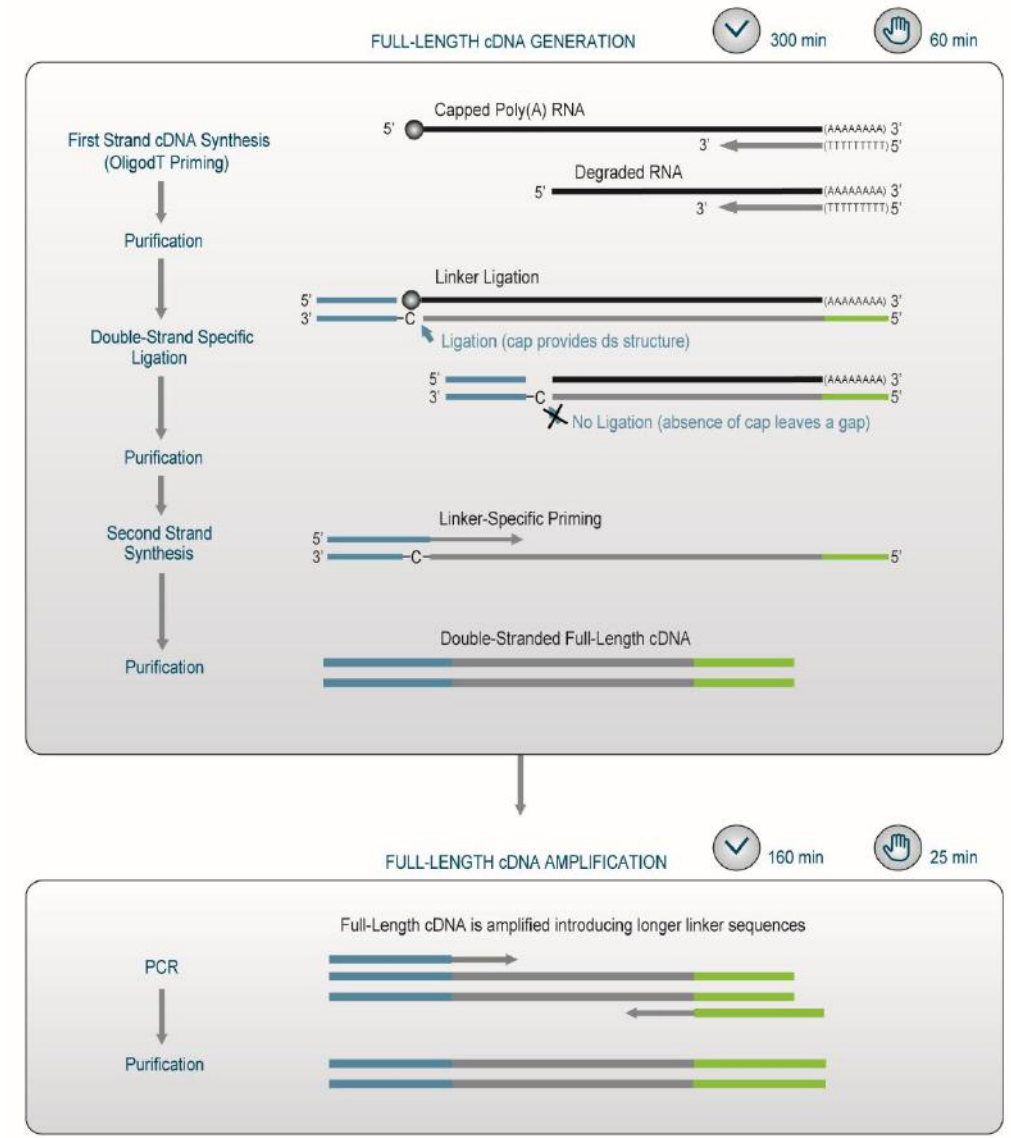
NGS Controls	
Spike-in RNA Controls » SIRV-Set 1 (Iso Mix E0, E1, E2) » SIRV-Set 2 (Iso Mix E0) » SIRV-Set 3 (Iso Mix E0 / ERCC)	**SIRV Suite Spike-In Analysis Software**

Services		
Library Preparation » QuantSeq 3′ mRNA-Seq » QuantSeq on SLAMseq RNA » QuantSeq on Blood-RNA (Globin Block) » Small RNA-Seq	**Automation and Sequencing**	**Data Analysis and Reporting**

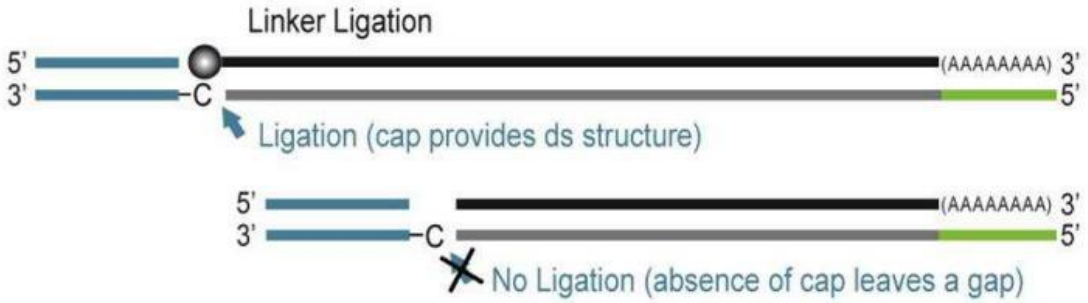
TELO™ PRIME

Full mRNA - precise ends

TeloPrime Full-Length cDNA Amplification Kit



Lexogen's unique - Cap Dependent Linker Ligation (CDLL)



Poly(A) Tail and cap → **ligation** → **second strand synthesis**

TeloPrime - Summary

- **Exceptional 5’-cap specificity** for full-length cDNA
- **Faithful representation** of the **mRNA transcriptome**
- **Preservation** of **short** and **long RNA** molecules
- Input amounts of **1 ng to 2 µg** of total RNA
- All-in-one protocol – only **60 minutes hands-on** time
- **Extensive tests** on mouse tissue (e.g., liver, lung etc.), Universal Human Reference RNA and Human Brain Reference RNA
- **Target specific application** – exchange Reverse Transcription, PCR Forward, and PCR Reverse Primers with your own primers of interest (e.g., gene-specific)
- **Various downstream applications** such as NGS library generation (incl. PacBio), microarray probe generation, RACE, cloning, and normalization

Customer publications

Global survey of cis-regulation in mammalian translation *Dissertation

Jingyi Hou

Department of Biology, Chemistry and Pharmacy of Freie Universität Berlin, Germany

Thyroglobulin Represents a Novel Molecular Architecture of Vertebrates

Guillaume Holzer, Yoshiaki Morishita, Jean-Baptiste Fini, Thibault Lorin, Benjamin Gillet, Sandrine Hughes, Marie Tohmé, Gilbert Deléage, Barbara Demeneix, Peter Arvan and Vincent Laudet

JBC.M116.719047. doi: 10.1074/jbc.M116.719047

cDNA Library Enrichment of Full Length Transcripts for SMRT Long Read Sequencing

Maria Cartolano, Bruno Huettel, Benjamin Hartwig, Richard Reinhardt, Korbinian Schneeberger

PLoS ONE 11(6):e0157779. doi:10.1371/journal.pone.0157779

Pervasive isoform-specific translational regulation via alternative transcription start sites in mammals

Xi Wang, Jingyi Hou, Claudia Quedenau, Wei Chen

Molecular Systems Biology (2016) 12, 875, DOI 10.15252/msb.20166941

Find the latest TeloPrime publications at https://www.lexogen.com/publications/#teloprimepub

Product portfolio in the RNA-Seq workflow

Sampling	RNA Preparation	NGS Library Prep	NGS Data Analysis
SLAMseq Metabolic RNA Labeling Kit » Explorer Modules » Kinetics Modules	**SPLIT RNA Extraction Kit**	**Expression Profiling** » QuantSeq 3′ mRNA-Seq Library Prep Kits » QuantSeq-Flex Targeted RNA-Seq Library Prep Kit	**QuantSeq Data Analysis** » QuantSeq Pipeline on Blue- bee® Genomics Platform » SLAMdunk Pipeline for QuantSeq-SLAMseq Data on Bluebee® Genomics Platform » QuantSeq Pipeline in Partek® Flow®
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MIX²

Accurate Analysis of RNA-Seq Data

Mix² features – Your benefits

Accurate estimation of isoform concentration

Get more relevant cases of differentially expressed isoforms and a reduced number of false positives

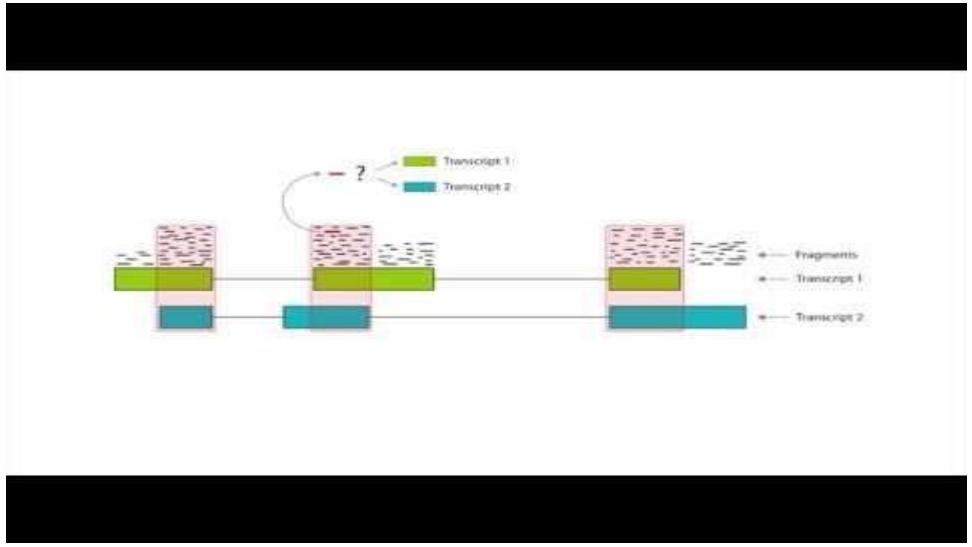
Identification of bias types

Detect and classify bias types

Fast run times

Speed up your data analysis and profit from a small memory footprint

Watch Mix² explained by our bioinformatics experts!



https://www.youtube.com/watch?v=9s5fOWf-s2s

Andreas Türk, PhD
Head of Bioinformatics, Lexogen

Customer publications

Tristetraprolin binding site atlas in the macrophage transcriptome reveals a switch for inflammation resolution

Vitaly Sedlyarov, Jörg Fallmann, Florian Ebner, Jakob Huemer, Lucy Sneezum, Masa Ivin, Kristina Kreiner, Andrea Tanzer, Claus Vogl, View ORCID ProfileIvo Hofacker, Pavel Kovarik

doi: 10.15252/msb.20156628

Find the latest Mix² publications at https://www.lexogen.com/publications/#mix2pub

Free Trial Kits Available

- **QuantSeq 3’** (4)*
- **SENSE** mRNA and total (4)*
- **SPLIT** (3)*
- **RiboCop** (4)*
- **TeloPrime** (4)*

- *Applications support is always available

Modules and Add-ons

Enabling Complete Transcriptome Sequencing

Questions?

Visit our website: ww.lexogen.com – FAQ

Contact us: +43 1345 1212-41 or info@lexogen.com