Advancements in NGS Library Preparation for Challenging Samples

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SMARTer® NGS portfolio

Encompasses three key technologies, all featuring **high sensitivity** and a **streamlined workflow**

	DNA SEQUENCING	TARGETED SEQUENCING	WHOLE TRANSCRIPTOME ANALYSIS
SMART® Technology	• ChIP-seq • Meth-seq	• Targeted RNA-seq • Small RNA-seq • Immune profiling	• Single-cell/ultra-low-input RNA-seq • Total RNA-seq
ThruPLEX® Technology	• Illumina library construction • Targeted sequencing • ChIP-seq	• Targeted DNA-seq with major enrichment platforms	
PicoPLEX® Technology	• Whole genome amplification • Aneuploidy/CNV detection		

New additions to the SMARTer NGS portfolio

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Evolution of DNA library prep

Back in the day
Conventional

>1 µg

1. Repair ends
2. Clean up
3. Adenylate ends
4. Clean up
5. Ligate adapters
6. Clean up
7. Size select
8. Amplify library

Long and tedious!

Illumina®
TruSeq® Nano

100 ng or 200 ng

1. Repair ends
2. Size select
3. Adenylate ends
4. Ligate adapters
5. Clean up
6. Amplify library

90 min hands on

4.5 hr total

4 transfer steps

Takara Bio
SMARTer ThruPLEX

0.05–200 ng

1. Repair ends
2. Ligate adapters
3. Amplify library

15 min hands on

2 hours total

0 transfer steps

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ThruPLEX enabled pioneers in cfDNA research to make groundbreaking findings

- Kitzman *et al.* Noninvasive Whole-Genome Sequencing of a Human Fetus. *Science Translational Medicine* (2012).
  - **Jay Shendure lab: Howard Hughes Medical Institute and the Department of Genome Sciences at the University of Washington**

- Murtaza *et al.* Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature* (2013).
  - **Nitzan Rosenfeld lab: Cancer Research UK Cambridge Institute**

RESEARCH ARTICLE

GENOMICS

Noninvasive Whole-Genome Sequencing of a Human Fetus

Jacob O. Kitzman,[1]* Matthew W. Snyder,[1] Mario Ventura,[1,2] Alexandra P. Lewis,[1] Ruolan Qiu,[1] LaVone E. Simmons,[3] Hilary S. Gammill,[3,4] Craig E. Rubens,[3,4] Donna A. Santillan,[5] Jeffrey C. Murray,[6] Holly K. Tabor,[6,8] Michael J. Bamshad,[1,7] Evan E. Eichler,[9,10] Jay Shendure[1]*

Analysis of cell-free fetal DNA in maternal plasma holds promise for the development of noninvasive prenatal genetic diagnostics. Previous studies have been restricted to detection of fetal trisomies, to specific paternally inherited mutations, or to genotyping common polymorphisms using material obtained invasively, for example, through chorionic villus sampling. Here, we combine genome sequencing of two parents, genome-wide maternal haplotyping, and deep sequencing of maternal plasma DNA to noninvasively determine the genome sequence of a human fetus at 18.5 weeks of gestation. Inheritance was predicted at 2.8 × 10^9 parental heterozygous sites with 98.1% accuracy. Furthermore, 39 of 44 de novo point mutations in the fetal genome were detected, albeit with limited specificity. Subsampling these data and analyzing a second family trio by the same approach indicate that parental haplotype blocks of ~300 kilo–base pairs combined with shallow sequencing of maternal plasma DNA is sufficient to substantially determine the inherited complement of a fetal genome. However, ultradeep sequencing of maternal plasma DNA is necessary for the practical detection of fetal de novo mutations genome-wide. Although technical and analytical challenges remain, we anticipate that noninvasive analysis of inherited variation and de novo mutations in fetal genomes will facilitate prenatal diagnosis of both recessive and dominant Mendelian disorders.

INTRODUCTION

LETTER

Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA

Muhammed Murtaza[1]*, Sarah-Jane Dawson[1,2]*, Dana W. Y. Tsui[1]*, Davinia Gale[1], Tim Forshew[1], Anna M. Piskorz[1], Christine Parkinson[1,3], Suet-Feung Chin[1], Zoya Kingsbury[1], Alvin S. C. Wong[1], Francesco Marass[1], Sean Humphray[2], James Hadfield[2], David Bentley[2], Tan Min Chin[4,5], James D. Brenton[1,3] , Carlos Caldas[1,3]† & Nitzan Rosenfeld[1]

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ThruPLEX: the gold standard for ChIP-seq

Widely cited in high-impact publications performing ChIP-seq to profile transcription factor binding sites:

- Liu, Y. *et al.* Transcriptional landscape of the human cell cycle. *PNAS* (2017).
- Warrick, J. I. *et al.* FOXA1, GATA3 and PPARγ cooperate to drive luminal subtype in bladder cancer: A molecular analysis of established human cell lines. *Sci. Reps.* (2016).
- Cejas, P. *et al.* Chromatin immunoprecipitation from fixed clinical tissues reveals tumor-specific enhancer profiles. *Nat. Med.* (2016).

Recent ChIP-seq Publications using ThruPLEX Technology

Our collaborators and customers are constantly making scientific breakthroughs. Here are the latest published results obtained using ThruPLEX DNA-seq for chromatin immunoprecipitation sequencing (ChIP-seq).

1. Baejen, C. *et al.* Genome-wide analysis of RNA Polymerase II termination at protein-coding. *Genes. Mol. Cell* **66,** 1–12 (2017).

   This paper used ChIP-seq, ChIP-qPCR, and other functional genomic methods to understand how RNA Pol II termination occurs in yeast. ThruPLEX DNA-seq kit was used to generate libraries for ChIP-seq. The author showed that the 3′-transition in budding yeast requires the Pol II elongation factor Spt5, and that polymerase II release from DNA requires the Rat1 exonuclease.

   Read now »

2. Maatouk, D.M. *et al.* Genome-wide identification of regulatory elements in Sertoli cells. *Development* **144,** 720–30 (2017).

   This study used ChIP-seq, DNaseI-seq, and RNA-seq to identify regulatory elements in mouse Sertoli cells during sex determination. ThruPLEX DNA-seq kit was used to prepare ChIP-seq libraries from FACS-sorted mouse Sertoli cells. By overlapping DNaseI-seq peaks with the chromatin landscape for H3K27ac, the authors were able to identify enhancers active only in Sertoli cells during the early stages of sex determination.

   Read now »

3. Liu, Y. *et al.* Transcriptional landscape of the human cell cycle. *PNAS* **114,** 3473–78 (2017).

   This paper investigated the transcriptional landscape across the cell cycles using a combination of ChIP-seq, DNase-seq, RNA-seq, and GRO-seq. ThruPLEX DNA-seq kit was used to prepare libraries for ChIP-seq and DNase-seq. Using the MCF-7 breast cancer cell line as a model, the authors revealed lag between transcription and steady-state RNA expression at the cell-cycle level. Other findings highlighted the importance of transcriptional and epigenetic dynamics during cell-cycle progression.

   Read now »

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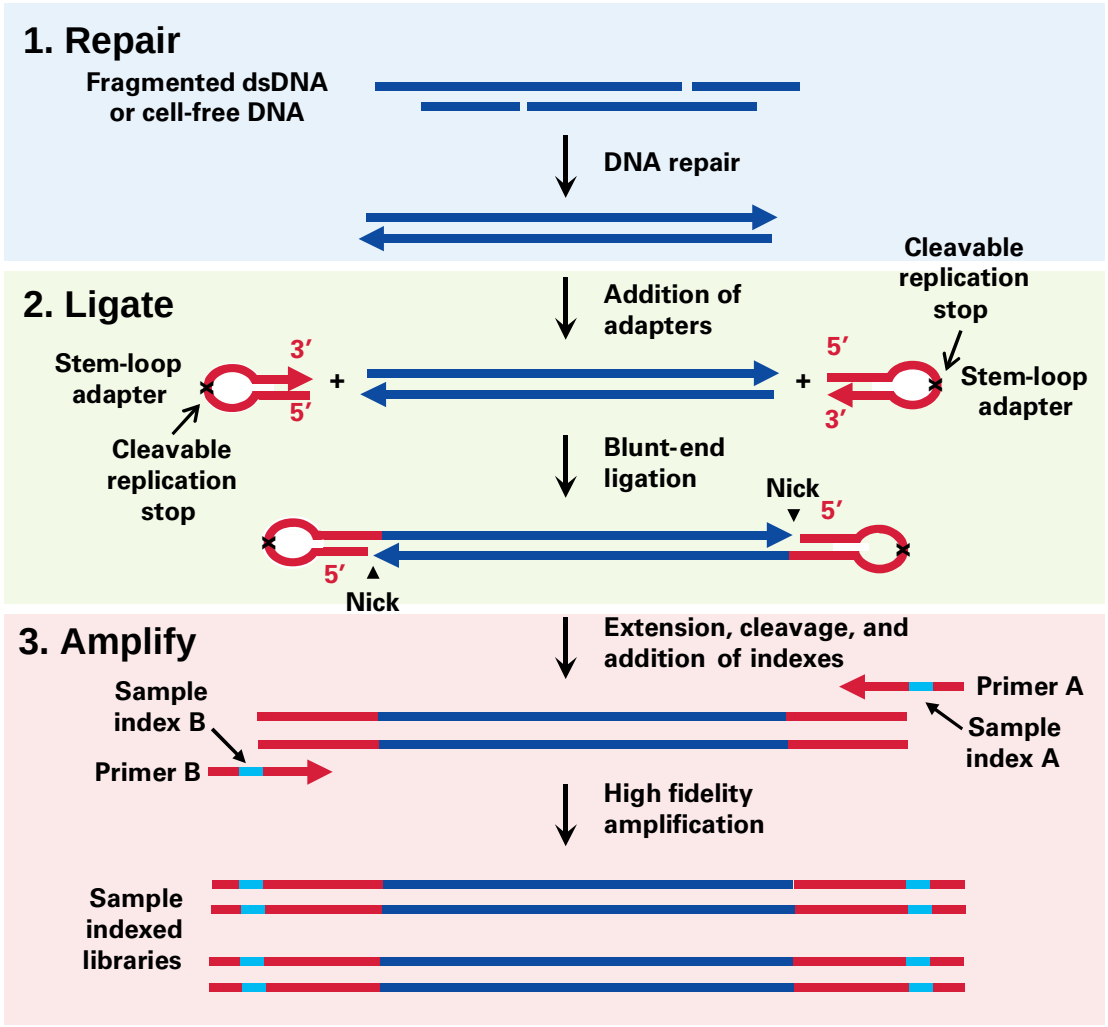
ThruPLEX technology



Superior performance and single-tube workflow resulting from innovative adapter design and highly efficient ligation reaction

- Innovative degradable stem-loop adapters
- Highly efficient single-stranded blunt-end ligation
- Excess adapter molecules degraded
- Post-ligation purification step eliminated

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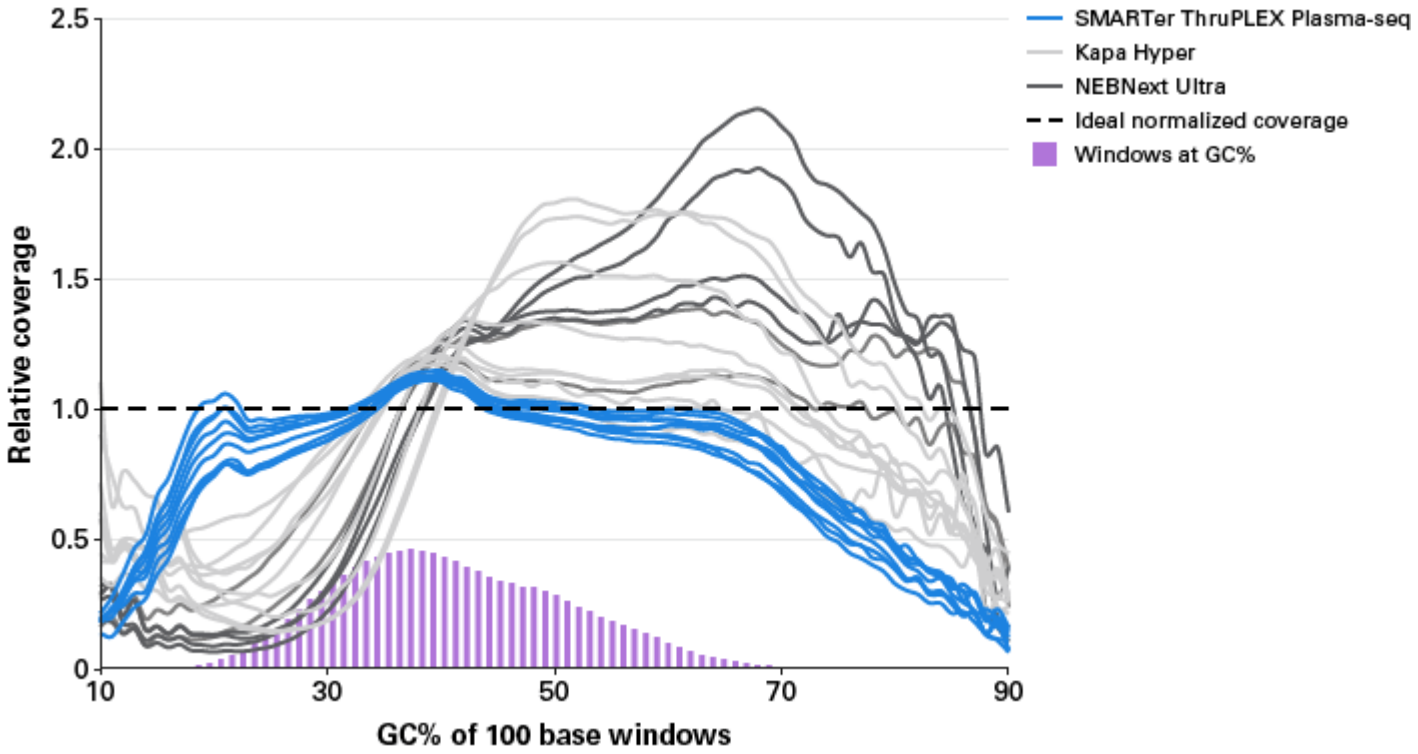
SMARTer ThruPLEX Plasma-seq Kit
Optimized specifically for cell-free DNA

Single-tube workflow and minimal pipetting operations translate into highly reproducible sequencing results



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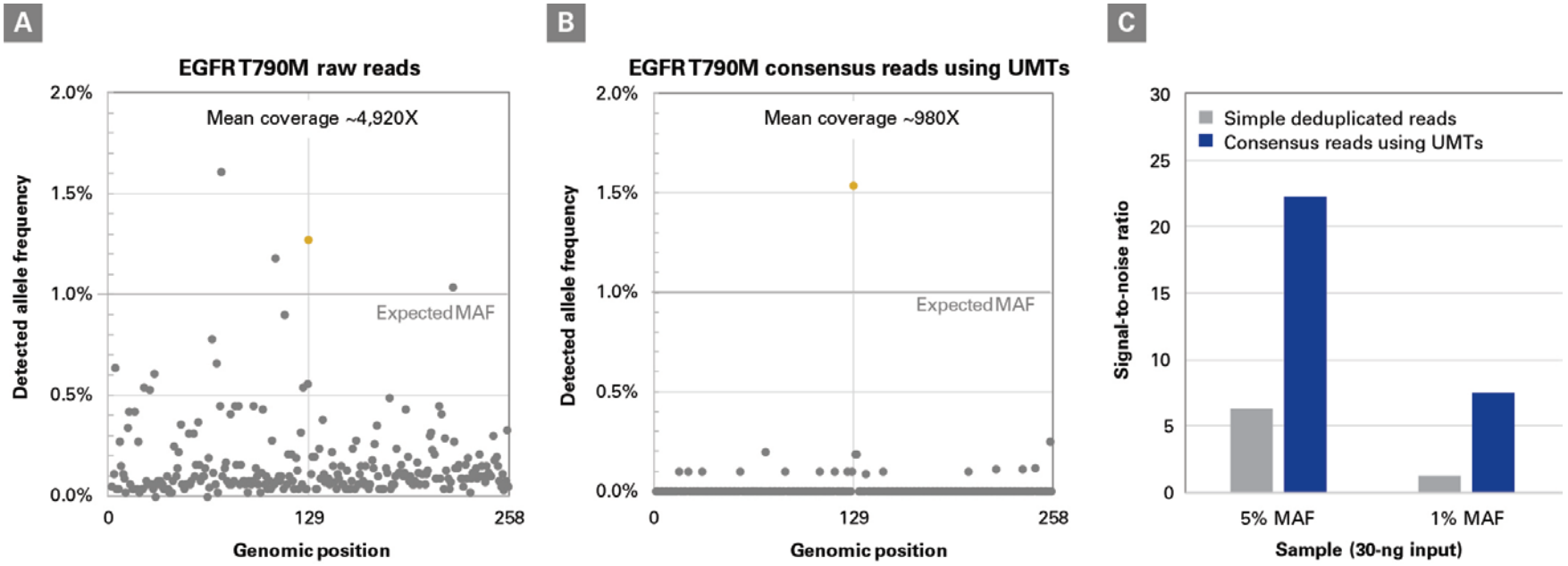
SMARTer ThruPLEX Tag-seq Kit

Confident rare variant detection with unique molecular tags

Unique molecular tags incorporated during ligation to remove PCR and sequencing errors, resulting in confident detection of rare variants



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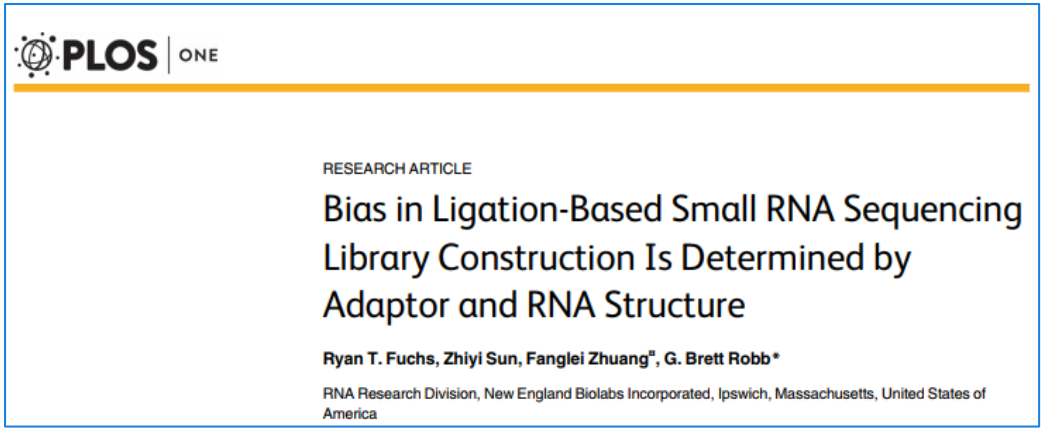
MicroRNA-seq challenges

- Most state-of-the-art ligation-based technologies for miRNA-seq suffer from ligation-induced bias
- This results in inaccurate representation of the biological state of the sample


PLOS ONE

RESEARCH ARTICLE

Bias in Ligation-Based Small RNA Sequencing Library Construction Is Determined by Adaptor and RNA Structure

Ryan T. Fuchs, Zhiyi Sun, Fanglei Zhuang, G. Brett Robb*

RNA Research Division, New England Biolabs Incorporated, Ipswich, Massachusetts, United States of America

Jackson, T. J. *et al.* Evaluating bias-reducing protocols for RNA sequencing library preparation. *BMC Genomics* (2014).

Zhuang F. *et al.* Small RNA expression profiling by High-Throughput Sequencing: Implications of Enzymatic Manipulation. *J. Nucleic Acids* (2012).

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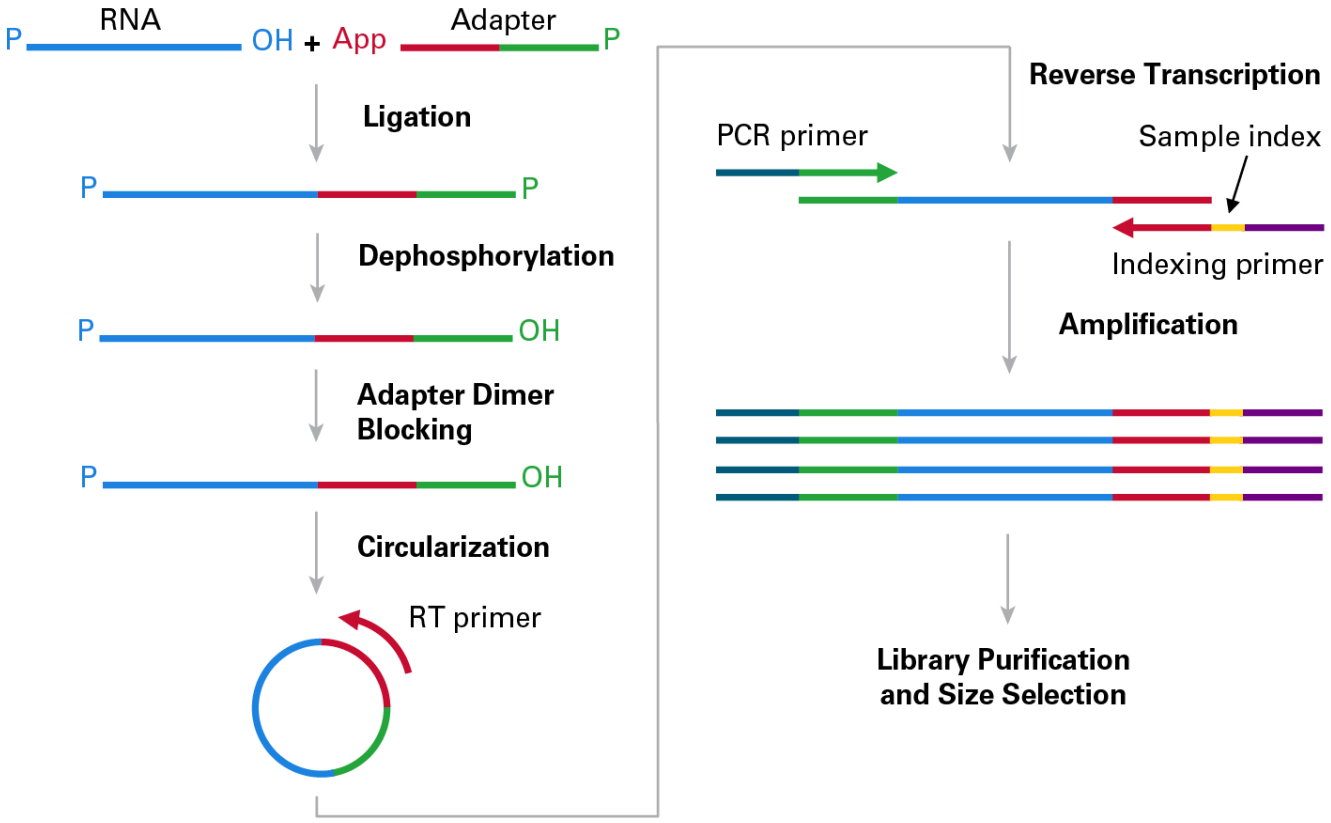
MAGIC: minimize ligation-induced bias

MAGIC technology:
Mono-**A**dapter li**G**ation and **I**ntramolecular **C**ircularization



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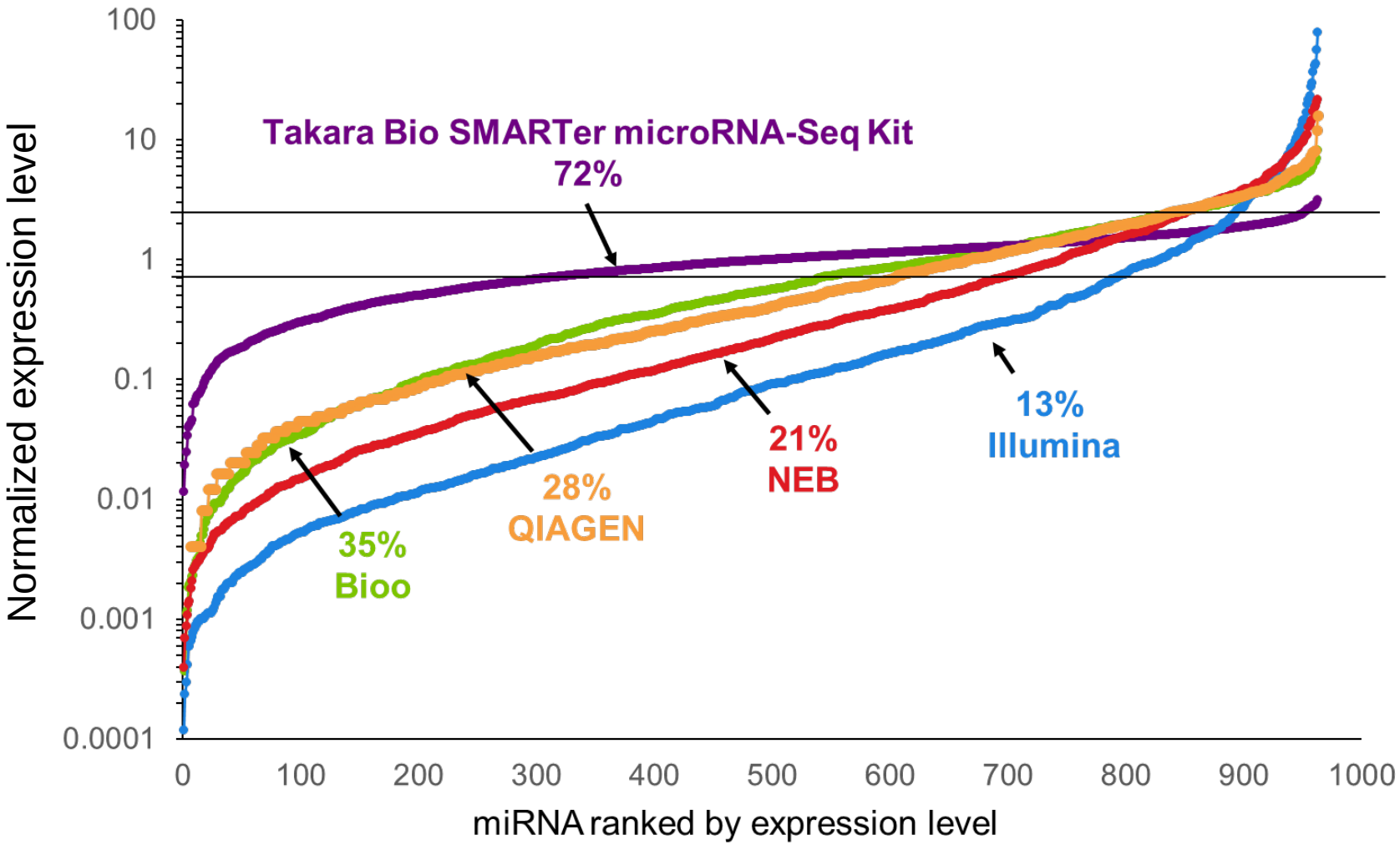
SMARTer microRNA-Seq Kit

Accurate expression profile of microRNAs



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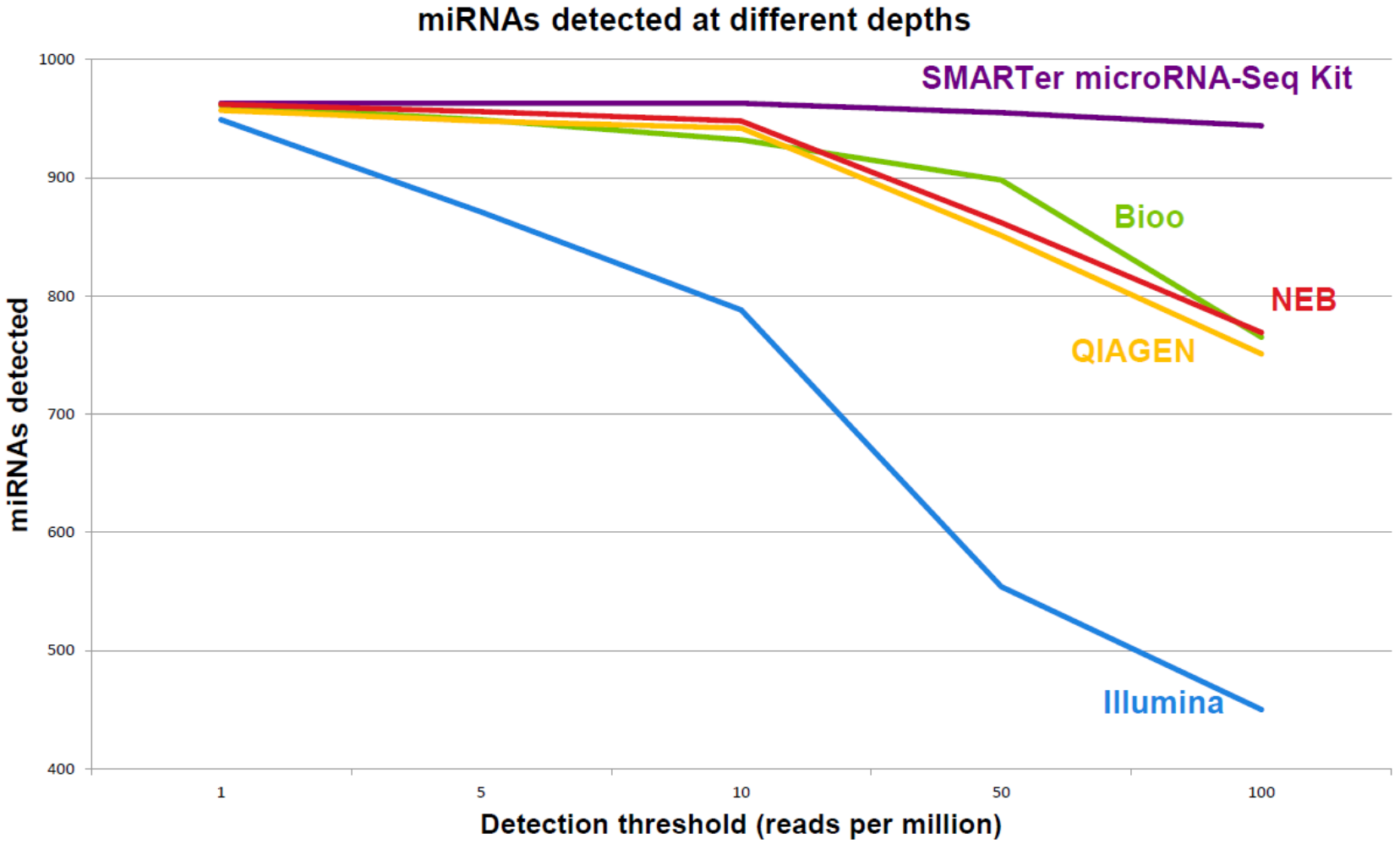
SMARTer microRNA-Seq Kit
Detects more microRNAs



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What else is new from Takara Bio?

- **SMARTer PicoPLEX Gold Single Cell DNA-seq Kit—**accurate and simultaneous detection of SNVs and CNVs from single cells
- **SMART-Seq® Stranded Kit—**random-primer-based single-cell RNA-seq kit for detecting coding and noncoding RNA and providing strand information of transcripts
- **SMARTer™ Apollo™ Library Prep System—**low to medium throughput benchtop automation system for NGS library preparation
- **SMARTer ICELL8® Single-Cell System—**open platform single-cell automation system for SMART-Seq library prep, TCR profiling, and more

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Thank you!

- Visit us at **Booth #300**
- Poster presentations
  - Confident detection of low-frequency mutations in cell-free DNA using SMARTer ThruPLEX technology with unique molecular tags
  - An unbiased and highly reproducible method for constructing microRNA NGS libraries for accurate expression profiling
  - A SMARTer solution to stranded single-cell RNA-seq
  - High-throughput single-cell transcriptomics with SMART-Seq technology

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