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Genomics & Bioinformatics

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Fred Hutch's Shared Resources are catalysts for lifesaving discoveries. This uniquely centralized program of 15 specialized core facilities and scientific services drives advances by integrating dedicated experts and cutting-edge technologies across the entire research pipeline, from basic science to clinical trial.

MAS-Seq for 10x Single Cell 3'

Multiplexed arrays sequencing (MAS-Seq) is a method in which cDNA fragments are concatenated into long sequencing libraries (Figure 1)[1]. MAS-Seq greatly increases the throughput of RNA sequencing on PacBio sequencers and provides isoform-level resolution. The Genomics and Bioinformatics Shared Resource (G&BSR) is now offering library preparation and sequencing services using the PacBio MAS-Seq for 10x Single Cell 3' Kit (PacBio PN:102-659-600).

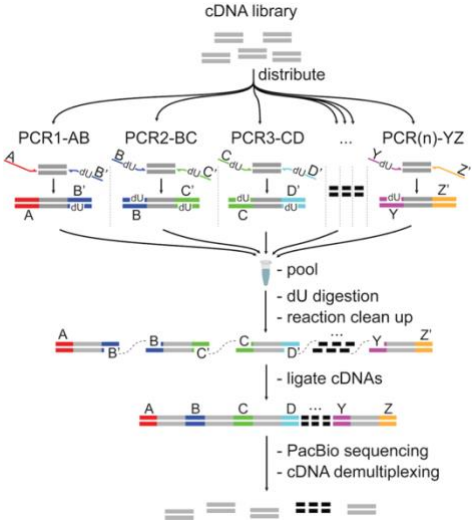

Figure 1 MAS-Seq Workflow. Adapted from Al'Khafaji et al. (Nat. Biotechnol. 2023).

In a pilot study conducted by the G&BSR, eight unique samples were prepared using the PacBio MAS-Seq for 10x Single Cell 3' Kit. Initially, cDNA was prepared using the 10x Genomics Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (10x Genomics PN: 1000121/1000128) with a targeted recovery of 10,000 cells per sample. MAS-seq libraries were then prepared and sequenced using one 8M SMRT cell per library on a PacBio Sequel IIe. Data were processed by SMRT Link using a purpose-built workflow.

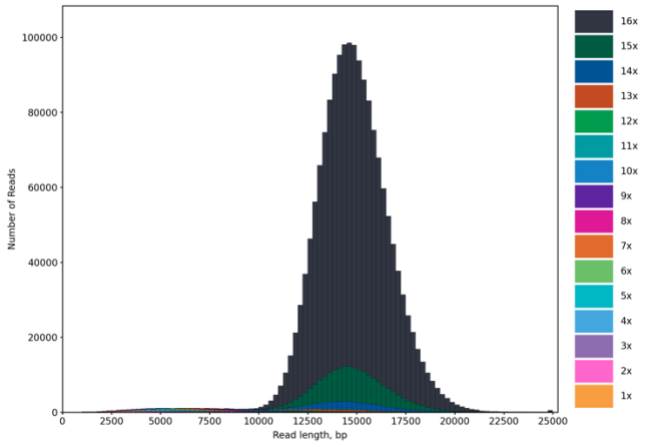

Figure 2 MAS-Seq read lengths. The read lengths indicate that the majority of input cDNA molecules were incorporated into 16-mer MAS-Seq arrays.

The results of the pilot study demonstrated modal HiFi read lengths of approximately 15 kilobases, supporting that the 16-mer MAS-Seq PCR arrays were successfully generated (Figure 2). An average of 1,858,009 ± 175,861 HiFi reads were generated per sample which were segmented into 28,905,499 ± 2,736,005 segmented reads, corresponding to the initial cDNA libraries, which were 884 ± 48 bp in length.

LEARN MORE

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Additionally, QC metrics relevant to single-cell RNA-seq analyses were collected (Table 1). These data demonstrated successful detection of an average of 400,421 ± 30,787 unique isoforms per sample.

Table 1 MAS-Seq Output Metrics.

Metric	*Mean*	*Median*	*SD*	*Min*	*Max*
Number of Cells	5572	6188	3394	1336	10010
Unique Genes Detected	21890	21582	715	21266	23114
Median Genes/Cell	1414	1125	769	672	2581
Unique Isoforms Detected	400421	392242	30787	364503	445411
Median Isoforms/Cell	1780	1356	1067	776	3444

The new MAS-Seq service offered through the G&BSR is a fully integrated sample-to-answer pipeline. Users may either submit disassociated cell suspensions or cDNA libraries generated using the 10x Genomics Chromium Next GEM Single Cell 3' Reagent Kits v3.1. If you are interested in submitting samples for MAS-Seq, please contact us at genomics@fhcrc.org.

References

1. Al'Khafaji, A.M., Smith, J.T., Garimella, K.V. *et al.* High-throughput RNA isoform sequencing using programmed cDNA concatenation. *Nat Biotechnol* (2023).