BioSkryb GENOMICS

Cells explored. Answers revealed.

Enabling Routine Single-cell Genomics

The cell is the basic unit of life. Yet, most of our knowledge of genetic diversity has been derived from bulk molecular analyses. Single-cell approaches are necessary to improve our understanding of complex biological systems. Recent technological advances have enabled routine single-cell transcriptomics, but many challenges remain in the interrogation of single cell genomes. One of these is an efficient and reliable method for whole-genome amplification (WGA).[1]

WGA enables genomic analysis from single cells and other minute biological specimens. However, existing methods only cover a fraction of the genome, or suffer from significant biases, experimental artifacts and poor reproducibility.

Primary Template-directed Amplification (PTA) is a novel, isothermal WGA method that reproducibly captures >95% of the genomes of single cells, in a controlled and more uniform and accurate manner than existing approaches.[2] This improves variant calling sensitivity and specificity, lowers sequencing costs, and facilitates bioinformatic analysis. PTA is a core technology that enables a wide variety of emerging single-cell genomics applications, including:

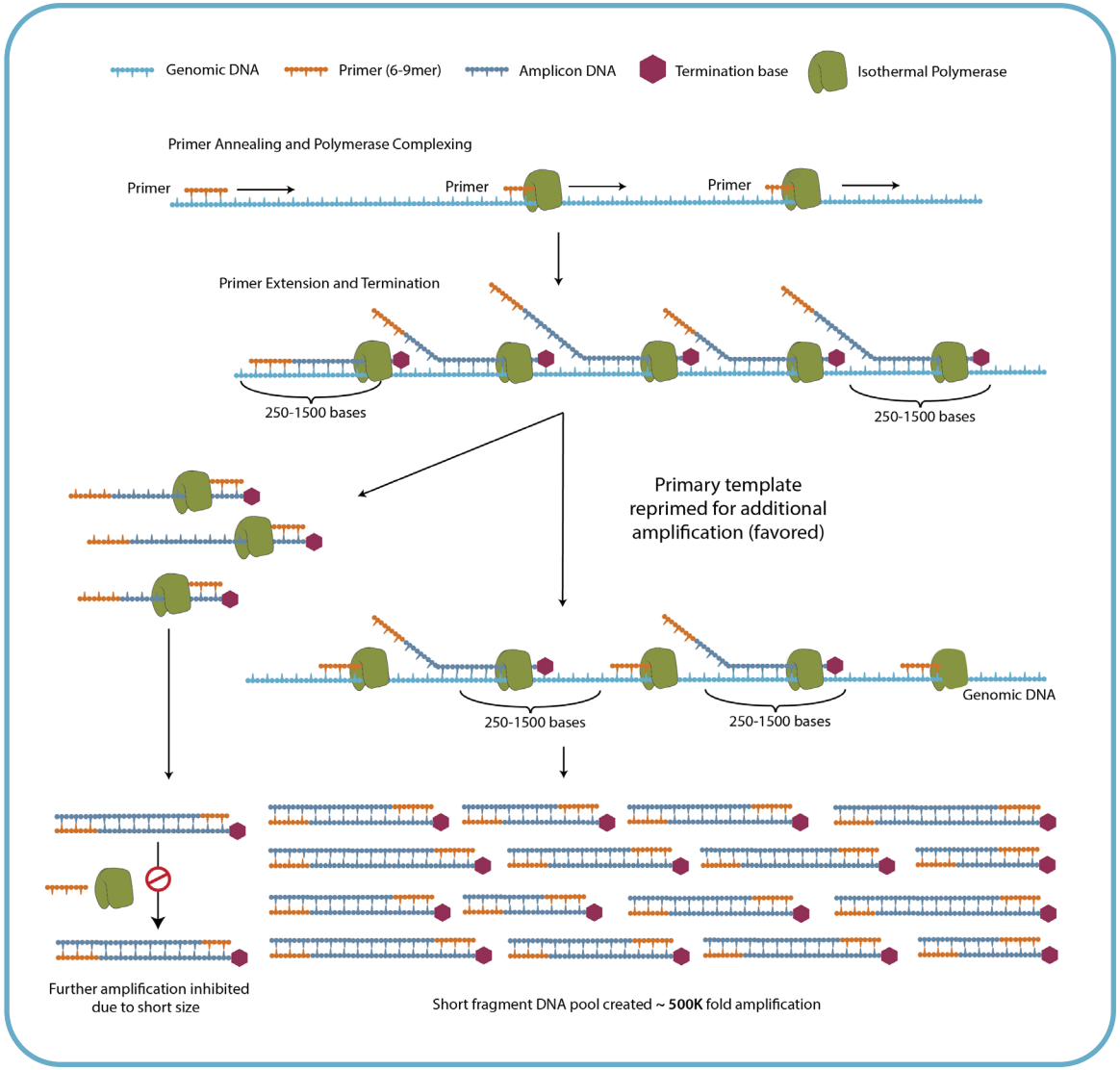
- accurate variant analysis (SNPs, indels, SNVs and CNVs) of single cells and sub-nanogram samples
- the detection and characterization of minimal residual disease (MRD)
- quantitative, genome-wide assessment of CRISPR/Cas9-mediated genome editing at single-cell resolution
- genome assembly and characterization of rare and unculturable microbes

Technology

Primary Template-directed Amplification (PTA)[3]

takes advantage of the processivity, strand displacement activity, and low-error rate of phi29 DNA polymerase. An innovative reaction setup employs exonuclease-resistant terminators to create relatively short, double-stranded amplification products that are poor templates for subsequent cycles of amplification. This transforms the reaction from an exponential into a quasi-linear process, during which the majority of amplicons are generated from the primary template.

Unlike multiple displacement amplification (MDA), PTA limits the exponential propagation of priming and amplification biases, allelic skewing and other errors from daughter molecules. This results in improved uniformity, coverage and reproducibility, and significantly improved variant call rates.[2]



How PTA works. PTA may be performed directly from single cells (collected by FACS, microfluidic or other methods), multiple cells, or ultra-low inputs of DNA (>4 pg– 10 ng). After denaturation, random primers (6- to 9-mers) are annealed. Extension with phi29 and a proprietary nucleotide pool results in amplicons of ~250 to >1,500 bp in length. The relatively small size of these amplicons favors subsequent priming off the primary template, thereby limiting the exponential propagation of biases and errors in daughter molecules. In addition, the PTA chemistry suppresses the formation of experimental artifacts such as chimeric molecules and non-specific priming.2 PTA reaction products are double-stranded, and may be converted to libraries for multiplexed sequencing on Illumina® or other short-read platforms without fragmentation.

BioSkryb GENOMICS

Applications

Minimal Residual Disease (MRD) Detection and Characterization

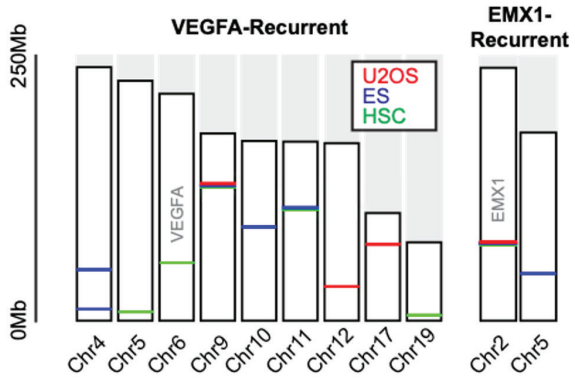
Intra-tumor heterogeneity is a key driver of poor outcomes in cancer intervention and management.[4] MRD is the term used for rare (typically <1% of mononuclear cells) and genetically diverse populations of leukemic cells that persist in bone marrow or peripheral blood during or after treatment. MRD testing is critical for treatment selection and effective disease management. However, current methods are not sensitive enough to detect very rare clones, and do not provide genetic information on specific clones that may cause drug resistance or relapse.

PTA forms the core of a novel immunogenotyping workflow that combines FACS and NGS to enable extremely sensitive MRD detection (as little as one leukemia cell within a background of one million cells), as well as genetic characterization of rare clones. By overcoming the data quality issues traditionally associated with WGA, this workflow is capable of associating the genotypes of clones (clonotypes) to clinically relevant phenotypes.[5]

Quantitative, genome-wide assessment of CRISPR/Cas9-mediated genome editing in single cells

Off-target gene editing can have impacts ranging from confounding and non-reproducible research results to genotoxic effects in vivo. Established methods for measuring off-target effects largely rely on bulk sampling. The improved data quality and scalability of PTA enables quantitative, genome-wide assessment of both on- and off-target editing at single-cell resolution.

	+VEGFA gRNA			+EMX1 gRNA	
	Unique	Recurrent		Unique	Recurrent
U2OS	9	2	1 (all) 1 (ES &HSC)	1	0
ES	3	3		0	1
HSC	4	2		1	0



Assessment of genome editing at single-cell resolution. CRISPR-Cas9 mediated gene editing was performed on three cell types: an osteosarcoma cell line (U20S), primary hematopoietic stem cells (HSC), and embryonic stem cells (ES). Two previously characterized guide RNAs, one known to be precise (EMX1), and one known to display high levels of off-target activity (VEGFA)6 were employed. Untreated cells and cells transfected with Cas9 only were included as controls. Single cells were isolated and subjected to WGA with the ResolveDNA™ Whole Genome Amplification Kit. WGA products were converted to dual-indexed libraries and sequenced on an Illumina® NovaSeq™ 6000 System. Indels were called using GATK4, whereas svABA was used for calling structural variants (SVs).

As expected, only a small number of off-target events were detected in EMX1-edited cells, whereas the off-target rate and cell-to-cell variability was much higher for VEGA-edited cells. The total number of off-target indel locations unique to one cell, or found in multiple cells (recurrent) are given in the table. The chromosomal locations of recurrent indels are shown in the graph (red, blue or green lines). SV data is not shown.

BioSkryb GENOMICS

Solutions & Products

Complete workflow solutions for single-cell and ultra-low input genomics applications

BioSkryb is developing complete sample-to-analysis workflow solutions to support low- and high-throughput single-cell and ultra-low input genomics applications. Our PTA-based ResolveDNA™ Whole Genome Amplification Kits form the core of these workflows. The ResolveDNA portfolio also includes:

- equipment and consumables for cell sorting (available in different kit configurations)
- Bead Purification Kits, optimized for efficient cleanup and optimal recovery of WGA reaction products and NGS libraries
- a choice of magnets for plate- or tube-based workflows, validated for use with our Bead Purification Kits

We are in the process of expanding our portfolio to include reagents for NGS library construction, target enrichment (exome and gene panels), multi-omics analysis, and bacterial whole-genome sequencing. In addition, we are pursuing collaborations with instrument manufacturers to offer additional solutions for cell sorting, as well as automated sample processing.

BioSkryb is also developing innovative computational tools. The BaseJumper Bioinformatics Platform uses standardized best practices for sequencing analysis, and enables easy visualization of sequence alignments, coverage, SNV calling, and data quality metrics across multiple cells or samples.

Contact us for a full product catalog, or to inquire about our early access program.

References:

1 Gawad, C *et al. Nat. Rev. Genet.* 2016; 17: 175. doi:10.1038/nrg.2015.16.
2 Gonzalez, V *et al*. Manuscript in review.
3 Method for Nucleic Acid Amplification. WO/2019/148119.
4 Iacobuzio-Donahue, CA *et al. Nat. Cancer* 2020; 1: 3. doi:10.1038/s43018-019-0002-1.
5 Unpublished data.
Fu, Y *et al. Nat. Biotechnol.* 2013; 31: 822. doi:10.1038/nbt.2623.

(919) 370-0841

info@bioskryb.com | www.bioskryb.com

BioSkryb GENOMICS