BioSkryb GENOMICS

Technical Note

Enrichment Workflows for Single Cell Genome Analysis

Cells explored. Answers revealed.

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Integration of Targeted Enrichment Methods for Single-cell Genomic Studies Using the ResolveDNA Product Solution

BioSkryb Genomics has established multiple workflows to enable low DNA input and single-cell genomics. These workflows highlight how next generation amplification, library preparation and sequencing technologies yield high quality single-cell targeted panel data.

Introduction

Heterogeneity within and between cellular populations dictates the fate of all tissues in both normal development and disease pathogenesis. The development of single-cell approaches has revealed amazing diversity across tissues. While the majority of methods to define cellular variation are based on the transcriptomics[1,2], ascertaining genomic diversity enables the understanding of the underlying blueprint of cellular heterogeneity. Numerous biological studies have demonstrated that accurate identification of genetic variation in single cells is essential for understanding the role of mutation in normal development and in disease[3,4,5]. Key to detecting this diversity is the ability to faithfully replicate the genomes that are not detectable reliably at the single cell level without prior amplification. The development of Primary Template-directed Amplification (Figure 1) for individual cells and low DNA inputs allows for amplification with unprecedented uniformity, providing revolutionary sequencing breadth and sensitivity[6].

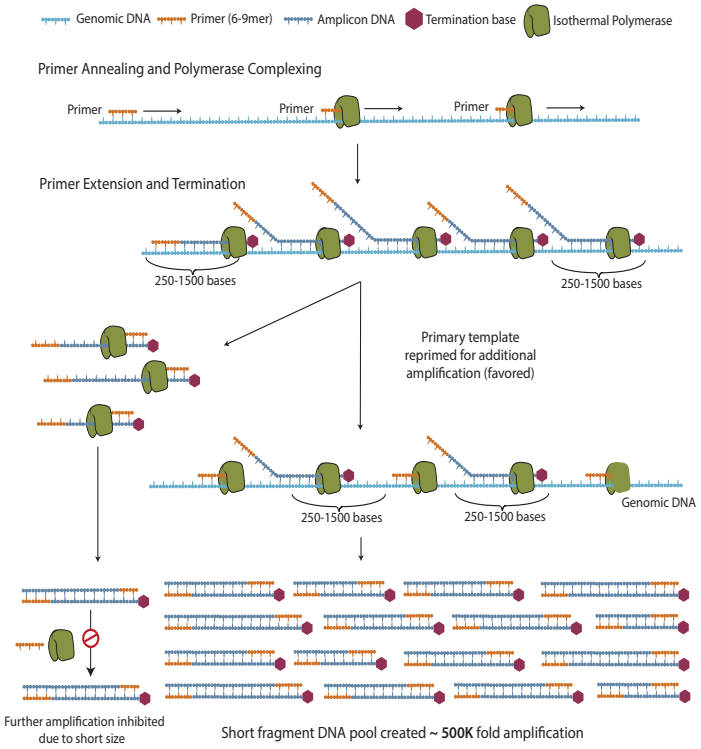

Figure 1. The principle of PTA. ResolveDNA provides unbiased amplification by utilizing random priming combined with a novel approach to produce a true representation of original sample template. The use of proprietary nucleotides prevents the production of long amplicons, which are kinetically unfavored to be re-copied during the amplification reaction. By limiting the size of the produced amplicon, primers are re-directed to the primary template.

While whole genome sequencing (WGS) using next generation sequencing (NGS) uncovers striking single nucleotide variation (SNV) across the entire genome, much of intergenic and intronic regions remain unannotated. Due to this and the cost of WGS, more cost effective approaches, such as whole exome sequencing, (WES) are desirable. WES has broad coverage of all annotated and expressed genes and is able to interrogate the impact of genetic variation. Alternatively, user-selected specific regions of the genome for high depth NGS analysis provide a cost effective approach allowing increased cellular

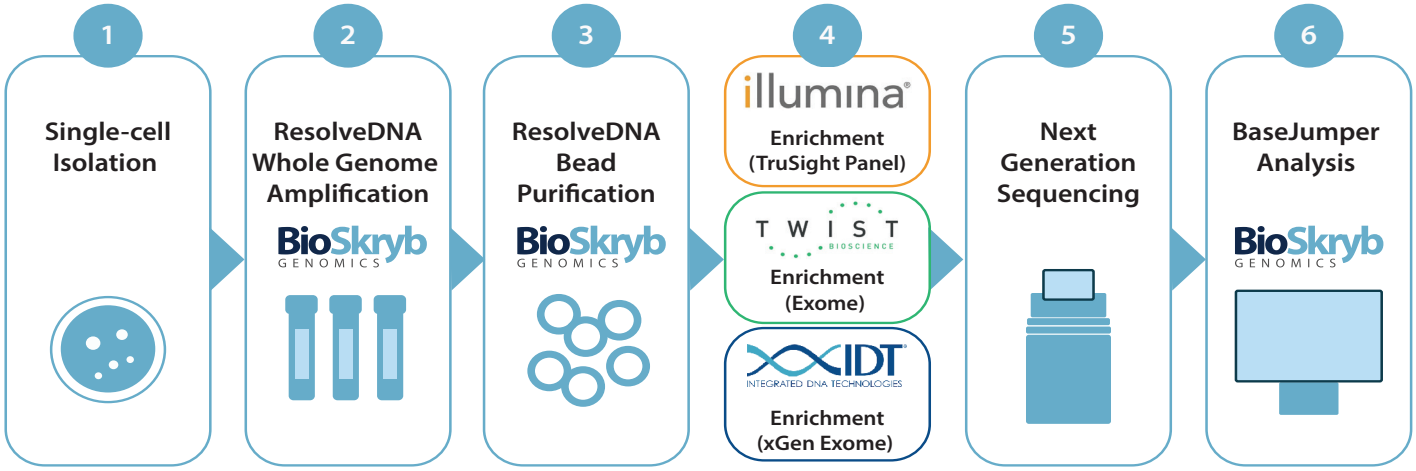

Figure 2. ResolveDNA™ Targeted Enrichment Workflow: Isolated single cells undergo Primary Template-directed Amplification, followed by tagmentation or enzymatic-fragmentation-based library preparation. Tagmentation-based libraries can then undergo hybrid capture with Illumina TruSight panels or with IDT exome panels, while enzymatically fragmented libraries are compatible with TWIST and IDT exome probe sets, library preparation, next generation sequencing, and analysis with BaseJumper software.

throughput and broad genomic analysis of a subset of genes to the entire exome. As a result, BioSkryb Genomics has paired several downstream enrichment products (Figure 2) with our ResolveDNA[8] whole genome amplification product solution for both experimental models and clinical samples[9].

Each workflow provided here, is further detailed in a discrete BioSkryb Genomics Technical note which will guide the user on the process of generating enriched NGS-ready libraries for single cell genomic studies. All Techincal Notes can be found at BioSkryb.com.

Illumina DNA Prep for Enrichment

A workflow demonstrating both WGS and genome enrichment using Illumina DNA preparation for Enrichment with the TruSight One Expanded panel containing 6700 genes, with ~16.5 megabase of coverage in the genome (~0.5% of the comprehensive genome).

Twist BioSciences Human Core Exome Enrichment

A workflow which utilizes double-stranded DNA (dsDNA) probes within a comprehensive target enrichment kit for exome and targeted sequencing. Using dsDNA as opposed to single-stranded DNA captures all specified sequences uniformly. The TWIST Human Core Exome covers nearly the entire exome (~34 megabases; ~1.0 % of the comprehensive genome).

Integrated DNA Technologies V2 Exome

To provide increased depth of coverage and enable high multiplexing of samples, the xGen Exome Research Panel v2 targets only the coding sequences of human coding genes in the RefSeq 109 database. The xGen Exome Research Panel v2 consists of 415,115 individually synthesized and quality-controlled xGen Lockdown Probes. The panel spans a 34 Mb target region (19,433 genes) of the human genome and covers 39 Mb of probe space.

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