Next generation sequencing-based detection

Introduction to barcode-based single-cell targeted genome sequencing

A promising new approach for high-throughput single-cell sequencing uses molecular barcodes to tag the nucleic acids of individual cells confined to emulsion droplets. Although, it is now feasible to perform single-cell RNA-seq on thousands of cells using this type of approach, high-throughput single-cell DNA sequencing using droplet microfluidics has not been demonstrated on eukaryotic cells. This is primarily due to the challenges associated with efficiently lysing cells, freeing genomic DNA from chromatin and enabling efficient PCR amplification in the presence of high concentrations of crude lysate. To overcome these obstacles and enable the characterization of genetic diversity within cancer cell populations, we developed a novel multi-step microfluidic droplet workflow that enables efficient and massively-parallel single-cell PCR-based genomic barcoding. The microfluidic workflow first encompasses individual cells in droplets, lyses the cells and prepares the lysate for genomic DNA amplification using proteases. Following this lysate preparation step, the proteases are inactivated with heat and droplets containing the genomes of individual cells are then paired with molecular barcodes and/or PCR amplification reagents.

Single-cell sequencing with molecular barcodes

Bulk VAFs: 0.2

Single-cell: 0.01

Sensitive detection of cells with PACS

Semi-quantitative real-time PCR was performed to detect the presence of marker genes in cell lines. The PACS workflow was compared with standard RT-PCR workflow.

Targeted sequencing of bone marrow biopsies

Looking for targeted assays to detect genetic engineering!

Platform can be configured for almost any cell type
- Up to 100 targets (250 bp amplions)
- High sensitivity detection
- 5,000-10,000 cell throughput per run

4-color multiplex TaqMan detection and sorting

Overview of PACS workflow

PACS capability and key microfluidics steps

1. Generate single-cell TaqMan reactions
   - A. Cell encapsulation and lysis
   - B. PCR reagent addition / lyte dilution
2. Thermocycle droplets
3. Fluorescence sorting & recovery

TaqMan-based detection and sorting

Introduction to PCR-activated cell sorting

PCR-activated cell sorting (PACS) is a novel cytometry method that uses single-cell TaqMan PCR reactions performed in microfluidic droplets to identify and isolate cell subtypes with high-throughput. The technology is able to analyze more than 100,000 cells in parallel for the presence of specific combinations of transcripts, spike variants, non-coding RNAs or genomic DNA and accurately sorts the cell material for further molecular characterization.

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