Platform for high sensitivity genome analysis with droplet microfluidics

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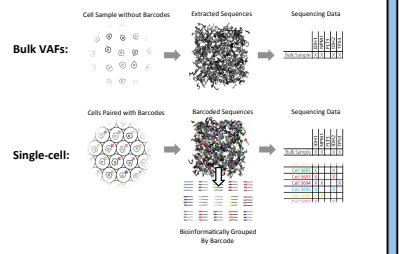
Next generation sequencing-based detection

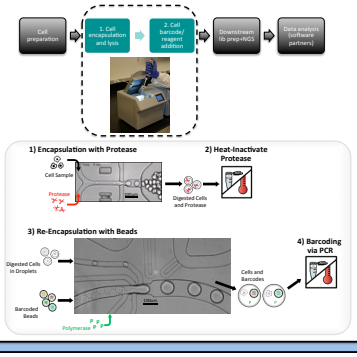
Introduction to barcode-based single-cell targeted genome sequencing

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 encapsulates 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



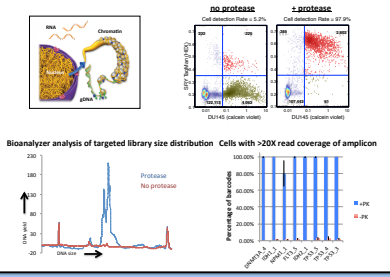
Single-cell sequencing workflow



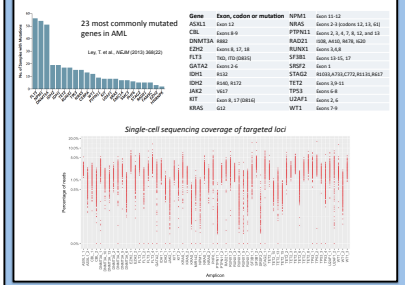
Two-step workflow requirement for barcoding

Access to gDNA restricted by chromatin

TaqMan detection of gDNA w/ 2-step workflow

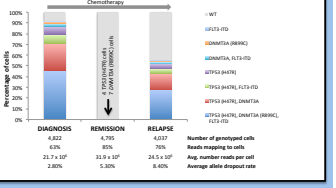


Targeted single-cell sequencing of 62 genomic loci



Gene	Exon, codon or mutation	Gene	Exon, codon or mutation
ASXL1	Exon 12	NPM1	Exon 11-12
CBL	Exons 8-9	NRAS	Exons 2-3 (codons 12, 13, 61)
DNMT3A	R882	PTPN11	Exons 2, 3, 4, 7, 8, 12, and 13
EZH2	Exons 8, 17, 18	RAD21	H38, A410, R478, R620
FLT3	TKD, ITD (D835)	RUNX1	Exons 3,4,8
GATA2	Exons 2-6	SF3B1	Exons 13-15, 17
IDH1	R132	SRSF2	Exon 1
IDH2	R140, R172	STAG2	R1010,A,T31,C772,R1110,R617
JAK2	V617	TET2	Exons 3,9-11
KIT	Exon 8, 17 (D816)	TP53	Exons 4-8
KRAS	G12	U2AF1	Exons 2, 6
		WT1	Exons 7-9

Targeted sequencing of bone marrow biopsies



	DIAGNOSIS	REMISSION	RELAPSE
Number of genotyped cells	4,822	4,795	4,037
Reads mapping to cells	63%	85%	76%
Avg. number reads per cell	21.7 x 10^6	31.9 x 10^6	24.5 x 10^6
Average allele dropout rate	2.80%	5.30%	8.40%

Looking for targeted assays to detect genetic engineering!

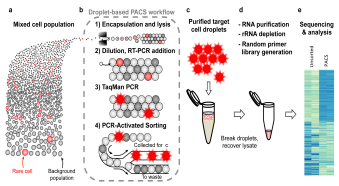
- Platform can be configured for almost any cell type
- Up to 100 targets (250 bp amplicons)
- High sensitivity detection
- 5,000-10,000 cell throughput per run

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, splice variants, non-coding RNAs or genomic DNA and accurately sorts the cell material for further molecular characterization.

Overview of PACS workflow



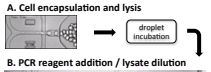
PACS capability and key microfluidics steps

Current capabilities:

- >100,000 single-cell reactions per run
- 4-color TaqMan-based sorting
- Detect transcripts, mutations, non-coding RNAs, splice variants
- Multiplex antibodies and PCR targets

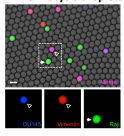
1. Generate single-cell TaqMan reactions

A. Cell encapsulation and lysis



B. PCR reagent addition / lysate dilution

2. Thermocycle droplets



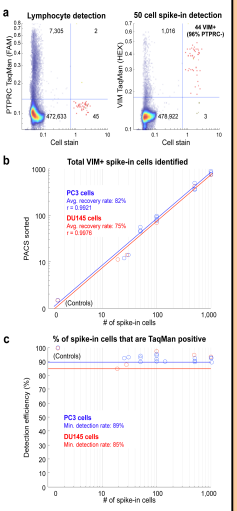
3. Fluorescence sorting & recovery

Sensitive detection of cells with PACS

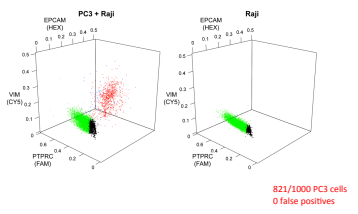
50 prostate cancer PC3 cells ~10,000 B-lymphocytes

Representative PACS cancer cell spike in data. 50 PC3 cells were spiked into ~10,000 Raji cells and sorted with the PACS single-cell RT-PCR workflow. 84% of the PC3 cells that were spiked in were detected based on the presence of vimentin expression and the exclusion of CD45 expression.

Combined cancer cell spike in data demonstrates the potential of PACS for the identification and analysis of rare cells

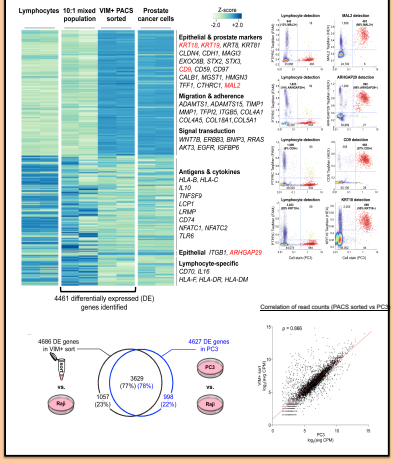


4-color multiplex TaqMan detection and sorting



RNA-Seq on PACS sorted lysate

Transcriptome analysis of PACS-sorted cells. (a) Hierarchical clustering of the 4461 genes differentially expressed (DE) between VIM+-sorted droplets and the Raji:PC3 (10:1) starting heterogeneous population. The expression of the same 4461 genes in the pure lymphocyte (Raji) and prostate cancer cell (PC3) populations are shown for comparison. The genes in red were independently verified as being differentially expressed in PC3 cells. (b) Venn diagram showing the number of DE genes in common between prostate cancer PC3 cells and VIM+-sorted material when each is independently compared to the lymphocyte Raji cell line.



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