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Single Cell Protein Profiling by Microdroplet Barcoding and Next Generation Sequencing

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Abstract

DNA barcoding of individual cells combined with next generation sequencing enables high-throughput parallel analysis of biomolecules at the single-cell level. Encoding protein identity with DNA barcoding of specific antibody binders achieves sequencing-based protein quantitation by converting protein signals into DNA signals. Here we describe how to prepare DNA-barcoded antibodies and connect protein identities to cellular identities using droplet microfluidics. This approach allows for multiplex single cell protein analysis compatible with single cell transcriptomic and mutational profiling methods.

Keywords

Next generation sequencing; Droplet microfluidics; Proteomics; DNA Barcoding; Single cell analysis

1. Introduction

Single cell sequencing with DNA barcoding is revolutionizing biology [1, 2]. DNA barcoding of cellular analytes can be achieved by isolating individual cells, adding DNA barcodes that are unique to each cell, converting target analytes into DNA molecules that are compatible with barcodes and joining analyte DNA with the barcodes. Conversion of intracellular DNA or RNA molecules to barcode-compatible DNA is conceptually straightforward and has been demonstrated by utilizing the properties of nucleic acid processing enzymes such as DNA polymerase, ligase and/or reverse transcriptase [3-9]. Unlike nucleic acids, the primary sequence of proteins cannot be easily converted back to an encoding DNA sequences using any known enzyme. Therefore, protein analysis at the single cell level has relied on the use of antibodies that bind specifically to target proteins. Flow cytometry uses fluorescently labeled antibodies to convert protein signals into fluorescence signals [10]. Because of the spectral overlap between fluorophores, the number

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of analytes that can be simultaneously measured by flow cytometry is limited to tens of protein species [11]. Mass cytometry increases the number of protein analytes measured in a single experiment to over one hundred by utilizing antibodies labeled with heavy metal isotopes [11-13]. The limiting factor for mass cytometry's multiplexability is not the resolution of mass spectrometry but rather the number of heavy metal elements available for antibody labeling. Additional increases in multiplexibility can be achieved using antibodies conjugated with DNA tags. The enormous encoding capacity of DNA enables analysis of thousands of proteins in a single experiment why remaining compatible with genomic and transcriptomic profiling of the same cell. Here we demonstrate that proteins can be quantified at the single cell level by converting protein signals into DNA signals using DNA-tagged antibodies; this connects protein identities to cellular identities by merging DNA-barcoded droplets with cell-encapsulating droplets and performing in-droplet strand overlap extension (SOE) PCR. We were able to measure expected surface protein expression patterns (CD3 and CD19) of approximately 10,000 individual cells from a mixture of two cell lines (Jurkat and Raji) with minimal crosstalk [14]. Several investigators have also demonstrated similar approaches enabling multiomic measurements from single cells [15-17].

2. Materials

Prepare all solutions using ultrapure water (resistivity of 18 MΩ·cm at 25 °C) and analytical grade reagents. Prepare and store all reagents at room temperature unless otherwise indicated. Diligently follow all waste disposal regulations when disposing waste materials for both biological and chemical wastes.

2.1 Cell culture

1. Culture medium: RPMI-1640 medium supplemented with 5% fetal bovine serum (FBS) and antibiotics (50 IU/mL penicillin and 50 μg/mL streptomycin).
2. Cell lines: Jurkat cells (clone E6-1) and Raji cells. Culture cells at 37 °C in the presence of 5% CO_2 and maintain cell density below 3×10^6 cells/mL by replacing medium.

2.2 Antibody-DNA conjugation

1. 100 μM 5′ thiol-modified DNA oligos (*see* Note 1)
   a. CD3 oligo

      5′-CAAGCAGAAGACGGCATACGAGATNNNNNNNNGTTATAA CTCTGCGTTGATACCACTGC-3′
   b. CD19 oligo

      5′-CAAGCAGAAGACGGCATACGAGATNNNNNNNNCAATTAA CTCTGCGTTGATACCACTGC-3′
2. Reducing agent: 1 M dithiothreitol (DTT)
3. DNA purification column: DNA Clean & Concentrator® (Zymo Research)

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4. Thiol reaction buffer (10x): 10x PBS pH 7.2
5. Crosslinker: SM(PEG)$_6$ (Thermo Scientific/Pierce) (Fig. 1a; *see* Note 2). Prepare 2.5 mM solution in anhydrous dimethyl sulfoxide (DMSO) immediately before reaction.
6. Amine reaction buffer (10x): 100 mM HEPES pH 8.2
7. Antibodies: LEAF-purified anti-human CD3 and CD19 antibodies (BioLegend)
8. Antibody purification kit: Nab® Protein A/G Spin Kit (Thermo Scientific)

2.3 Cell staining and in-droplet lysis

1. FACS buffer: Cold PBS pH 7.2 supplemented with 2% FBS
2. Blocking buffer: FACS buffer supplemented with 2% goat serum, 2% mouse serum and 1 μg/mL salmon sperm dsDNA. Keep blocking buffer on ice.
3. Fc block buffer: Blocking buffer supplemented with 25 μg/mL of Human BD Fc Block® (BD Biosciences)
4. Cell loading buffer: FACS buffer supplemented with 17% OptiPrep® (*see* Note 3).
5. Lysis buffer: 100 mM Tris pH 8 with 2% Tween 20 and 1.5 mg/mL Proteinase K.
6. Oil phase: HFE 7500 supplemented with 2% (w/w) fluorosurfactant (PEG-PFPE amphiphilic block copolymer surfactant from Ran Biotechnologies).
7. Co-flow device (Fig. 2a left; *see* Note 4).
8. Injection system: 3-mL syringes, 27-gauge needles, polyethylene tubing (I.D. 0.38 mm, O.D. 1.09 mm; PE/2 from Scientific Commodifies, Inc.), and syringe pumps (*see* Note 5).
9. PCR machine

2.4 Preparation of barcode droplets

1. Barcode oligos
   a. Barcode template:

      5′-GCGACCACCGAGATCTACACTATAGCCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNNNNGCAGTGGTATCAACGCAG AG-3′

   b. Forward primer (P5):

      5′-AATGATACGGCGACCACCGAGATCTACAC-3′

   c. Reverse primer (Homology):

      5′-CTCTGCGTTGATACCACTGC-3′

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2. Droplet PCR master mix: Platinum Multiplex PCR Master Mix (Thermo Fisher Scientific), 2% (w/v) Tween 20, 2% (w/v) PEG 6000.
3. FC40 fluorinated oil supplemented with 5% (w/w) fluorosurfactant.
4. Single-inlet droplet maker device (Fig. 2a right; *see* Note 4).

2.5 Droplet merge and SOE-PCR

1. Adaptor primers
   a. Forward primer (P5-short):
      5′-AATGATACGGCGACCACCGA-3′
   b. Reverse primer (P7):
      5′-CAAGCAGAAGACGGCATACGAGAT-3′
2. FAM-conjugated TaqMan® probe: 5′-AGATCGGAAGAGCGTCGTGTAGG-3′
3. Triple-merge device (Fig. 2b; *see* Note 4).
4. Droplet coalescence reagent: 1*H*,1*H*,2*H*,2*H*-perfluoro-1-octanol (perfluorooctanol)
5. Exonuclease I
6. qPCR system with FAM/GFP channel

2.6 Library Preparation and NGS

1. DNA quantitation kits: Qubit® dsDNA HS Assay Kit (Thermo Fisher), NEBNext® Library Quant Kit for Illumina (New England BioLabs)
2. 2100 Bioanalyzer (Agilent)
3. MiSeq sequencer (Illumina)

3. Methods

Carry out all procedures at room temperature unless otherwise specified (Fig. 3).

3.1 Antibody-DNA conjugation

1. Reduce thiol groups of the 5′ thiol-modified DNA oligo by adding 10 μL of 1 M DTT to 100 μL of the 100 μM oligo solution.
2. Transfer 15 μL of the reduced oligo to a DNA purification column, spin for purification, and elute in 15 μL of water.
3. Add 1.5 μL of 10x thiol reaction buffer. Add 0.5 μL of 2.5 mM SM$(PEG)_6$ crosslinker and incubate for 20 min at room temperature.
4. Transfer the entire volume to a new DNA purification column, spin for purification, and elute in 15 μL of water.

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5. Add 1.5 μL of 10x amine reaction buffer. Add 10 μL of 1 mg/mL antibody and incubate for 30 min at room temperature.
6. Transfer the entire volume to the antibody purification kit, following the manufacturer's protocol (*see* Note 6).
7. Store conjugated antibody solution at 4 °C (*see* Note 7).

3.2 Cell staining and in-droplet cell lysis

1. Determine the cell density of each cell culture. Combine 5×10^5 Jurkat and Raji cells and wash with FACS buffer.
2. Resuspend cells in 100 μL of blocking buffer and incubate cells for 30 minutes on ice.
3. Centrifuge cells at 300 RCF for 3 min and resuspend in 100 μL of Fc block buffer. Incubate for 10 minutes at room temperature.
4. Add 1 μg of each conjugated antibody to the cell suspension and incubate for 30 minutes on ice. Wash cells 5 times with 1 mL of FACS buffer.
5. Resuspend cells in 100 μL of cell loading buffer.
6. Introduce cell suspension and lysis buffer to a co-flow device to generate droplets using the injection system at the following flow rates: 200 μL/hour for cell suspension and lysis buffer, and 600 μL/hour for oil phase (*see* Note 8).
7. Collect cell-containing droplets in PCR tubes.
8. Incubate droplets for 30 minutes at 55 °C.
9. Inactivate proteinase K by incubating at 95 °C for 15 min.
10. Store cell lysate droplets on ice.

3.3 Preparation of barcode droplets

1. Prepare 2.1 fM barcode template solution in droplet PCR master mix. Add 200 nM of forward (P5) and reverse (Homology) primers to make barcode solution (*see* Note 9).
2. Introduce barcode solution to a single-inlet droplet maker using the injection system at the following flow rates: 300 μL/hour for barcode solution and 900 μL/hour for oil phase (*see* Note 5).
3. Collect droplets in PCR tubes.
4. Thermocycle to perform in-droplet digital PCR as follows: initiation (95°C for 2 min), amplification (35 cycles of 95 °C for 30 s, 60 °C for 90 s, 72 °C for 20 s), and final extension (72 °C for 10 min).
5. Store barcode droplets on ice.

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3.4 Droplet merge and SOE-PCR

1. Add 200 nM of forward (P5-short) and reverse (P7) primers to droplet PCR master mix to make SOE-PCR mix.
2. Transfer barcode droplets and cell lysate droplets to syringes for device injection.
3. Introduce barcode droplets, cell lysate droplets and SOE-PCR mix to a triple-merge device (*see* Note 10) using the injection system at the following flow rates: 30 μL/hour for barcode droplets and cell lysate droplets, 300 μL/hour for droplet PCR master mix, and 500 μL/hour for oil phase.
4. When droplet formation and pairing become stable, turn on 100–200 V of alternating electric field to induce droplet coalescence in the electrode region of the device.
5. Collect droplets in PCR tubes.
6. Exchange oil phase with FC40 supplemented with 5% fluorosurfactant.
7. Thermocycle to perform in-droplet SOE-PCR as follows: initiation (95°C for 2 min), amplification (11 cycles of 95 °C for 30 s, 60 °C for 90 s, 72 °C for 20 s), and final extension (72 °C for 10 min).
8. Coalesce droplets by adding 100 μL perfluorooctanol and centrifuge at 1000 RCF for 1 min.
9. Transfer aqueous phase to a new PCR tube.
10. Add 0.1x volume of Exonuclease I and incubate at 37 °C for 15 minutes.
11. Inactivate enzymes at 80 °C for 15 minutes.
12. Transfer solution to a new DNA purification column, spin for purification, and elute with water.
13. Perform a second round of bulk PCR using the same primers and thermal cycling.
14. Purify PCR product using a new DNA purification column.

3.5 Sequencing and data analysis

1. Determine DNA concentration using DNA quantitation kits (*see* Note 11).
2. Dilute library to 15 pM and sequence using MiSeq (single read, 45 cycles).
3. Load fastq files using ShortRead R package. Apply quality control filters to homology (up to 2 mutations allowed) and cell barcode sequences (Hamming distance filtering; Fig. 4a and 4b).
4. Count the number of unique molecular identifiers (UMIs) that belong to each antibody tag sequences: CD3 (TTATAAC) and CD19 (TTAATTG) (Fig. 4c; *see* Note 12).

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4. Notes

1. The underlined sequences correspond to the reverse complements of antibody-specifying sequences: CD3 (TTATAAC) and CD19 (TTAATTG).

2. Store crosslinker solid cold and desiccated at −20 °C. Warm to room temperature before opening and avoid freeze-thaw cycles. This crosslinker is heterobifunctional, designed to react with two different nucleophilic groups on the antibody. *N*-hydroxysuccinimide ester reacts rapidly with amines above pH 7.0. Maleimides react most rapidly with sulfhydryl groups under the same condition. Use of this reagent enables conjugation of thiol-modified DNA oligo to amine-rich antibody proteins.

3. OptiPrep® is 60% (w/v) iodixanol solution in water, which slows down cell sedimentation during droplet generation by increasing density of cell loading buffer.

4. We fabricate polydimethylsiloxane (PDMS) microfluidic devices in the lab using soft lithography [18]. A detailed procedure and video recording can be found in [14, 19]. At a minimum, soft lithography based on photoresist chemistry requires a chemical fume hood, a spincoater for silicon wafers, a UV irradiation source, an incubation oven (temperature ranges between 50 °C and 100 °C) and a temperature-controlled hot plate. Nano/microfabrication facilities are typically equipped with these instruments. Also, there are commercial manufacturers of microfluidic devices (such as Dolomite Microfluidics and uFluidix).

5. Please refer to video recording in [20] for general guidelines on droplet handling and microfluidic device operation.

6. Successful conjugation and purification can be confirmed by SDS-PAGE stained with a protein-binding dye and/or a DNA-binding dye (Fig. 1b and 1c).

7. Conjugated antibodies are commercially available. TotalSeq® antibodies (Biolegend) are compatible with Chromium® droplet workflows (10x Genomics). AbSeq® antibodies are compatible with BD Rhapsody® system (BD Biosciences). DNA + Protein panels are compatible with Tapestri® system (Mission Bio).

8. Water-in-oil droplet generation should become stable before starting collection of droplets. Video images from a high-speed camera on a microscope can be used to monitor droplet formation. Also, a small volume of generated droplets can be collected in a microscopy slide and inspected for size uniformity. Using a co-flow device with a 38-μm nozzle at the specified flow rates, droplets are generated at 7 million droplets per hour, and droplet size should be approximately 47 μm in diameter.

9. Barcode template concentration should be adjusted to match droplet size. Our single-inlet device (30-μm nozzle) makes 53-μm diameter droplets (78 pL). 2.1 fM is equivalent to one template molecule per 10 droplets, thus ensuring single-molecule template for digital PCR.

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10. The triple-merge device uses electric field to induce droplet coalescence (Fig. 2b, blue box). Before introducing aqueous and oil phases to the device, both electrode and moat layers should be filled with 1 M NaCl solution to serve as saltwater electrodes and electric field barriers, respectively [21]. It is crucial to eliminate air bubbles inside the microchannels and tubing between electrodes and the voltage source (*see* Note 5).

11. DNA quantitation results can vary depending on the methods, especially for short DNA amplicons at low concentrations. We recommend using multiple DNA quantitation methods and comparing results to determine the final dilution factor for sequencing library.

12. Bioinformatic analysis pipeline is not included in this chapter. Please refer to ref. [14] for detailed description of quality control filters and amplification bias correction.

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References

1. Papalexi E, Satija R (2018) Single-cell RNA sequencing to explore immune cell heterogeneity. Nature Reviews Immunology 18:35–45. 10.1038/nri.2017.76
2. Baslan T, Hicks J (2017) Unravelling biology and shifting paradigms in cancer with single-cell sequencing. Nature Reviews Cancer 17:557–569. 10.1038/nrc.2017.58 [PubMed: 28835719]
3. Picelli S, Faridani OR, Björklund Åk, et al. (2014) Full-length RNA-seq from single cells using Smart-seq2. Nature Protocols 9:171–181. 10.1038/nprot.2014.006 [PubMed: 24385147]
4. Fan HC, Fu GK, Fodor SPA (2015) Combinatorial labeling of single cells for gene expression cytometry. Science 347:1258367. 10.1126/science.1258367 [PubMed: 25657253]
5. Klein AM, Mazutis L, Akartuna I, et al. (2015) Droplet barcoding for single cell transcriptomics applied to embryonic stem cells. Cell 161:1187–1201. 10.1016/j.cell.2015.04.044 [PubMed: 26000487]
6. Macosko EZ, Basu A, Satija R, et al. (2015) Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets. Cell 161:1202–1214. 10.1016/j.cell.2015.05.002 [PubMed: 26000488]
7. Zheng GXY, Terry JM, Belgrader P, et al. (2017) Massively parallel digital transcriptional profiling of single cells. Nature Communications 8:14049. 10.1038/ncomms14049
8. Lan F, Demaree B, Ahmed N, Abate AR (2017) Single-cell genome sequencing at ultra-high-throughput with microfluidic droplet barcoding. Nature Biotechnology 35:640–646. 10.1038/nbt.3880
9. Pellegrino M, Sciambi A, Treusch S, et al. (2018) High-throughput single-cell DNA sequencing of acute myeloid leukemia tumors with droplet microfluidics. Genome Res 28:1345–1352. 10.1101/gr.232272.117 [PubMed: 30087104]
10. Robinson JP, Roederer M (2015) Flow cytometry strikes gold. Science 350:739–740. 10.1126/science.aad6770 [PubMed: 26564833]
11. Bendall SC, Nolan GP, Roederer M, Chattopadhyay PK (2012) A deep profiler's guide to cytometry. Trends in Immunology 33:323–332. 10.1016/j.it.2012.02.010 [PubMed: 22476049]

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12. Bendall SC, Simonds EF, Qiu P, et al. (2011) Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum. Science 332:687–696. 10.1126/science.1198704 [PubMed: 21551058]

13. Bodenmiller B, Zunder ER, Finck R, et al. (2012) Multiplexed mass cytometry profiling of cellular states perturbed by small-molecule regulators. Nature Biotechnology 30:858–867. 10.1038/nbt.2317

14. Shahi P, Kim SC, Haliburton JR, et al. (2017) Abseq: Ultrahigh-throughput single cell protein profiling with droplet microfluidic barcoding. Scientific Reports 7:44447. 10.1038/srep44447 [PubMed: 28290550]

15. Stoeckius M, Hafemeister C, Stephenson W, et al. (2017) Simultaneous epitope and transcriptome measurement in single cells. Nature Methods 14:865–868. 10.1038/nmeth.4380 [PubMed: 28759029]

16. Peterson VM, Zhang KX, Kumar N, et al. (2017) Multiplexed quantification of proteins and transcripts in single cells. Nature Biotechnology 35:936–939. 10.1038/nbt.3973

17. Mimitou EP, Cheng A, Montalbano A, et al. (2019) Multiplexed detection of proteins, transcriptomes, clonotypes and CRISPR perturbations in single cells. Nature Methods 16:409–412. 10.1038/s41592-019-0392-0 [PubMed: 31011186]

18. Xia Y, Whitesides GM (1998) Soft Lithography. Angewandte Chemie International Edition 37:550–575. 10.1002/(SICI)1521-3773(19980316)37:5<550::AID-ANIE550>3.0.CO;2-G [PubMed: 29711088]

19. Cole RH, Tran TM, Abate AR (2015) Double Emulsion Generation Using a Polydimethylsiloxane (PDMS) Co-axial Flow Focus Device. JoVE (Journal of Visualized Experiments) e53516. 10.3791/53516

20. Demaree B, Weisgerber D, Lan F, Abate AR (2018) An Ultrahigh-throughput Microfluidic Platform for Single-cell Genome Sequencing. JoVE (Journal of Visualized Experiments) e57598. 10.3791/57598

21. Sciambi A, Abate AR (2014) Generating electric fields in PDMS microfluidic devices with salt water electrodes. Lab on a Chip 14:2605–2609. 10.1039/c4lc00078a [PubMed: 24671446]

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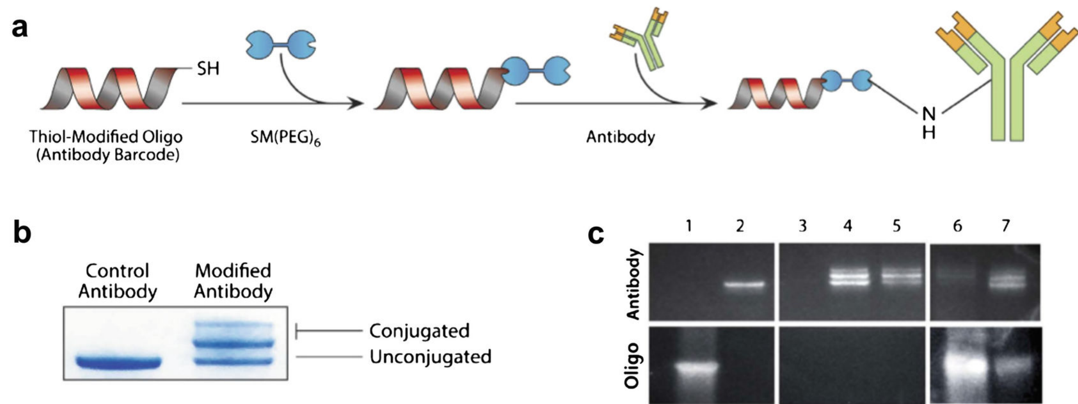

Fig. 1.

Antibody-DNA conjugation. (a) SM(PEG)$_6$, a heterobifunctional crosslinker, is used to link thiol-modified DNA oligo with antibody via amine groups. (b) SDS-PAGE gel image stained with a protein-binding dye (SimplyBlue® SafeStain) confirms conjugation reaction for CD3 antibody as indicated by additional bands at a higher molecular weight region. The pattern suggests that the largest fraction is from a single conjugation and a smaller fraction is composed of multiply conjugated antibodies. (c) Gel electrophoresis stained with a DNA-binding dye (SYBR Green) shows successful purification of DNA-labeled antibody using the antibody purification kit: 1 = DNA oligo only; 2 = unconjugated CD3 antibody; 3–5 = first, second and third elution fractions from the antibody purification kit; 6 = flow-through from the antibody purification kit; 7 = conjugated antibody before purification (Reproduced from ref. [14], licensed under the Creative Commons license http://creativecommons.org/licenses/by/4.0/).

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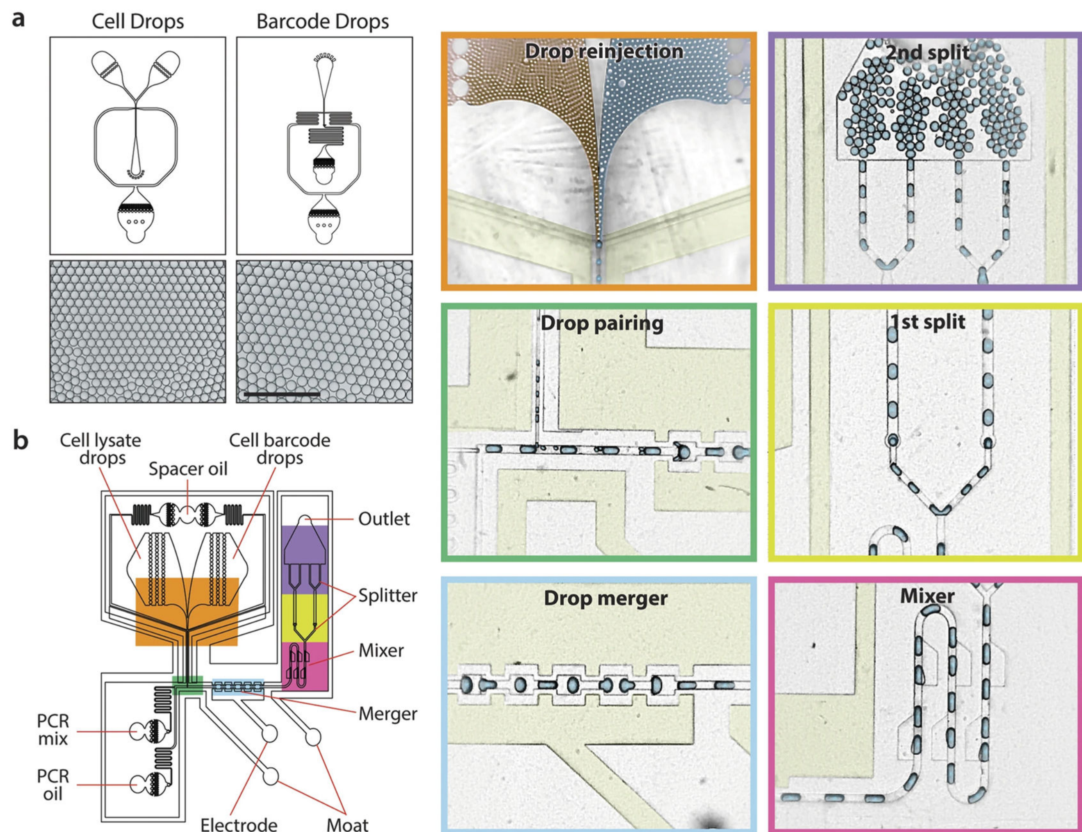

Fig. 2.

Microfluidic devices for single cell protein profiling. (a) A co-flow device with two inlets (left) is used to encapsulate single cells with a lysis buffer; a single-inlet droplet maker (right) is used to make barcode droplets from single-molecule templates via digital PCR. (b) Cell lysate droplets and barcode droplets are paired and merged with PCR reagent droplets using a triple-merge device. Each colored rectangular region is shown in expanded micrographs on the right, showing droplet generation and manipulation process (Reproduced from ref. [14]).

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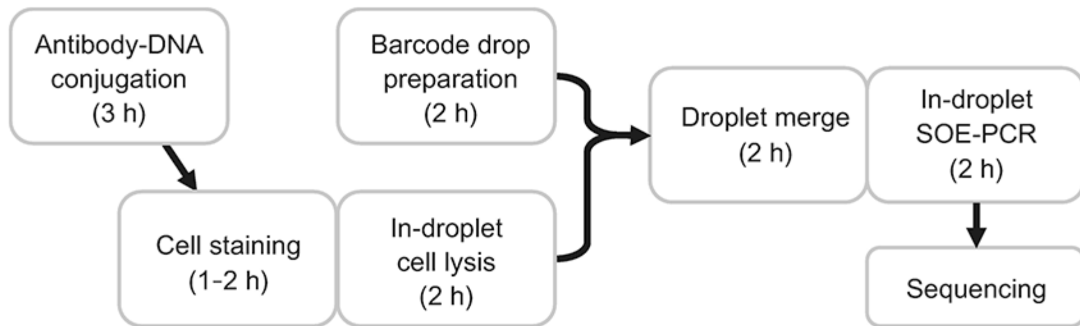

Fig. 3.

Protocol workflow and estimated duration of each step (excluding device fabrication time). DNA-tagged antibodies and sequencing library molecules can be stored, therefore providing safe stopping points. Produced droplets should be processed immediately to minimize the loss of cellular identity due to droplet coalescence. Barcode droplet preparation step can be performed in parallel to cell droplet steps if multiple droplet stations and PCR machines are available.

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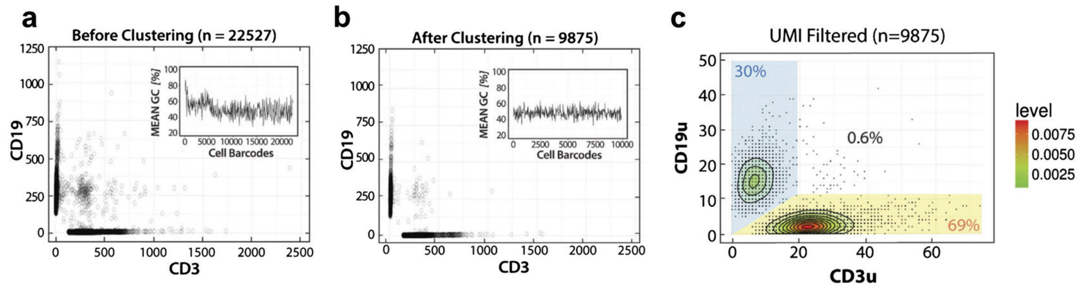

Fig. 4.

Measurement of protein levels from single cell sequencing data. (a) The read counts for CD3 and CD19 antibody tags include a large number of "double positives" before quality filtering. (b) After Hamming distance filtering, most of double positive cell barcodes are removed. (c) UMI-corrected antibody tag counts reveal two distinct populations of cells: $CD3^{high}CD19^{low}$ (T cell signature for Jurkat) and $CD3^{low}CD19^{high}$ (B cell signature for Raji) (Reproduced from ref. [14]).