PrimeFlow RNA Assay - Simultaneous detection of RNA and protein by flow cytometry

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11/8/18
PrimeFlow combines gene AND protein expression

- Flow cytometry → Enrich specific cell subsets
- Invitrogen PrimeFlow RNA Assay
- qPCR → Gene expression analysis
Single cell gene analysis power

**qPCR**

- **CCR5**: 100%
- **CCL5**: 13, 2

**Primeflow**

- **CCR5**: 66, 19
- **CCL5**: 100 cells

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Proprietary & Confidential
Why Single-Cell Gene-Expression Analysis?

**Today**

*Blended average of all events*

**Future**

*See distinct and relevant events*

We need to understand the RED cell and the BLUE cell individually to truly understand biological systems and **create better therapies**
What Is Branched DNA (bDNA)?
Anatomy of Branched DNA (bDNA)

400 labels / bDNA x 20 bDNAs / mRNA = 8000-fold signal amp!

**Target Probe**
- Mix of oligos binding to specific RNA target region
- Each oligo pair secures one bDNA “tree”
Specificity Is Derived from Paired Oligo Probes

![Diagram showing specificity analysis with paired oligo probes](image)

**Fluorescence signal**

- **Count**
- **10^0** to **10^5**

**MFI**

- **No probe**
- **GAPDH left**
- **GAPDH right**
- **GAPDH left + right**
- **DAPB**

**Legend**

- Red: GAPDH left + right
- Yellow: GAPDH left oligos
- Green: GAPDH right oligos
- Blue: DapB
- Black: No probe
Highly specific probes distinguish human from mouse RNA

Mixture of human and murine CD4 T cells

Human-specific CD45 antibody and β2-microglobulin mRNA probe

Branched DNA v. Traditional FISH

Image-based transcriptomics in thousands of single human cells at single-molecule resolution

Nico Battich, Thomas Stoeger & Lucas Pelkmans
PrimeFlow RNA Assay Workflow

**Design**
Probes design, panel design & bDNA

**Stain**
Detect surface & intracellular proteins

**Hybridize**
Detect up to 4 RNA transcripts

AF™ 488 Type 4
AF™ 568 Type 10
AF™ 647 Type 1
AF™ 750 Type 6
RNA detection compatibility with the Attune™ NxT

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AlexaFluor™ 488
AlexaFluor™ 568
AlexaFluor™ 647
AlexaFluor™ 750
Probe types exhibit different assay sensitivities. The highest sensitivity is determined to be ~10 copies/cell for HMBS mRNA in U937 cells when using type 1 and 10 probes.
PrimeFlow offers unique possibilities beyond pure protein or gene expression analysis.

- Investigate Gene/Cell heterogeneity
- Investigate Transcription & Translation Kinetics

No flow cytometry antibody? No problem

Detect virus in infected cell subsets

Singe-cell detection of miRNA on your flow cytometer!
PrimeFlow™ Applications
Mapping transcription/translation kinetics at the single-cell level

![Graph showing IFNγ and TNFα kinetics](image)

- **CD8+ protein**
- **CD8+ mRNA**
- **CD8- protein**
- **CD8- mRNA**

*Data Collected on Attune*
Robust results with rare, hard to isolate cell types

Characterize Rare Tumor-infiltrating CD8⁺ T-cell Inhibitory IgA⁺⁺ B cells

*PMA/Iono/LPS treatment

CD45⁺ cells: 10-20%  IgA⁺CD19⁺ cells: 2-30% of CD45⁺ cells (0.2-6% in total)

22-40% of IgA⁺ cells express IL-10 : 0.04-3 % in total (500-20000 cells / mouse)

Shalapour et al., Nature. 2015 Apr 29. doi: 10.1038/nature14395

*Mouse Prostate cancer model
Single-cell gene expression data from pure FoxP3+ population

Samples from patients with non-small cell lung cancer

Akimova et al., 2017, JCI Insights
Single-cell gene expression data from pure FoxP3+ population

Samples from patients with non-small cell lung cancer

Akimova et al., 2017, JCI Insights
No antibody? No problem!

IL-21 in Idiopathic thrombocytopenic purpura (ITP)

Porichis et al., 2014, Nature Communications
Study viral RNA with flow cytometry

- EBV/KSHV co-infection promotes tumorigenesis in mouse model

McHugh et al., 2017, Cell Host & Microbe 22, 61–73
Evaluation of telomere in five normal human donors

![Diagram of telomere structure with donor samples](image)

**Donor 1**
- Lymphocytes
- Telomere – AF647

**Donor 2**
- Lymphocytes
- Telomere – AF647

**Donor 3**
- Lymphocytes
- Telomere – AF647

**Donor 4**
- Lymphocytes
- Telomere – AF647

**Donor 5**
- Lymphocytes
- Telomere – AF647

**Monocytes**

**Donor 1**
- Monocytes
- Telomere – AF647

**Donor 2**
- Monocytes
- Telomere – AF647

**Donor 3**
- Monocytes
- Telomere – AF647

**Donor 4**
- Monocytes
- Telomere – AF647

**Donor 5**
- Monocytes
- Telomere – AF647
Localization of telomere signal

Human PBMC
Anti-CD3-FITC + telomere Type 1

DAPI  CD3  telomere  overlay
Equipment Needed for the PrimeFlow RNA Assay

- **Incubator with heat block inside**
  - Validated to maintain 40 ± 1°C
  - Metal heat block for 1.5 mL microcentrifuge tube

- **Refrigerated swinging bucket centrifuge**
  - Swinging bucket centrifuge with adaptors for 15 mL conical tubes and 1.5 mL microcentrifuge tubes; (optional: with refrigeration to 4°C)

- **Flow cytometer**
  - Three lasers: blue (488 nm), yellow–green (561 nm), red (633 nm or similar)
  - Detection optics optimized for FITC, Invitrogen™ eBioscience™ PE-eFluor™ 610 (PE-Texas Red™), APC, and Invitrogen™ eBioscience™ APC-eFluor™ 780 (APC-CyR® 5)

- **ViewRNA Temperature Validation Kit**
  - NIST-traceable thermometer, with temperature probe in a 1.5 mL microcentrifuge tube; (Cat. No. QV0523)
The PrimeFlow probes search tool is available at

thermofisher.com/primeflow
Information we need:

• Accession number for the gene of interest

• Fluorochrome preference: Alexa Fluor 647, Alexa Fluor 488, Alexa Fluor 750, or Alexa Fluor 568

We will check design viability for the target probes
bDNA technology – probe design

YOUR gene of interest

ViewRNA proprietary algorithm

Gene specific probe is comprised of ~12-46 oligos
Branched DNA Technology Platforms

PrimeFlow Assay

ViewRNA Family

Quantigene family

B(ranched) DNA
ViewRNA Cell versus ViewRNA Cell Plus

ViewRNA Cell : Detection of human CTCs (pancreatic origin)


ViewRNA Cell Plus: Detection of SKBR3 cells spiked in PBMC

Data courtesy of RARECTYE
ViewRNA ISH Tissue Assay - Pancreas

With Insulin Probe

No Probe Negative Control

Image courtesy of: Ann Dongtao Fu, University of Florida
Questions?

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