

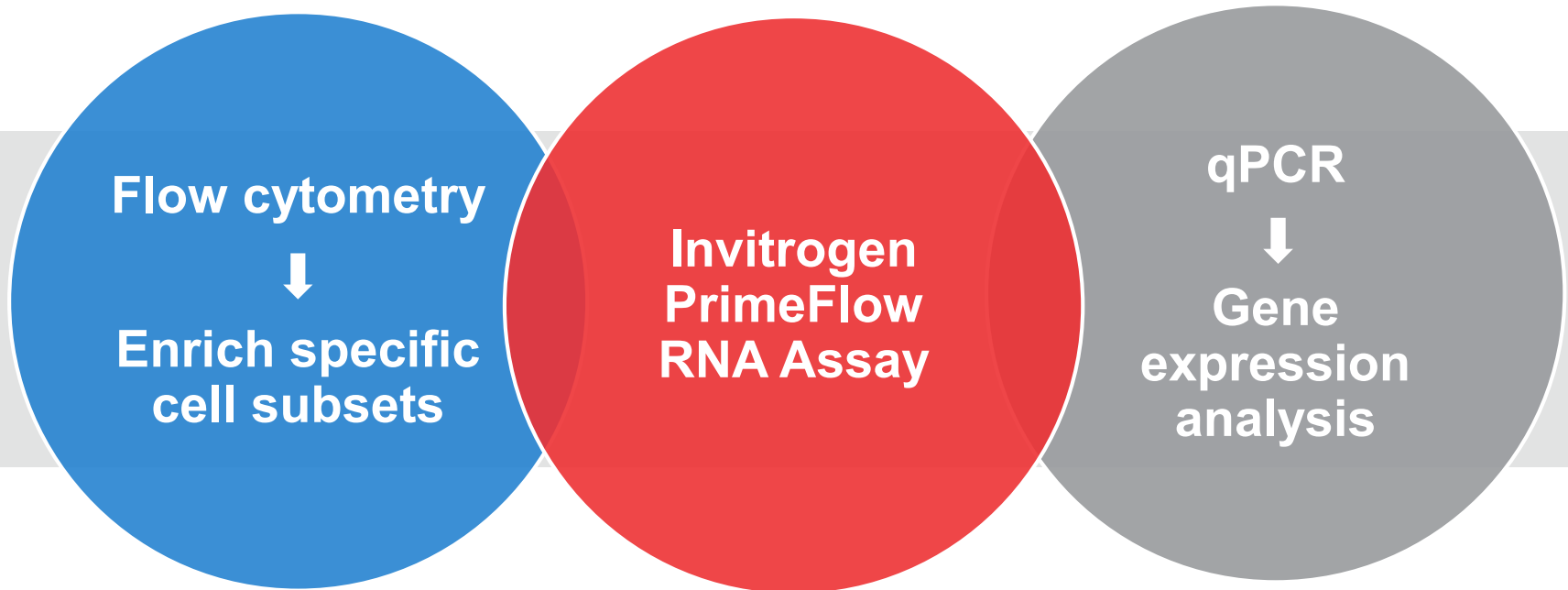


PrimeFlow RNA Assay - Simultaneous detection of RNA and protein by flow cytometry

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Field Application Scientist
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11/8/18

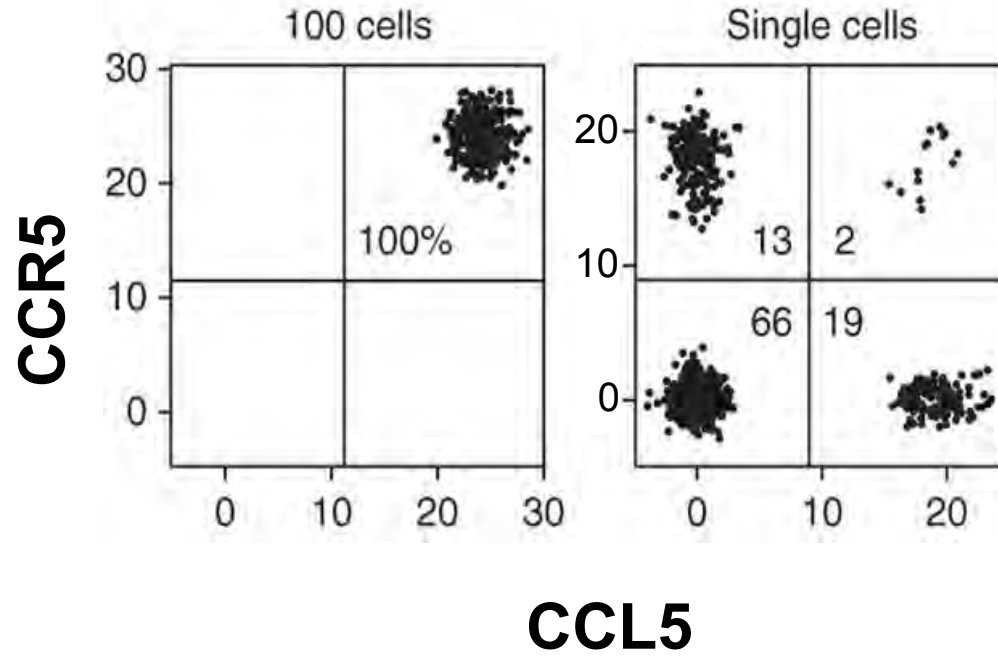
PrimeFlow combines gene AND protein expression



Single cell gene analysis power

qPCR

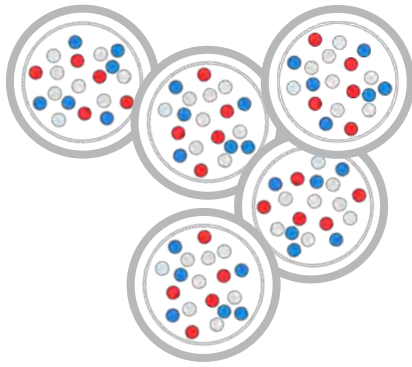
Primeflow



Why Single-Cell Gene-Expression Analysis?

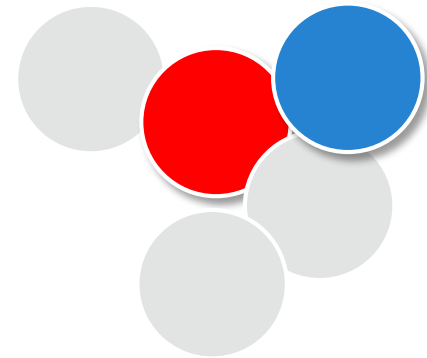
Today

Blended average of all events



Future

See distinct and relevant events



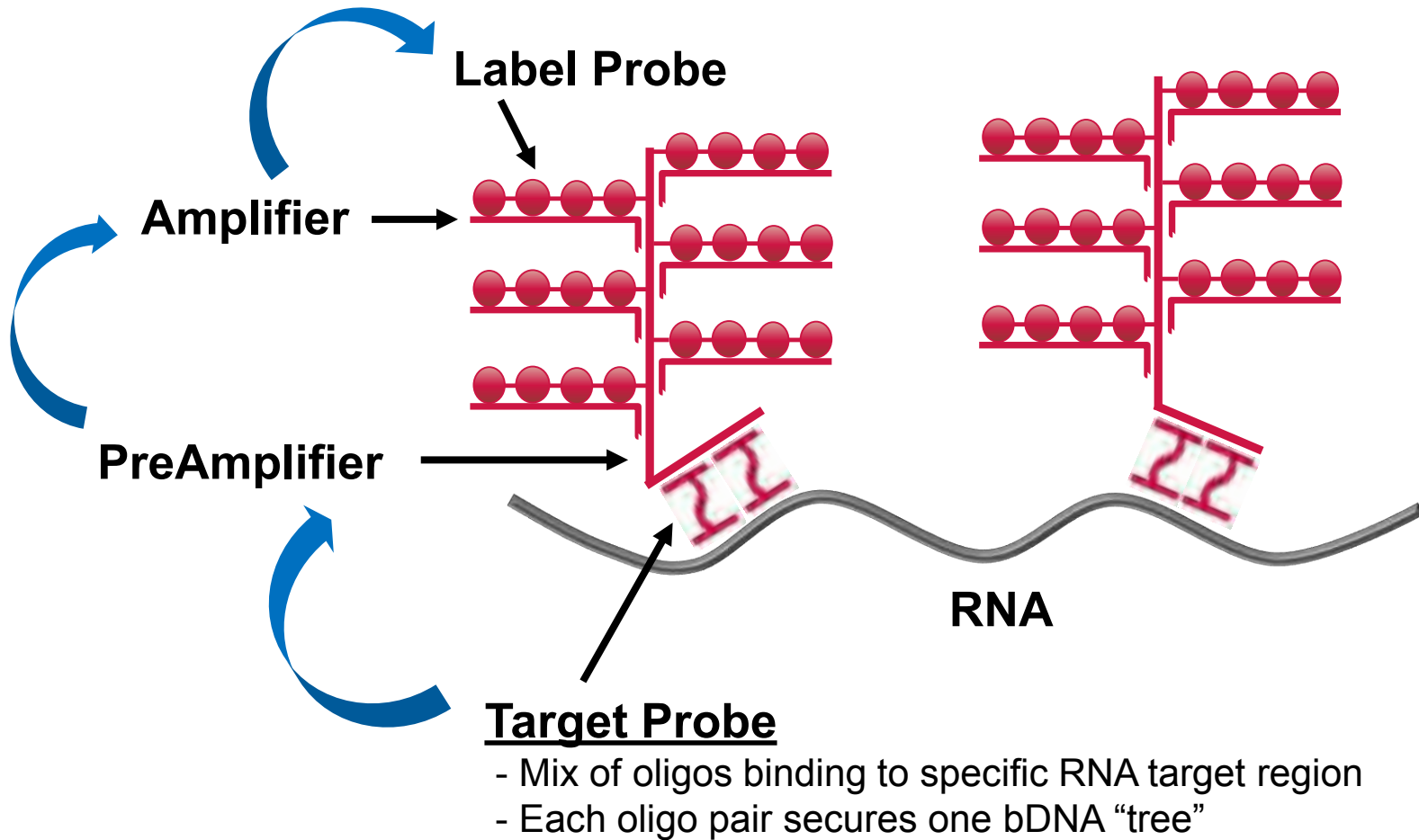
We need to understand the RED cell and the BLUE cell individually to truly understand biological systems and **create better therapies**

What Is Branched DNA (bDNA)?

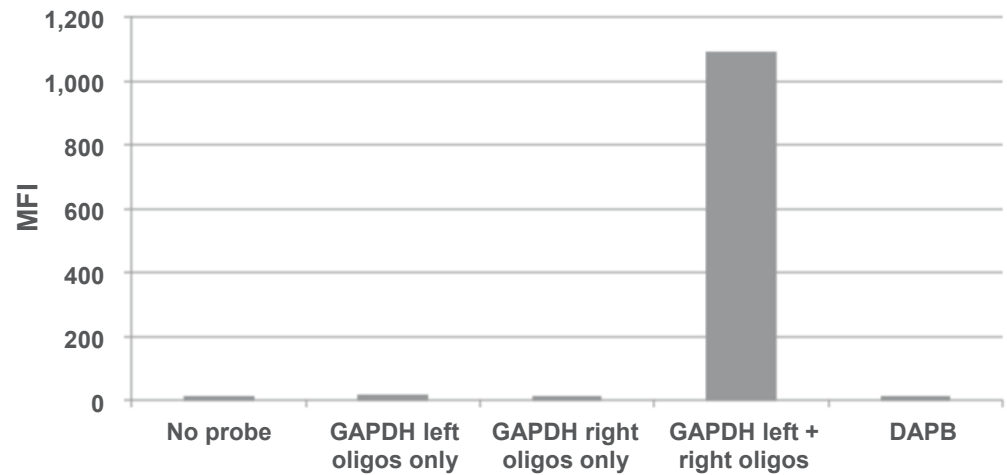
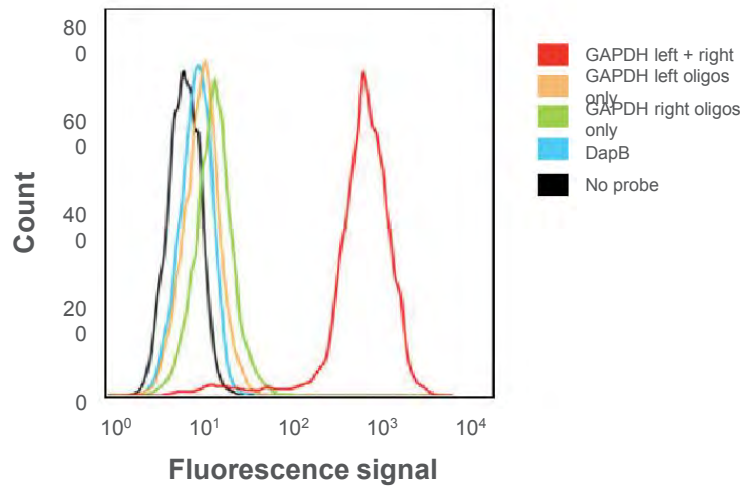
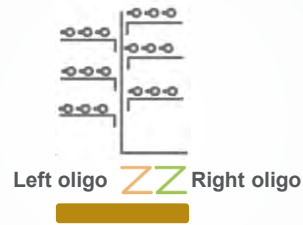


Anatomy of Branched DNA (bDNA)

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

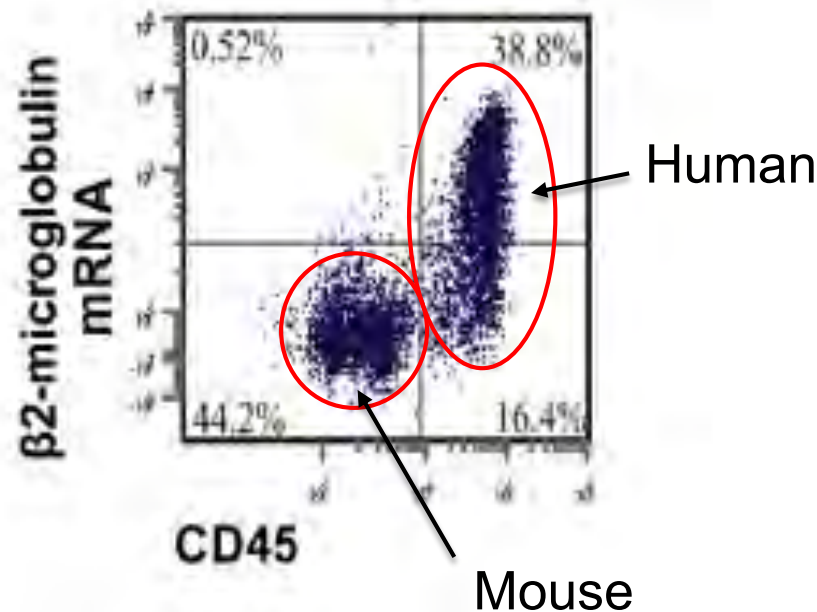


Specificity Is Derived from Paired Oligo Probes



Highly specific probes distinguish human from mouse RNA

Mixture of human and murine CD4 T cells



Human-specific CD45 antibody and $\beta 2$ -microglobulin mRNA probe

Mashiko et al., J Allergy Clin Immunol. 2015 Mar 16. pii: S0091-6749(15)00175-X. doi: 10.1016/j.jaci.2015.01.033

Branched DNA v. Traditional FISH

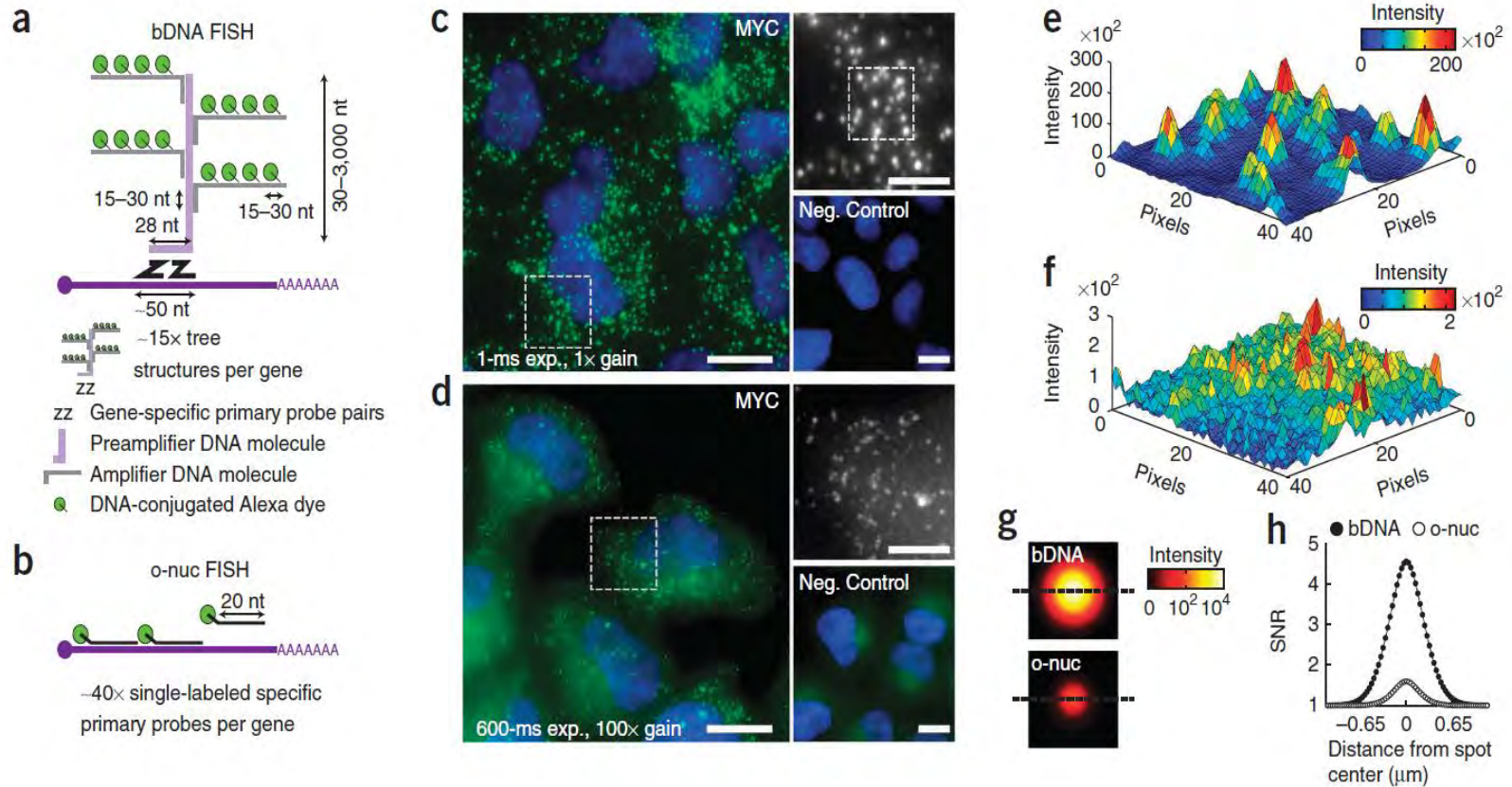
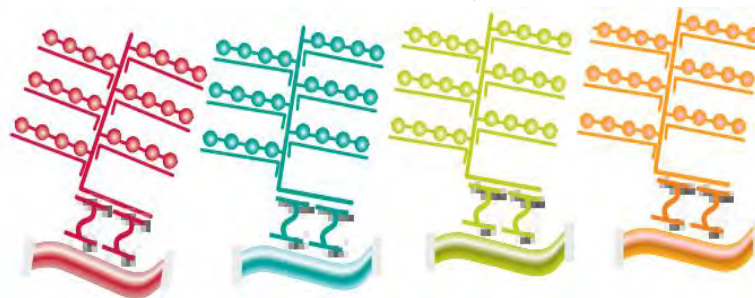
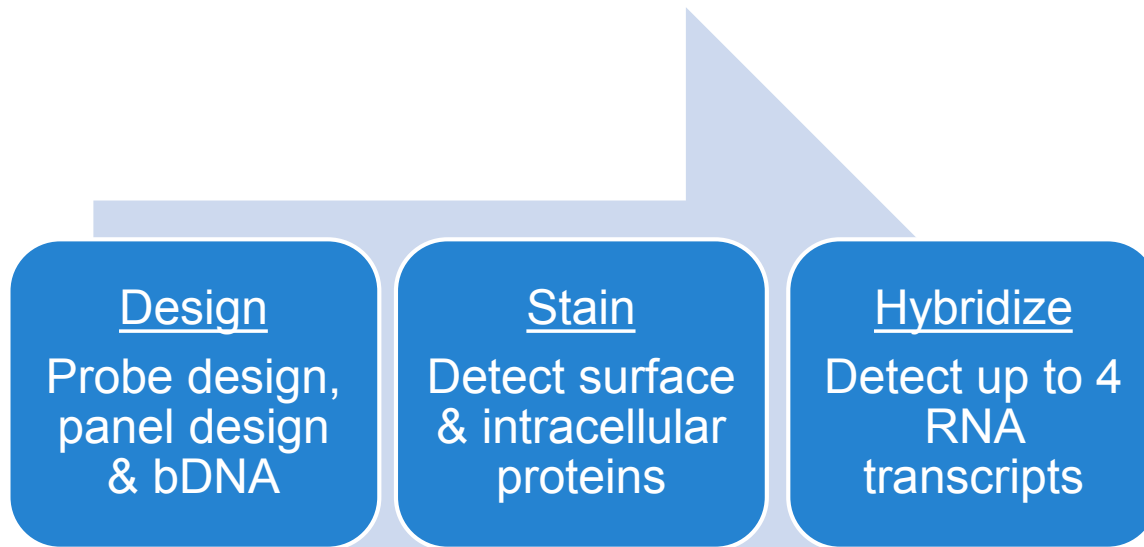


Image-based transcriptomics in thousands of single human cells at single-molecule resolution

Nico Battich, Thomas Stoeger & Lucas Pelkmans
Nature Methods 10, 1127–1133 (2013)

PrimeFlow RNA Assay Workflow



AF™ 488
Type 4

AF™ 568
Type 10

AF™ 647
Type 1

AF™ 750
Type 6

RNA detection compatibility with the Attune™ NxT



Configuration									
Excitation laser	Channel	Antibody labels	Blue	Blue/ Yellow	Blue/ Violet	Blue/ Red	Blue/ Violet/ Yellow	Blue/ Red/ Violet	Blue/ Red/ Yellow/ Violet
Violet-405 nm	VL1	eFluor® 450			▲		▲	▲	▲
	VL2	eFluor® 506			▲		▲	▲	▲
	VL3	eVolve™ 605			▲		▲	▲	▲
	VL4	Qdot® 705			▲		▲	▲	▲
Blue-488 nm	BL1	FITC	▲	▲	▲	▲	▲	▲	▲
		Alexa Fluor® 488	▲	▲	▲	▲	▲	▲	▲
	BL2	PE	▲	▲	▲	▲	▲	▲	▲
	BL3	PE-Cyanine5							
		PE-Cyanine5.5	▲	▲	▲	▲	▲	▲	▲
		PerCP-Cyanine5.5							
		PerCP-eFluor® 710							
	BL4	PE-Cyanine7	▲		▲	▲		▲	
Yellow-561 nm	YL1	PE		▲			▲		▲
	YL2	PE-eFluor® 610		▲			▲		▲
	YL3	PE-Cyanine5		▲			▲		▲
		PE-Cyanine5.5		▲			▲		▲
	YL4	PE-Cyanine7		▲			▲		▲
Red-637 nm	RL1	APC				▲		▲	▲
		eFluor® 660				▲		▲	▲
	RL2	Alexa Fluor® 700				▲		▲	▲
	RL3	APC-eFluor® 780				▲		▲	▲



AlexaFluor™ 488



AlexaFluor™ 568



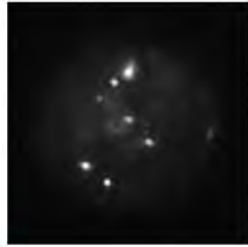
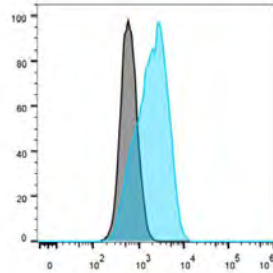
AlexaFluor™ 647



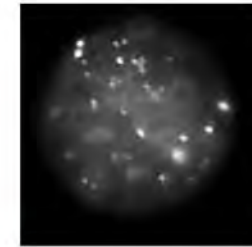
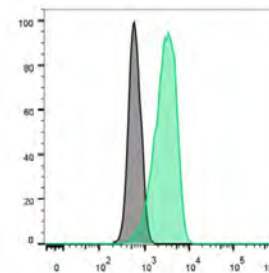
AlexaFluor™ 750

Sensitivity of Messenger RNA Detection by PrimeFlow RNA Assay

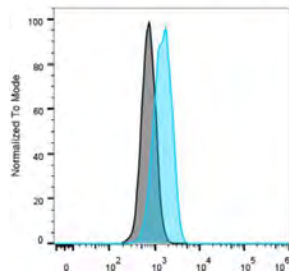
HMBS
Type 1



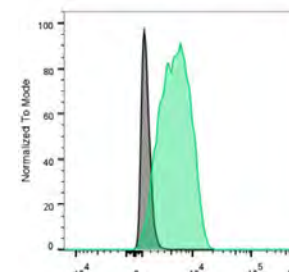
POLR2
A Type
4



HMBS
Type
10

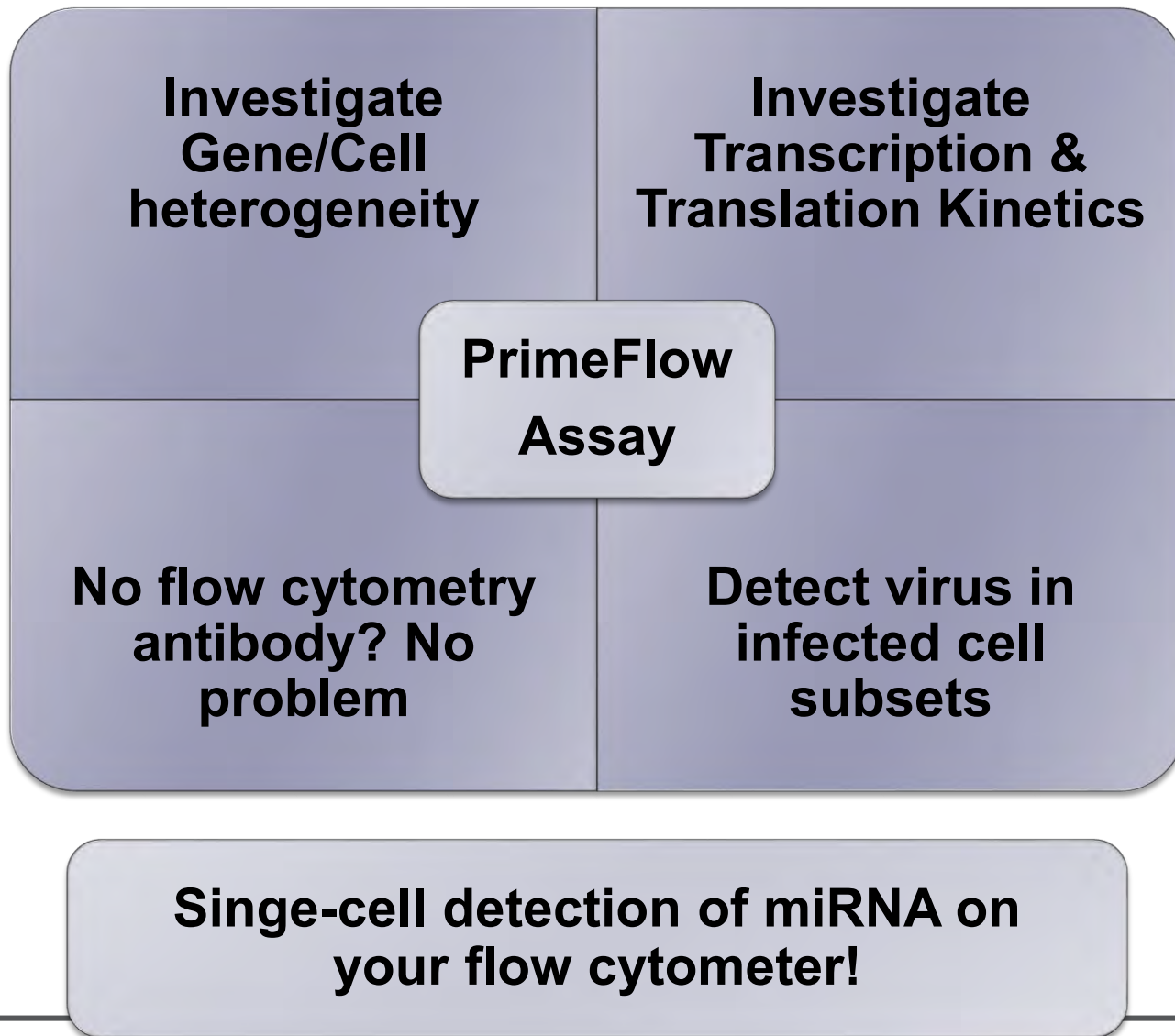


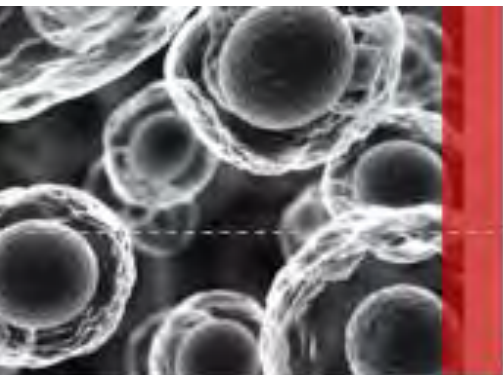
POLR2
A Type
6



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.

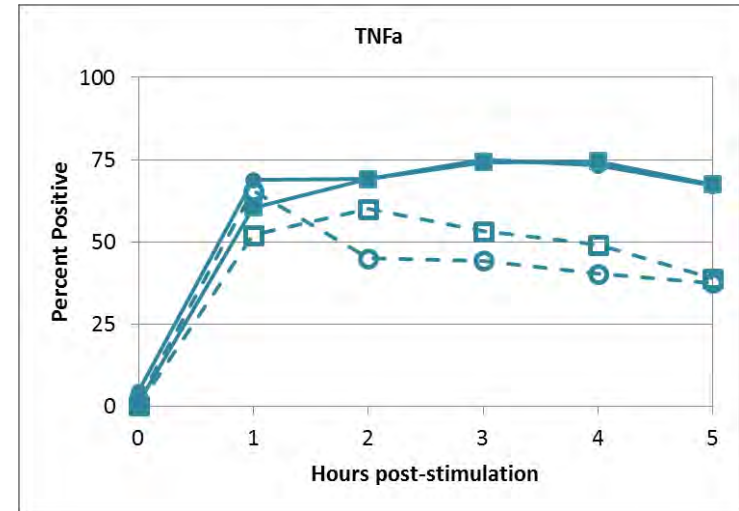
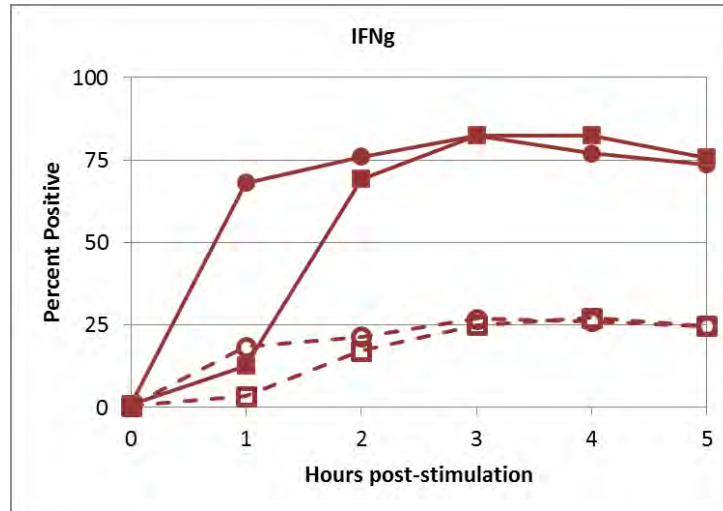
PrimeFlow offers unique possibilities beyond pure protein or gene expression analysis





PrimeFlow™ Applications

Mapping transcription/translation kinetics at the single-cell level

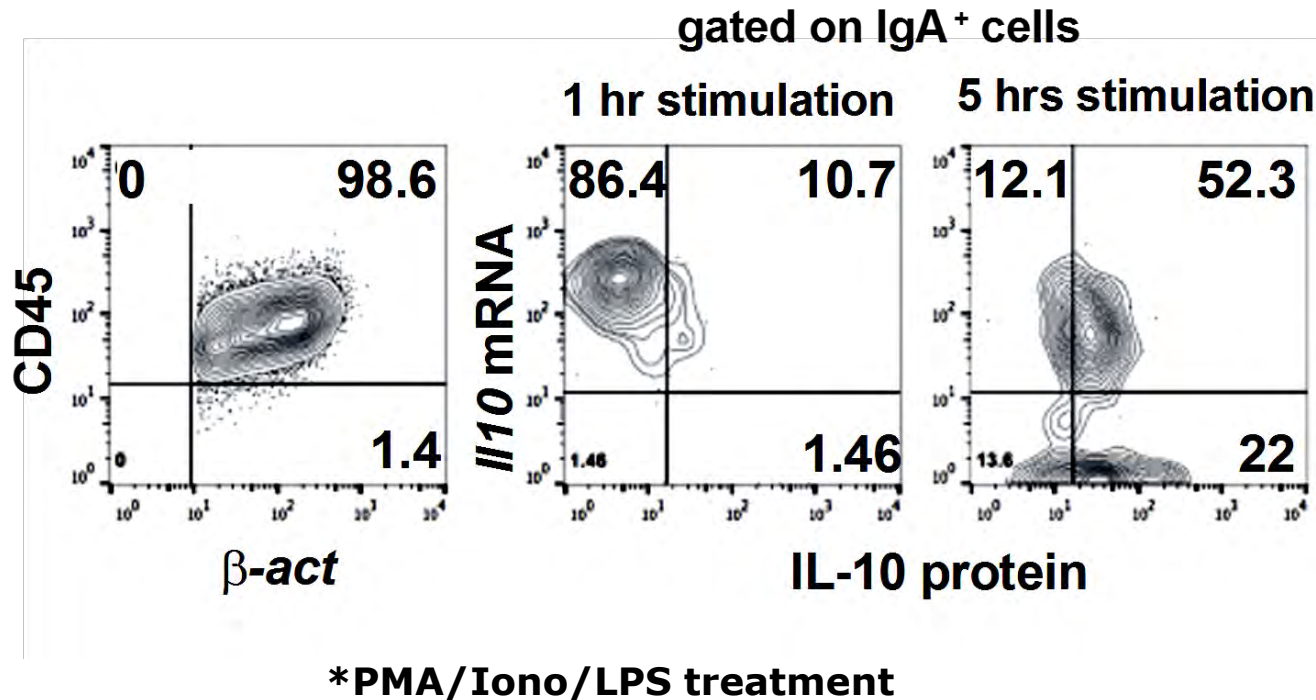


—■— CD8+ protein
—●— CD8+ mRNA
- - □ - - CD8- protein
- - ○ - - CD8- mRNA

—■— CD8+ protein
—●— CD8+ mRNA
- - □ - - CD8- protein
- - ○ - - CD8- mRNA

Robust results with rare, hard to isolate cell types

Characterize Rare Tumor-infiltrating CD8⁺ T-cell Inhibitory IgA^{hi} B cells



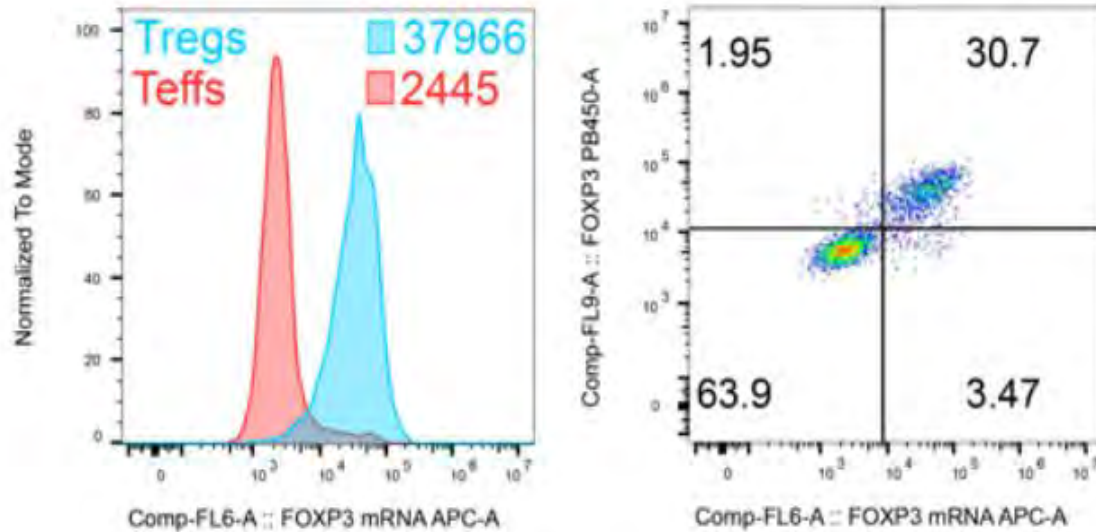
CD45⁺ cells: 10-20% IgA⁺CD19⁺ cells: 2-30% of CD45⁺ cells (0.2-6% in total)

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

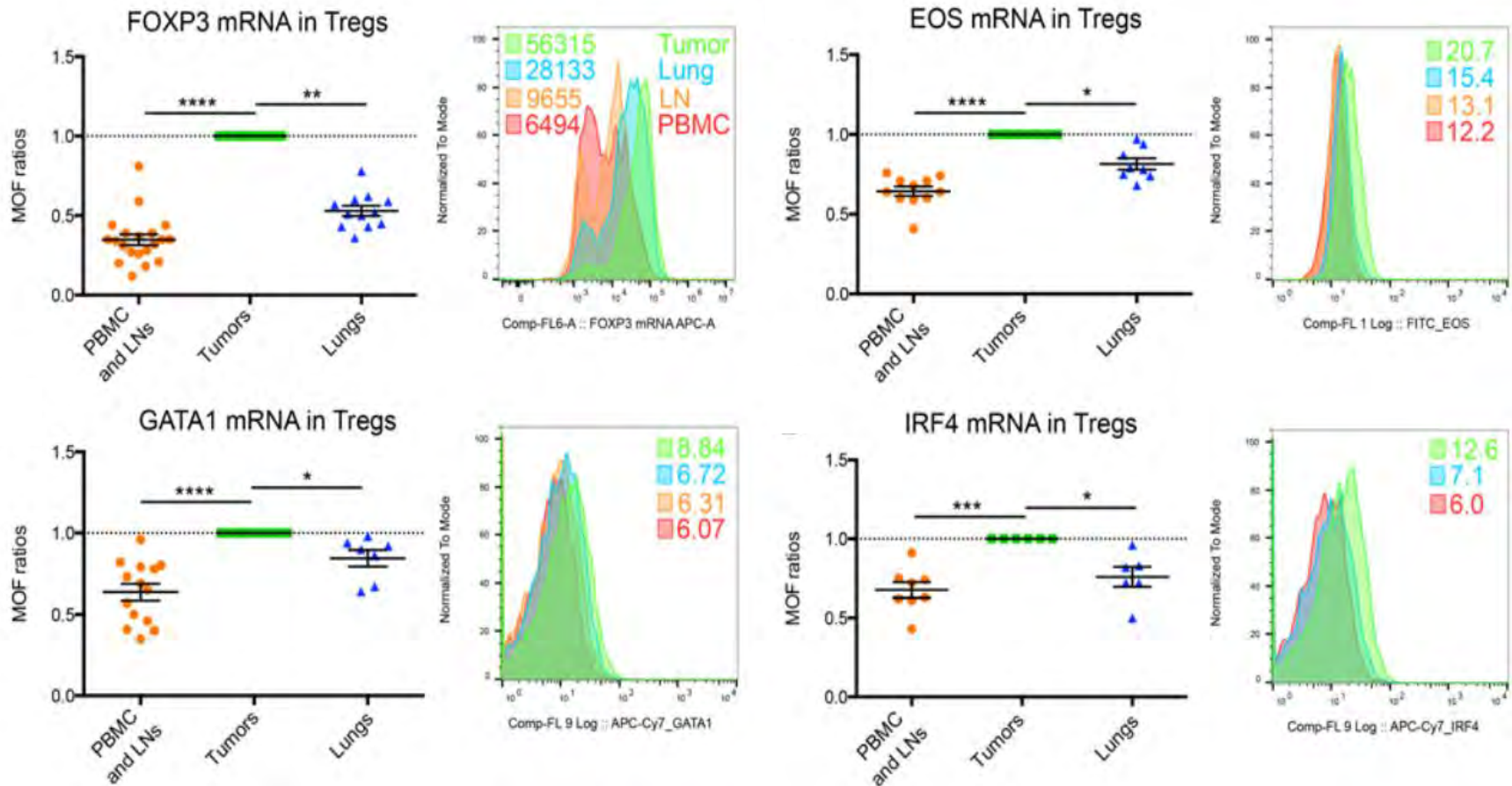
Single-cell gene expression data from pure FoxP3+ population



Samples from patients with non-small cell lung cancer

Akimova et al., 2017, JCI Insights

Single-cell gene expression data from pure FoxP3+ population



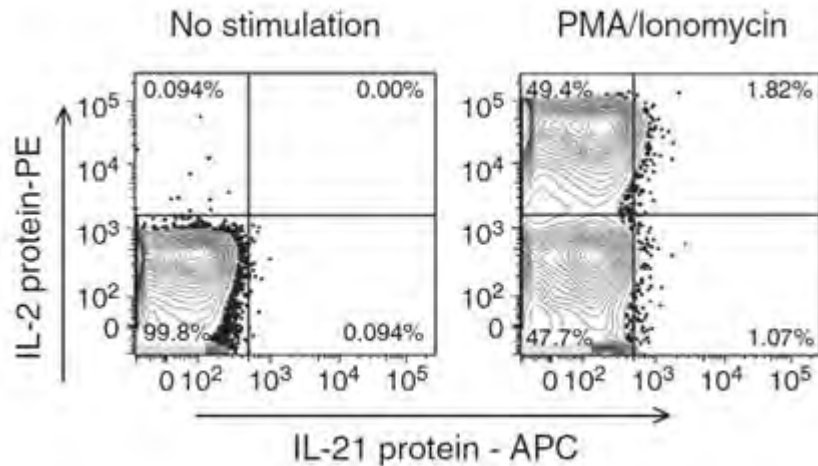
Samples from patients with non-small cell lung cancer

Akimova et al., 2017, JCI Insights

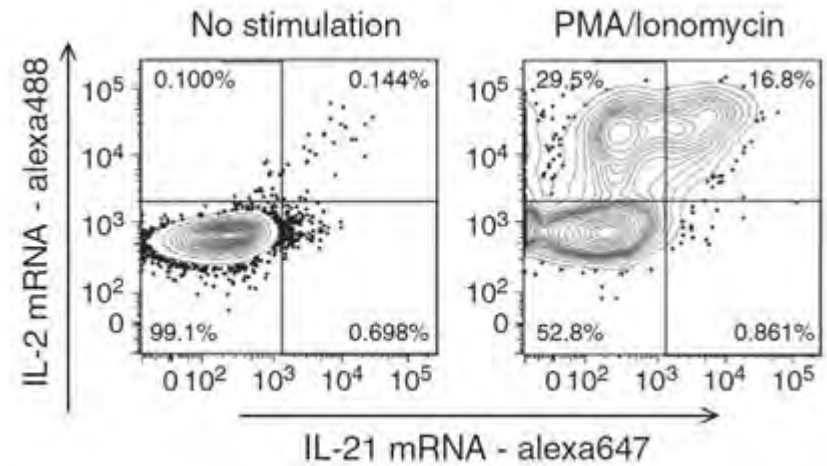
No antibody? No problem!

IL-21 in Idiopathic thrombocytopenic purpura (ITP)

Intracellular cytokine staining

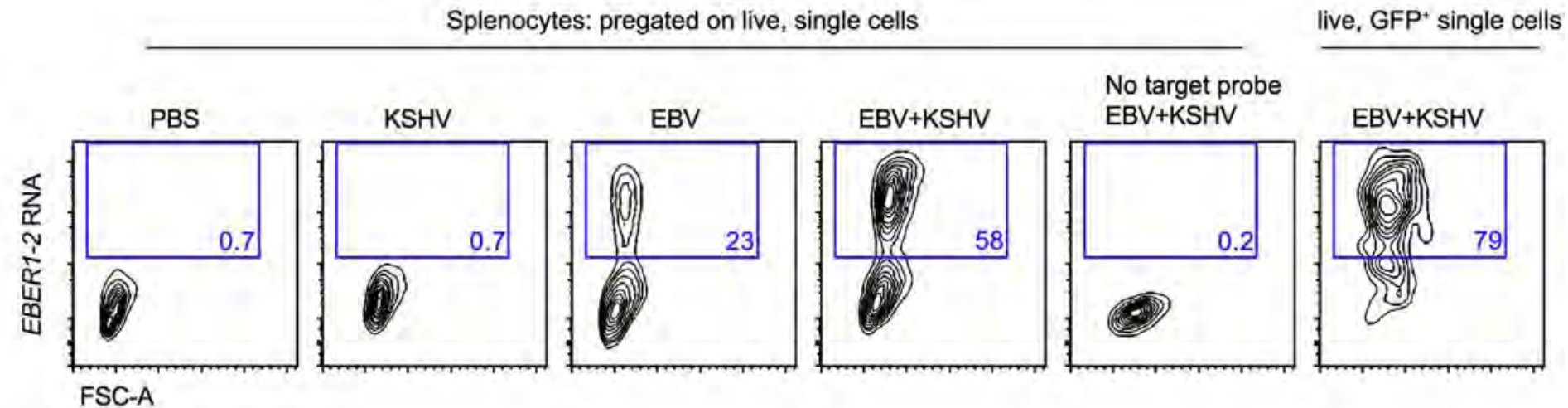


PrimeFlow RNA Assay

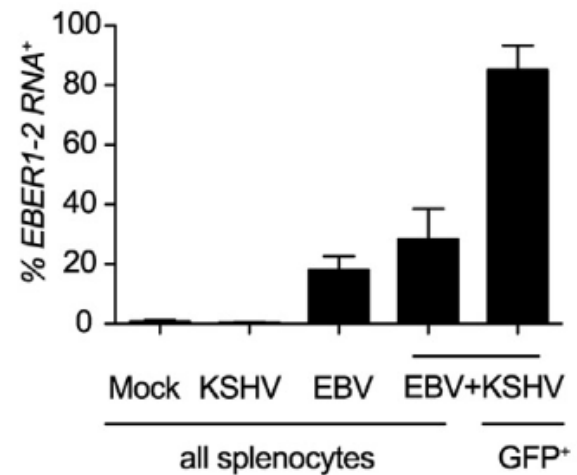


Porichis *et al.*, 2014, *Nature Communications*

Study viral RNA with flow cytometry

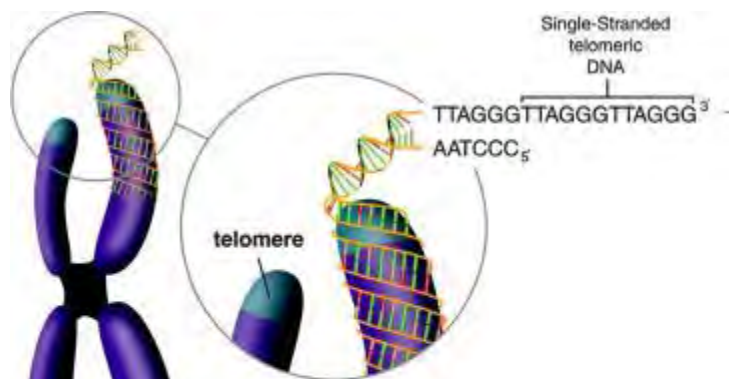


- EBV/KSHV co-infection promotes tumorigenesis in mouse model

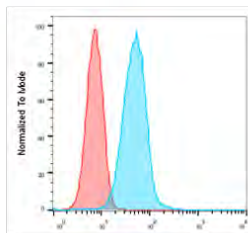


McHugh et al., 2017, Cell Host & Microbe 22, 61–73

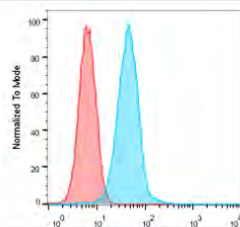
Evaluation of telomere in five normal human donors



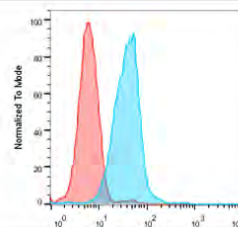
Donor 1
Lymphocytes



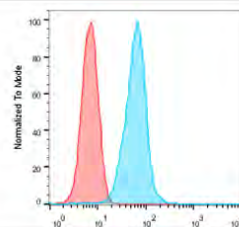
Donor 2



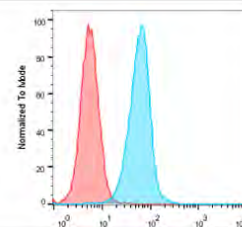
Donor 3



Donor 4

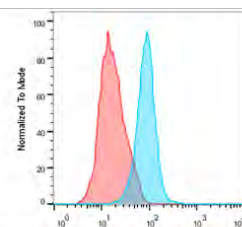
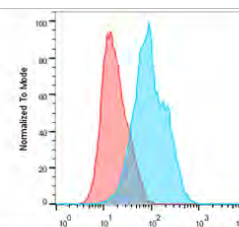
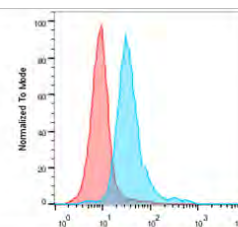
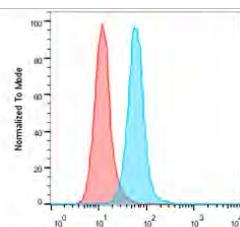
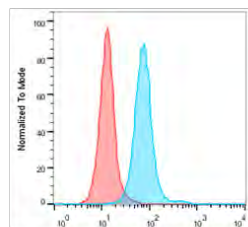


Donor 5



Telomere - AF647

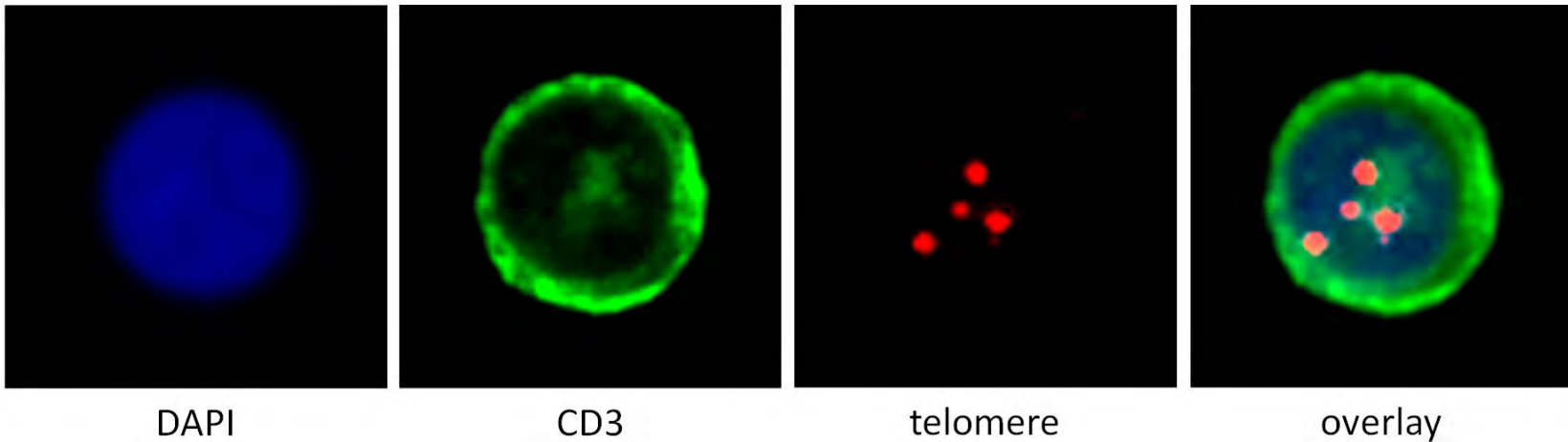
Monocytes



Telomere - AF647

Localization of telomere signal

Human PBMC
Anti-CD3-FITC + telomere Type 1



Equipment Needed for the PrimeFlow RNA Assay



Incubator with heat block inside

- Validated to maintain $40 \pm 1^\circ\text{C}$
- 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)

Search for Available (In-Stock) Probe Sets

The PrimeFlow probes search tool is available at

thermofisher.com/primeflow

Search for available in-stock probe sets

1. **Gene Expression** (selected)

2. **PrimeFlow** (selected)

3. **What species do you want to target? (Select one or more)**

4. **Search**

What species do you want to target? (Select one or more)

[Hs] Human [Mm] Mouse [Rn] Rat More (24) All

Enter target information ?
e.g., Gene Symbol, Array Probe ID, Assay ID...

Enter / Upload Multiple Targets

5 Fluorochrome label option

Change Your Search

Narrow Your Results

Gene
IL1B

Species
(Hs) Human

Detection Label

- ☒ Alexa Fluor 488
- ☐ Alexa Fluor 568
- ☐ Alexa Fluor 647
- ☐ Alexa Fluor 750

Product Subtype

[Reset Filters](#)

Assay ID	Gene	Species	Transcripts	Detection Label	Type	Product Type	Availability	Catalog #
VA1-11609-PF	IL1B	Human	2 RefSeqs	Alexa Fluor 647	1	PrimeFlow Probe Set	Made to Order	PF-204
							Price (USD):	40 tests
							Check your price	Add To Cart
View Assay on Map								
View Details								
VA4-15609-PF	IL1B	Human	2 RefSeqs	Alexa Fluor 488	4	PrimeFlow Probe Set	Made to Order	PF-204
							Price (USD):	40 tests
							Check your price	Add To Cart
View Assay on Map								
View Details								
VA10-3252155-PF	IL1B	Human	2 RefSeqs	Alexa Fluor 568	10	PrimeFlow Probe Set	Made to Order	PF-210
							Price (USD):	100 tests
							Check your price	Add To Cart
View Assay on Map								
View Details								

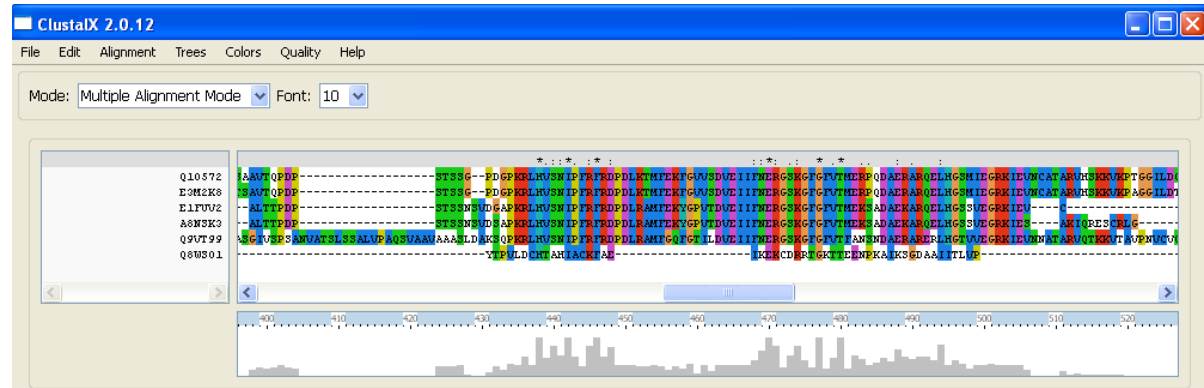
Information we need:

- 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

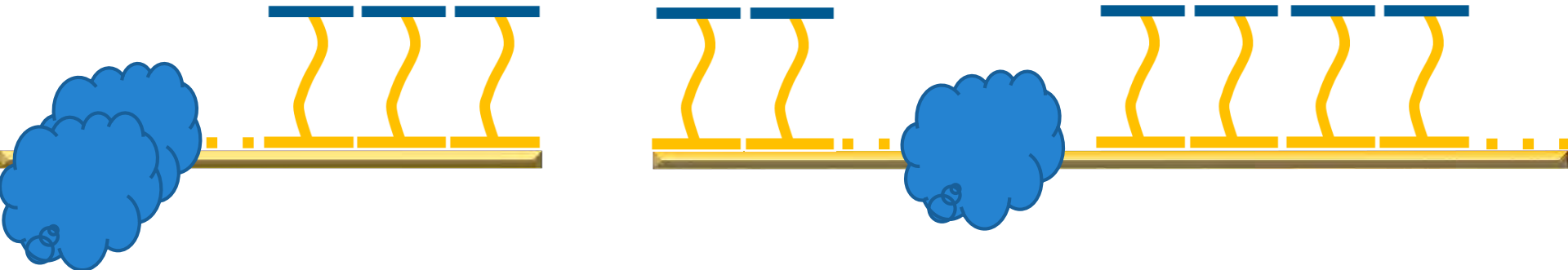
YOUR gene of interest



ViewRNA proprietary algorithm



Gene specific probe is comprised of ~12-46 oligos





PrimeFlow Assay

ViewRNA Family



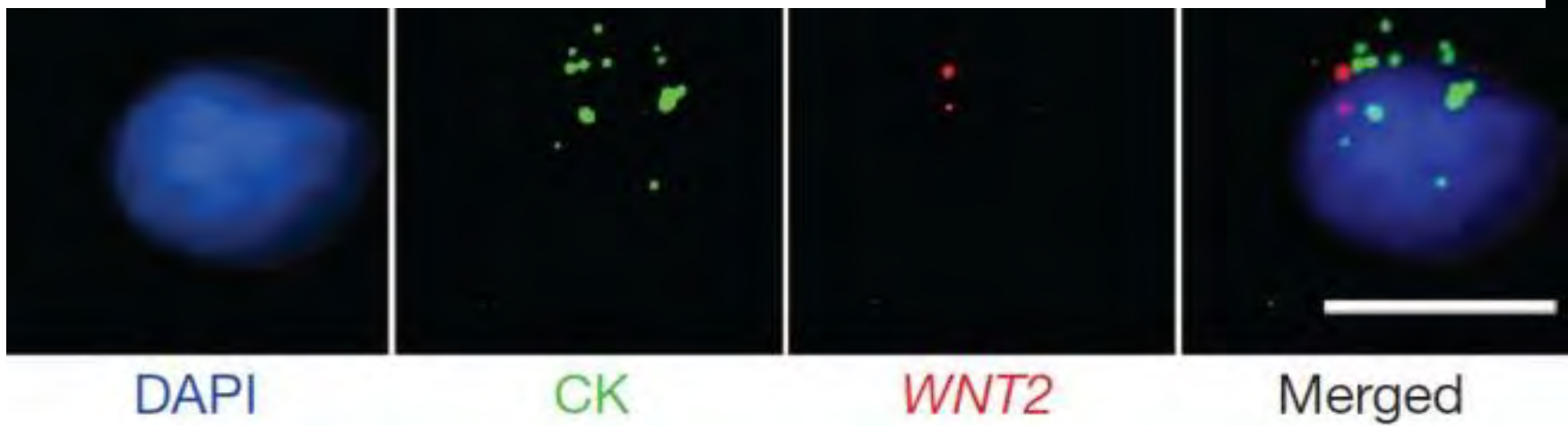
B(ranched)
DNA



Quantigene family

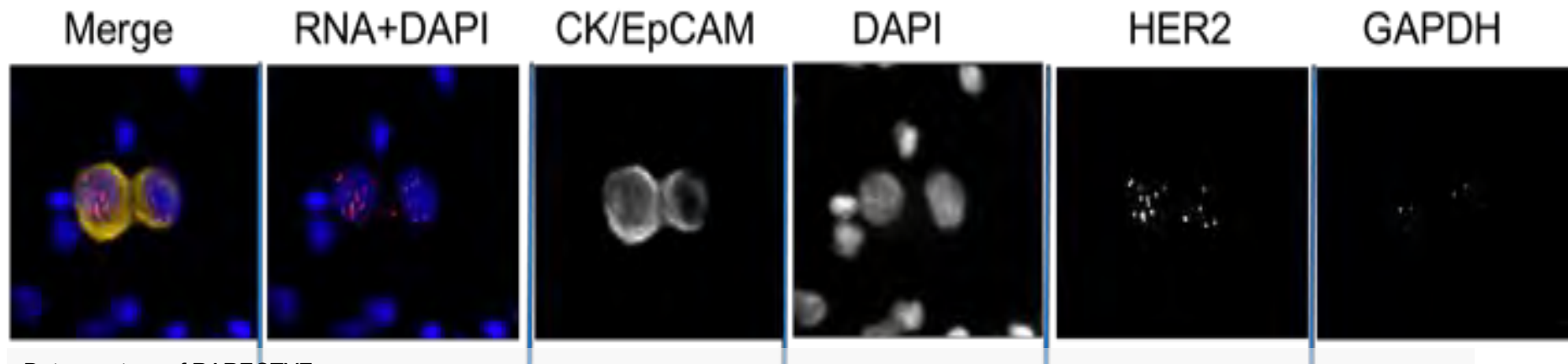
ViewRNA Cell versus ViewRNA Cell Plus

ViewRNA Cell : Detection of human CTCs (pancreatic origin)



Yu, M. *et al.*, RNA sequencing of pancreatic CTCs implicates WNT signaling in metastasis. *Nature*, **487**(7408):510-3 (2012).

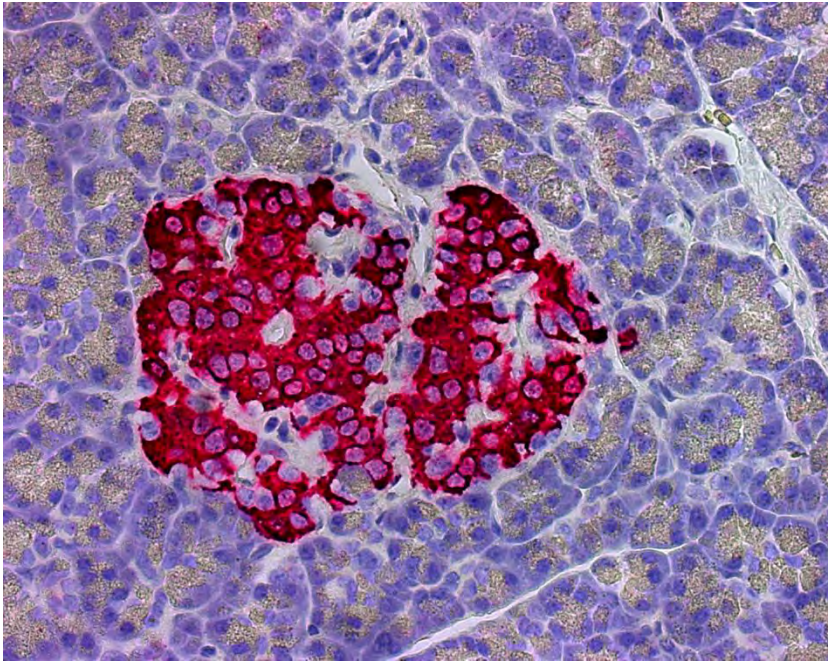
ViewRNA Cell Plus: Detection of SKBR3 cells spiked in PBMC



Data courtesy of RARECTYE

ViewRNA ISH Tissue Assay - Pancreas

With Insulin Probe



No Probe Negative Control

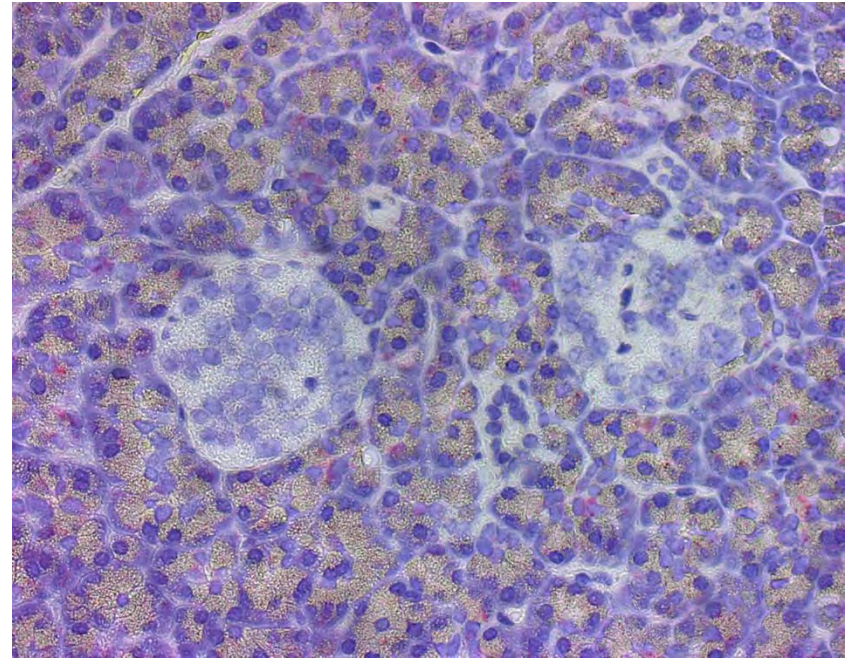


Image courtesy of: Ann Dongtao Fu, University of Florida

Questions?

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