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Extras of introduction to
Single-cell RNA-seq

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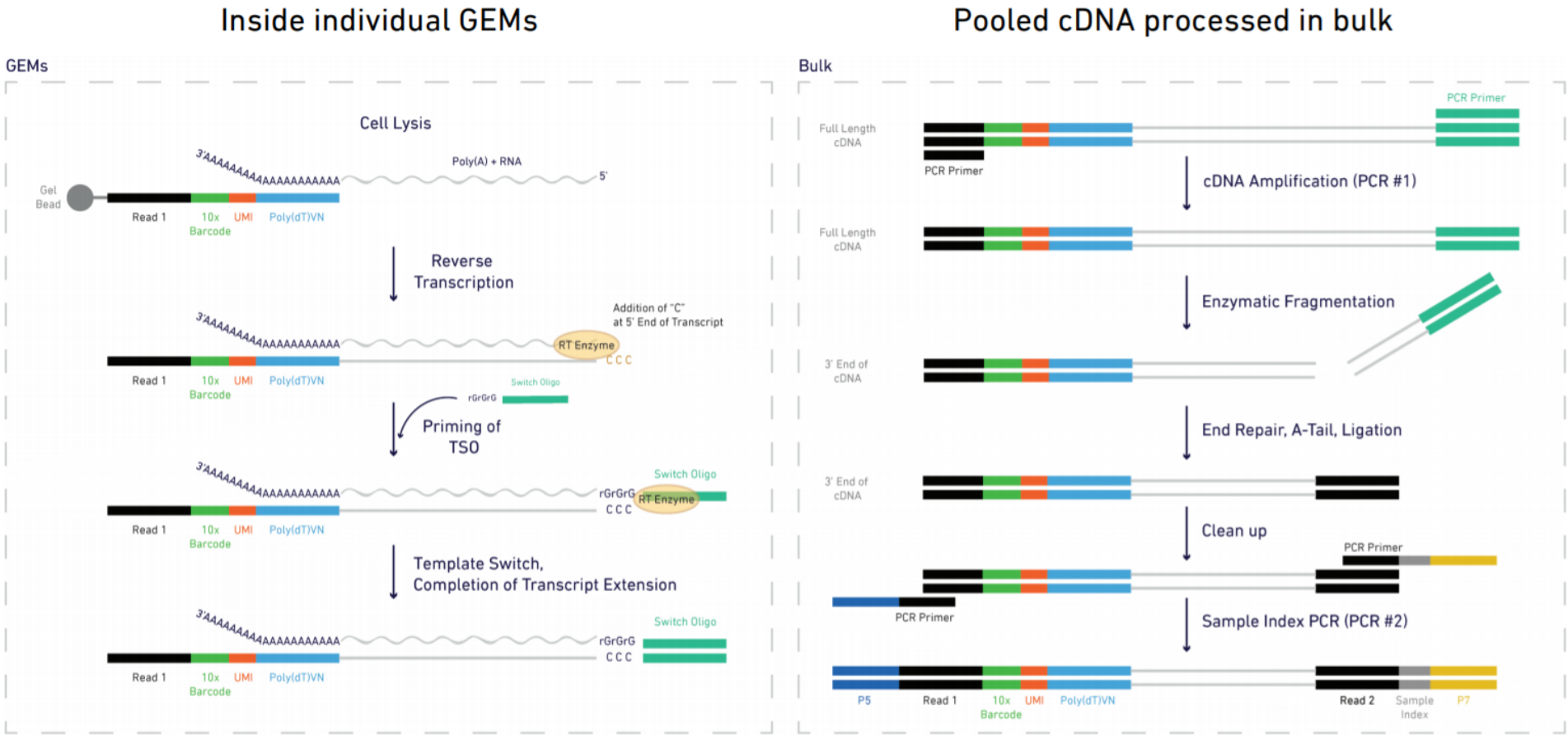
Latest things: single-nuclei RNA-seq

- Not much different in sequencing and preparation
- Can be done with frozen samples
- People do that a lot with brain biobanks

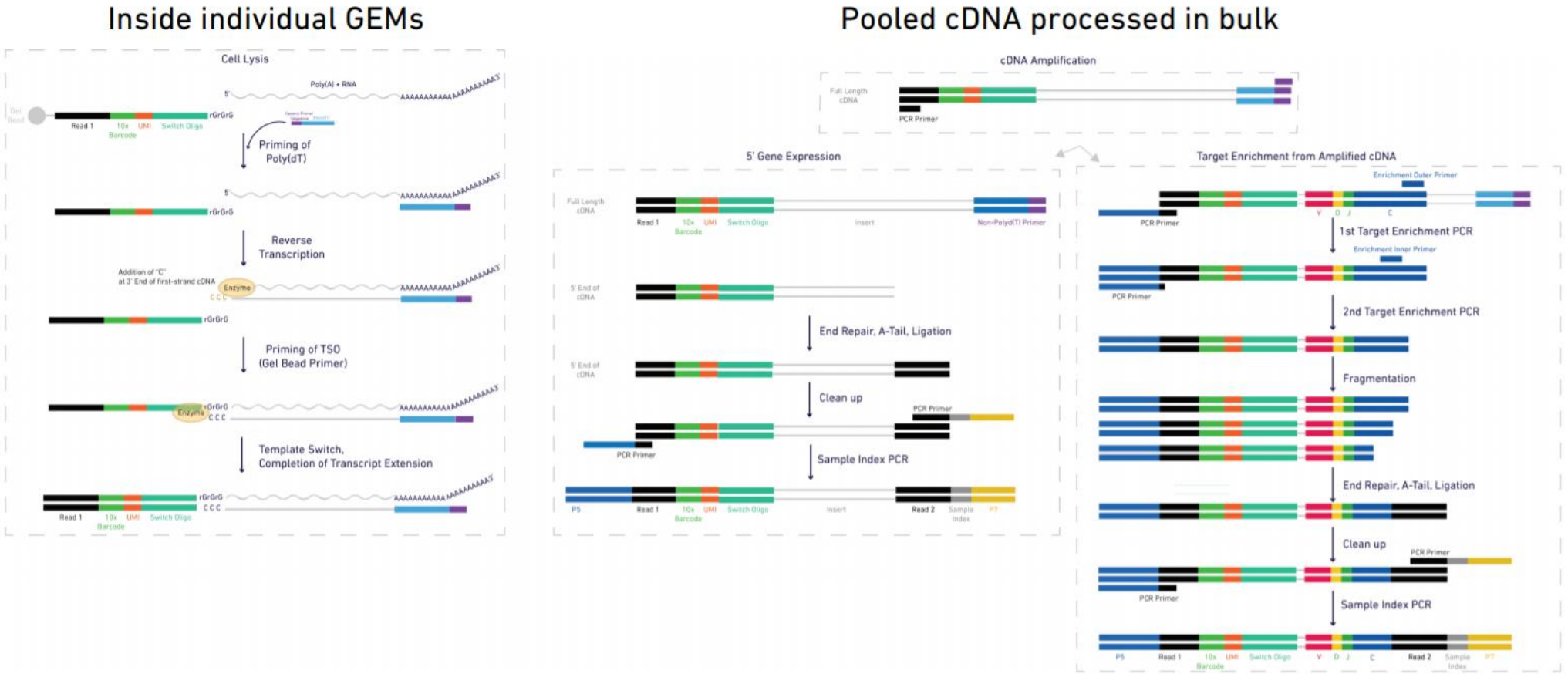
Latest things: 5’ scRNA-seq allows TCR/IG capture

- 5’ scRNA-seq is different from 3’ scRNA-seq
- 5’ scRNA-seq allows us to get TCR and IG repertoires of the cells

3’ scRNA-seq


Inside individual GEMs
Pooled cDNA processed in bulk
GEMs
Bulk
Cell Lysis
Poly(A) + RNA
5'
Gel Bead
Read 1
10x Barcode
UMI
Poly(dT)VN
Reverse Transcription
Addition of "C" at 5' End of Transcript
RT Enzyme
CCC
Switch Oligo
rGrGrG
Priming of TSO
Template Switch, Completion of Transcript Extension
PCR Primer
Full Length cDNA
cDNA Amplification (PCR #1)
Enzymatic Fragmentation
3' End of cDNA
End Repair, A-Tail, Ligation
Clean up
Sample Index PCR (PCR #2)
P5
Read 2
Sample Index
P7

5’ scRNA-seq


Inside individual GEMs
Cell Lysis
Poly(A) + RNA
Read 1
10x Barcode
UMI
Switch Oligo
rGrGrG
Priming of Poly(dT)
Reverse Transcription
Addition of 'C' at 3' End of first-strand cDNA
Enzyme
CCC
Priming of TSO (Gel Bead Primer)
Template Switch, Completion of Transcript Extension
Pooled cDNA processed in bulk
cDNA Amplification
Full Length cDNA
PCR Primer
5' Gene Expression
Non-Poly(T) Primer
End Repair, A-Tail, Ligation
Clean up
Sample Index PCR
P5
Read 2
Target Enrichment from Amplified cDNA
Enrichment Outer Primer
1st Target Enrichment PCR
Enrichment Inner Primer
2nd Target Enrichment PCR
Fragmentation
End Repair, A-Tail, Ligation
Clean up
Sample Index PCR

Latest things: scRNA-seq + surface protein expression

BRIEF COMMUNICATIONS

nature biotechnology

Multiplexed quantification of proteins and transcripts in single cells

Vanessa M Peterson[1,5], Kelvin Xi Zhang[2,5], Namit Kumar[1], Jerelyn Wong[3], Lixia Li[1], Douglas C Wilson[3], Renee Moore[4], Terrill K McClanahan[3],

the standard 10x Genomics single-cell (sc)RNA-seq platform[3], which is a droplet-based system designed for 3′ digital counting of mRNA in thousands of single cells.

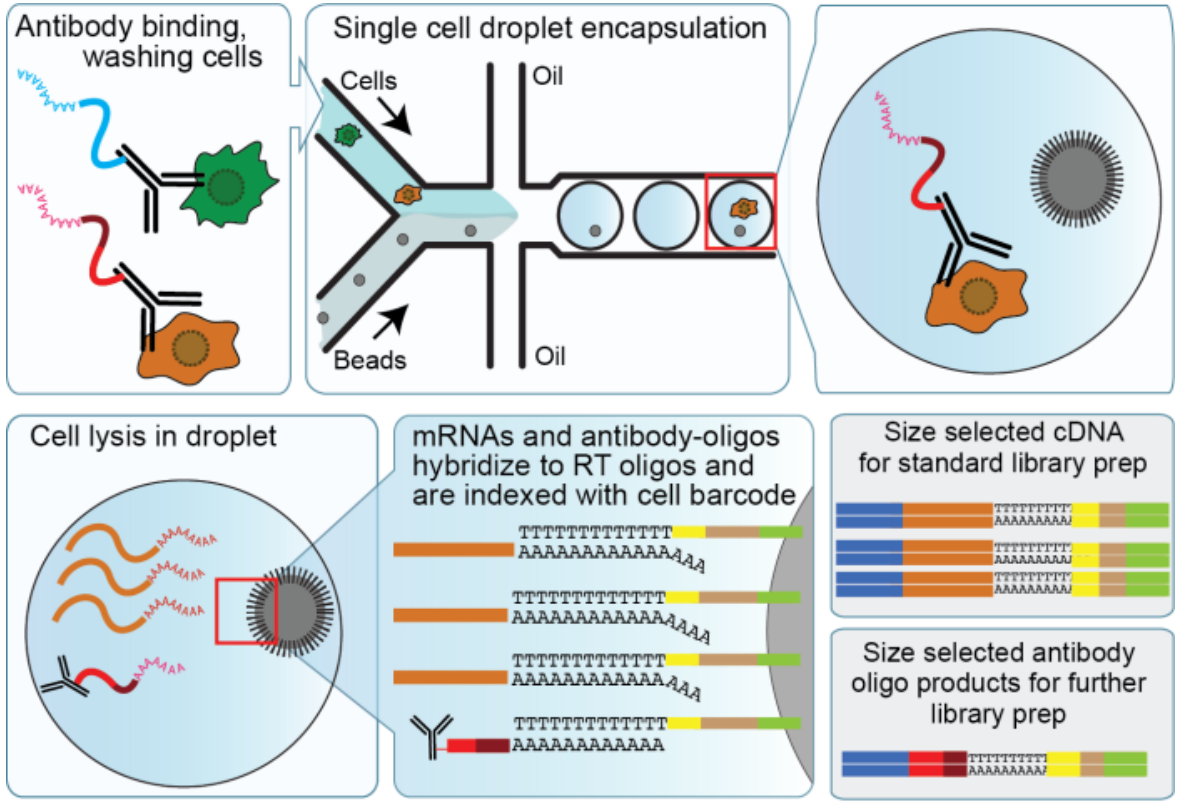
REAP-seq leverages the DNA polymerase activity of the reverse transcriptase to simultaneously extend the primed AbB with the poly(dT) cell barcode and synthesize complementary DNA from mRNA in the same reaction. Exonuclease I is then used to degrade any excess unbound single-stranded oligonucleotides from the protein double-stranded (ds) DNA (~155 bp) products to prevent crosstalk between AbBs and cell barcodes from different cells (**Supplementary Fig. 4**). Dextran sulfate was added to AbB labeling buffer to reduce non-specific binding of nega-

Brief Communication

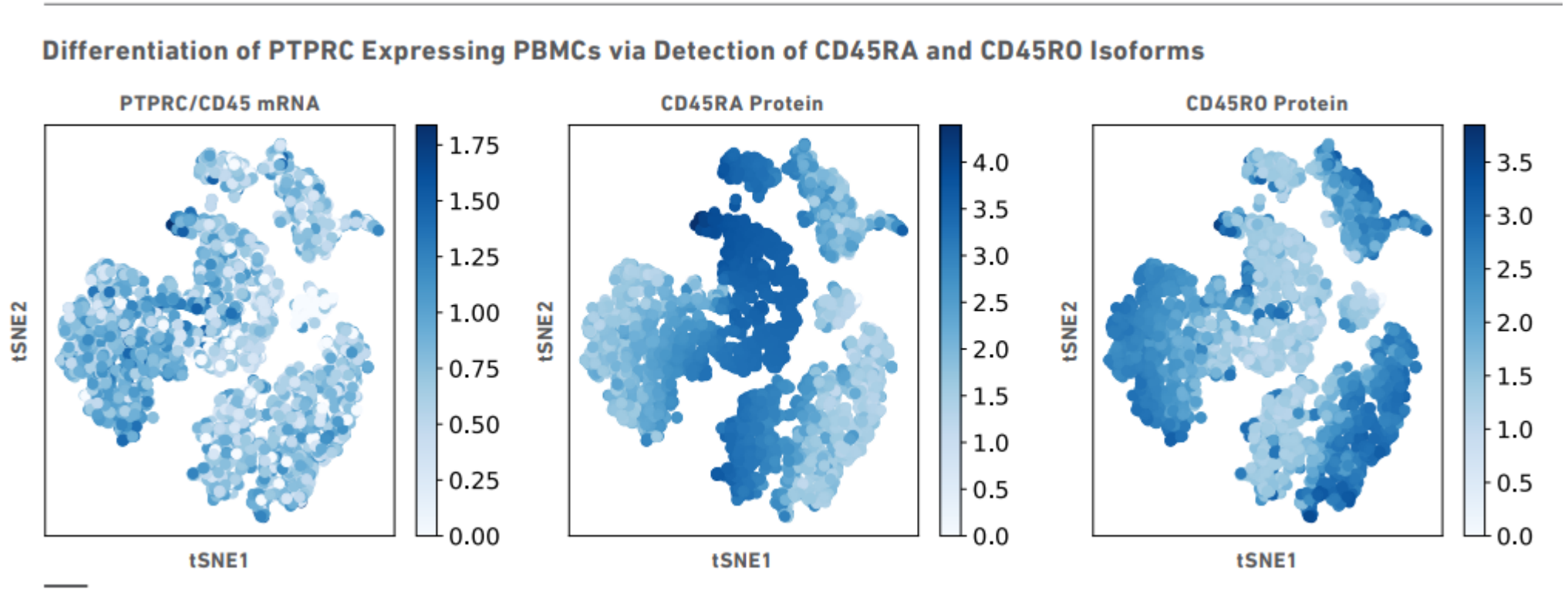
Simultaneous epitope and transcriptome measurement in single cells

Marlon Stoeckius ✉, Christoph Hafemeister, William Stephenson, Brian Houck-Loomis, Pratip K Chattopadhyay, Harold Swerdlow, Rahul Satija & Peter Smibert

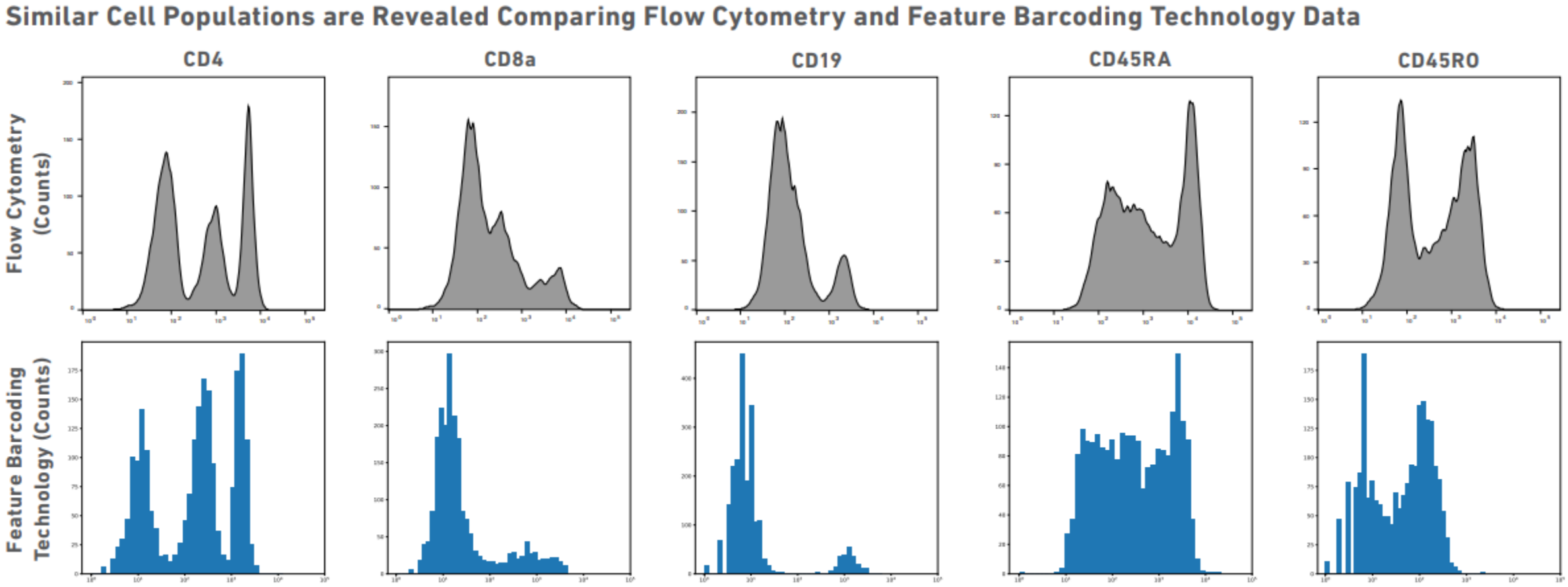
Latest things: scRNA-seq + surface protein expression



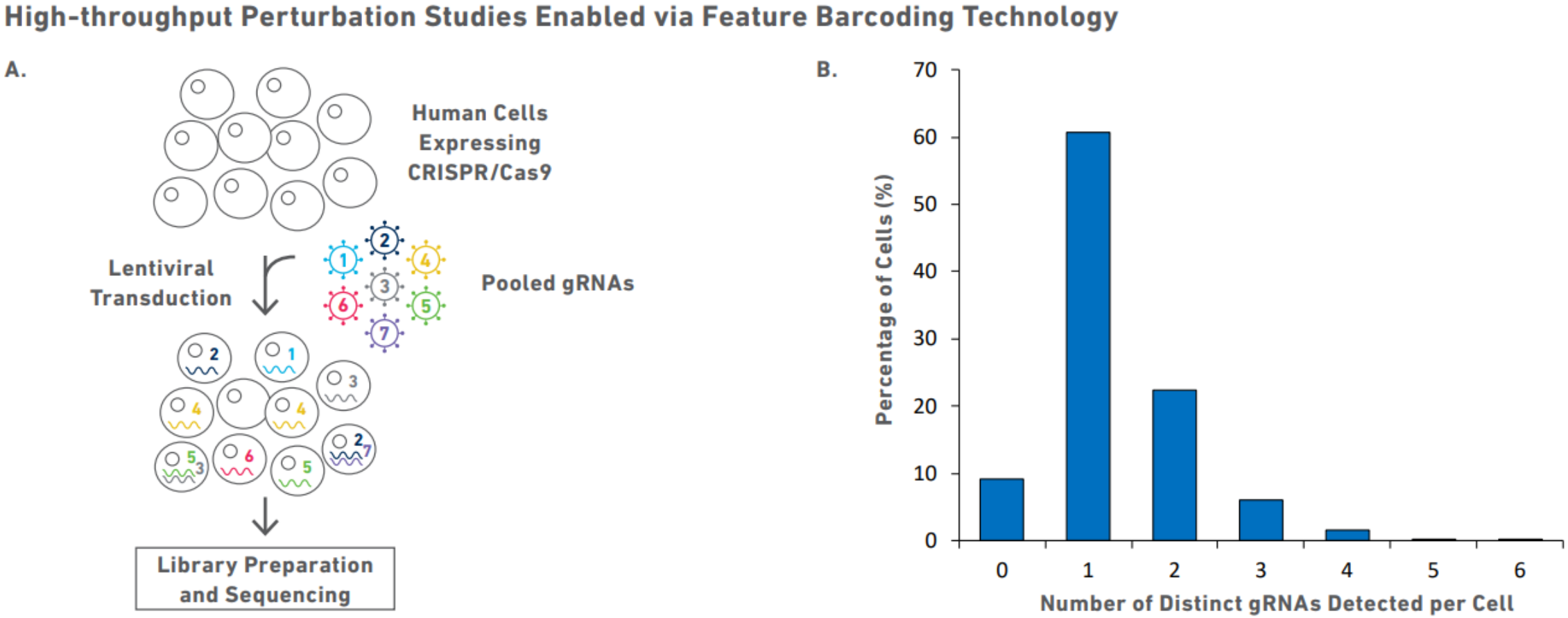
Latest things: scRNA-seq + surface protein expression



Latest things: scRNA-seq + surface protein expression

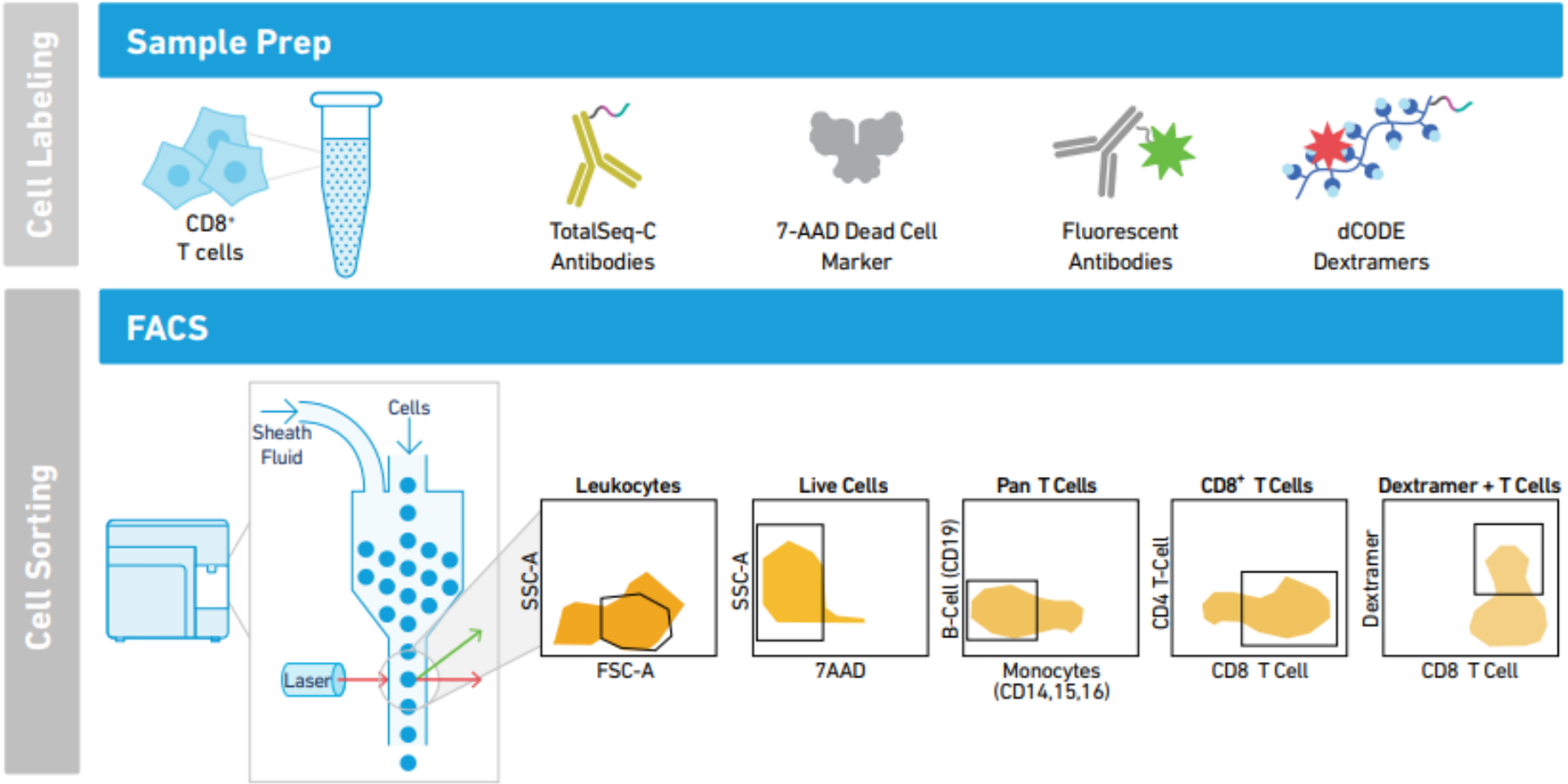


Latest things: scRNA-seq + pooled CRISPR screening



A. K562 cells expressing CRISPR/Cas9 were transduced with a pool of 18 gRNA's (8 targeting: 4 genes, 2 targets per gene and 10 non-targeting guides) before 10260 cells were profiled with the Chromium Single Cell Gene Expression Solution. **B.** 60% of the cells profiled were found to contain a single gRNA, 30% of cells were found to contain 2 - 6 gRNAs and the remaining 10% of cells did not contain a gRNA.

Latest things: all the things combined



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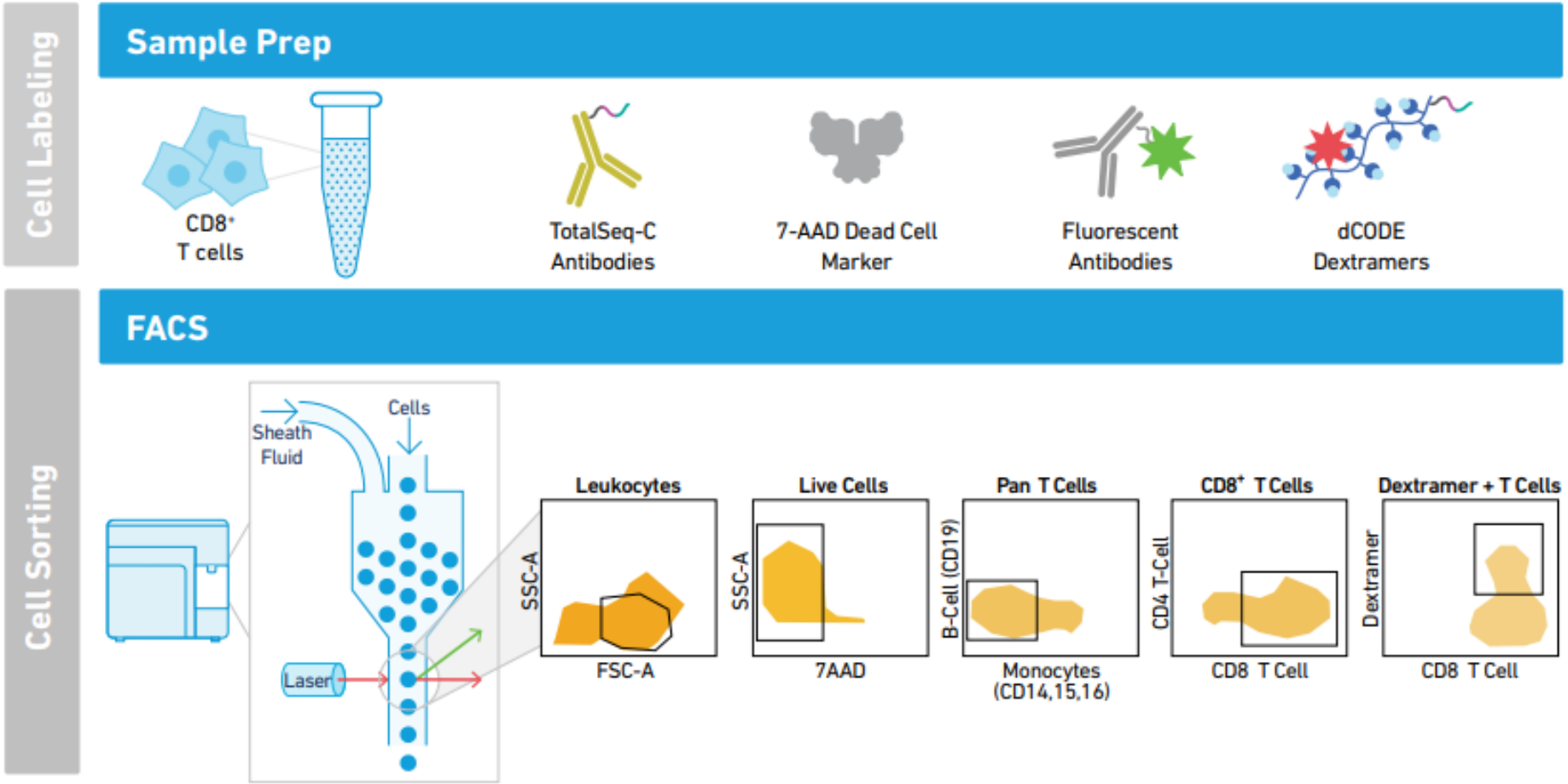

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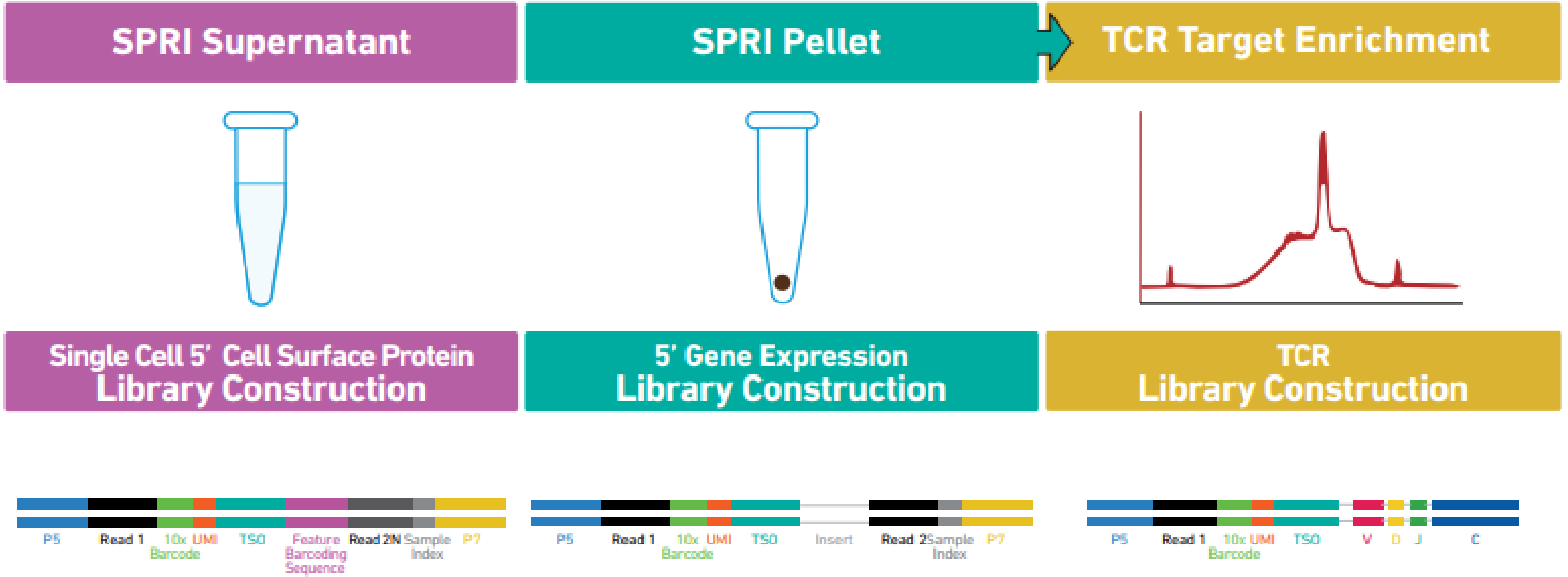
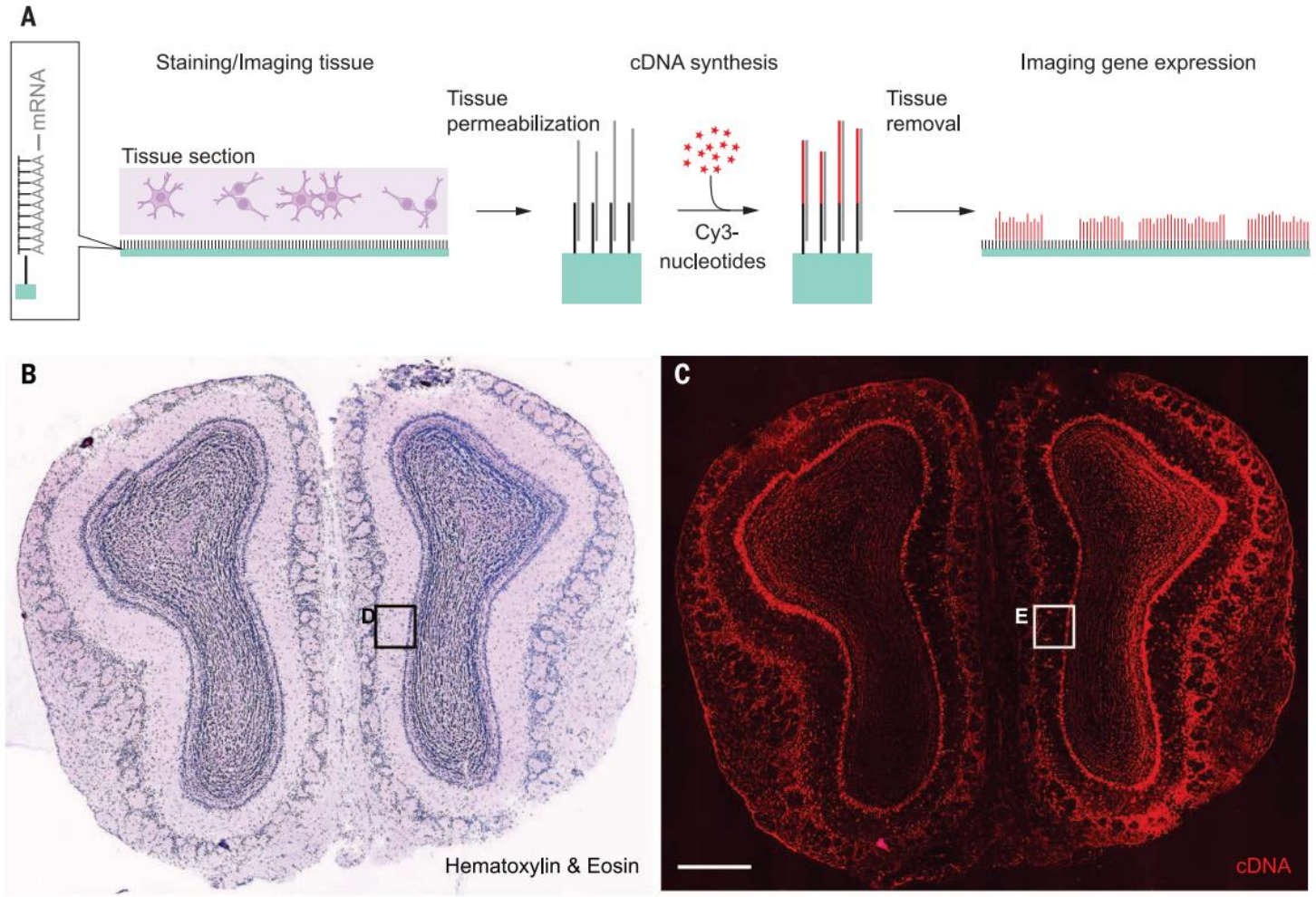


Latest things: spatial transcriptomics

https://science.sciencemag.org/content/353/6294/78



Latest things: spatial transcriptomics

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