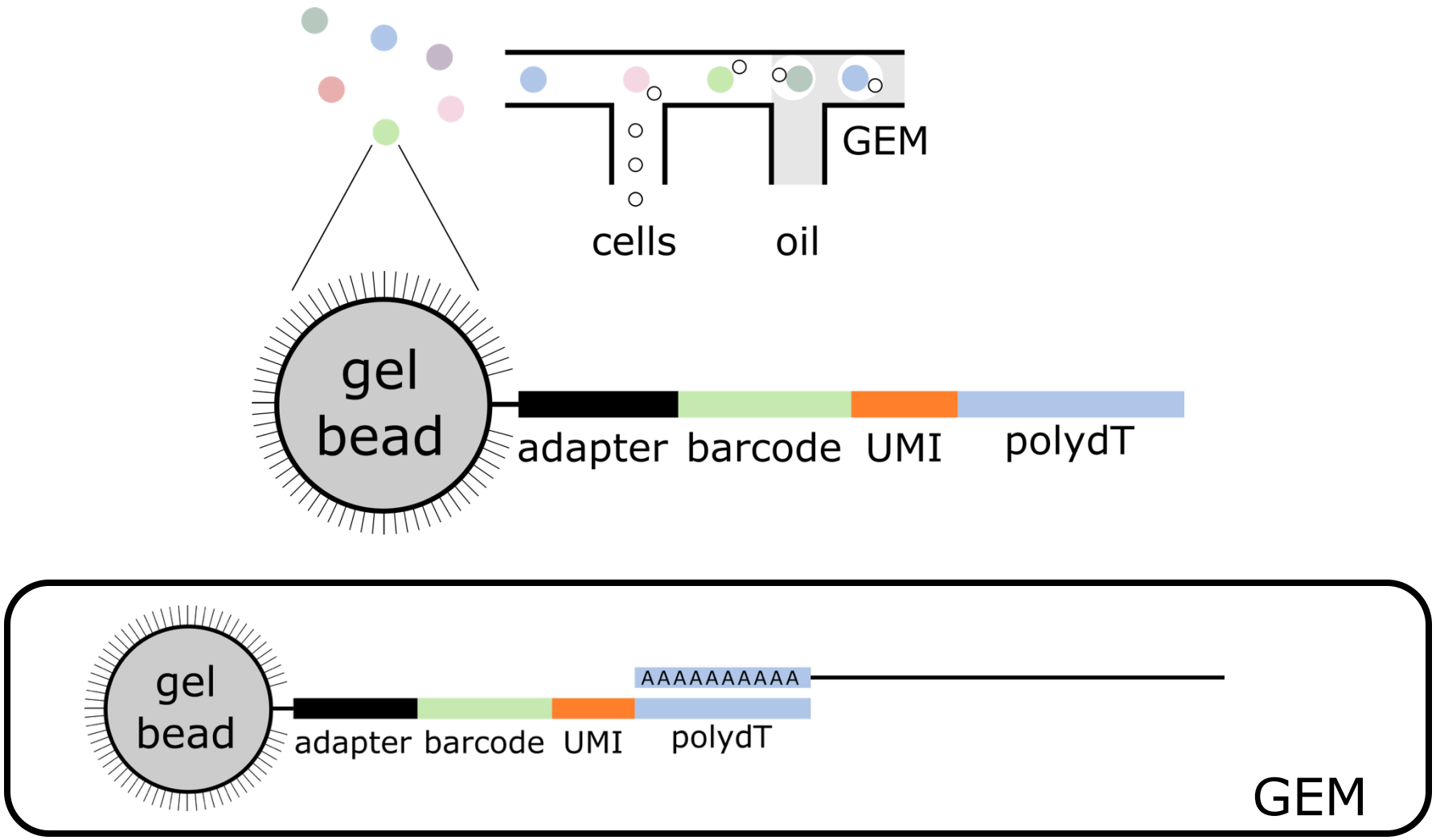
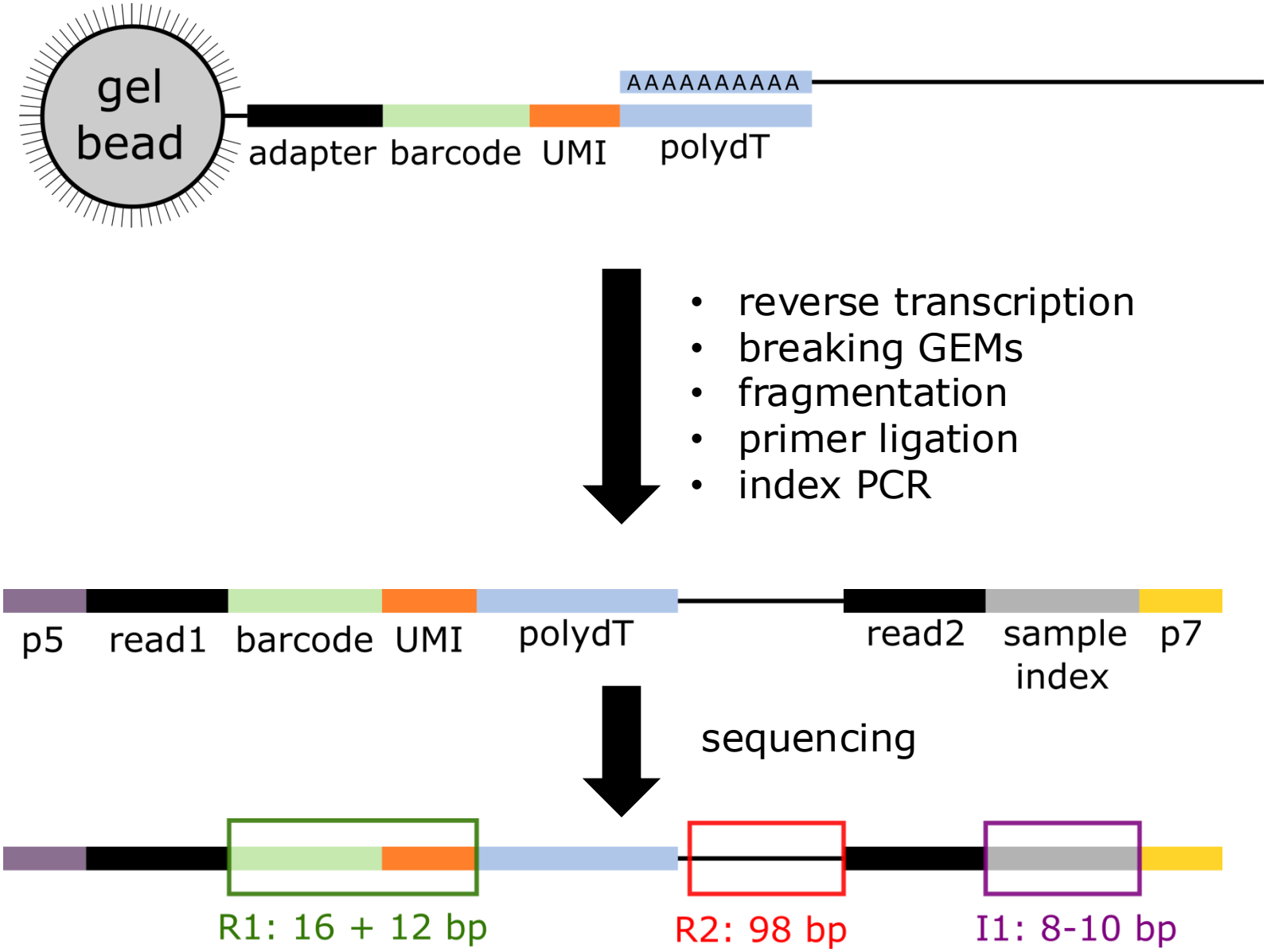
Single cell transcriptomics

10x genomics Chromium



All captured **transcripts** from **single** cell:
identical + **unique** barcode


gel bead
adapter
barcode
UMI
AAAAAAAAAA
polydT
• reverse transcription
• breaking GEMs
• fragmentation
• primer ligation
• index PCR
p5
read1
barcode
UMI
polydT
read2
sample index
p7
sequencing
R1: 16 + 12 bp
R2: 98 bp
I1: 8-10 bp

Sequencing output

sample# read type

```
ETV6-RUNX1_1_S1_L001_I1_001.fastq.gz
ETV6-RUNX1_1_S1_L001_R1_001.fastq.gz
ETV6-RUNX1_1_S1_L001_R2_001.fastq.gz
```

sample ID lane

- Dual indexing: second index in I2
- Indexes can also be added to fastq titles

After sequencing (pre-processing)

1. Assign reads to cell
2. Alignment
3. Quantification: # UMI/gene
4. Cell calling

For 10x all with `cellranger count`

Alternatives:
STARSolo
Alevin

cellranger references

- Human & mouse: download pre-built from 10x website
- Other organisms: custom reference with **`cellranger mkref`**
- Exogenous marker genes (e.g. GFP): add sequence to both fasta and gtf
- Features (e.g.) hashing or surface-proteins: feature barcode reference csv

extensive documentation:
https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger

Why count UMI (and not read alignments?)

- UMI: Unique Molecular Identifier:
  - Identifies each molecule (i.e. sequence) uniquely
- Molecules from a common PCR template -> carry the same UMI
- By counting UMI: correct for PCR duplicates

Cellranger report

ETV6-RUNX1_1

Alerts

The analysis detected ⚠ 1 warning.

	Alert	Value	Detail
⚠	Fraction of RNA read bases with Q-score >= 30 is low	59.4%	Fraction of RNA read bases with Q-score >= 30 should be above 65%. A lower fraction might indicate poor sequencing quality. This is Read 1 for the Single Cell 3' v1 chemistry and Single Cell 5' paired end, Read 2 for the Single Cell 3' v2/v3 chemistry and Single Cell 5' R2-only)

Summary | Analysis

3,091
Estimated Number of Cells

68,259
Mean Reads per Cell

1,717
Median Genes per Cell

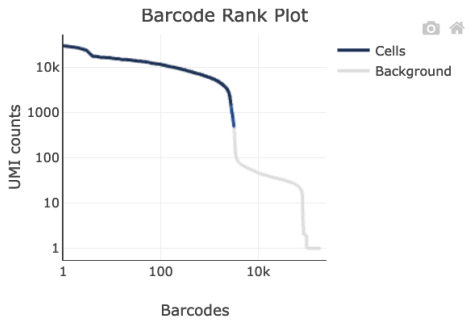
Sequencing

Number of Reads	210,987,037
Number of Short Reads Skipped	0
Valid Barcodes	98.2%
Valid UMIs	100.0%
Sequencing Saturation	84.4%
Q30 Bases in Barcode	96.4%
Q30 Bases in RNA Read	59.4%
Q30 Bases in UMI	96.5%

Mapping

Reads Mapped to Genome	95.8%
Reads Mapped Confidently to Genome	92.9%
Reads Mapped Confidently to Intergenic Regions	5.2%
Reads Mapped Confidently to Intronic Regions	25.5%
Reads Mapped Confidently to Exonic Regions	62.2%
Reads Mapped Confidently to Transcriptome	58.2%
Reads Mapped Antisense to Gene	1.2%

Cells

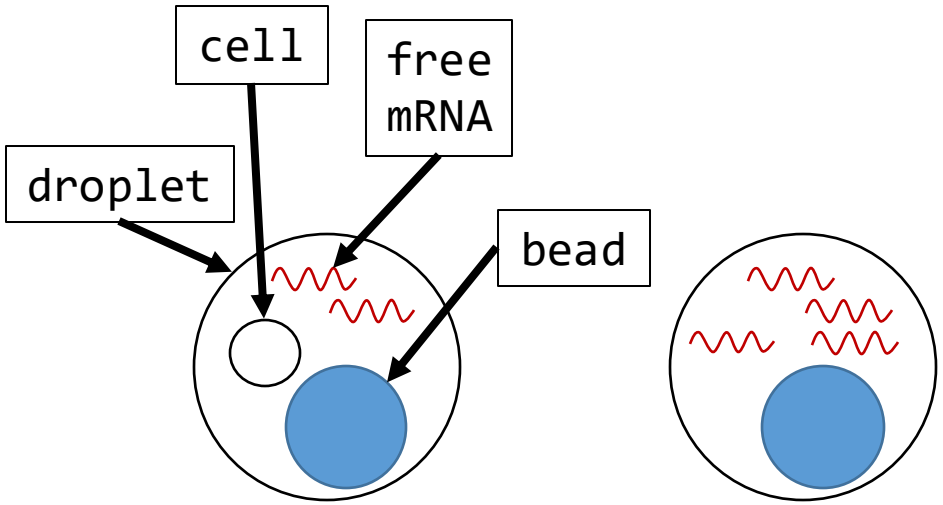


Estimated Number of Cells	3,091
Fraction Reads in Cells	91.1%
Mean Reads per Cell	68,259
Median Genes per Cell	1,717
Total Genes Detected	18,334
Median UMI Counts per Cell	4,825

Sample

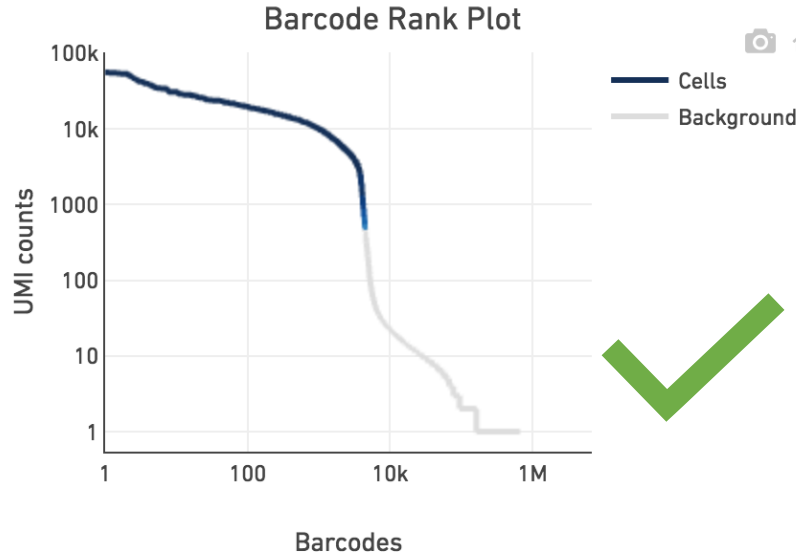
Sample ID	ETV6-RUNX1_1
Sample Description	
Chemistry	Single Cell 3' v2
Include introns	False
Reference Path	…nger/refdata-cellranger-GRCh38-3.0.0
Transcriptome	GRCh38-3.0.0
Pipeline Version	cellranger-6.0.1

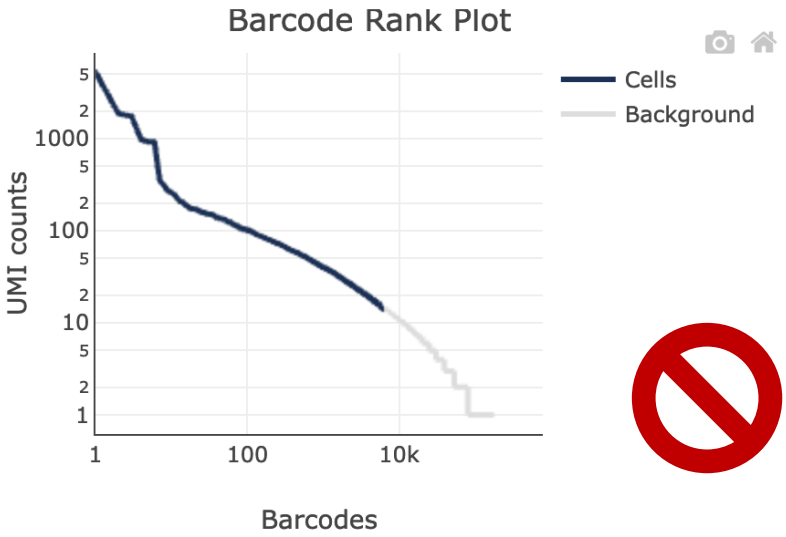
Cell calling



Cell captured

Only background captured



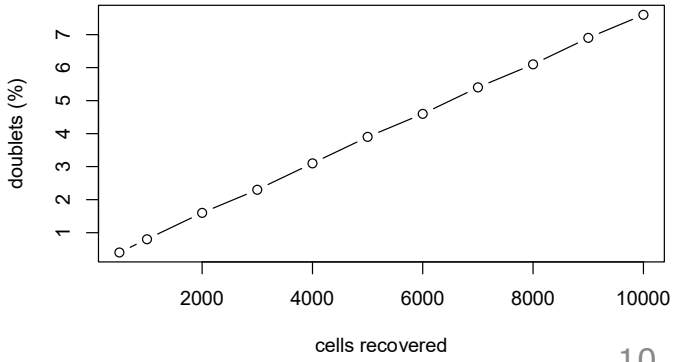


Background ‘cells’: low #UMI/barcode

Other important parameters

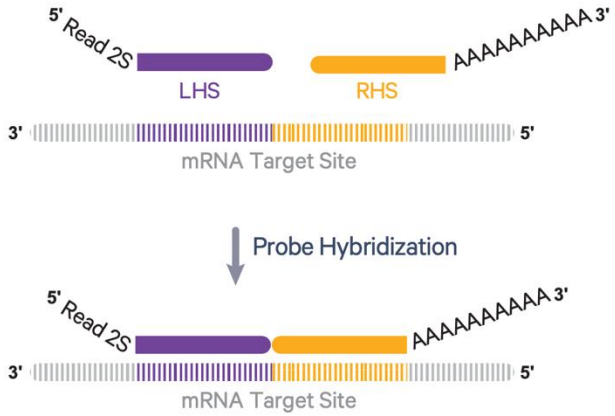
- Number of cells (<8% doublet rate):
  - <20k/channel (chromium X)
  - <10k/channel (chromium controller)
- Reads per cell: 20k – 50k (depends on application)
- Sequencing saturation

$$saturation = 1 - \frac{\#\ unique\ reads}{\#\ reads}$$



10x single cell flex

- FFPE fixed cells
- Based on probe hybridzation:
  - Specificy through ligation
  - ~3 probes/gene
  - Only human and mouse
  - Hybridized probes are sequenced
- 16 barcoded probe sets – allows for multiplexing!



More info here

10x + PacBio sequencing

- Kinnex single cell RNA kit - compatible with 5’ and 3’ 10x protocols
- Full length transcripts = isoforms!
- Similar price range to short reads (Revio)