Single Cell RNA Analysis

Biocore Bootcamp

University of Massachusetts
UMASS. Medical School

Bioinformatics Core

Overview

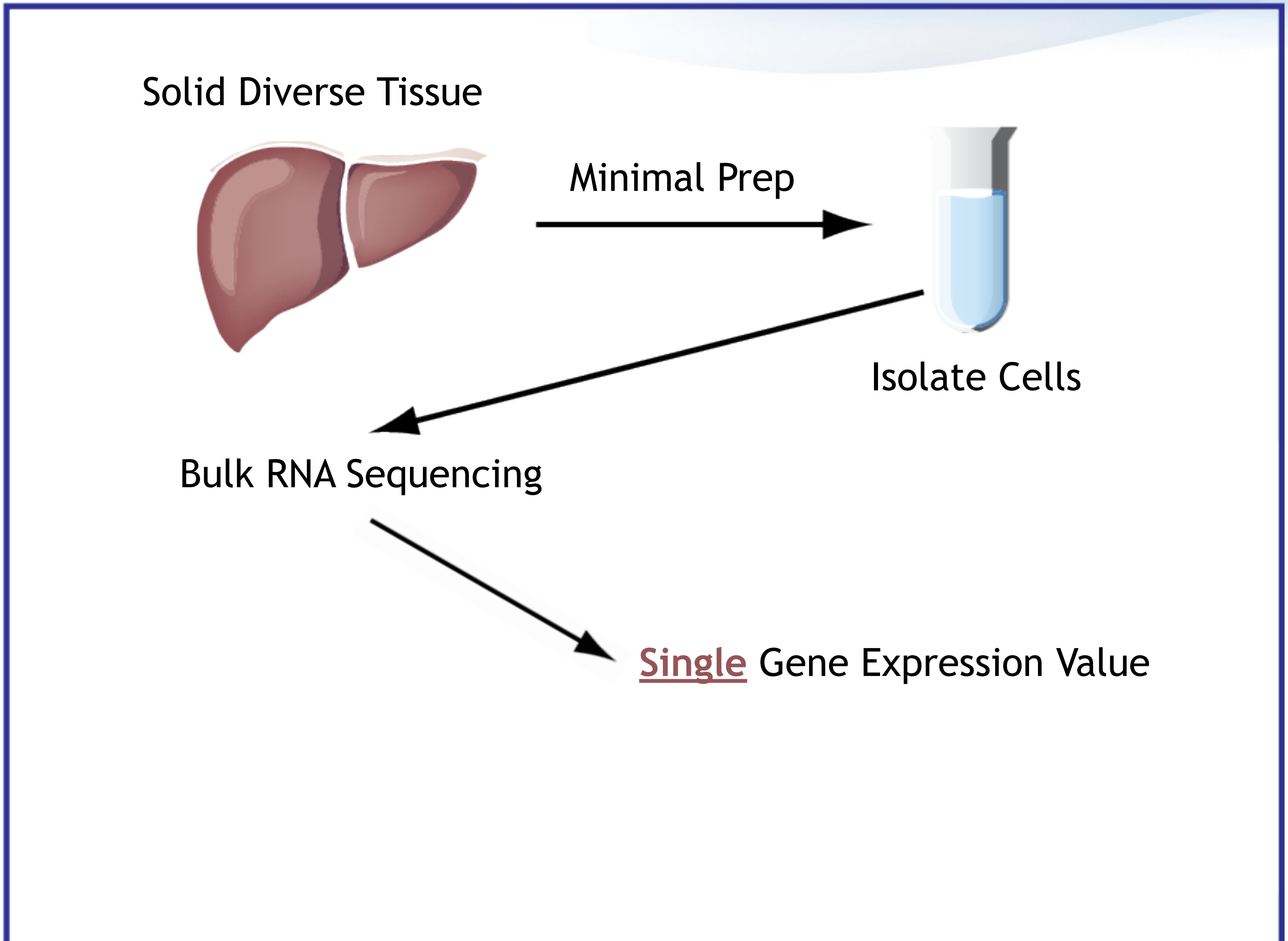
1) Why single cell?

2) inDrops Technology

3) Dolphinnext pipelines

4) Data Structures

Why Single Cell??



Solid Diverse Tissue

Minimal Prep

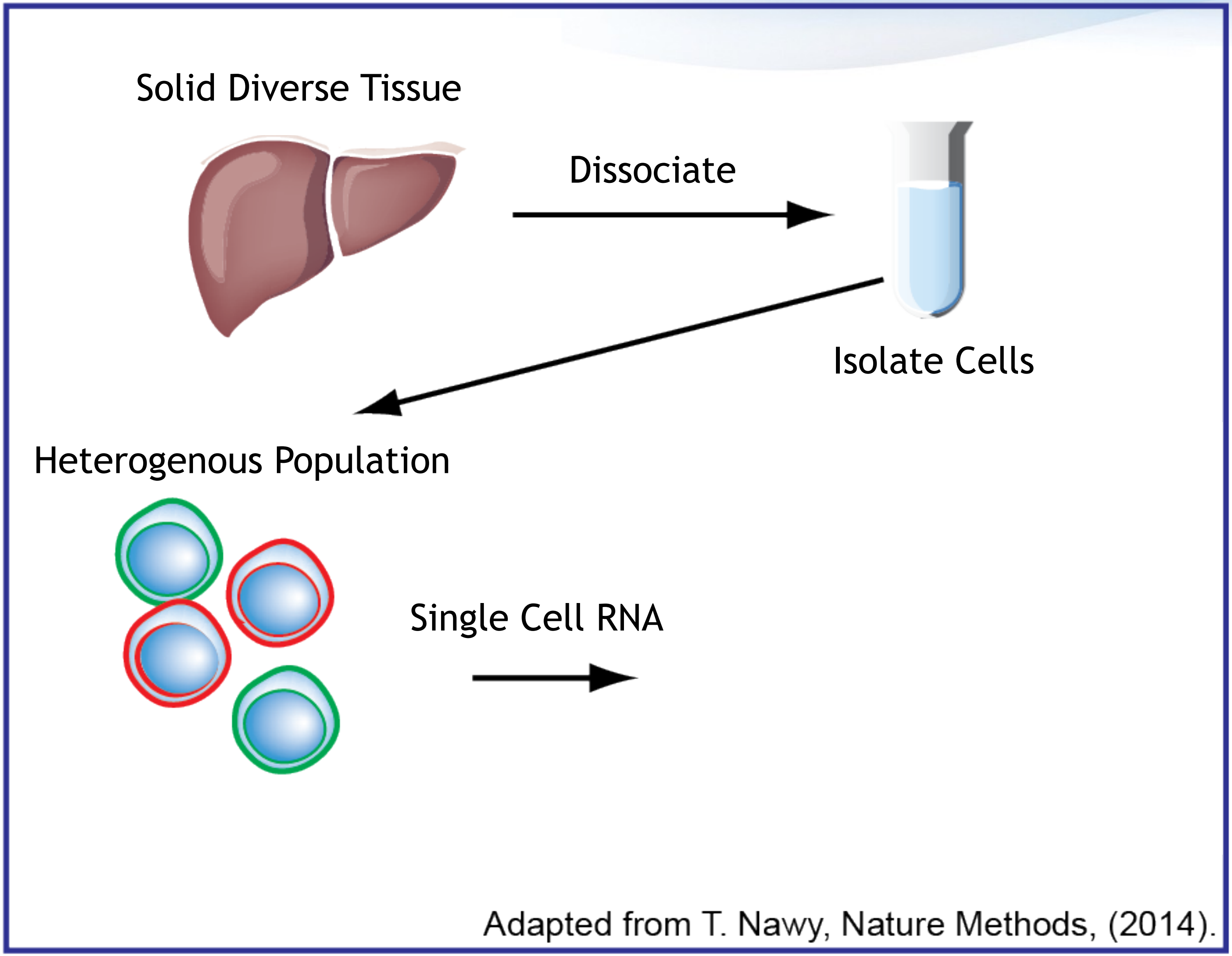
Isolate Cells

Bulk RNA Sequencing

Single Gene Expression Value

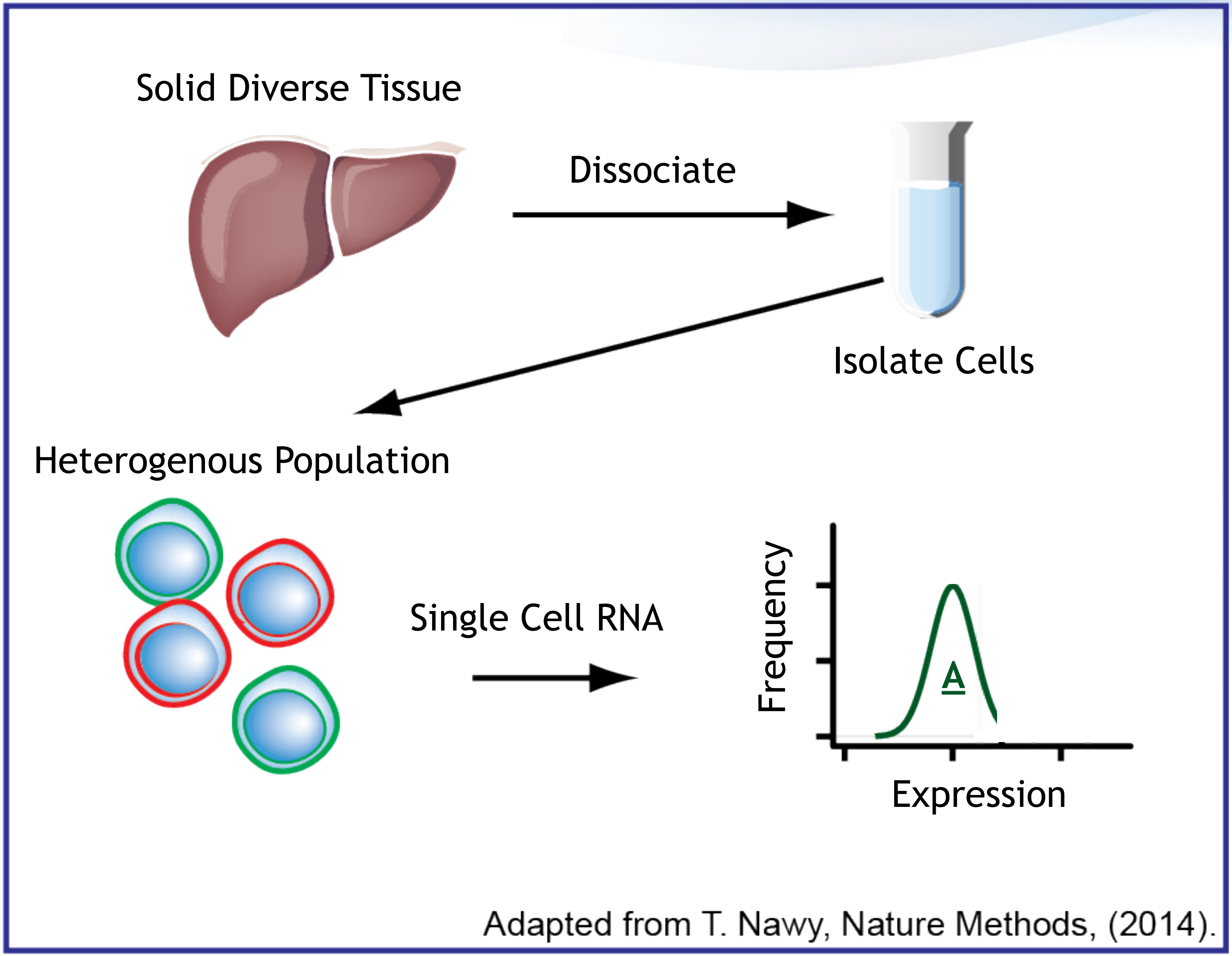
Adapted from T. Nawy, Nature Methods, (2014).

Why Single Cell??



Adapted from T. Nawy, Nature Methods, (2014).

Why Single Cell??



Solid Diverse Tissue

Dissociate

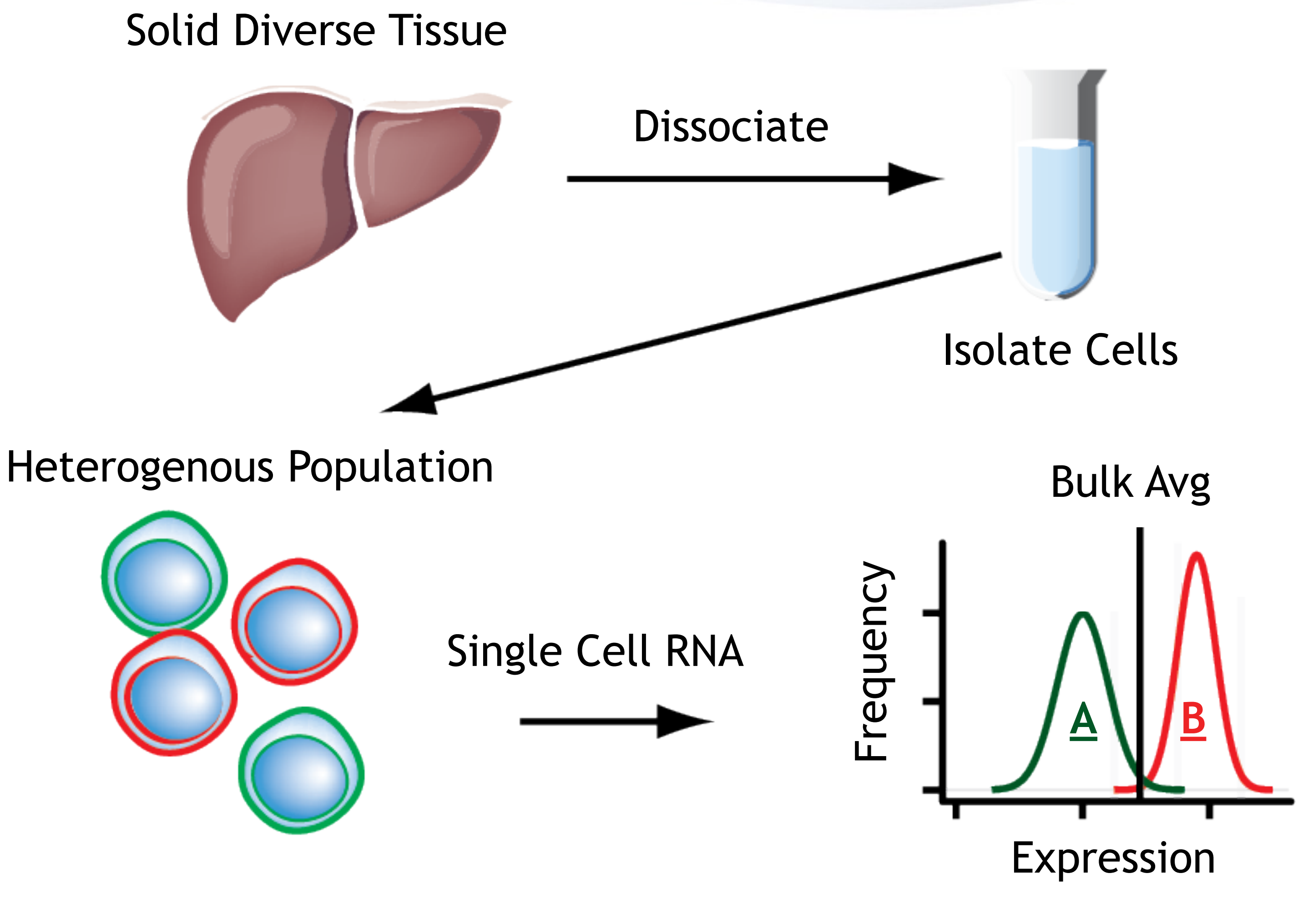
Isolate Cells

Heterogenous Population

Single Cell RNA

Adapted from T. Nawy, Nature Methods, (2014).

Why Single Cell??



Solid Diverse Tissue

Dissociate

Isolate Cells

Heterogenous Population

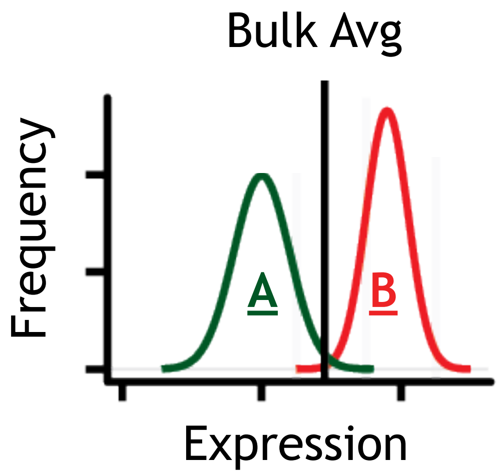
Single Cell RNA

Adapted from T. Nawy, Nature Methods, (2014).

Why Single Cell??

- Not all problems necessitate scRNA-seq

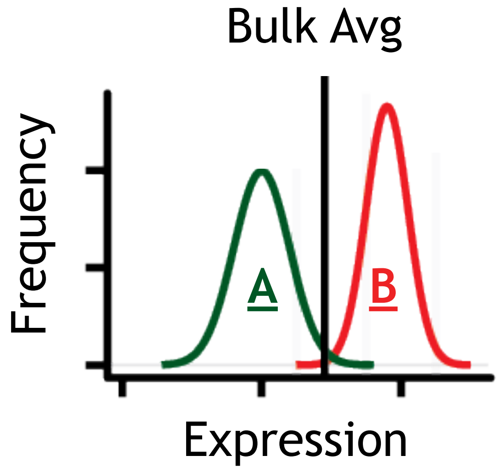
- It is well suited when populations are heterogeneous



Adapted from T. Nawy, Nature Methods, (2014).

Why Single Cell??

- Not all problems necessitate scRNA-seq
- It is well suited when populations are heterogeneous
- It is a powerful tool for studying intra and inter cell type variations in gene expression
- Useful for unbiased discovery



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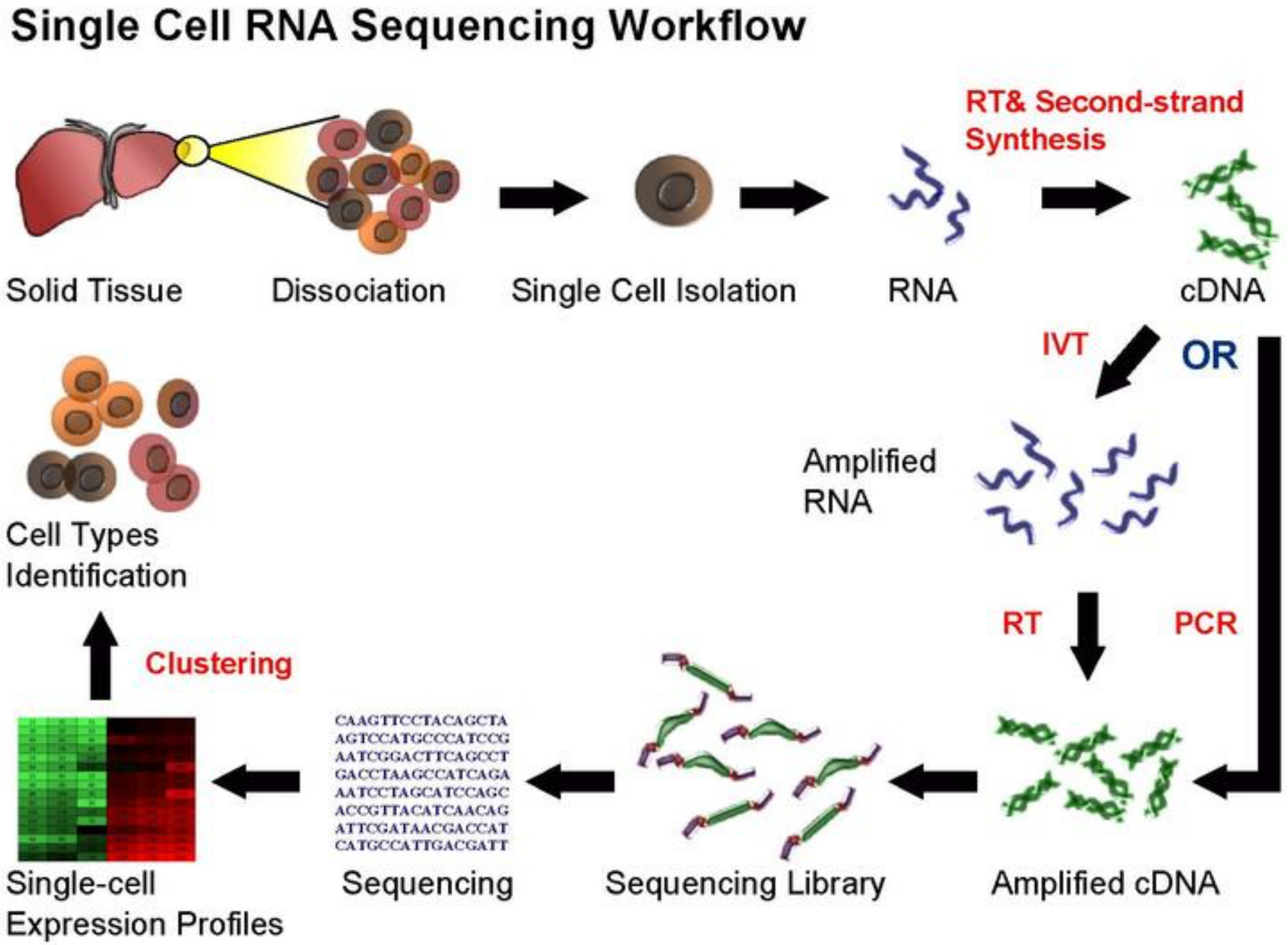
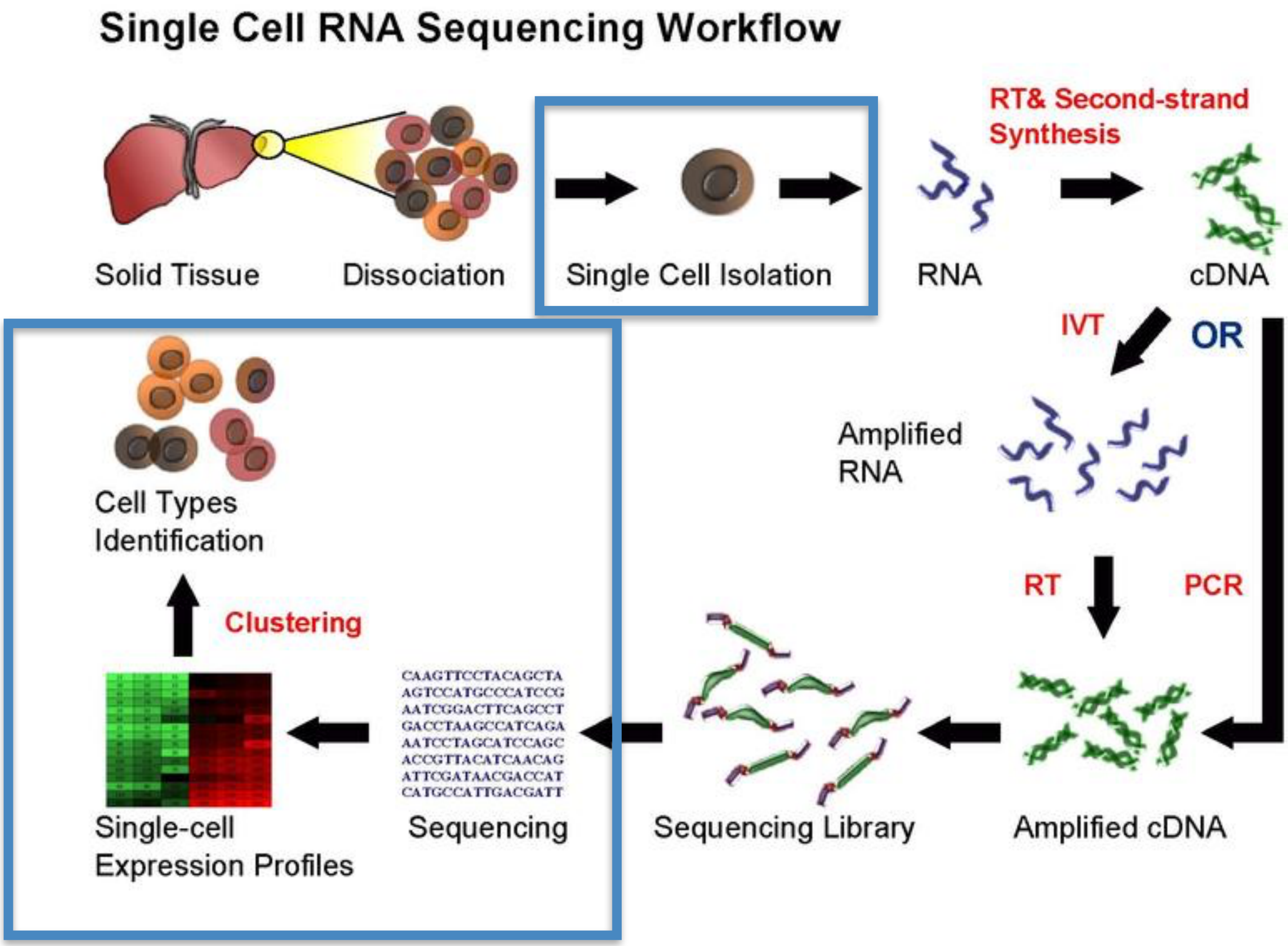
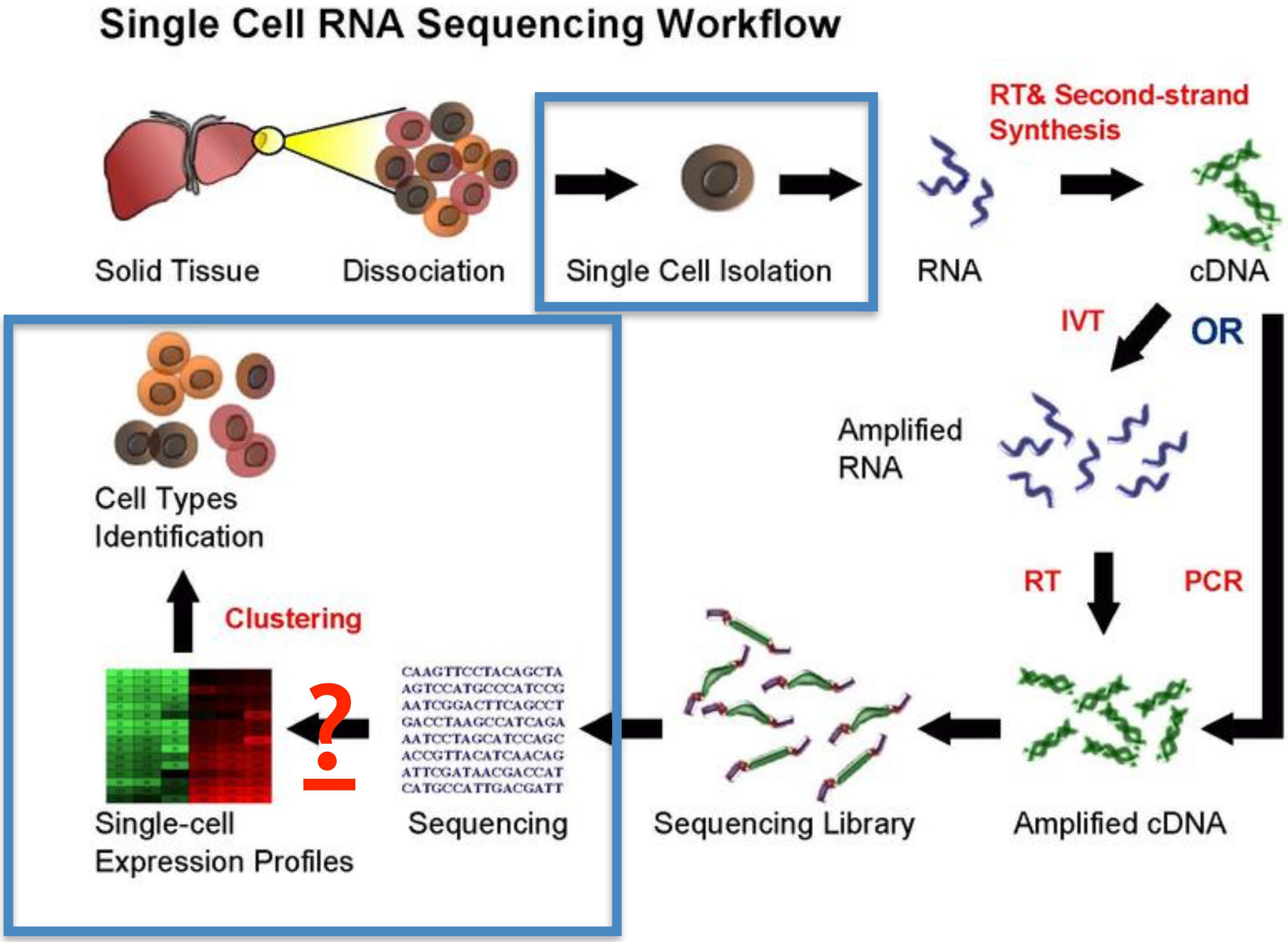
Single Cell Workflows



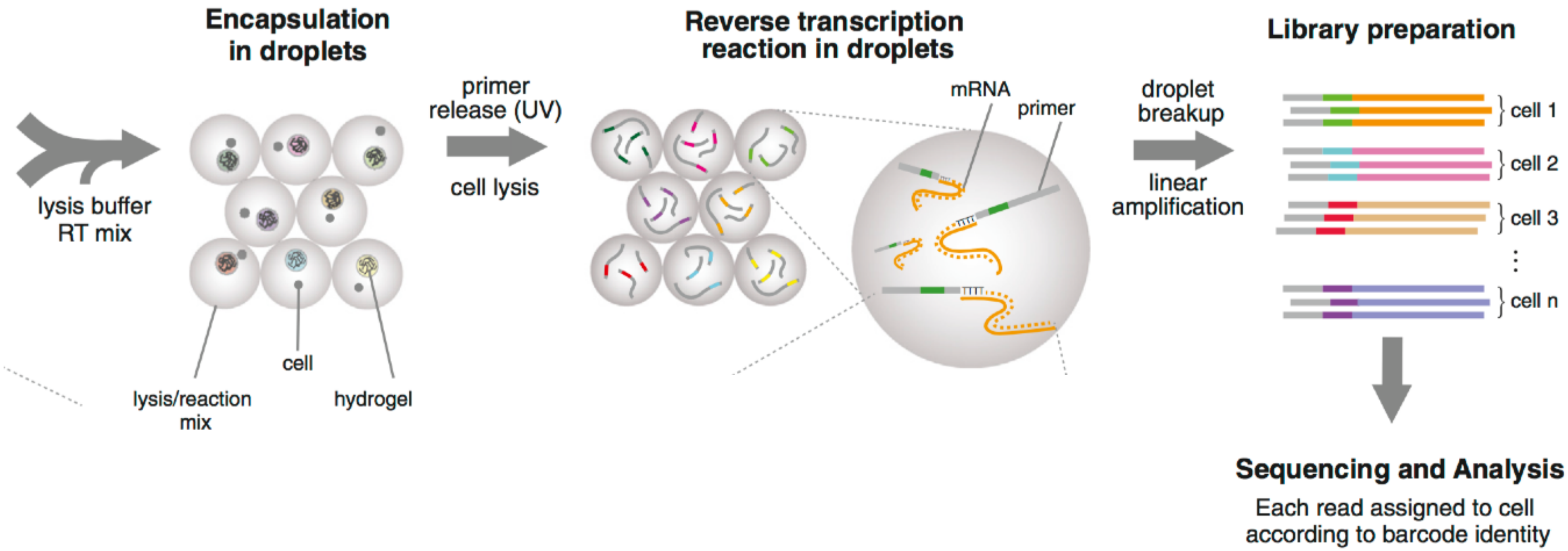
Single Cell Workflows



Single Cell Workflows

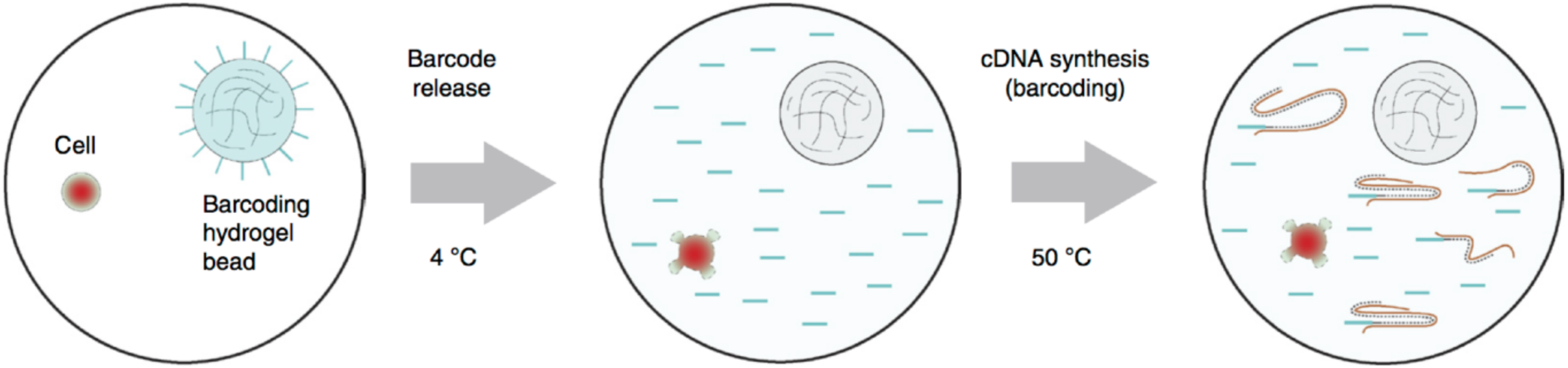


inDrops Technology



Zilionis, Rapolas, et al. "Single-cell barcoding and sequencing using droplet microfluidics." Nature Protocols

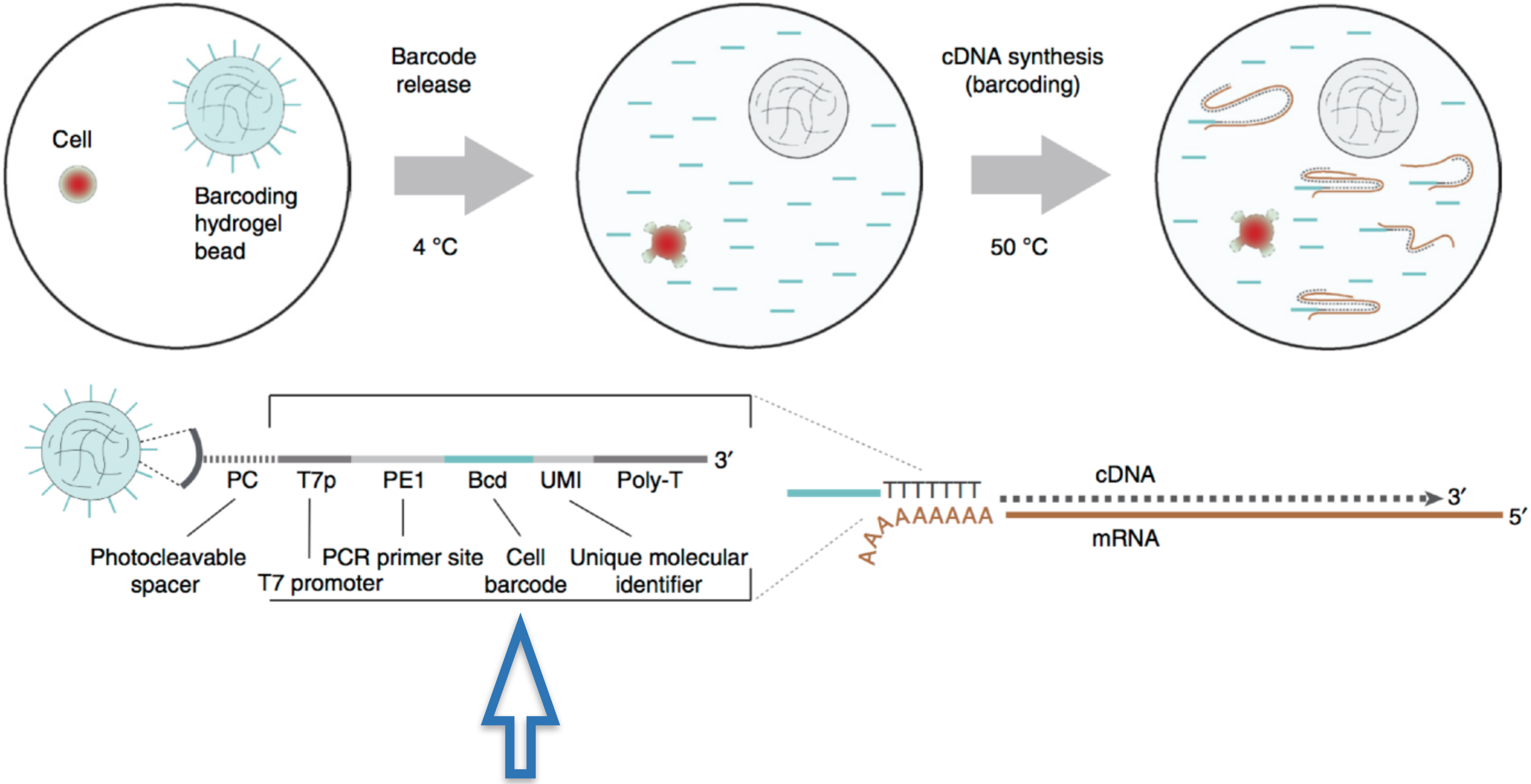
inDrops Technology



?

Zilionis, Rapolas, et al. "Single-cell barcoding and sequencing using droplet microfluidics." Nature Protocols

inDrops Technology



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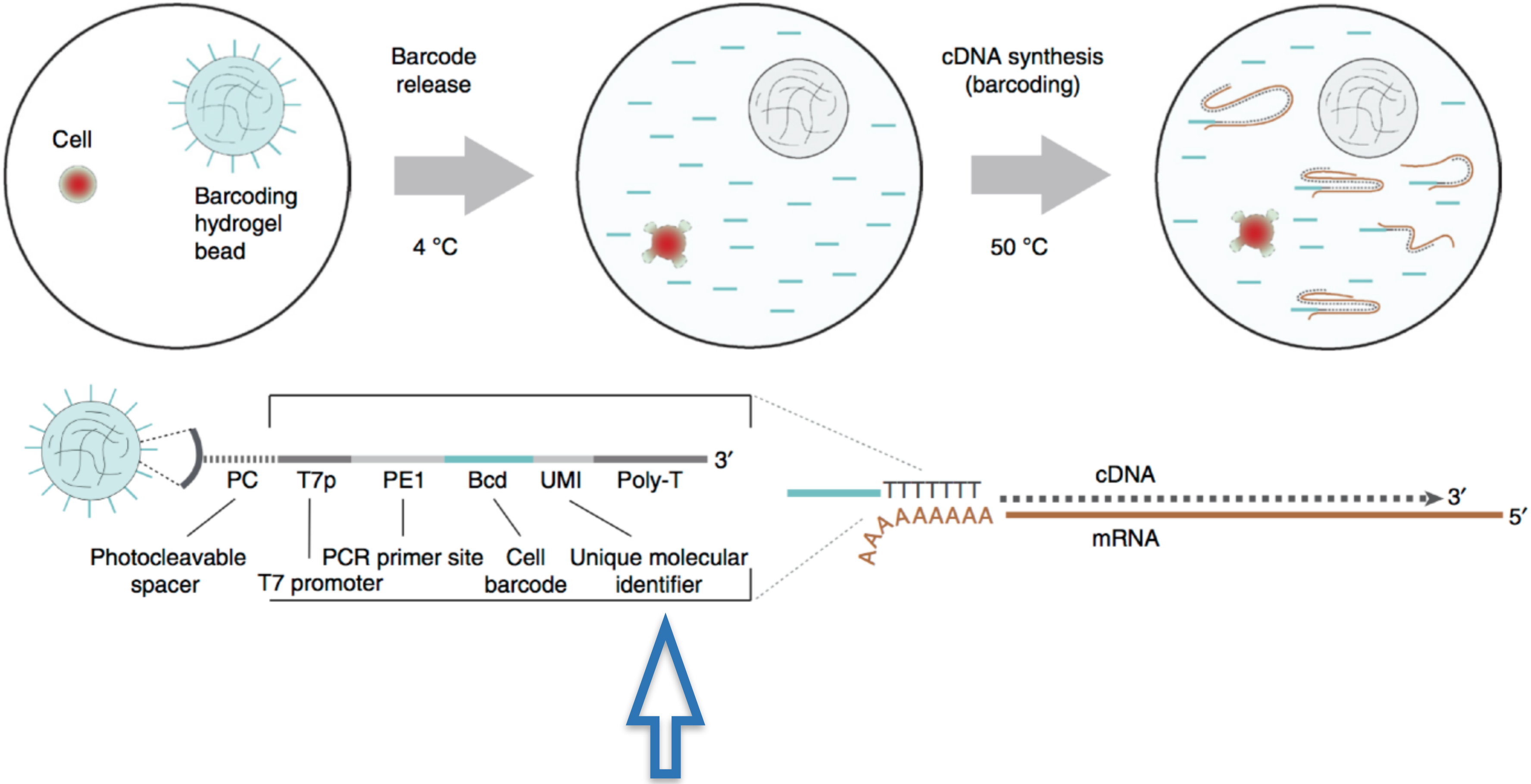
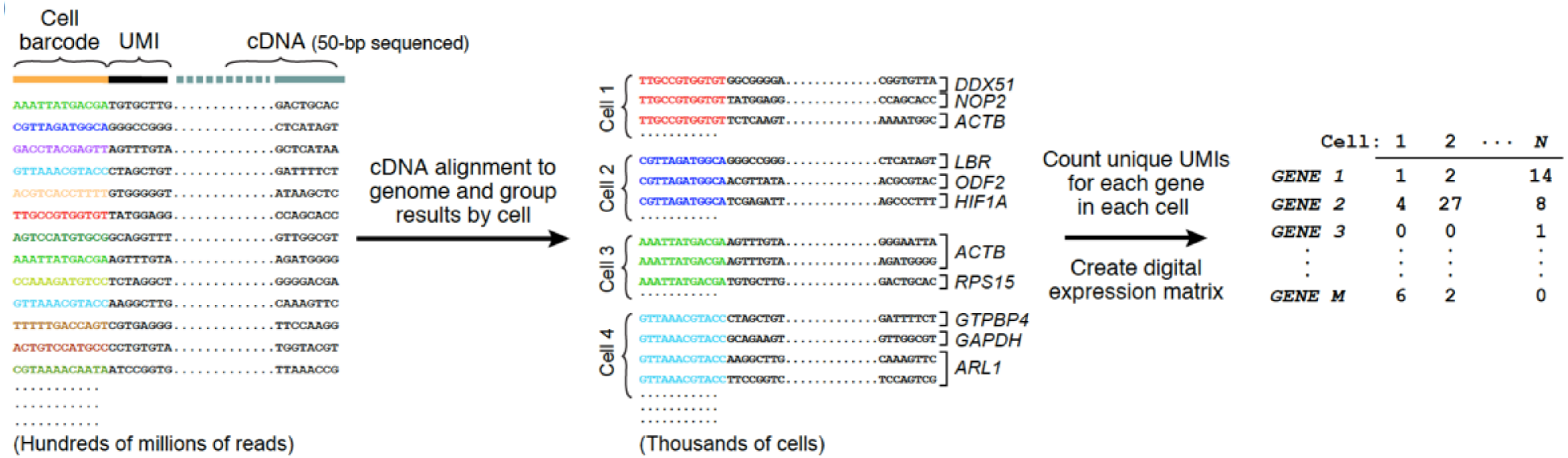
inDrops Technology



Zilionis, Rapolas, et al. "Single-cell barcoding and sequencing using droplet microfluidics." Nature Protocols

inDrops Data Processing


Cell barcode
UMI
cDNA (50-bp sequenced)
AAATTATGACGATGTGCTTG.............GACTGCAC
CGTTAGATGGCAGGGCCGGG.............CTCATAGT
GACCTACGAGTTAGTTTGTA.............GCTCATAA
GTTAAACGTACCCTAGCTGT.............GATTTTCT
ACGTCACCTTTTGTGGGGGT.............ATAAGCTC
TTGCCGTGGTGTTATGGAGG.............CCAGCACC
AGTCCATGTGCGGCAGGTTT.............GTTGGCGT
AAATTATGACGAAGTTTGTA.............AGATGGGG
CCAAAGATGTCCTCTAGGCT.............GGGGACGA
GTTAAACGTACCAAGGCTTG.............CAAAGTTC
TTTTTGACCAGTCGTGAGGG.............TTCCAAGG
ACTGTCCATGCCCCTGTGTA.............TGGTACGT
CGTAAAACAATAATCCGGTG.............TTAAACCG
(Hundreds of millions of reads)
Cell 1
TTGCCGTGGTGTGGCGGGGA.............CGGTGTTA
TTGCCGTGGTGTTATGGAGG.............CCAGCACC
TTGCCGTGGTGTTCTCAAGT.............AAAATGGC
DDX51
NOP2
ACTB
Cell 2
CGTTAGATGGCAGGGCCGGG.............CTCATAGT
CGTTAGATGGCAACGTTATA.............ACGCGTAC
CGTTAGATGGCATCGAGATT.............AGCCCTTT
LBR
ODF2
HIF1A
Cell 3
AAATTATGACGAAGTTTGTA.............GGGAATTA
AAATTATGACGAAGTTTGTA.............AGATGGGG
AAATTATGACGATGTGCTTG.............GACTGCAC
ACTB
RPS15
Cell 4
GTTAAACGTACCCTAGCTGT.............GATTTTCT
GTTAAACGTACCGCAGAAGT.............GTTGGCGT
GTTAAACGTACCAAGGCTTG.............CAAAGTTC
GTTAAACGTACCTTCCGGTC.............TCCAGTCG
GTPBP4
GAPDH
ARL1
(Thousands of cells)

cDNA alignment to genome and group results by cell

Count unique UMIs for each gene in each cell

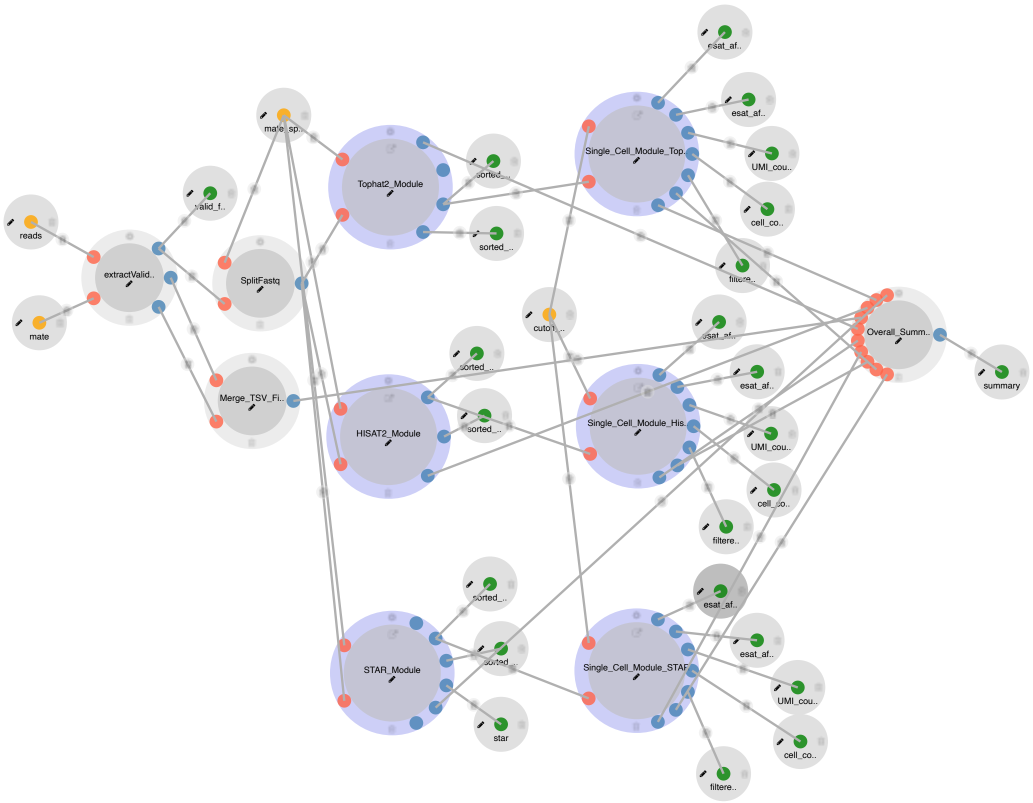
Create digital expression matrix

Cell:	1	2	...	N
GENE 1	1	2		14
GENE 2	4	27		8
GENE 3	0	0		1
⋮	⋮	⋮		⋮
GENE M	6	2		0

Macosko, Evan Z., et al. "Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets."

Dolphinnext Pipelines

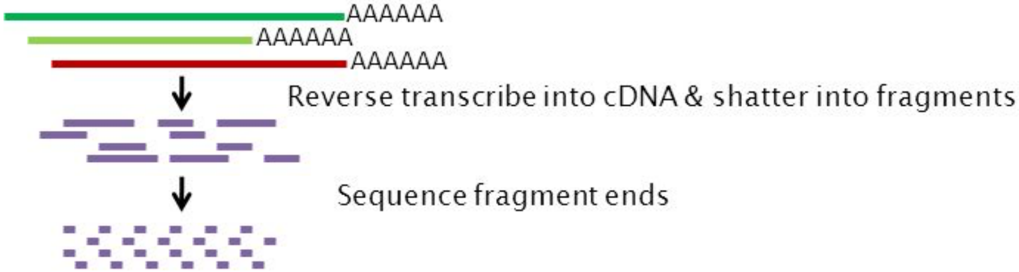
To process the FASTQs that the instrument generates into a gene expression matrix involves many steps, which can be run as a continuous pipeline with dolphinnext



Dolphinnext Pipelines : bcl2fastq

1) bcl2fastq

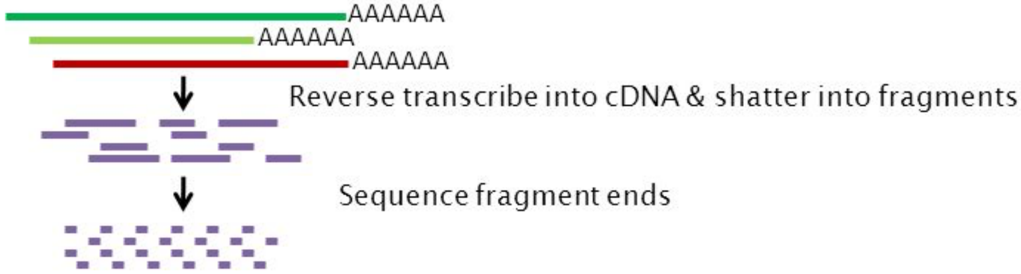
- valid sample barcode



```
@NS500602:550:HM2TFBGX5:1:11101:7989:1090:CAAACATTGGCCCAAT:CTGACTTA 1:N:0:TATAGACG
TTCCCNCTAGACATTTCTGTGCATAGATTTTTGGTGTGTTTACATAGTCGTTATTCTG
AAAAA#EEEEEEEEEEEEEEEEEEEEEAEEEEE/EAAEEEEEEEEAEEEEEEEEEA6/
```

Dolphinnext Pipelines : extract valid reads

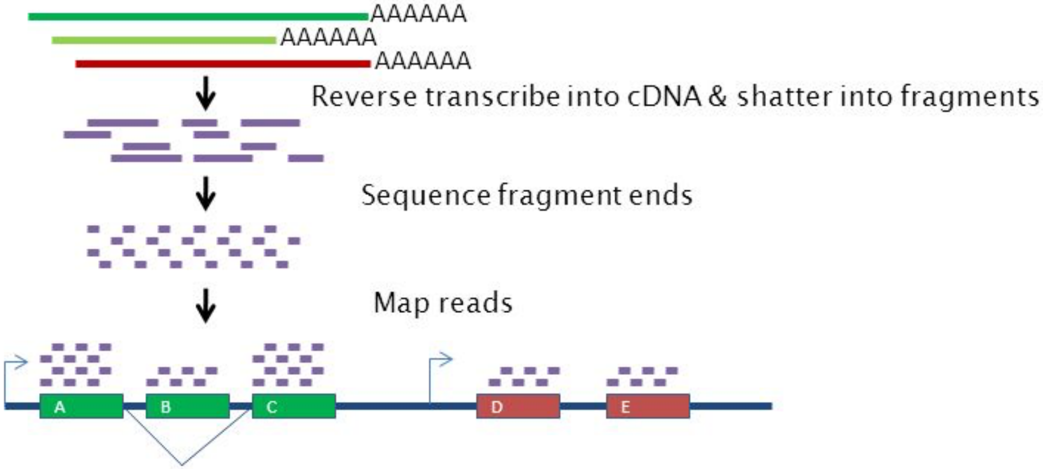
1) bcl2fastq
2) extract reads
   - valid cell barcode
   - valid UMI



```
@NS500602:550:HM2TFBGX5:1:11101:7989:1090:CAAACATTGGCCCAAT:CTGACTTA 1:N:0:TATAGACG
TTCCCNCTAGACATTTCTGTGCATAGATTTTTGGTGTGTTTACATAGTCGTTATTCTG
AAAAA#EEEEEEEEEEEEEEEEEEEEAEEEEEE/EAAEEEEEEEEAEEEEEEEEEA6/
```

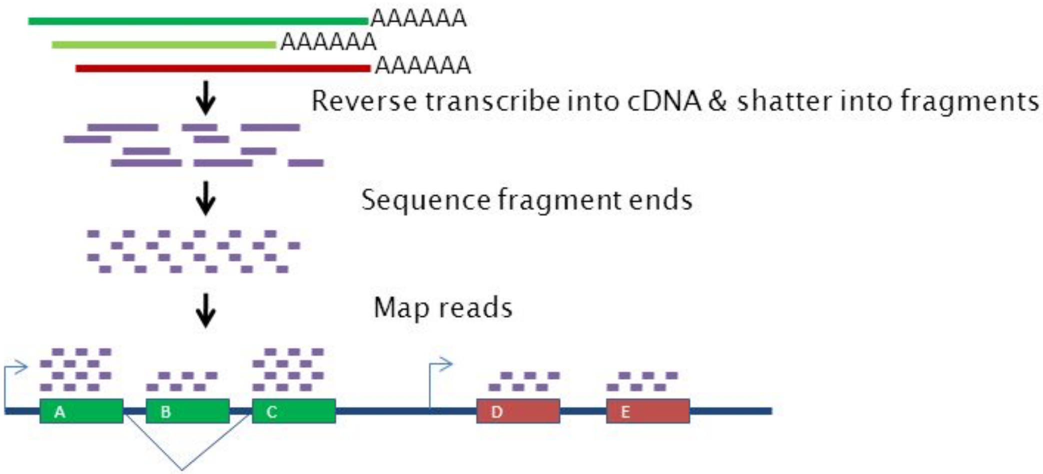
Dolphinnext Pipelines : alignment

1) bcl2fastq
2) extract reads
3) align



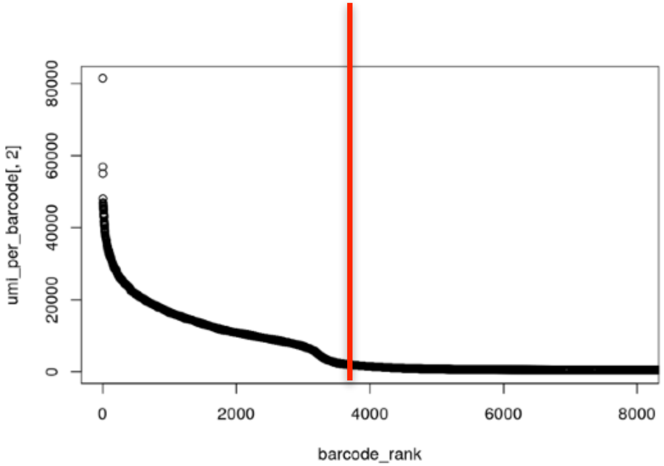
Dolphinnext Pipelines : filtering

1) bcl2fastq
2) extract reads
3) align
4) filter
   - num reads per cell barcode


Reverse transcribe into cDNA & shatter into fragments

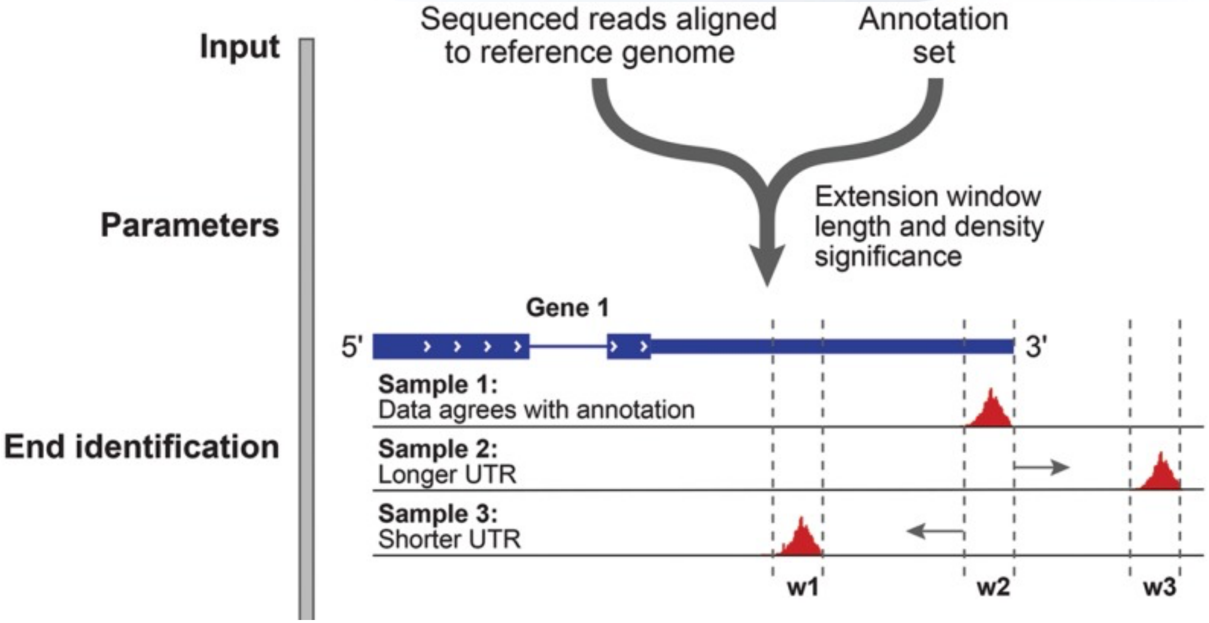
Sequence fragment ends

Map reads



Dolphinnext Pipelines : ESAT

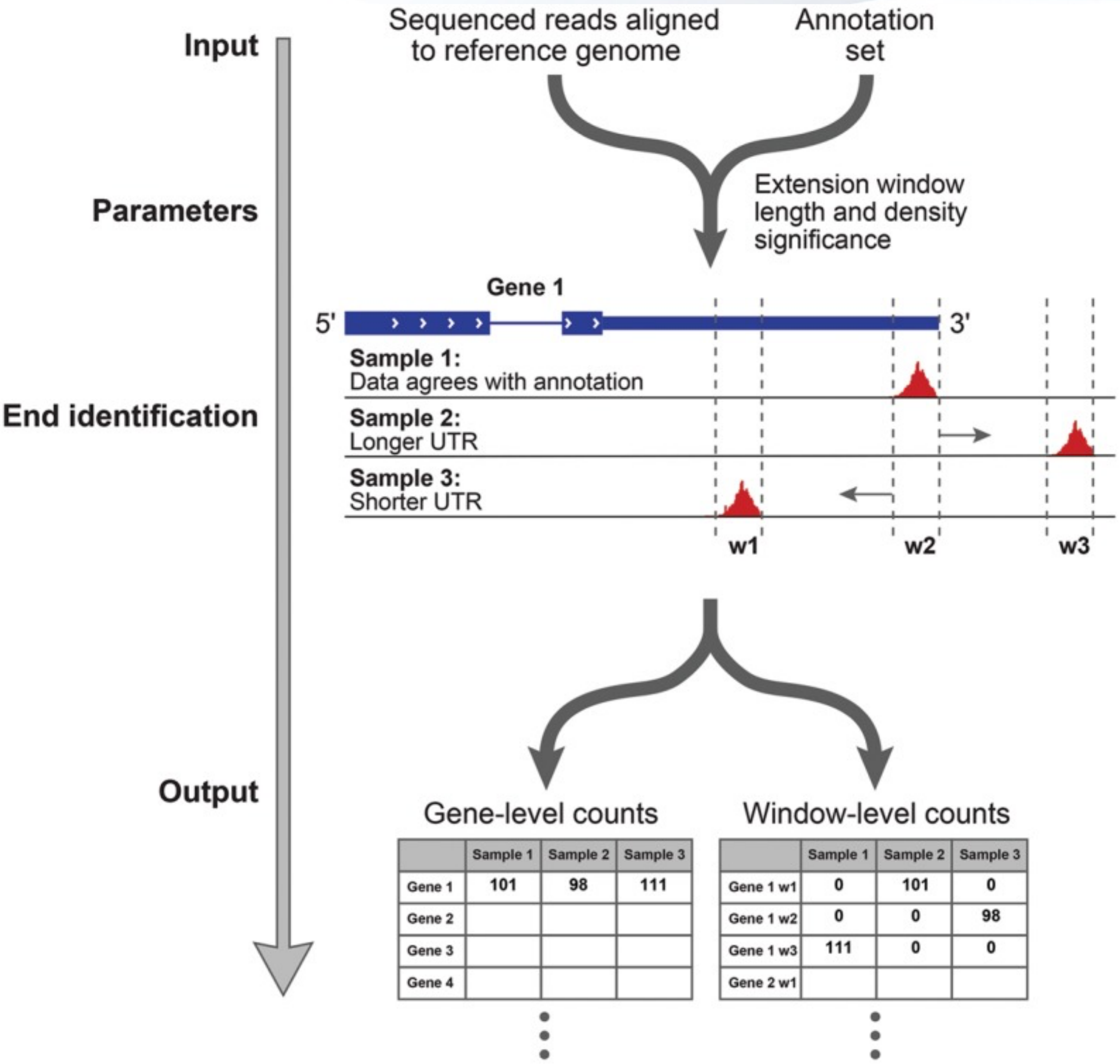
1) bcl2fastq
2) extract reads
3) align
4) filter
5) ESAT


Input
Parameters
End identification
Sequenced reads aligned to reference genome
Annotation set
Extension window length and density significance
Gene 1
5'
3'
Sample 1:
Data agrees with annotation
Sample 2:
Longer UTR
Sample 3:
Shorter UTR
w1
w2
w3

Derr, Alan, et al. "End Sequence Analysis Toolkit (ESAT) expands the extractable information from single-cell RNA-seq data."

Dolphinnext Pipelines : ESAT

1) bcl2fastq
2) extract reads
3) align
4) filter
5) ESAT



	Sample 1	Sample 2	Sample 3
Gene 1	101	98	111
Gene 2			
Gene 3			
Gene 4			

	Sample 1	Sample 2	Sample 3
Gene 1 w1	0	101	0
Gene 1 w2	0	0	98
Gene 1 w3	111	0	0
Gene 2 w1			

Derr, Alan, et al. "End Sequence Analysis Toolkit (ESAT) expands the extractable information from single-cell RNA-seq data."

Data Structures : UMI Table

	Cell 1	Cell 2	Cell 3	Cell 4…
Gene 1	42	43	10	9
Gene 2	25	24	2	3
Gene 3	10	9	100	98
Gene 4…	40	39	4	5
SUM	117	115	116	115

Bulk = Average of all genes across all cells

Bulk = proportion of each cell type *
cell types average expression profile

Data Structures : UMI Table

Group A

	Cell 1	Cell 2	Cell 3	Cell 4…
Gene 1	42	43	10	9
Gene 2	25	24	2	3
Gene 3	10	9	100	98
Gene 4…	40	39	4	5
SUM	117	115	116	115

Group B

Data Structures : UMI Table

+1000 Cells

+10000 Genes

	Cell 1	Cell 2	Cell 3	Cell 4…
Gene 1	42	43	10	9
Gene 2	25	24	2	3
Gene 3	10	9	100	98
Gene 4…	40	39	4	5
SUM	117	115	116	115

Data Structures : UMI Table

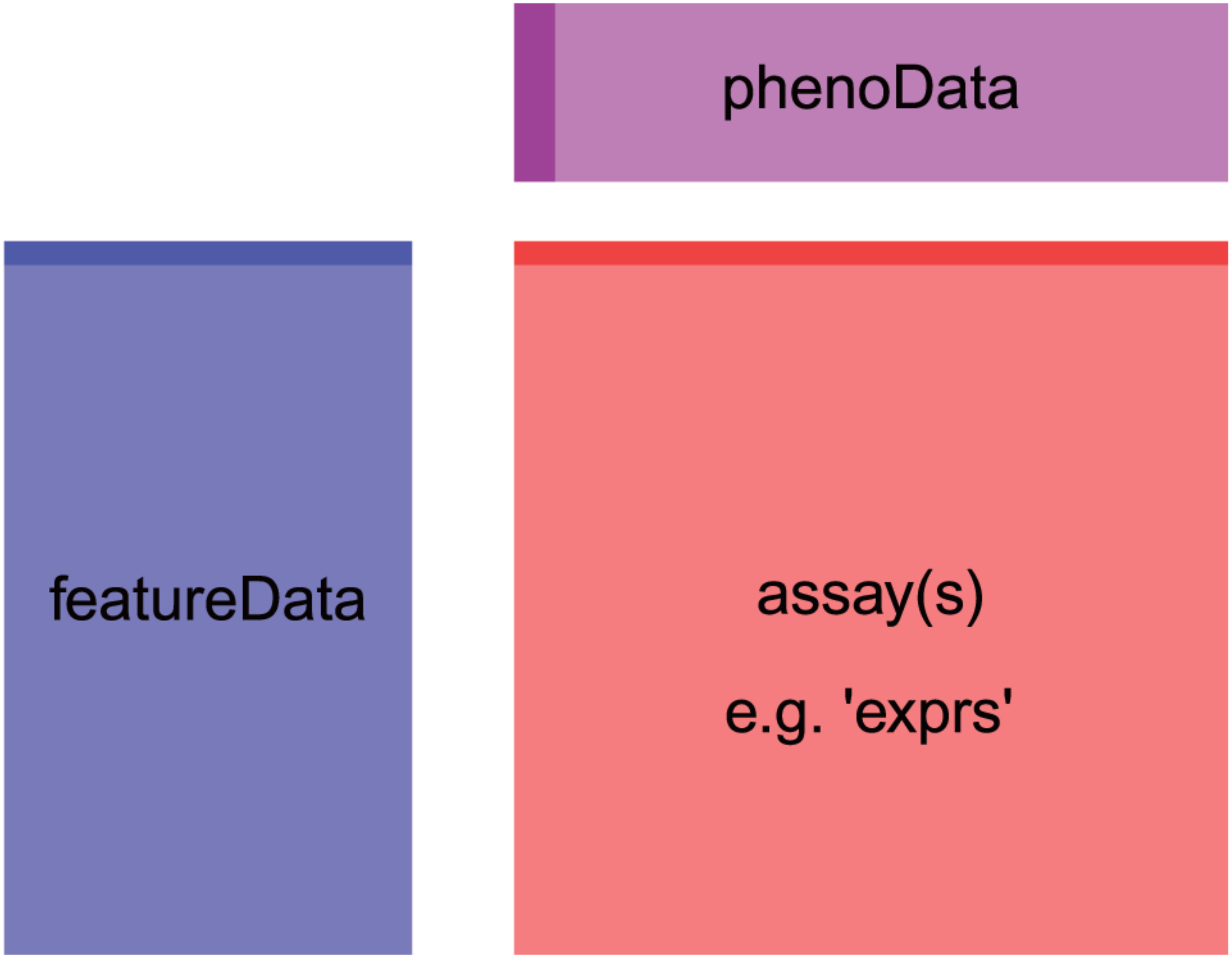
+1000 Cells

+10000 Genes

	Cell 1	Cell 2	Cell 3	Cell 4…
Gene 1	42	43	10	9
Gene 2	25	24	2	3
Gene 3	10	9	100	98
Gene 4…	40	39	4	5
SUM	117	115	116	115

What if we want to store information about these cells or genes??

Data Structures : Expression Set Class



Data Structures : exprs()

Exprs

	0hrA_TGACGGACAAGTAATC	0hrA_ATGGGCACACCTTGCC	0hrA_TCGAAGCTGTTGCACG
0610007P14Rik	0	2.10985244161277	0
0610009B22Rik	0	0	1.72247211812165
0610009O20Rik	0	0	0
0610010B08Rik	0	0	0
0610010F05Rik	0	0	0

Genes

Cells

Data Structures : pData()

pData

	size_factor	UMI_sum	x	y	iPC_Comp1	iPC_Comp2…	iPC_Comp12	Cluster	Timepoint
0hrA_TGACGGACAAGTAATC	1.47	1604.45	0.31	0.72	-0.02	0.01	-0.01	Cluster4	0hr
0hrA_ATGGGCACACCTTGCC	1.21	1333.71	0.93	0.33	-0.02	0.01	-0.01	Cluster3	0hr
0hrA_TCGAAGCTGTTGCACG	1.30	1102.09	0.68	0.13	-0.02	0.02	-0.00	Cluster2	0hr
0hrA_TGTTTGAGTCGGTTCG	1.63	1210.53	0.86	0.32	-0.02	0.02	-0.00	Cluster3	0hr
0hrA_TAAATAGGCACAAGGC	0.43	1714.06	0.90	0.29	-0.02	0.01	-0.01	Cluster3	0hr
0hrA_GATTAGACGGGAACCT	0.64	946.06	0.48	0.59	-0.02	0.01	0.00	Cluster1	0hr

Normalization tSNE PCA Clustering Metadata

Data Structures : fData()

fData

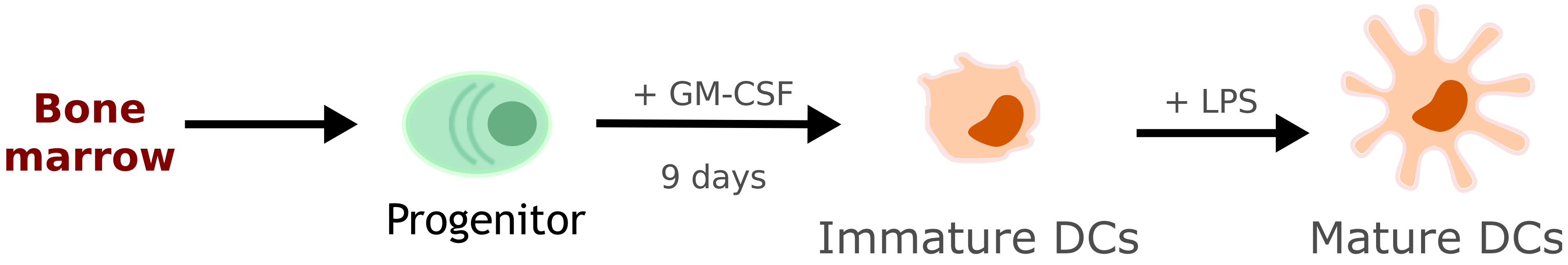
	C1_score	C2_score	C3_score	C1_bulk	C2_bulk	C3_bulk	0hr_score	1hr_score	4hr_score
0610007P14Rik	9704	704	2572	0.05	0.23	0.19	1187	5052	10278
0610009B22Rik	5293	642	1181	0.04	0.11	0.10	503	11045	6766
0610009O20Rik	7535	2732	7733	0.00	0.01	0.01	6310	7579	4016
0610010B08Rik	3184	5176	9845	0.00	0.01	0.00	8161	5170	3772
0610010F05Rik	75	11158	8698	0.11	0.01	0.04	10373	662	4595
0610010K14Rik	3888	5066	2634	0.07	0.10	0.12	1774	4074	10087

Cluster Markers

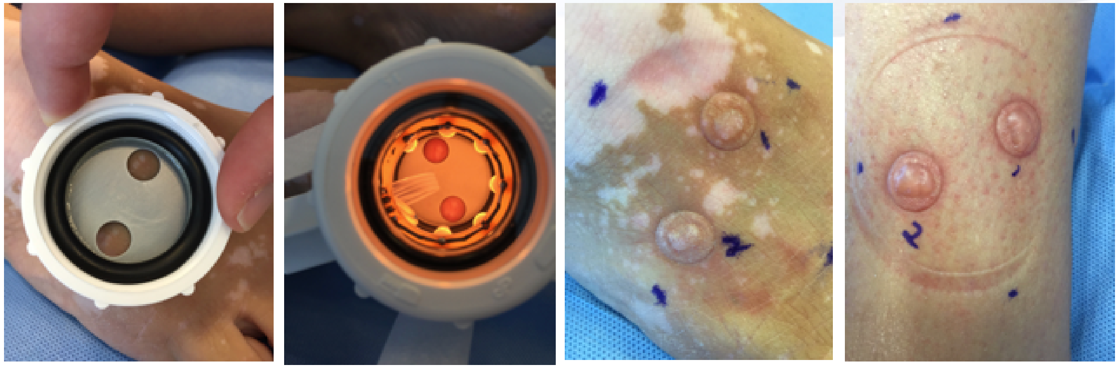
Aggregated Bulk

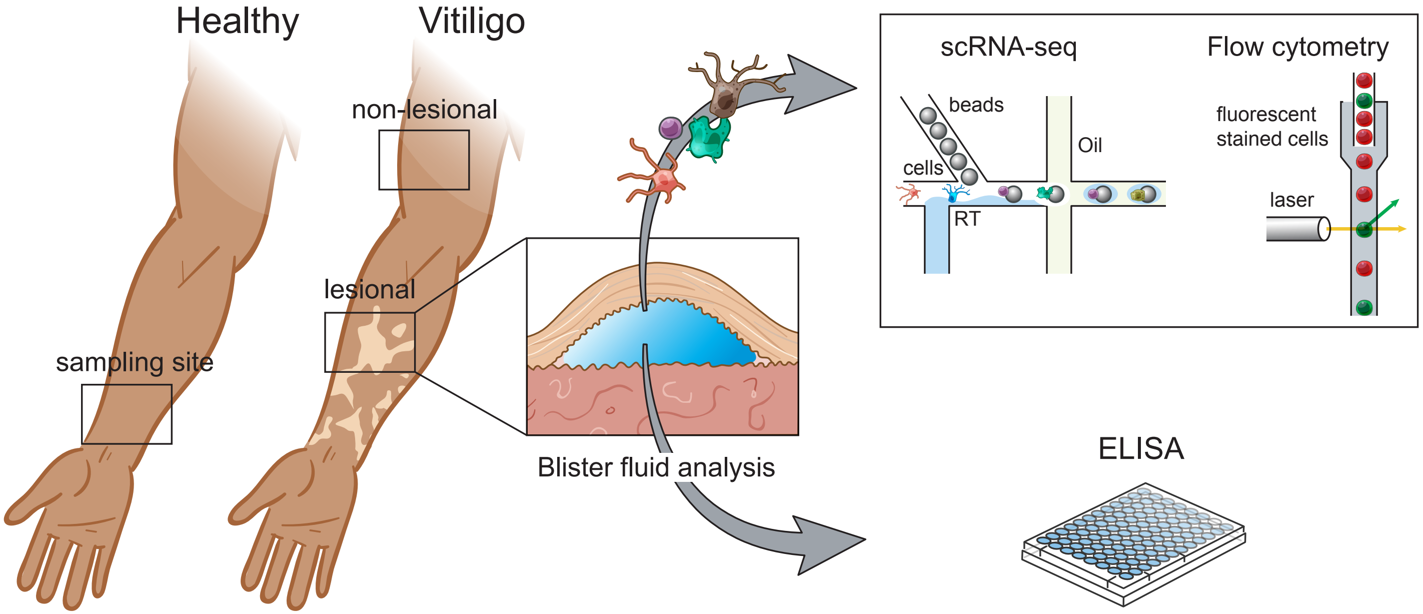
Timepoint Markers

BMDC Data Set



Skin Data Set




Healthy
Vitiligo
non-lesional
lesional
sampling site
Blister fluid analysis
scRNA-seq
beads
cells
Oil
RT
Flow cytometry
fluorescent
stained cells
laser
ELISA