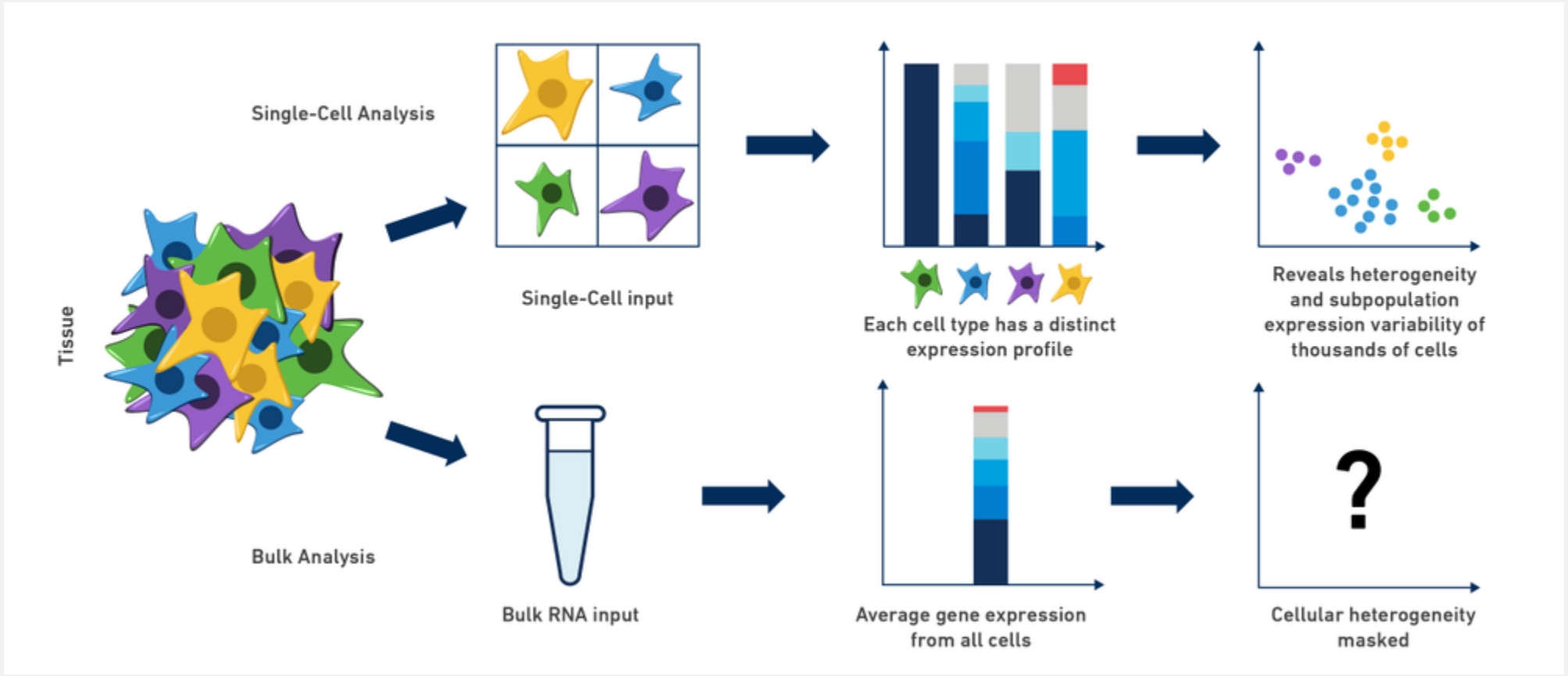
SINGLE CELL SEQUENCING

Joseph Powell

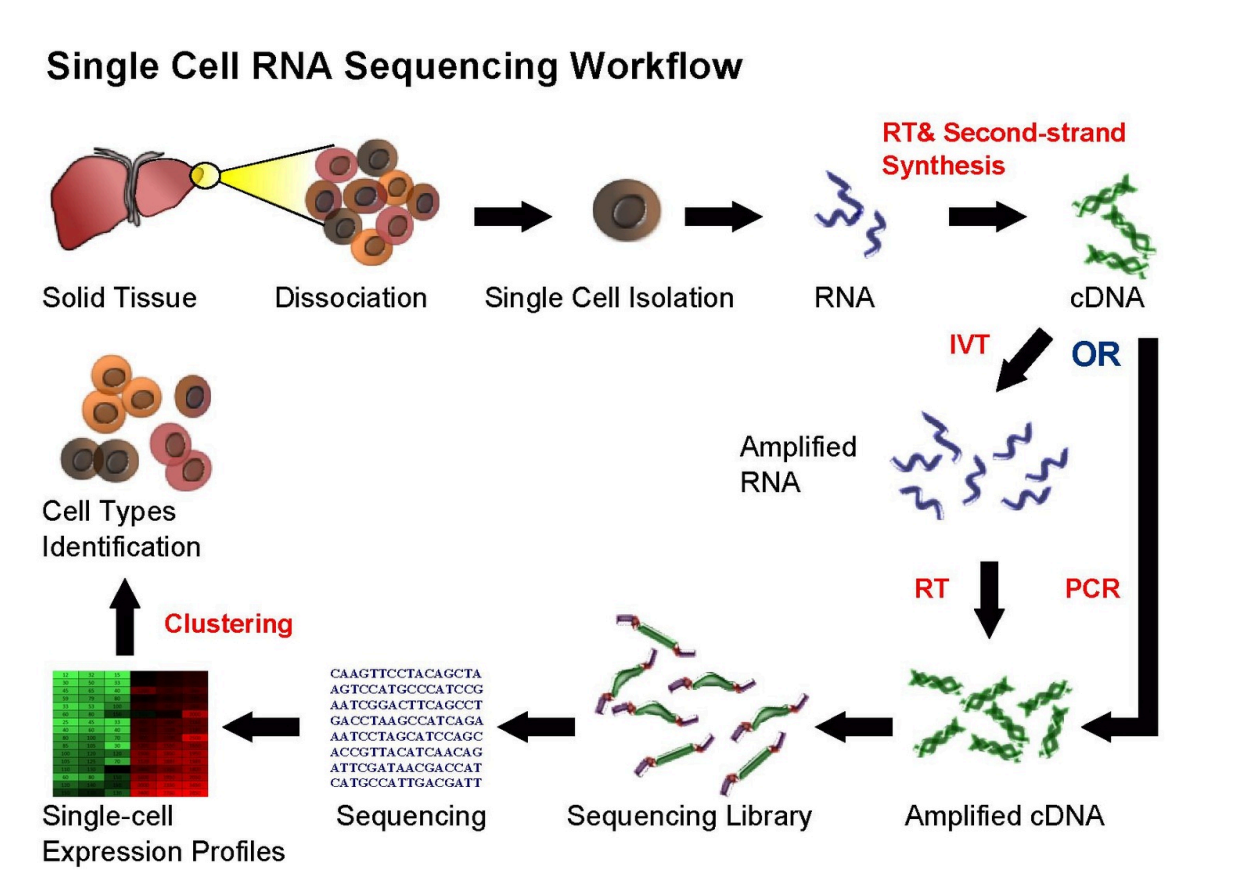
SISG- 2018

CONTENTS

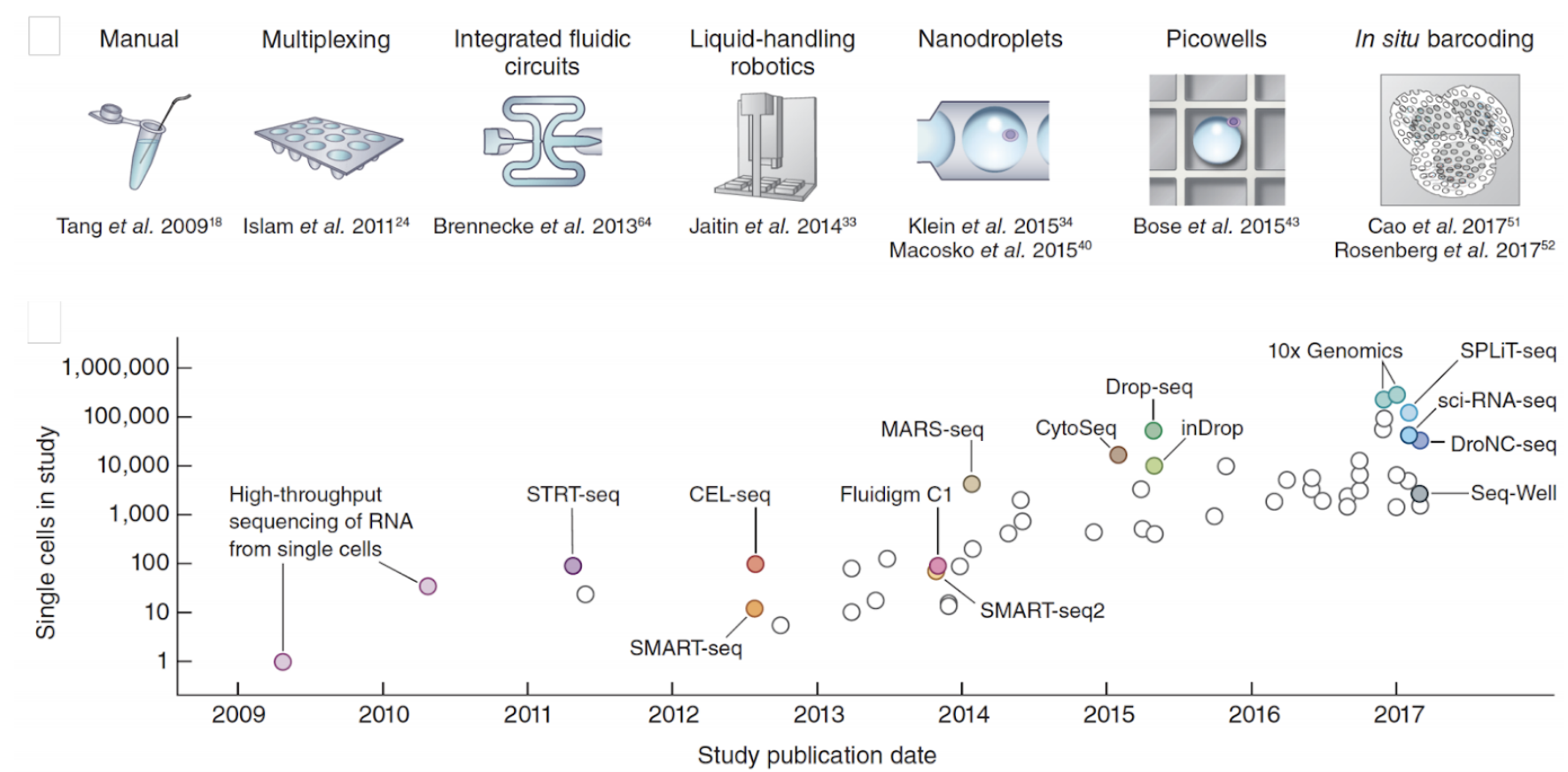
WHY SINGLE CELLS?



TYPICAL WORKFLOW

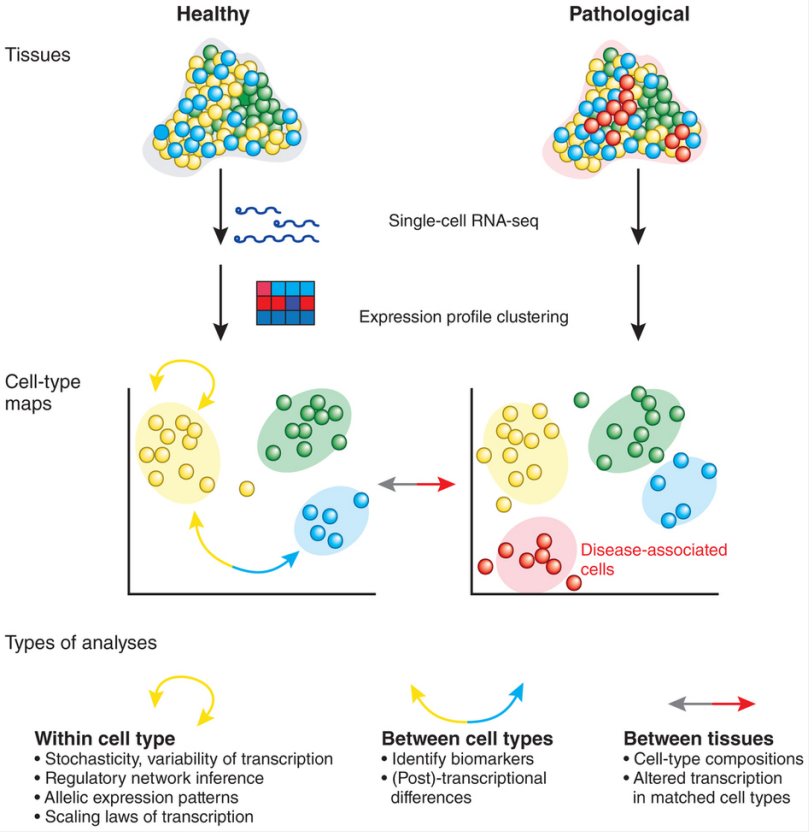


TECHNOLOGY HAS PROGRESSED RAPIDLY



CONSIDERATIONS FOR SINGLE CELL EXPERIMENTS

- Experimental design
- Cell Prep
- Library Prep
- Sequencing
- Bioinformatics
- Analysis



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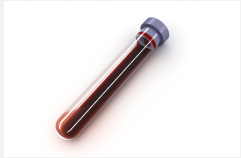
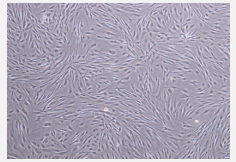
What is the source of cells?





- Blood, primary tissue, cell lines, preserved tissue?
- How much starting material is there?
- Human/non-human

- The critical point is **getting single cells**
- ***and keeping them that way***

- **Complete dissociation is required**
  - Cell Strainers
  - FACS

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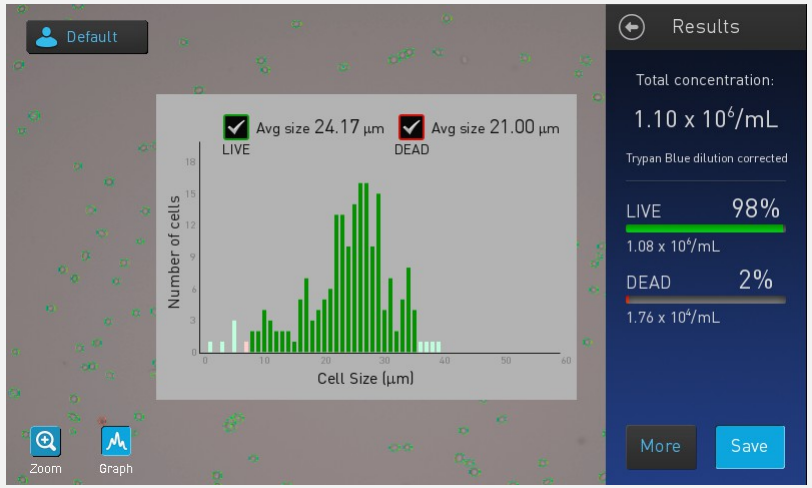
- How big are they?
- How sensitive are they?
  - Handling (e.g. pipetting force)
  - Enzymatic dissociation (timing and harshness)
  - FACS pressure/nozzle gauge
  - Post-dissociation viability?



- Will they lyse in the reaction buffer?
- Are they sticky/liable to clump?

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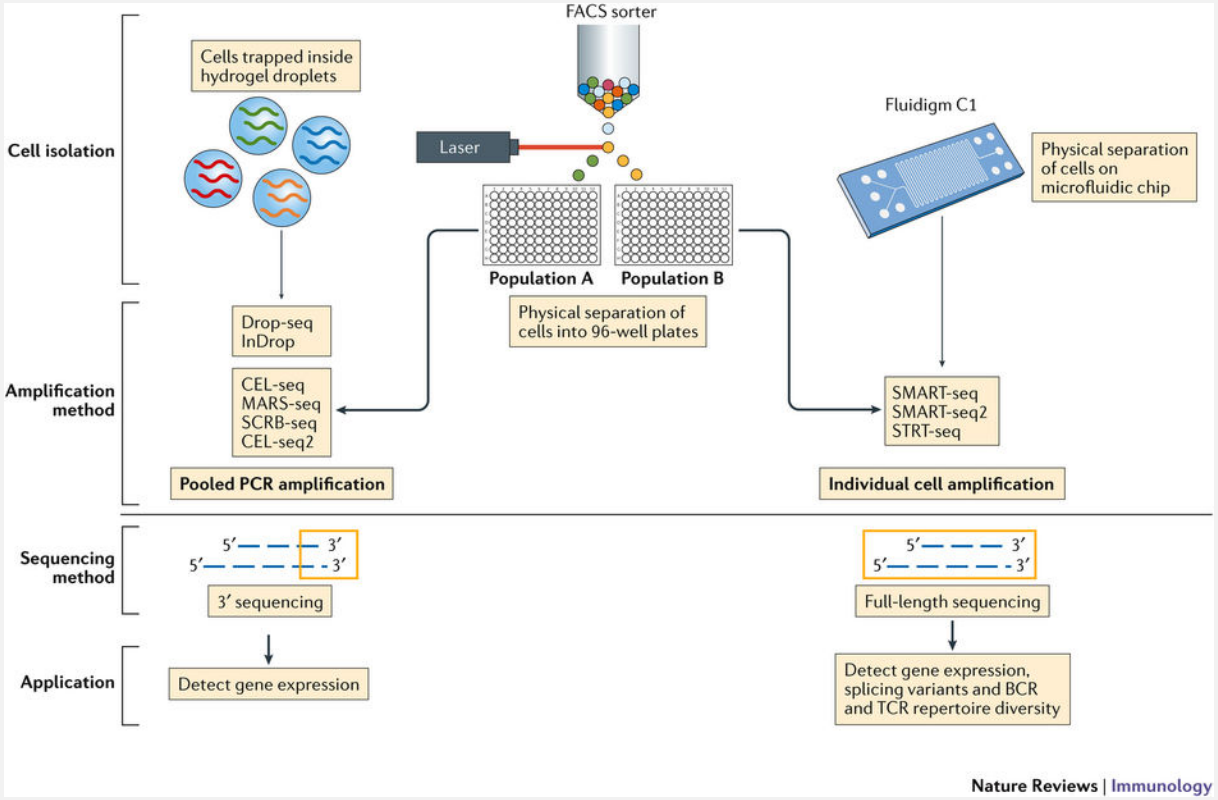

Papalexi & Satija NRI 2018

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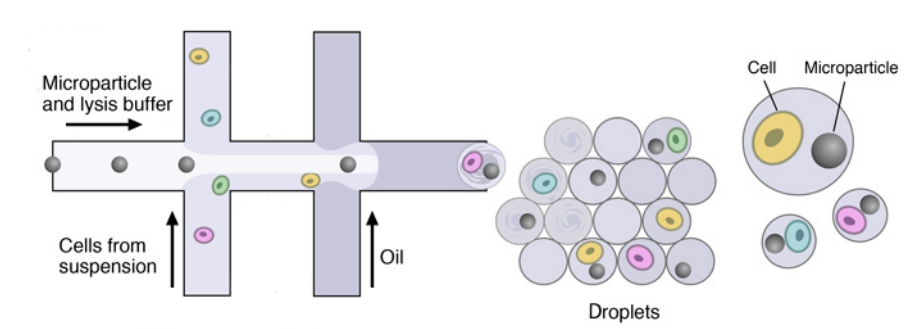
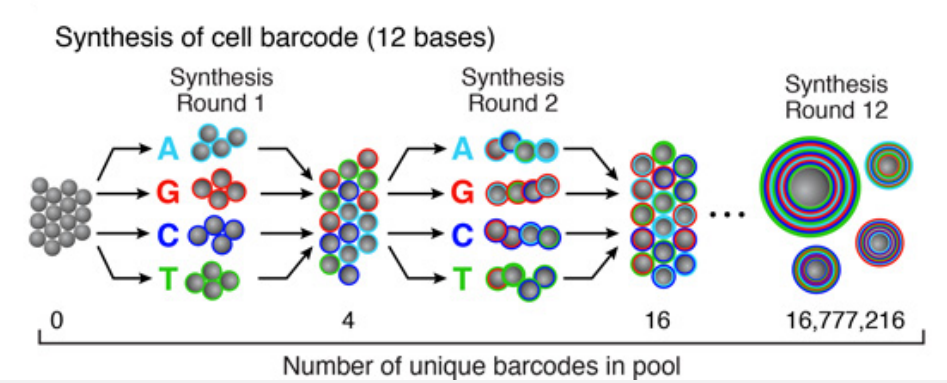
Microfluidic systems



Barcodes



CONSIDERATIONS FOR SINGLE CELL EXPERIMENTS

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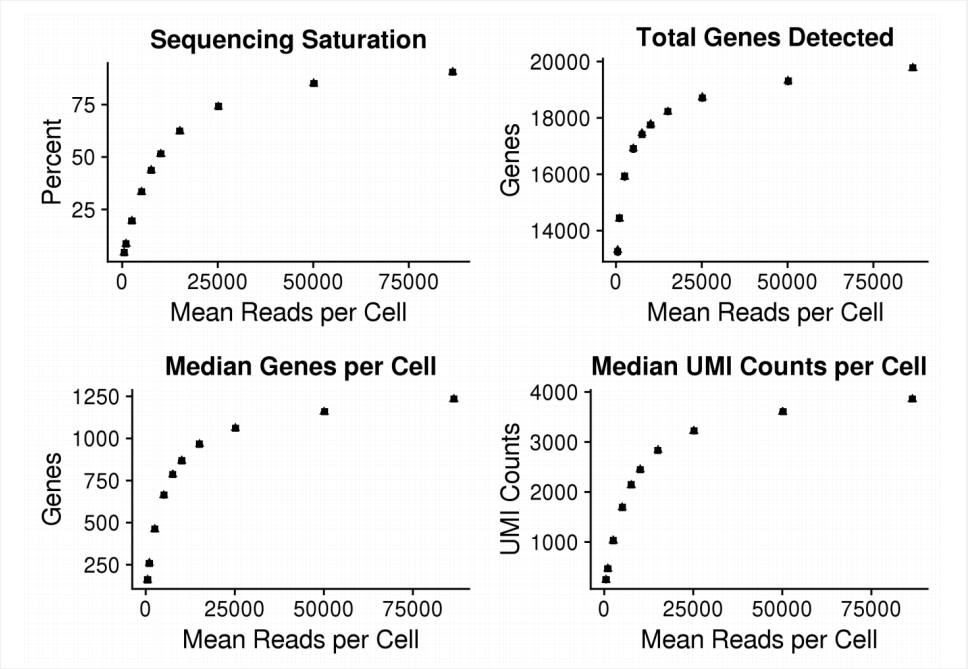
How deep should I sequence?



Costs

Per 10,000 reads* per cell

NextSeq	5-10 cents
HighSeq2500	10-15 cents
NovaSeq S2	3-5 cents
NovaSeq S4	2-4 cents

*With 3' scRNA-seq

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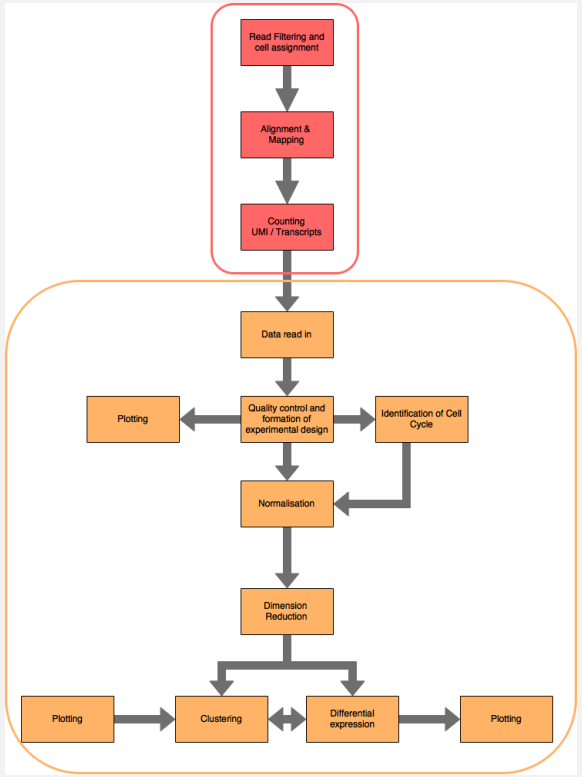

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- Experimental design
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- **Bioinformatics**
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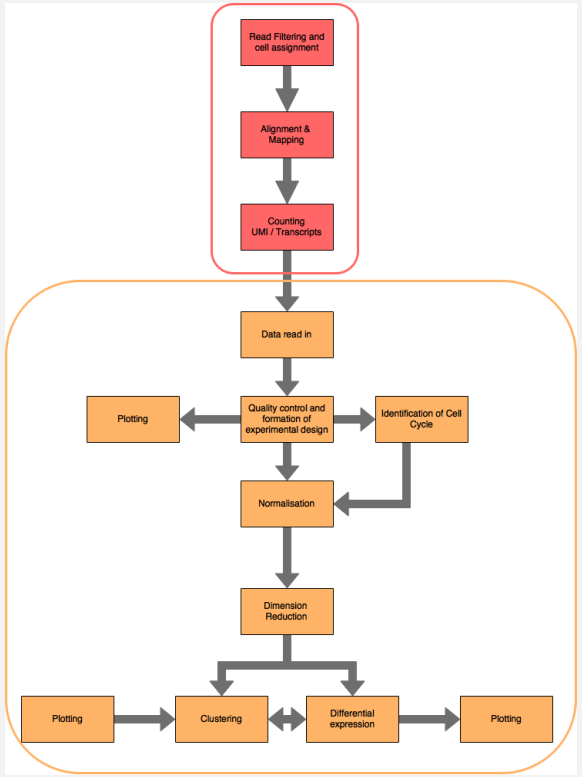

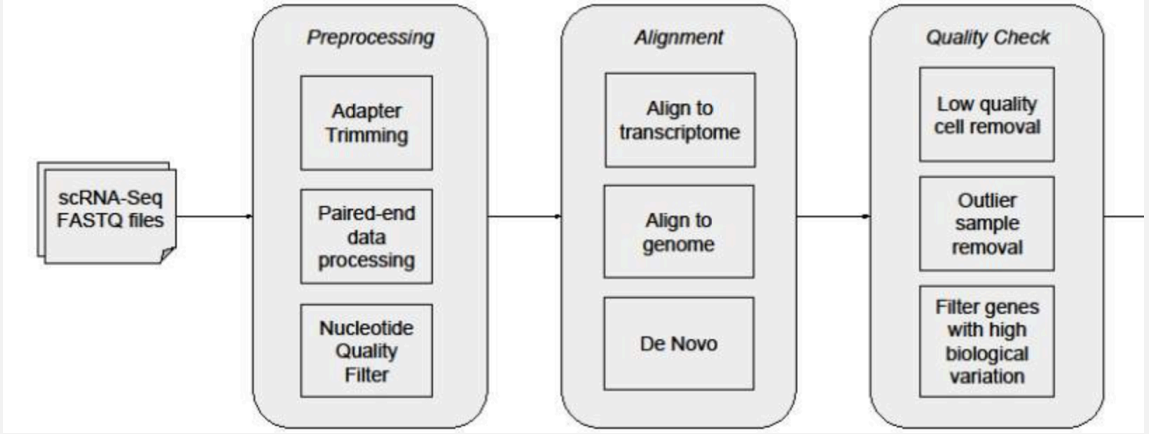

https://github.com/LuyiTian/scPipe

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- **Bioinformatics**
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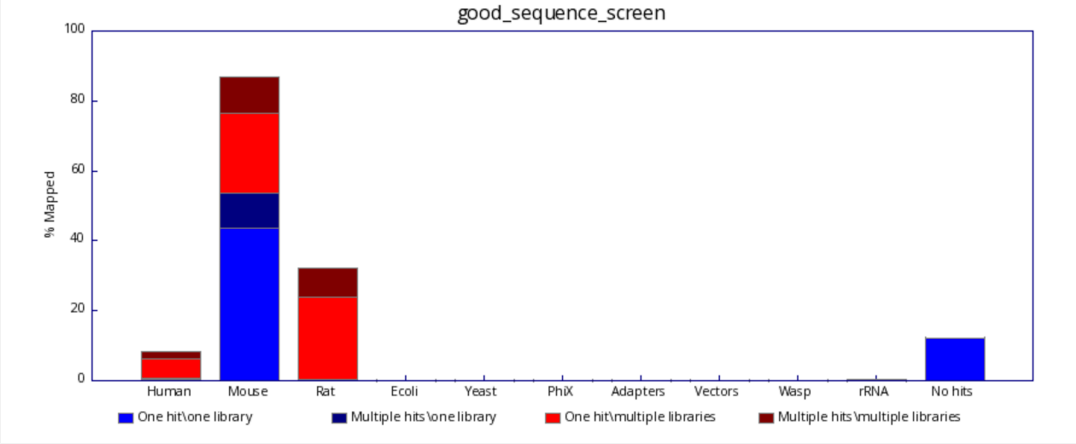
Contamination screen



FastQ Screen

Function	FastQ Screen allows you to screen a library of sequences in FastQ format against a set of sequence databases so you can see if the composition of the library matches with what you expect.
Language	Perl
Requirements	Unix-based operating system Bowtie or Bowtie2 or BWA gzip (optional) SamTools (optional) GD::Graph (optional) Bismark (bisulfite mapping only)
Code Maturity	Stable - has been working in production for some time
Code Released	Yes, under GPL v3 or later.
Initial Contact	Steven Wingett

Download Now

CONSIDERATIONS FOR SINGLE CELL EXPERIMENTS

- Experimental design
- Cell Prep
- Library Prep
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Bulk

← Samples →

↓ Genes→									
85	97	58	52	48	84	79	62	61	61
116	45	74	95	57	91	66	57	57	68
50	76	61	64	65	70	78	95	52	89
15	67	67	67	68	76	36	60	92	121
61	116	84	85	43	71	89	81	62	40
105	65	76	48	89	78	88	67	85	42
61	84	70	97	49	66	77	40	61	92
47	75	58	62	89	40	50	100	66	75
62	87	61	85	86	56	49	65	78	95
102	86	60	46	75	66	31	88	41	99

scRNA

8	0	0	0	0	0	7	6	0	0
0	0	0	5	0	0	0	0	0	0
0	7	0	0	11	0	0	0	0	0
5	0	0	0	8	11	0	7	8	0
4	9	5	0	0	0	0	0	0	0
10	3	0	0	0	5	0	0	0	8
0	0	0	0	0	0	0	10	9	8
6	6	0	7	0	0	3	0	0	0
0	0	0	0	0	1	0	5	5	0
0	0	0	0	7	6	0	0	0	0

Plus you will get lots of it!

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- Experimental design
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Bulk

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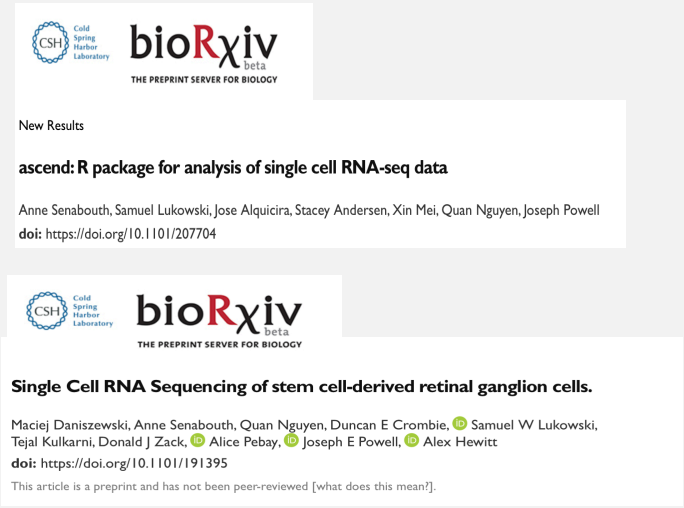
scRNA

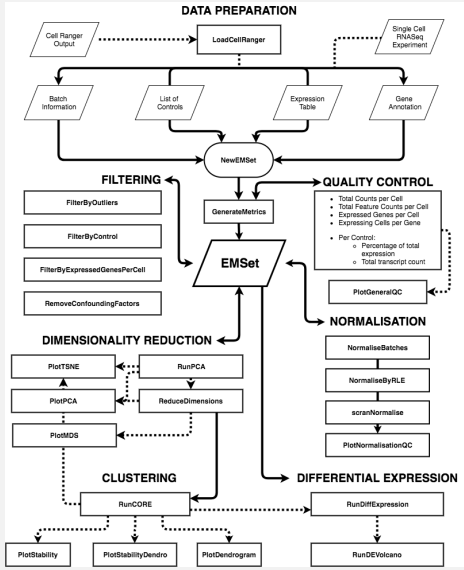
8	0	0	0	0	0	7	6	0	0
0	0	0	5	0	0	0	0	0	0
0	7	0	0	11	0	0	0	0	0
5	0	0	0	8	11	0	7	8	0
4	9	5	0	0	0	0	0	0	0
10	3	0	0	0	5	0	0	0	8
0	0	0	0	0	0	0	10	9	8
6	6	0	7	0	0	3	0	0	0
0	0	0	0	0	1	0	5	5	0
0	0	0	0	7	6	0	0	0	0

Plus you will get lots of it!

ascend::a flexible integrated software package for single cell analysis

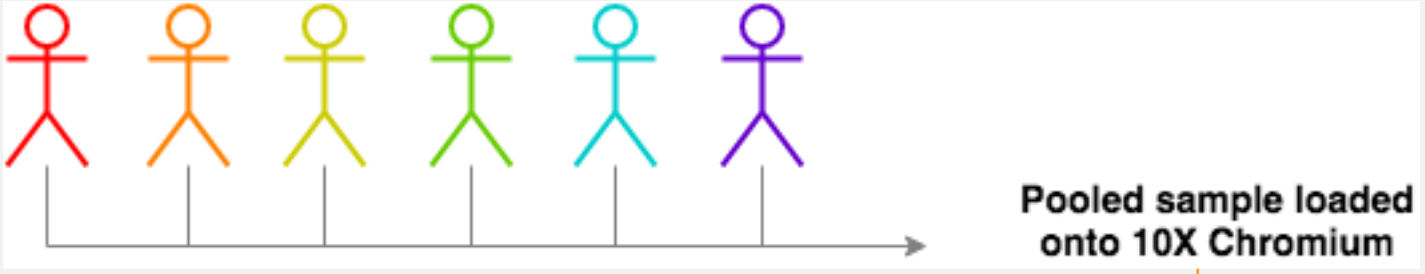
https://github.com/IMB-Computational-Genomics-Lab/ascend

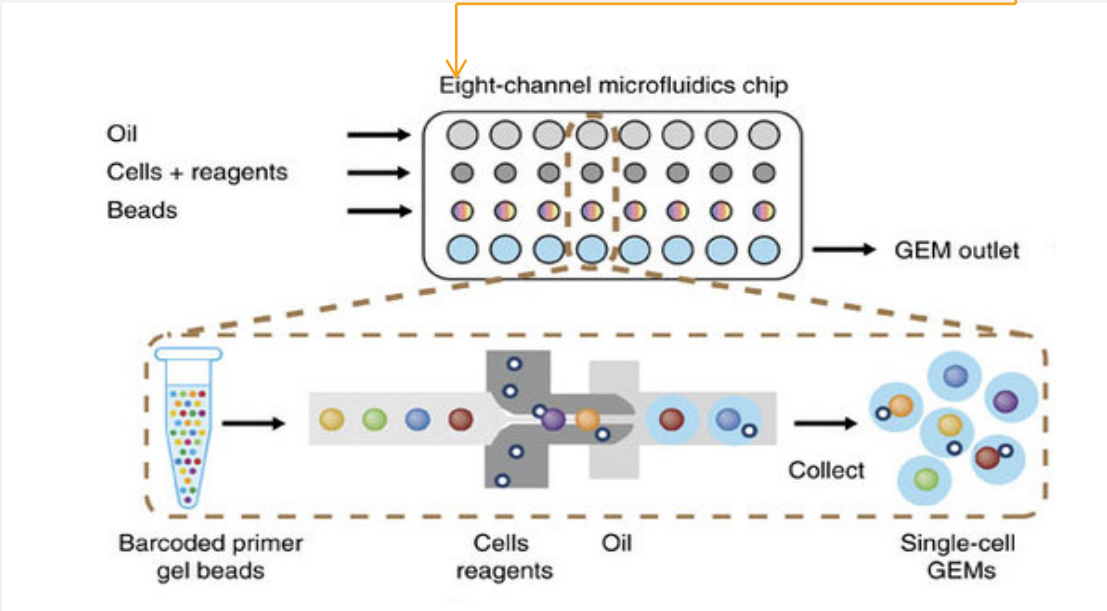




SINGLE CELL MEETS POPULATION GENETICS

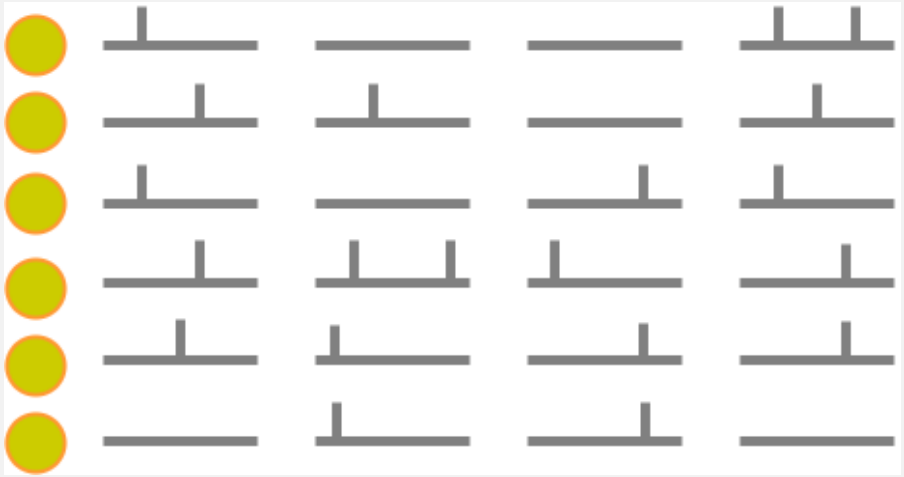
MULTIPLEXING SAMPLES FOR SINGLE CELL LIBRARY PREP



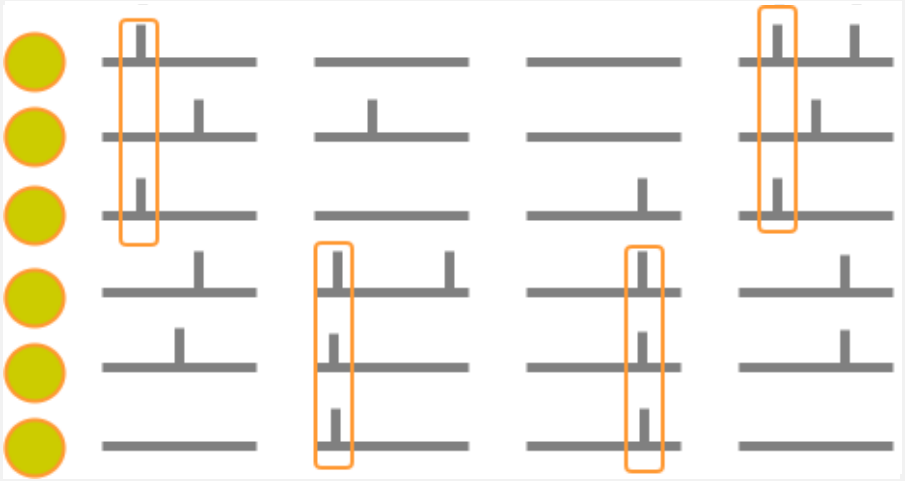


MULTIPLEXING SAMPLES FOR SINGLE CELL LIBRARY PREP

Call SNPs from the 3’ reads



MULTIPLEXING SAMPLES FOR SINGLE CELL LIBRARY PREP



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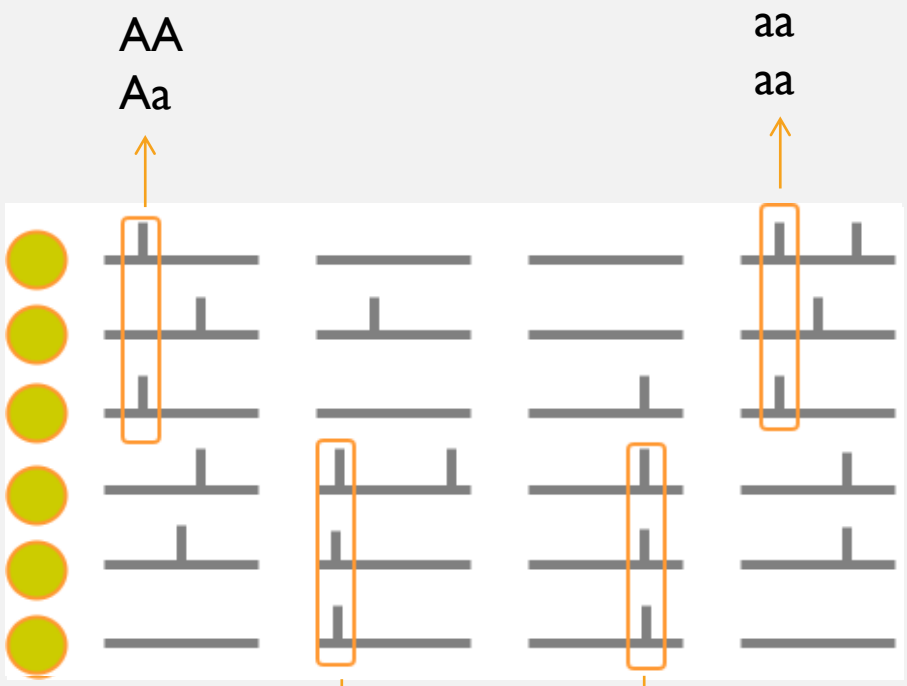

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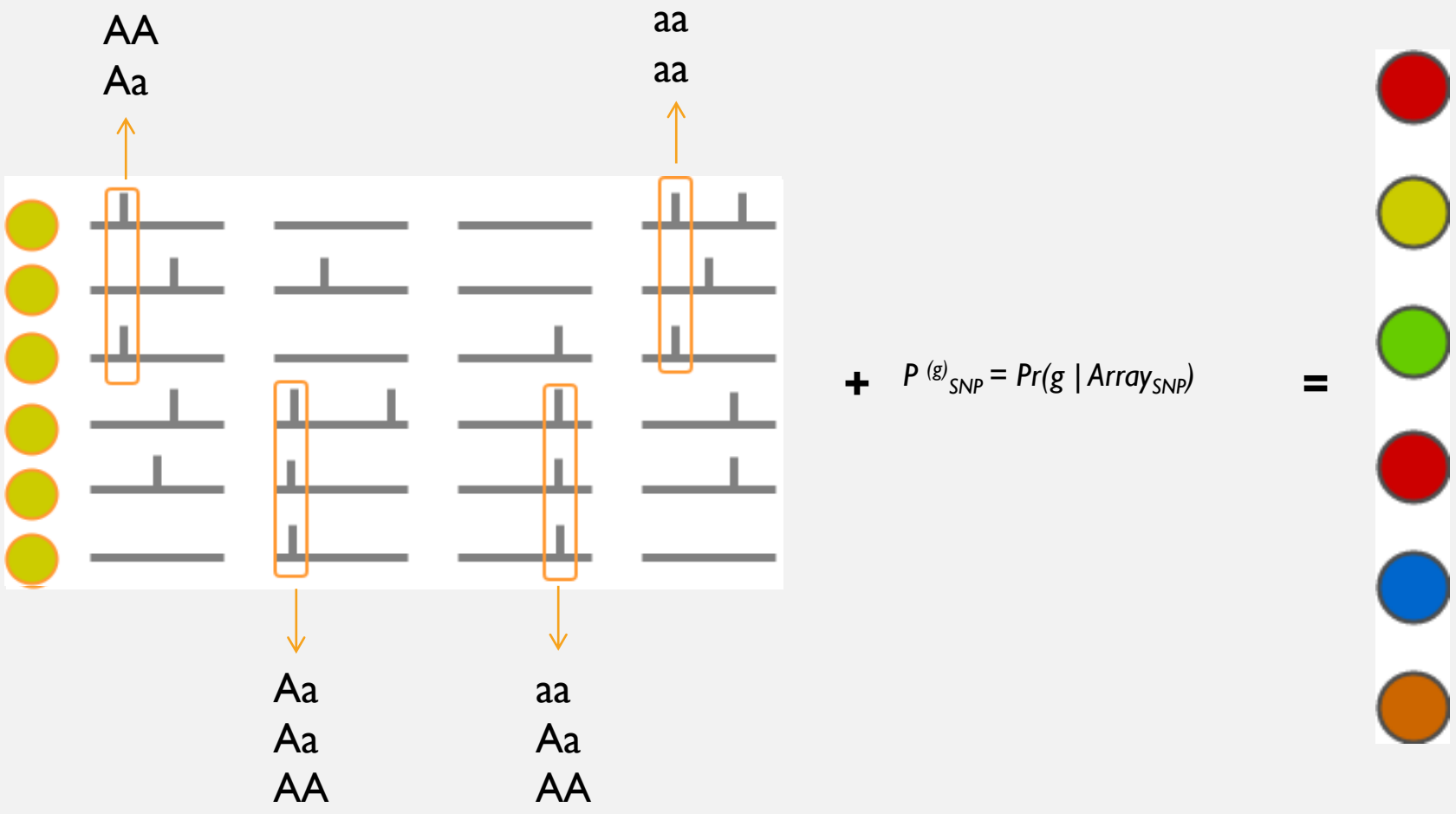

THANK YOU

- Email me: j.powell@garvan.org.au
- Twitter: @JP_Garvan