

Controls & Standards for High-Throughput Single B Cell Sequencing and Functional Screening

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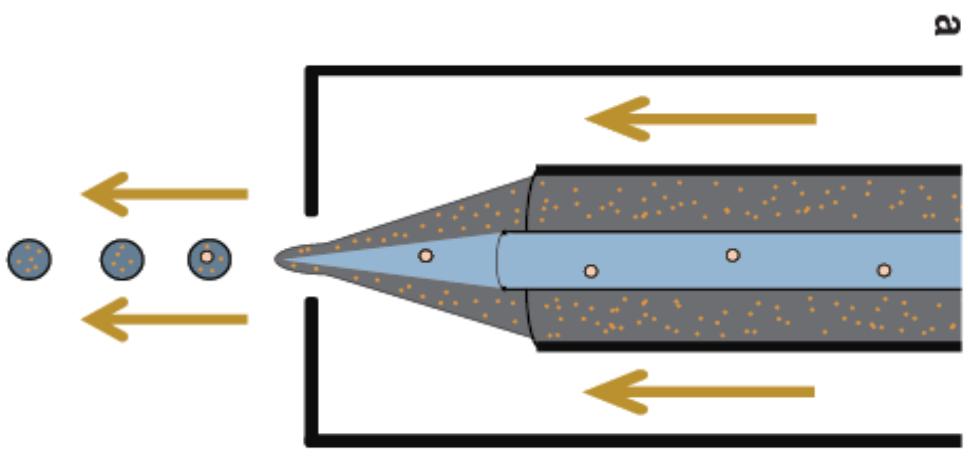
Pharmaceutical Chemistry, Chemical Engineering
The University of Kansas



What did you make?

paired antibody sequence → antibody function

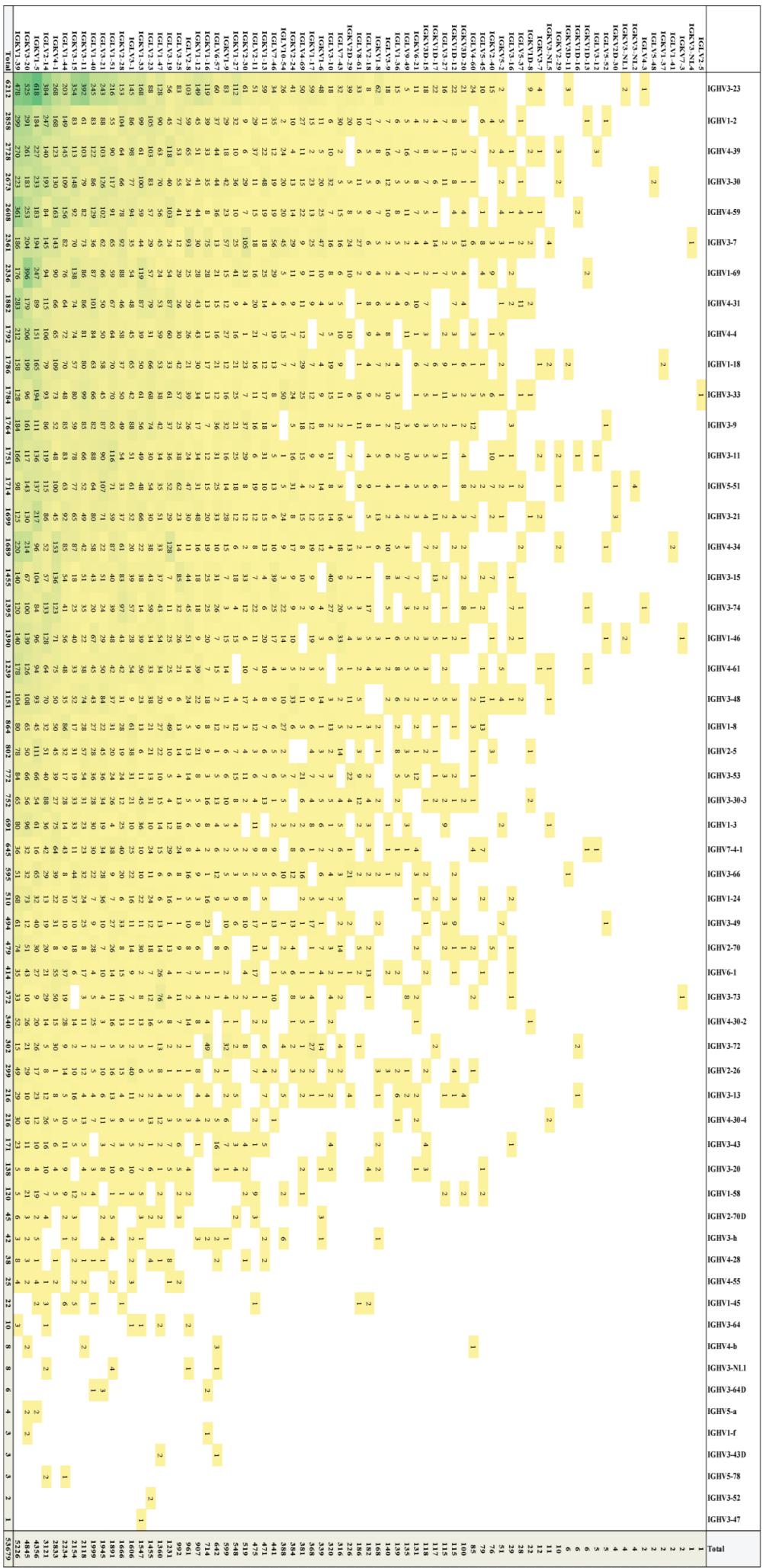
High-throughput Paired Heavy:Light Sequencing



Single-cell Emulsification

DeKosky et al., *Nat Biotechnol*, 2013
DeKosky et al., *Nat Medicine*, 2015
McDaniel & DeKosky et al., *Nat Prot*, 2016
Lagerman et al., *J Biosci Bioeng*, 2019

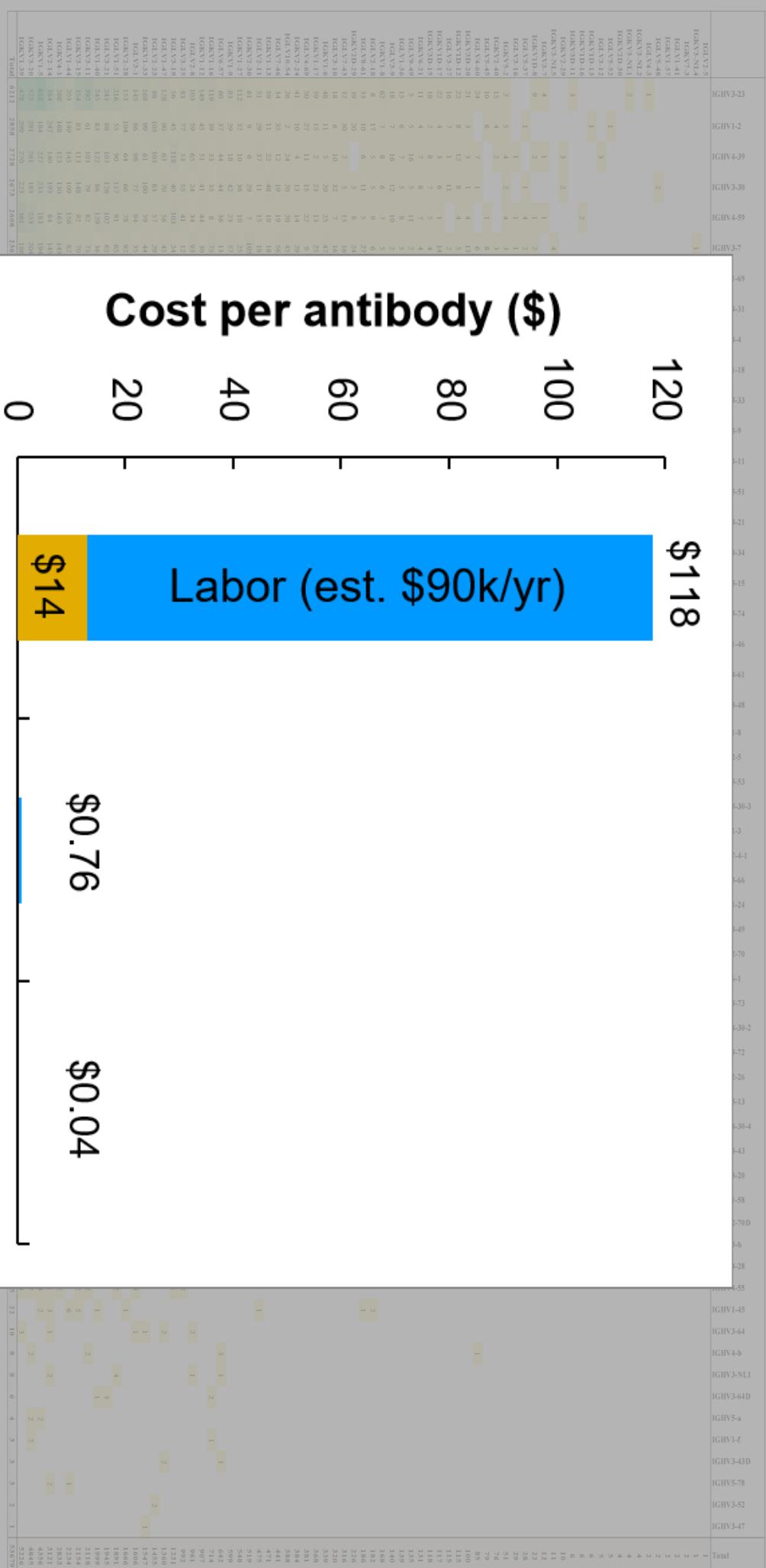
Light V-Genes



Heavy V-Genes

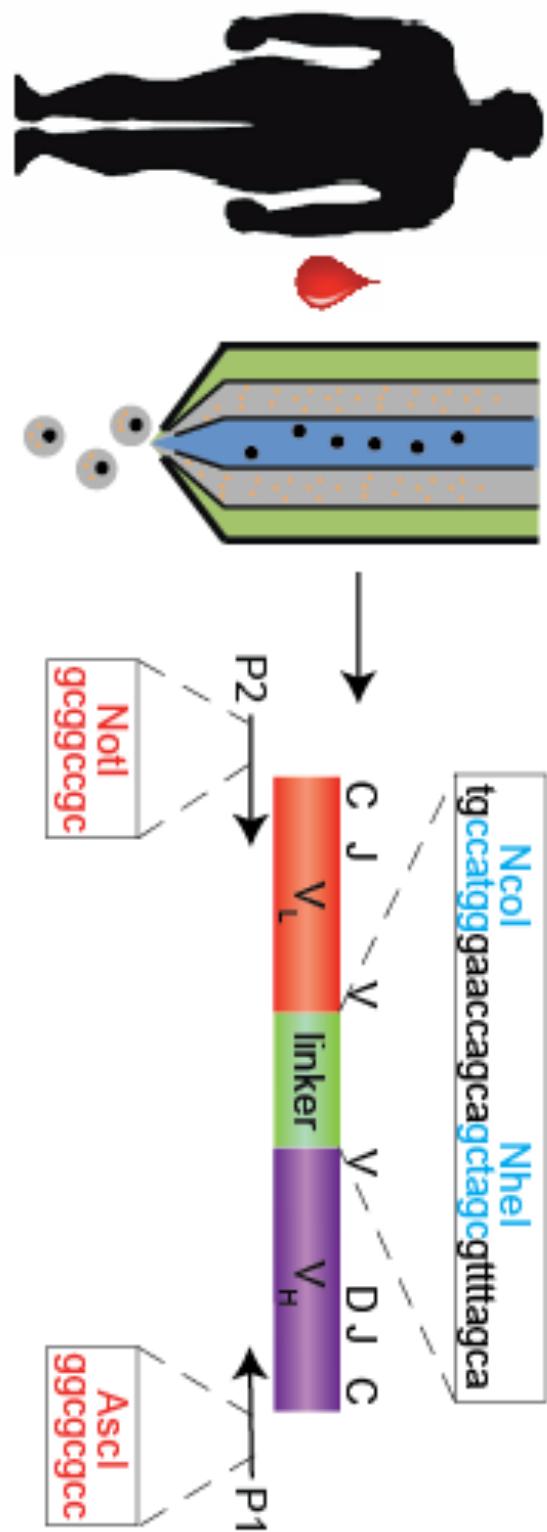
- 129,000 distinct VH:VL clusters recovered in a single day (96% H3 clustering)
- 36,468 cross-confirmed VH:VL pairs / 37,995 VH in both replicates → 98.0% VH:VL pairing precision

Light V-Genes

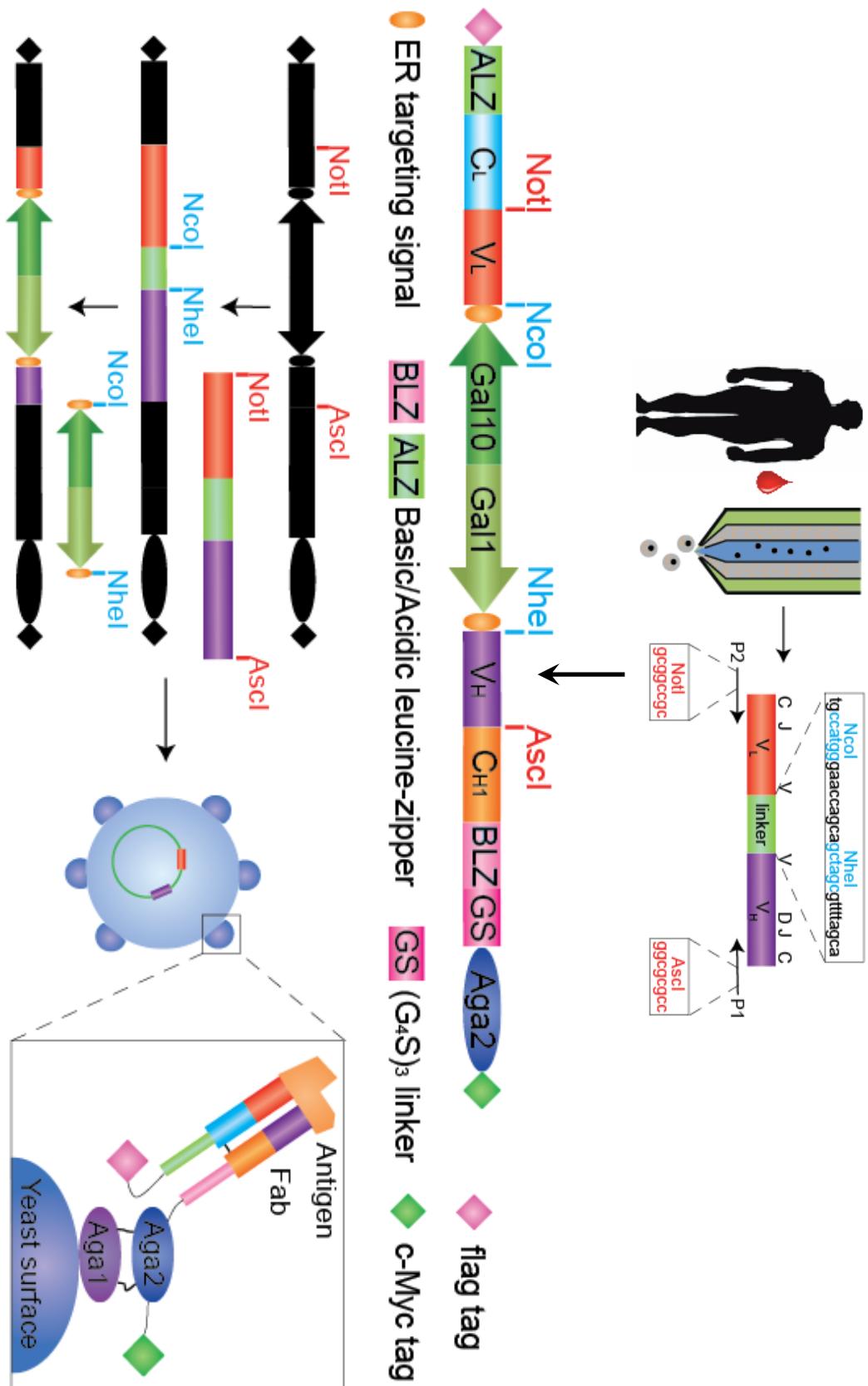


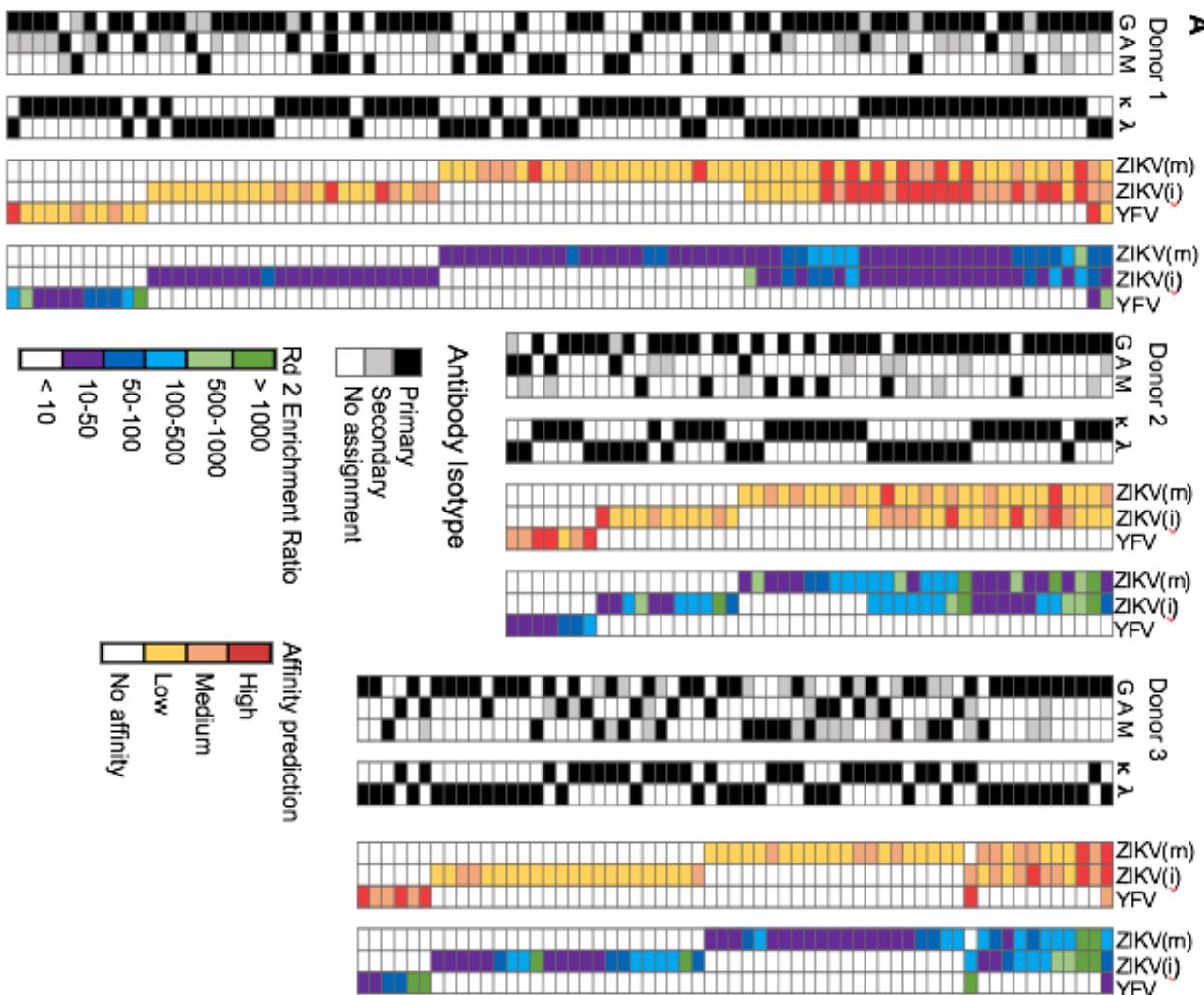
- 129,000+ unique VH:VL pairings
- 36,468 cross-confirmed VH:VL pairs / 37,995 VH in both replicates
→ 98.0% VH:VL pairing precision

Functional Antibody Repertoire Analysis

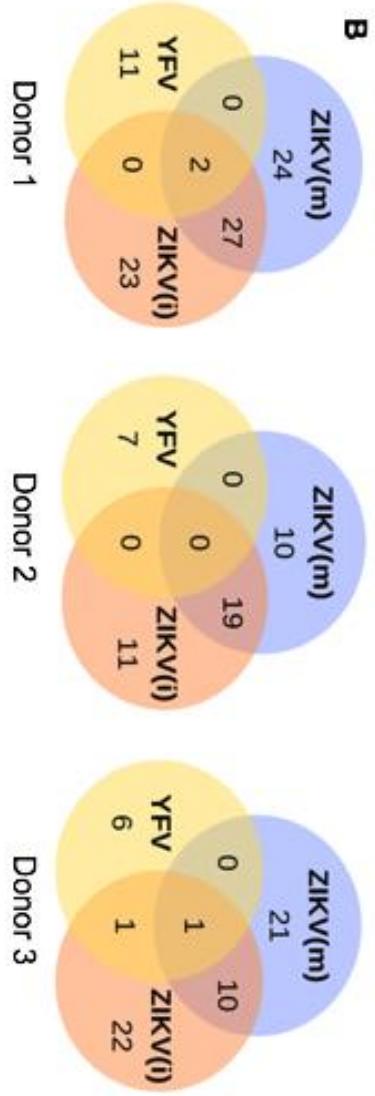


Functional Antibody Repertoire Analysis



A

Anti-ZIKV mAb Profiling



195 native human antibody lineages mapped for affinity and flavivirus Ag specificity.

>90% functional accuracy predictions in 24-mAb panel

Potent neutralizers (<2 ng/mL IC₅₀)

Method of design

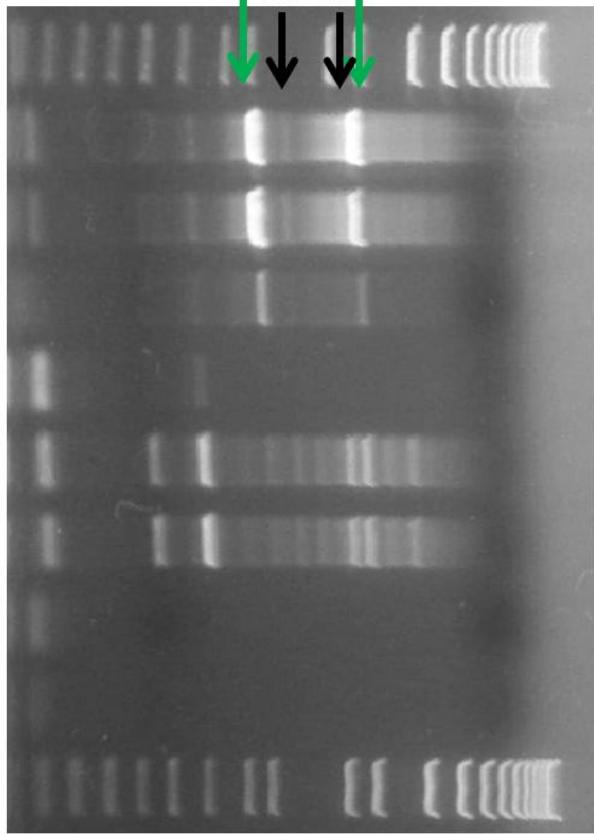
one standard after another

Standards for Single-Cell H:L BCR Seq

1. Mix two immortalized cell lines together with known VH:VL
2. Spike an immortalized cell line(s) into an unknown human immune repertoire
3. Blinded control with single-cell RT-PCR from a matched patient
4. Mix a panel of known cells together (10-ish)
5. Compare two different biological replicates from the same individual

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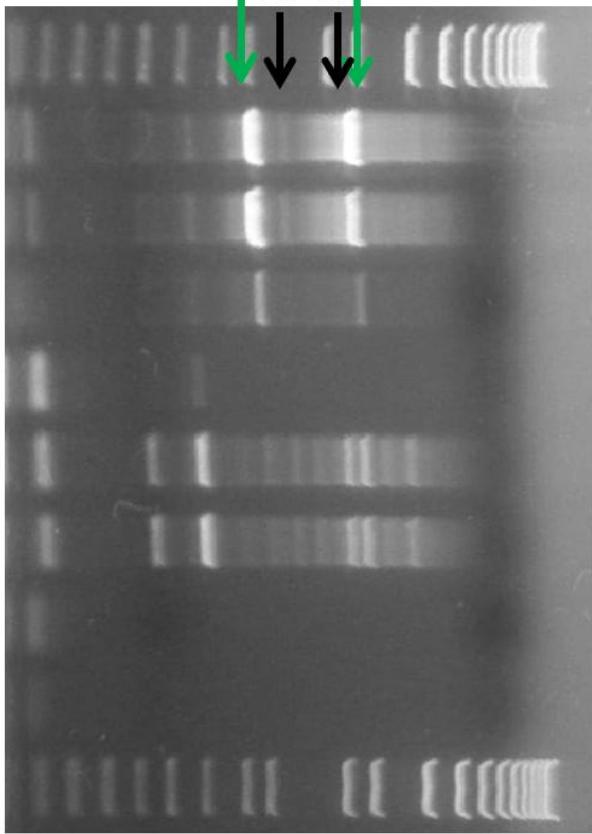
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12/16/2011

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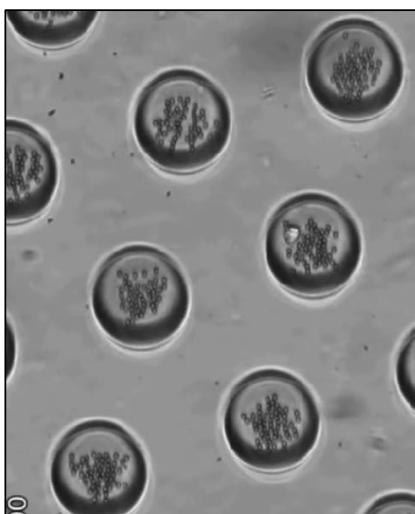
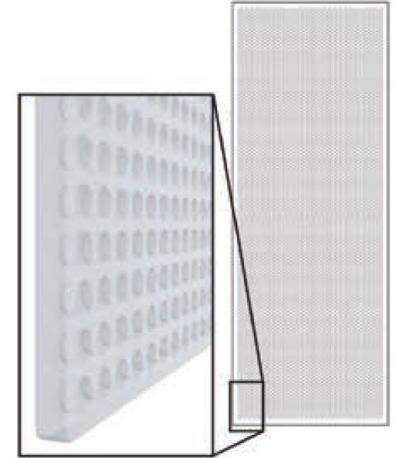


Similar: mix human/mouse lines in Drop-seq

12/16/2011

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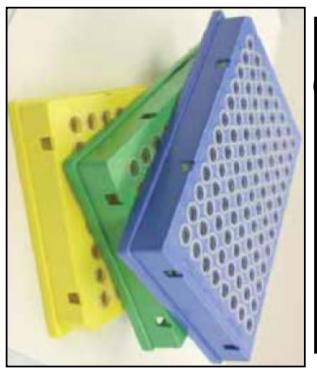
GGGTGGGGAGGCGACTT
GGGTATCGGTTCGGAG
TGCTATCGGTATAAA
TACTCTAACCGGTATAAT
GGAGATAAGCGGTATAAT
GGGIGGGGAGGGCGACTT
GGGTATCGGTTCGGAG
TGCTATCGGTATAAA
TACTCTAACCGGTATAAT
GGAGATAAGCGGTATAAT
TGCTATCGGTTCGGAG
TACTCTAACCGGTATAAT
GGAGATAAGCGGTATAAT

Known cell lines → sequenced correctly?

Standards for Single-Cell H:L BCR Seq

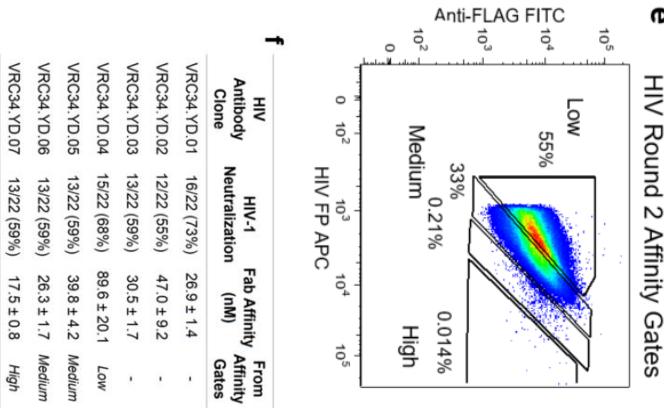
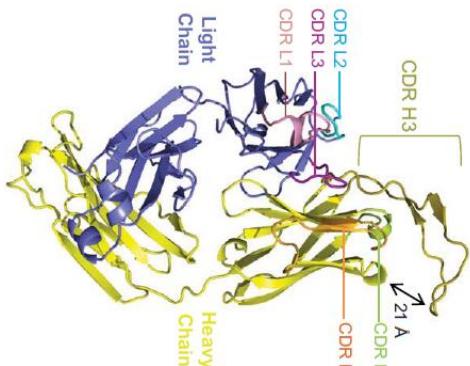
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Single-cell RT-PCR



NGS

Sequence data from NGS showing reads from a single-cell RT-PCR sample. The sequence is: GGGTGGCGAGCCGACTT, GGCTATCGGTATAA, TGCATACGGTATAA, TACTCIAAGCGTATAAT, GGAGATAAACCGTATAAT, GGGGGGGAGCGACTT, TCTATCGGTTCGGAG, TACTCIAAGCGTATAAT, CGAGATAAACCGTATAAT, TGCATACGGTTCGGAG, TACTCIAAGCGTATAAT, GGAGATAAACCGTATAAT.



Agree?

DeKosky et al, *Nature Biotechnol* 2013

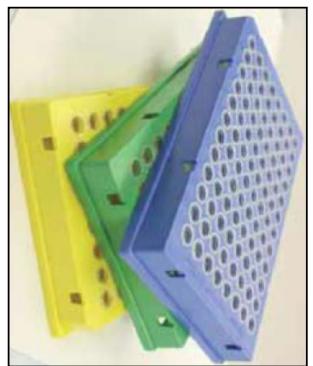
Doria-Rose et al, *Nature* 2014

Wang & Dekosky et al, *Nature Biotechnol* 2018

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3. **Blinded control with single-cell RT-PCR from a matched patient**
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Single-cell RT-PCR



NGS

```
GGGTGGCGAGCCGACTT  
GGCTATCGGTTCGGAG  
TGCTATAGCGTTATAA  
TACTCTAACGGTATAT  
GAAGATAAACGGTATAT  
GGGGGGGAGCGACTT  
GGGGGGGAGCGACTT  
TCTCTCGGTTCGGAG  
TACTCTAACGGTATAT  
GGAGATAAGGGTATAT  
TGCTATCGGTTCGGAG  
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		Heavy Chain											
		1H	2H	3H	4H	5H	6H	7H	8H	9H	10H	11H	Total
		1L	1,842	4	20	13	18	16	39	20	49	6	2,031
		2L	0	4,916	34	31	59	41	102	127	146	28	5,492
		3L	0	2	6,251	9	38	25	116	60	118	13	6,634
		4L	21	75	14,592	81	158	348	189	397	75	51	16,014
		5L	5	15	97	41	16,204	99	192	231	277	86	17,266
		6L	2	12	92	37	64	16,427	358	180	404	62	17,661
		7L	9	13	218	72	112	180	203	1,320	78	45	23,565
		8L	4	39	85	71	242	145	365	32,393	506	79	34,001
		9L	4	29	182	105	116	186	1,335	323	35,391	109	46
		10L	12	24	944	189	1,597	1,080	3,519	1,898	4,291	8,535	37,826
		11L	32	66	1,153	272	1,258	1,655	6,405	6,567	6,185	555	22,187
Total		1,931	5,147	9,151	15,432	19,789	20,012	34,094	42,191	49,084	9,626	14,494	38,274
													220,951

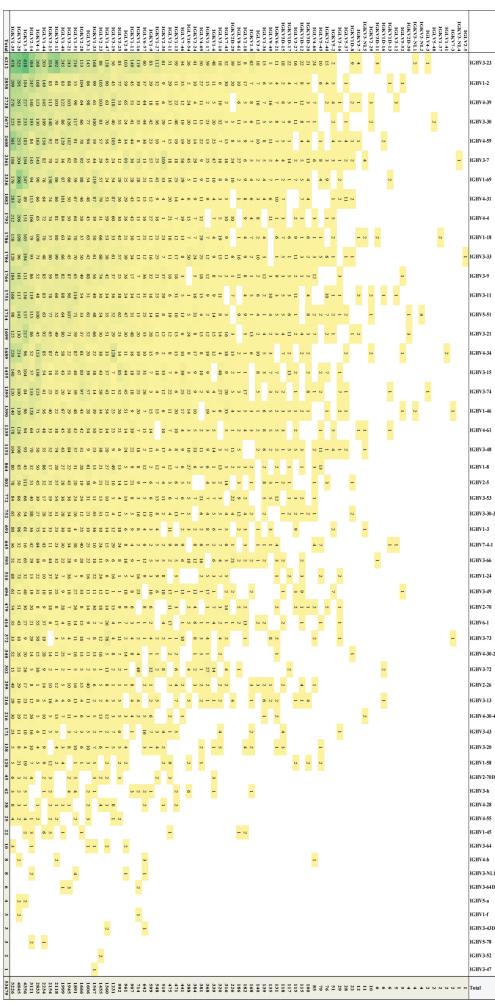
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$$P_{Land2} = \frac{TP_{Land2}}{(TP_{Land2} + FP_{Lor2})} \quad P_{Land2} = P_1 \times P_2 = P^2$$

$$P_{Land2} = P^2 \frac{TP_{Land2}}{(TP_{Land2} + FP_{Lor2})}$$

$$P = \sqrt{\frac{TP_{Land2}}{TP_{Land2} + FP_{Lor2}}}$$



Dekosky et al, *Nature Medicine* 2014

Lagerman & López et al, *J Biosci Bioeng* 2019

Oct 2020 Case Study

1. Mix two immortalized cell lines together with known H:L
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Experiment A

Experienced lab staff

Standard nozzle emulsification
95,000 BCR clusters/sample (2 samples)
97%/98% pairing precision

Experiment B

New lab staff

Microfluidics #1 (small droplets)
3,900 clusters
96% pairing precision

Microfluidics #2 (larger droplets)

17,000 clusters
74% pairing precision

October 2020

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Experiment B

New lab staff

Microfluidics #1 (small droplets)
3,900 clusters
96% pairing precision

→Conducted post-hoc
estimate of # cells per
droplet.

Small droplets – **9%**
occupancy, perfect.

Microfluidics #2 (larger droplets)
17,000 clusters
74% pairing precision

Large droplets – **36%**
occupancy, 50/50 split

October 2020

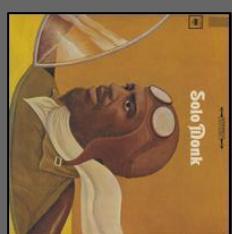
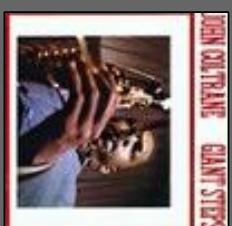
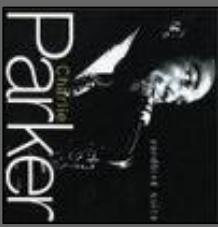
conclusions / take-home

- 1. new methods development starts and ends with standards**
- 2. infectious disease is better for early technologies than autoimmune diseases and cancer (difficult models)**

conclusions / take-home

1. new methods development starts and ends with standards
2. infectious disease is better for early technologies than autoimmune diseases and cancer (difficult models)
3. standards are fun! you learn so much!!

we love the standards



University of Kansas

Tiffany Nguyen	Bharat Madan	George Georgiou
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Jacy Wolfe	Amy Laflin	
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thank you!

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