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

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What did you make?
High-throughput Paired Heavy:Light Sequencing

- Single cell Emulsification
  - Lagerman et al., J Biosci Bioeng. 2019
  - McDaniel & DeKosky et al., Nat Prot. 2013
  - DeKosky et al., Nat Medicine. 2015
Heavy V Genes

Light 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

DeKosky et al., Nat Medicine, 2014
• 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
Functional Antibody Repertoire Analysis

Wang & DeKosky et al., Nature Biotechnology, 2018
Potent neutralizers (>2 ng/mL IC50) mapped for flavivirus Ag specificity. Native human antibody lineages mapped for affinity and functional accuracy predictions in 24-mAb panel. >90% functional accuracy. Fahad et al., In 2nd Revision.

Anti-ZIKV mAb Profiling
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
1. Mix two immortalized cell lines together with known H:L BCR Seq

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DeKosky et al., Nature Biotechnol 2013
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Similar: mix human/mouse lines in Drop-seq

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DeKosky et al. Nature Biotechnol 2013

Known cell lines sequenced correctly?
1. Mix two immortalized cell lines together with known H:L
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 (10-15)
5. Compare two different biological replicates from the same individual

Standards for Single-Cell BCR Seq

Agree?
Standards for Single-Cell H:L BCR Seq

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Wang & DeKosky et al, Nature Biotechnol 2013
DeKosky et al, Nature Biotechnol 2014
Doria-Rose et al, Nature 2014
Wang & DeKosky et al, Nature Biotechnol 2018
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DeKosky et al, Nature Medicine 2014
Lagerman & Lóp et al, J Biosci Bioeng 2019
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Microfluidics #2 (larger droplets)
17,000 clusters
74% pairing precision

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

New Lab Staff
Experiment A

95,000 BCR clusters/sample (2 samples)
77%/98% pairing precision

Standard nozzle emulsification

New Lab Staff
Experiment B

Microfluidics #1
3,900 clusters
96% pairing precision

Microfluidics #2
17,000 clusters
74% pairing precision
1. Mix two immortalized cell lines together with known H:L
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 (10-15h)
5. Conducted post-hoc estimate of # cells per droplet

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Experienced lab staff</th>
<th>New lab staff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microfluidics #1 (small droplets)</td>
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<td>95,000 BCR clusters/sample</td>
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</tr>
<tr>
<td>Microfluidics #2 (larger droplets)</td>
<td>97%/98% pairing precision</td>
<td>96% pairing precision</td>
</tr>
<tr>
<td>47,000 clusters</td>
<td>95,000 BCR clusters/sample</td>
<td></td>
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</tbody>
</table>

Conclusion:
- Large droplets – 36%
- Small droplets – 9%

Conducted post-hoc estimate of # cells per droplet.
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
thank you!
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