Identifying αβ T cell clones via pooling and b-matching

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Itinerary

1. $\alpha \beta$ T cells and T cell receptors (TCRs)
   1. What are they?
   2. How are they formed?

2. Identifying TCRs

3. Reconstructing clones
   1. Our approach
   2. Results and future work
αβ T cell
How are TCRs formed?

Multiple rearrangements?

• Up to 30% of T cells contain two in-frame α-recombinants (dual α)
  • Only 10% of T cells express both

• Up to 2-10% contain two in-frame β-recombinants (dual β)
  • Only 1-3% of T cells express both

High-throughput pairing of T cell receptor α and β sequences

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Identifying TCRs: PairSEQ

T cells are distributed across a 96-well plate and assigned well-specific barcodes.

Barcoded cDNAs are sequenced together.

TCR pairs are identified by finding sequences that occupy the same wells.

Benefits

1. Can identify hundreds of thousands of TCRs
2. Uses existing high-throughput sequencing technologies

1. Is it possible to identify entire clones, not just pairs?
2. Can clone recognition be done efficiently?
RESEARCH ARTICLE

Identifying T Cell Receptors from High-Throughput Sequencing: Dealing with Promiscuity in TCRα and TCRβ Pairing

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T cells are sampled onto 96-well plates at 10-300 cells/well

cDNA libraries are created from each well with RT-PCR

Unpaired CDR3α and CDR3β are sequenced in each well

A resampling strategy is used to obtain a list of possible TCR pairs by repeatedly performing steps (i), (ii), and (iii)

(i) A subset of wells is randomly selected for steps (ii) and (iii)

(ii) Association scores are calculated for every α and β chain across all wells in the subset

\[ S_{ij} = \sum_{k=1}^{W} \left( \frac{\delta_k^{ij}}{c_{\alpha}^{ij}} + \frac{\delta_k^{ij}}{c_{\beta}^{ij}} \right) \]

(iii) Scores are used to select likely αβ pairs within each well

<table>
<thead>
<tr>
<th>( \alpha_1 )</th>
<th>( \alpha_2 )</th>
<th>( \alpha_3 )</th>
<th>( \alpha_4 )</th>
<th>( \alpha_5 )</th>
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<tr>
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<td>4.3</td>
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<td>( \beta_2 )</td>
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<td>0.2</td>
<td>1.0</td>
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<td>( \beta_3 )</td>
<td>1.0</td>
<td>0.2</td>
<td>60.2</td>
<td>0.3</td>
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<td>( \beta_4 )</td>
<td>3.2</td>
<td>30.1</td>
<td>0.1</td>
<td>0.4</td>
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Pairs from Step D present in more than a threshold proportion of replicates are candidate TCRs

Clonal frequencies are estimated with maximum-likelihood and used to distinguish β-sharing and dual TCRs

The output is a list of single and dual TCR clones with their respective clonal frequencies

Observations

1. Many sequences appear in the same subset of wells.
   - These aren’t uniquely identifiable!
   - Don’t consider sequences directly, match similar well subsets instead

2. Modeling dual clones is easy!
   - All recombinants should appear together most of the time
   - Known generative probabilities
   - Up to two recombinants of each type

3. Modeling chain-sharing is difficult!
   - Recombinants do not appear together most of the time
   - Unknown generative probabilities
   - Amount of sharing unknown

TCR pairs are identified by finding sequences that occupy the same wells.
Our approach
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4. Identify a b-matching.
5. Output b-matching edges connecting uniquely identifiable well sets.
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4. Repeat for alphas.
5. Run b-matching procedure as before.
ALPHABETR Simulator

• Models PairSeq experiments
  • Number of clones, ~1000
  • Number of wells, 96 and 480
  • Dual alpha and dual beta rates, 0.3 and 0.06
  • Proportion of chains shared
• Has a few shortcomings:
  • Does not model T cell maturation, e.g. dual beta vs. dual alpha reuse
  • Is not real data.
Results - 480 wells

Clone identification (W=480, with sharing)

Pair identification (W=480, with sharing)

Clone identification (W=480, without sharing)

Pair identification (W=480, without sharing)
Results - 96 wells

Clone identification (W=96, with sharing)

Pair identification (W=96, with sharing)

Clone identification (W=96, without sharing)

Pair identification (W=96, without sharing)
Results - Dual alphas

Dual alpha identification (W=480, with sharing)

Dual alpha identification (W=480, without sharing)

Dual alpha identification (W=96, with sharing)

Dual alpha identification (W=96, without sharing)
Results - Running time

Running time (W=480)

Running time (W=96)
### Results - b-matching dual identification

#### W = 480

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<tr>
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<tbody>
<tr>
<td><strong>PPV</strong></td>
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<tr>
<td>Dual alpha</td>
<td>0.9189</td>
<td>0.9985</td>
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<td>Dual beta</td>
<td>0.7236</td>
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<td>Dual dual</td>
<td>0.8840</td>
<td>0.9624</td>
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<tr>
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#### W = 96

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<td>0.9363</td>
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<tr>
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<tr>
<td>Dual dual</td>
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<tbody>
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<td></td>
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<tr>
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<td>0.0676</td>
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</tr>
<tr>
<td>Dual dual</td>
<td>0.0219</td>
<td>0.1250</td>
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Future work

1. Incorporate chain-sharing into b-matching model.
2. Run b-matching procedure on real PairSeq data.
3. Generate and analyze larger datasets using ALPHABETR simulator.
4. Implement original PairSeq binomial model to analyze datasets.
Questions?