

Statistical analysis of an antibody repertoire

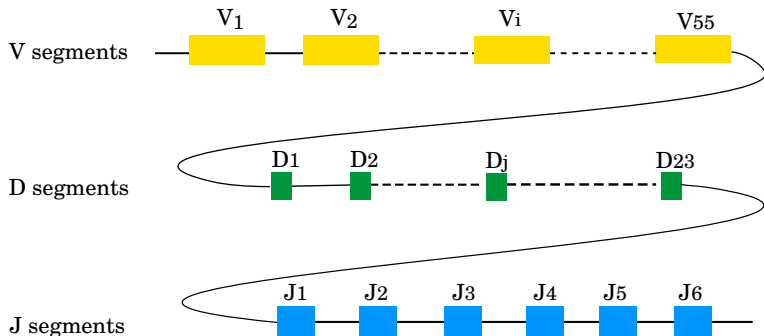
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September 13, 2015

- 1 Introduction
- 2 Cleavage and specific gene segments
- 3 Two types of palindromes

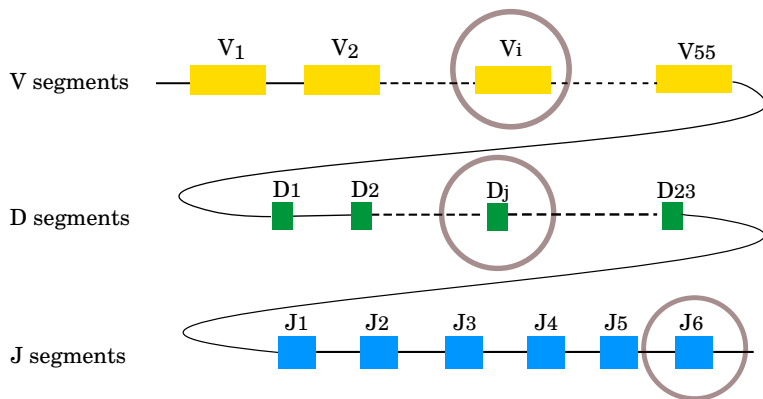
Introduction: V(D)J-recombination

B-cells gene locus:



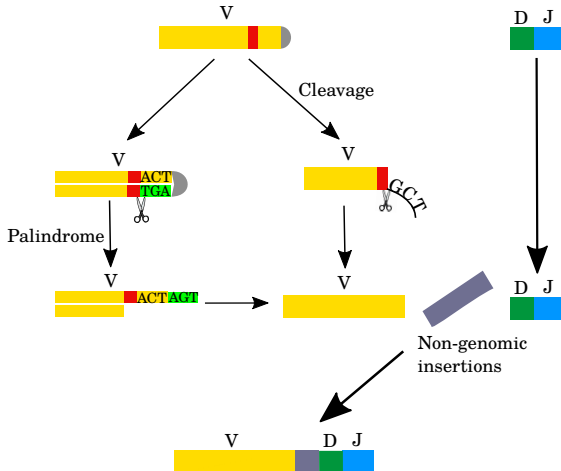
Introduction: V(D)J-recombination

Segment of each type is selected:



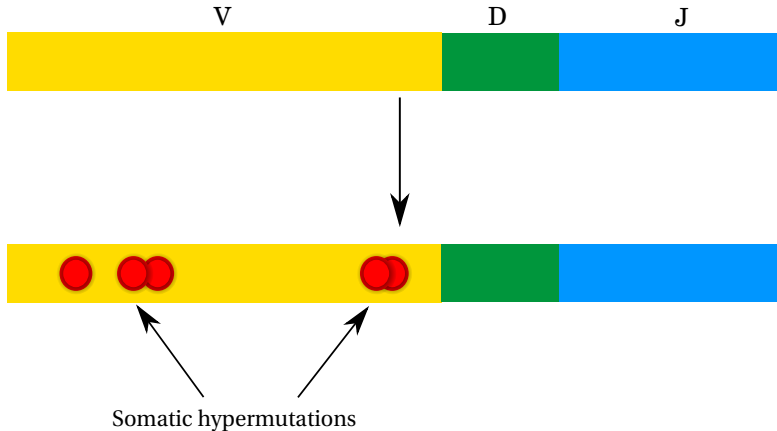
Introduction: V(D)J-recombination

3 types of biochemical events: *palindrome*, *cleavage*, *non-genomic insertion*.



Introduction: V(D)J-recombination

Further optimization of antibody affinity is achieved through extensive mutations referred as *somatic hypermutations*:



Because we do not know the deterministic nature of the V(D)J-recombination, it is reasonable to consider this transform as a **random** (stochastic) process.

Hence the analysis of somatic recombination can be done in statistical and simulation terms.

Motivation: comparing different antibody repertoires

B-cells:


- Comparison of Antibody Repertoires against *Staphylococcus aureus* in Healthy Individuals and in Acutely Infected Patients.
- Comparison of the antibody repertoire generated in healthy volunteers following immunization with a monomeric recombinant gp120 construct derived from a CCR5/CXCR4-using human immunodeficiency virus type 1 isolate with sera from naturally infected individuals.

T-cells:

- Donor Unrestricted T Cells: A Shared Human T Cell Response.
- Exhaustive T-cell repertoire sequencing of human peripheral blood samples reveals signatures of antigen selection and a directly measured repertoire size of at least 1 million clonotypes.

Motivation: simulation of a repertoire

Appropriate statistical model of somatic recombinations potentially improves IgSimulator, making it more “realistic”.

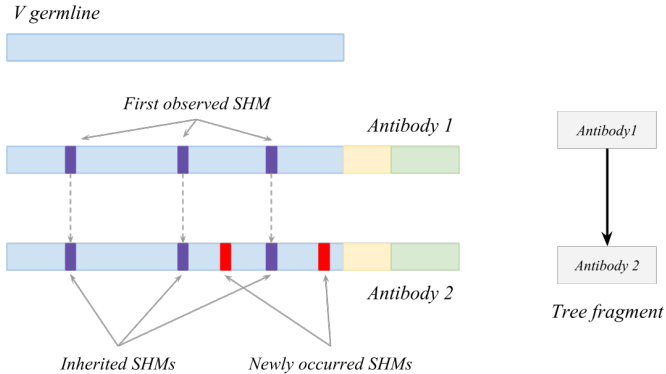


IgSimulator

Statistical
Model

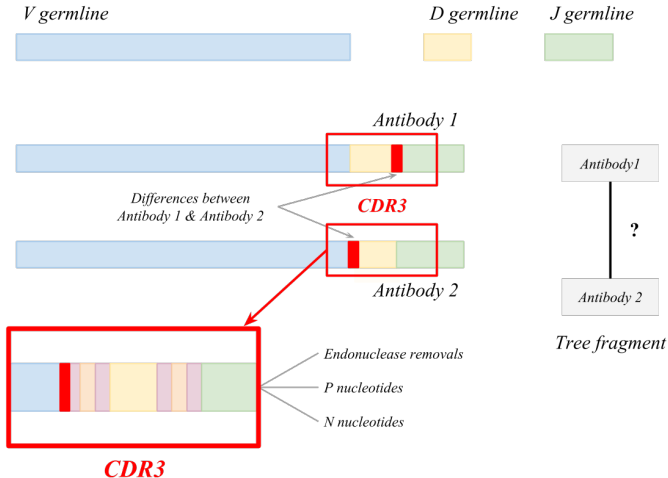
Motivation: Clonal trees

Clear situation:

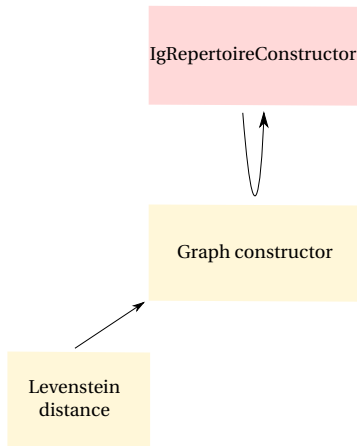


Motivation: Clonal trees

Arguable situation:

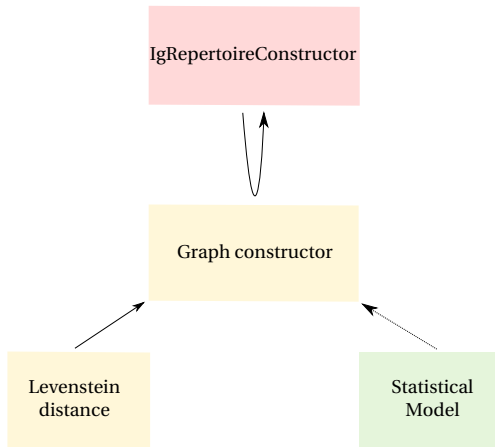


Motivation: IgRepertoireConstructor



The current release of the IgRepertoireConstructor uses Levenstein distance to construct edges in the graph.

Motivation: IgRepertoireConstructor



The statistical model could suggest a more delicate approach.

There are lots of tasks. To name a few:

- What is the correlation between D-J and V-DJ joining?
- Is there any correlation between the *cleavage* / *palindromes* and specific gene segments?
- What are the properties of the *non-genomic insertion*?

An article about the distribution law of CDR3 generating recombinations for T-cells:

Anand Murugana, Thierry Morab, Aleksandra M. Walczakc and Curtis G. Callan — 2012:

- Analysis is focused on nonproductive CDR3s.
- Suggested model sets joint distribution over the set of discrete variables: *identities* of V-, D-, J- genes, number of *deletions* from the end of a segment, *palindromic* nucleotides and *non-genomic insertion* at the end of a gen.

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- 2865(!) parametr to estimate.

Questions about the paper:

- Is the suggested model really adequate (including the problem of potential overfitting)?
- Are the results statistically significant?
- Are similar results true for B-cells?

Two types of events

The goals

- correlations between *palindromes* and specific gene segments,
- properties of the *non-genomic insertion*

include the task of distinguishing “accidental” and “biological” events and hence require some additional knowledge about the structure of the repertoire.

Considering that and the questions about the article let's firstly concentrate on a simpler problem of seeking **correlation between cleavage and specific gene segments.**

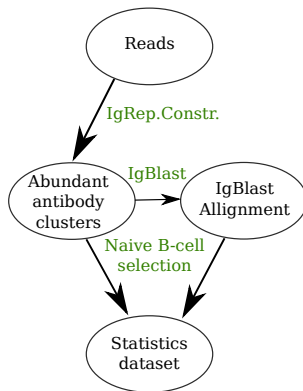
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Problem: In the datasets not only V(D)J-recombinations effects are reflected, but also the result of secondary mutations.

Cleavage and specific gene segments

Problem: In the datasets not only V(D)J-recombinations effects are reflected, but also the result of secondary mutations.

- Consider a merged pairs dataset.
- Use IgRepertoireConstructor for this dataset and consider **highly abundant antibody clusters** of the constructed repertoire.
- Apply IgBlast to reads from highly abundant clusters.
- **Downsampling**: consider only such reads, that have alignment score of their segments not less than a **threshold** according to IgBlast.



Cleavage and specific gene segments: V-genes

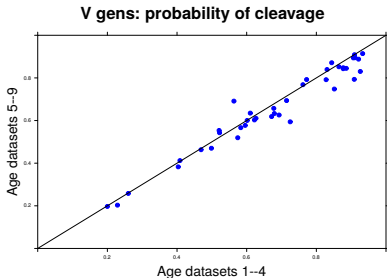


Figure : Age datasets. The point — is the gen. Pearson correlation is 0.98.

Permutation test: significance of the correlation

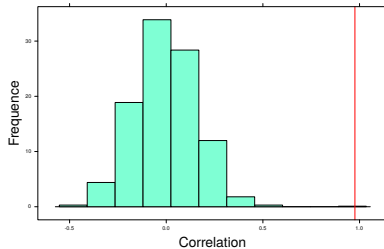


Figure : Histogram of statistics of permutation test that shows the significance of Pearson correlation.

Cleavage and specific gene segments: V-genes

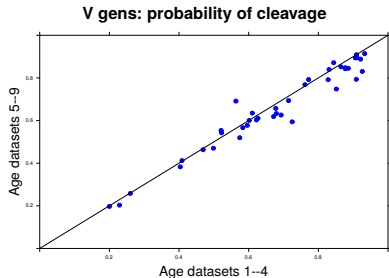


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Permutation test: significance of the correlation

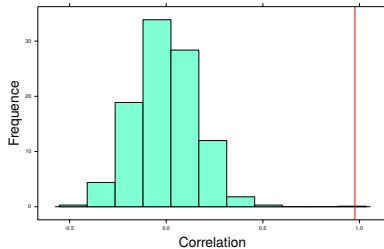


Figure : Histogram of statistics of permutation test that shows the significance of Pearson correlation.

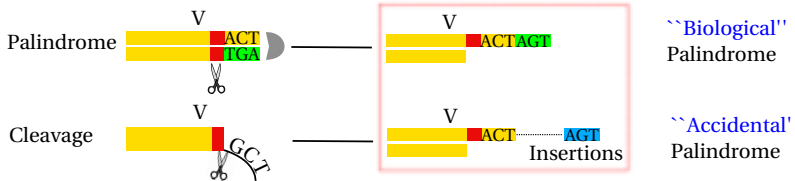
Hence reads in the dataset are dependent standard pooled Z-test for equal proportions is not applicable.

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Two types of palindromes

If a *cleavage* took place, then no “biological” *palindrome* can happen.

An “accidental” *palindrome* can still happen due to the *non-genomic insertions*.



Two types of palindromes

- The simplest model is that any nucleotide ξ in the sequence is distributed **uniformly**:

$$\mathbb{P}(\xi = x) = \frac{1}{4} \text{ where } x \in \{ 'A', 'C', 'G', 'T' \}.$$

- In that model the length η of an “accidental” palindrome has $\text{Geom}(3/4)$ distribution, so

$$\mathbb{P}(\eta = n) = \frac{3}{4^{n+1}} \text{ for all } n \in \mathbb{N}_0.$$

Two types of palindromes

Emperical (Age-datasets) and Geom(3/4) distribution in log scale:

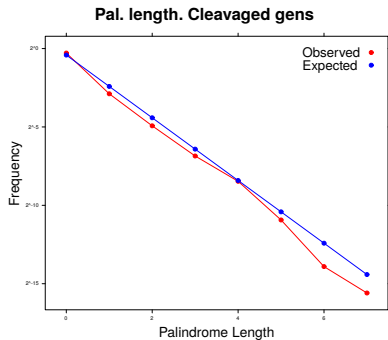


Figure : The mean of length is 0.33.

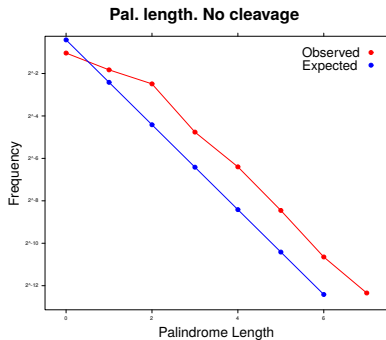


Figure : The mean of length is 0.82.

Two types of palindromes

Emperical (Age-datasets) and Geom(3/4) distribution in log scale:

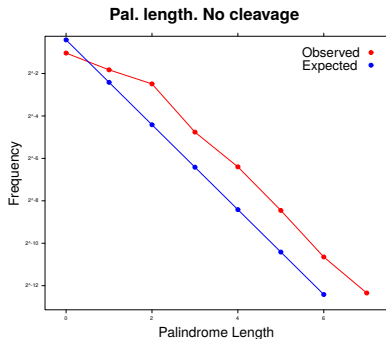
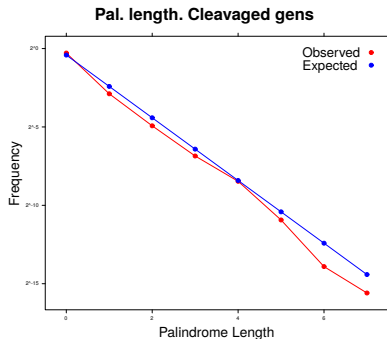


Figure : The mean of length is 0.33.

Figure : The mean of length is 0.82.

Hence reads in the dataset are dependent goodness-of-fit χ^2 -test is not applicable.

- To find out the distribution of “biological” palindrome length.
- To construct more adequate model for nucleotide distribution.

Statistical analysis of an antibody repertoire is very promising research with lots of applications. Some of them:

- Comparing antibodies repertoires.
- Simulation of a repertoire.
- Edges in clonal trees.
- Improvement of `IgRepertoireConstructor`.

Our goals:

- Construct an adequate statistical model of the V(D)J-recombination.
- Find out correlations between segments of genes.
- Find out correlations between various biological events, including *cleavage*, *palindromes* and *non-genomic-insertions*.