

Statistical analysis of full-length antibody repertoire using immunosequencing data

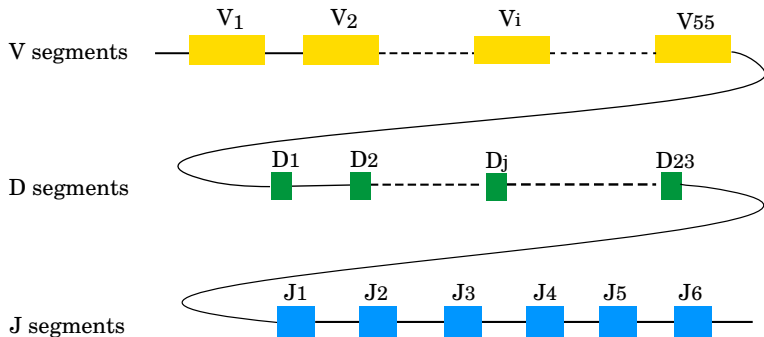
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September 13, 2015

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

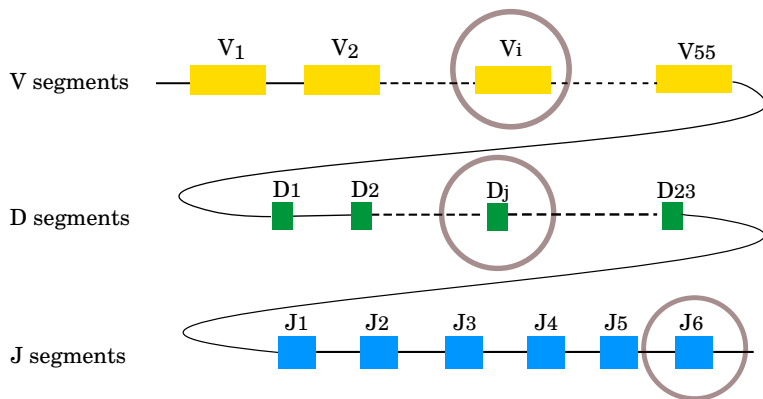
Introduction: V(D)J-recombination

Immunoglobulin heavy chain locus:



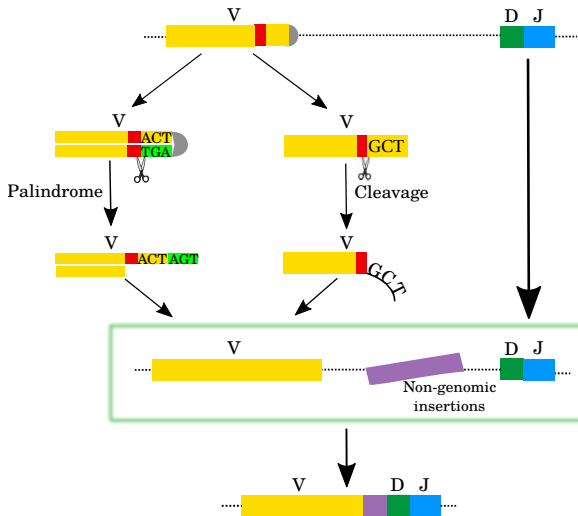
Introduction: V(D)J-recombination

Segment of each type is selected:



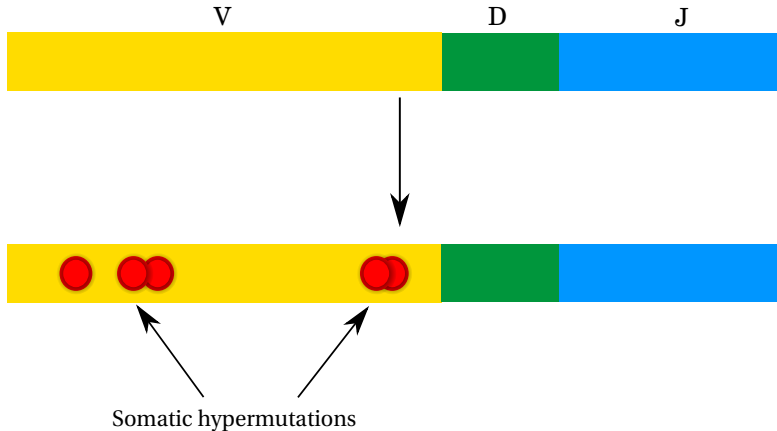
Introduction: V(D)J-recombination

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*:



Introduction: V(D)J-recombination

Because we do not know the deterministic nature of the V(D)J-recombination, it is reasonable to consider it 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 — Agnieszka Dryla *et al.*, CVI, 2005.
- 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 — Beddows S. *et al.*, Journal of Virology, 1999.

T-cells:

- Donor Unrestricted T Cells: A Shared Human T Cell Response — Van Rhijn I, Moody DB, Journal of Immunology, 2015.
- 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 — Warren RL *et al.*, Genome Research, 2011.

Motivation: simulation of a repertoire

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

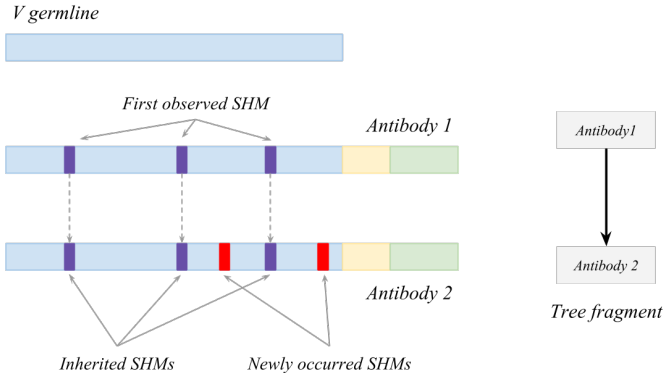


IgSimulator

Statistical
Model

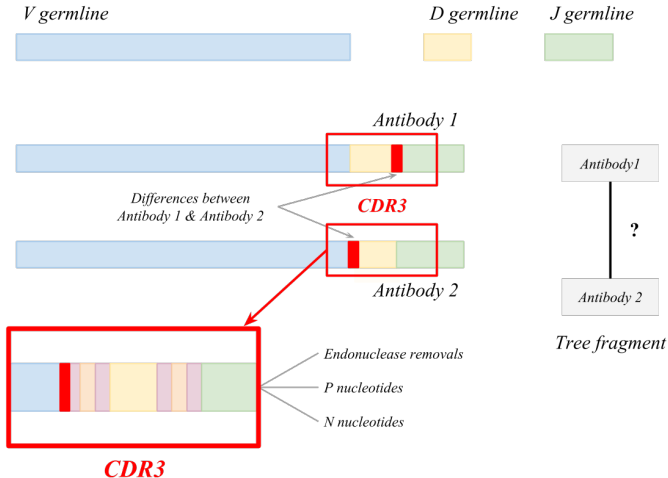
Motivation: Clonal trees

Clear situation:

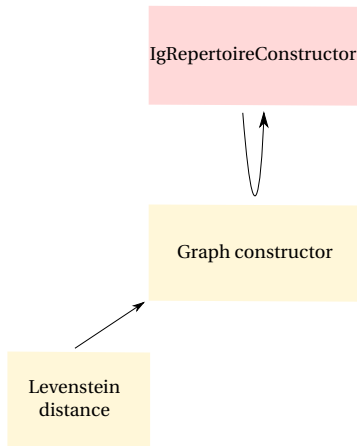


Motivation: Clonal trees

Arguable situation:

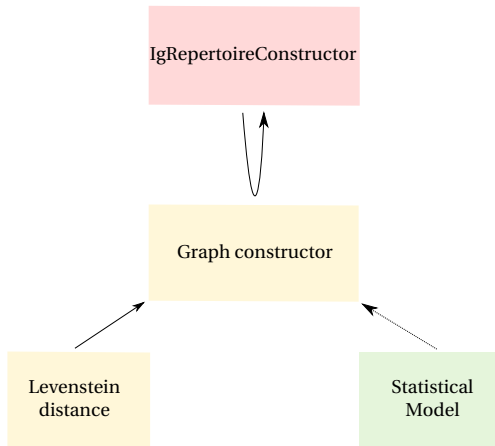


Motivation: IgRepertoireConstructor



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 gene.

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- 2865(!) parameters 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 “biological” (meaningful) from “accidental” (random) observations and hence require additional knowledge about repertoire structure.

Considering that first we decided to concentrate on a problem of computation of correlation between **cleavage and specific gene segments**.

Unlike two problems above *cleavage* can always be detected precisely according to the alignment.

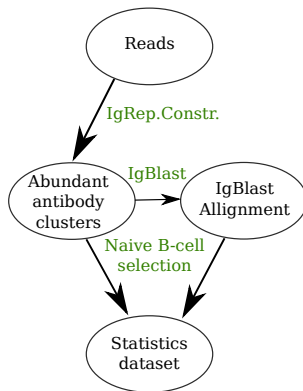
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Problem: In the datasets not only V(D)J-recombinations effects are reflected, but also the result of secondary mutations.

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 full-length 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**: reads with alignment score of the segments not less than a **threshold**.



Cleavage and specific gene segments: V-genes

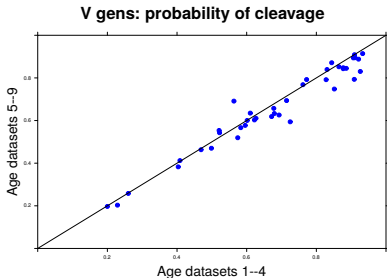


Figure : Age datasets. The point — is the gene. Pearson correlation is 0.98.

Permutation test: significance of the correlation

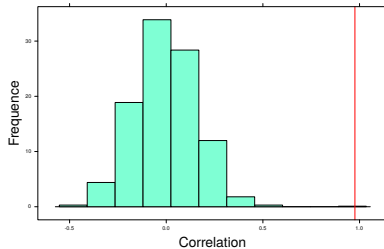


Figure : Histogram of statistics of permutation test that shows the significance of Pearson correlation.

Cleavage and specific gene segments: V-genes

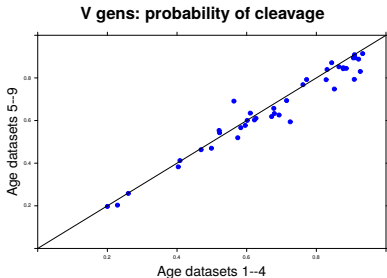


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Permutation test: significance of the correlation

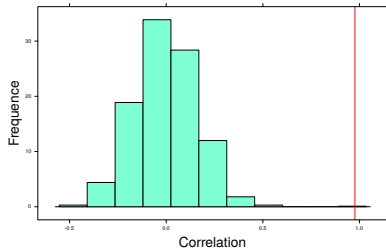


Figure : Histogram of statistics of permutation test that shows the significance of Pearson correlation.

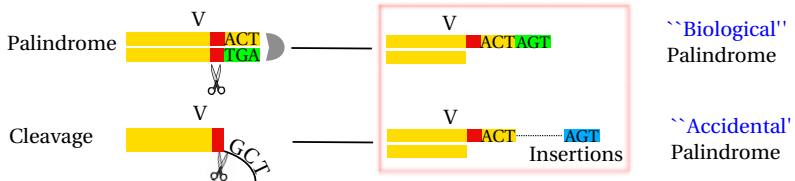
Hence reads in the dataset are dependent standard pooled Z-test for equal proportions is not applicable.

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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:

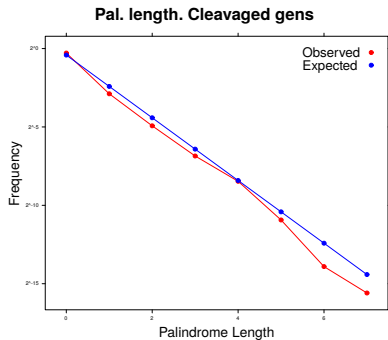


Figure : The mean of length is 0.33.

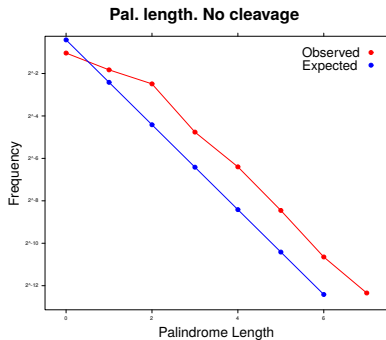


Figure : The mean of length is 0.82.

Two types of palindromes

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

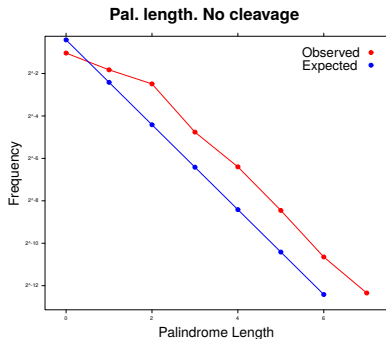
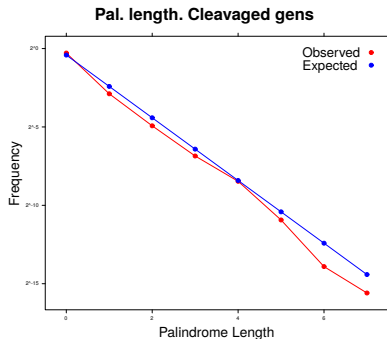


Figure : The mean of length is 0.33. Figure : The mean of length is 0.82.

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*.