

Immune repertoire annotation

A RepSeq data analysis tutorial

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Skoltech, MA03172 course [Term 2, 2017-2018]

December 5, 2017

Outline

Introduction

Basic RepSeq analysis methods

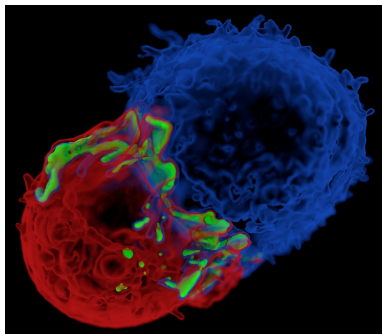
Getting started

Interactive part

The assignment

T-cell receptor

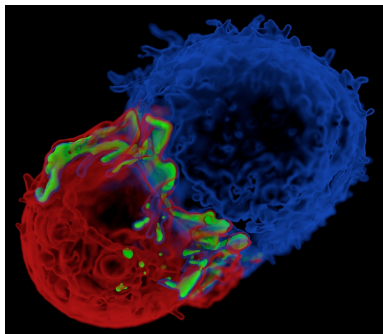
T-cell:APC contact



From James and Vale, Nature
2012,
<https://valelab.ucsf.edu/images/>

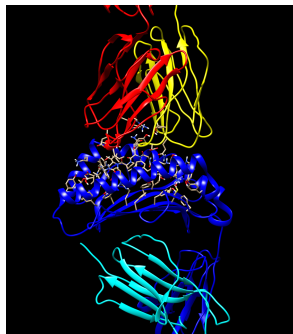
T-cell receptor

T-cell:APC contact



From James and Vale, Nature
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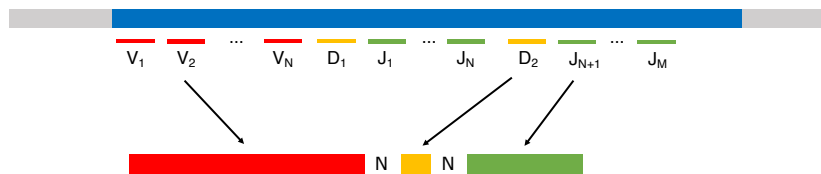
TCR:pMHC structure



PDB:1ao7, rendered using
UCSF chimera, colored by
chain

VDJ rearrangement

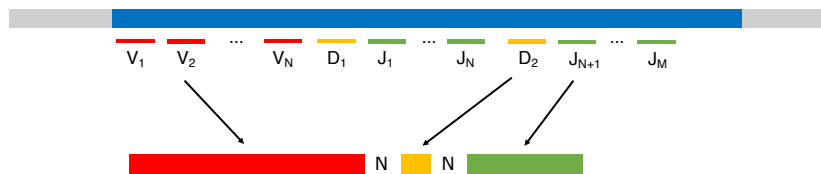
An example schema for TCR β locus



Variable, Diversity and Joining are chosen at random, V-D and D-J junctions are filled with non-template N bases.

VDJ rearrangement

An example schema for TCR β locus



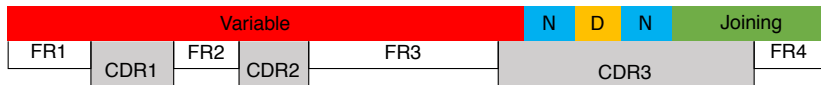
Variable, Diversity and Joining are chosen at random, V-D and D-J junctions are filled with non-template N bases.

VDJ rearrangement mechanism can be efficiently recaptured with a probabilistic model [Murugan et al. PNAS 2012]

$$\begin{aligned}
 P(\sigma) &= P(V)P(D, J) \\
 &\times P(\#del_V|V)P(\#del_J|J)P(\#del_{D5}, \#del_{D3}|D) \\
 &\times P(\#ins_{VD})P(\#ins_{DJ}) \prod_{i \in ins_{VD}} P(b_i|b_{i-1}) \prod_{i \in ins_{DJ}} P(b_i|b_{i+1})
 \end{aligned}$$

TCR regions

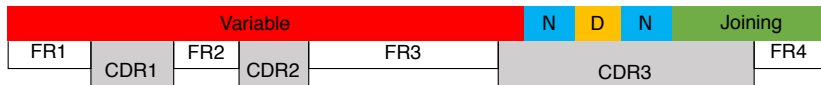
A TCR chains consists of the following regions:



In total there are four framework (FRs) and three complementarity determining regions/loops (CDRs).

TCR regions

A TCR chains consists of the following regions:

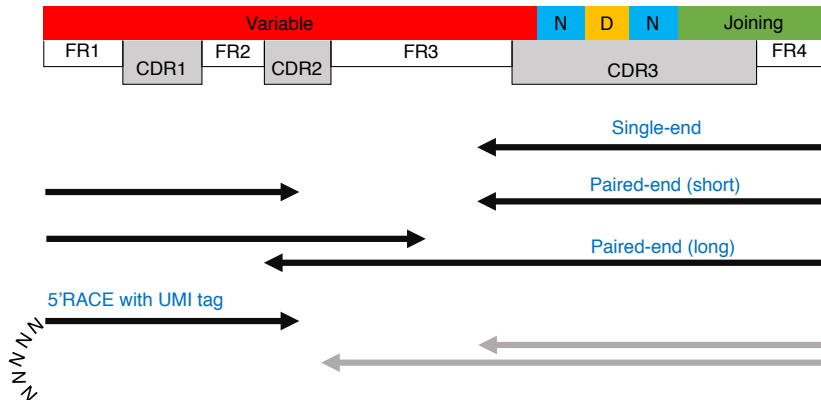


In total there are four framework (FRs) and three complementarity determining regions/loops (CDRs).

The likely functions of these regions are:

- ▶ FR regions maintain TCR secondary structure and (possibly) play role in MHC binding
- ▶ CDR1,2 are germline encoded and play role in antigen recognition, as well as (possibly) MHC binding
- ▶ CDR3 plays a major role in antigen recognition and is extremely variable

TCR repertoire sequencing



An example of a RepSeq dataset

After all pre-processing steps:

- ▶ Read grooming (filtering, etc)
- ▶ UMI-based assembly (for molecular barcoded data)
- ▶ V-D-J mapping and clonotype assembly

An example of a RepSeq dataset

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- ▶ Read grooming (filtering, etc)
- ▶ UMI-based assembly (for molecular barcoded data)
- ▶ V-D-J mapping and clonotype assembly

We finally get clonotype frequency tables that look like

Index	Frequency	Count	CDR3AA	V	D	J	CDR3NT
1	1.0%	3913	CSA GG L G STDTQYF	TRBV20-1	TRBD1	TRBJ2-3	TGCAGT GCTG GGGGGC TCGGTAGCACAGATACGCAGTATTTT
2	0.90%	3440	CAS NSG SSYNEQFF	TRBV5-1	TRBD2	TRBJ2-1	TGCGCCAGCA ATAG CGGGAGCTCCTACAATGAGCAGTCTTTC
3	0.79%	3021	CSA RQG NQPQHF	TRBV20-1	TRBD1	TRBJ1-5	TGCAGT GCGC SACAGGGGAATCAGCCCCAGCATTTT
4	0.65%	2490	CASSQE PGGE QFF	TRBV4-1	TRBD2	TRBJ2-1	TGCGCCAGCAGCCAAGAGCCGGGGGGGAGCAGTCTTTC
5	0.61%	2336	CASSY GM NTEAFF	TRBV6-6	TRBD2	TRBJ1-1	TGTGCCAGCAGTTACGGGATGAACACTGAAGCTTTCTTT
6	0.52%	1992	CASSQ GGR APHTQYF	TRBV4-3	TRBD2	TRBJ2-3	TGCGCCAGCAGCCAAGGGGGGAGGGCCCCCATACGCAGTATTTT
7	0.49%	1871	CASSQS QGS SYEQYF	TRBV5-1	TRBD1	TRBJ2-7	TGCGCCAGCAGCCA AAAGTCA AGGGGGGTCTACGAGCAGTACTTC
8	0.48%	1847	CASSR PKSGRS GELFF	TRBV11-2	TRBD2	TRBJ2-2	TGTGCCAGCAGCCGACCCAAGAGCGGGAGAAAGTGGGGAGCTGTTTTTT

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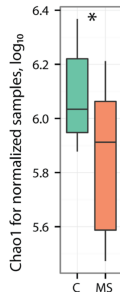
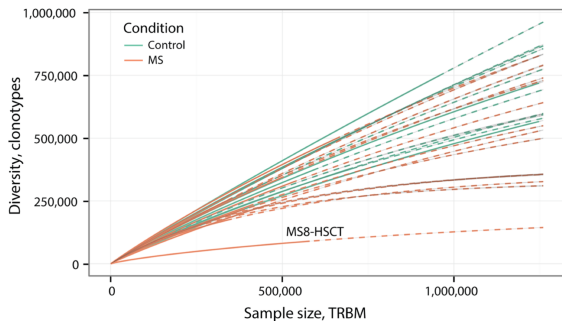
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Diversity analysis

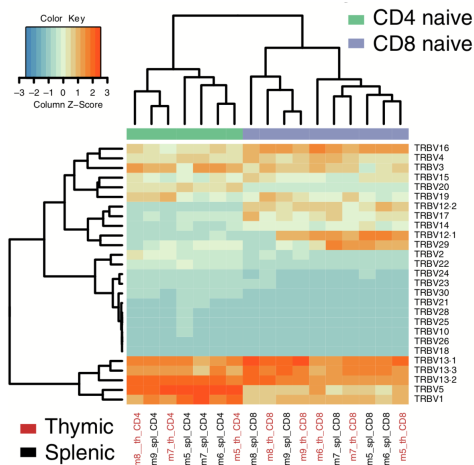
Inspired by species richness/diversity analysis in ecology.
Useful to tell naive T-cell samples from antigen-experienced T-cells containing expanded clones.



Shugay et al. PLoS Comp Biol 2015

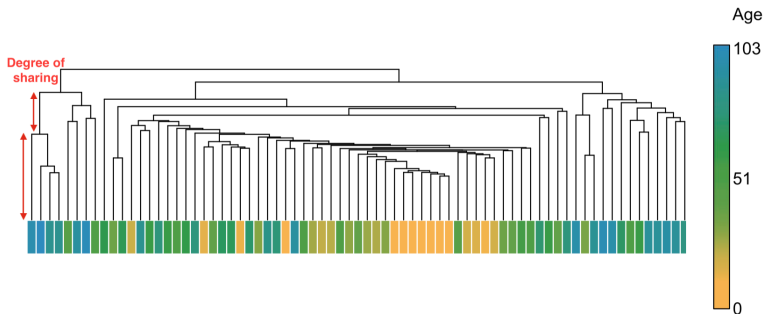
Variable segment usage

Similar to conventional gene expression analysis: segment profile can be useful for distinguishing different subsets of T-cells.



Clonotype sharing

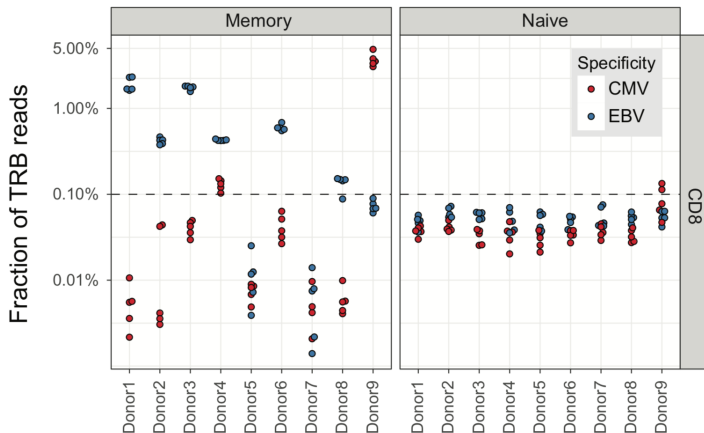
The overlap/co-incidence of hypervariable CDR3 region sequences in different samples. Useful for determining sample origin and comparative analysis of immune repertoires in general.



Britanova et al. J Immunol 2016

TCR sequence annotation

Using a curated database of TCRs with known antigen specificity (VDJdb, vdjdb.cdr3.net). Directly searching for specific TCRs/determining the specificity profile of a repertoire.



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Downloading data

Navigate to

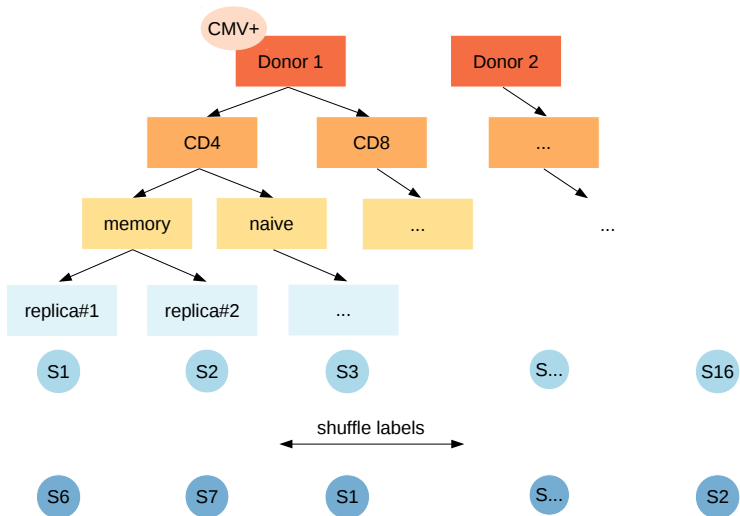
`github.com/antigenomics/repseq-annotation-tutorial`
and download the data + code bundle as zip

The screenshot shows the GitHub repository page for `antigenomics / repseq-annotation-tutorial`. The repository is described as "RepSeq data mining basics in R". It has 17 commits, 1 branch, 0 releases, 1 contributor, and is licensed under CC-BY-SA-4.0. The "Clone or download" button is highlighted with a red circle. Below the repository information, a list of files and their commit history is shown:

File	Commit Message	Time Ago
<code>datasets</code>	Shuffle datasets, add PDF	14 days ago
<code>slides</code>	2prev	42 minutes ago
<code>.gitignore</code>	Slides WIP	14 days ago
<code>LICENSE</code>	Add license	14 days ago
<code>README.md</code>	readme upd	33 minutes ago
<code>samples.png</code>	upd	44 minutes ago

Dataset layout

Datasets were generated as shown in the figure below



Executing R code

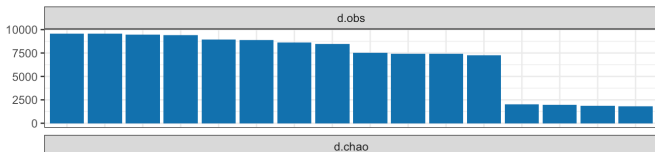
Open the tutorial1.Rmd in RStudio, it can be found in the root folder of the bundle.

```
108 Plot diversity values
109
110 ~~~{r message=FALSE}
111 diversity %>%
112   melt %>%
113   # set what values we are going to plot
114   # fct_reorder reorders sample id by value
115   ggplot(aes(x=fct_reorder2(sample_id, variable, value), y=value)) +
116   # we'll make a bar plot
117   geom_bar(stat = "identity", fill = "#0570b0") +
118   # show each index on different subplot
119   facet_wrap(~variable, scales = "free_y", ncol = 1) +
120   xlab("") + ylab("Diversity index") +
121   theme_bw()
122 ~~~
```



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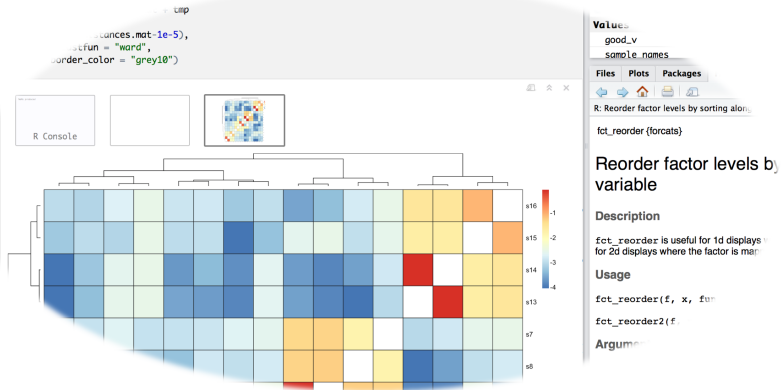
Basic RepSeq analysis methods

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The assignment

Using the analysis results we've obtained we need to assign feature labels to each sample. Namely, you need to fill the table with the following structure:

sample	donor	subset	phenotype	CMVstatus
s1	D1	CD4	memory	CMV-
s2	D2		naive	CMV+
s3	D1	CD8	naive	CMV-
...

Details

Table filling rules:

- ▶ Column names should match those on previous slide
- ▶ Sample id should be one of $s_1..s_{16}$
- ▶ Two distinct donor IDs should be used, naming doesn't matter
- ▶ Subset should be either **CD4** or **CD8**
- ▶ Phenotype should be either **memory** or **naive**
- ▶ CMV status should be either **CMV+** or **CMV-**
- ▶ Unknown/ambiguous fields should be left blank

A hint

While you can unambiguously assign CD4/8 and memory/naive labels, as well as point out biological replicates of the same sample, assigning donor labels is tricky.

First, it is impossible to link CD4-CD8 cells of the same donor. Same for CMV status, that is unambiguous only for CD8+ memory T-cells. Therefore I expect that you mark donors in the way they will distinguish samples/replicas coming from the same and different donors.

I.e. there is no problem if donor labels are swapped between CD4 and CD8 T-cells as far as they point to distinct donors for CD4 or CD8 T-cells coming from different donor and the same donor for replicas.

Feedback

Send me filled tables to ____@gmail.com:

- ▶ As plain text tab-delimited files
- ▶ Mail title should start with REPSEQ-TUTORIAL.
- ▶ Attachment name should be in your-name.assignment.txt format.

Thanks for your attention!

These slides and a PDF file containing compiled analysis results can be found in `slides/` and root folders of the data and code bundle.