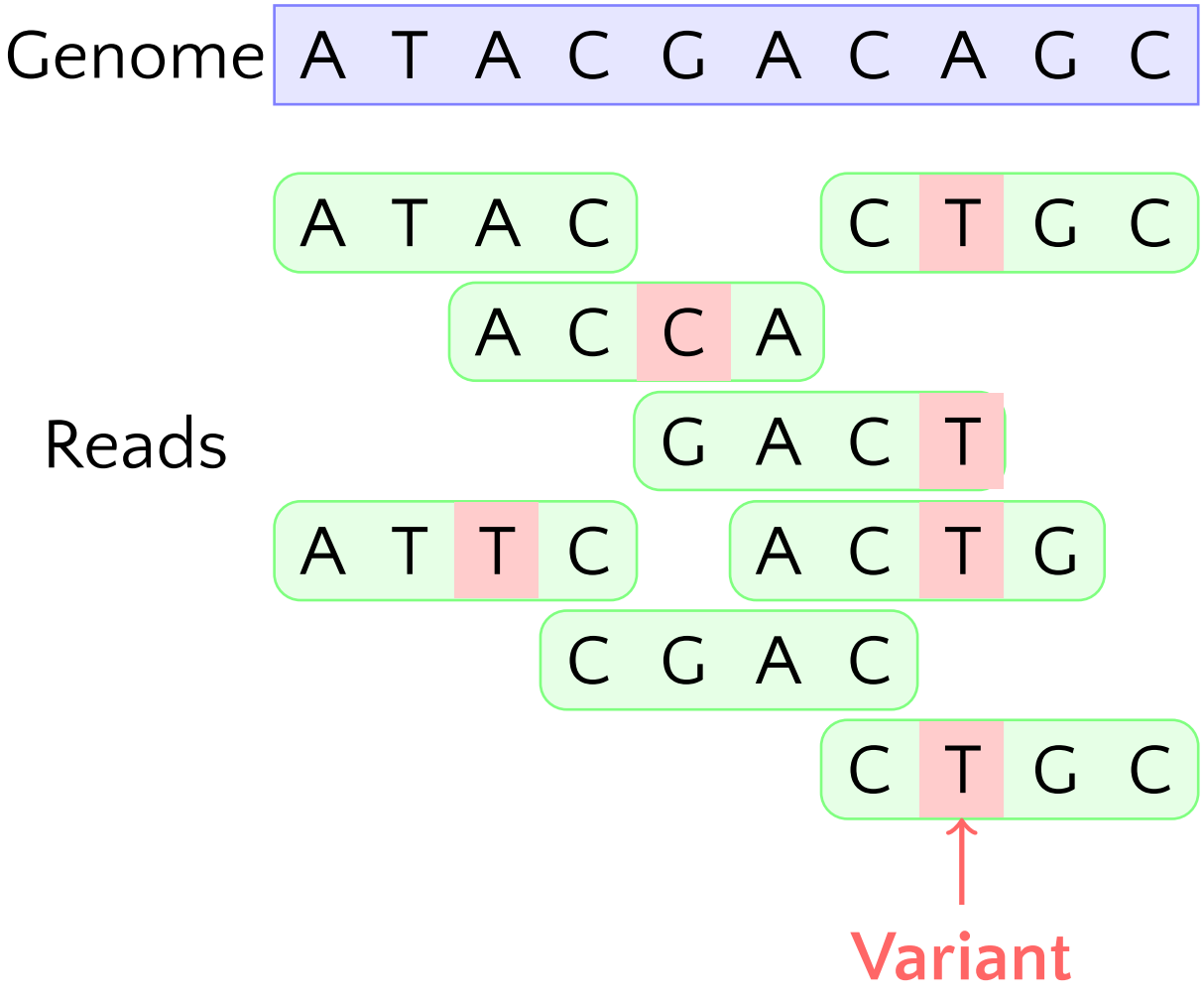
How string algorithms process high-throughput sequencing data in clinical practice

Mikaël Salson

CRIStAL (CNRS, U. Lille)

A classical task: variant calling



Efficient string algorithms matter

Let's compare two sequences

A T A C T G A

T A C G A C

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The optimal* solution is

A T A C T G A
  T A C – G A C

* Optimal in terms of minimising the number of differences

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This takes $\geq$**30 microseconds** for two 300nt sequences

* Optimal in terms of minimising the number of differences

Sequencing data is produced at a (very) high throughput

$10^8 - 10^{10}$

reads per run

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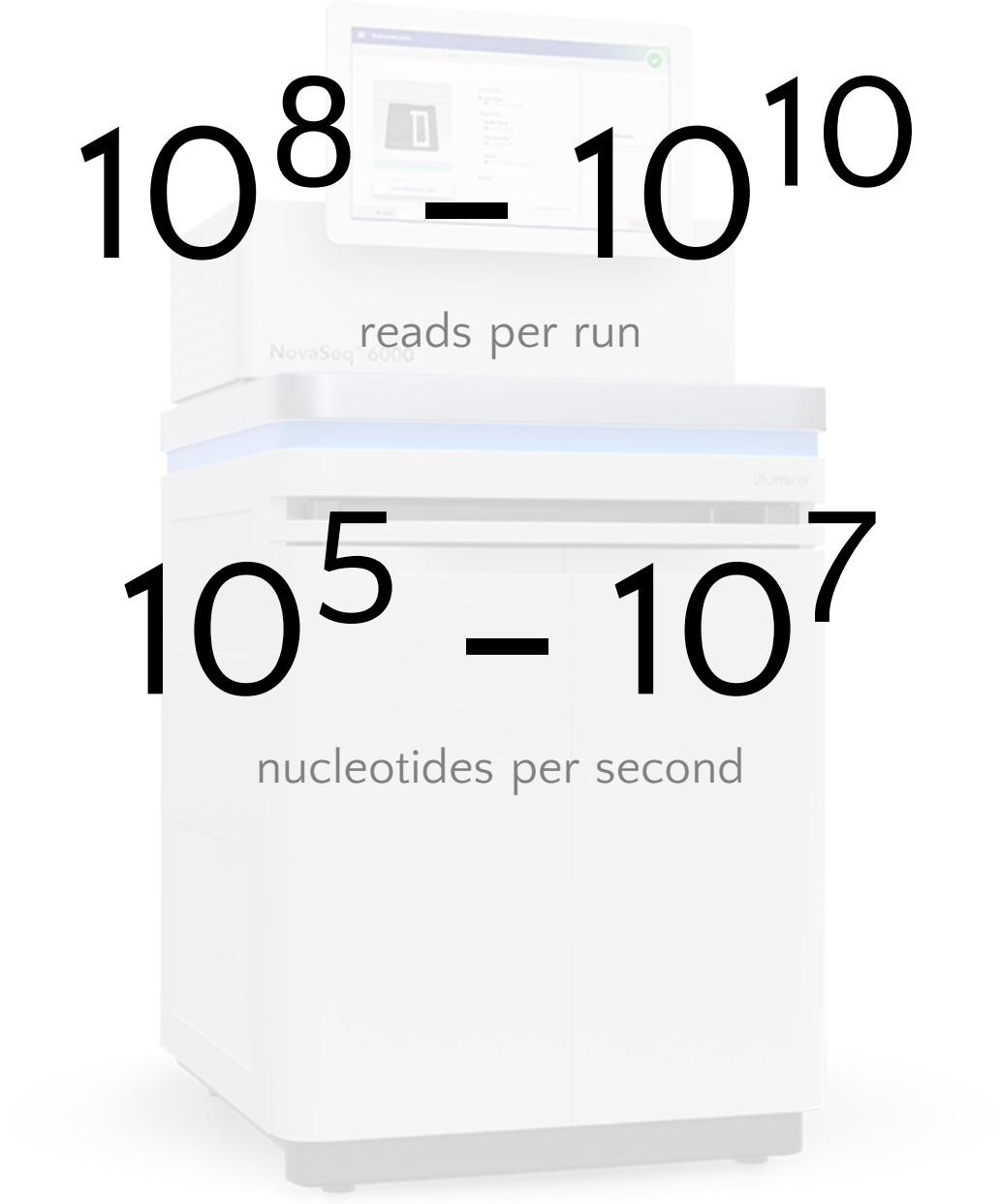

$10^5 - 10^7$

nucleotides per second

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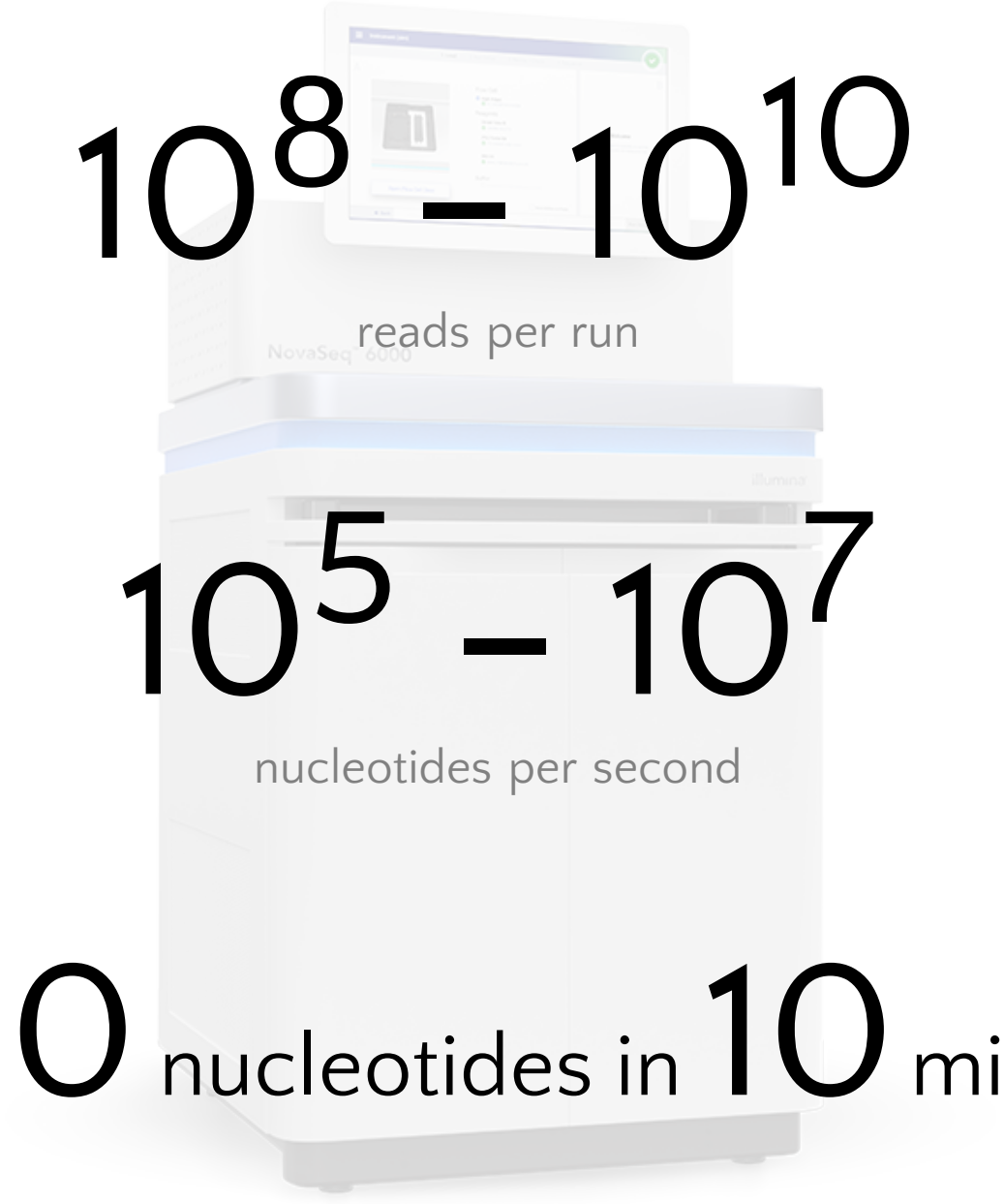

$10^8 - 10^{10}$

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nucleotides per second

up to 300 nucleotides in 10 microseconds

Comparing sequences could be longer than sequencing

Processing the data could be
10 times longer than sequencing

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It's not. How?

How to process 10^5 reads per second?

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Costly
Wasteful
Not very challenging

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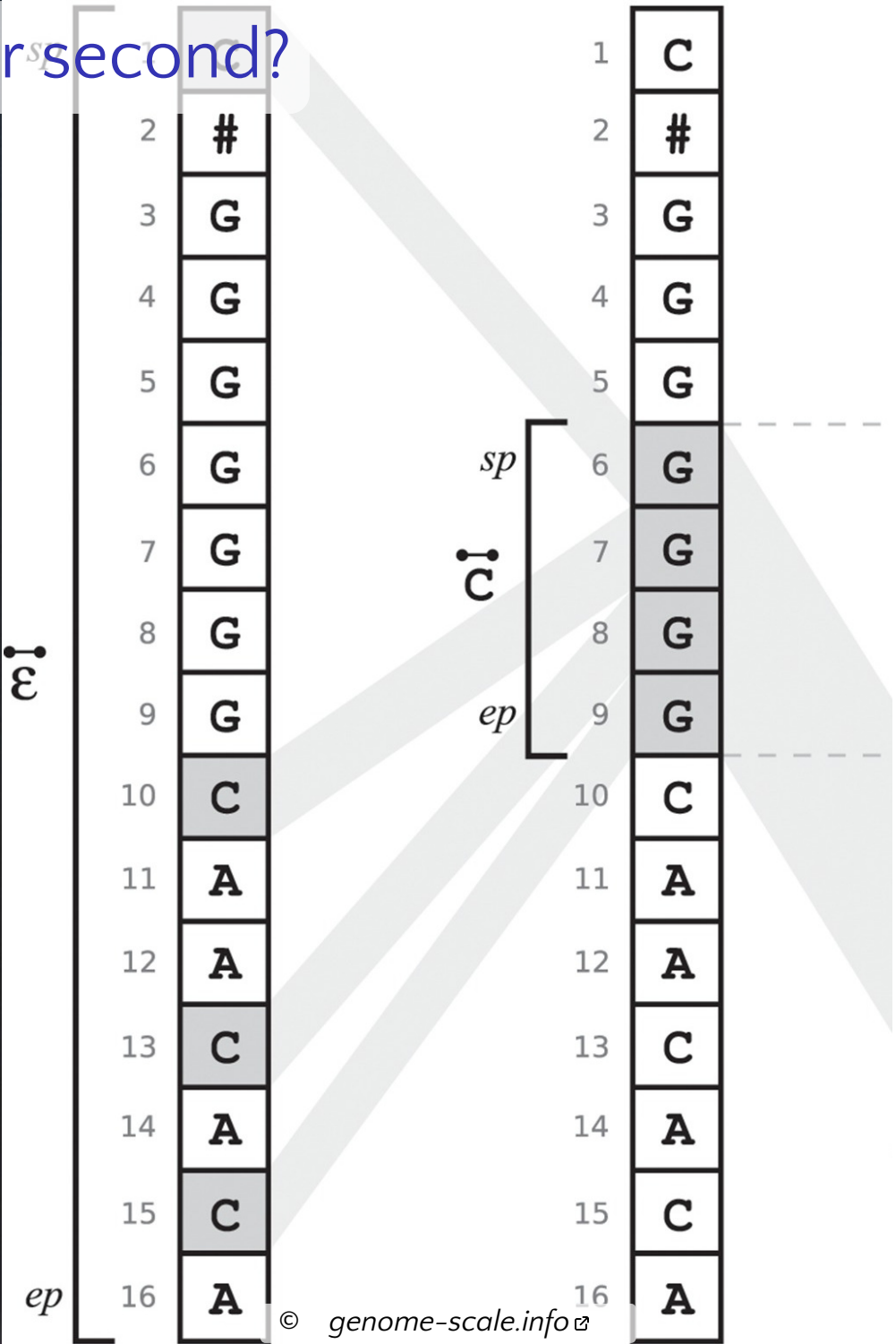

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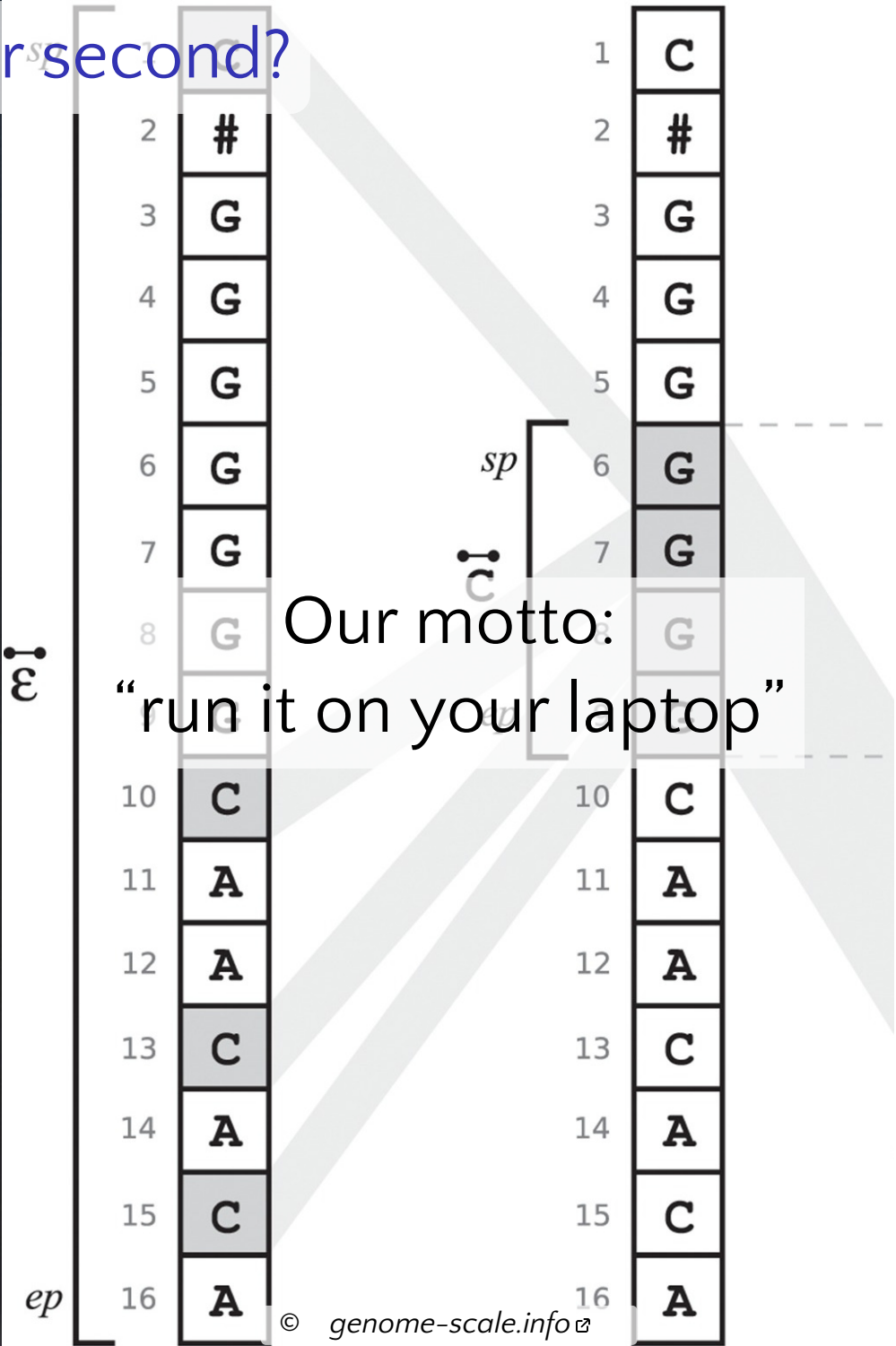

Our motto:
"run it on your laptop"

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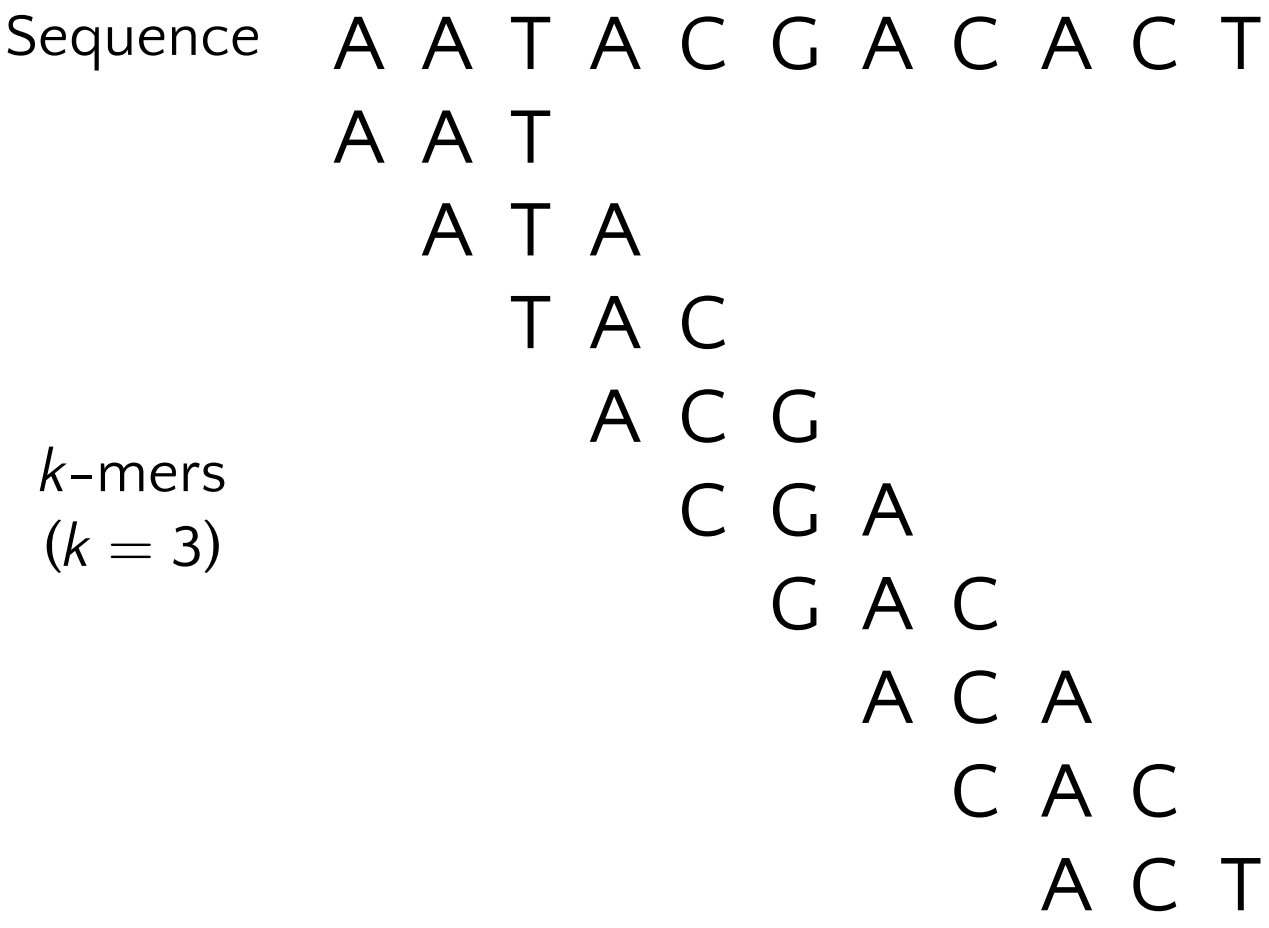
One solution: splitting sequences in k-mers

Sequence A A T A C G A C A C T

One solution: splitting sequences in k-mers



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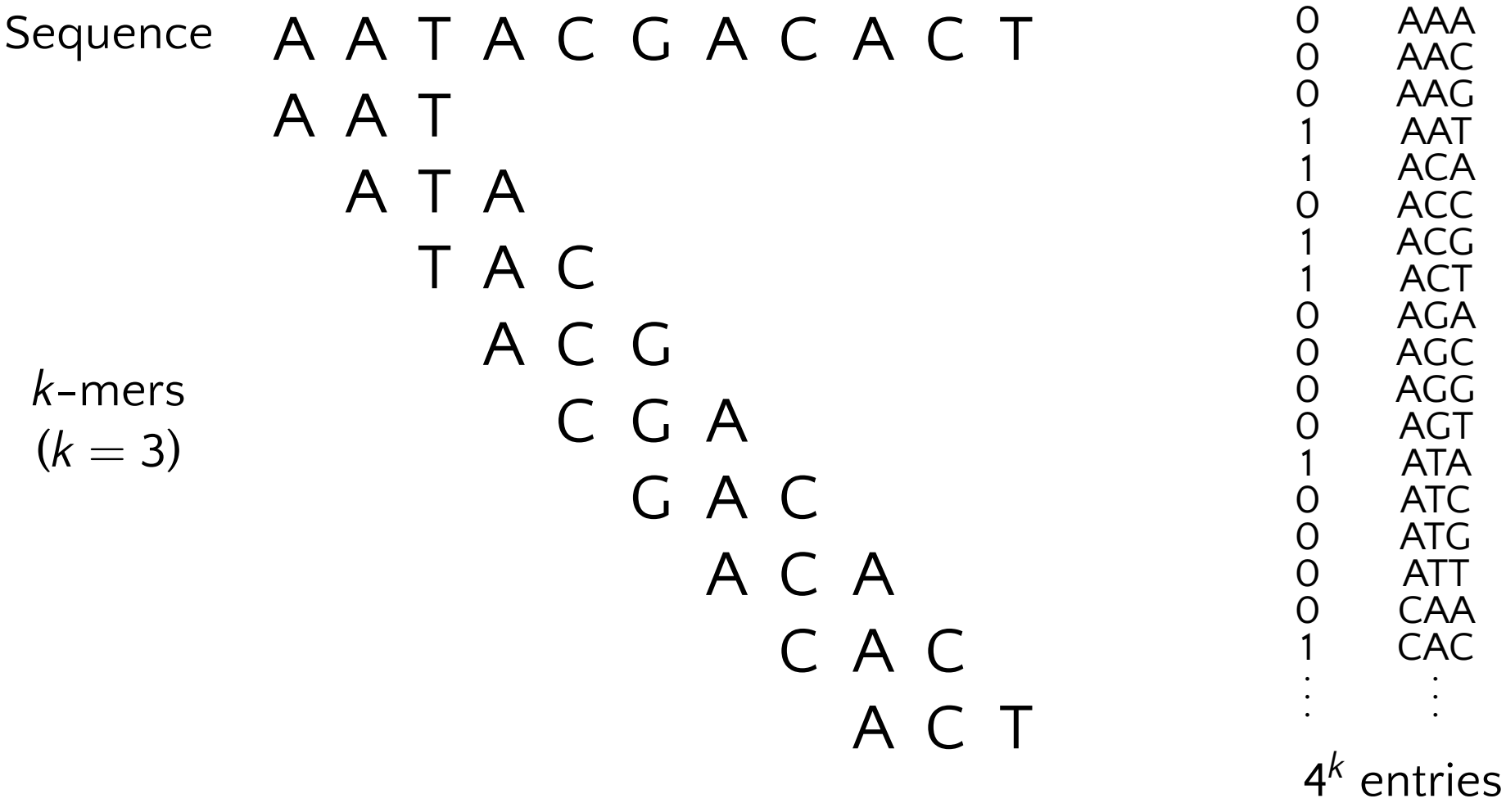
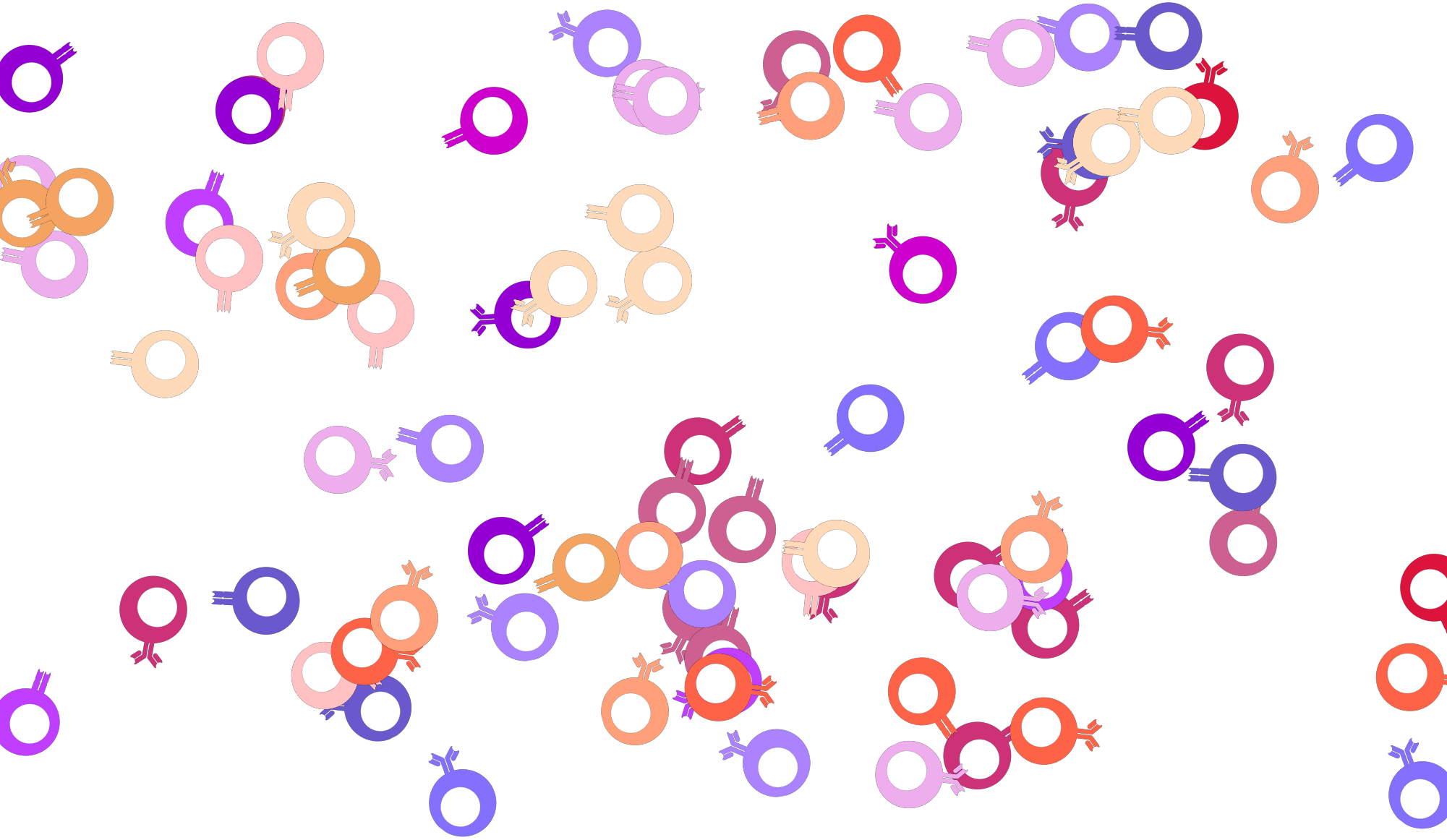


Studying immune repertoires with *k*-mers



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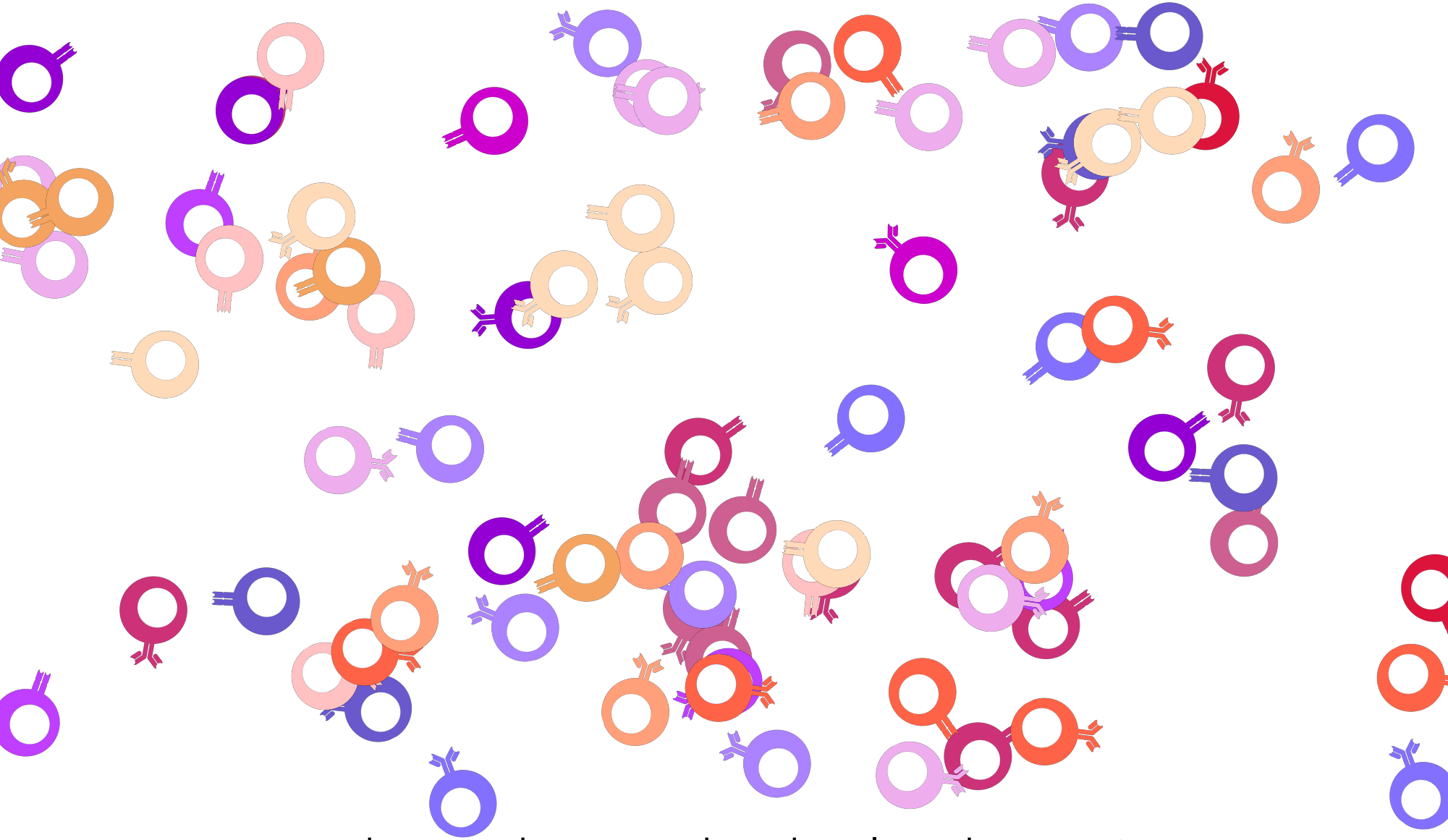
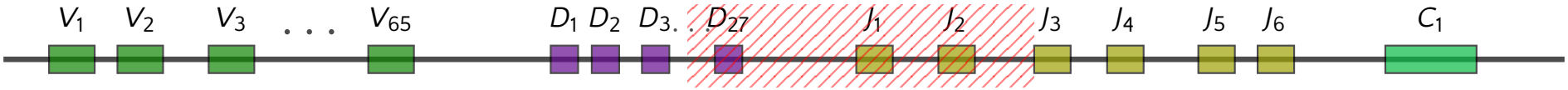


What are the most abundant lymphocytes?

Counting lymphocytes through their V(D)J recombinations

Counting lymphocytes through their V(D)J recombinations

On a lymphoblast genome…



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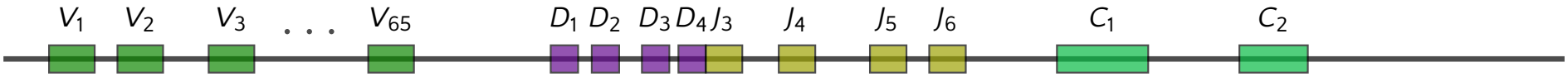

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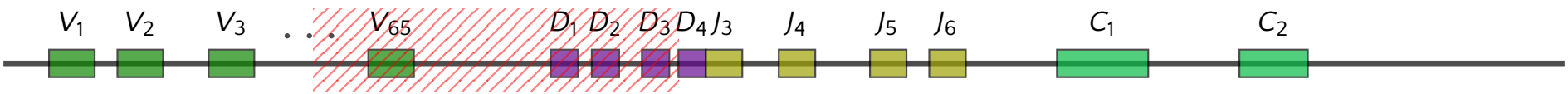

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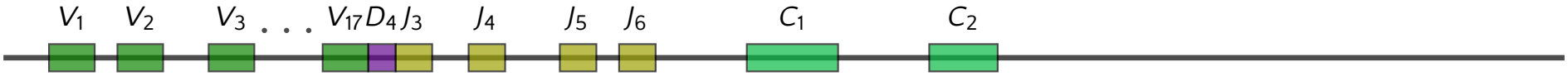

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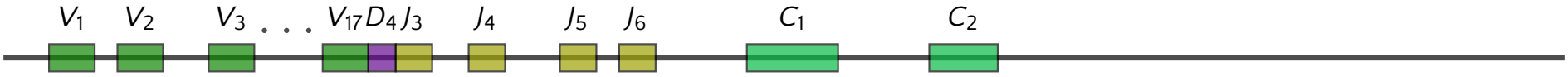
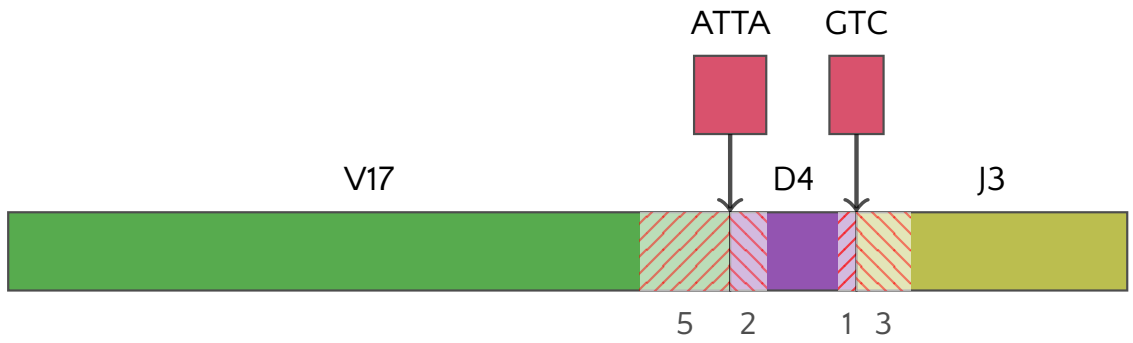


150 – 500nt



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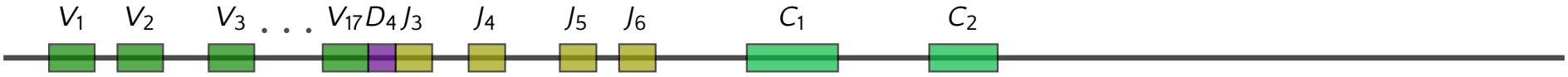

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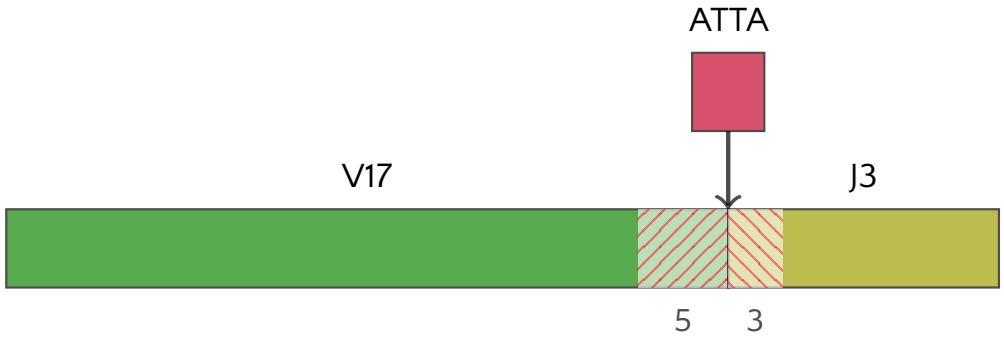

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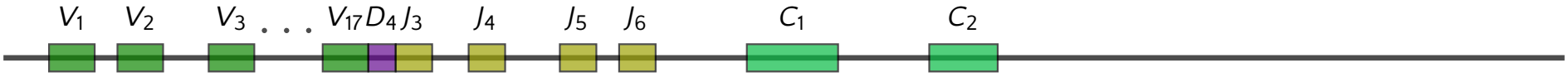

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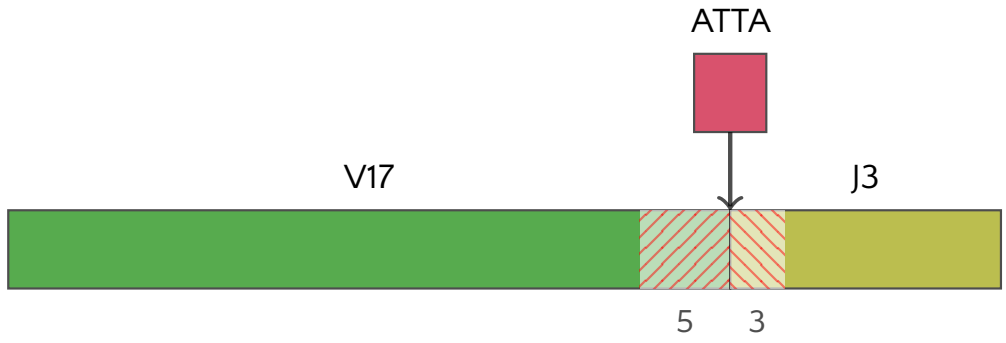

V17 -5/4/-3 J3

Counting lymphocytes through their V(D)J recombinations

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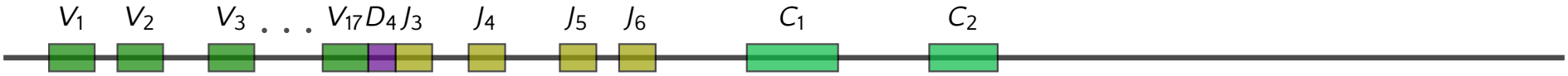

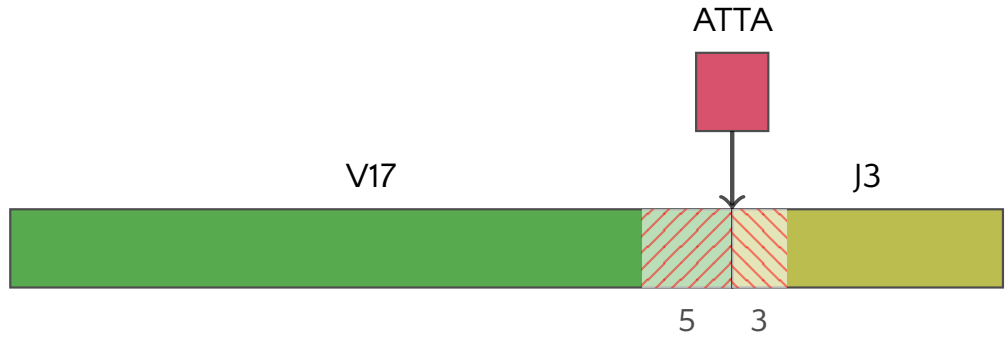

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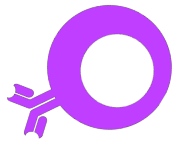
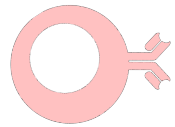
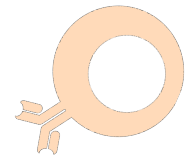


V5 -2/3/-4 J2
1.2 %



V3 -1/12/-1 J5
0.7 %



V1 -2/0/-7 J3
0.9 %

. . .

Two solutions to detect V(D)J recombinations

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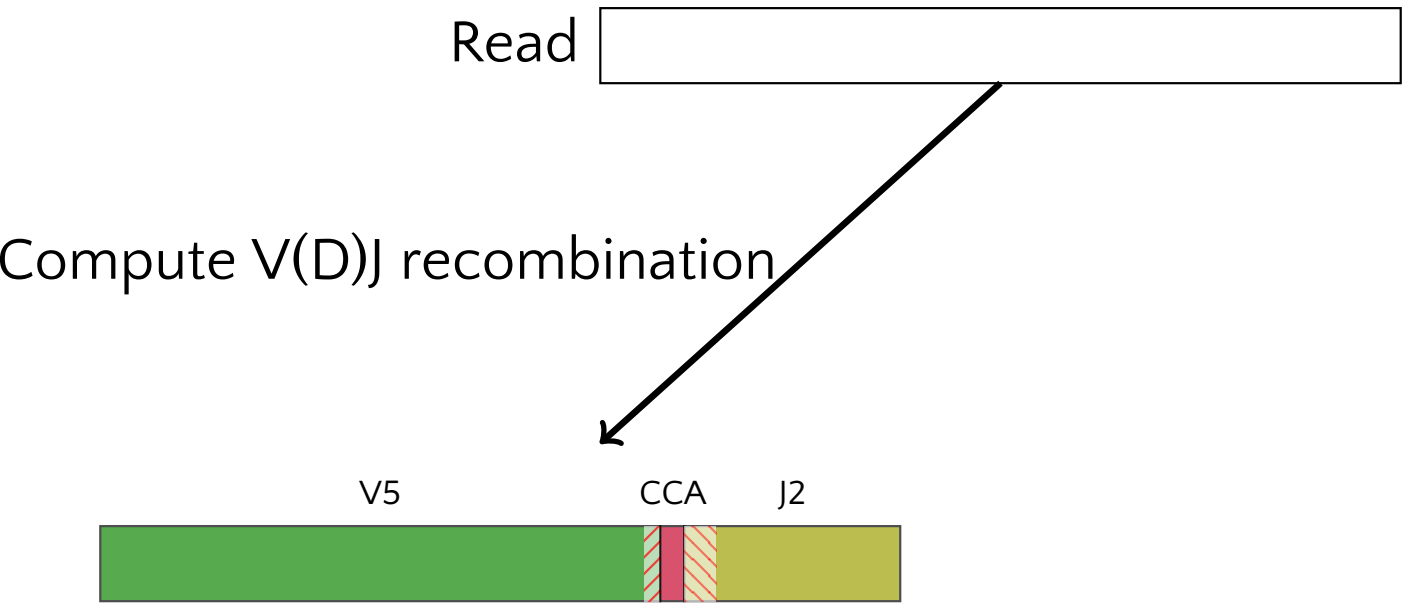

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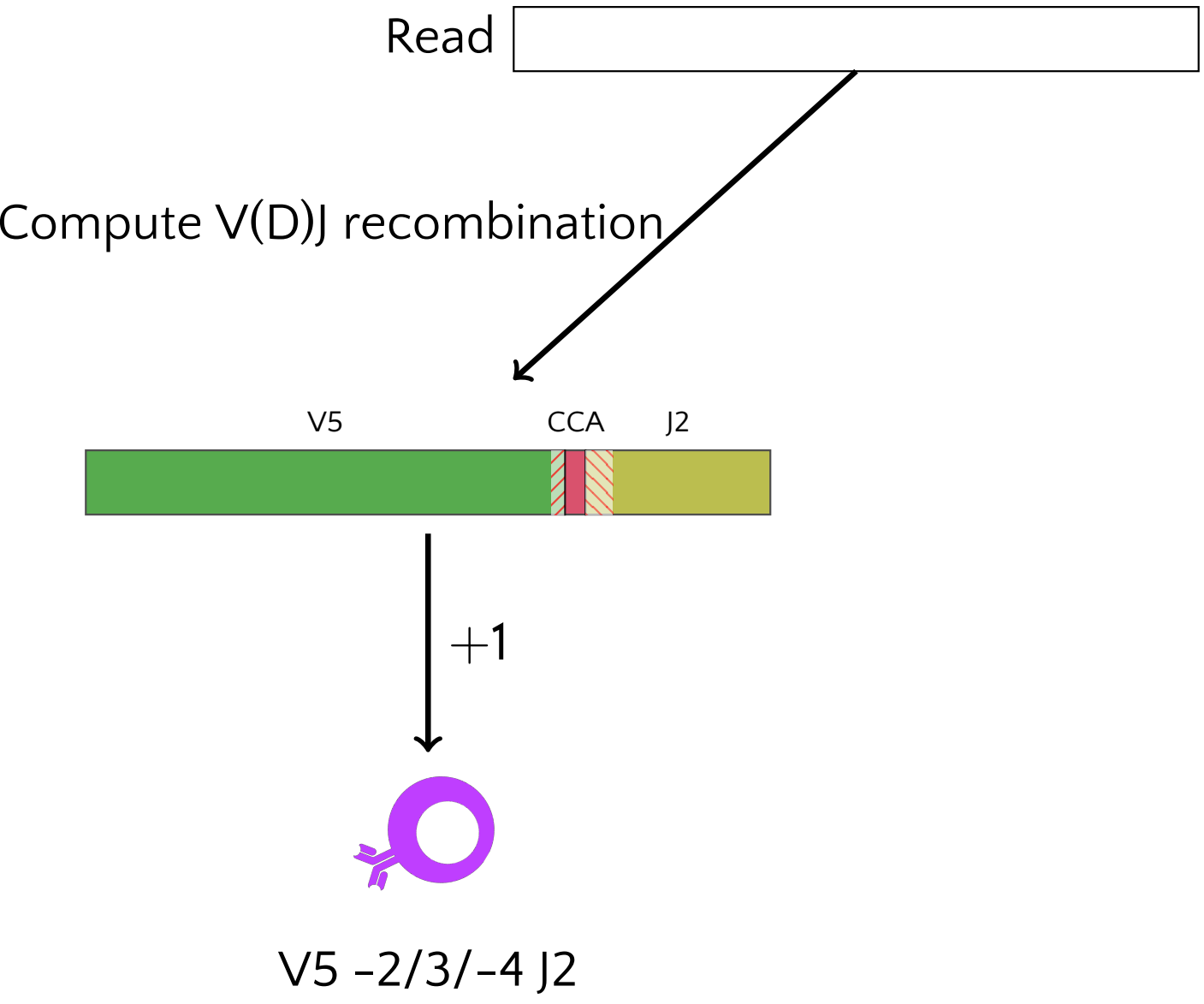

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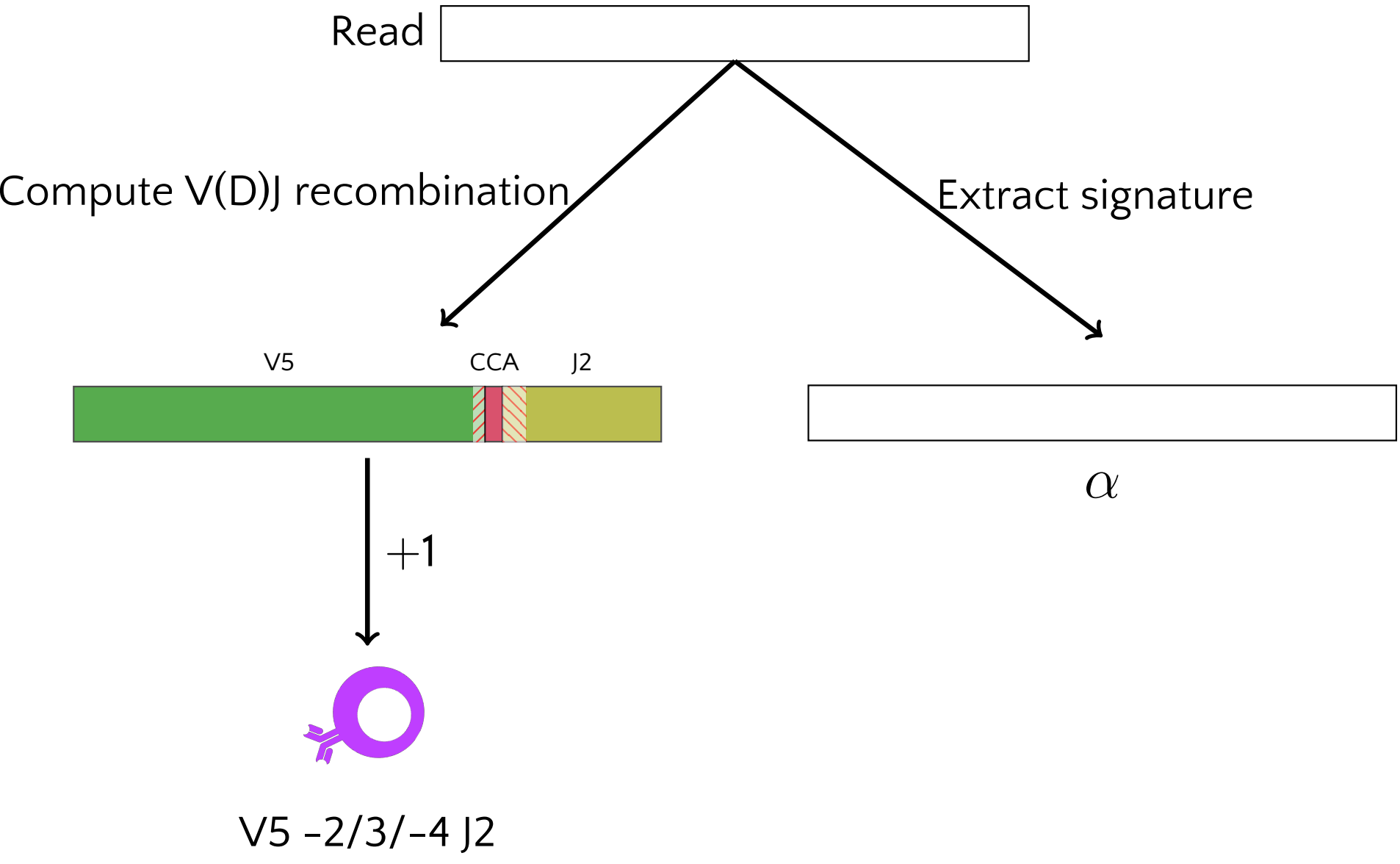

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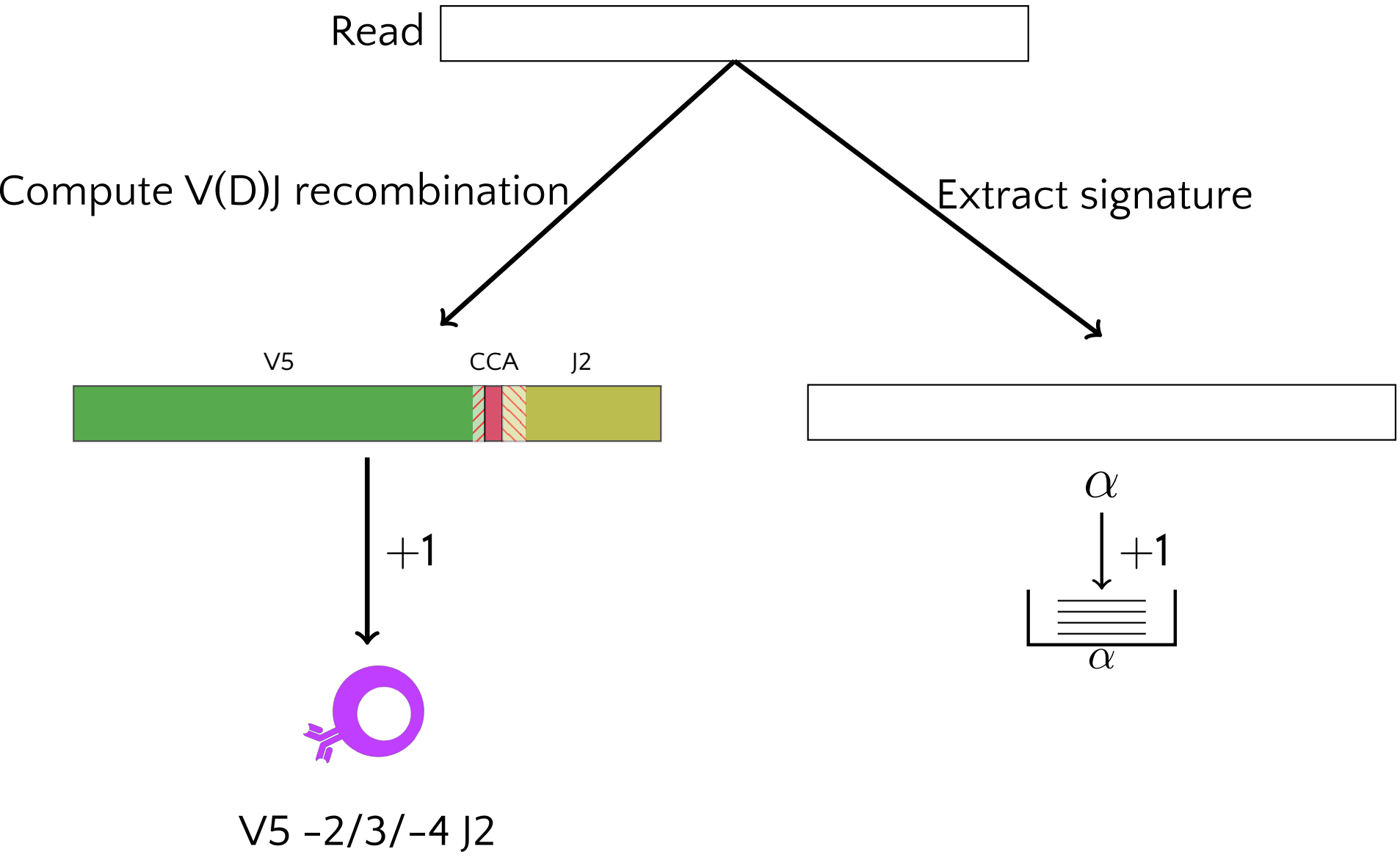

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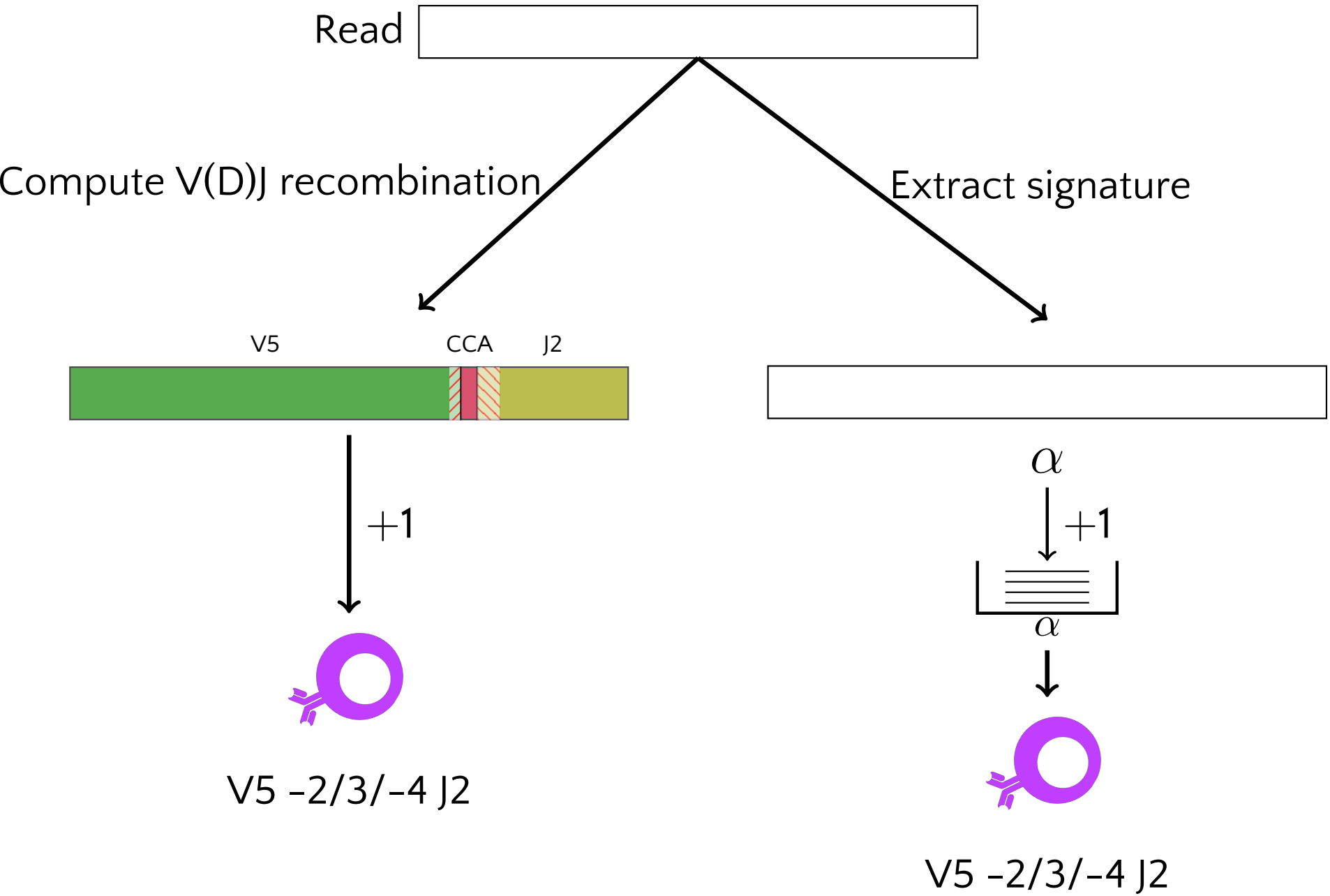

Extracting V(D)J recombination signatures with k-mers

ACACGGCCGTGTATTACTGTGCGAGAGAGCTGAATACTTCCAGCACTGGGGCC

Extracting V(D)J recombination signatures with *k*-mers

parts of V genes

```
ACAC CACG ACGG CGGC GGCC
GCCG TCTT CTTC TTCC TCCA
CCAA CAAC AACC ACCT CCTT
CTTG TTGG TGGA GGAC  ...
```

parts of J genes

```
ATAC TACT ACTG CCAG CAGC
AGCA GCAC TGGG GGGC GGCA
GCAA CAAG AAGA AGAG GAGT
AGTT GTTG TTGG  ...
```

```
ACACGGCCGTGTATTACTGTGCGAGAGAGCTGAATACTTCCAGCACTGGGGCC
```

Extracting V(D)J recombination signatures with *k*-mers

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AGTT GTTG TTGG  ...
```

Signature

Vidjil – Ultrafast V(D)J recombination detection

A story started in 2011 as a collaboration with Lille hospital

Mathieu Giraud
CRIStAL

Vidjil – Ultrafast V(D)J recombination detection

A story started in 2011 as a collaboration with Lille hospital

Mathieu Giraud
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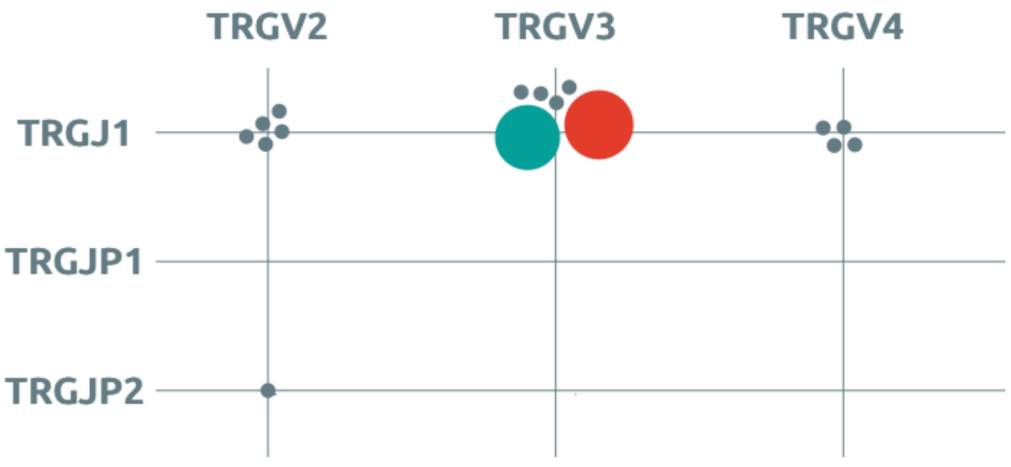
Marc Duez

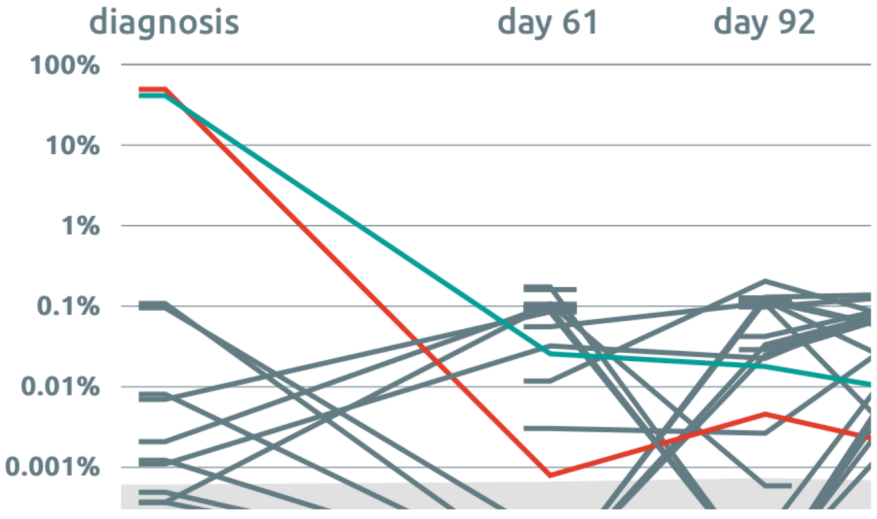
Florian Thonier

Tatiana Rocher

Ryan Herbert

Vidjil – Also a ready-to-use web application





merge align › to IMGT/V-QUEST › to IgBlast › to Blast

4 clones, 738 494 reads (90.53%)

✖ TRGV3 1/11/2 J1	49.30%	★	i	GCCACCTGGGACAGCTCCC-TT-GTTC--ATTATAAGAAACTCTTTGGCAGTG
✖ TRGV3 4/1/2 J1	41.23%	★	i	GCCACCTGGG--A--T--A--T---T--ATTATAAGAAACTCTTTGGCAGTG
✖ TRGV3 3/16/3 J1	0.0021%	★	i	GCCG-CTTGGA-ACCCCAATTTGGTACGGGTTATAAGAAACTCTTTGGCAGTG
✖ TRGV3 5/4/2 J1	+	★	i	GCCACCTGGG---GC--CA-AT---T--A-TA--AGAAACTCTTTGGCAGTG

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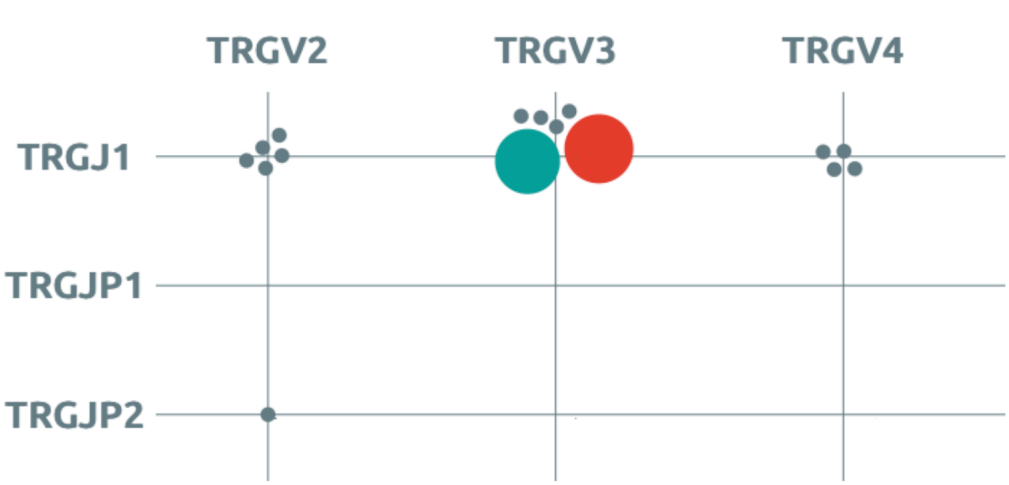

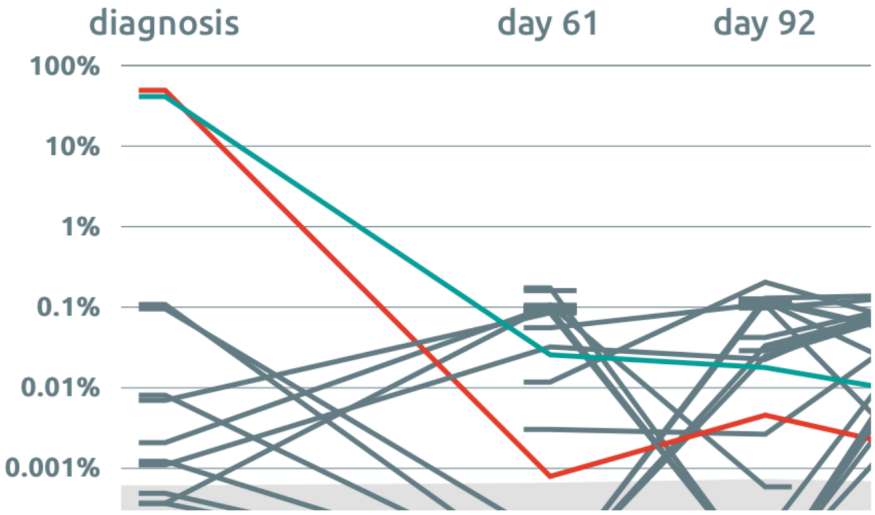

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✕ TRGV3 3/16/3 J1	0.0021%	★	i	GCCG-CTTGGA-ACCCCAATTTGGTACGGGTTATAAGAAACTCTTTGGCAGTG
✕ TRGV3 5/4/2 J1	+	★	i	GCCACCTGGG---GC--CA-AT---T--A-TA--AGAAACTCTTTGGCAGTG

Vidjil 3 min

MiXCR 50 min

On 2M reads

Vidjil is used throughout the world

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A public web server accessible to anyone

`app.vidjil.org`

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`gitlab.vidjil.org`

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A nonprofit consortium to support and enhance the software

`vidjil.net`

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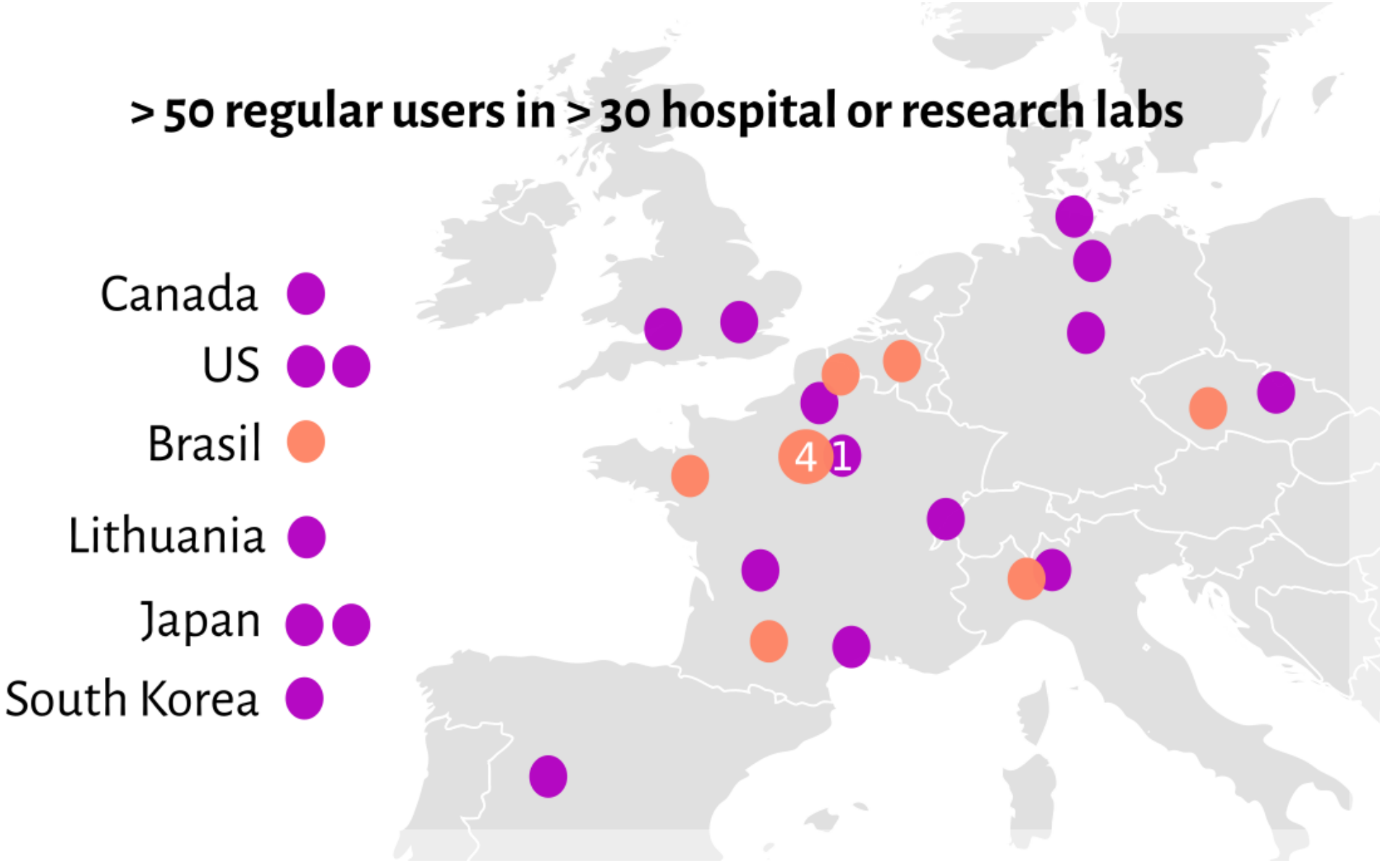

Why do the algorithms matter?

52 Vidjil launched every day

Median value for 2019. D1=6, D9=166

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33 seconds for each job

Median value for 2019. D1=1, D9=433

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Median value for 2019. D1=6, D9=166

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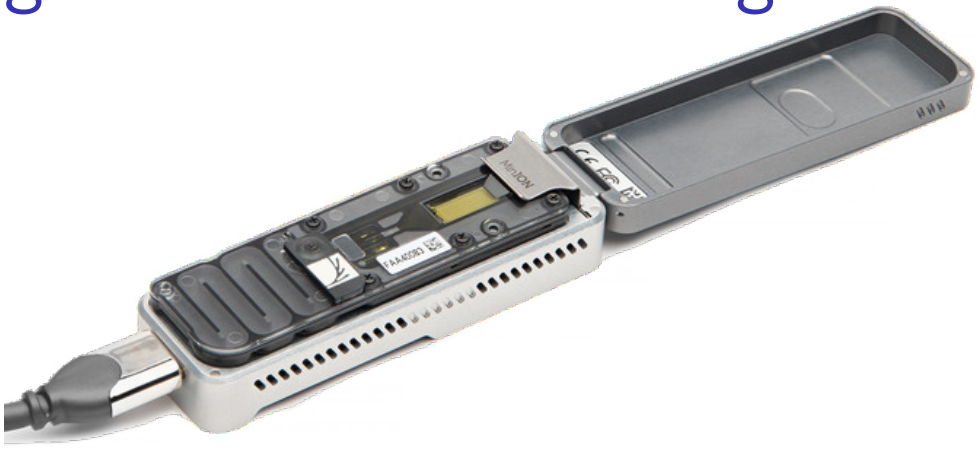
Median value for 2019. D1=1, D9=433

For years, the server was less powerful than my 5-year old laptop…

What string algorithms can still bring to Vidjil?



Long reads, amplification-free...but 10-15 % of errors

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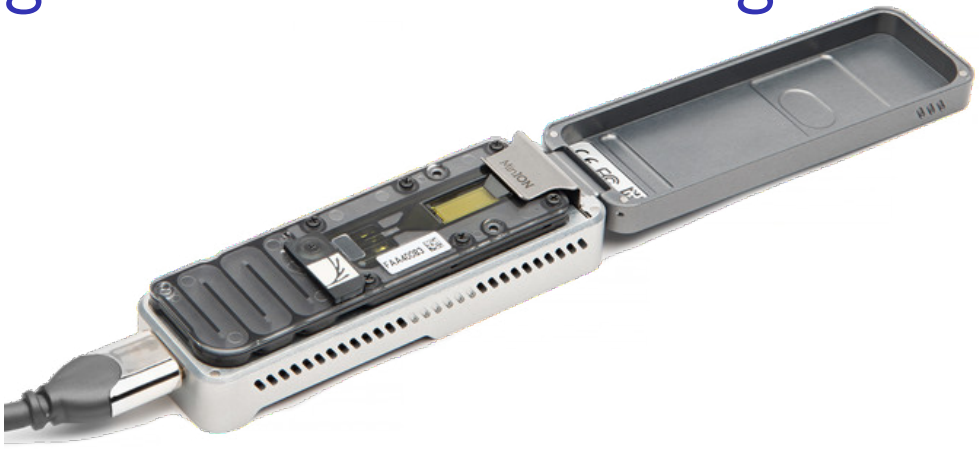

Long reads, amplification-free…but 10-15 % of errors

genome A A G A A G A C C C T

read A A T A C G A C A C T

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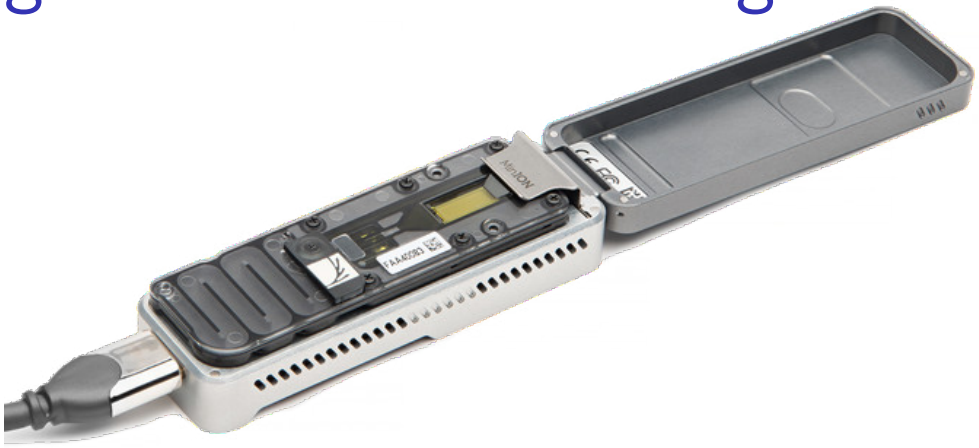

Long reads, amplification-free…but 10-15 % of errors

```
genome  A A G A A G A C C C T
read    A A T A C G A C A C T
        A A T A
          A T A C
            T A C G
              A C G A
                C G A C
                  G A C A
                    A C A C
                      C A C T
```

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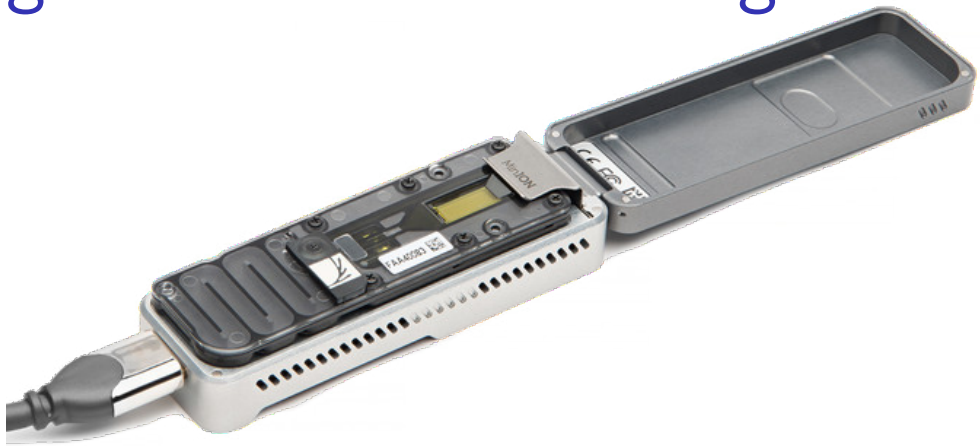

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read    A A T A C G A C A C T
        A A T A
          A T A C
            T A C G
              A C G A
                C G A C
                  G A C A
                    A C A C
                      C A C T
```

No k-mer in common between the read and the genome

01^*0 seeds: finding similarities beyond the k-mers

We want to accept a possible error in a k-mer

01^*0 seeds: finding similarities beyond the k-mers

We want to accept a possible error in a k-mer

Split the k-mer in two parts

01^*0 seeds: finding similarities beyond the k-mers

We want to accept a possible error in a k-mer

Split the k-mer in two parts

k-mer k-mer

01^*0 seeds: finding similarities beyond the k-mers

We want to accept a possible error in a k-mer

Split the k-mer in two parts

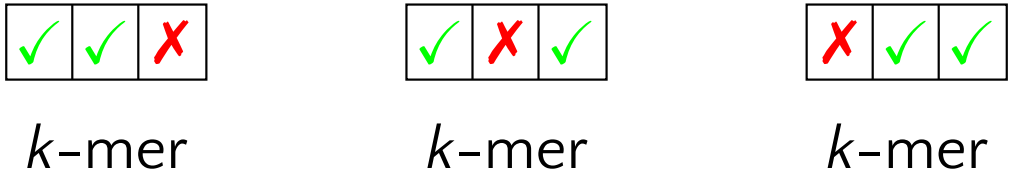
Tolerate one error, more efficiently: split in 3 parts

01^*0 seeds: finding similarities beyond the k-mers

We want to accept a possible error in a k-mer

Split the k-mer in two parts

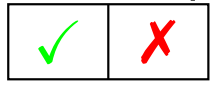
Tolerate one error, more efficiently: split in 3 parts



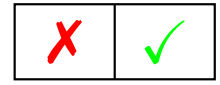
01^*0 seeds: finding similarities beyond the k-mers

We want to accept a possible error in a k-mer

Split the k-mer in two parts

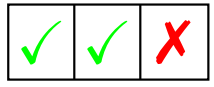


k-mer

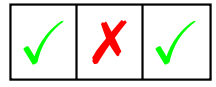


k-mer

Tolerate one error, more efficiently: split in 3 parts



k-mer



k-mer

k-mer

With e errors, split in $e + 2$ parts.

There will always be two parts with 0 error with all interleaving parts having one error

Vidjil – from string algorithmics to clinical practice

Efficient string algorithms (really) matter

We need good theoretical research to conceive practical tools

Why using a computing cluster when a laptop is enough?

Strong interdisciplinary collaborations can have great impacts

Even if not very successful when applying for grants…