

Synthetic and functional genomics of viruses

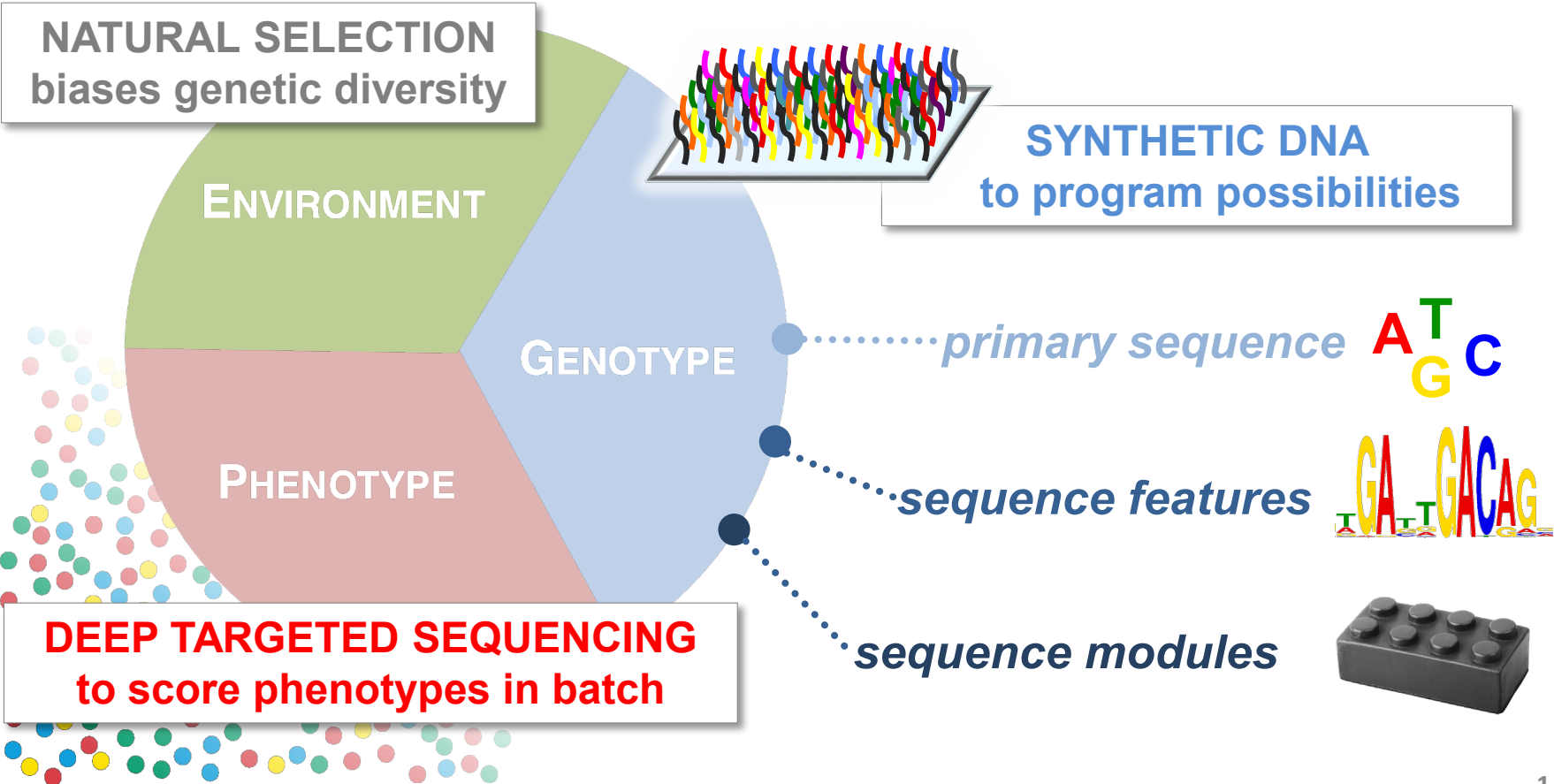
Guillaume Cambray

✉ cambray.guillaume@gmail.com

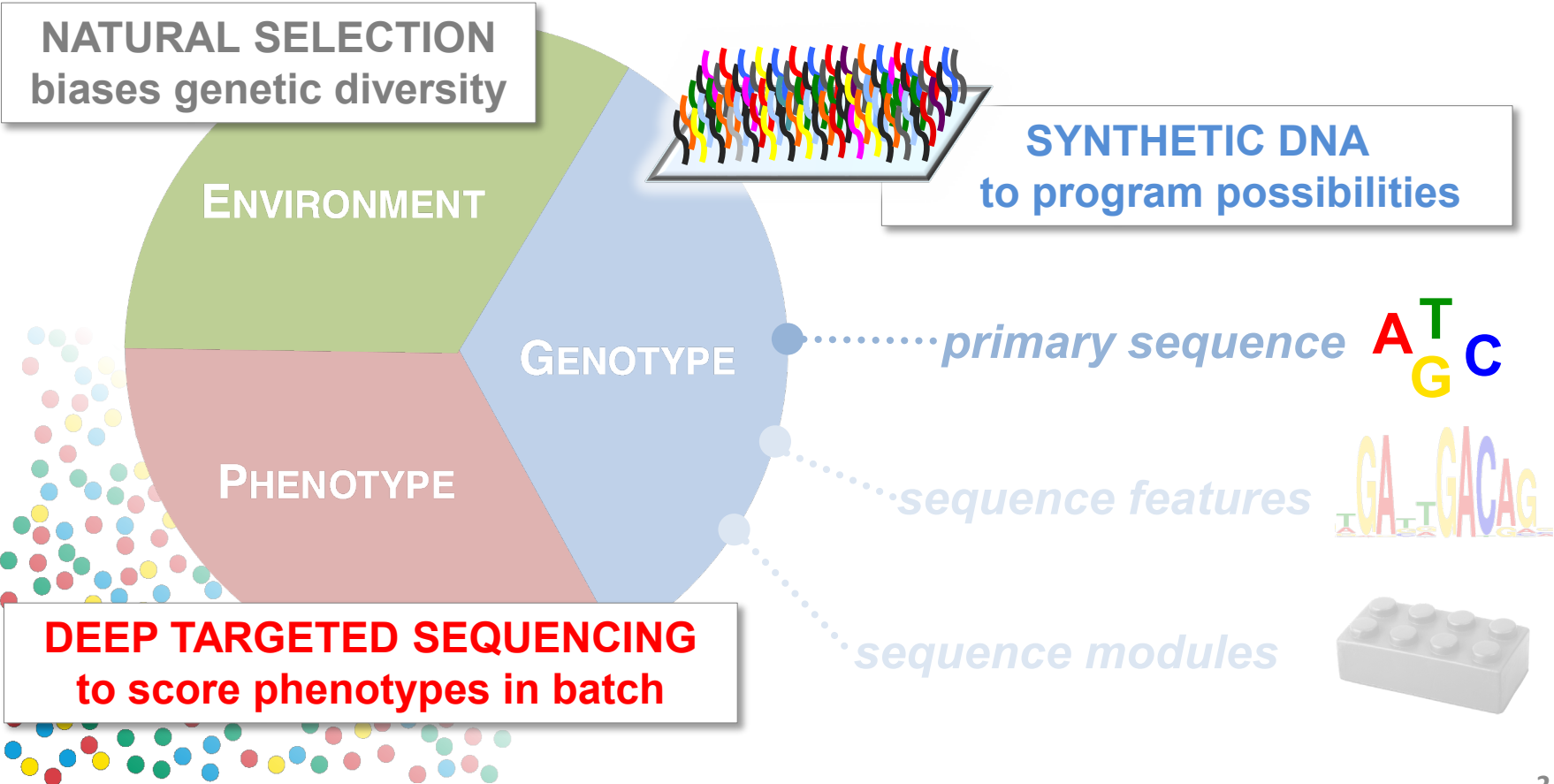
🐦 [@GiomCambray](https://twitter.com/GiomCambray)

**Journée thématique reseau Vectopole Sud
Montpellier – 24.11.23**

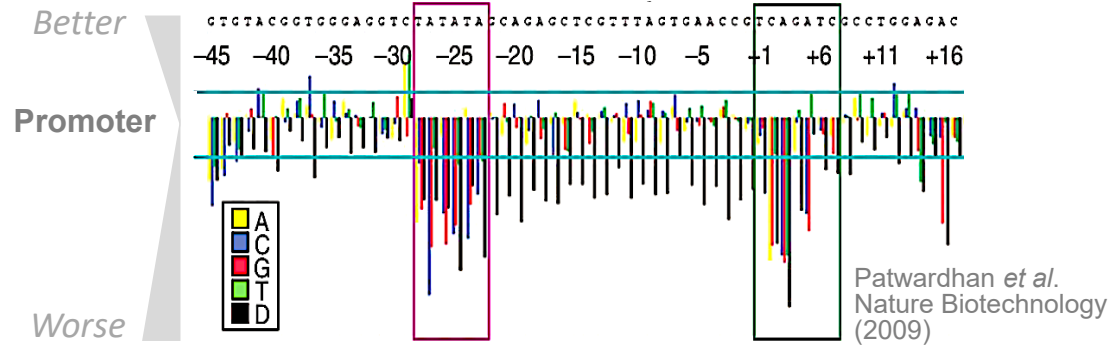
Manipulating molecular systems and sequence space



Manipulating molecular systems and sequence space

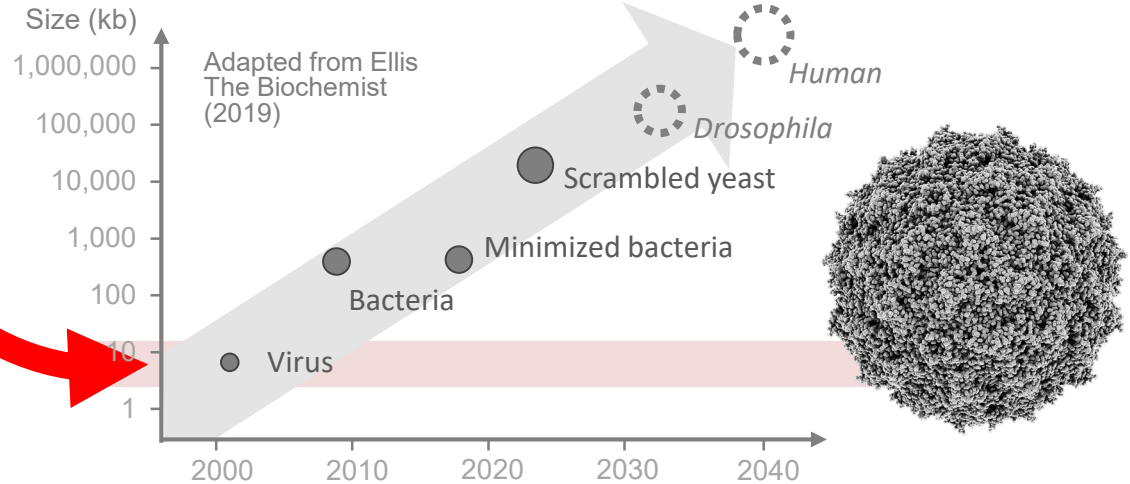


Toward genome-wide mutational scanning

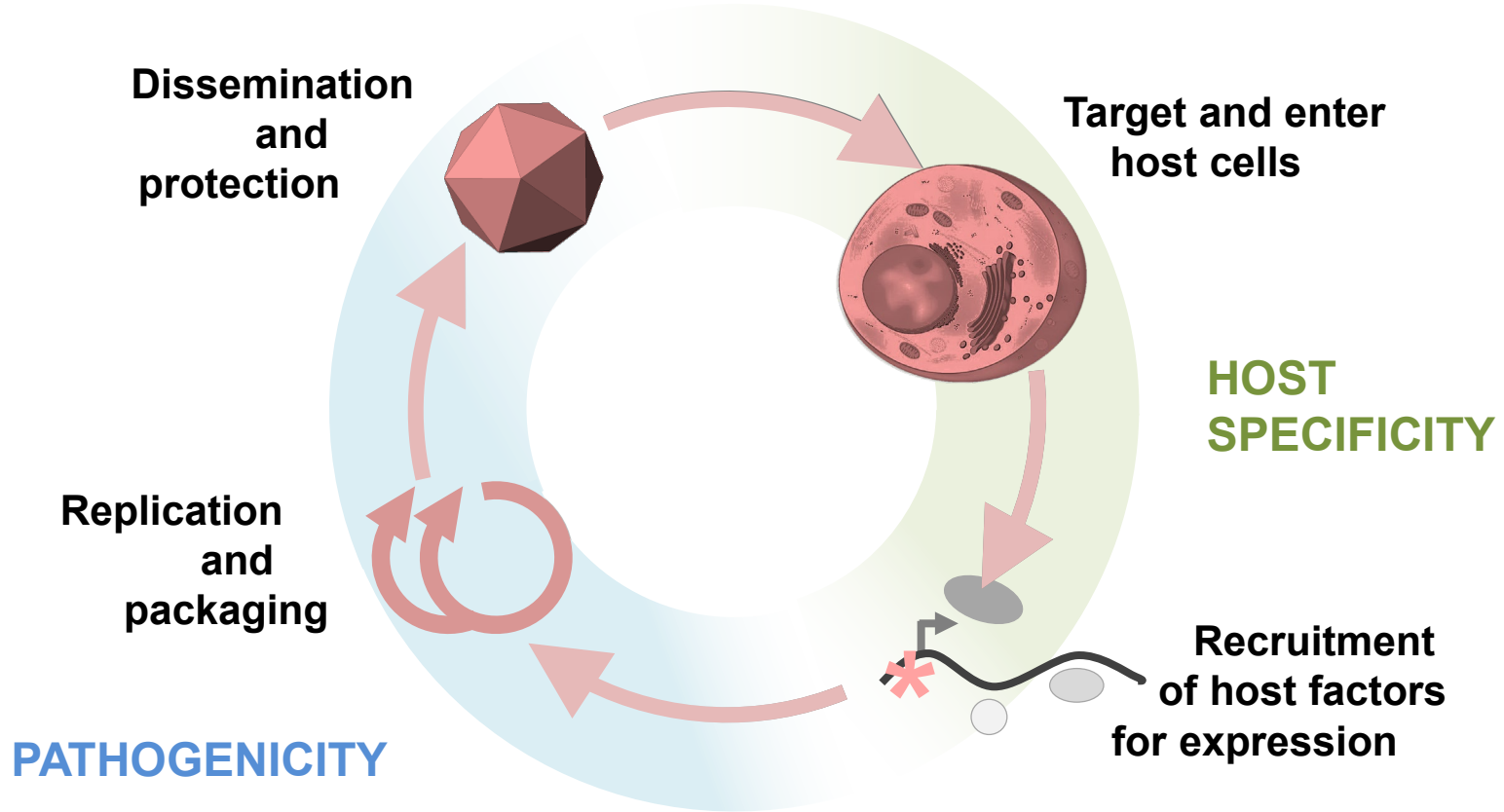


**Deep
mutational
scanning**

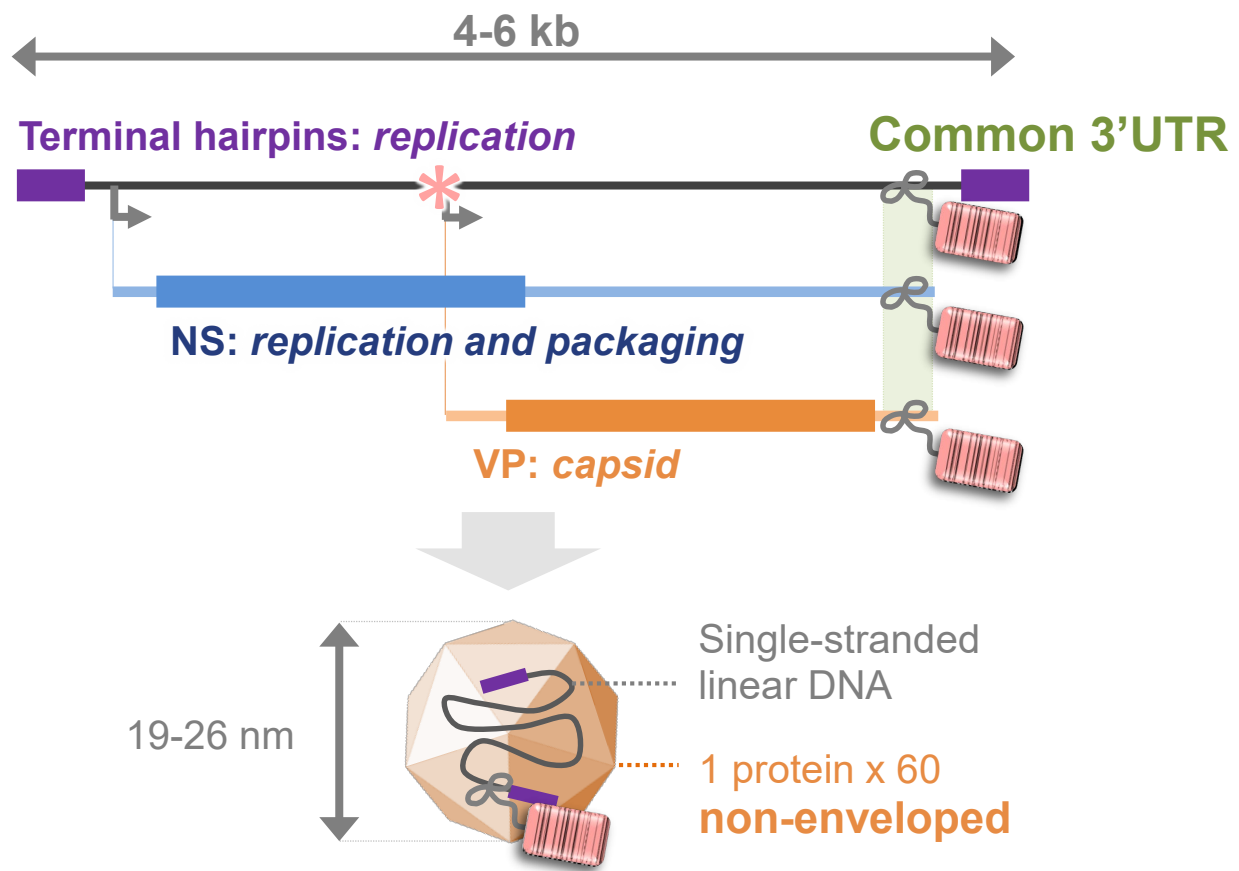
**Synthetic
Genomics**



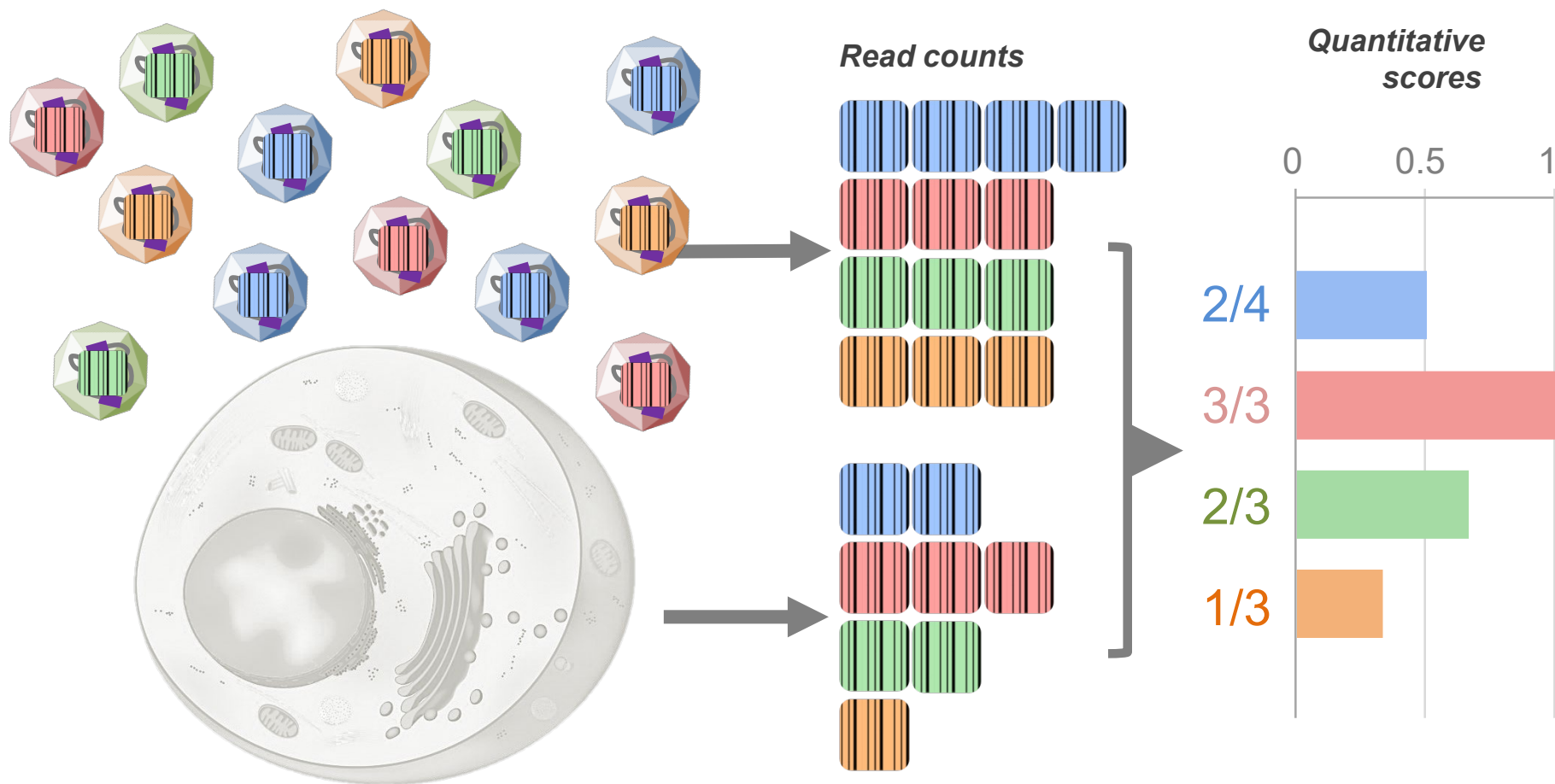
Small genome yet complex life cycle



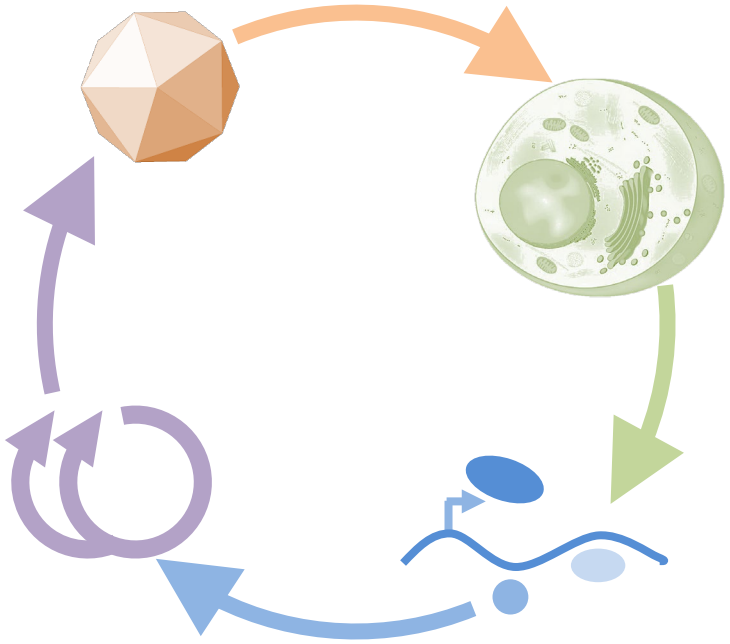
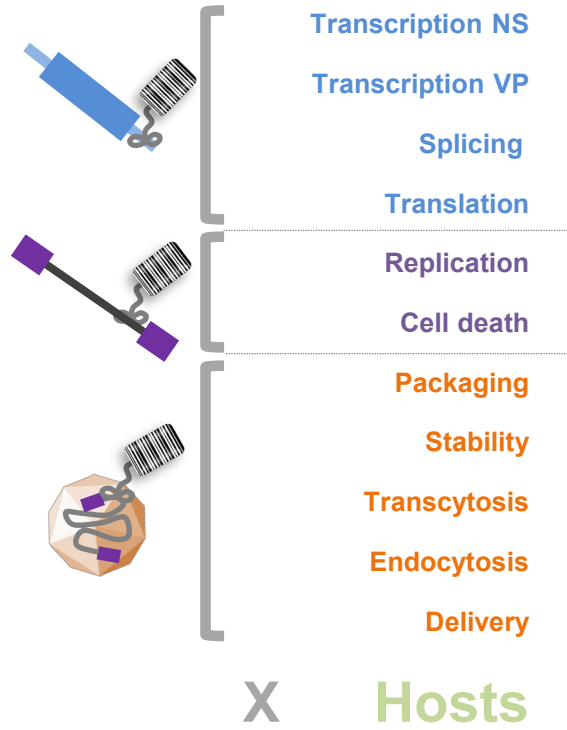
Densoviruses afford an elegant barcoding strategy



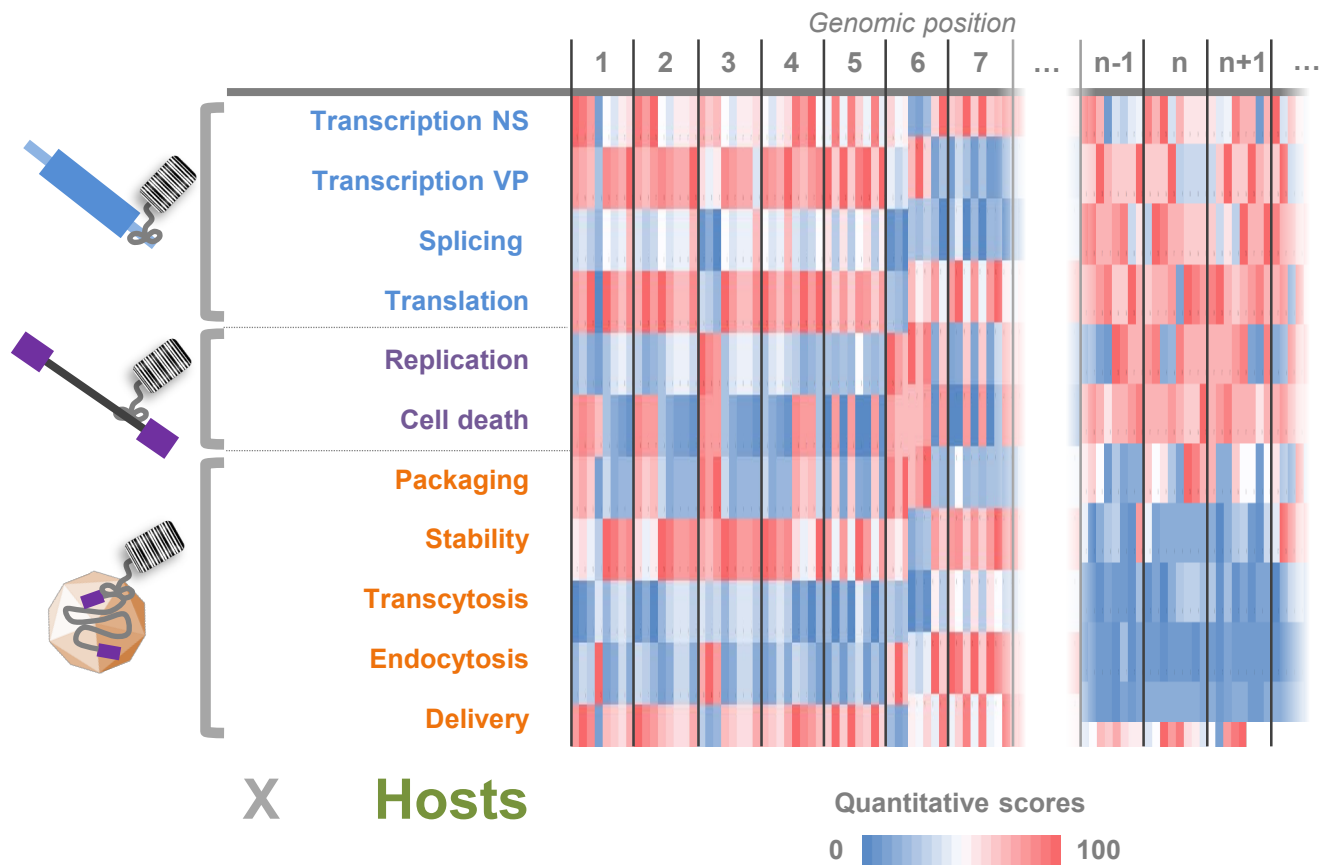
An example of barcode-based screening: *quantifying cell entry*



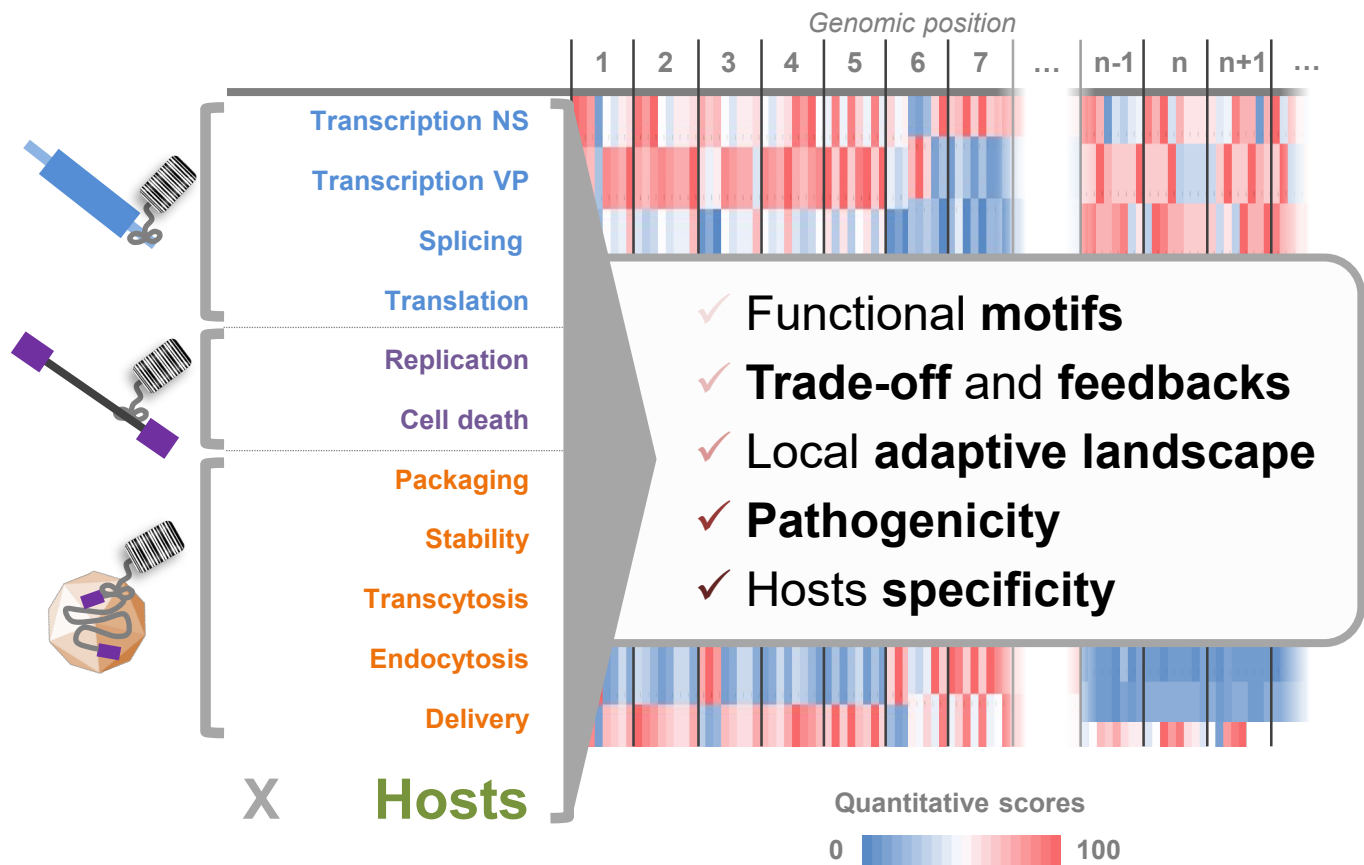
Deconstructing and reconstructing viral life cycle



Deconstructing and reconstructing viral life cycle

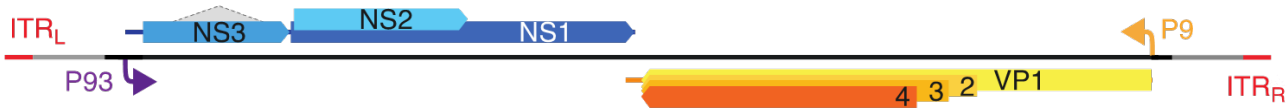


Deconstructing and reconstructing viral life cycle



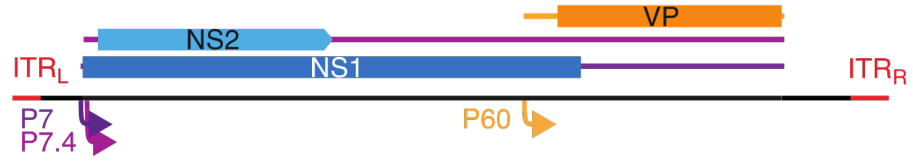
Reprogramming specificity of densoviruses

JcDV
6,032 nts



Fall armyworm

AaIDV
4,179 nts



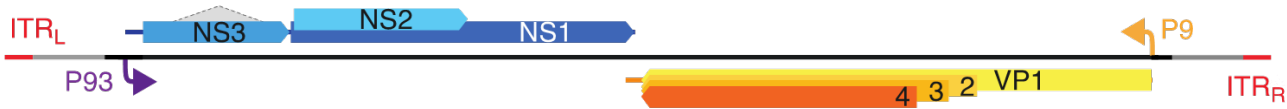
Tiger mosquito

Biological control

- Safety
- Reprogramming
- Pathogenicity
- Specificity
- Durability

Reprogramming specificity of densoviruses

JcDV
6,032 nts

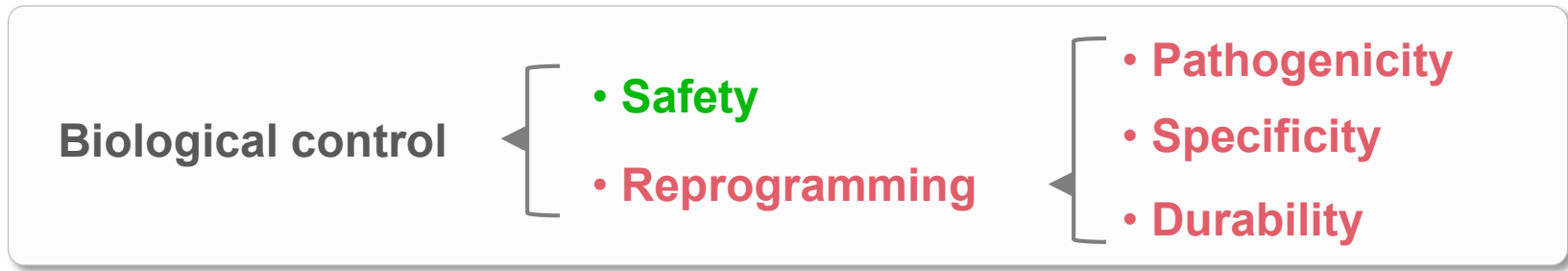


Fall armyworm

AaIDV
4,179 nts

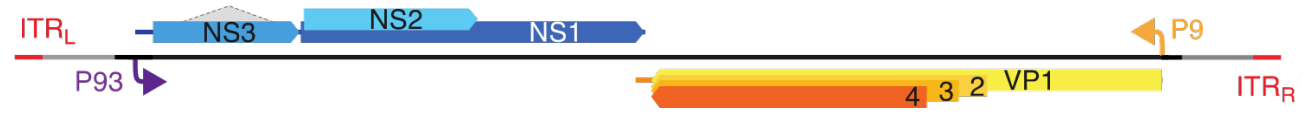


Tiger mosquito



Reprogramming specificity of densoviruses

JcDV
6,032 nts



Fall
armyworm

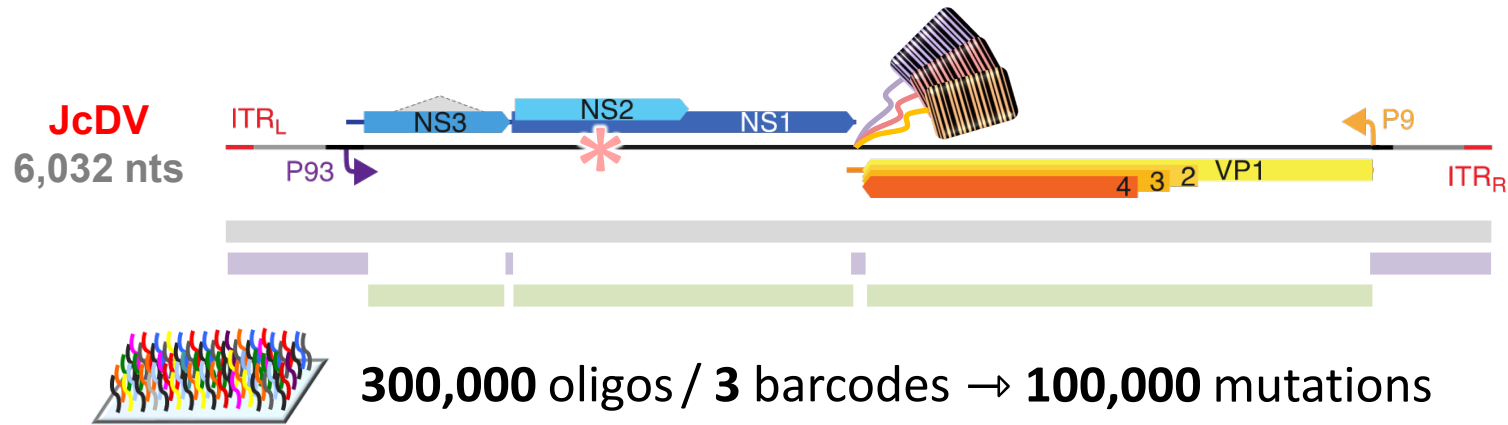


**Thomas
Labadie**
Post-doc



**Agah
Ince**
IE

Mutational strategy : SNPs and beyond



Genome-wide

- single nucleotide **substitutions**

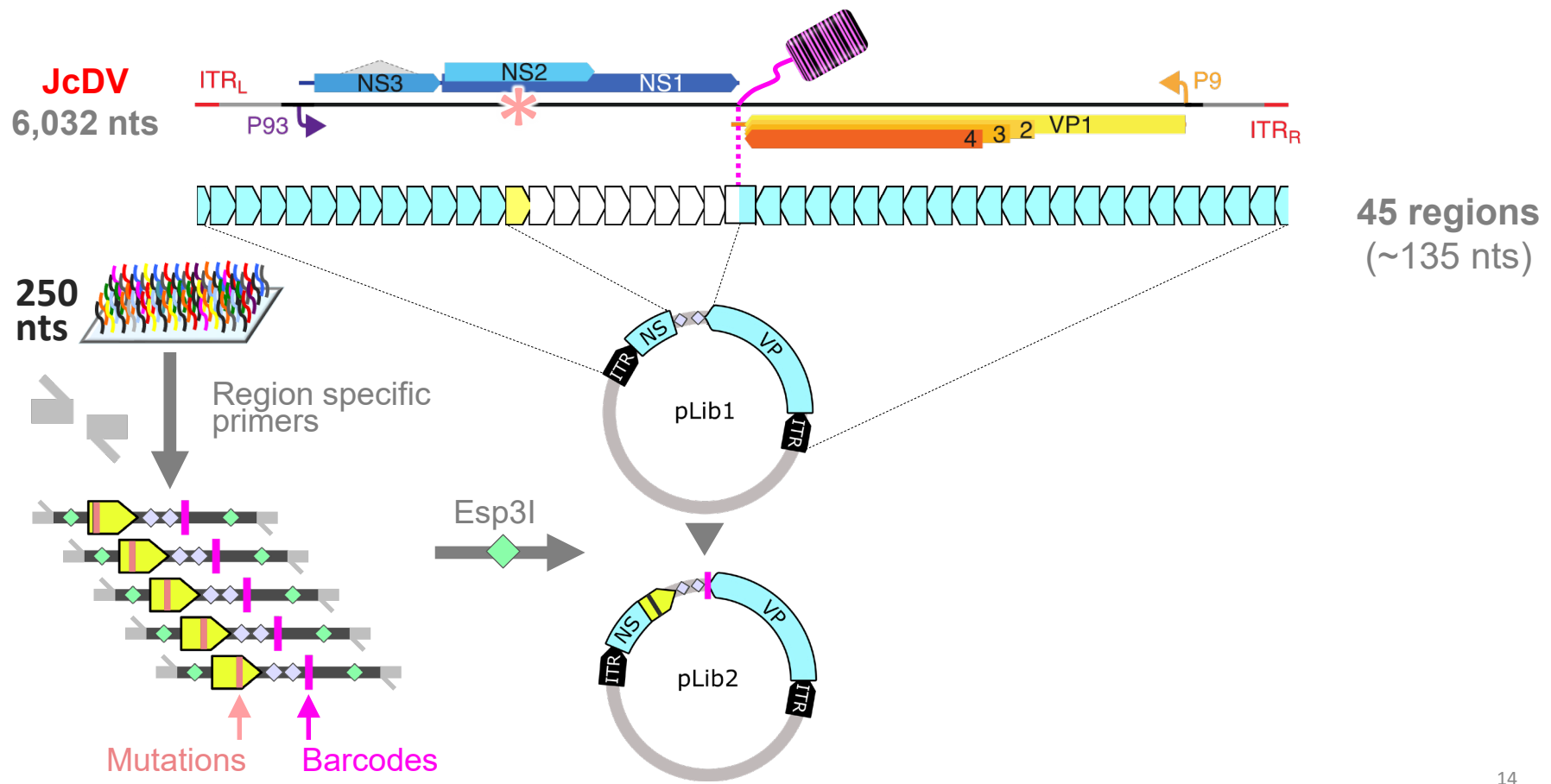
Non-coding

- **double** nucleotide **substitutions** around **Promoters**
- **single** nucleotide **insertions** and **deletions**
- **6-nucleotides deletions**
- **5 & 10-nucleotides insertions**

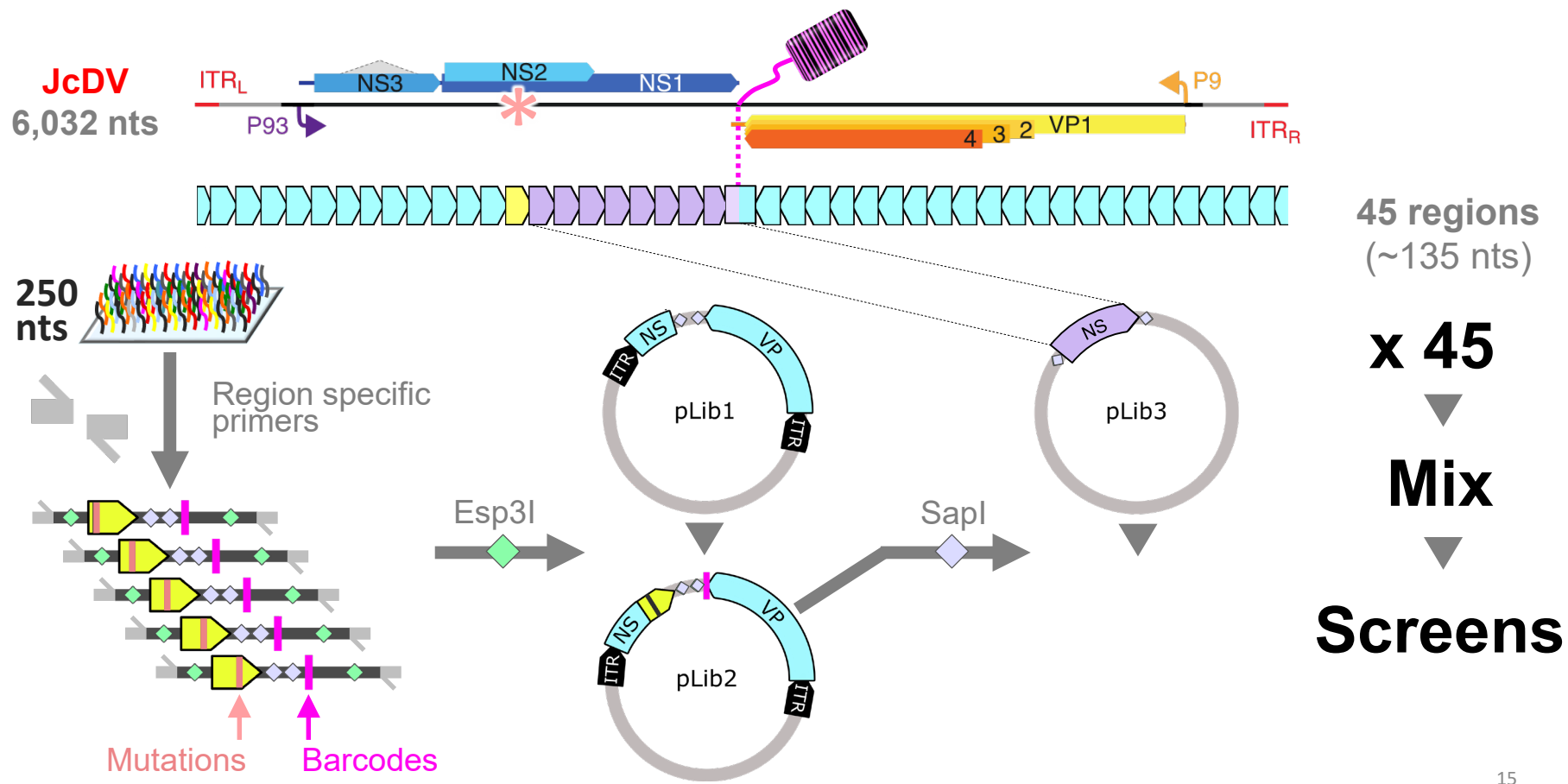
Coding

- **amino-acid substitutions** (best codon)
- **synonymous** codon **substitutions**
- **all codon substitutions** (**NS1**, **NS2**)
- codon **deletions**
- **amino-acid insertions** (best codon , **VP4**)
- C-tag & BsaI insertions (**4 codons**, **VP4**)

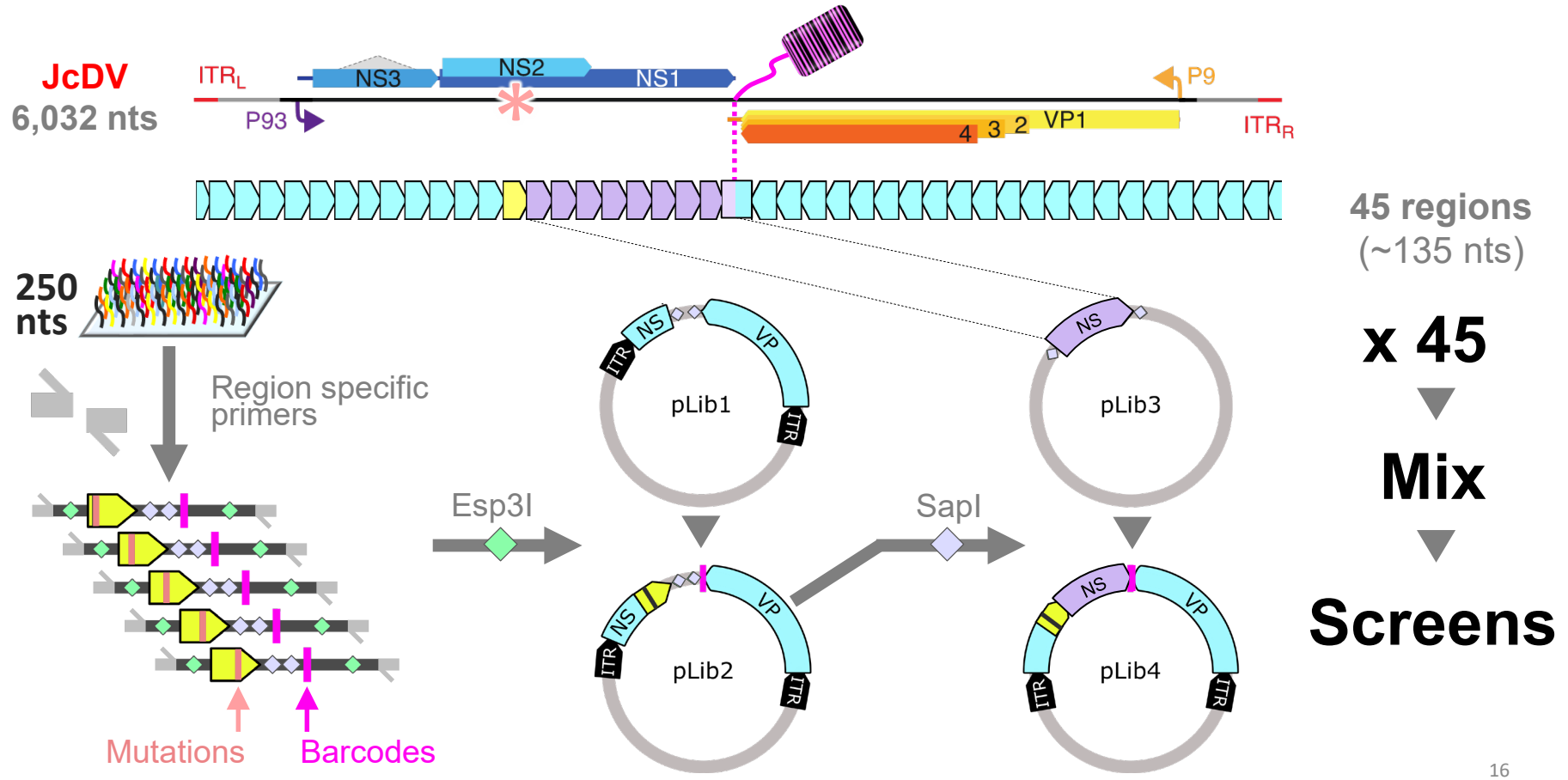
Linking mutations to barcodes : a complex construction strategy



Linking mutations to barcodes : a complex construction strategy

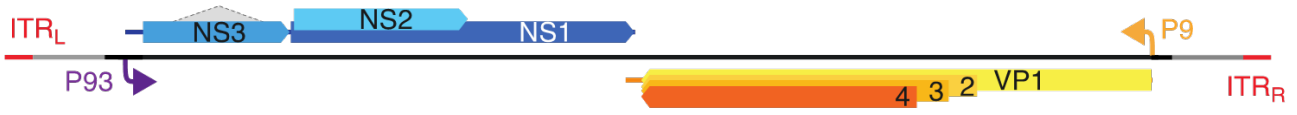


Linking mutations to barcodes : *easier said than done...!!!*



Linking mutations to barcodes : *easier said than done...!!!*

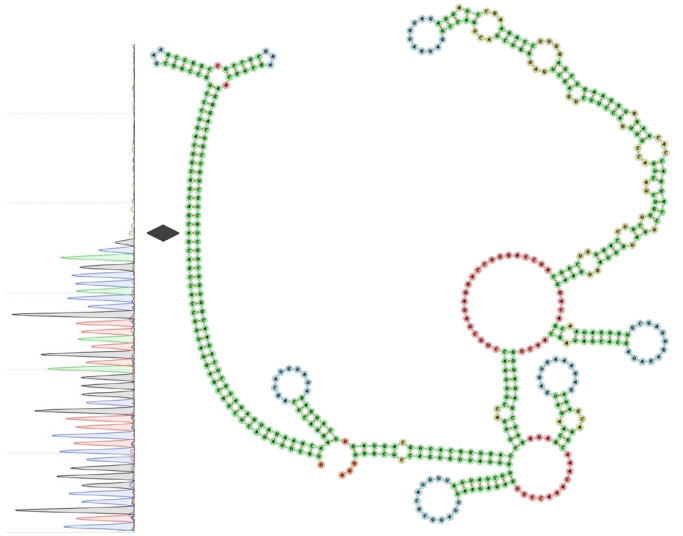
JcDV
6,032 nts



45 regions
(~135 nts)



471 nts Inverted Terminal Repeats



**130 nts Terminal Hairpins + other structures
required for replication and packaging**



Hard to replicate

50 nts direct repeats

Very unstable in *E. coli*

x 45



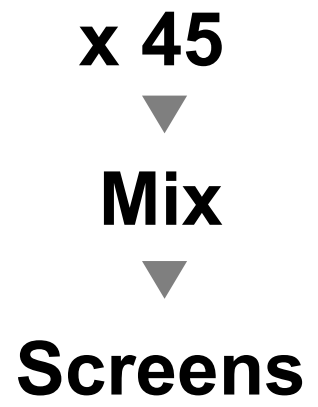
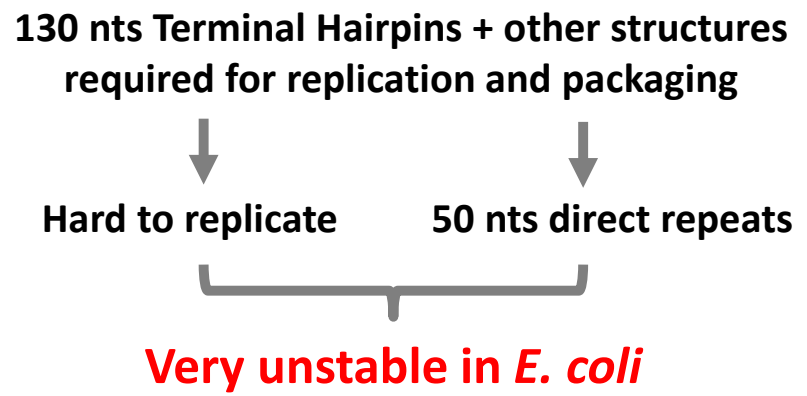
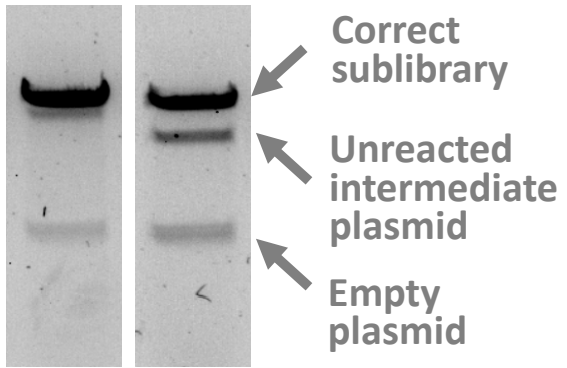
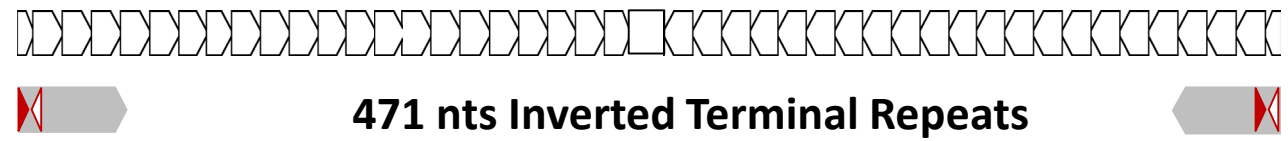
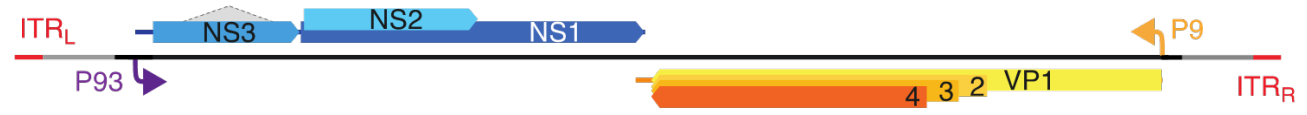
Mix



Screens

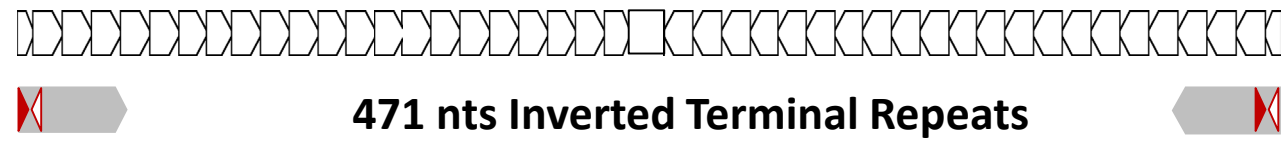
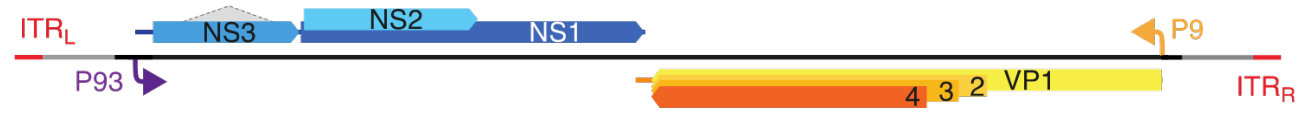
Linking mutations to barcodes : *easier said than done...!!!*

JcDV
6,032 nts



Linking mutations to barcodes : *easier said than done...!!!*

JcDV
6,032 nts



45 regions
(~135 nts)

130 nts Terminal Hairpins + other structures
required for replication and packaging

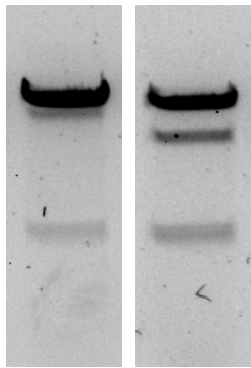
Hard to replicate 50 nts direct repeats

Very unstable in *E. coli*

x 45

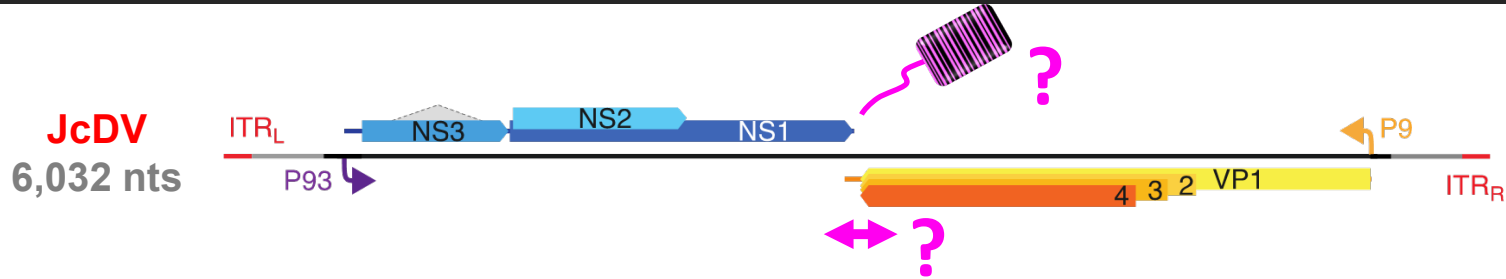
Mix

Screens



Correct sublibrary
Unreacted intermediate plasmid
Empty plasmid

A serendipitous side story about transcription fidelity



Screen for 16 nts barcodes location with low impact

Deep sequencing screens :

✓ *Viral replication*

✓ *RNA abundance*

✓ *Transcription termination*



DNA



RNA



High Errors
DNA << RNA

A pipeline to detect rare mutation

Errors = Mutations + Sequencing Errors

10^{-8} (DNA)

10^{-6} (RNA)

10^{-3}

(illumina)

reference



read 1



read 2



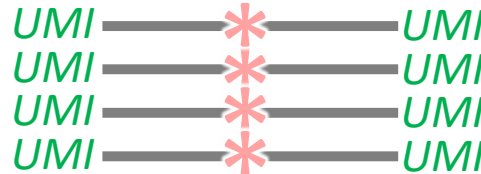
read merged



UMI-based

read cluster

(min = 3)



consensus

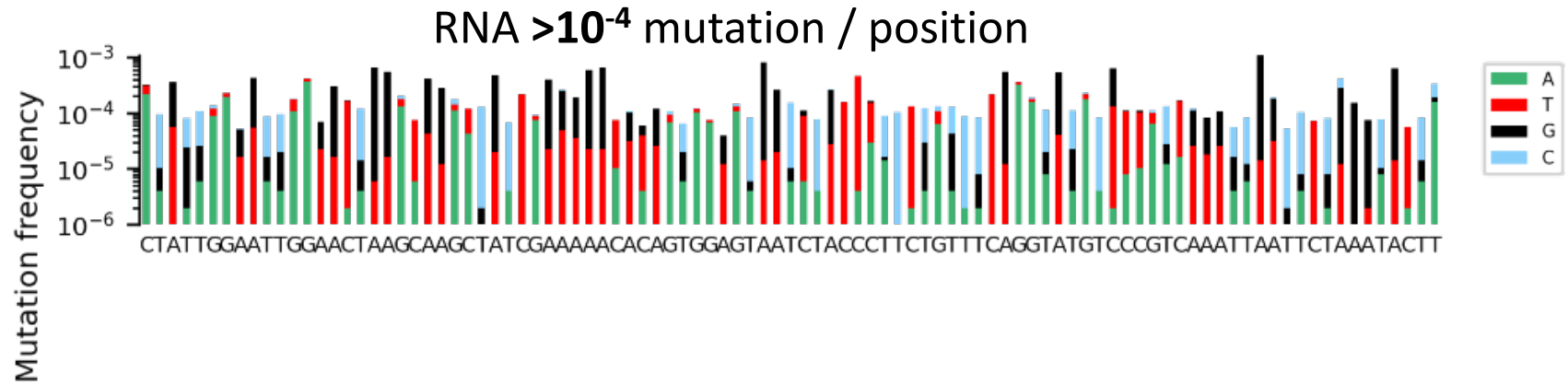


Universal **M**olecular **I**dentifier
(2x random 8 nts barcode)

→ Uniquely tag each molecule

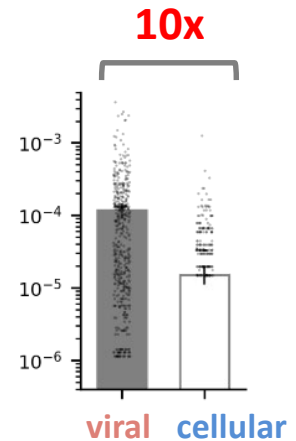
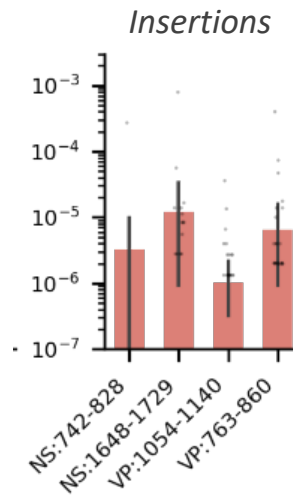
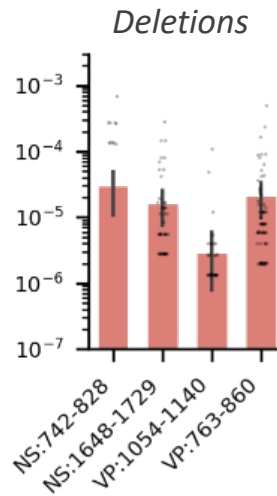
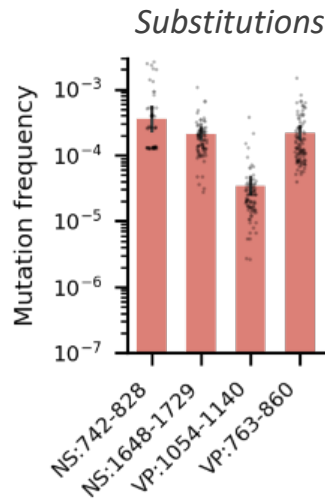
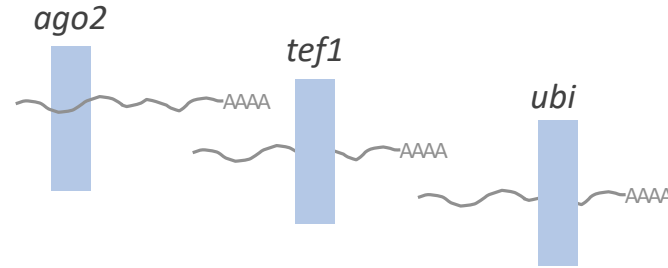
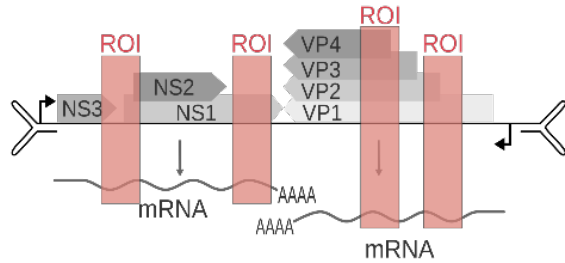
- ✓ Detect PCR duplicates
- ✓ Enable error correction

High RNA mutation rates in viral transcript

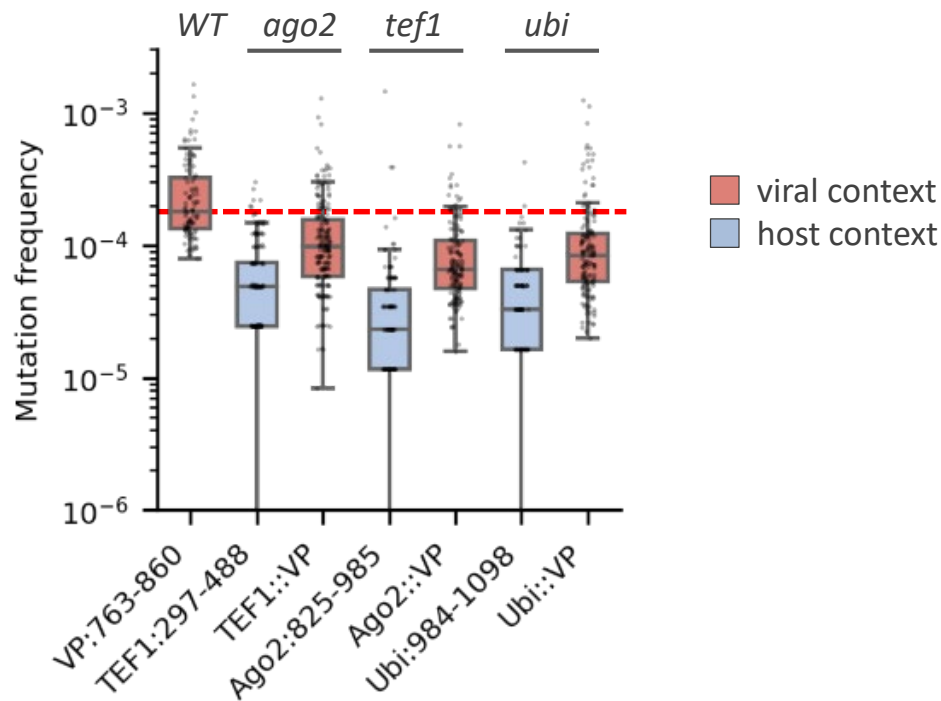
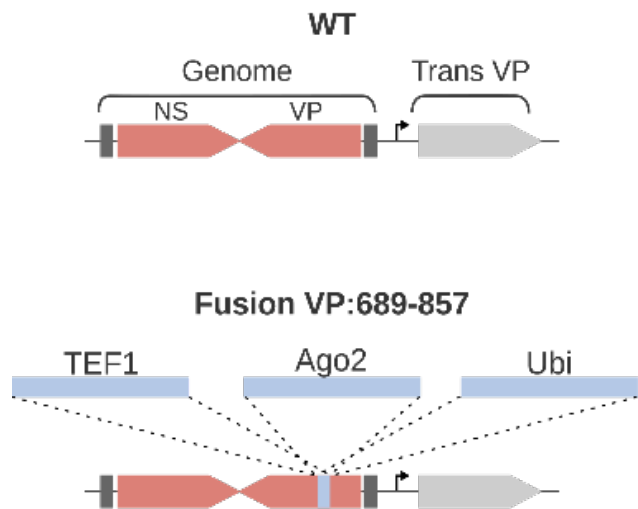


- ✓ Variations between positions
- ✓ Variation in mutation identities

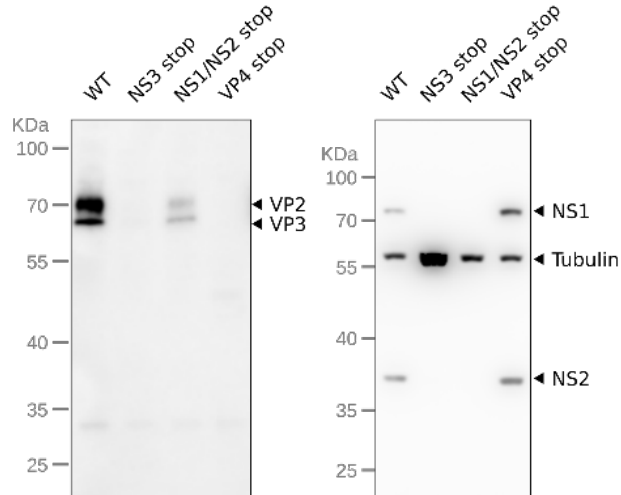
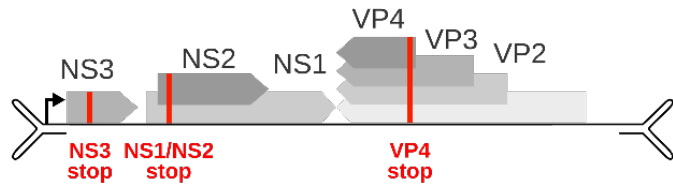
Viral more mutated than cellular transcripts



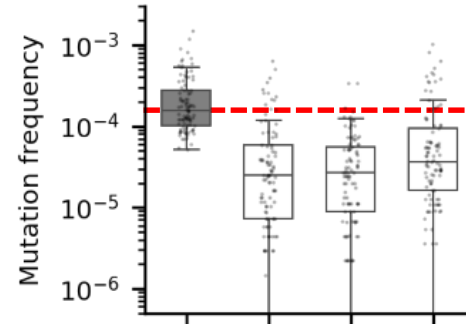
Viral context – not sequences – drives lower fidelity



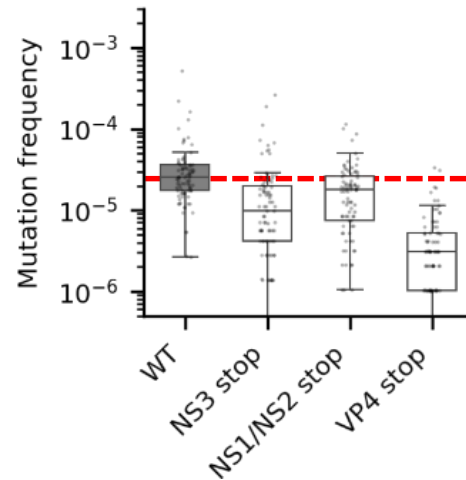
Viral proteins cause low transcription fidelity



➔ **Decreased VP in all mutants**



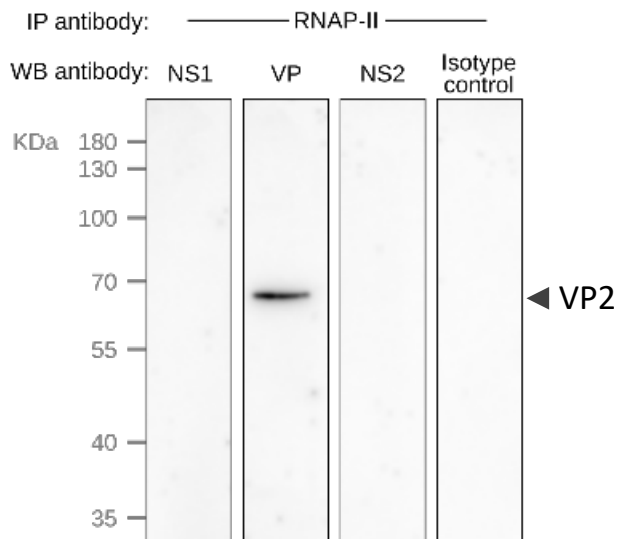
High mutated
viral region



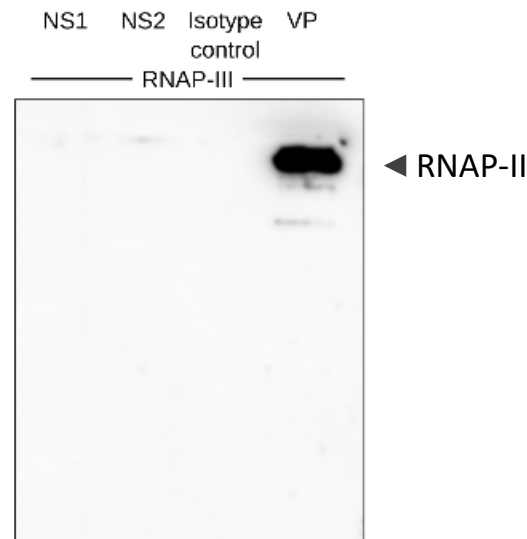
Low mutated
viral region

VP proteins interacts with host RNA polymerase (1)

Co-Immuno Precipitation with RNAP-II

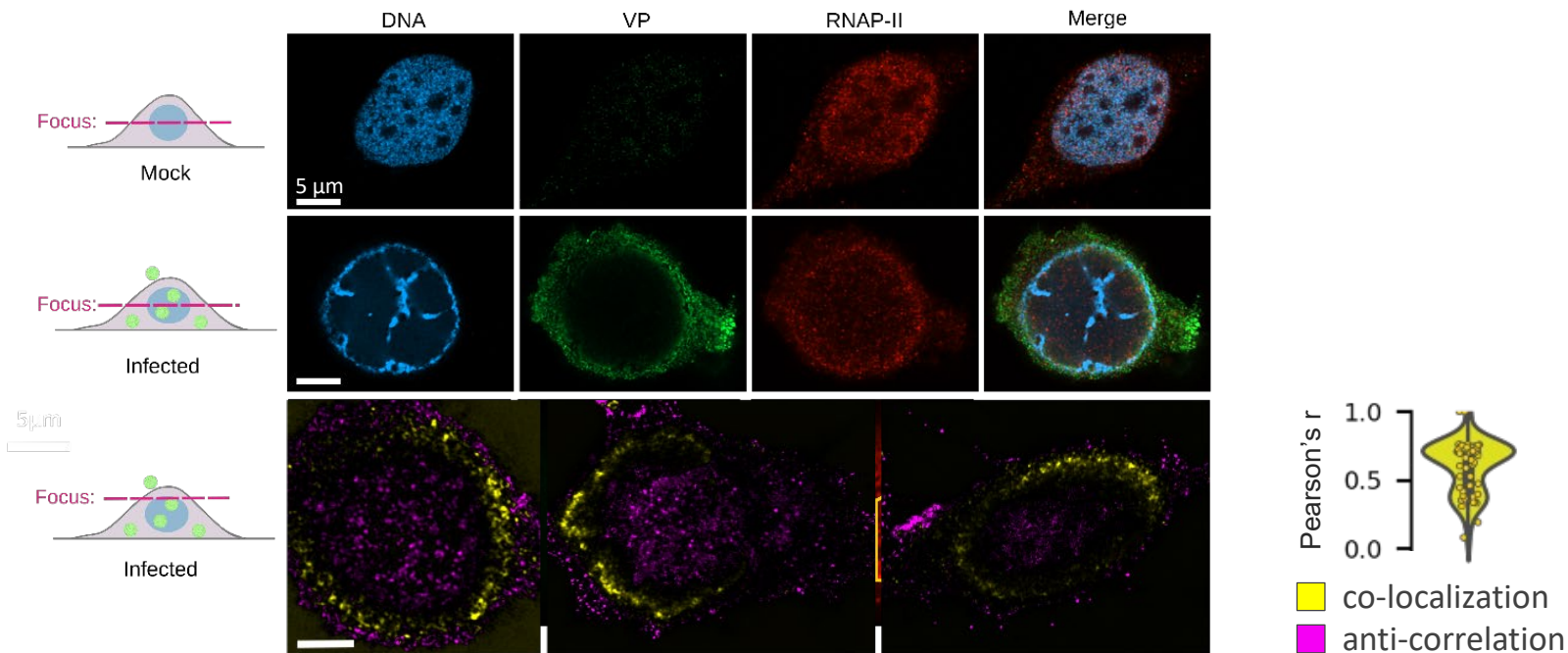


Co-Immuno Precipitation with viral proteins

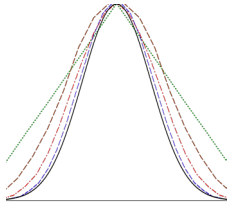


VP proteins interacts with host RNA polymerase (2)

Confocal immunofluorescence microscopy



Low transcription fidelity as a phenotypic mutator



- Increase genetic variation
- Non-heritable
- Cope with fluctuating environment

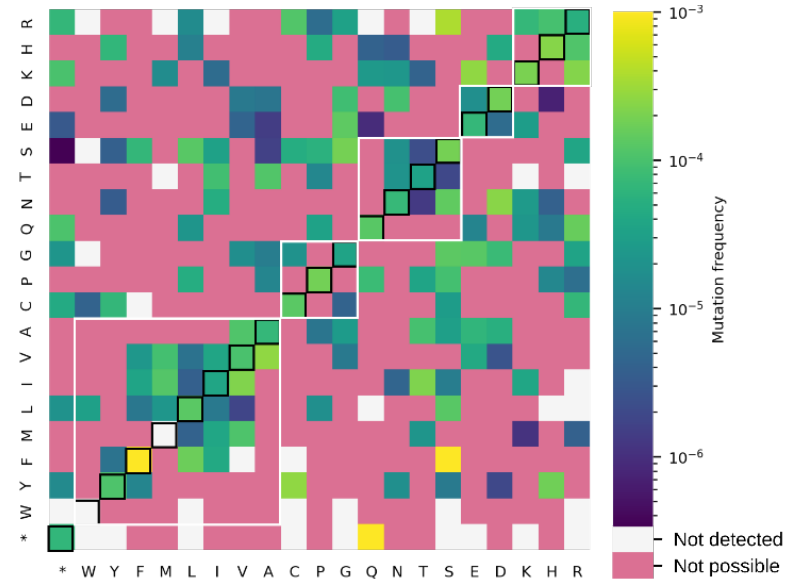
1 in 3 viral transcripts is mutated !

- Gene expression levels
- Immune evasion ?
- Protein (function, stability, ...)

3% VP4 proteins → 2 mutations / capsid

 Genome-wide mutational scanning data

Consequences for biocontrol ?



Team

Synthetic, Functional and Evolutionary Genomics

Thomas Labadie

Agah Ince



Elsa Fristot

Estelle Grosjean

Charbel Choufani

Alexandre Deloupy

Amani Ghousein

Lukas Brichet

Collaborators

Mylène Ogliastro

Anne-Sophie Gosselin



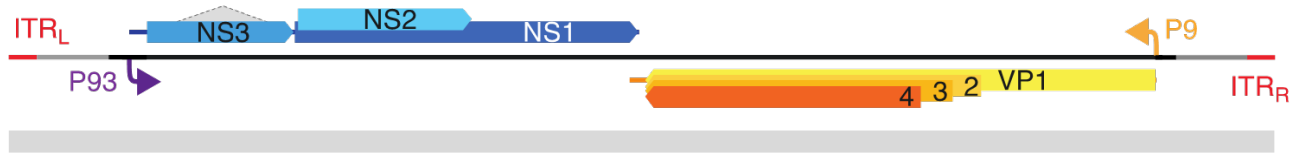
And you

for your attention !



Mutational strategy : SNPs and beyond

JcDV
6,032 nts



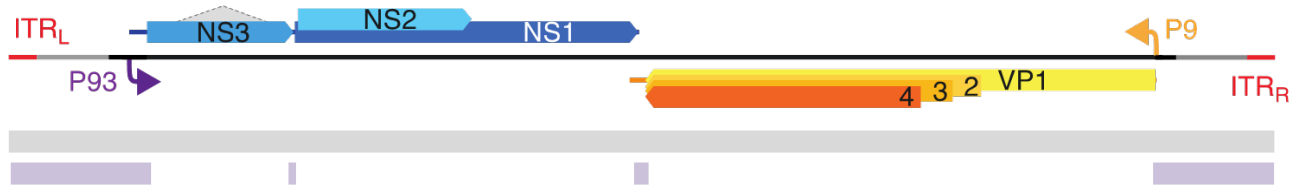
Genome-wide

- single nucleotide **substitutions**

...GCAT**T**AGAC..
 ▼
·
...GCAT**A**AGAC..
·
...GCAT**G**AGAC..
·
...GCAT**C**AGAC..
·

Mutational strategy : SNPs and beyond

JcDV
6,032 nts



Genome-wide

- single nucleotide **substitutions**

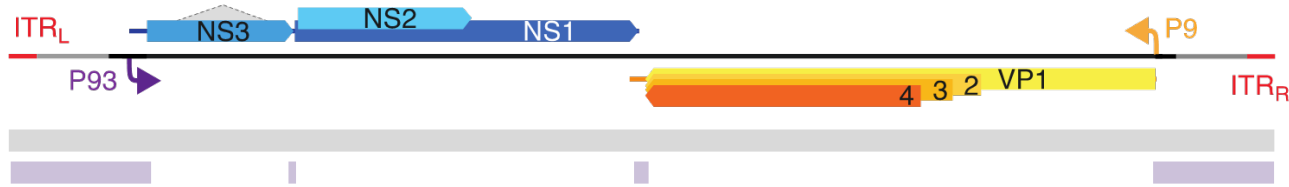
Non-coding

- **single** nucleotide **insertions** and **deletions**
- **double** nucleotide substitutions around **promoters**
- **6-nucleotides deletions**
- **5 & 10-nucleotides insertions**

```
...GCATTAGAC...  
      ▲  
...GCATTAGAC...  
      ▼  
...GCATTTAGAC...  
...GCATTAAGAC...  
...GCATTGAGAC...  
...GCATTCAGAC...
```

Mutational strategy : SNPs and beyond

JcDV
6,032 nts



Genome-wide

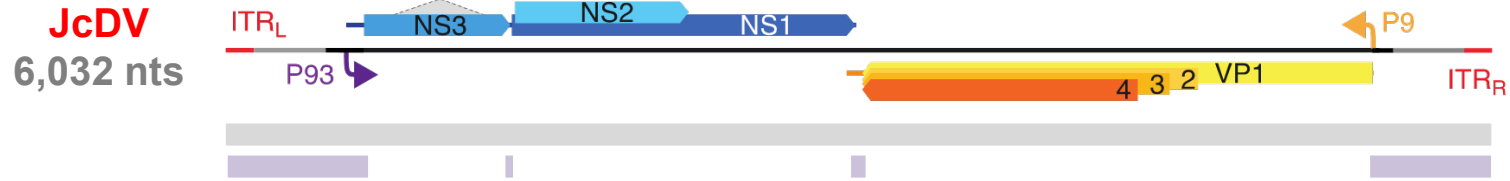
- single nucleotide **substitutions**

Non-coding

- **single** nucleotide **insertions** and **deletions**
- **double** nucleotide substitutions around **promoters**
- 6-nucleotides deletions
- 5 & 10-nucleotides insertions

···GCATTAGAC···
 ↓
· · ·GTATAAGAC···
·
···GTATGAGAC···
·
···GTATCAGAC···
·
···GAATAAGAC···
·
···GAATGAGAC···
·
···GAATCAGAC···
·
···GGATAAGAC···

Mutational strategy : SNPs and beyond



Genome-wide

- single nucleotide **substitutions**

Non-coding

- **single** nucleotide **insertions** and **deletions**
- **double** nucleotide substitutions around **promoters**
- **6-nucleotides deletions**
- 5 & 10-nucleotides insertions

...G**T**A**T**A**G**A**G**A**C**...

.

...G**T**A**T****G**A**G**A**C**...

.

...GC**A**T**T**A**G**A**C**...

..▼

...GC**A**T**T**A**G**A**C**...

.

...G**C**A**T**T**A**G**A**C...

.

...G**A**A**T**A**G**A**C**...

.

...G**A**A**T****G**A**G**A**C**...

.

...G**A**A**T****C**A**G**A**C**...

.

...G**C**A**T**T**A**G**A**C...

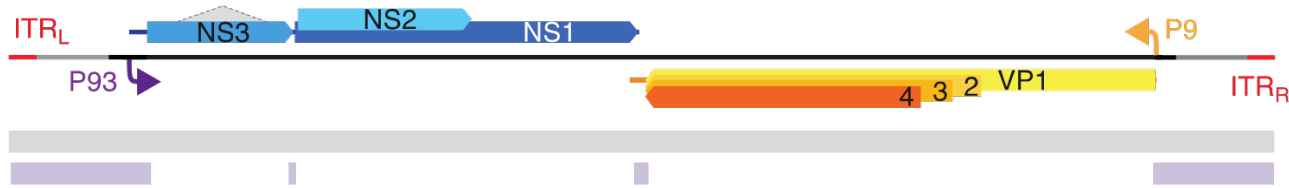
.

...G**G**A**T**A**G**A**C**...

54

Mutational strategy : SNPs and beyond

JcDV
6,032 nts



Genome-wide

- single nucleotide **substitutions**

Non-coding

- **single** nucleotide **insertions** and **deletions**
- **double** nucleotide substitutions around **promoters**
- **6-nucleotides deletions**
- **5 and 10-nucleotides insertions**

```
...GCATTAGAC...  
      ▼  
...GCATTAGAC...  
.  
AGAGA  
...GTATAAGAC...  
.  
...GTATGAGAC...  
.  
...GTATCAGAC...  
.  
...GAATAAGAC...  
.  
...GAATGAGAC...  
.
```