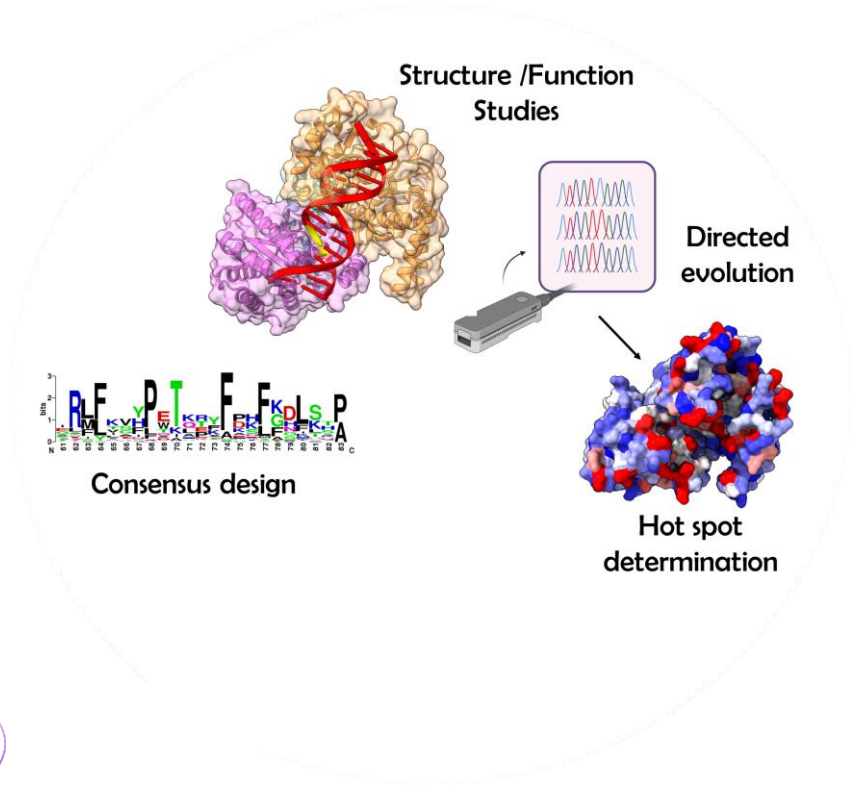
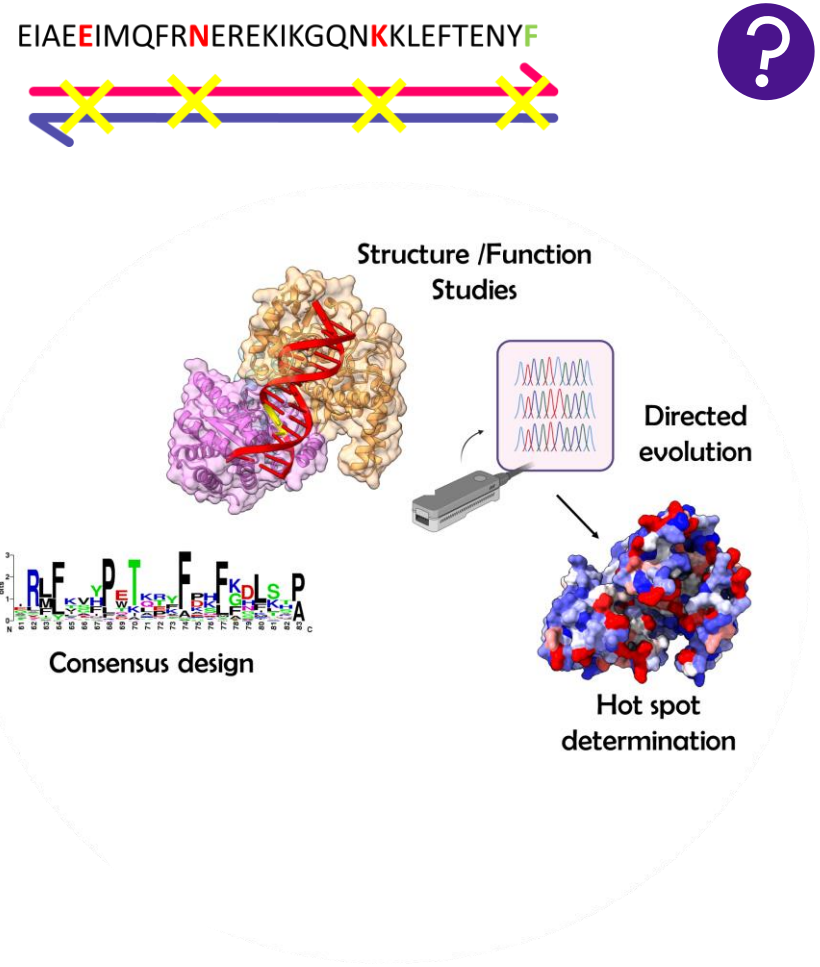
The Amuλett

Multi-Site Targeted Mutagenesis



Adèle Dramé-Maigné

The AmuλettMulti-Site Targeted Mutagenesis

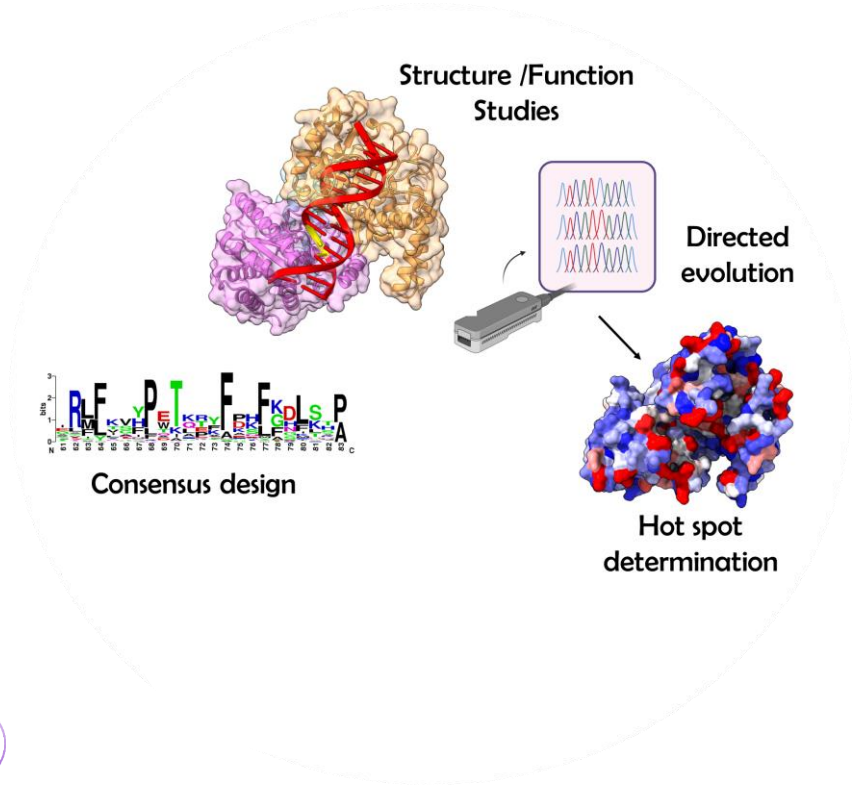


- Existing methods can be either time consuming and/or expensive (multiple PCRs, chemical reactions needed …)
- For a maximum efficiency : single strand template
- For a maximum efficacy : remove wild type

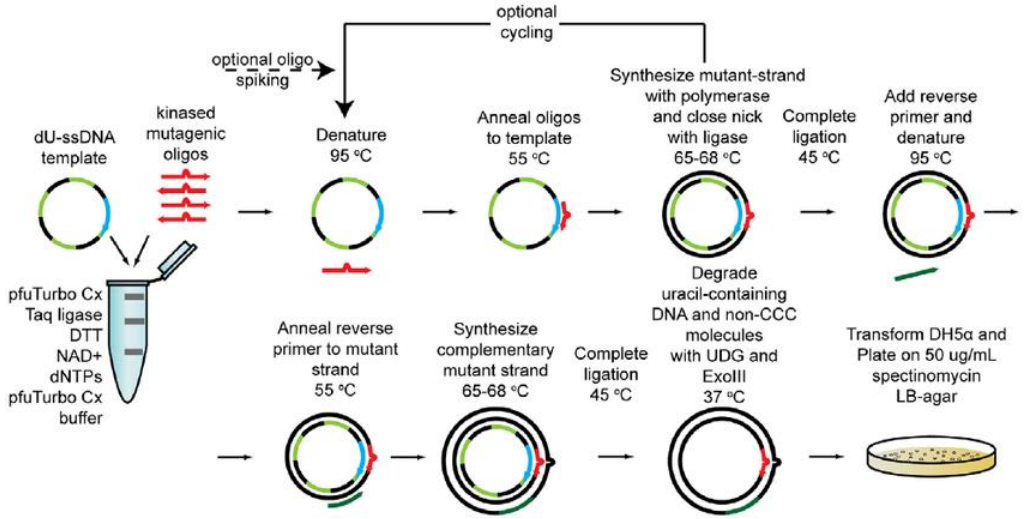
How to generate easily a transient single strand template ?

Adèle Dramé-Maigné

The AmuλettMulti-Site Targeted Mutagenesis

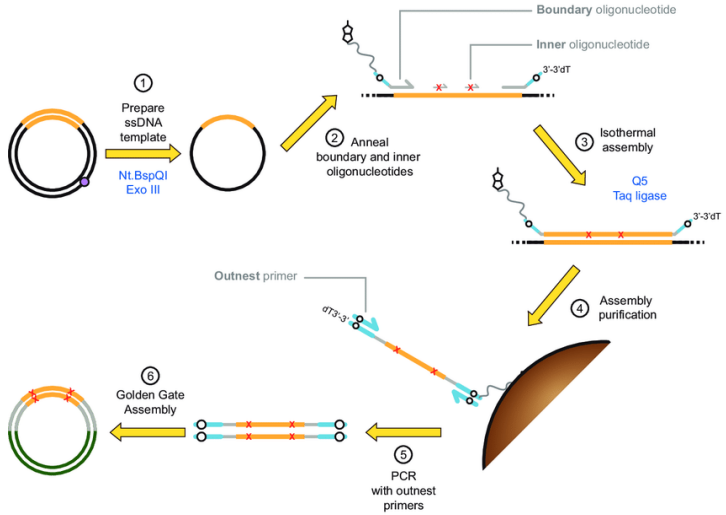


Pfunkel



Firnberg, Elad et al. PloS one 2012

Darwin Assembly

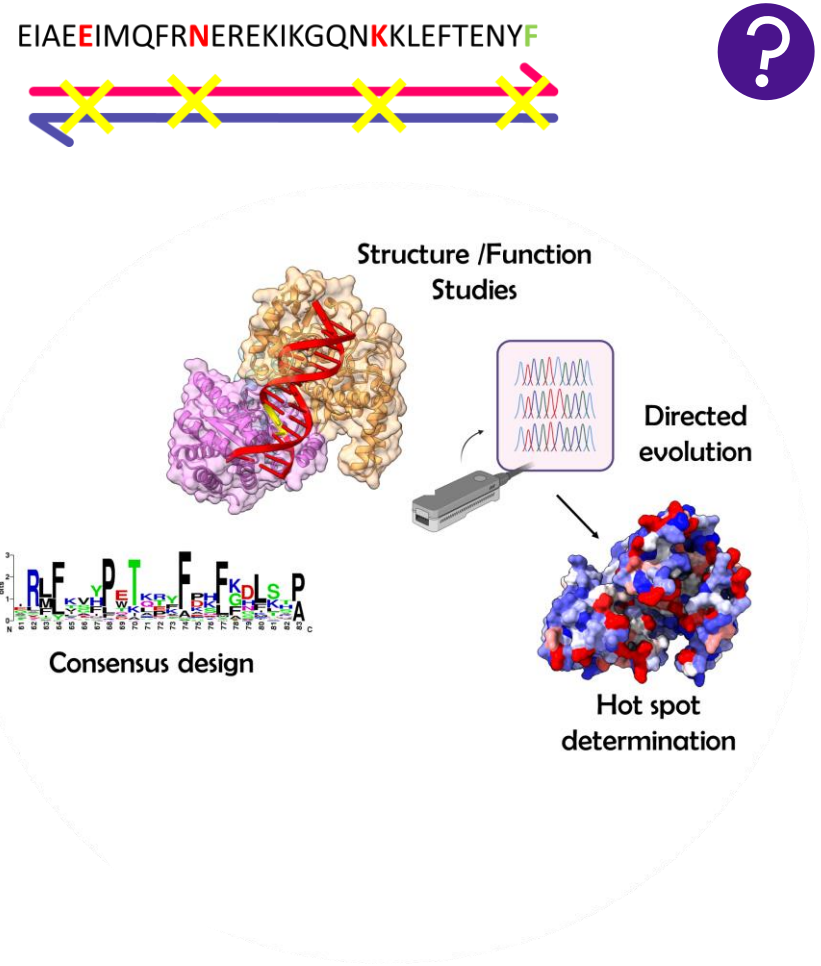


Christopher Cozens et al. NAR 2018

Adèle Dramé-Maigné

The AmuΛett

Multi-Site Targeted Mutagenesis



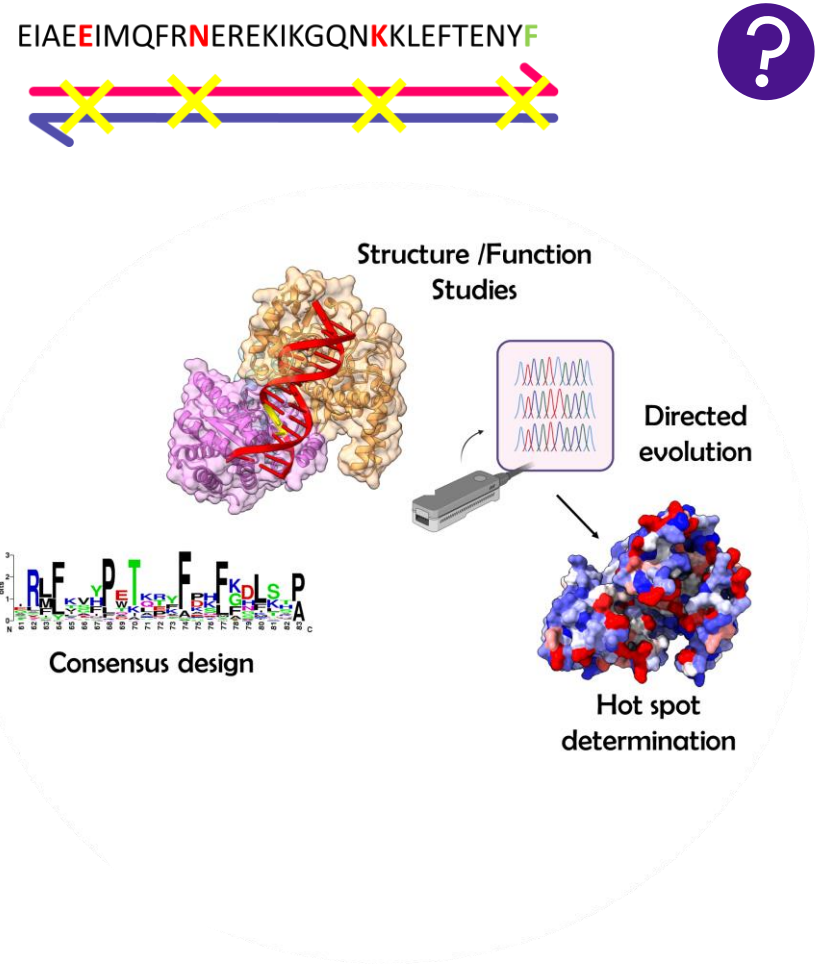
Pfunkel

- Need phages to produce the uracilated template
- Multiple steps

Darwin Assembly

- Need to have the right restriction sites in your plasmid
- Chemically modified oligos
- Plasmid not used directly for transformation

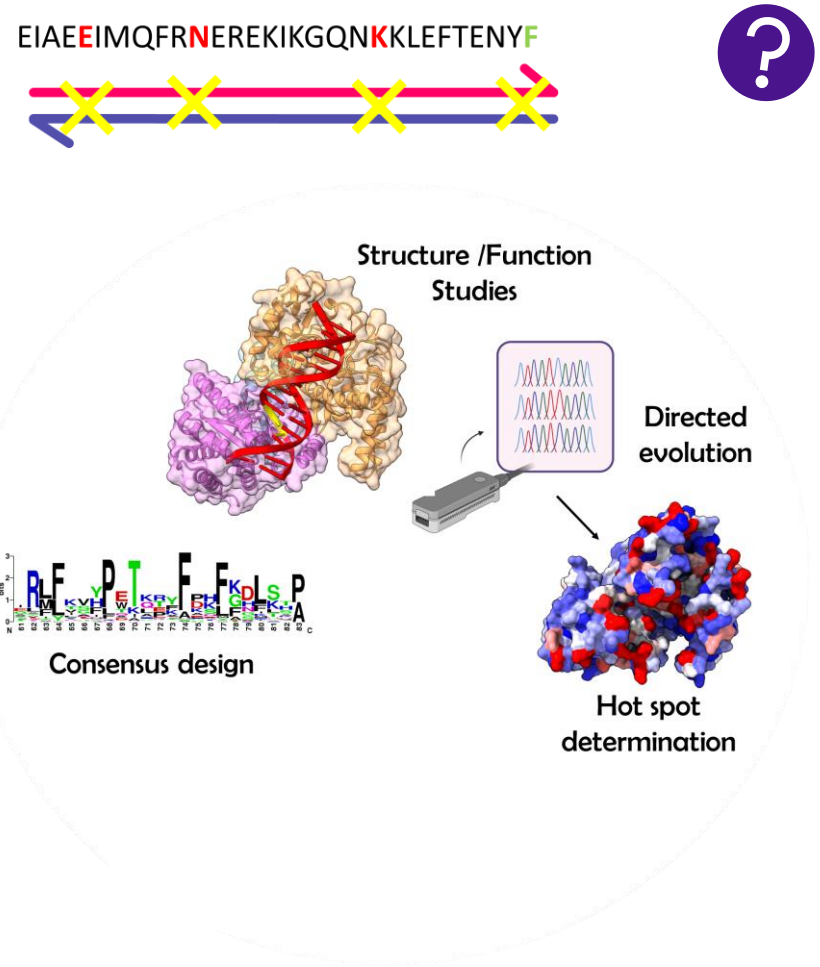
The AmuλettMulti-Site Targeted Mutagenesis



Annealing of
mutagenic oligos on
λ-exonulease generated
transient
template

Adèle Dramé-Maigné

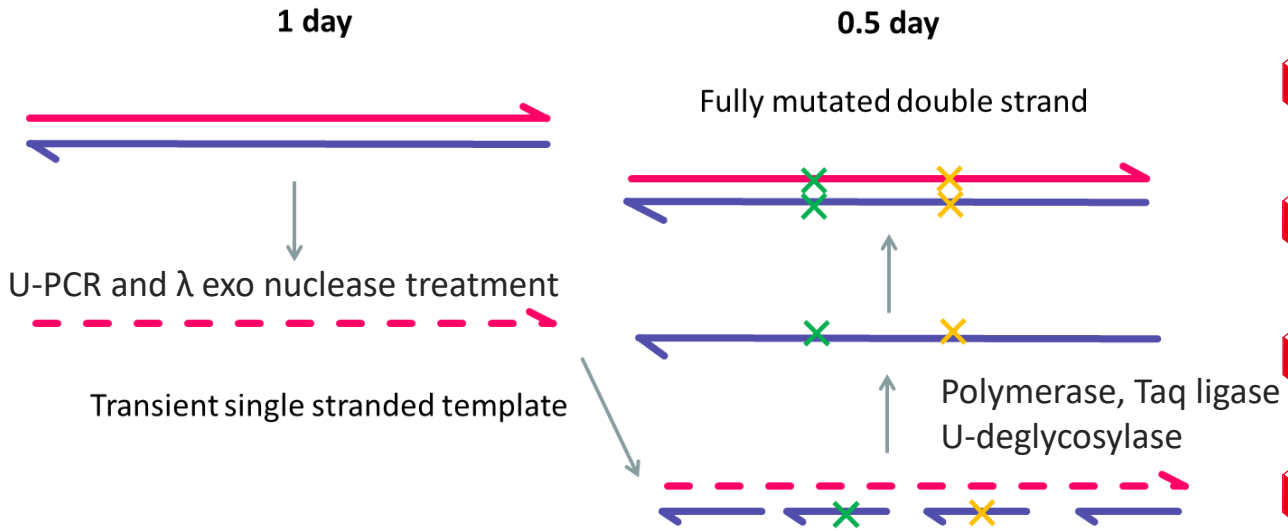
The AmuλettMulti-Site Targeted Mutagenesis



Fast **Cheap** **Efficient**

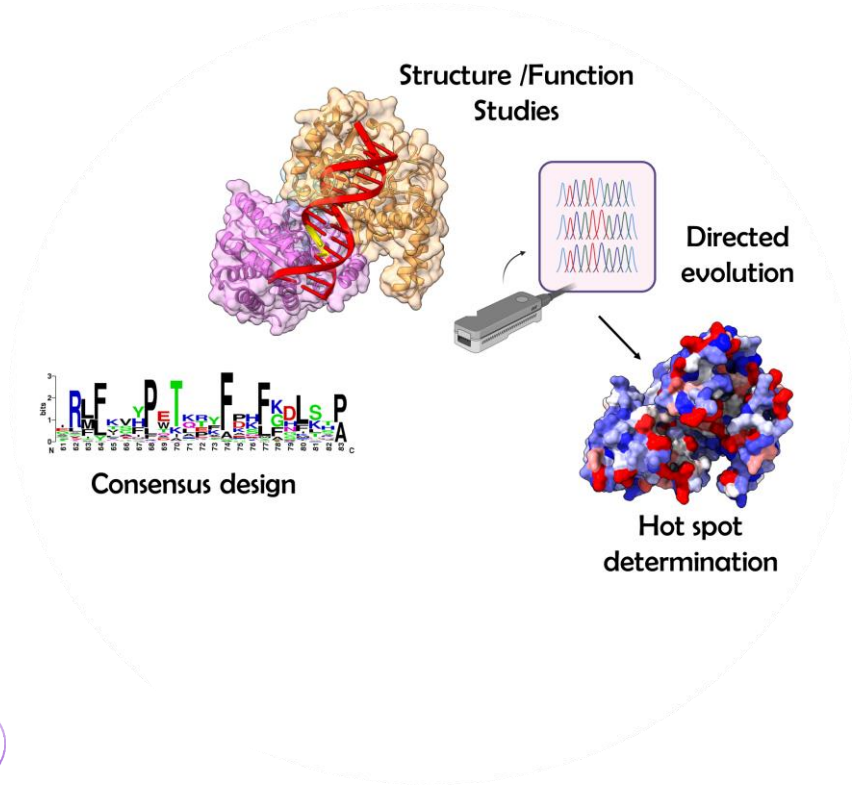
- Small oligos
- Few enzymes

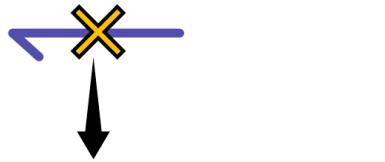
> 90% of mutations incorporated



Adèle Dramé-Maigné

The AmuλettMulti-Site Targeted Mutagenesis



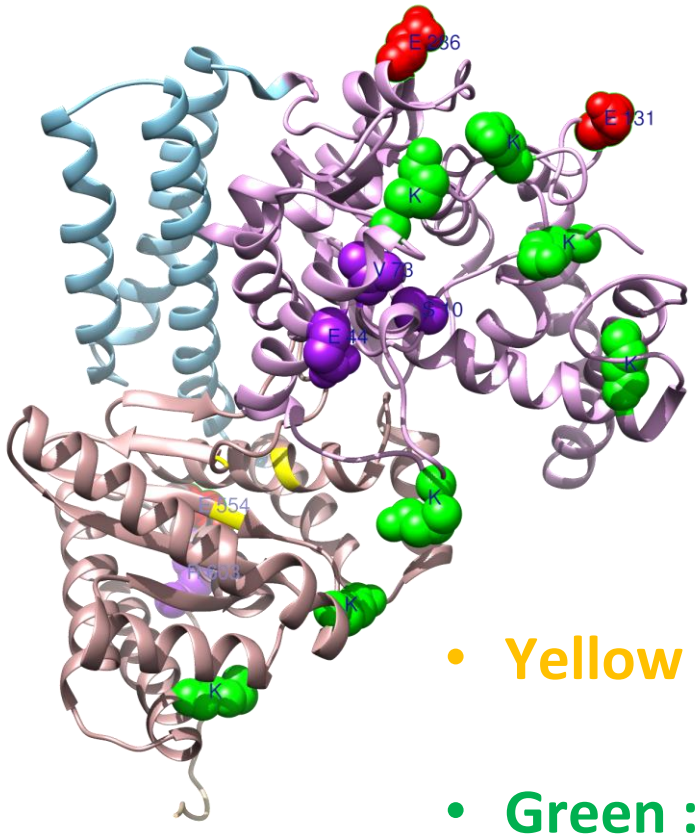


GCX → ALA
NNN → random
NNK → degenerate

Possibilities :
- Build mutants with specific mutations
- Perform alanine scanning
- Genrate library
  - Fully mutated at specific positions
  - Playing on primers ratio to obtain different mutation rate, mutate only x positions per gene

Adèle Dramé-Maigné

Exemple : Validation of mutations obtained with the PEN CSR



< 2 weeks

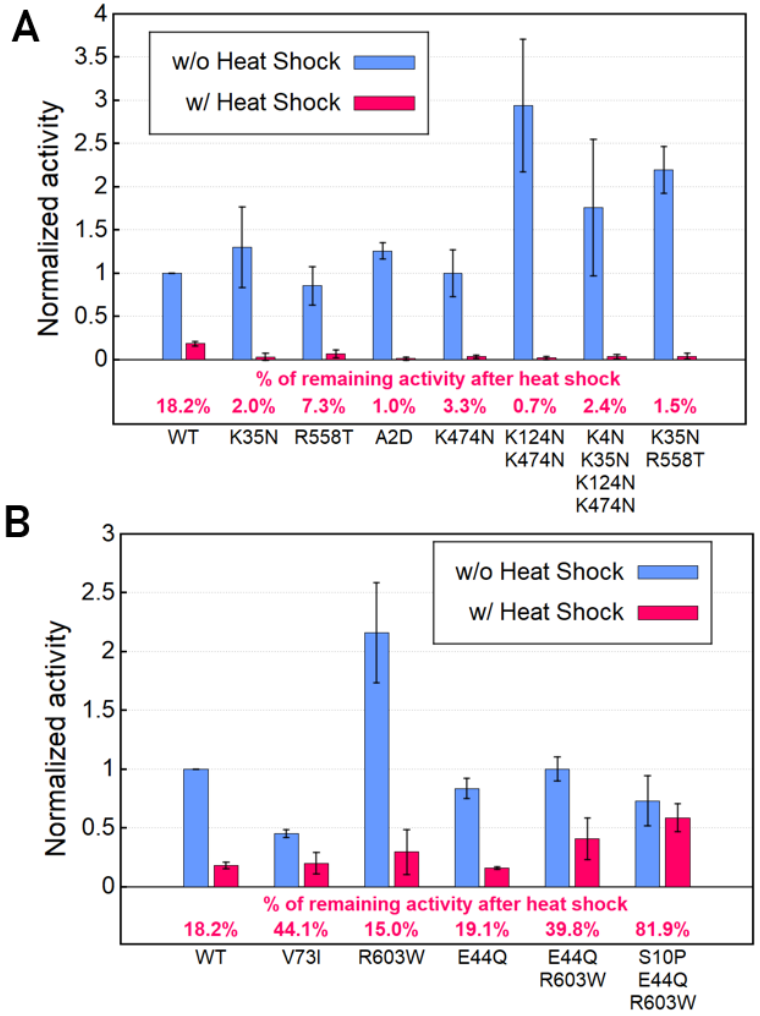
Up to 6 positions simultaneously mutated obtained and validated

- **Yellow** : Catalytic residues

According to NGS

- **Green :** Increased reaction speed
- **Purple :** Increased thermostability
- **Red :** Increased both

Variants lysate

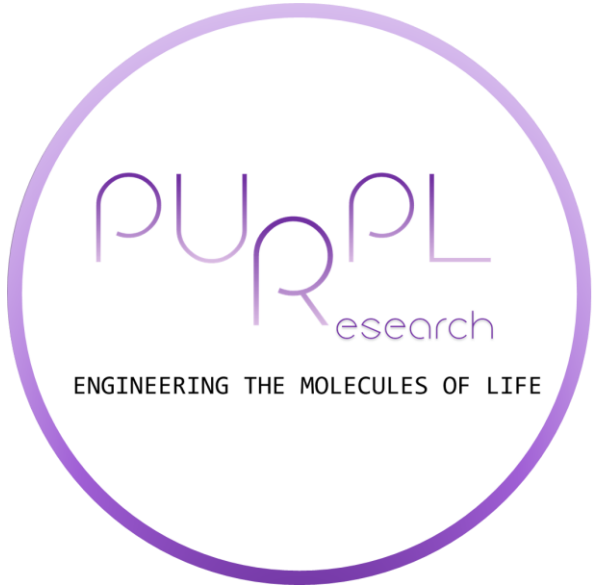


Nicking enzyme Nt.BstNBI PDB 2EWF

Adèle Dramé-Maigné

Thank you for your attention

Contact :



Adèle Dramé-Maigné

adele.dm@purpl-research.com

www.purpl-research.com