WIDEnzymes
Widening Synergies
for Novel Enzymes Development

Directed evolution of enzymes

Workshop #2 of WIDEnzymes

02.06.2025 – 06.06.2025

Department of Chemistry, Heraklion, Greece

Funded by the European Union

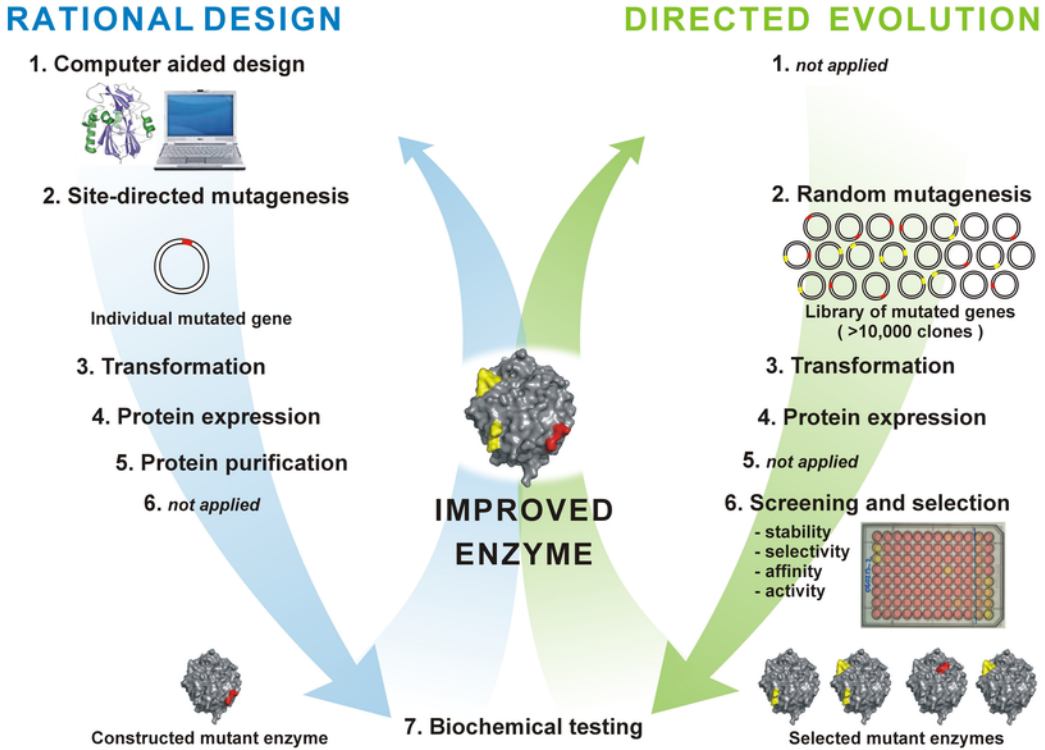
The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Workshop #2: Directed evolution of enzymes

Nobel Prize in Chemistry

2024

“for computational protein design and for protein structure prediction”



Nobel Prize in Chemistry

2018

“for the directed evolution of enzymes”

Funded by the European Union

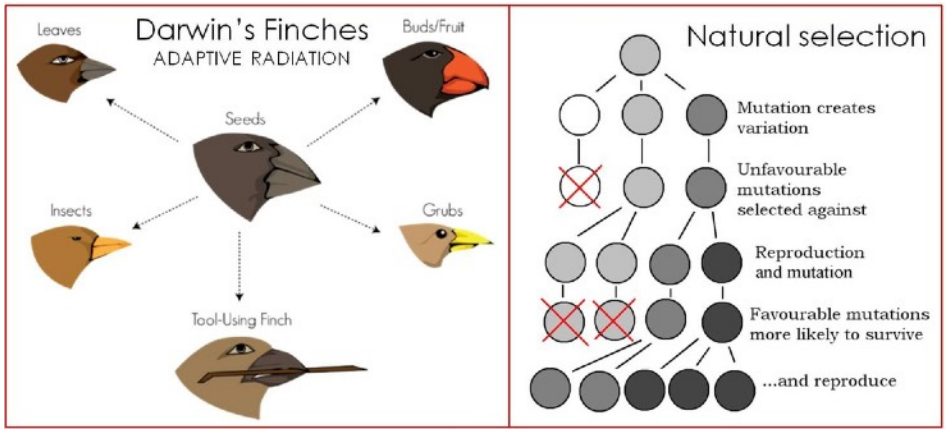
The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

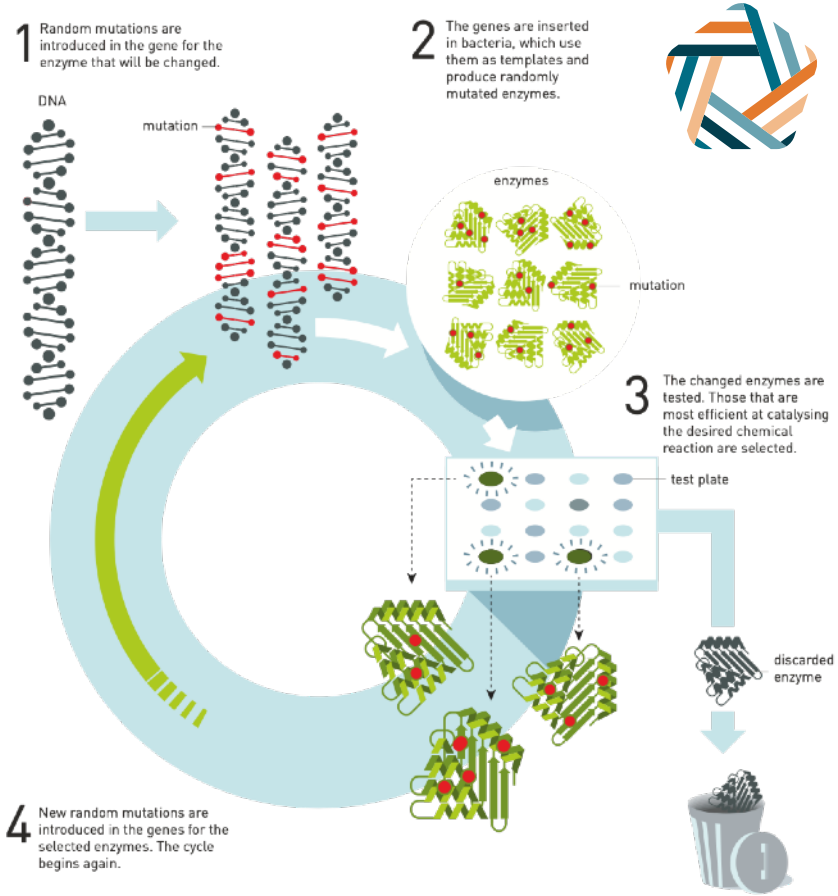
Directed evolution

Based on the two driving forces of natural evolution:

1) **Differentiation** through random mutagenesis

2) **Selection** of fitting phenotype



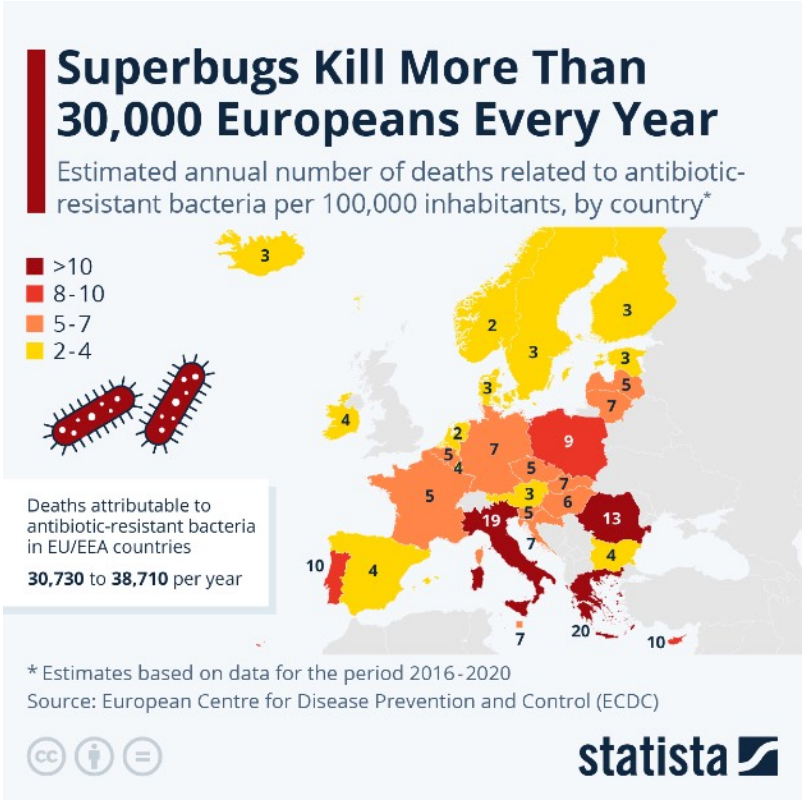


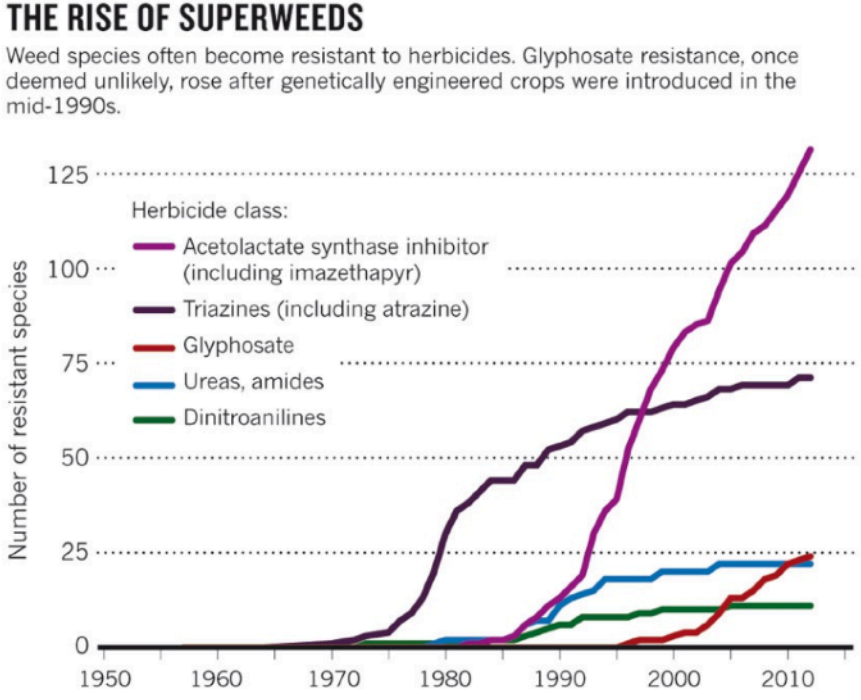
©Johan Jarnestad/The Royal Swedish Academy of Sciences

Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Directed evolution – examples from nature

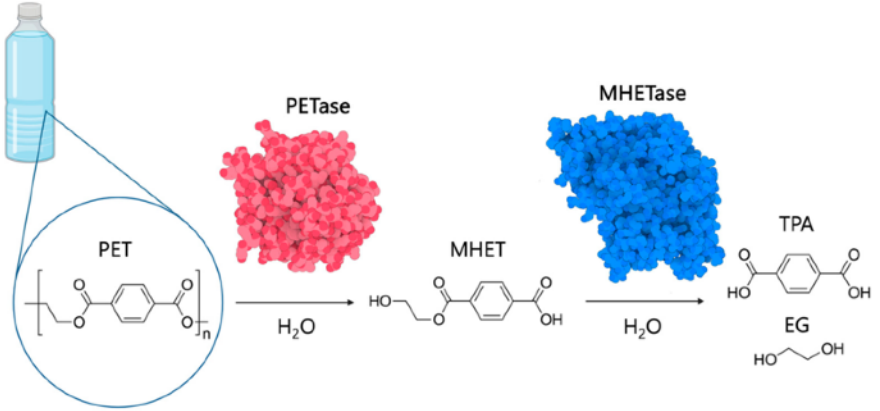


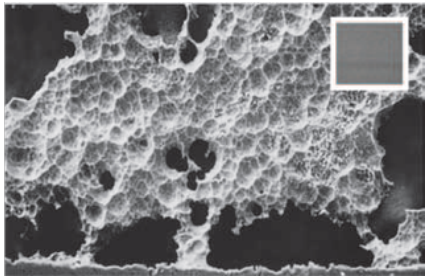


Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Directed evolution – examples from nature

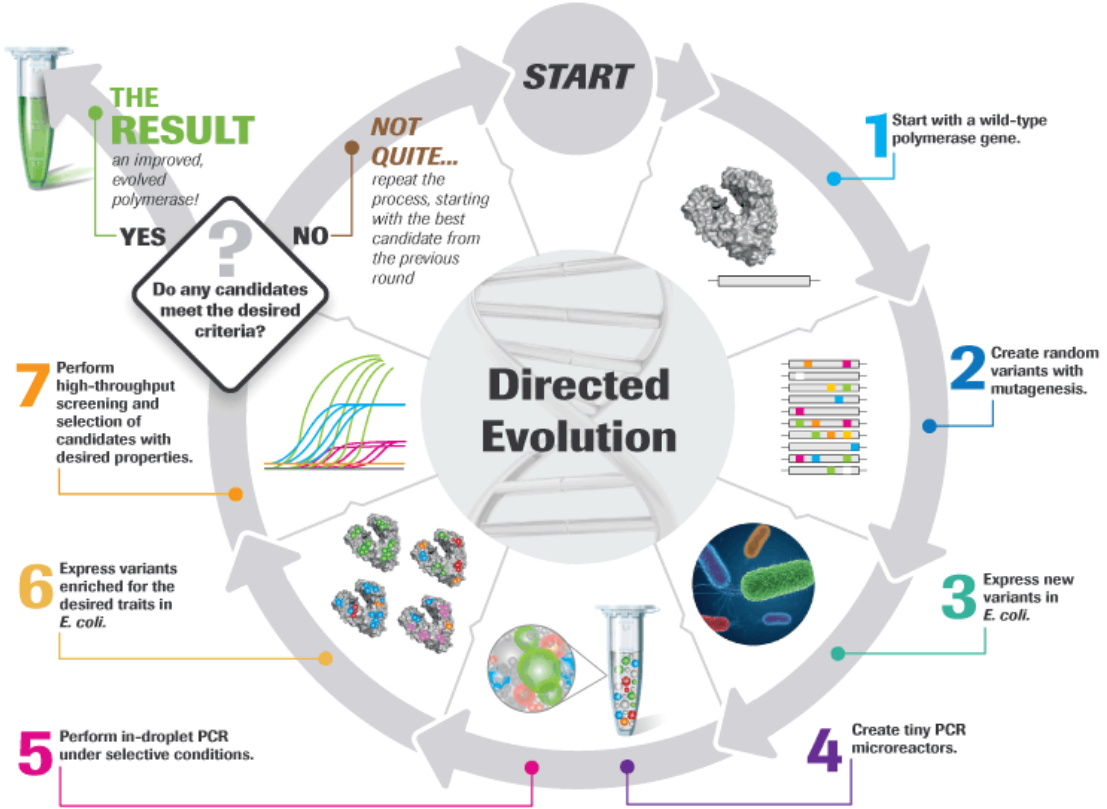




Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Directed evolution



Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Random mutagenesis

- No need of structure or mechanism
- Relatively easy methods of mutagenesis (UV irradiation, chemicals, error-prone PCR)

Points to consider:

- The error rate should be controlled and optimized
- Mutagenesis bias
- It is rare to get two consecutive mutations

What do we need?

- Gene of interest
- Functional expression in a (bacterial) host
- Efficient mutagenesis strategy
- Fast and accurate method of selection

Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

And old concept – or at least theory

These results provide favourable promise for the actual construction of a machine . Its principle of operation is new – instead of trying to screen a very large number of mutants in the hope of finding advantageous offspring, one may sample the mutant distribution of say only a thousand members and probe their value topology. This value topology – explored in an iterative procedure, just as one would go about exploring the surface of a planet – furnishes guidance about the best direction to continue the search, namely in the direction of highest peak density. A prerequisite of the procedure is to keep track of the branched genealogies during the successive generations of mutant spectra. The summary of our experience with RNA self-reproduction is that their optimal structures do evolve according to such a 'Darwinian logic'. (See especially Ref. 10) We can formulate the operation of this Darwinian logic as a procedure

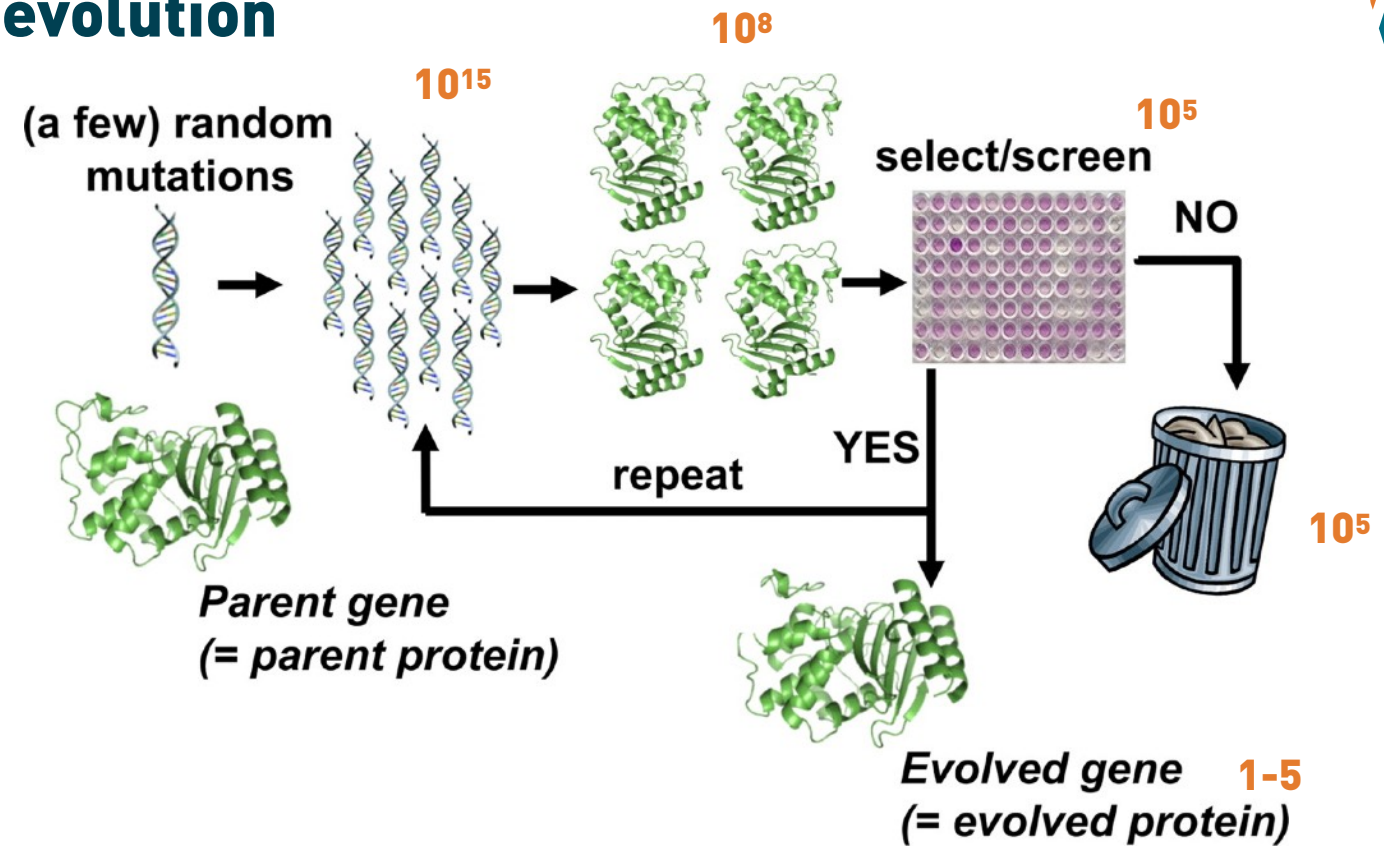
```
10 START WITH SELECTED GENOTYPE
20 LET IT REPRODUCE, MUTATING OCCASIONALLY
30 FORCE DIFFERENT GENOTYPES TO COMPETE
40 NATURAL SELECTION OF QUASI-SPECIES AROUND
   BEST-ADAPTED GENOTYPE OCCURS
50 WHEN ADVANTAGEOUS MUTANT APPEARS - GOTO 10
```

M. Eigen, W. Gardiner (1984), Pure Appl. Chem. 56, 967-978.

Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

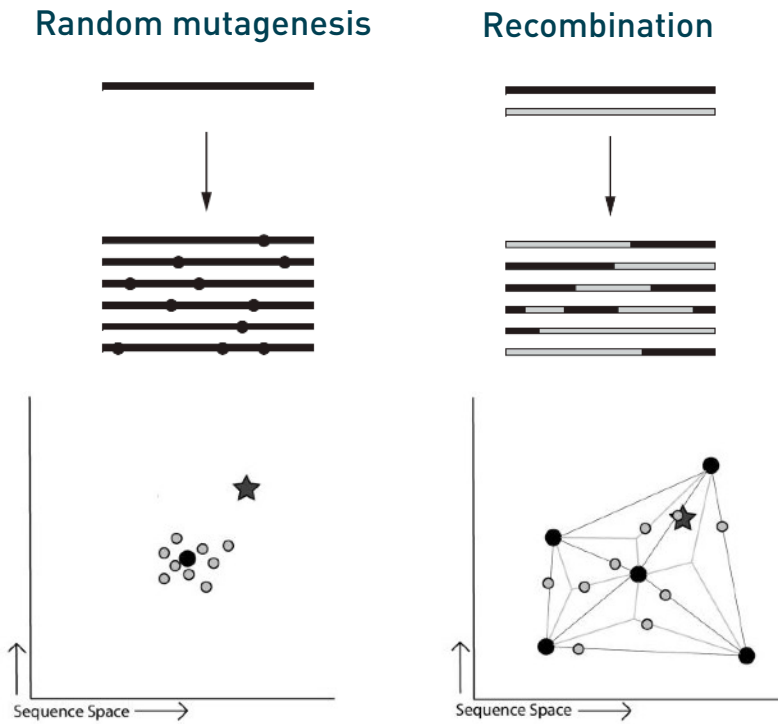
Directed evolution



Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Directed evolution techniques



Method	*Pros*	*Cons*
Error-prone PCR	Easy to perform, mutation rate adjustable	Non-biased amino acid substitutions Only point mutations accessible
Mutator strains	Easy to perform	Entire organism/plasmid is mutated Only point mutations accessible
DNA-shuffling	Modest sequence homology sufficient Several parent genes can be used Creation of chimeras possible Useful mutations are combined, harmful ones lost	Requires sequence homology
StEP	Similar to DNA-shuffling, more simple No fragment purification necessary	Requires sequence homology PCR protocol must be specifically adapted
SHIPREC	No sequence homology required	Low diversity library in single round (might be repeated) Limited to two parents of similar length Deletions/duplications possible
ITCHY	Similar to SHIPREC	Similar to SHIPREC
THIO-ITCHY	Similar to ITCHY, but more efficient/easier	Similar to ITCHY
GSSM	All single amino acid substitutions are covered	Technically out of reach for most researchers

Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Methods of random mutagenesis

Error-prone PCR

- PCR with low-fidelity polymerase
- ✓ Easy method, mutations in a targeted area, easy control of mutation rate
- ✗ Cloning issues, non-statistical change of nucleotides

DNA (gene) shuffling

- Digestion and recombination of gene fragments
- ✓ Mutations only in selected areas, cooperative effects from homologous genes
- ✗ Reproducibility issues, cloning issues

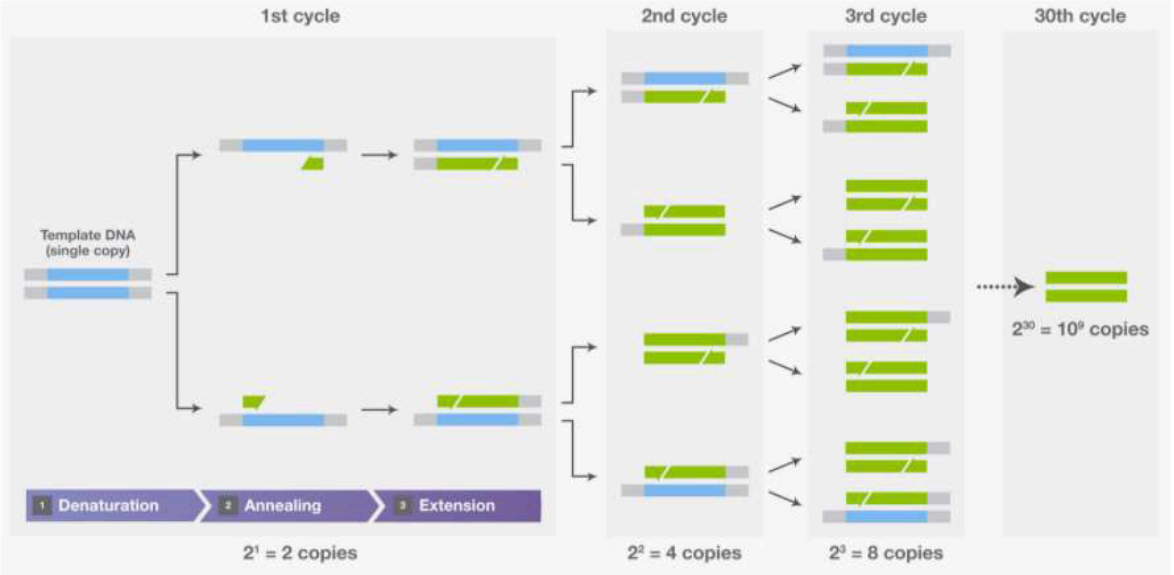
Strain mutagenesis via chemicals or UV

- DNA damages. The DNA repair mechanisms can introduce mutations
- ✓ Easy method, about 1 mutation per 1000 nucleotides
- ✗ Mutations are introduced all over the genome and/or plasmid

Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

PCR



Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Error-prone PCR

Randomization of Genes by PCR Mutagenesis

R. Craig Cadwell and Gerald F. Joyce

Developed by Leung et al. 1989 – modified by Cadwell & Joyce 1992

Based on a PCR at conditions that increase error rate
(low fidelity polymerase and non-optimal conditions)

Error-rate could be 0.5-3.0 % (or more)

False hypothesis:

All mutations happen at the same rate, thus all mutations are represented in a library equally

- Codon bias
- Polymerase bias
- Multiplication bias

Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Bias already was shown in the first works

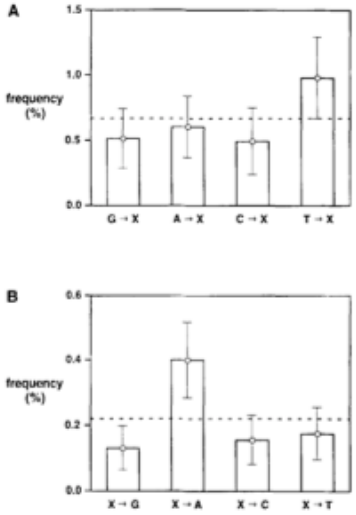


FIGURE 1 Frequency of various types of mutations under the preferred reaction condition for PCR mutagenesis. (*A*) Mutations of the form N → X; (*B*), Mutations of the form X → N; (N = G,A,C,T; X ≠ N). Frequencies refer to the mean number of mutations per base pair per PCR (30 cycles), corrected for base composition of the mutagenized gene. Error bars correspond to 95% confidence interval. Dashed horizontal line indicates expected value based on an overall error rate of 0.66% per position per PCR.

TABLE 1 Error Rate of the PCR Under Various Mutagenic Reaction Conditions[a]

[dGTP] mM	[dATP] mM	Nucleotides sequenced	Mutation rate (95% C.I.)	AT→GC / GC→AT	Transitions / Transversions
1.0	0.2	6,001	1.37 ± 0.29%	10	2.7
0.2	0.2	16,591	0.66 ± 0.13%	1	0.8
0.2	1.0	1,765	0.85 ± 0.43%	2	0.4
0.4	0.2	3,177	0.72 ± 0.29%	4	3.2

[a]Reaction conditions were as described in Materials and Methods, differing only in the concentration of dGTP and dATP. Mutation rate refers to the mean number of mutations per base pair per PCR (30 cycles). Frequencies of AT→GC and GC→AT mutations are corrected for base composition of the mutated gene.

FIGURE 3 Distribution of mutations within the gene that was amplified. (*Short arrow*) One occurrence; (*medium-length arrow*) two occurrences; (*long arrow*) three occurrences; no position was mutated more than three times. The DNA strand having the same sense as the RNA transcript is shown. Boxed regions correspond to primer binding sites.

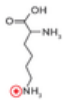
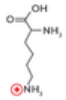
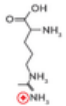
Funded by the European Union

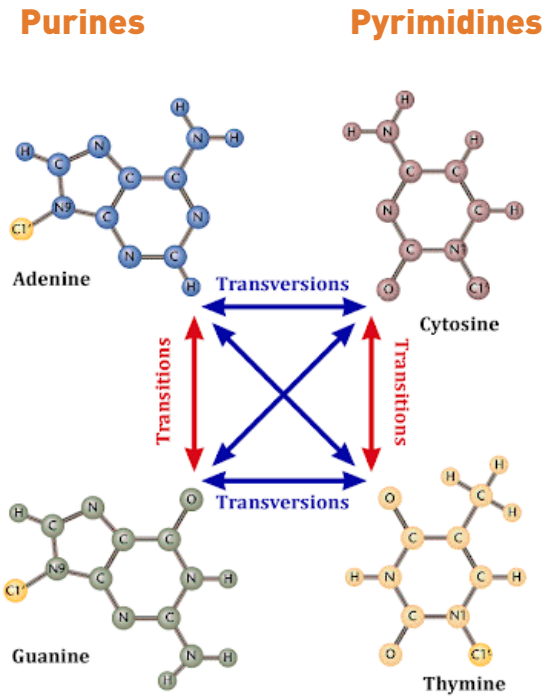
The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Mutations

Types of Mutations
(At the Protein level)

- Silent
- Nonsense
- Missense
  - Conservative
  - Non- Conservative

	No Mutation	Silent	Nonsense	Conservative	Non- Conservative
DNA Level					
mRNA Level					
Protein Level	 Lys	 Lys	STOP	 Arg	 Thr



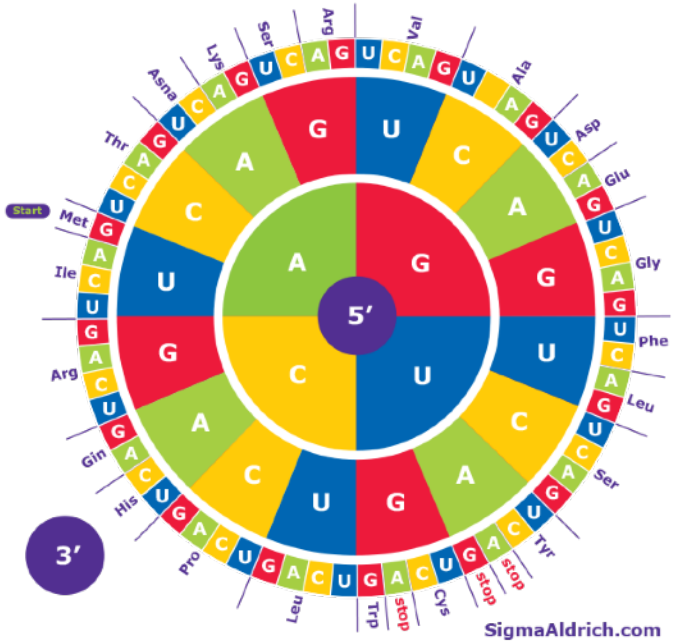
Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Codon bias

Degenerate genetic code

- $4^3 = 64$ codons
- Multiple codons encode one amino acid
- Statistically, less than 6 amino acids are accessible per codon with epPCR



Lutz & Patrick (2004) Curr Opin Biotechnol 15, 291.
Nevlon (2004) Nucl Acid Res 32, 1448.

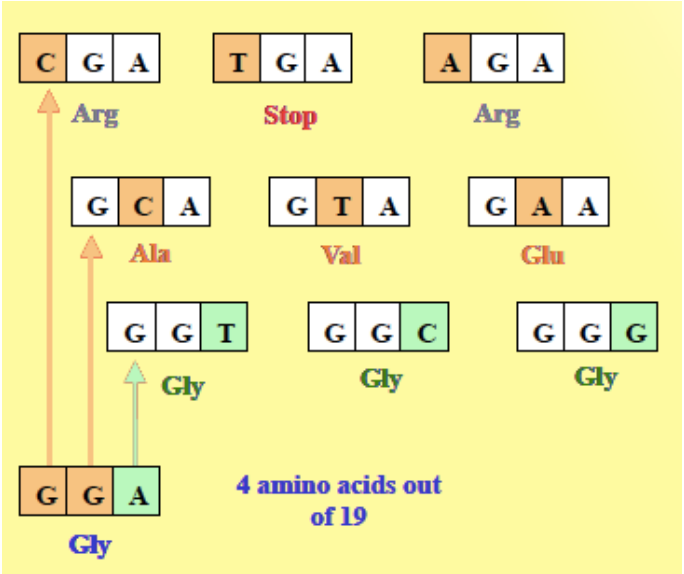
Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Codon bias

Degenerate genetic code

- $4^3 = 64$ codons
- Multiple codons encode one amino acid
- Statistically, less than 6 amino acids are accessible per codon with epPCR
- Difficult to have more than one mutation per codon
- Degeneracy leads to silent mutations



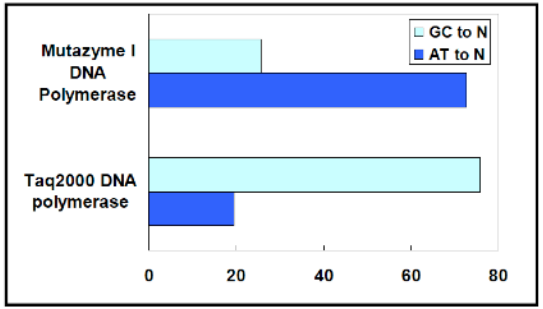
Lutz & Patrick (2004) Curr Opin Biotechnol 15, 291.
Nevlon (2004) Nucl Acid Res 32, 1448.

Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Polymerase bias

- Polymerases may have a preference for the bases they will mutate
- Under the same conditions, different polymerases may add different mutations
- Bias can be alleviated by working with several polymerases
- Bias can be used to understand the copy mechanism of the lagging and leading strands *Allen et al. (2011) Nucl Acid Res 39, 7020-7033*



Mutation frequencies and spectra resulting from *Taq*- and Mutazyme-based error-prone PCR and subsequent StEP shuffling

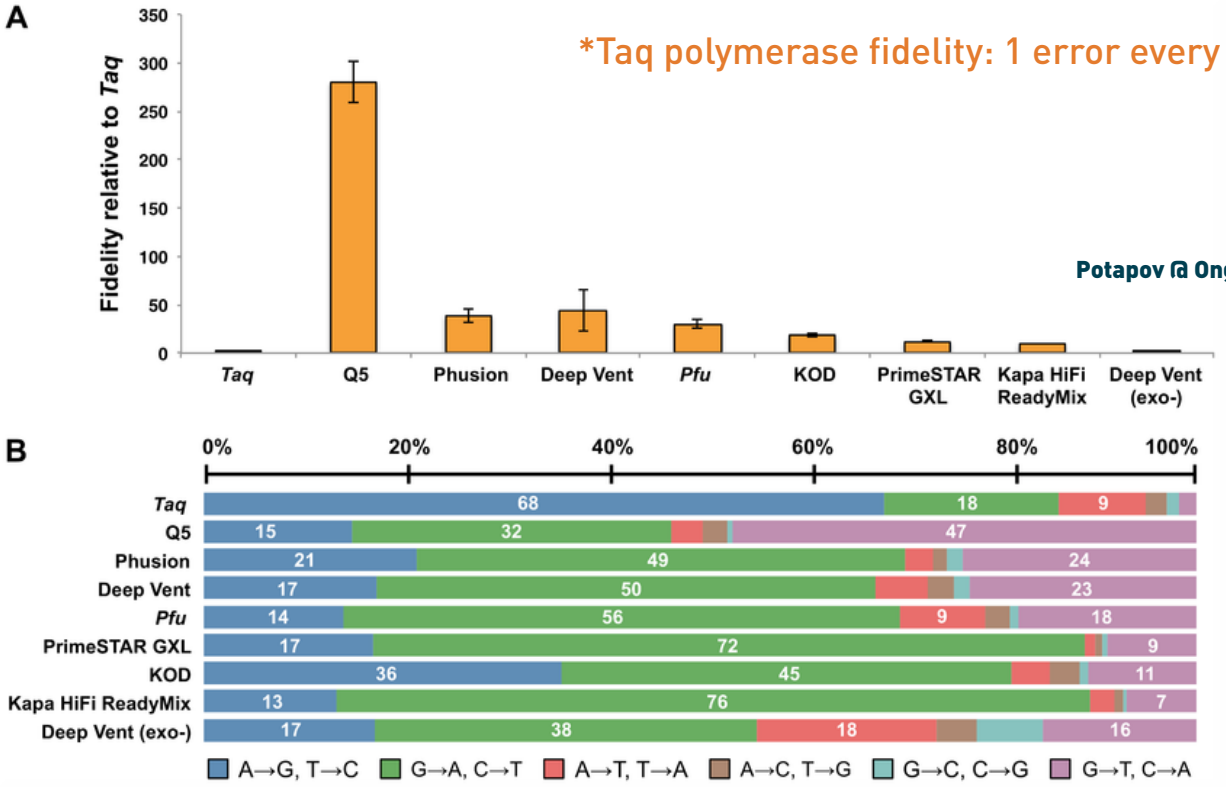
	Number of mutations (19,120 bp sequenced)	Mutation frequency[a]	Substitution (%)						Insertion (%)	Deletion (%)	Bias indicators			
			AT → GC	GC → AT	AT → TA	AT → CG	GC → CG	GC → TA			Ts/Tv	AT → GC/ GC → AT	AT changes (%)	GC changes (%)
Taq error-prone PCR	216	1.13% (0.98;1.29)	51.8	11.1	22.7	5.6	1.8	2.8	1.4	2.8	1.9	4.7	80.1	15.7
Mutazyme error-prone PCR	36	0.19% (0.13;0.26)	22.2	27.8	13.9	5.6	2.8	19.4	0.0	8.3	1.2	0.8	41.7	50
StEP shuffling	121	0.63%[b] (0.53;0.76)	50.4	13.2	17.4	5	4.1	4.1	0.8	5	2.1	3.8	72.8	21.4

Vanhercke et al. (2005) Anal Biochem 339, 9-14

Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

DNA polymerases and fidelity



*Taq polymerase fidelity: 1 error every 3700 nucleotides

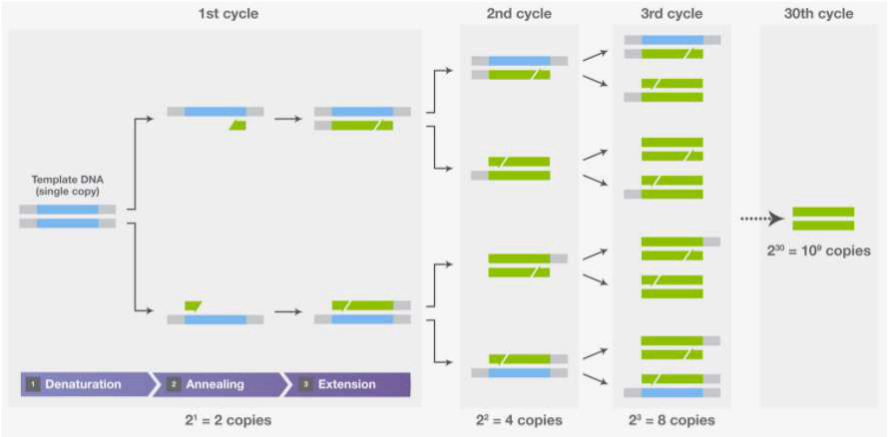
Potapov & Ong (2017) PLOS One 12, e016774.

Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Multiplication bias

- Exponential growth on DNA products in PCR
- Over-representation of mutations that happen in the first rounds
- Up to 25% of the library can carry mutations from the first round



Possible solutions:

- Less PCR cycles, with
- More reactions with less volume, to produce more libraries

Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Results of bias

Methods	Transitions		Transversions				InDel	T_s/T_v	Reference
	AT →GC	GC →AT	AT →TA	AT →CG	GC →CG	GC →TA			
Ideal method	16.7	16.7	16.7	16.7	16.7	16.7	0.0	0.5	Wong et al. (2006a), Wong et al. (2006b)
Taq/$MnCl_2$ and imbalanced dNTP concentration	38.1	19.0	27.8	4.0	0.0	6.3	4.8	1.5	Rasila et al. (2009)
Taq/8–oxo–dGTP and dPTP	65.4	27.5	1.6	4.4	0.0	0.0	1.1	15.5	Rasila et al. (2009)
Mutazyme/Amplicon	14.0	32.5	15.1	2.3	5.8	22.1	8.3	1.0	Rasila et al. (2009)
Mutazyme/Cycle	17.1	25.7	28.6	2.9	0.0	14.3	11.4	0.9	Rasila et al. (2009)
XL1–Red	0.0	60.0	10.0	0.0	0.0	0.0	30.0	6.0	Rasila et al. (2009)
NH_2OH–HCl	15.4	76.9	7.7	0.0	0.0	0.0	0.0	12.0	Rasila et al. (2009)
DuARChEM	13.5	26.9	1.9	5.8	42.3	9.6	0.0	0.7	Mohan and Banerjee (2008)
Taq/D_2O	50.0	37.5	12.5	0.0	0.0	0.0	0.0	7.0	Minamoto et al. (2012)
Taq/D_2O and $MnCl_2$	60.0	12.0	8.0	8.0	4.0	8.0	0.0	2.6	Minamoto et al. (2012)
dITP and endonuclease V	38.4	39.7	4.6	8.6	4.6	4.0	0.0	3.6	Wang et al. (2013c)
epRCA with Cre/*loxP* recombination	6.7	86.7	0.0	0.0	6.7	0.0	0.0	13.9	Huovinen et al. (2011)
SeSaM–Tv –II	15.2	18.3	1.8	1.8	33.9	29.1	0.0	0.5	Mundhada et al. (2011)
TaGTEAM	6.1	10.2	18.4	0.0	14.3	26.5	24.5	0.3	Finney–Manchester and Maheshri (2013)

Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

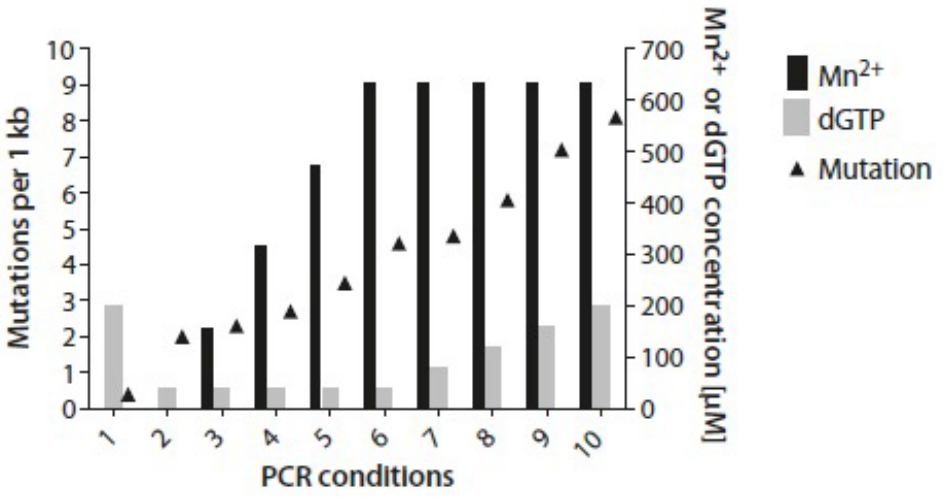
Methods to introduce mutations

- **Metal concentrations affect polymerase fidelity**
- **$MgCl_2$**: stabilizes non-complementary pairs.

  It is crucial for polymerase activity
- **$MnCl_2$**: is added to reduce polymerase specificity

  (related to annealing temperature too)
- Some polymerases are also affected

  from the concentration of the template



Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Methods to introduce mutations

- **Non-equal concentration of dNTPs**
- In typical PCR they are added equally

Vartanian et al. (1996) Nucl Acids Res 24, 2627-2631.

dNTP/μM C	T	A	G	Mn^{2+} mM	Colonies sequenced	No. mut.[a]	Ti/Tv[b]	N→A,T[c]	N→G,C[d]	Mutation frequency[e]
1	1000	50	50	–	37 $trim^R$	126	125/1	121	5	1.5×10^{-2}
1	1000	50	50	–	24 $ampi^R$	162	157/5	157	5	2.9×10^{-2}
3	1000	50	50	–	20 $trim^R$	24	22/2	24	0	5.2×10^{-3}
3	1000	50	50	–	20 $ampi^R$	29	27/2	28	1	6.3×10^{-3}
10	1000	50	50	–	20 $trim^R$	15	12/3	12	3	3.2×10^{-3}
10	1000	50	50	–	22 $ampi^R$	21	16/5	19	2	4.1×10^{-3}
5	1000	5	1000	–	18 $trim^R$	3	3/0	3	0	7.2×10^{-4}
5	1000	5	1000	–	18 $ampi^R$	0	0/0	0	0	$\leq 10^{-4}$
30	1000	30	1000	–	18 $ampi^R$	4	4/0	1	3	10^{-3}
30	1000	30	1000	0.5	34 $ampi^R$	755	521/234	256	499	10^{-1}
100	1000	100	1000	0.5	18 $trim^R$	19	16/3	8	11	4.5×10^{-3}
100	1000	100	1000	0.5	24ampiR	41	33/8	11	30	7.4×10^{-3}

Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Typical epPCR protocol

Step 1: Set up reactions for PCR

1.1 Add to a 0.2 ml PCR tube on ice:
- 100 ng template DNA
- 10 µl 10X polymerase buffer
- 10 µl 2 mM dNTP mix
- 1 µl 100 µM Forward primer
- 1 µl 100 µM Reverse primer
- 1 µl 5 U/µl polymerase
- water to 100 µl

Step 2: Generate additional template for mutagenesis

2.1 Run PCR with the following conditions:

94°C	5 min
10 cycles:	
94°C	30 sec
55-65°C	30 sec
72°C	30 sec to 5 min

Step 3: Perform mutagenic PCR

3.1 Add 1 µl 500 µM $MnCl_2$ to the PCR reaction Mix well

3.2 Return tube to thermocycler and continue PCR with the following conditions:
30 cycles:

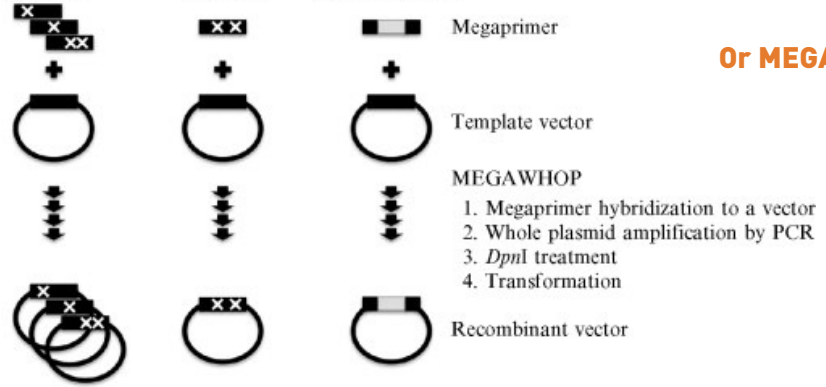
94°C	30 sec
55-66°C	30 sec
72°C	30 sec to 5 min
72°C	5-10 min
4°C	hold

Step 4: Subclone and sequence the PCR products

4.1 Run 10 µl of the PCR products on an agarose gel

4.2 Check the purity of the PCR products

- Single band of correct size → 4.2 Clean up PCR reaction using a PCR purification kit
- Multiple bands → 4.2 Run remainder of PCR reaction on an agarose gel. Isolate the correct band using a DNA gel extraction kit



Or MEGAWHOP

4.6 Transform competent *E. coli* and spread on selective plates Grow O/N at 37°C

4.7 Pick single colonies into 5 ml LB + antibiotic Grow O/N at 37°C with shaking

4.8 Isolate plasmid DNA using a plasmid miniprep kit Sequence inserts to determine whether they contain mutations

Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

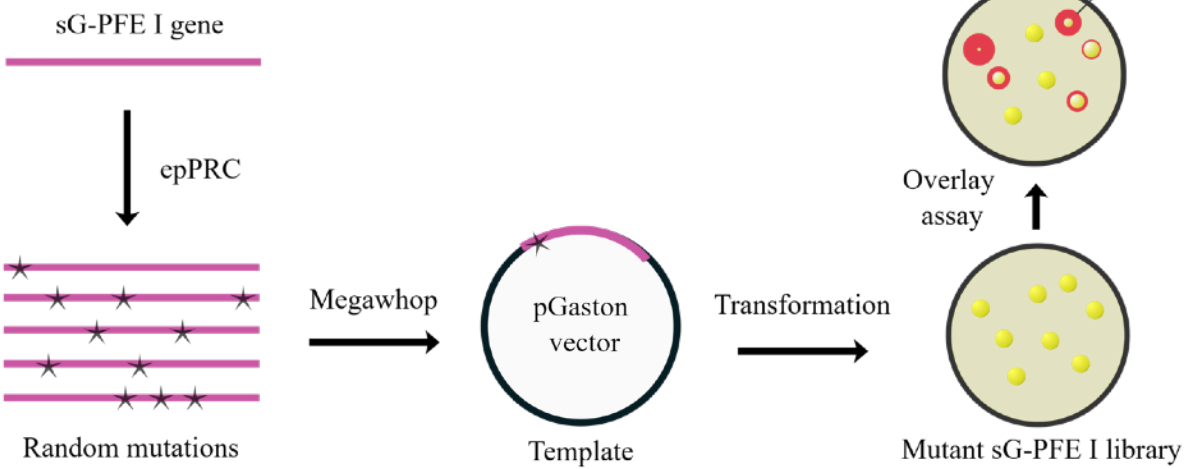
Points for consideration

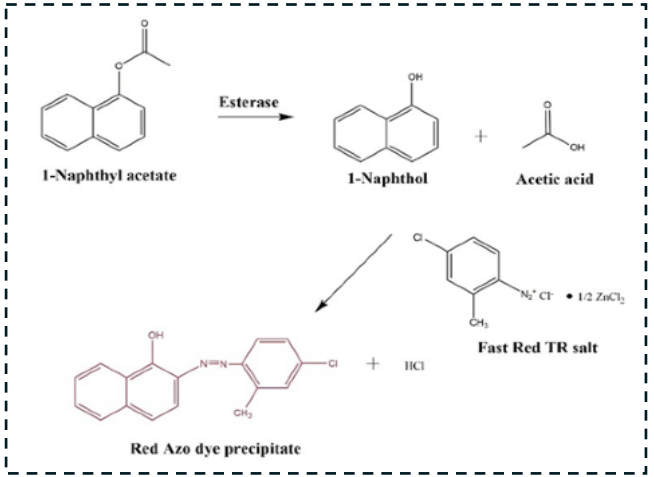
- How many mutations are needed for the desired phenotype?
- What is the best template for evolution?
- Is the protein selected for evolution evolvable?
- Are negative mutations masking my beneficial mutations?
- Do I screen more one library or do I go to a second round with the best variant?
- What assay do I use for preselection?

Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Our workshop

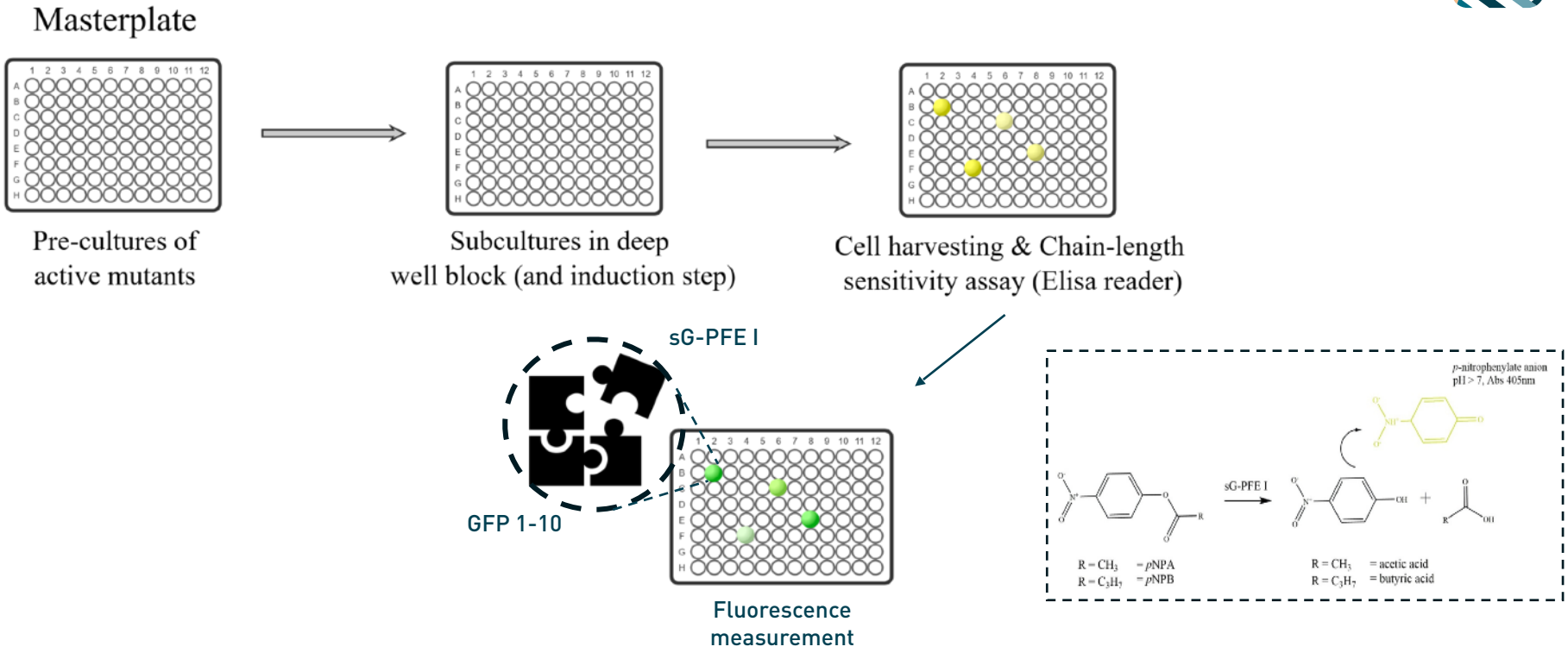




Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Our workshop



Funded by the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

WIDEnzymes
Widening Synergies
for Novel Enzymes Development

Thank you!

Funded by
the European Union

The WIDEnzymes project has received funding from the European Union's HORIZON-WIDERA-2023-ACCESS-04 programme under grant agreement No. 101159534. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.