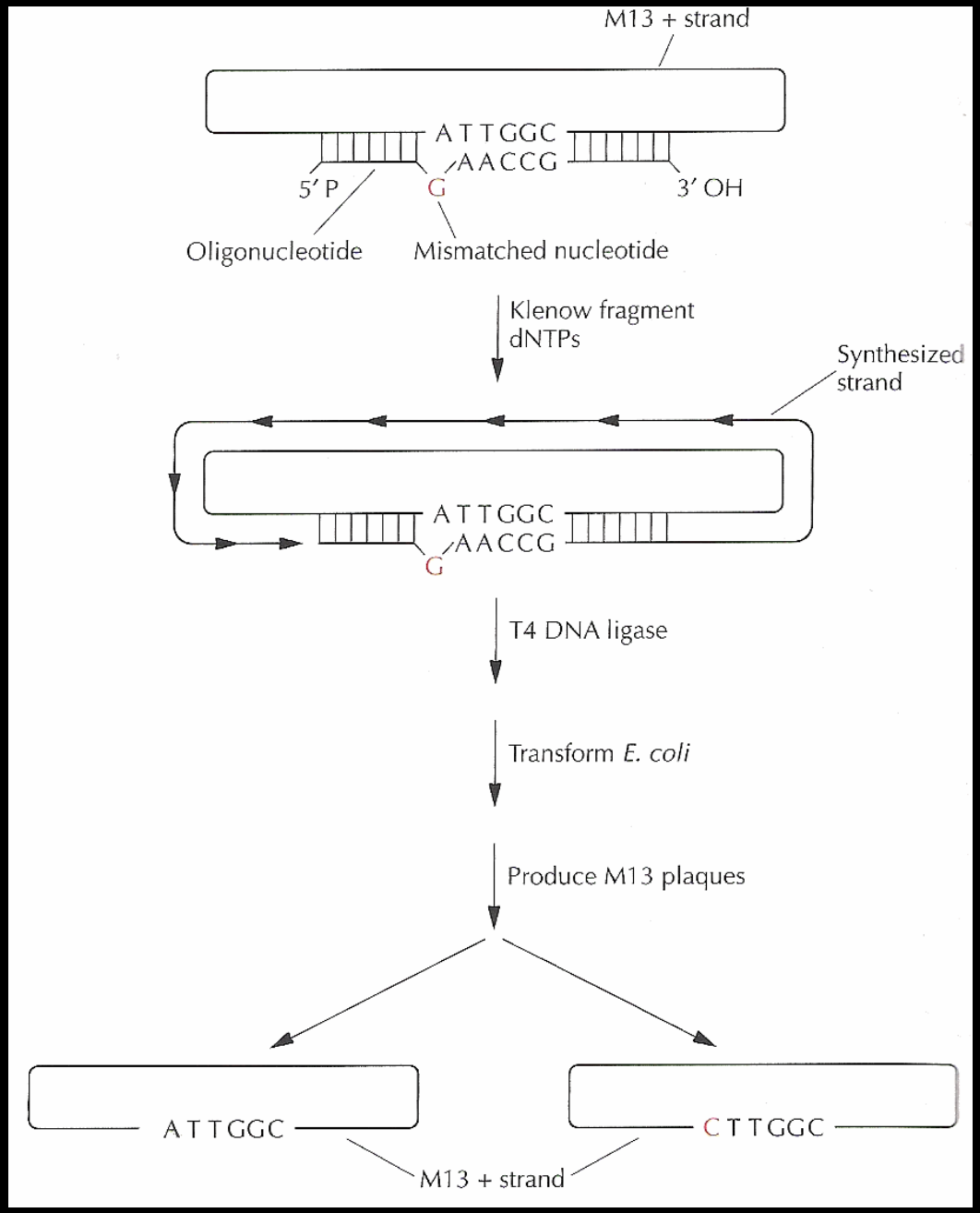
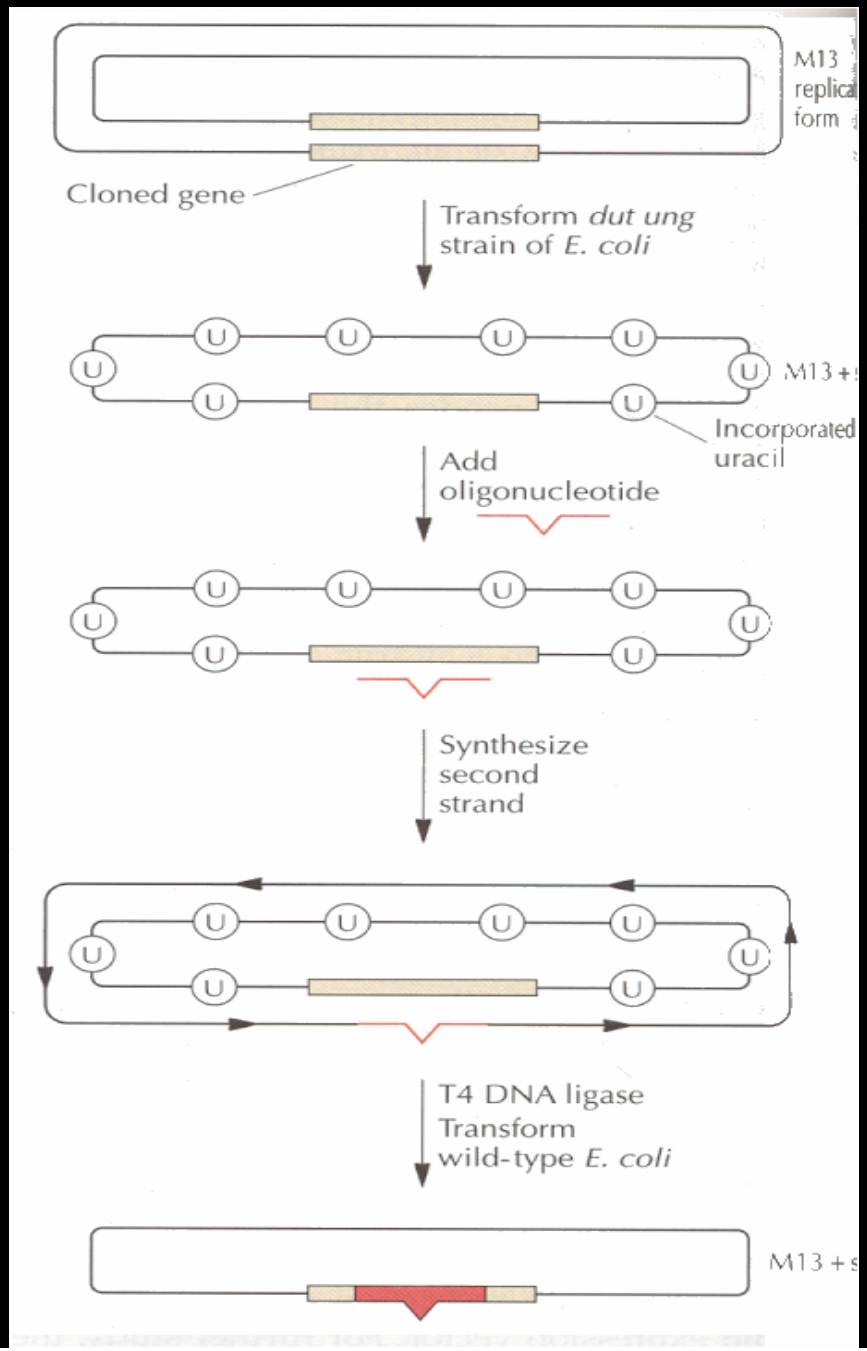
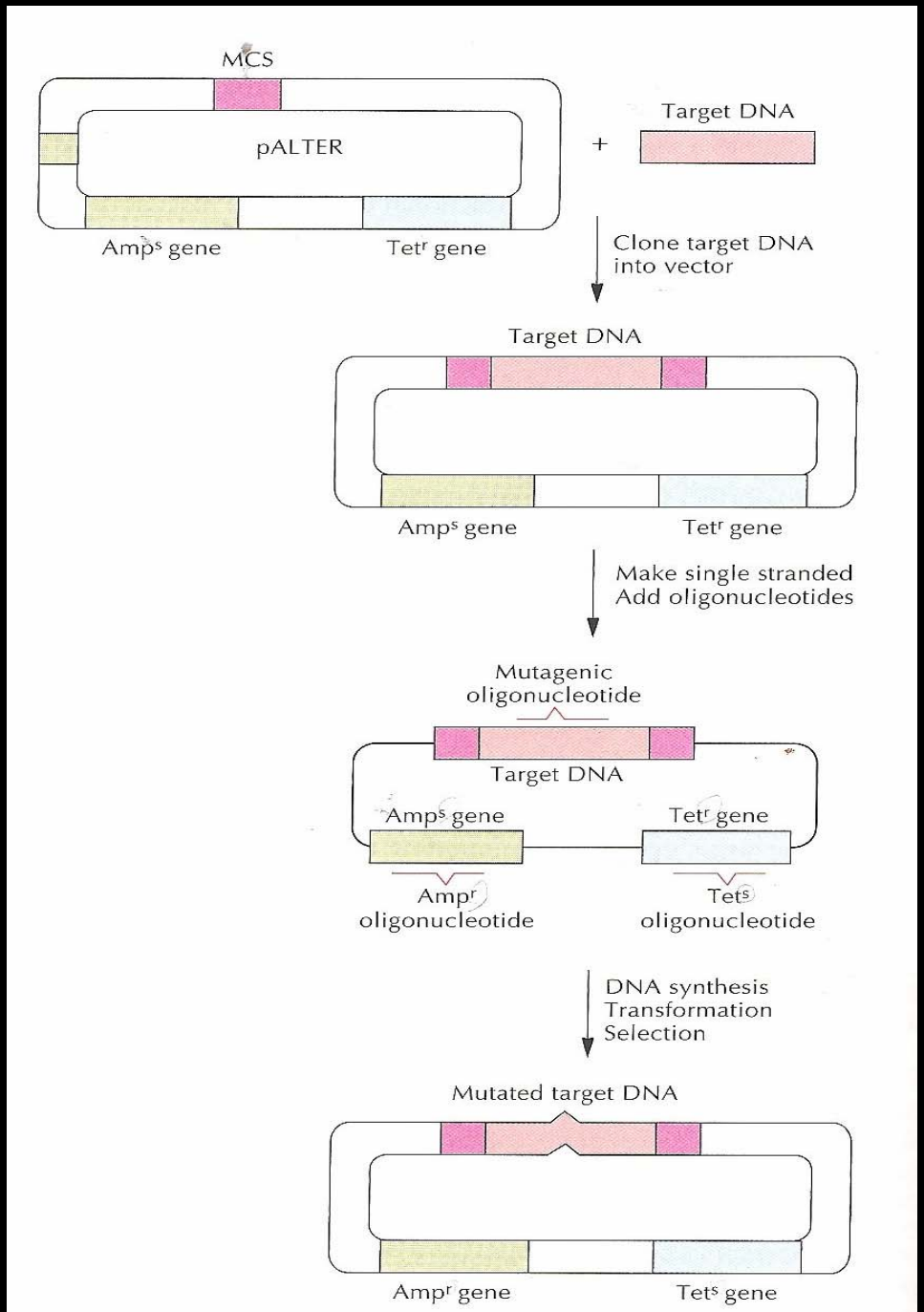
MUTAGÉNESISIS DIRIGIDA



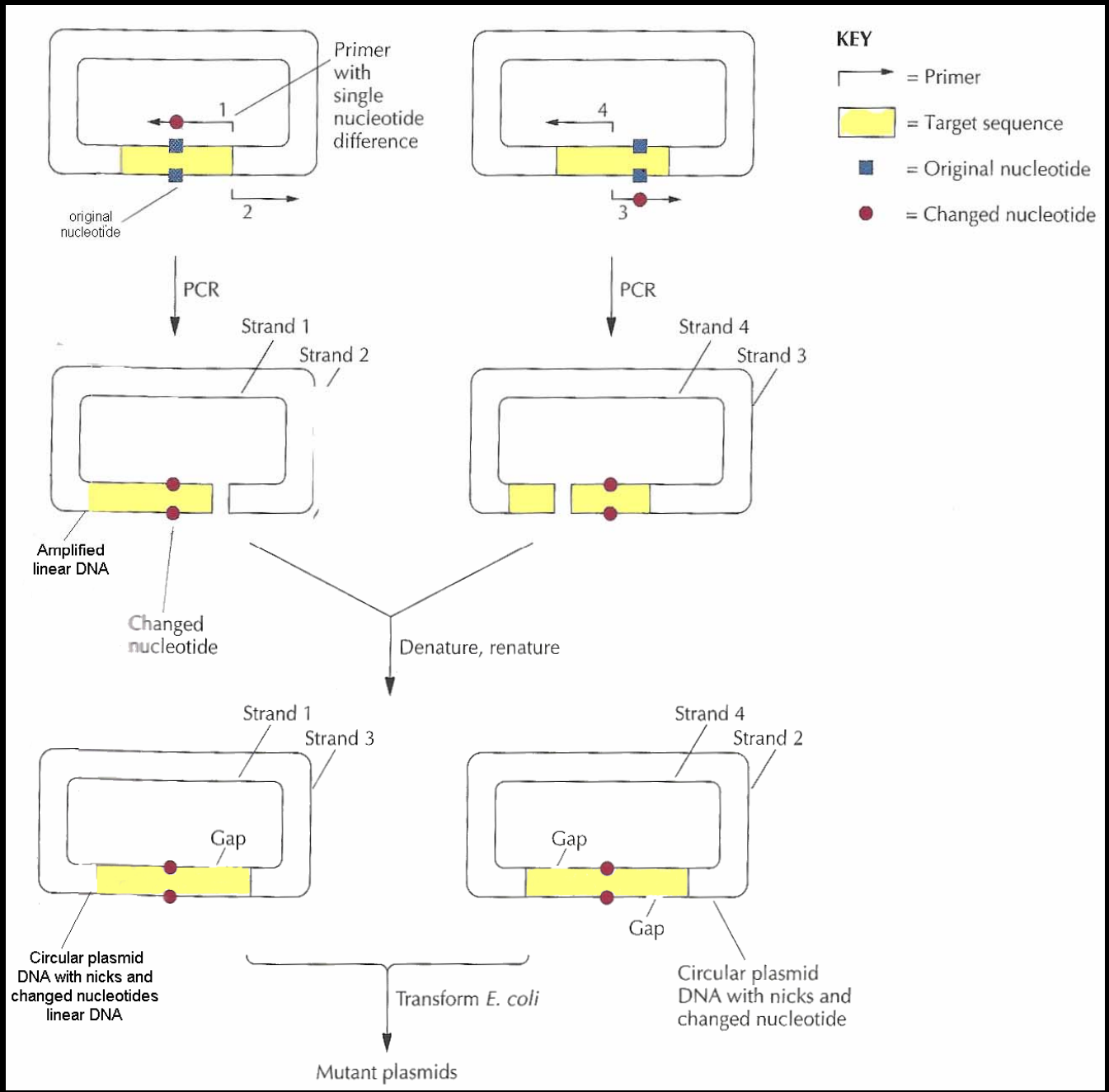
Mutagénesis dirigida utilizando el cromosoma del virus M13.



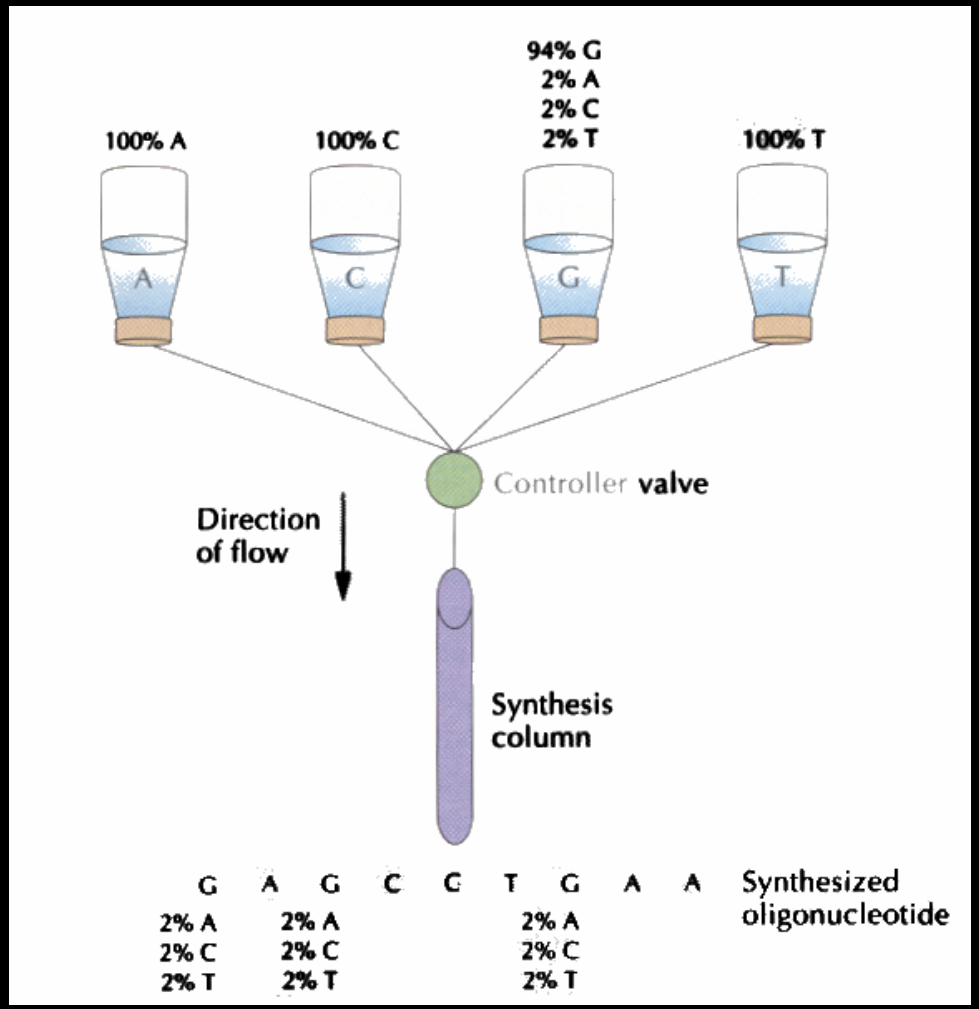
Mejora de la técnica utilizando M13



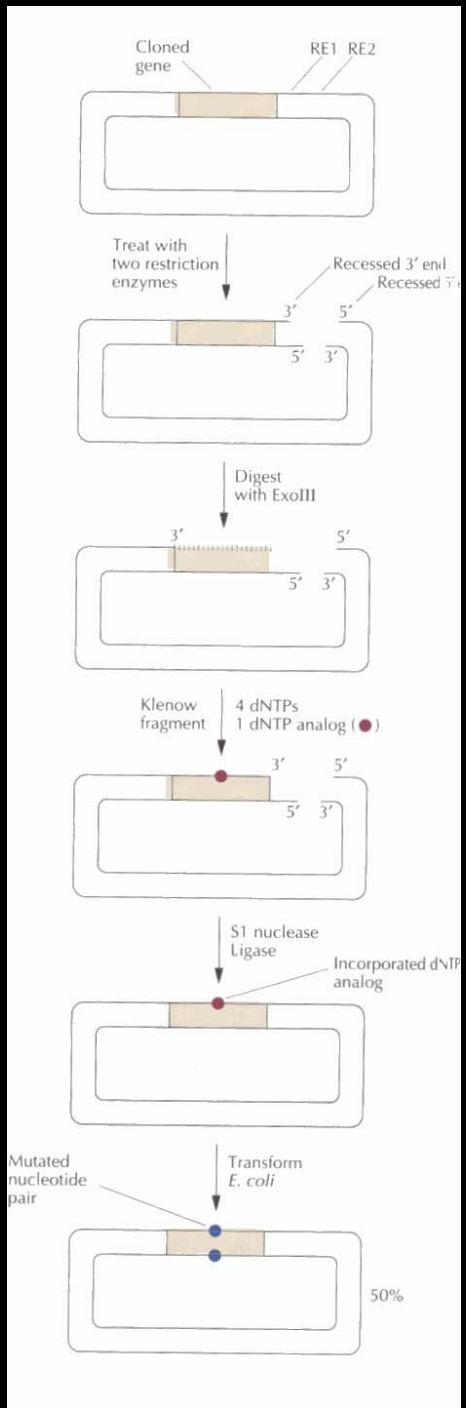
Mutagénesis dirigida utilizando plásmidos



Mutagénesis dirigida con PCR

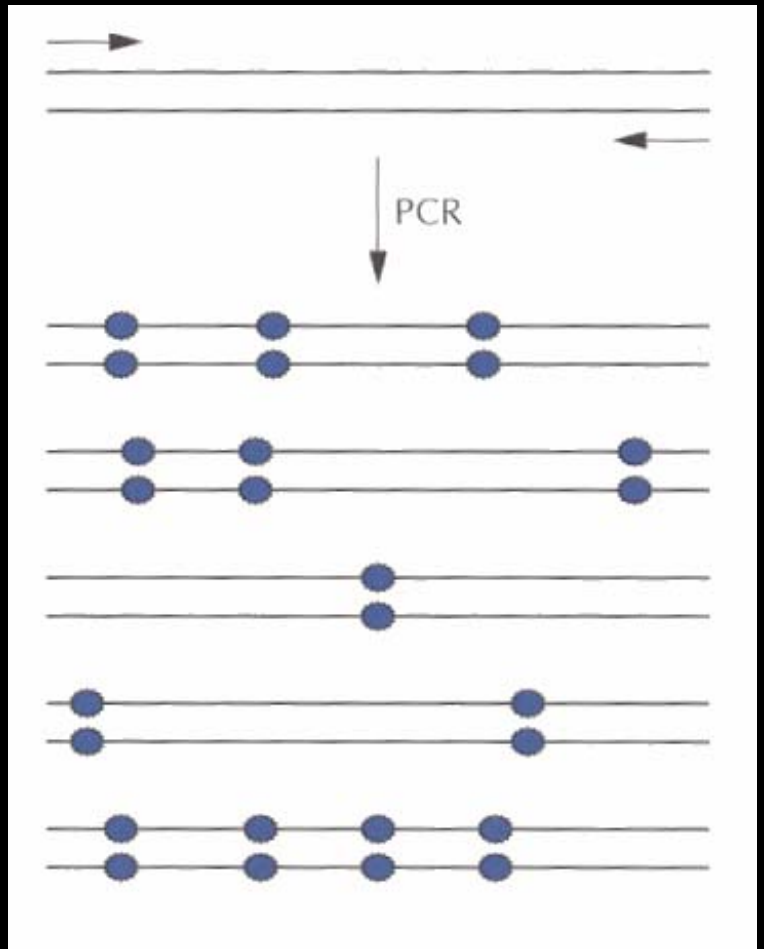


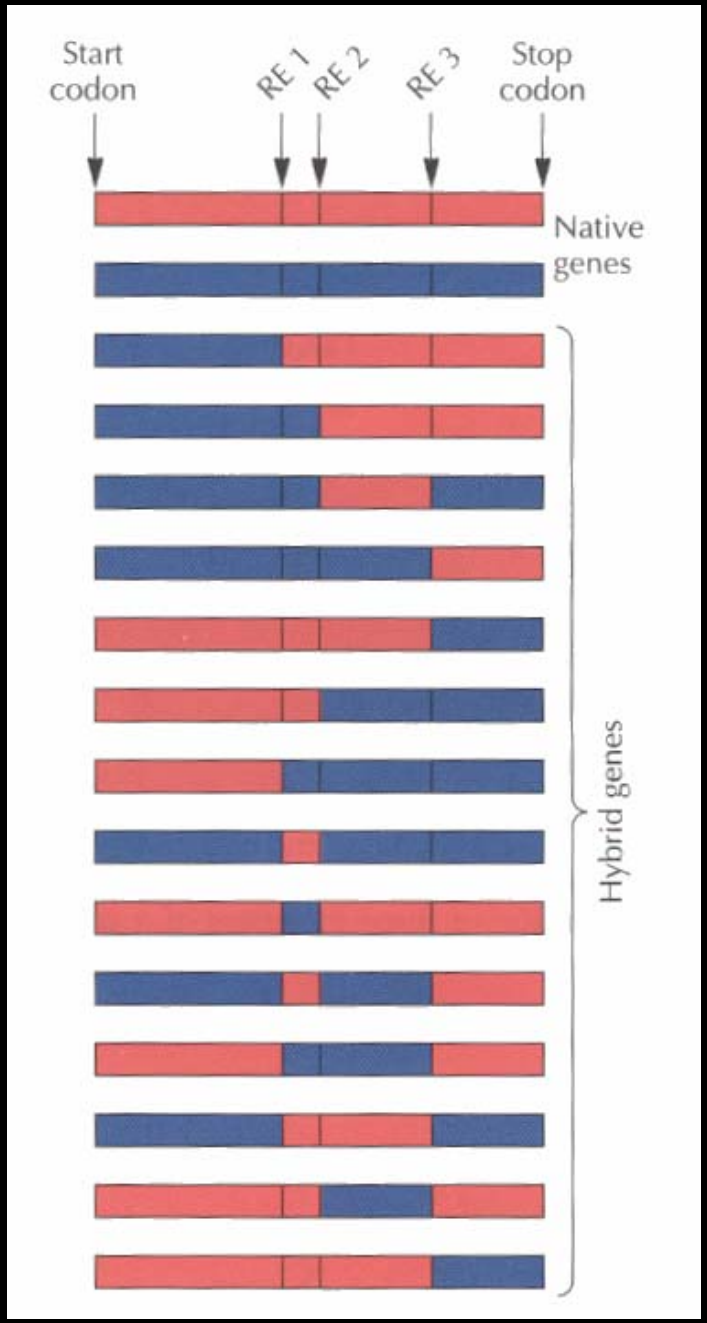
Mutagénesis aleatoria
Las sondas utilizadas no son específicas



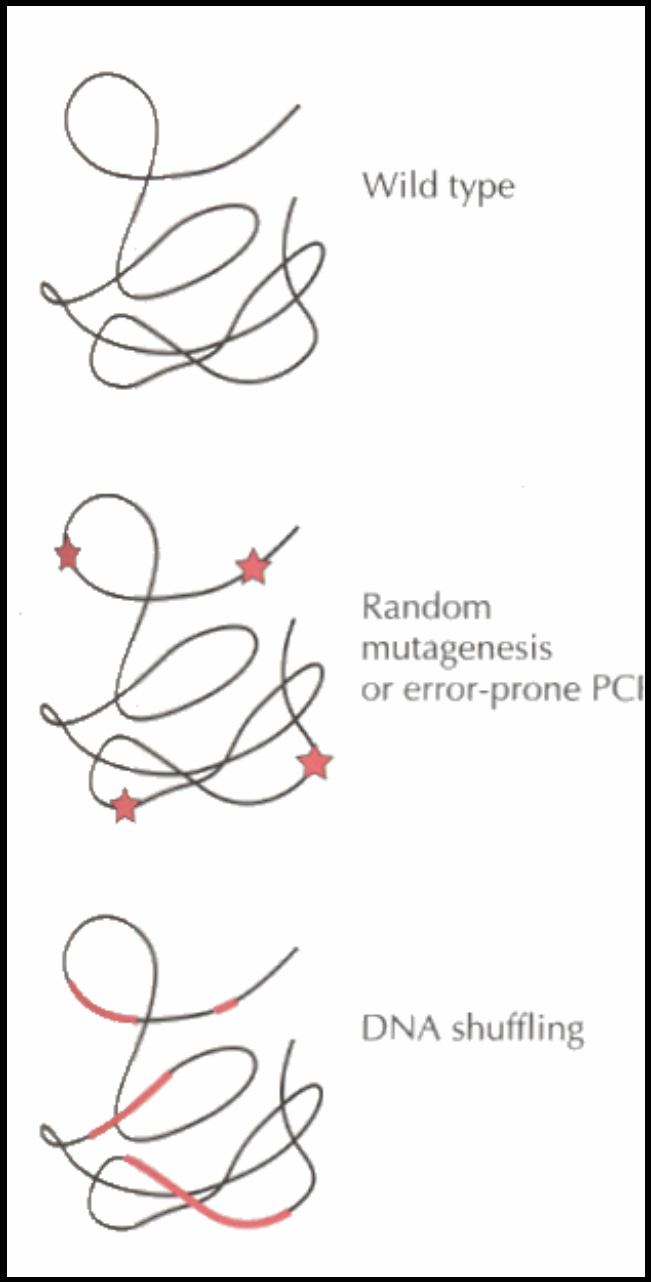
Mutagénesis aleatoria de un gene clonado

PCR con tendencia a incorporar basas equivocadas.





Generación de nuevos genes mezclando fragmentos de restricción (DNA shuffling)

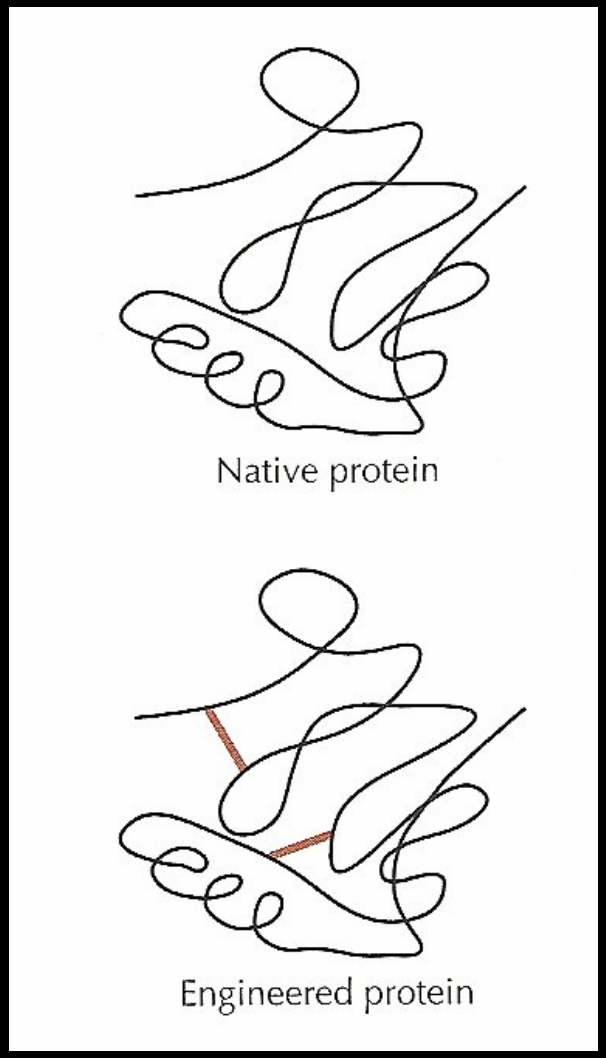


Comparación entre los cambios puntuales de la mutagénesis y los cambios en fragmentos de la mezcla de fragmentos de restricción

INGENIERÍA DE PROTEÍNAS

Some industrial enzymes and their commercial uses

Enzyme	Industrial use(s)
α-Amylase	Beer making, alcohol production
Aminoacylase	Preparation of L-amino acids
Bromelain	Meat tenderizer, juice clarification
Catalase	Antioxidant in prepared foods
Cellulase	Alcohol and glucose production
Ficin	Meat tenderizer, juice clarification
Glucoamylase	Beer making, alcohol production
Glucose isomerase	Manufacture of high-fructose syrups
Glucose oxidase	Antioxidant in prepared foods
Invertase	Sucrose inversion
Lactase	Whey utilization, lactose hydrolysis
Lipase	Cheese making, preparation of flavorings
Papain	Meat tenderizer, juice clarification
Pectinase	Clarifying fruit juices, alcohol production
Protease	Detergent, alcohol production
Rennet	Cheese making



Lisozima del virus T4.
Mutagénesis para incluir dos enlaces disulfuro

Properties of T4 lysozyme and six engineered variants

Enzyme	Amino acid at position:							No. of -S-S-	% Activity	T_m (°C)
	3	9	21	54	97	142	164			
wt	Ile	Ile	Thr	Cys	Cys	Thr	Leu	0	100	41.9
pwt	Ile	Ile	Thr	Thr	Ala	Thr	Leu	0	100	41.9
A	Cys	Ile	Thr	Thr	Cys	Thr	Leu	1	96	46.7
B	Ile	Cys	Thr	Thr	Ala	Thr	Cys	1	106	48.3
C	Ile	Ile	Cys	Thr	Ala	Cys	Leu	1	0	52.9
D	Cys	Cys	Thr	Thr	Cys	Thr	Cys	2	95	57.6
E	Ile	Cys	Cys	Thr	Ala	Cys	Cys	2	0	58.9
F	Cys	Cys	Cys	Thr	Cys	Cys	Cys	3	0	65.5

Adapted from Matsumura et al., *Nature* **342**:291–293, 1989.

wt, wild-type T4 lysozyme; pwt, pseudo-wild-type enzyme; A through F, six engineered cysteine variants; -S-S-, disulfide bonds; T_m, "melting" temperature (a measure of thermostability).

Sustitución de aminoácidos para aumentar la estabilidad térmica

Stability at 100°C of the yeast enzyme triosephosphate isomerase and its engineered derivatives

Enzyme	Amino acid at position:		Half-life (min)
	14	78	
Wild type	Asn	Asn	13
Variant A	Asn	Thr	17
Variant B	Asn	Ile	16
Variant C	Thr	Ile	25
Variant D	Asp	Asn	11

Adapted from Ahern et al., *Proc. Natl. Acad. Sci. USA* 84:675–679, 1987.

Enzyme stability is expressed as the half-life, or rate of enzyme inactivation, at 100°C. A longer half-life indicates a more stable enzyme.

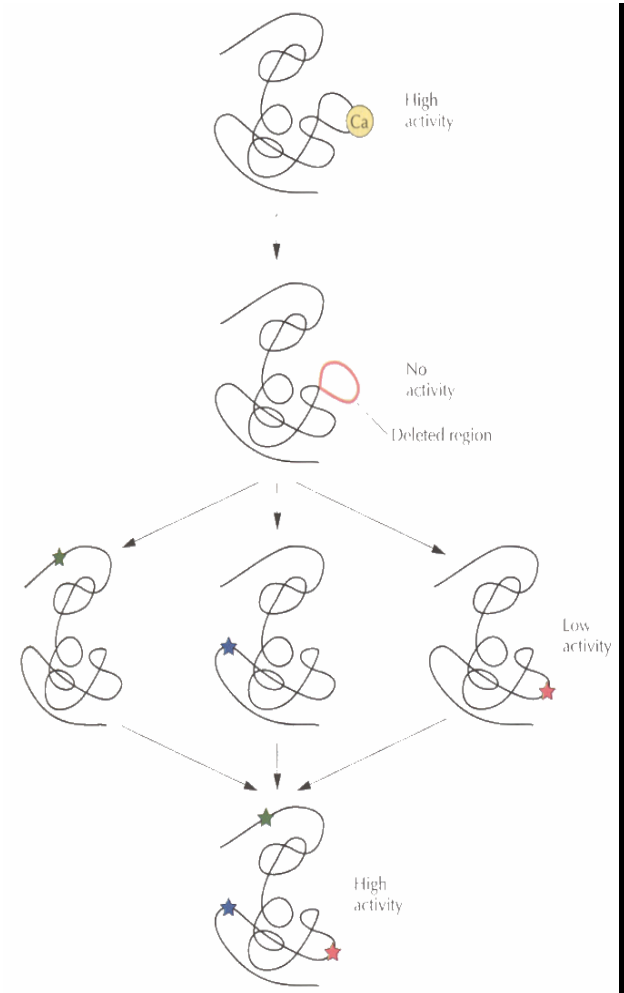
Sustitución de aminoácidos para mejorar la actividad de una enzima

Aminoacylation activity of native (Thr-51) and modified (Ala-51 and Pro-51) tyrosyl-tRNA synthetases

Enzyme	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m (s^{-1} M^{-1})
Thr-51	4.7	2.5	1,860
Ala-51	4.0	1.2	3,200
Pro-51	1.8	0.019	95,800

Adapted from Wilkinson et al., *Nature* 307:187–188, 1984.

The units for K_m, the binding constant of the enzyme for ATP, are millimolar units (mM); the units for k_{cat}, the catalytic rate constant, are reciprocal seconds (s^{-1}); and the units for k_{cat}/K_m, the catalytic efficiency, are s^{-1} M^{-1}.



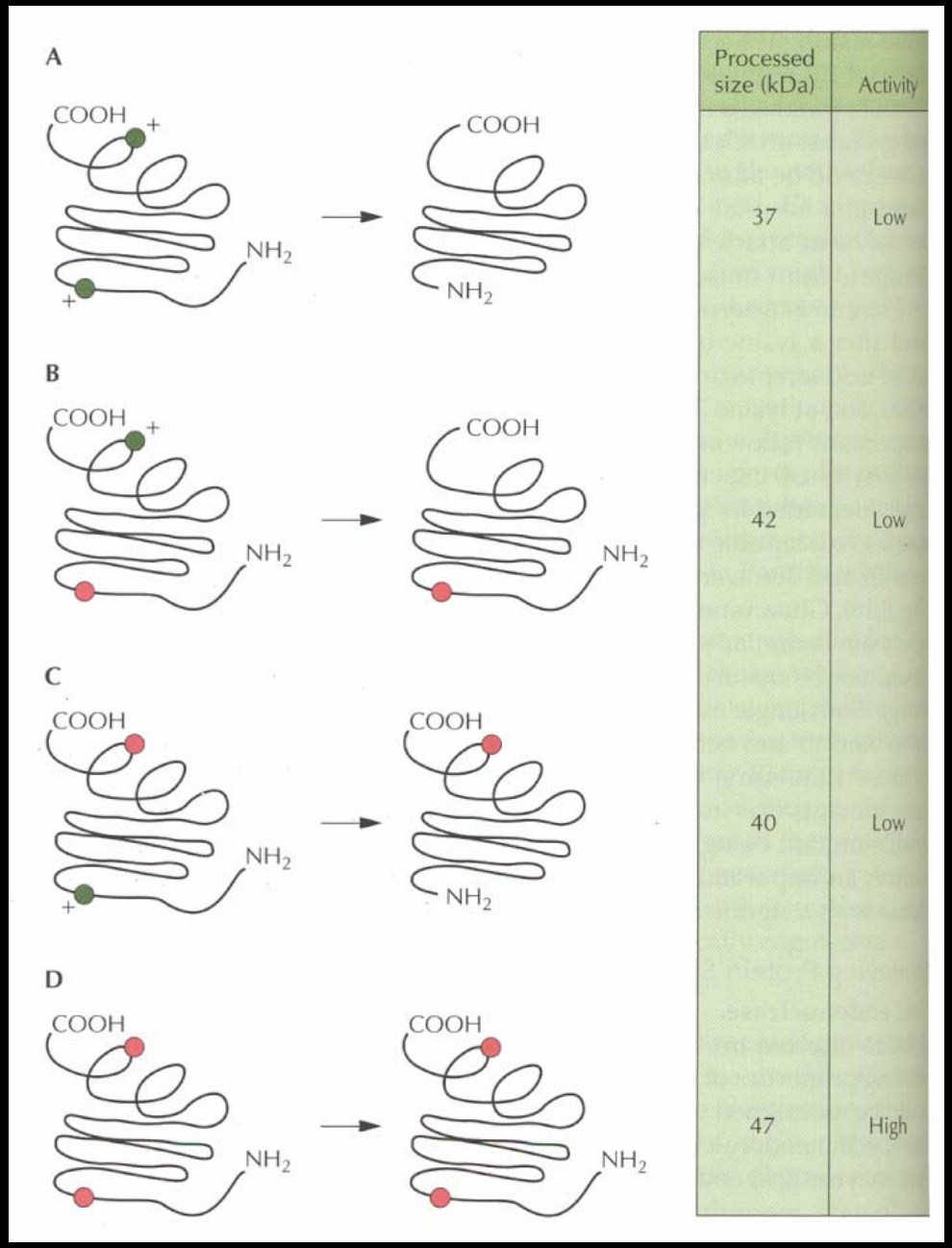
Modificación de la subtilisina, proteasa utilizada en limpiadores biodegradables.

Effect of random mutations of selected amino acid residues on the stability of subtilisin BPN′ lacking a calcium-binding domain

Region of protein	Amino acid residue	Stabilizing mutation	Fold increase in half-life
N terminus	2	Gln→Lys	2.0
	3	Ser→Cys	17.0
	4	None found	Nil
	5	Pro→Ser	1.2
Omega loop	41	Asp→Ala	1.5
	43	Lys→Asn	1.2
α-Helix	73	Ala→Leu	2.6
	74	None found	Nil
β-Pleat structure	206	Gln→Cys	17.0
	214	None found	Nil

Adapted from Strausberg et al., *Bio/Technology* 13:669–673, 1995.

The mutations at positions 3 and 206 to Cys occur in the same clone and provide such a high level of stability because of the formation of a disulfide bridge between these residues.



Processed size (kDa)	Activity
37	Low
42	Low
40	Low
47	High

Modificación de la estreptocinasa a la acción de una proteasa

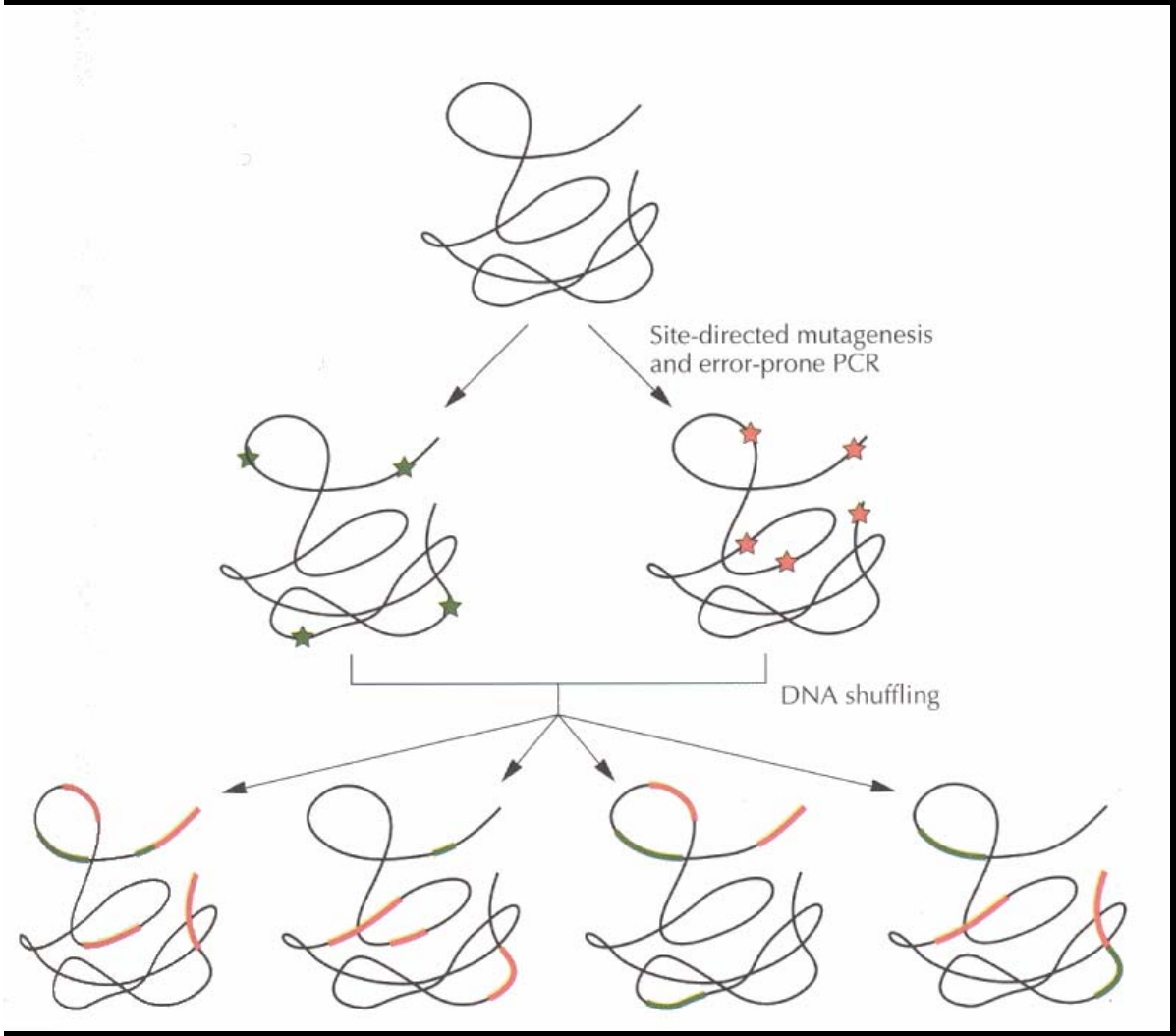
Modificación para aumentar la estabilidad y especificidad del activador del plasminógeno tisular

Stability and activity of various modified versions of tPA

tPA variant	Modifications	Stability in plasma	Fibrin binding	Activity in plasma	Activity vs clots
1	Thr (103)→Asn	10	0.34	0.68	0.56
2	LysHisArgArg(296–299)→AlaAlaAlaAla	0.85	0.93	0.13	1.01
3	Thr(103)→Asn, LysHisArgArg(296–299)→AlaAlaAlaAla	5.3	0.33	0.13	0.65
4	Thr(103)→Asn, Asn(117)→Gln	3.4	1.0	1.13	1.17
5	LysHisArgArg(296–299)→AlaAlaAlaAla, Asn(117)→Gln	1.2	1.33	0.16	1.38
6	Thr(103)→Asn, LysHisArgArg(296–299)→AlaAlaAlaAla, Asn(117)→Gln	8.3	0.87	0.06	0.85

Adapted from Keyt et al., *Proc. Natl. Acad. Sci. USA* **91**:3670–3674, 1994.

All of the values shown are normalized to the wild type. Plasma stability is the reciprocal of the time it takes for plasma clearance; larger numbers indicate a more stable derivative. Fibrin specificity is reflected by a high activity versus clots and a low activity in plasma.



Se pueden generar familias
De proteínas modificadas
utilizando las técnicas de
mutagénesis y mezcla de
fragmentos de restricción.