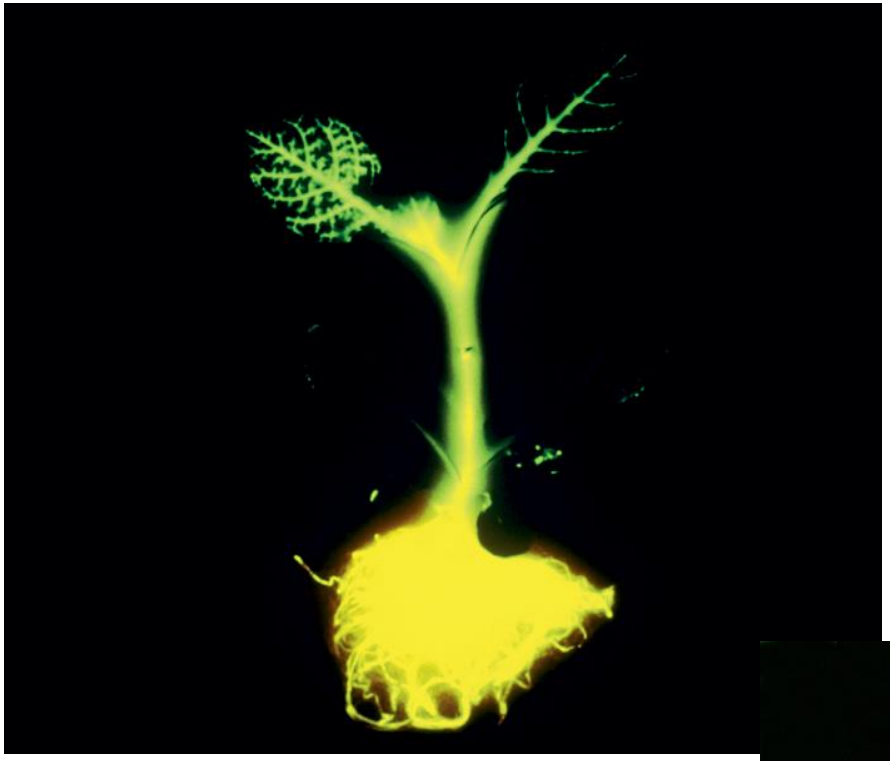
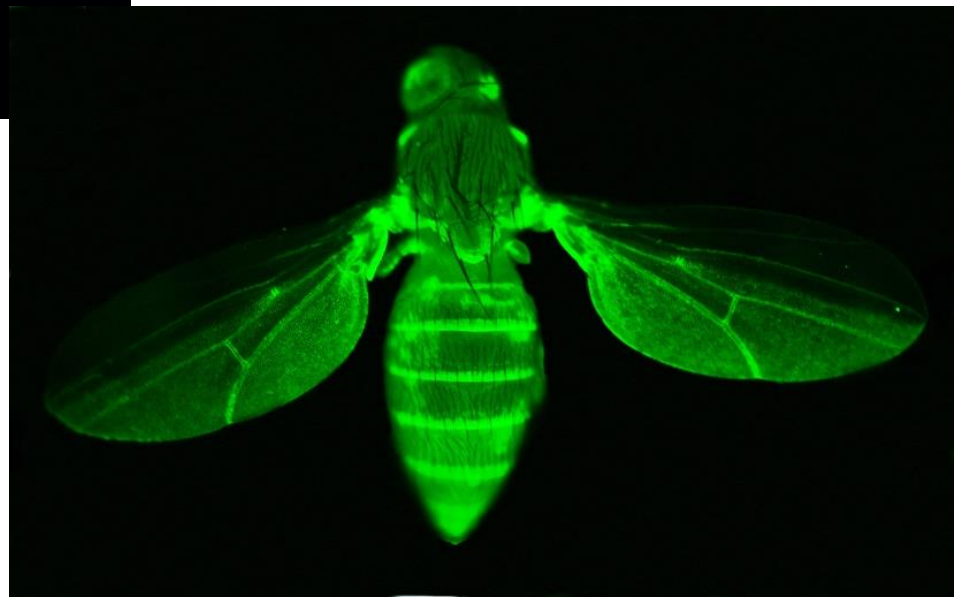
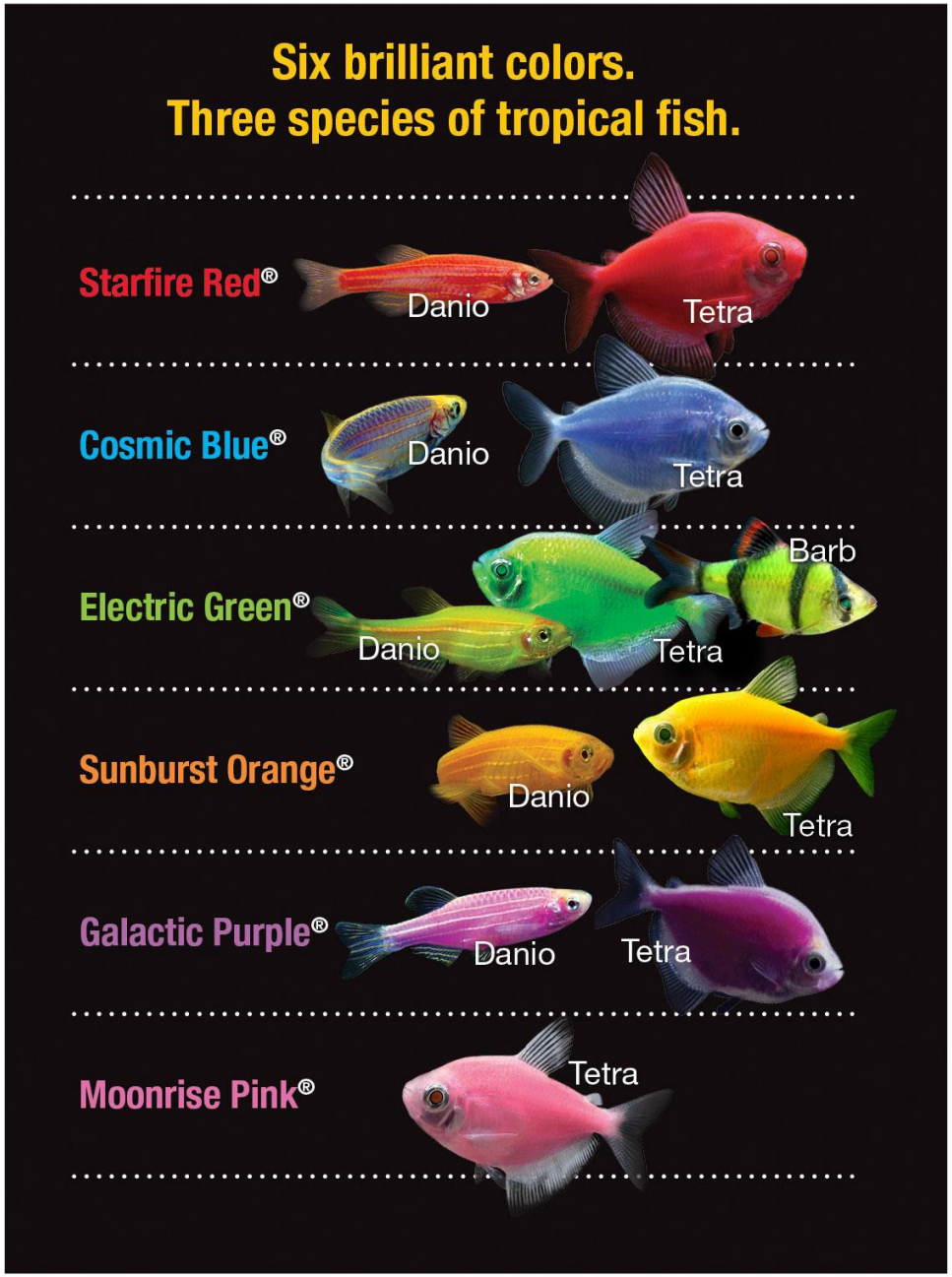
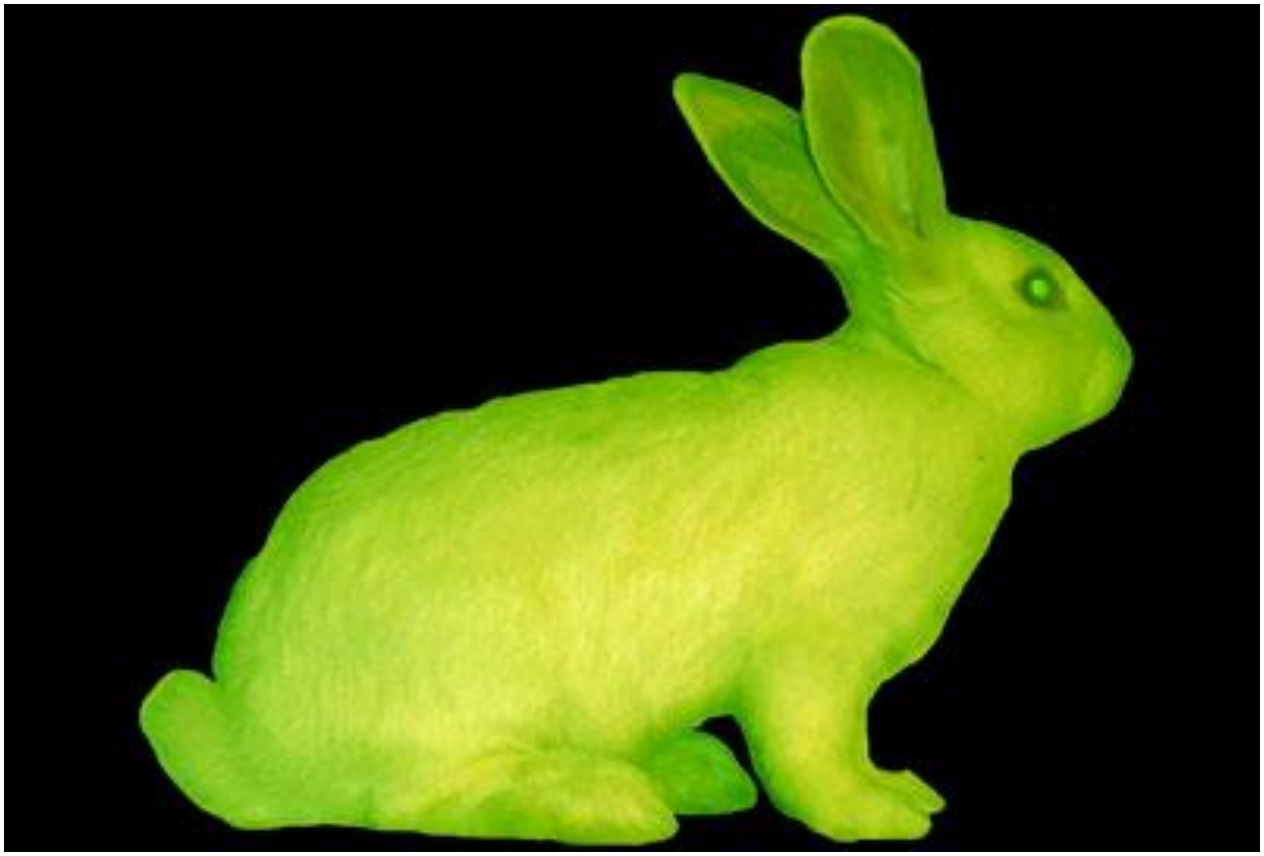
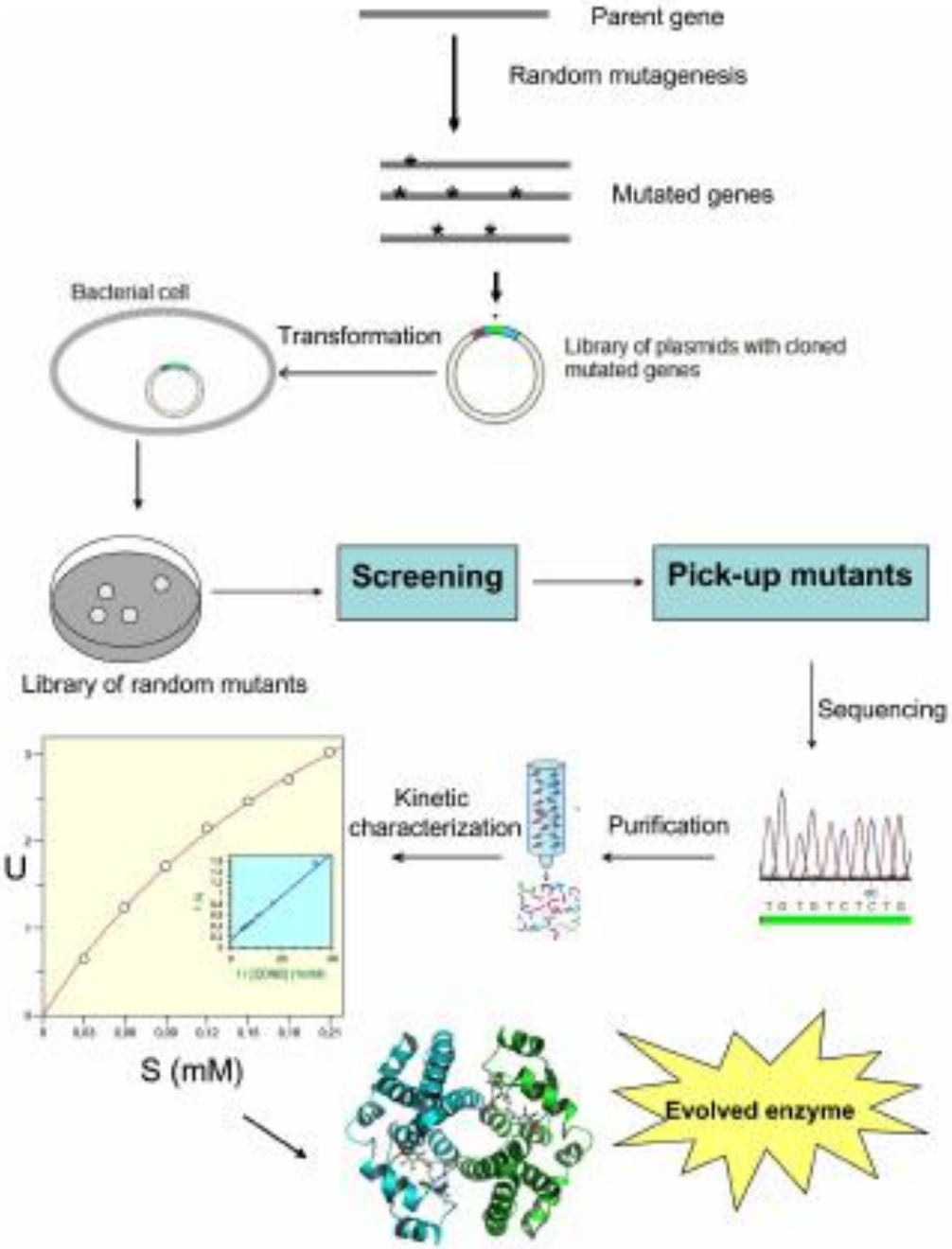




# Six brilliant colors.
# Three species of tropical fish.






Parent gene
Random mutagenesis
Mutated genes
Bacterial cell
Transformation
Library of plasmids with cloned mutated genes
Screening
Pick-up mutants
Library of random mutants
Sequencing
Kinetic characterization
Purification
U
S (mM)
Evolved enzyme

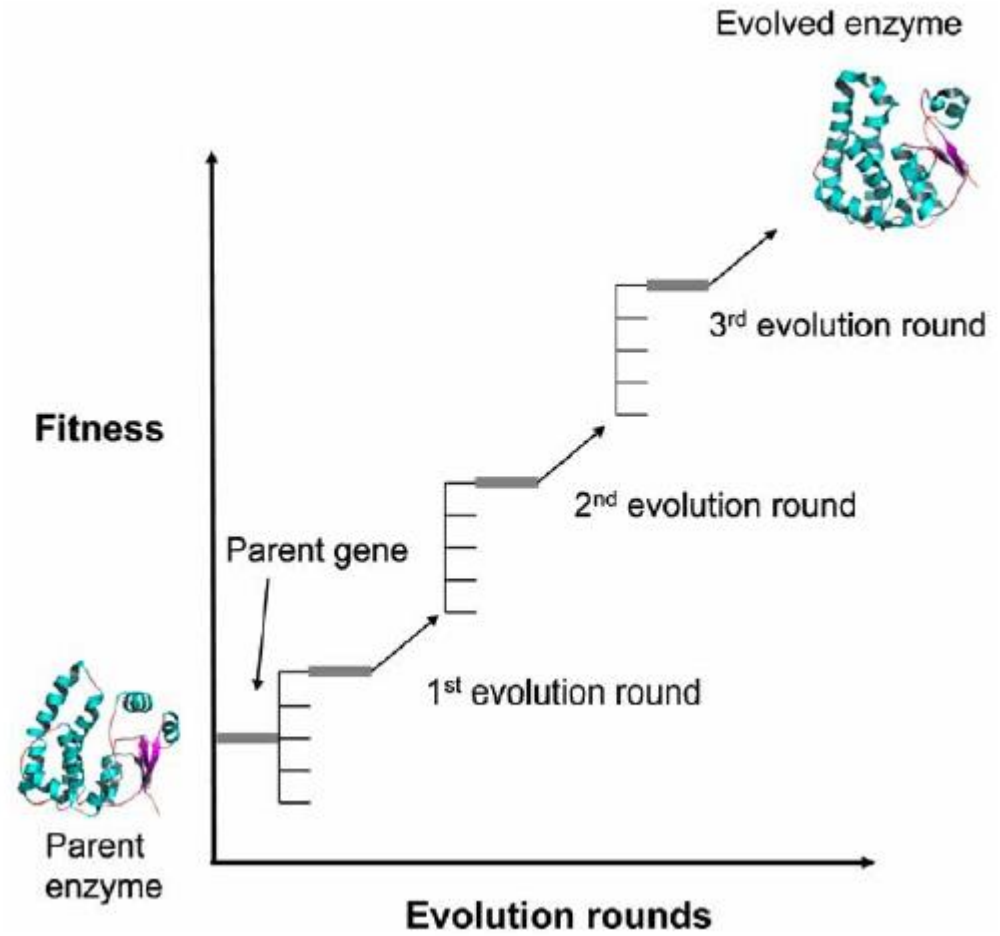
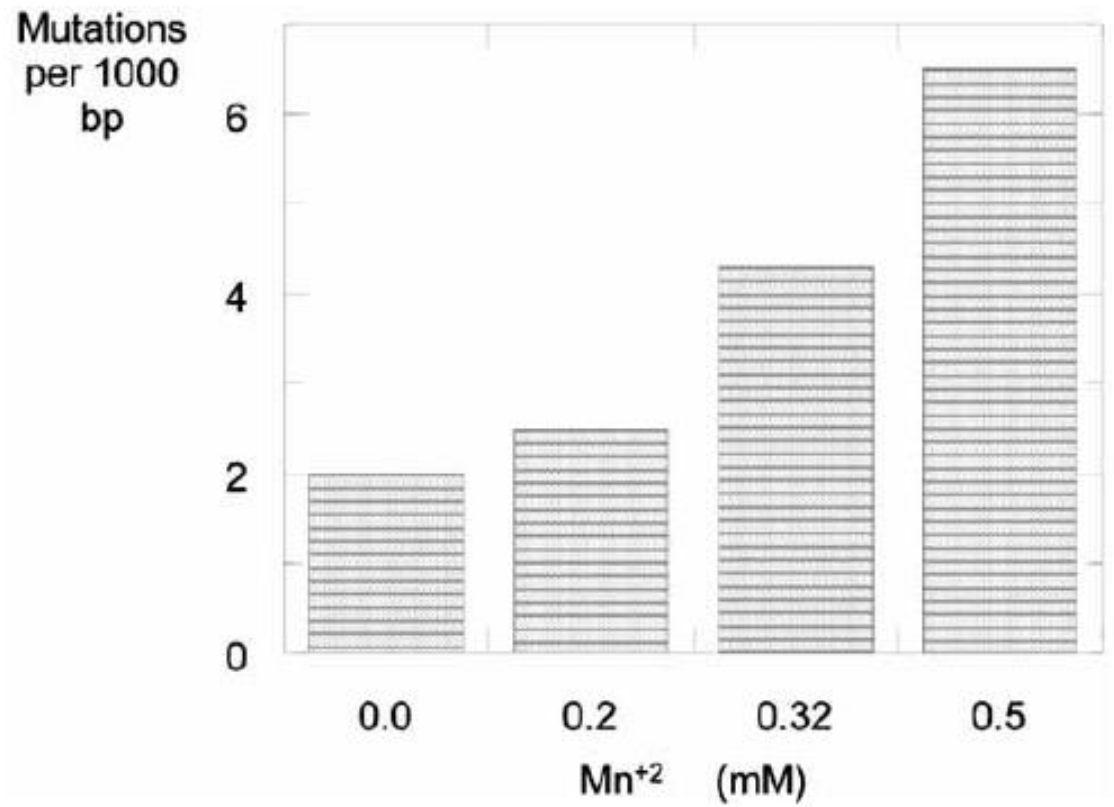
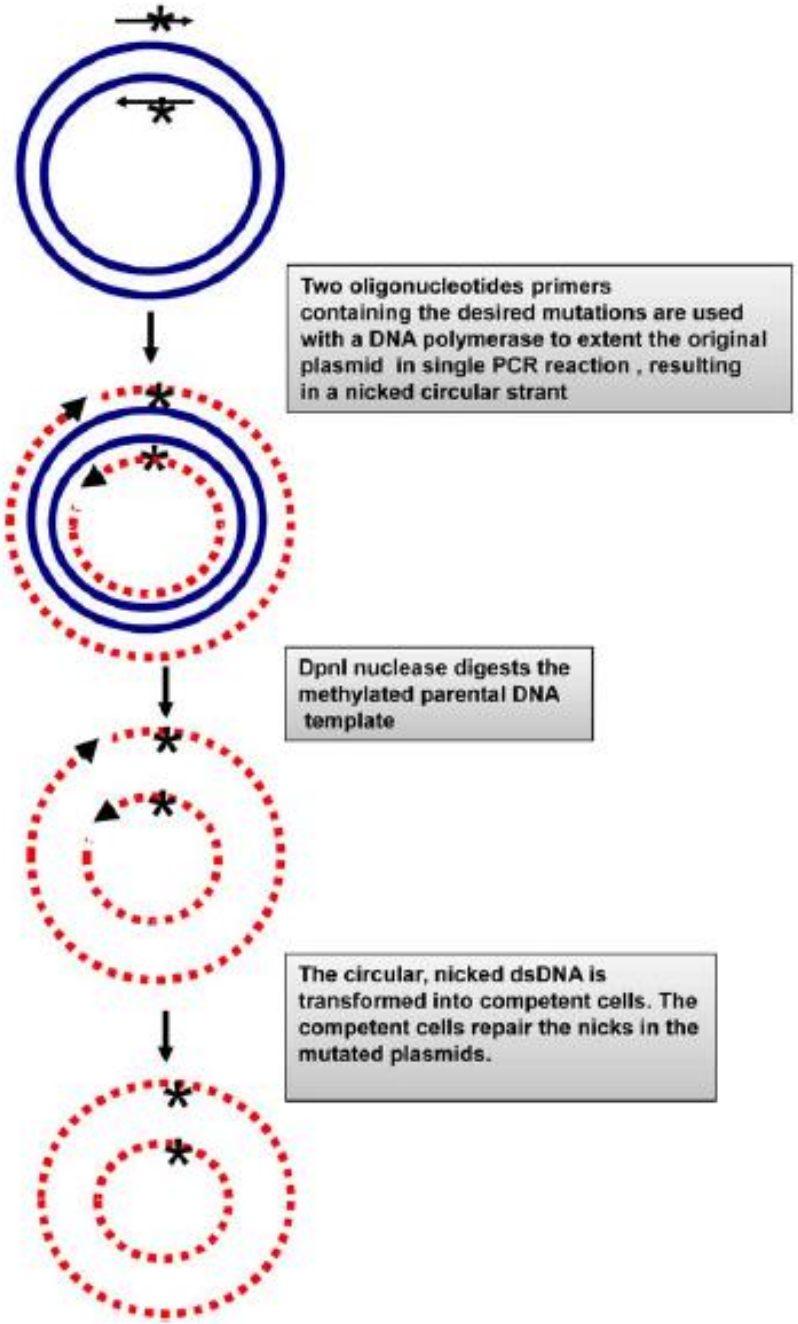
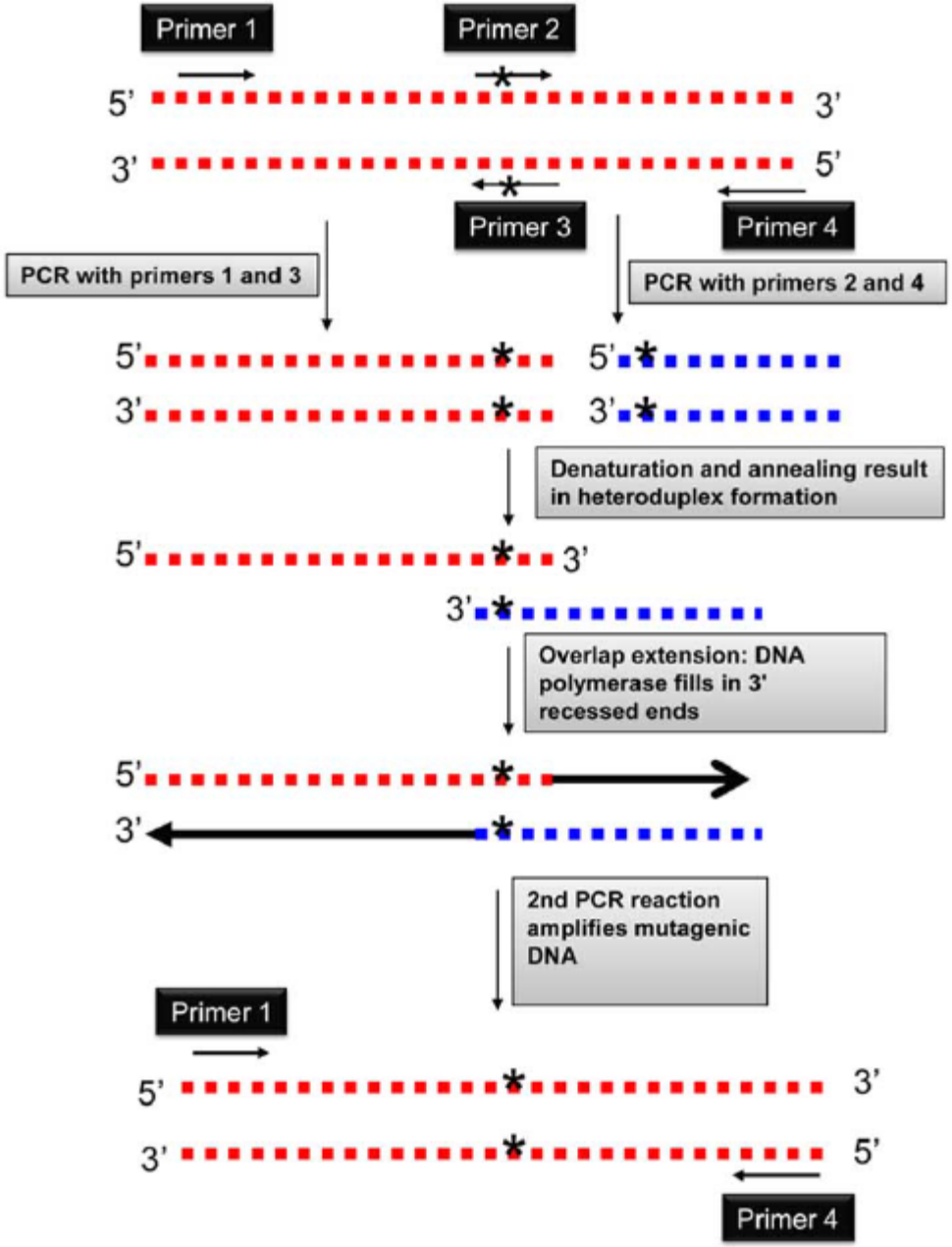

Evolved enzyme
3rd evolution round
Fitness
2nd evolution round
Parent gene
1st evolution round
Parent
enzyme
Evolution rounds

**Table 1. Frequently Used Chemical Mutagens and Mutation Type Caused**

| Chemical | Type of DNA Lesion | Mutation Type |
|---|---|---|
| ICR-170 | Intercalation | Frameshift |
| MMC, DEO | Interstrand cross-linking | Deletion |
| 4-NQO, DEB | DNA adducts | Base-pair substitution |
| MNNG, EMS, MNU | Alkylation | Base-pair substitution |
| NA, HA | Modification of bases | Base-pair substitution |
| 2AP | Base analogue | Base-pair substitution |
| MMS<br>MA<br>BS | Alkylation/strand breaks<br>Transition mutations<br>Reacts specifically with C in single-stranded regions of DNA | Several different types<br>GC to AT |


Mutations per 1000 bp
6
4
2
0
0.0
0.2
0.32
0.5
Mn+2 (mM)


Two oligonucleotides primers containing the desired mutations are used with a DNA polymerase to extent the original plasmid in single PCR reaction , resulting in a nicked circular strant
DpnI nuclease digests the methylated parental DNA template
The circular, nicked dsDNA is transformed into competent cells. The competent cells repair the nicks in the mutated plasmids.


Primer 1
Primer 2
5'
3'
3'
5'
Primer 3
Primer 4
PCR with primers 1 and 3
PCR with primers 2 and 4
5'
3'
5'
3'
Denaturation and annealing result in heteroduplex formation
5'
3'
3'
Overlap extension: DNA polymerase fills in 3' recessed ends
5'
3'
2nd PCR reaction amplifies mutagenic DNA
Primer 1
5'
3'
3'
5'
Primer 4

ACS chemical biology

Articles

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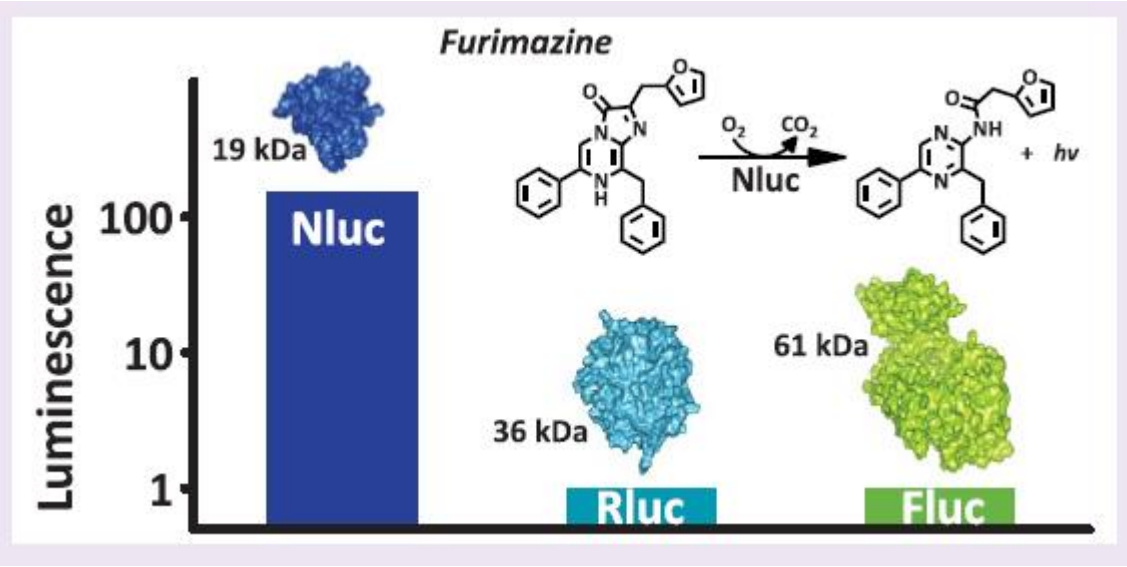
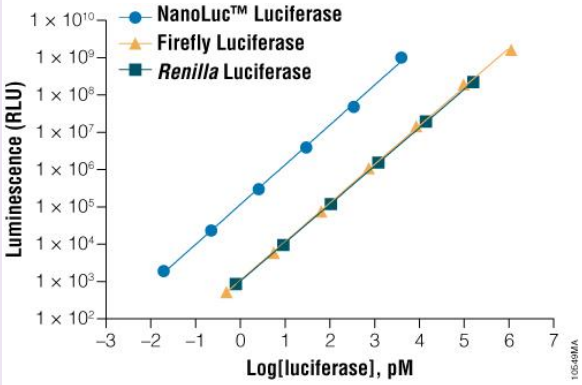
# Engineered Luciferase Reporter from a Deep Sea Shrimp Utilizing a Novel Imidazopyrazinone Substrate

Mary P. Hall,[†] James Unch,[‡] Brock F. Binkowski,[†] Michael P. Valley,[†] Braeden L. Butler,[†] Monika G. Wood,[†] Paul Otto,[†] Kristopher Zimmerman,[†] Gediminas Vidugiris,[†] Thomas Machleidt,[†] Matthew B. Robers,[†] Hélène A. Benink,[†] Christopher T. Eggers,[†] Michael R. Slater,[†] Poncho L. Meisenheimer,[‡] Dieter H. Klaubert,[‡] Frank Fan,[†] Lance P. Encell,*[,†] and Keith V. Wood[†]

[†]Promega Corporation, Madison, Wisconsin 53711 United States

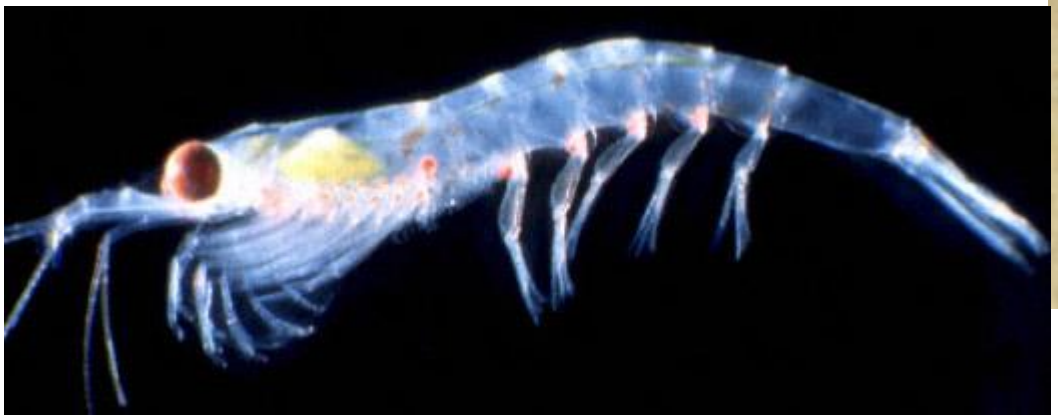
[‡]Promega Biosciences Incorporated, San Luis Obispo, California 93401 United States

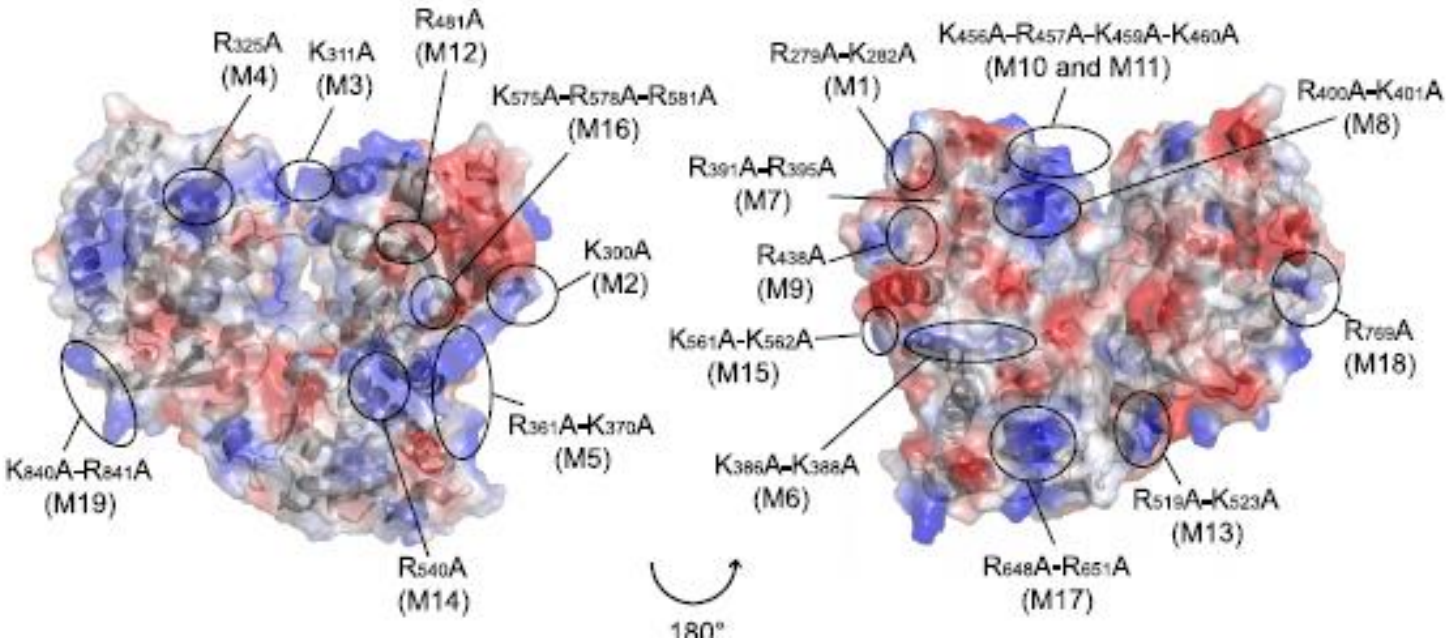


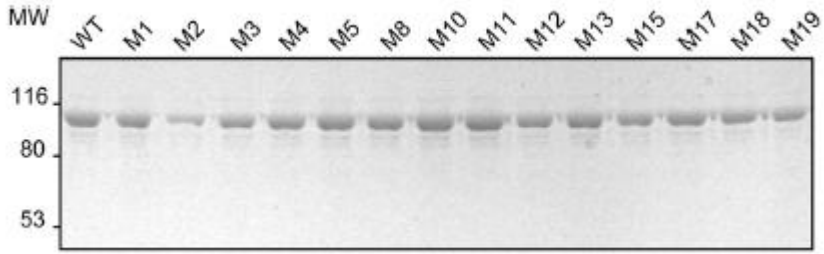



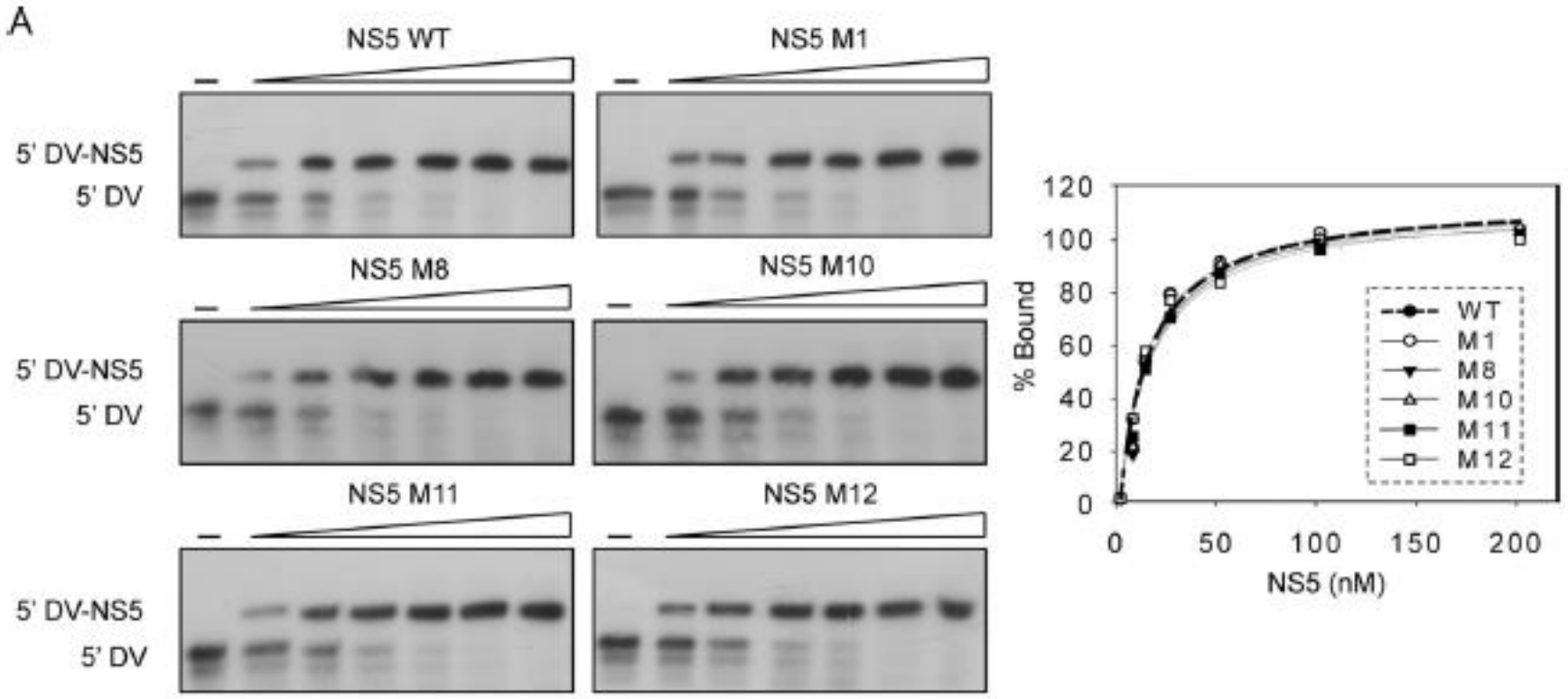

freedom evo
TECAN

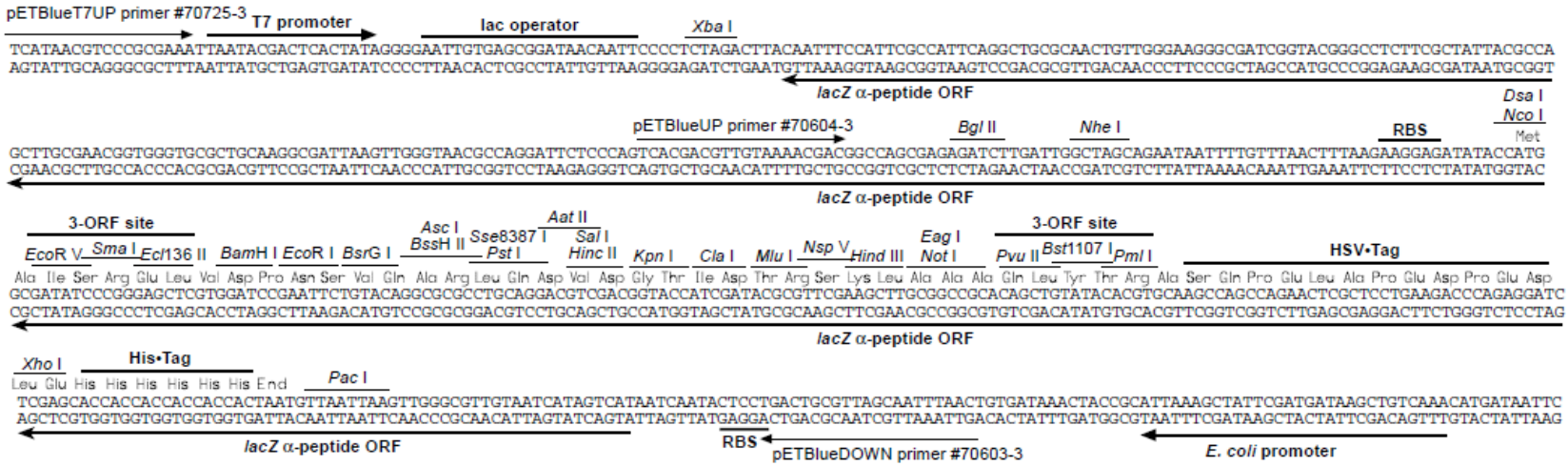

© BugDreams.com



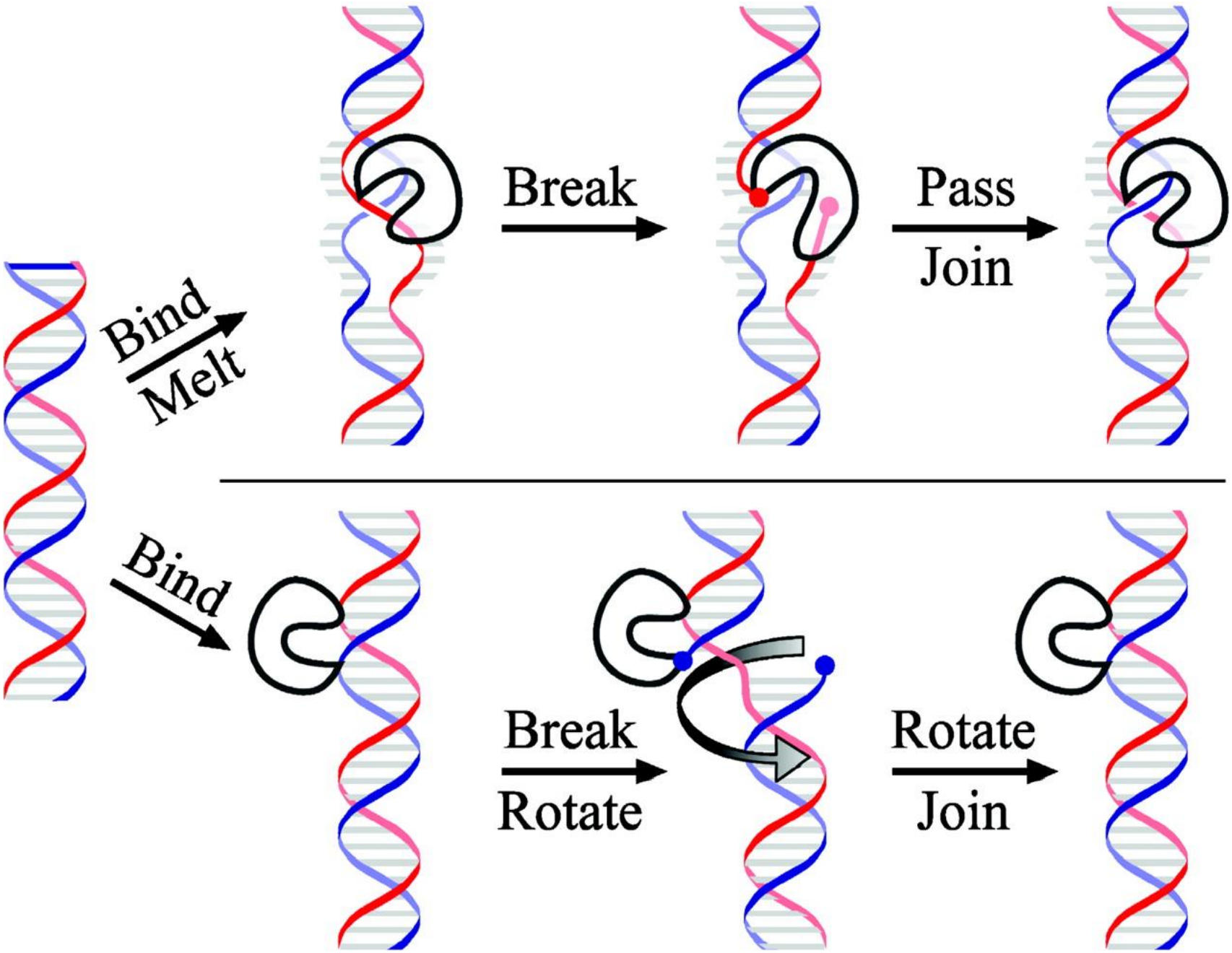

R325A
(M4)
K311A
(M3)
R481A
(M12)
K575A-R578A-R581A
(M16)
K300A
(M2)
R361A-K370A
(M5)
K840A-R841A
(M19)
R540A
(M14)
180°
R279A-K282A
(M1)
K456A-R457A-K459A-K460A
(M10 and M11)
R400A-K401A
(M8)
R391A-R395A
(M7)
R438A
(M9)
K561A-K562A
(M15)
R769A
(M18)
K388A-K388A
(M6)
R519A-K523A
(M13)
R648A-R651A
(M17)

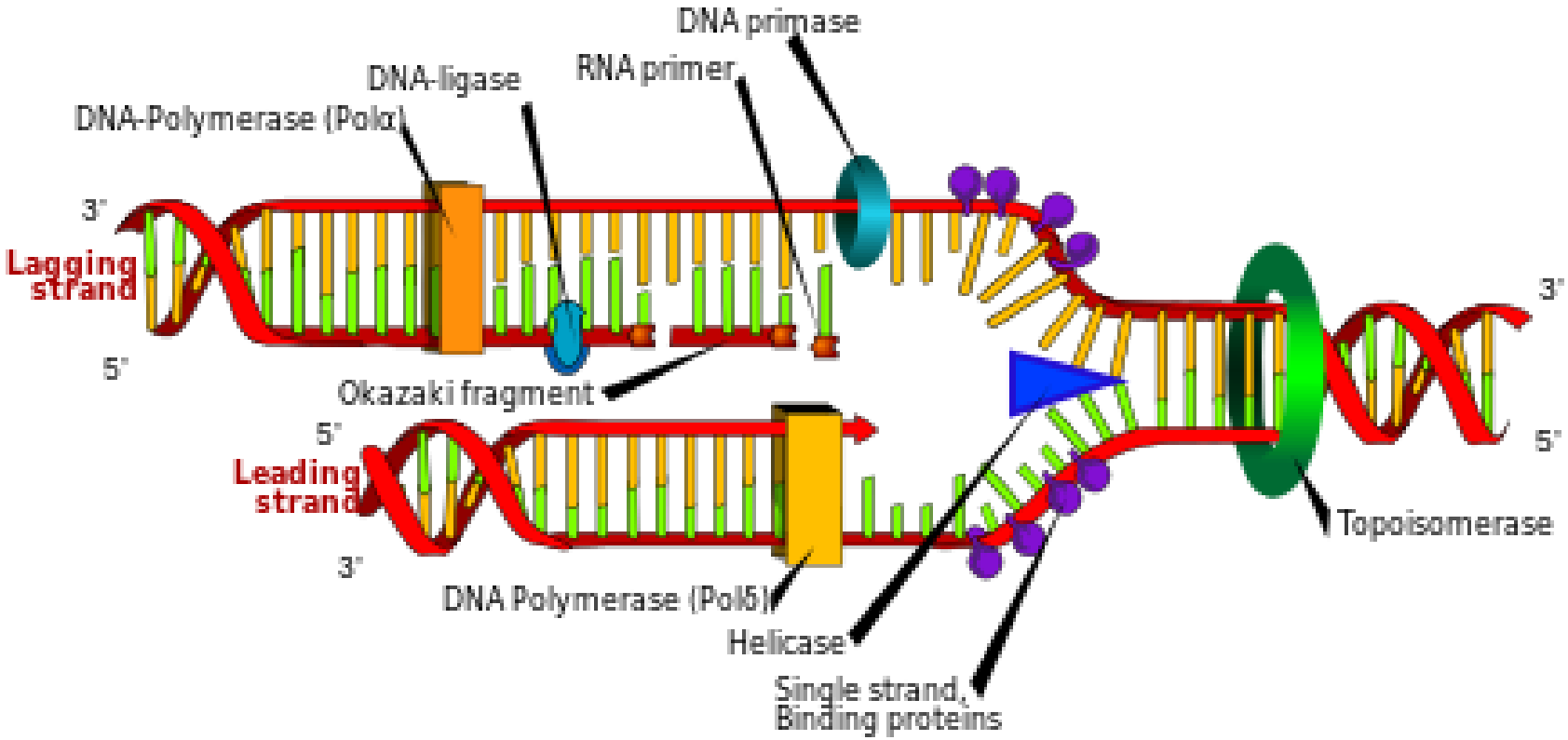

MW
WT
M1
M2
M3
M4
M5
M8
M10
M11
M12
M13
M15
M17
M18
M19
116
80
53


A
NS5 WT
NS5 M1
NS5 M8
NS5 M10
NS5 M11
NS5 M12
5' DV-NS5
5' DV
% Bound
NS5 (nM)
WT
M1
M8
M10
M11
M12
0
20
40
60
80
100
120
0
50
100
150
200




**pETBlue-2 cloning/expression region**


Bind
Melt
Break
Pass
Join
Bind
Break
Rotate
Rotate
Join


DNA primase
DNA-ligase
RNA primer
DNA-Polymerase (Polα)
3'
Lagging strand
5'
Okazaki fragment
5'
Leading strand
3'
DNA Polymerase (Polδ)
Helicase
Single strand Binding proteins
3'
5'
Topoisomerase

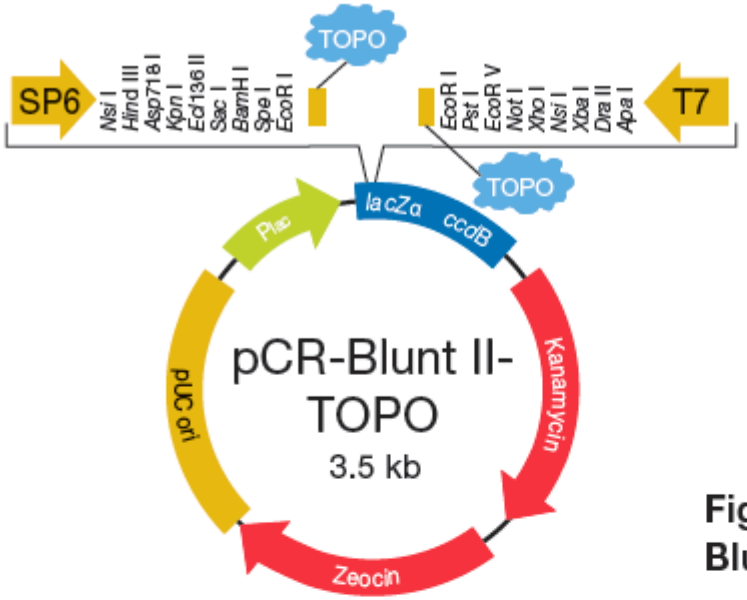



**Figure 6. The pCR-Blunt II-TOPO Vector.**

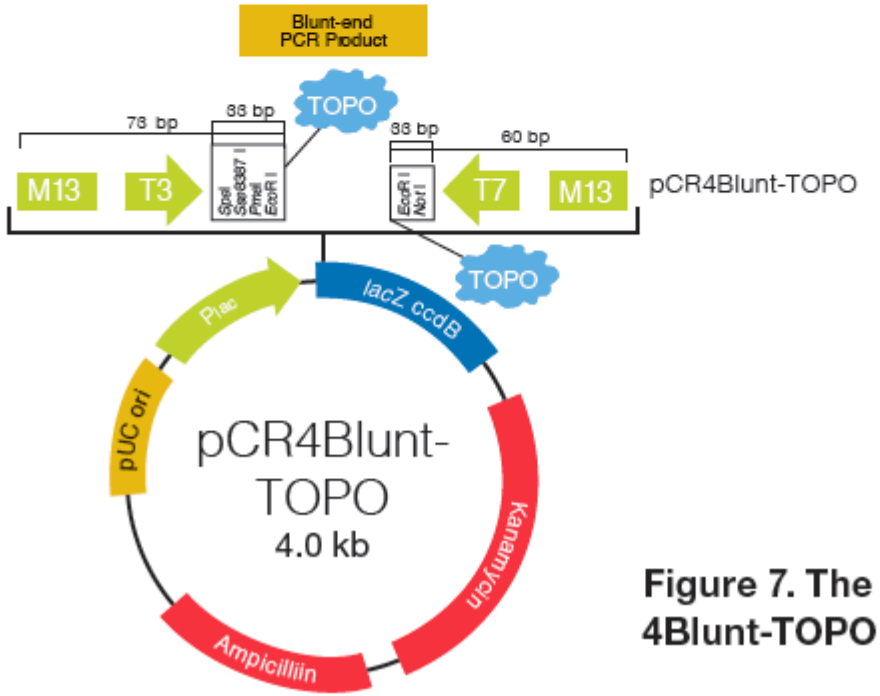
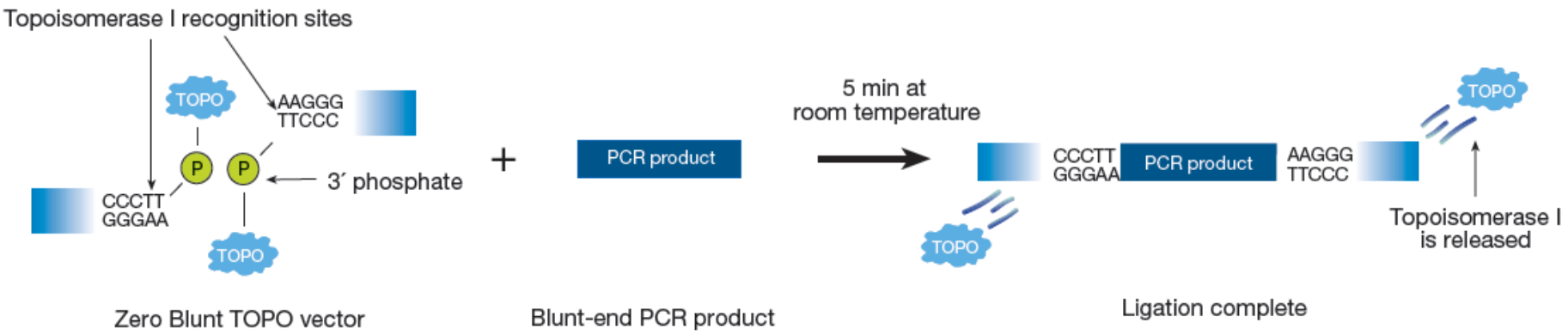
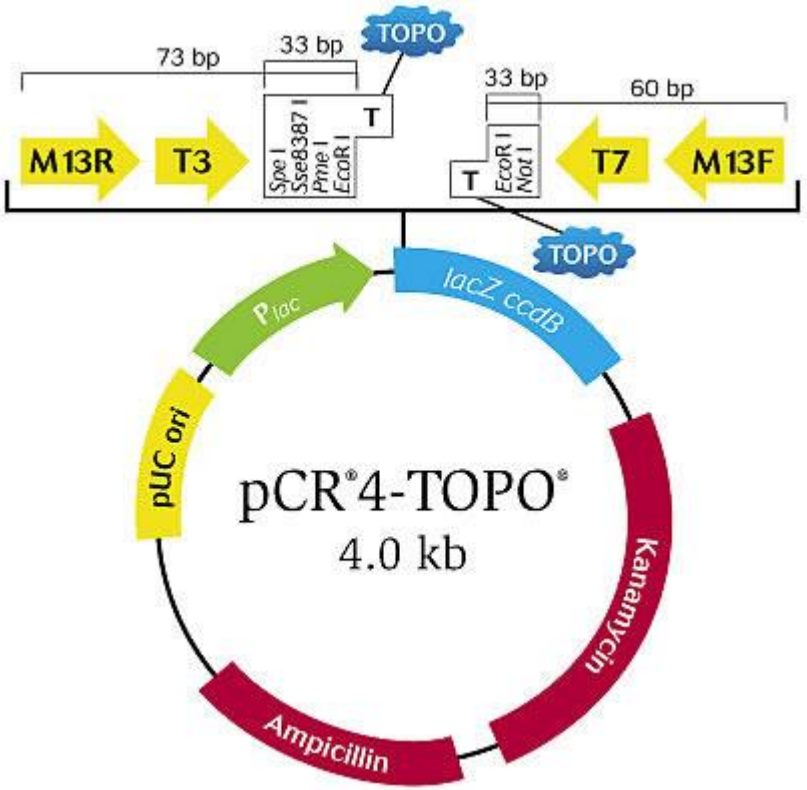
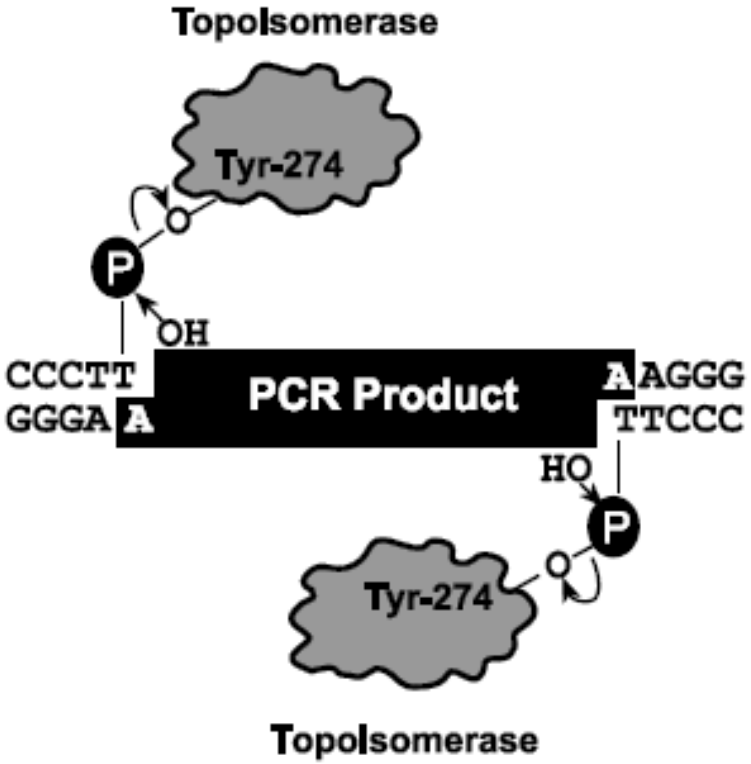
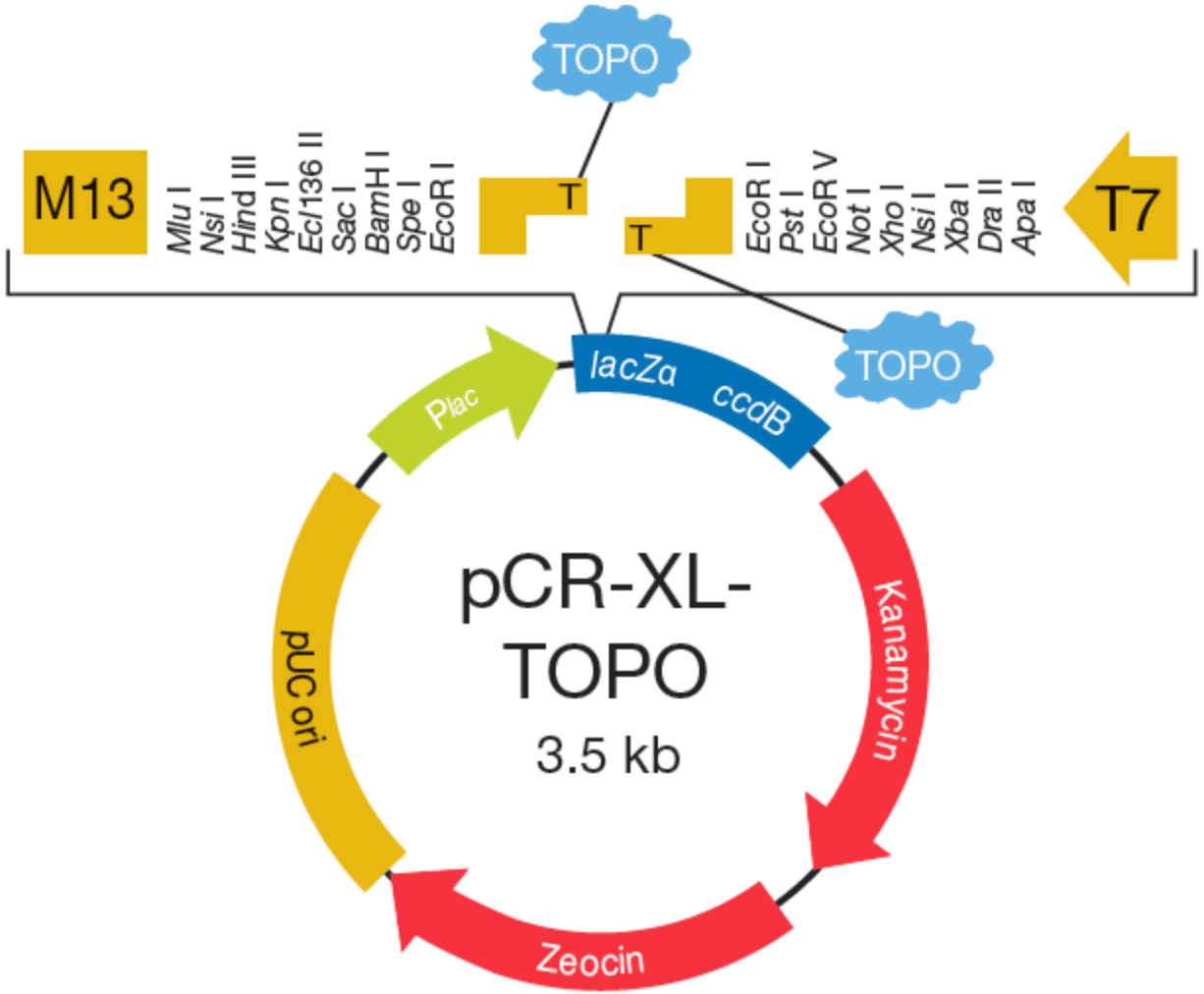



**Figure 7. The pCR-4Blunt-TOPO Vector.**


Topoisomerase I recognition sites
TOPO
AAGGG
TTCCC
P
P
3′ phosphate
CCCTT
GGGAA
TOPO
PCR product
5 min at
room temperature
TOPO
CCCTT
GGGAA
PCR product
AAGGG
TTCCC
TOPO
Topoisomerase I
is released
Zero Blunt TOPO vector
Blunt-end PCR product
Ligation complete


73 bp
33 bp
TOPO
33 bp
60 bp
M13R
T3
Spe I
Sse8387 I
Pme I
EcoR I
T
T
EcoR I
Not I
T7
M13F
TOPO
P lac
lacZ ccdB
pUC ori
pCR®4-TOPO®
4.0 kb
Kanamycin
Ampicillin


Topoisomerase
Tyr-274
P
OH
CCCTT
GGGA
A
PCR Product
A
AGGG
TTCCC
HO
P
Tyr-274
Topoisomerase


TOPO
M13
Mlu I
Nsi I
Hind III
Kpn I
Ecl136 II
Sac I
BamH I
Spe I
EcoR I
T
T
EcoR I
Pst I
EcoR V
Not I
Xho I
Nsi I
Xba I
Dra II
Apa I
T7
TOPO
lacZα
ccdB
Plac
pUC ori
Kanamycin
Zeocin
pCR-XL-
TOPO
3.5 kb

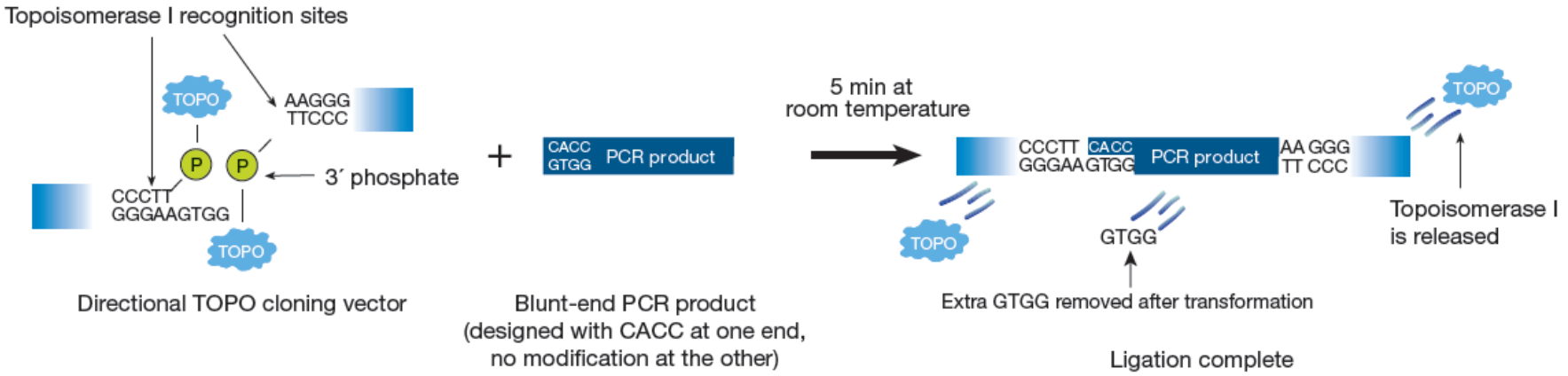
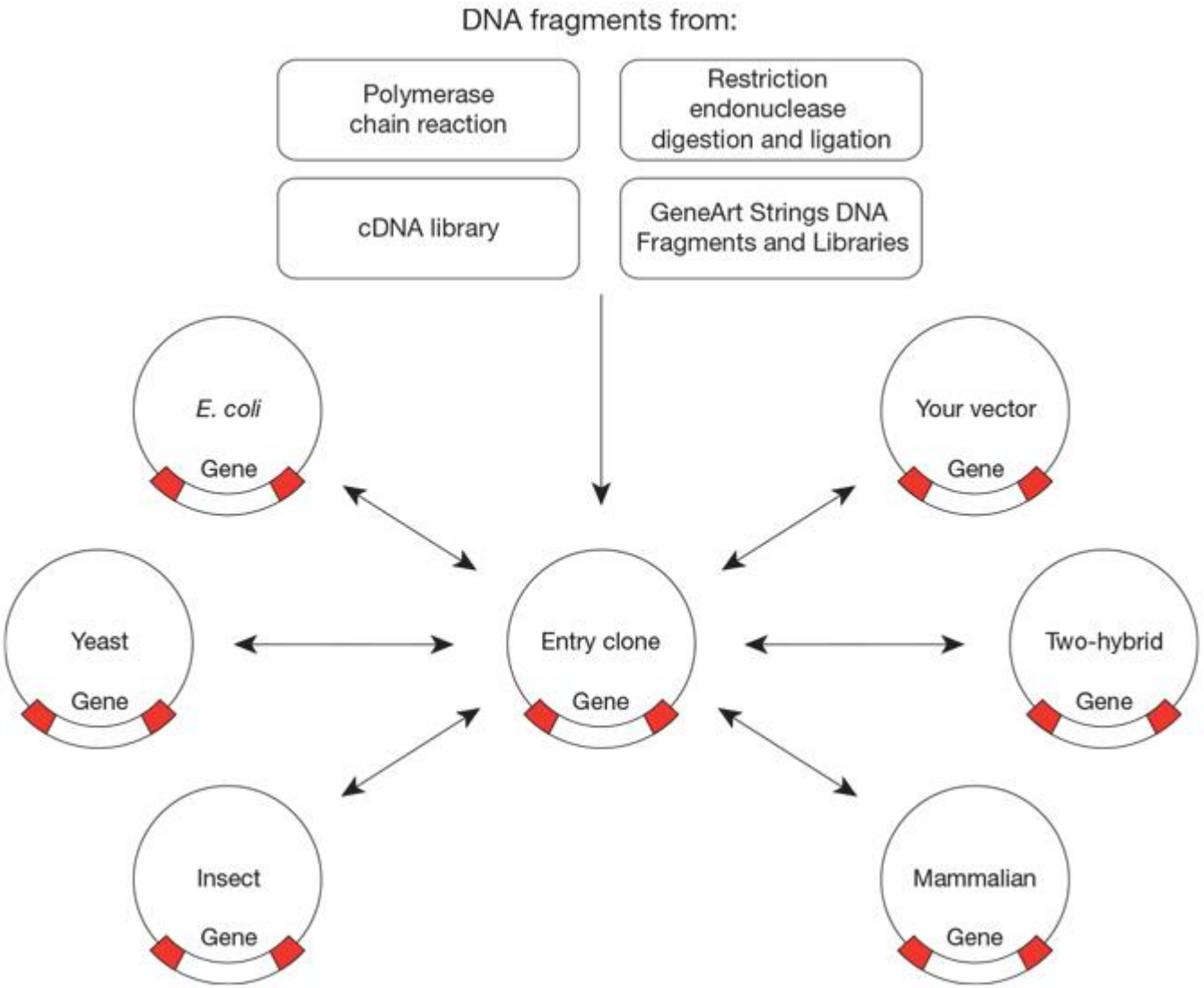
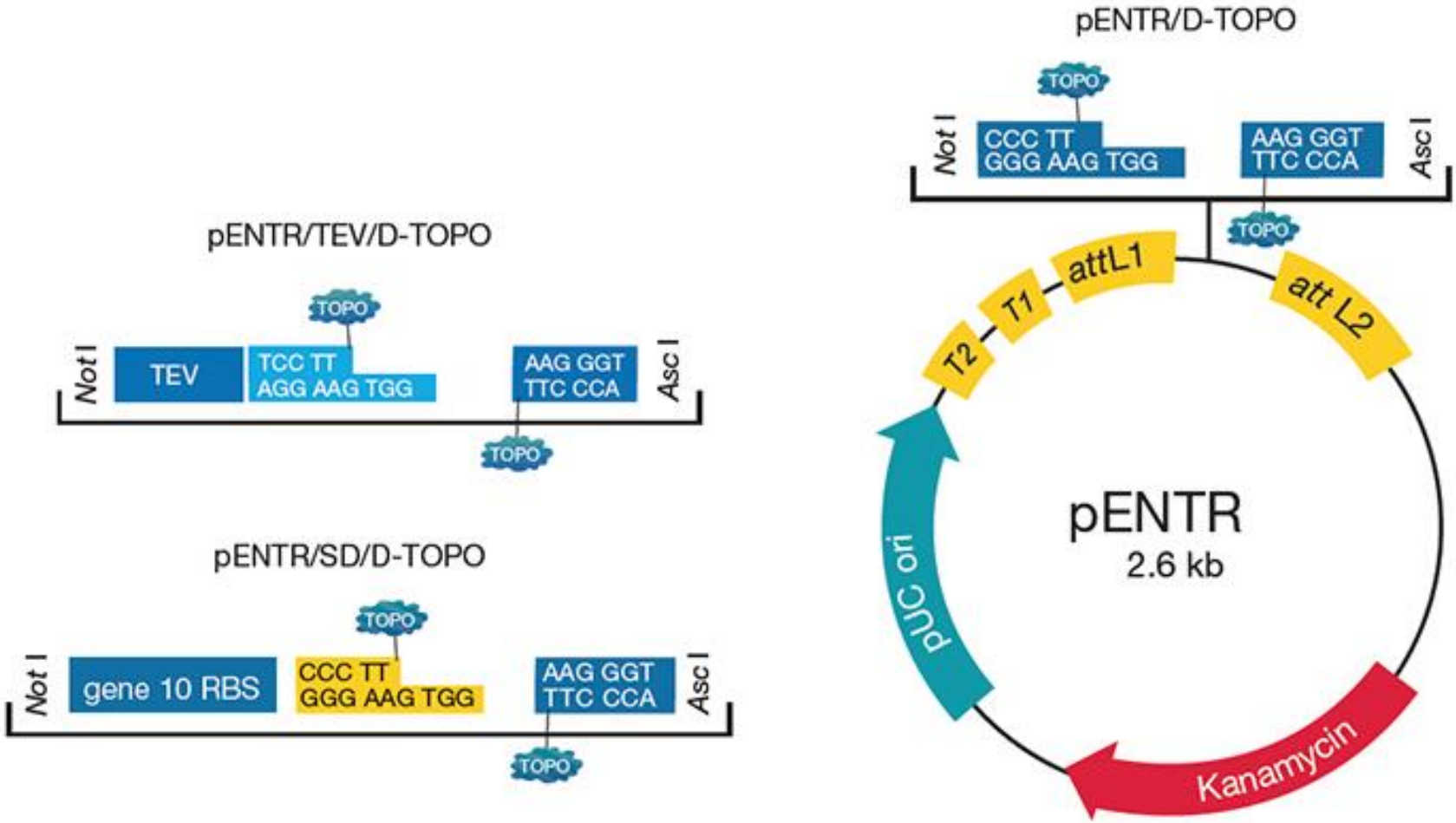


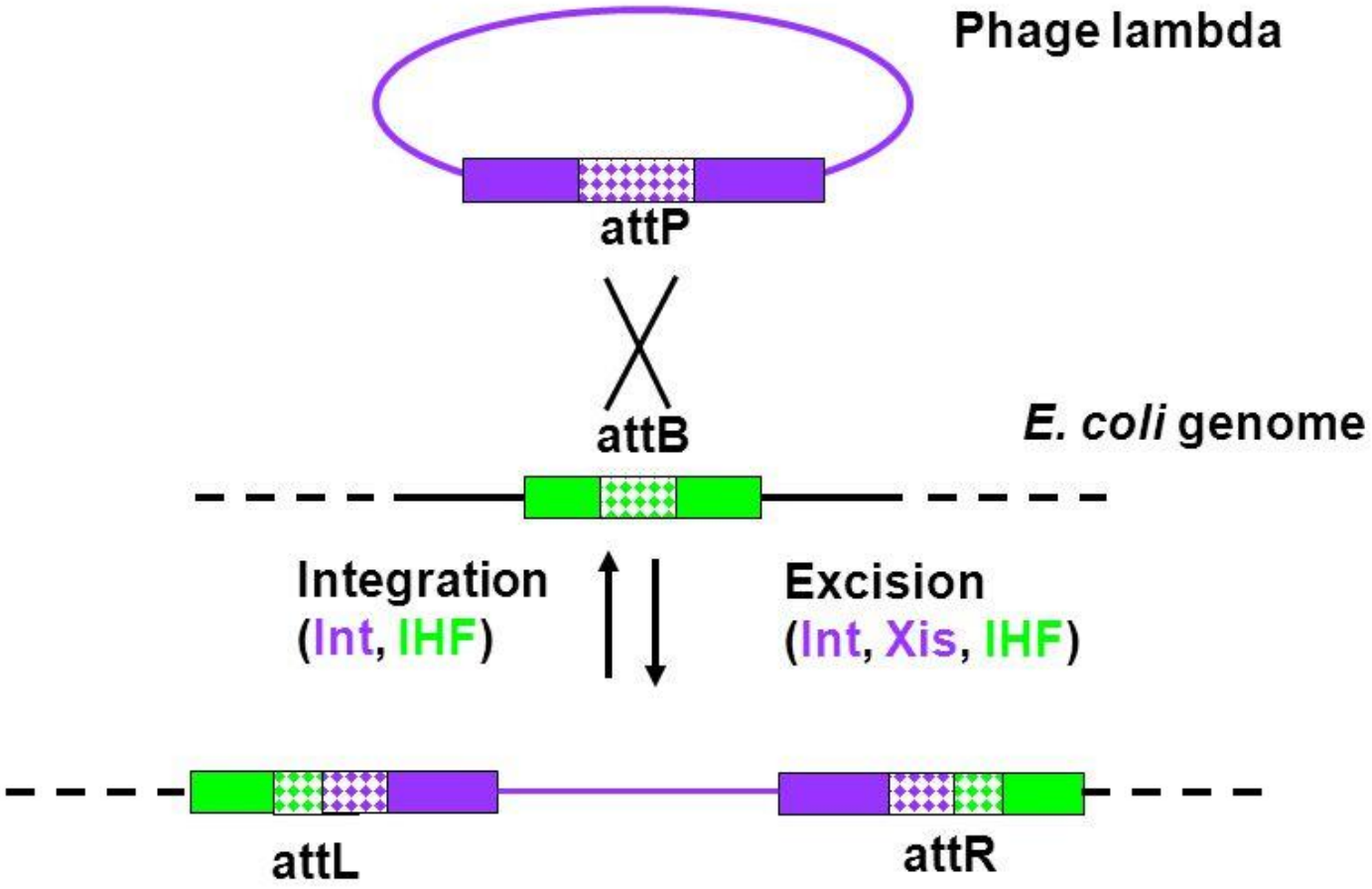

**Figure 10. Directional TOPO cloning of blunt-end DNA.**

# Gateway technology

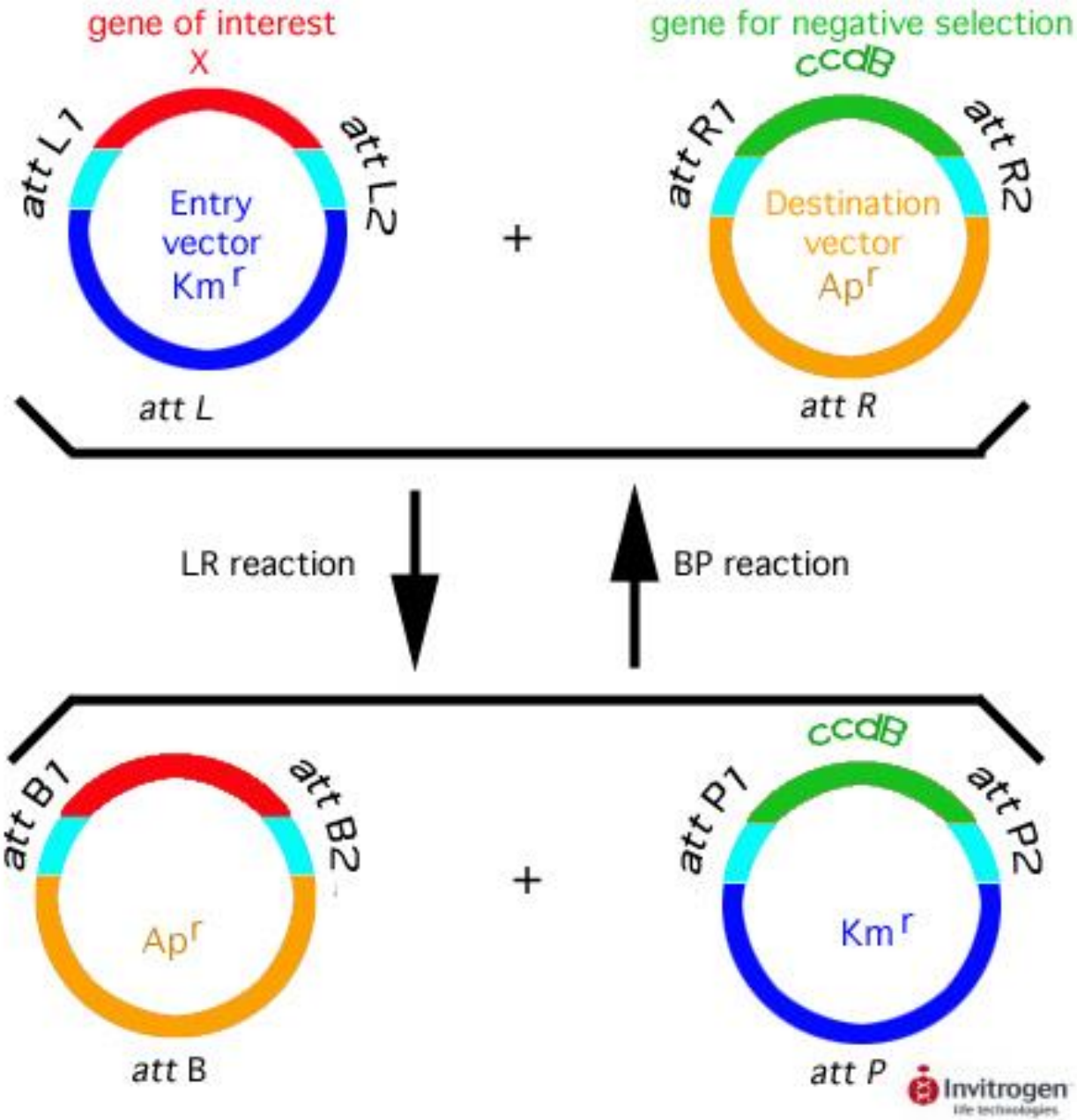



pENTR/TEV/D-TOPO
TOPO
Not I
TEV
TCC TT
AGG AAG TGG
AAG GGT
TTC CCA
Asc I
TOPO
pENTR/SD/D-TOPO
TOPO
Not I
gene 10 RBS
CCC TT
GGG AAG TGG
AAG GGT
TTC CCA
Asc I
TOPO
pENTR/D-TOPO
TOPO
Not I
CCC TT
GGG AAG TGG
AAG GGT
TTC CCA
Asc I
TOPO
attL1
T1
T2
att L2
pUC ori
pENTR
2.6 kb
Kanamycin

# Prophage Integration




Int (integrasa), IHF (factor de integración del huésped) y Xis (excisionasa)


gene of interest
X
att L1
att L2
Entry
vector
Km r
att L
+
gene for negative selection
ccdB
att R1
att R2
Destination
vector
Ap r
att R
LR reaction
BP reaction
att B1
att B2
Ap r
att B
+
ccdB
att P1
att P2
Km r
att P
Invitrogen
life technologies