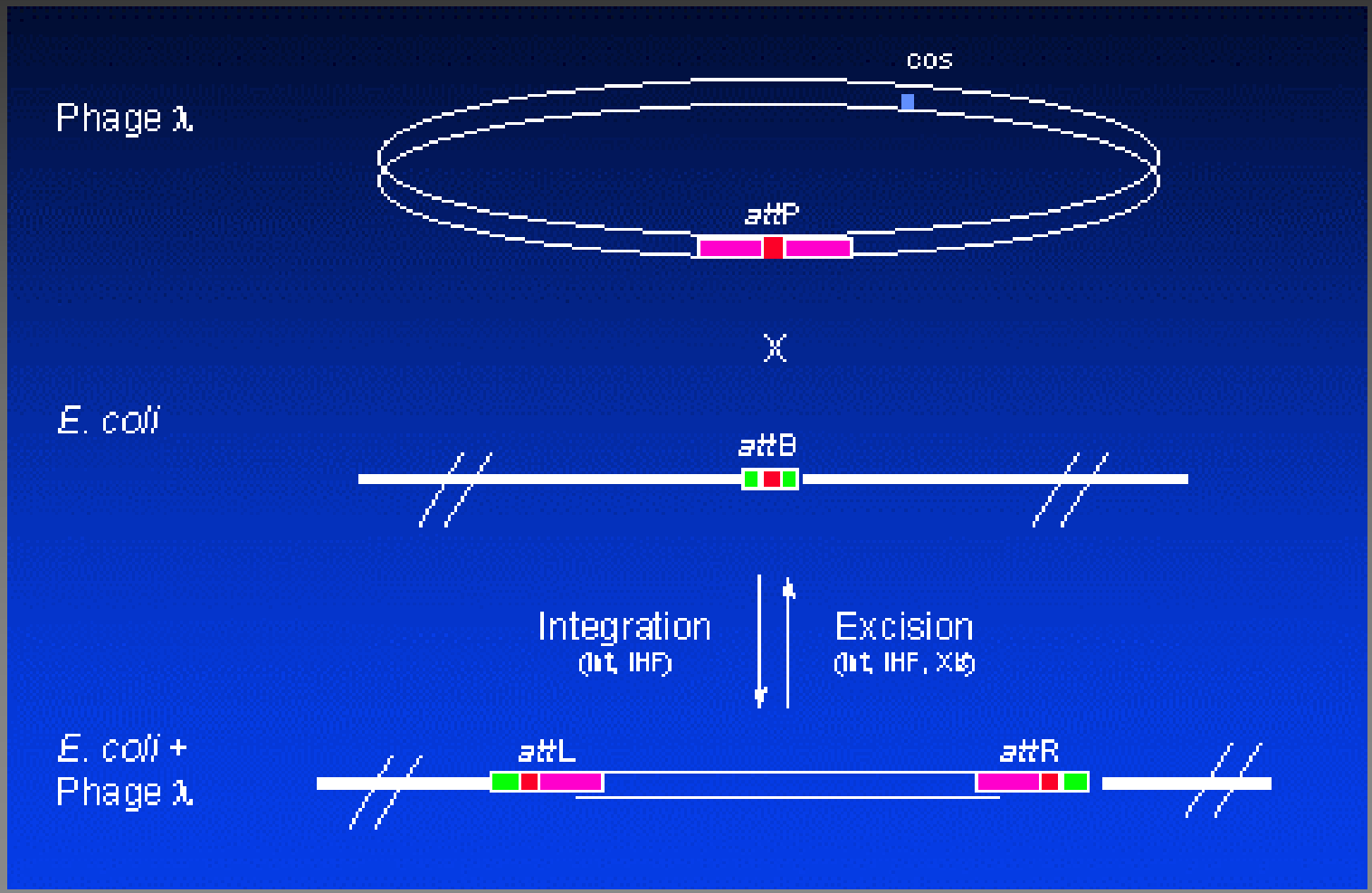
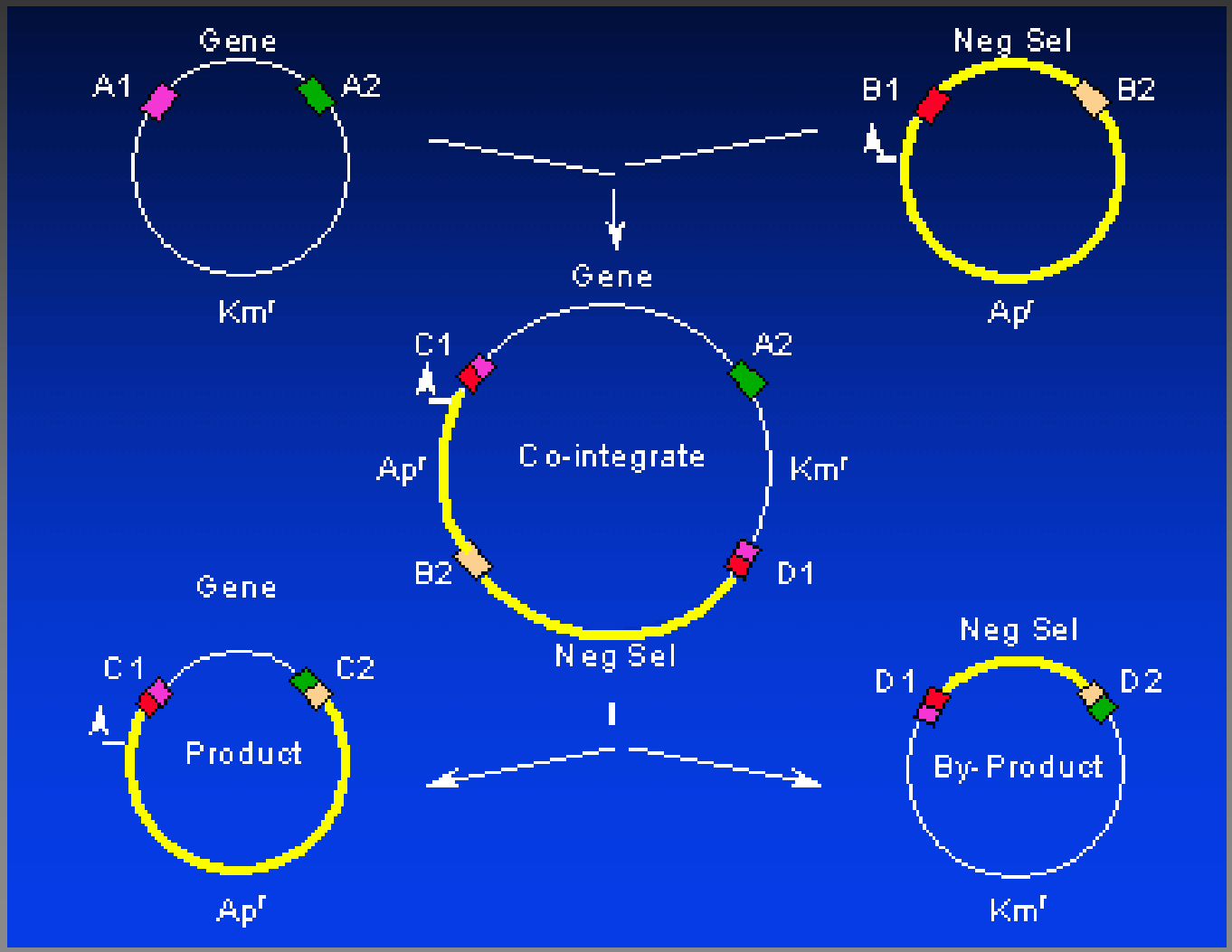
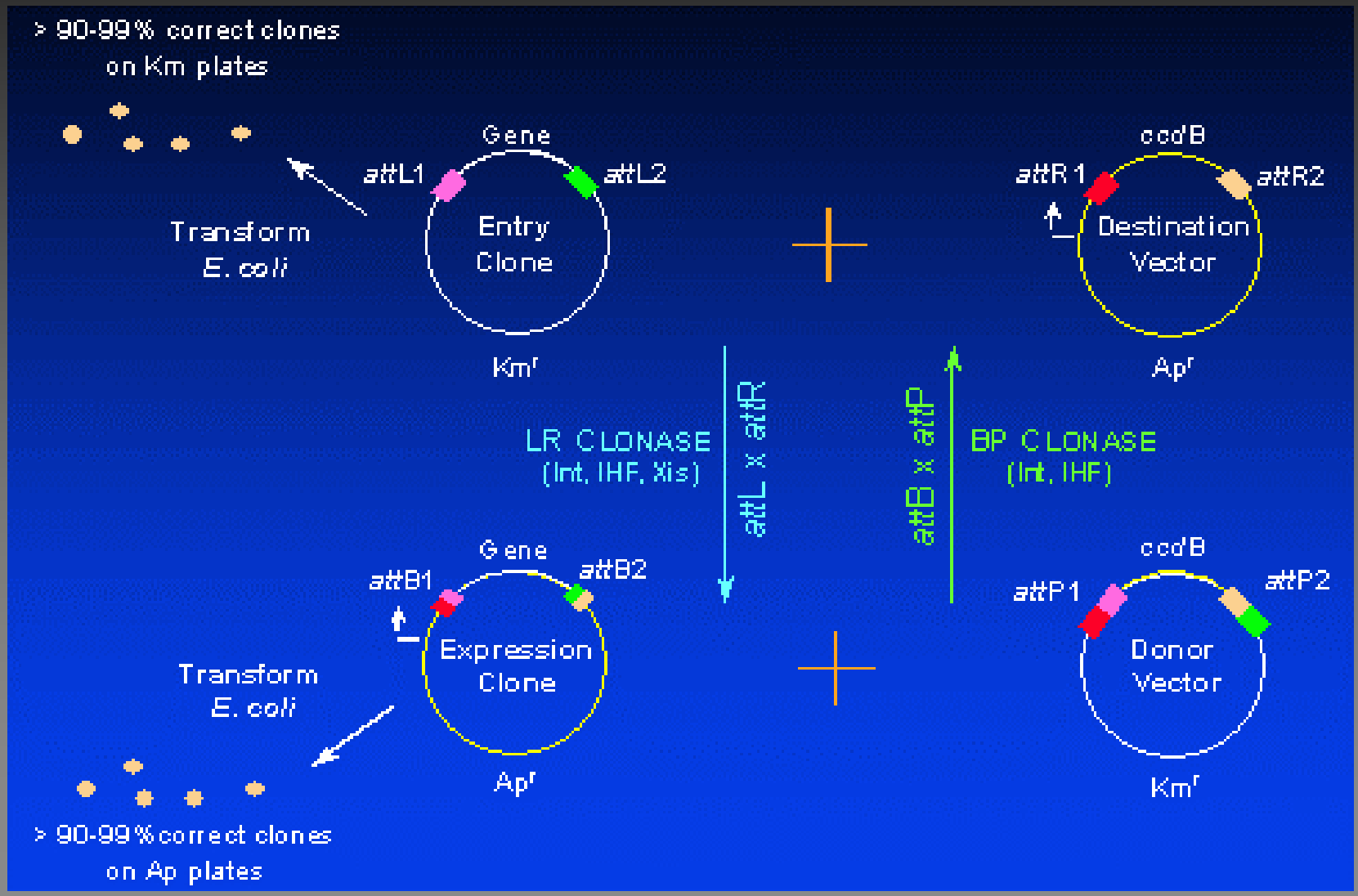
Recombinación de Fago Lambda



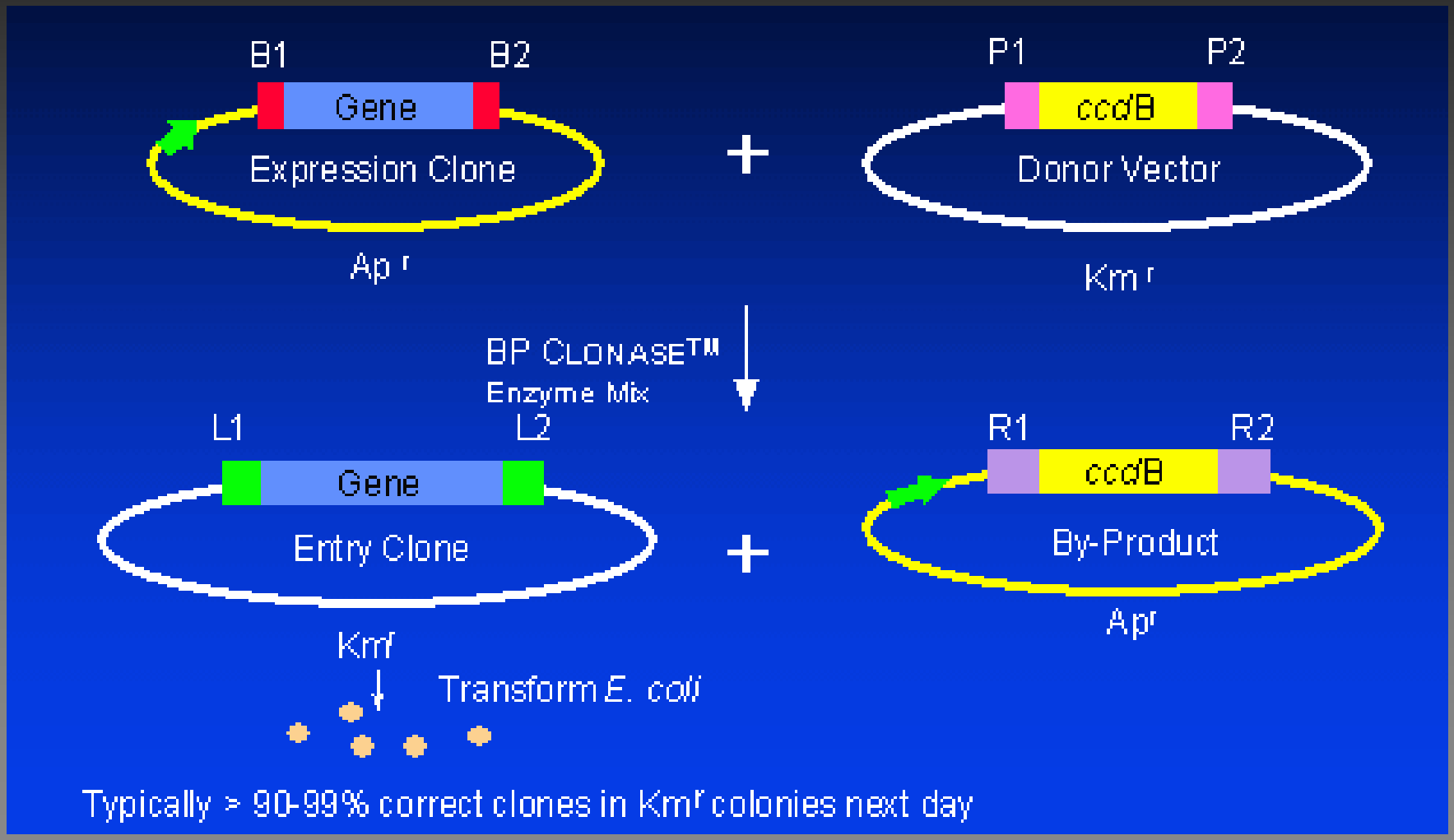
Usando recombinación sitio específica del sistema λ



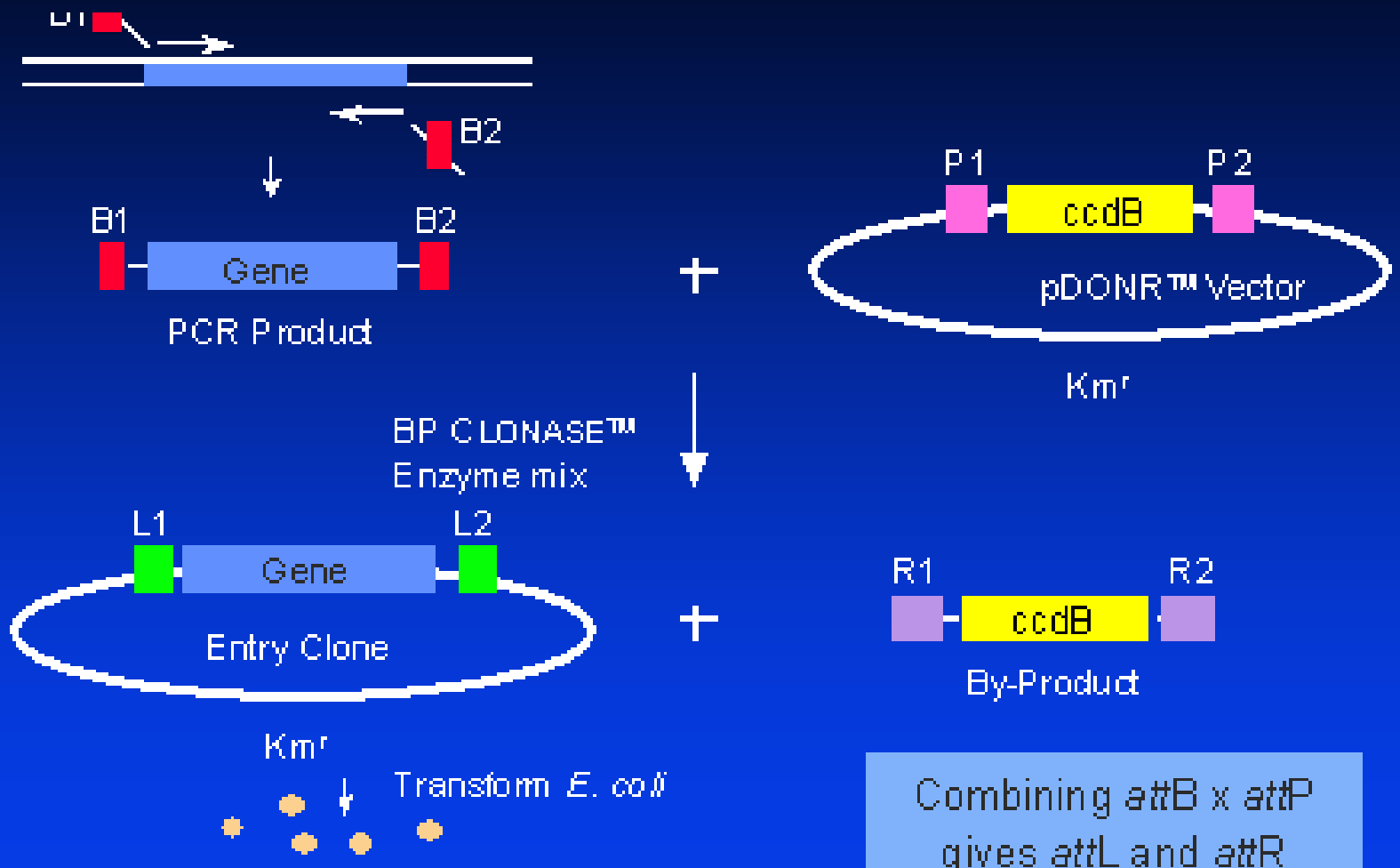
Sistema Gateway



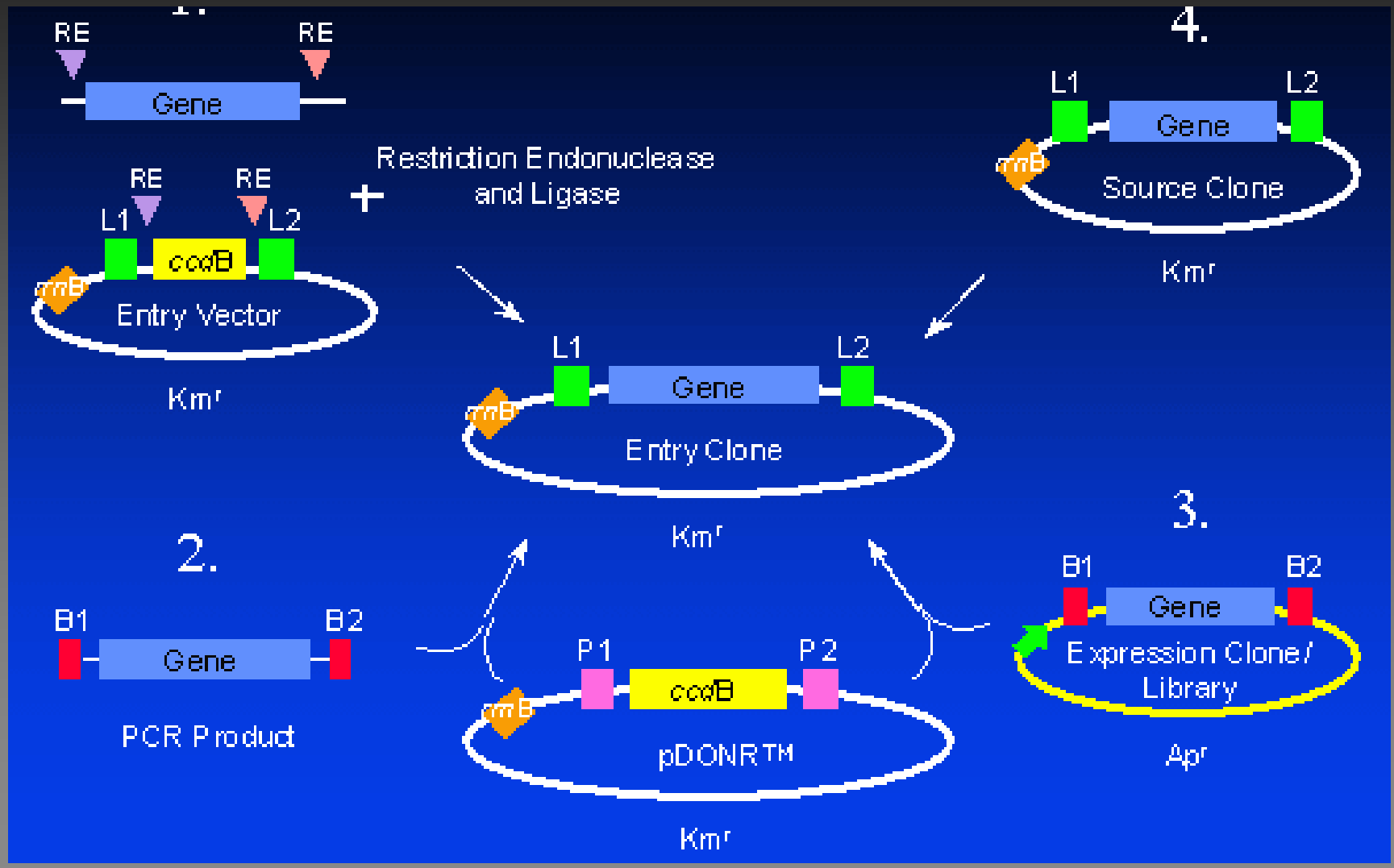
Reacción BP


B1
B2
Gene
Expression Clone
Ap r
P1
P2
ccdB
Donor Vector
Km r
BP CLONASE™
Enzyme Mix
L1
L2
Gene
Entry Clone
Km r
Transform E. coli
R1
R2
ccdB
By-Product
Ap r
Typically > 90-99% correct clones in Km r colonies next day

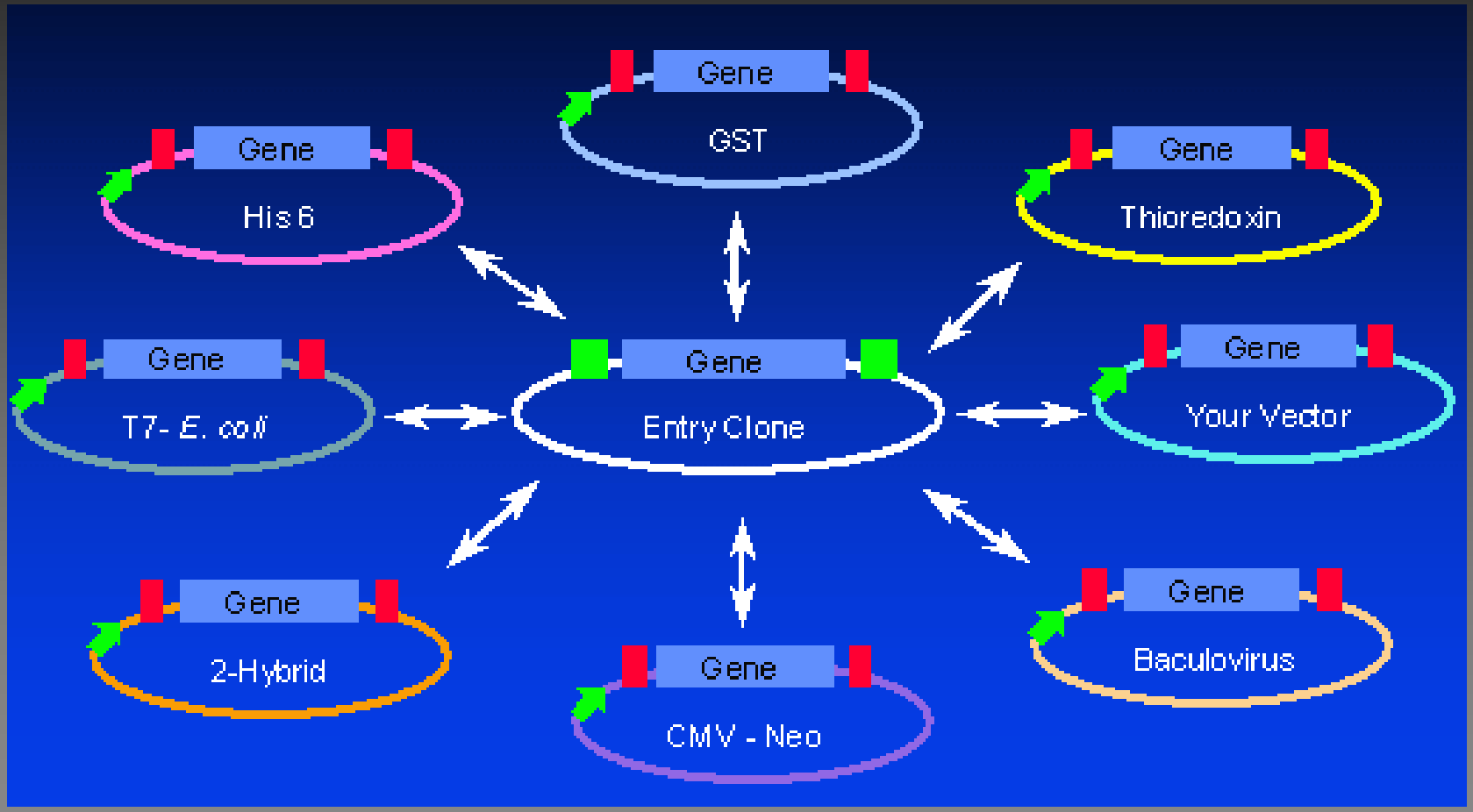
Clonado desde PCR



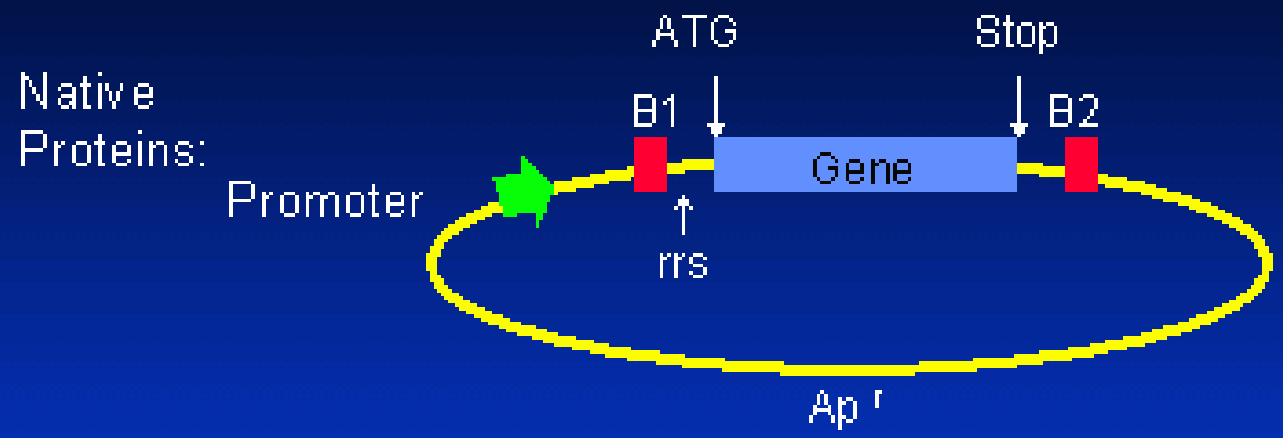
Generando el vector de entrada

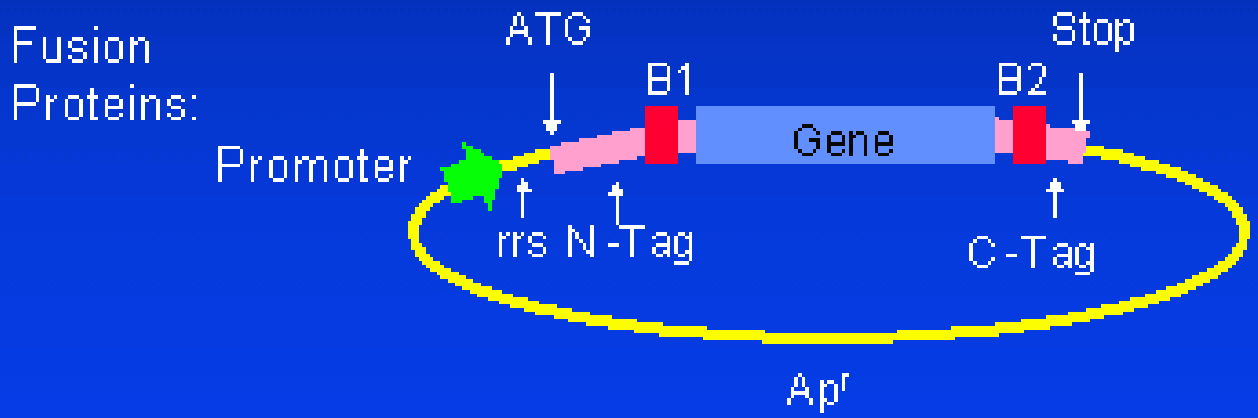


Subclonando desde el vector de entrada a multiples Vectores de expresión

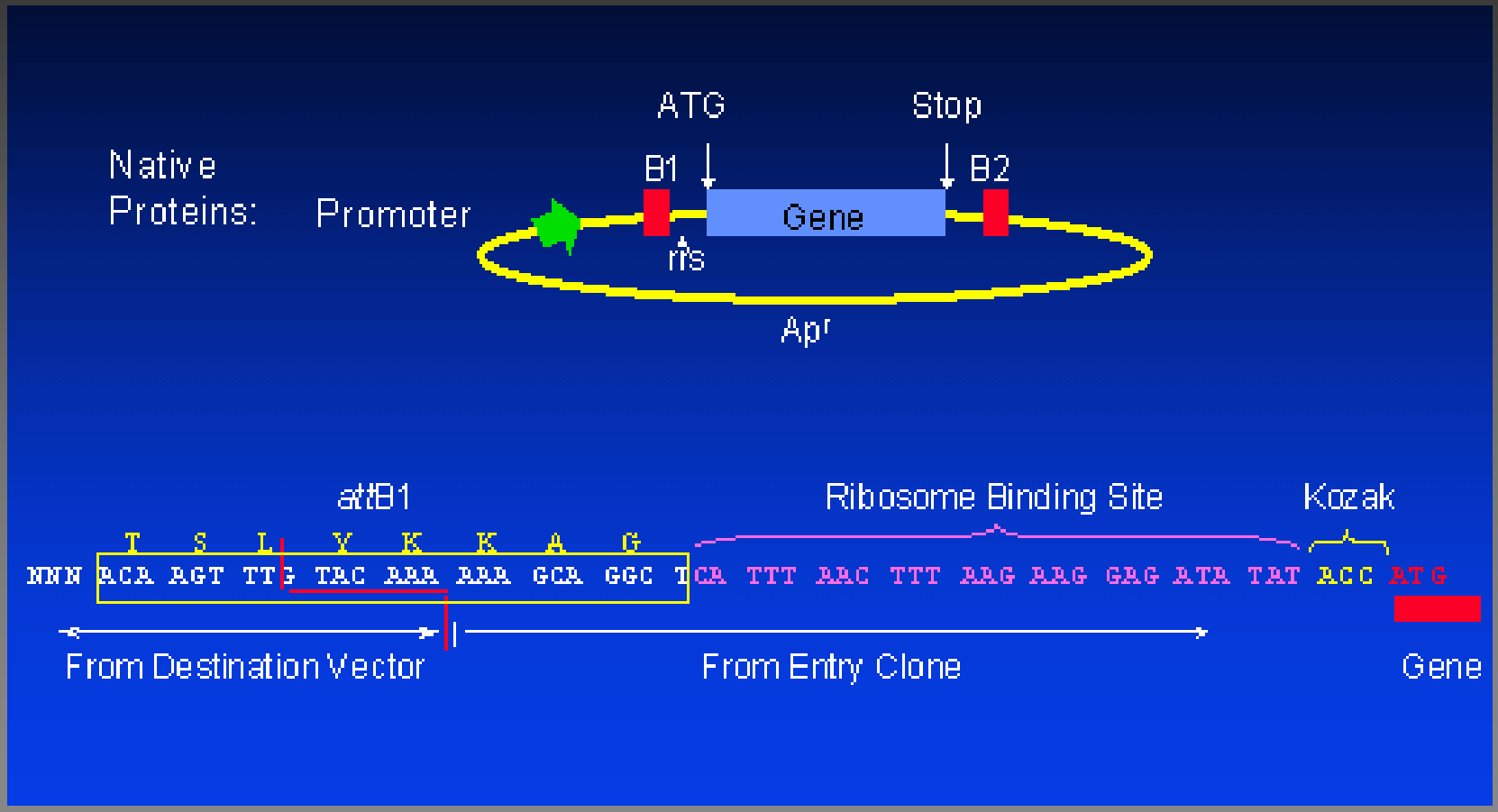


Vectores de expresión



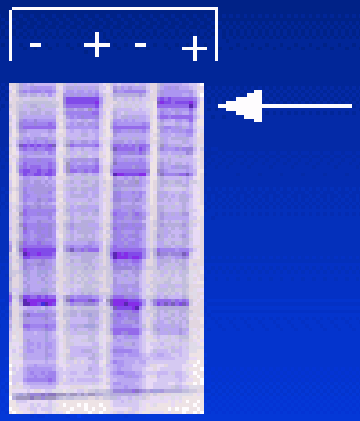


Región de recombinación en vector de expresión

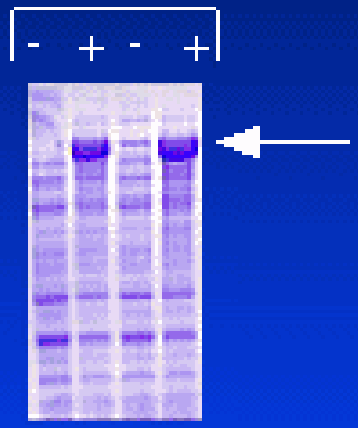


Optimización de la expresión de β-Gal en diferentes vectores de expresión

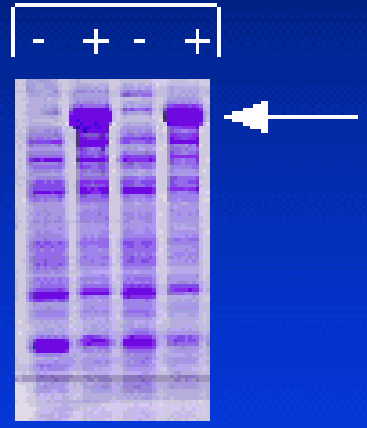
GST β-Gal Fusion



His6 β-Gal Fusion

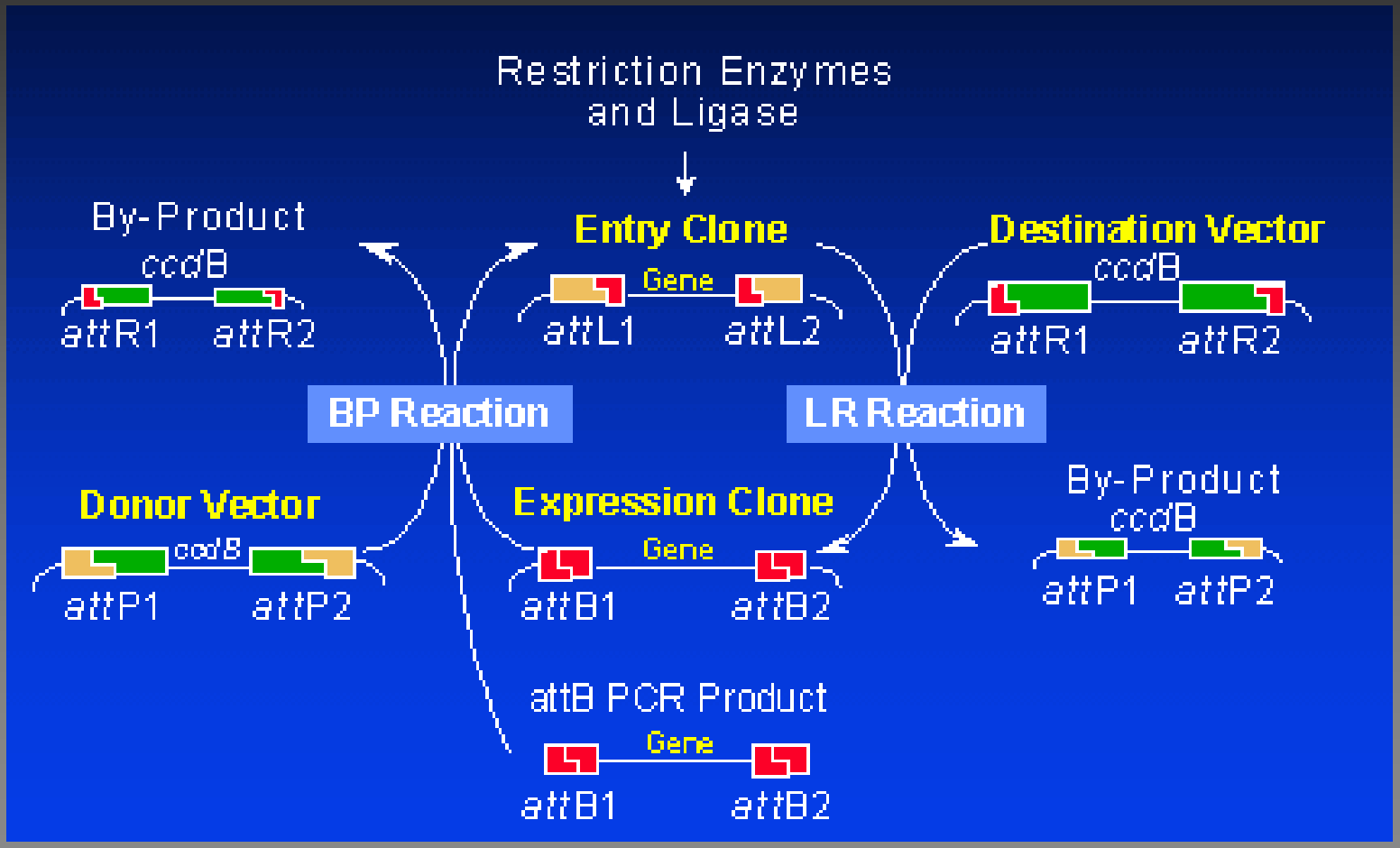


His6-Thioredoxin β-Gal Fusion



* *E. coli* strain BL21-SI (T7 promoter)

Esquema general del uso del sistema Gateway

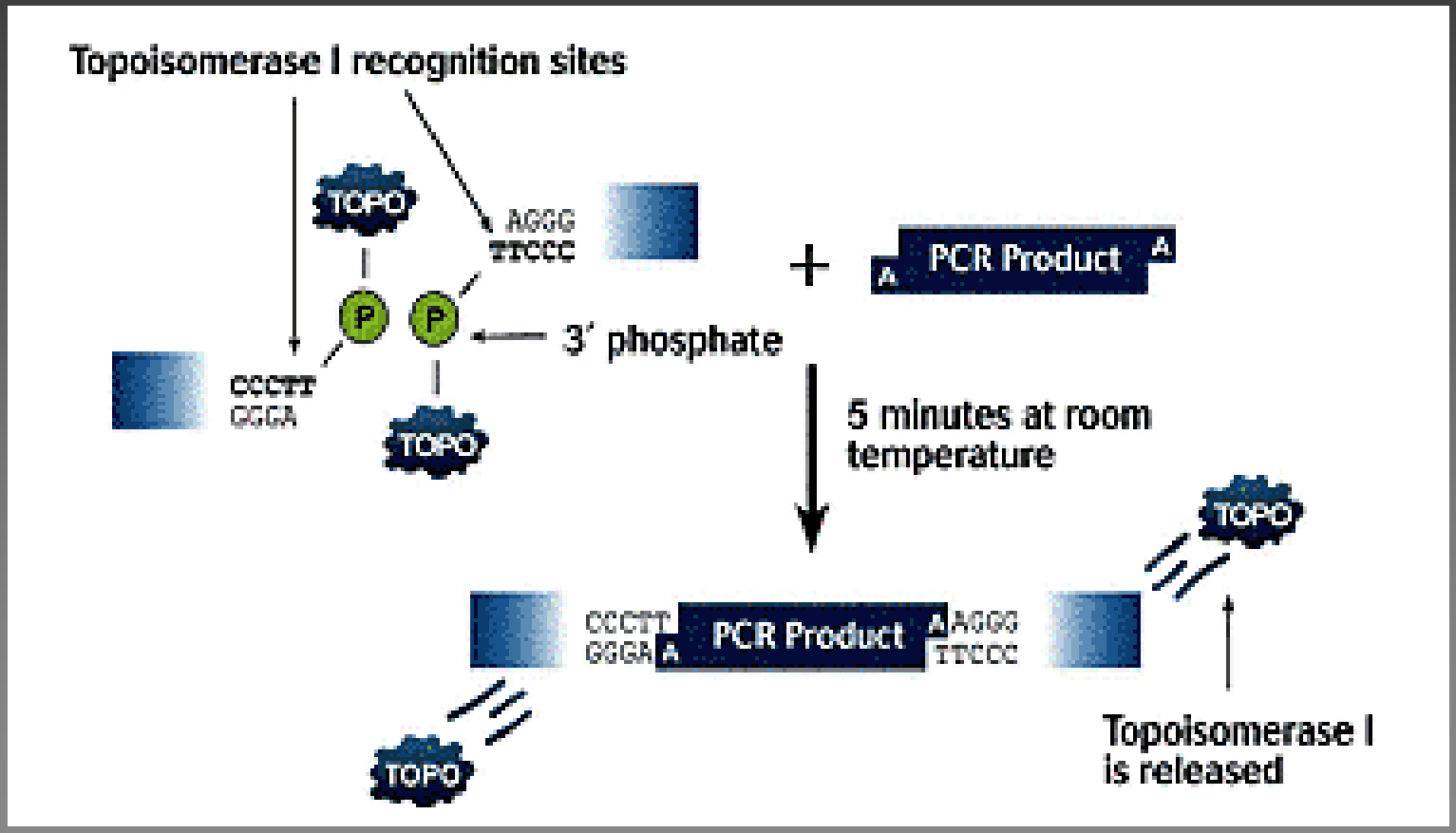


Esquema-pGem T

- Takes advantage of the terminal transferase activity of *Taq* DNA Polymerase
  - *Taq* adds single, 3´-A overhangs
  - TA Cloning® vectors are supplied linearized with single, 3´-T overhangs

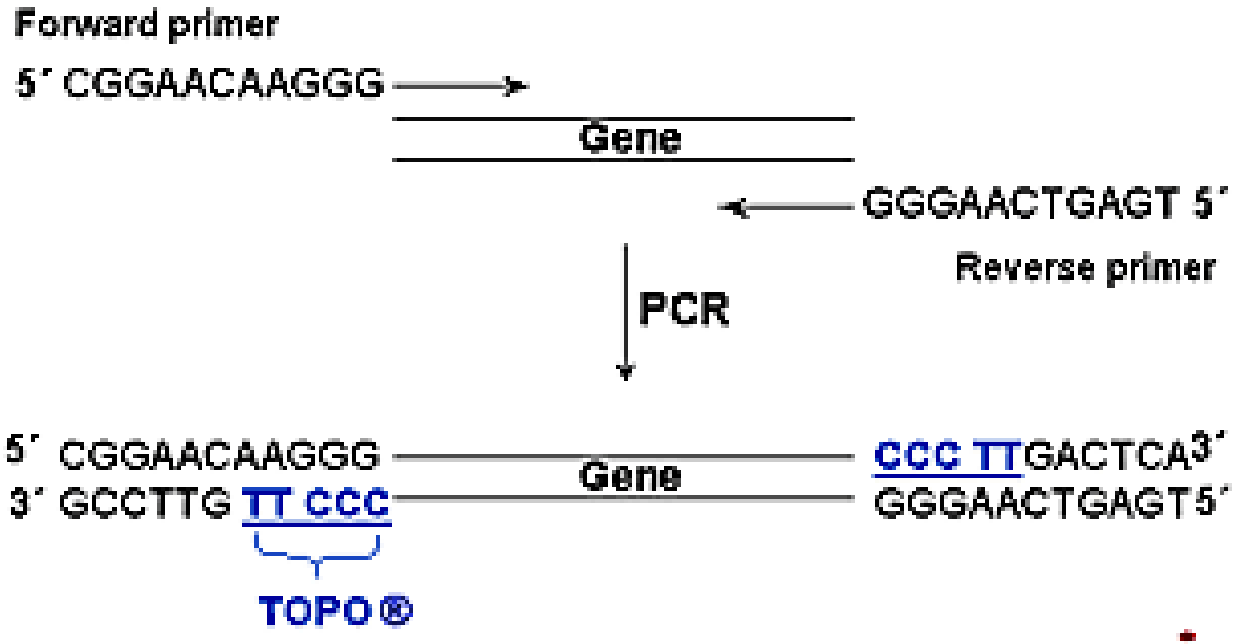
- Enables direct ligation at high efficiencies

Detalles - SistemaTopo



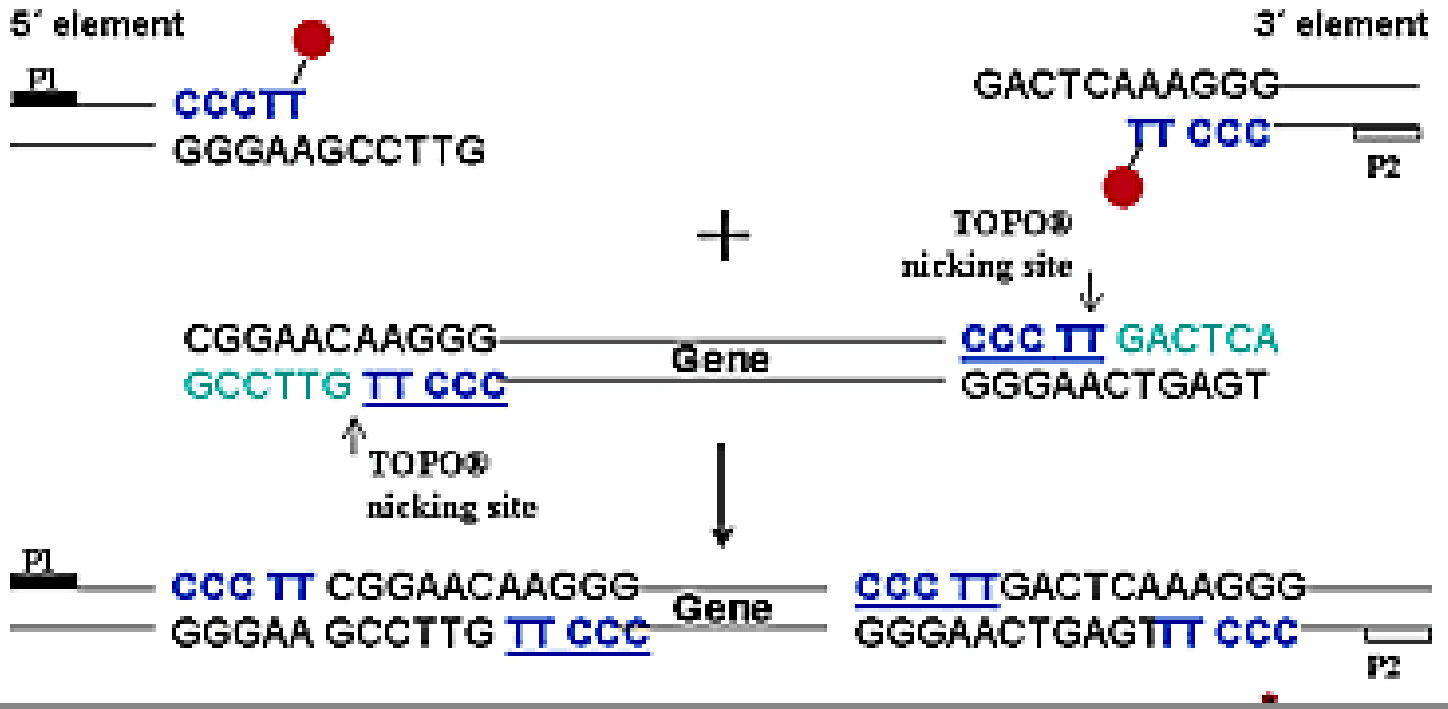
Paso 1

- Generate PCR product containing TOPO® site at each end



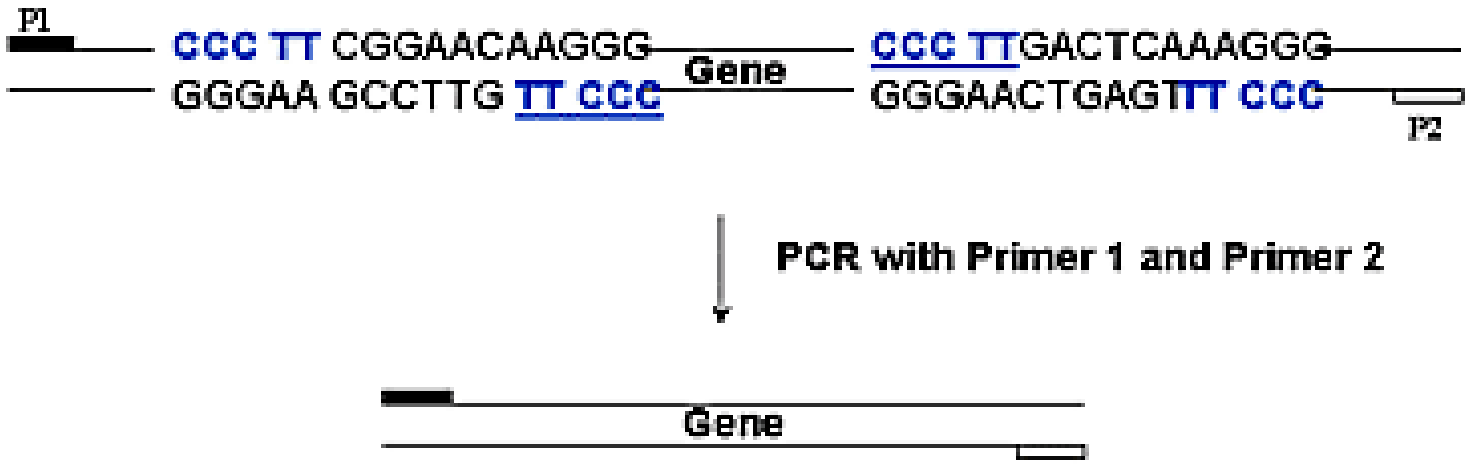
Paso 2

- Mix TOPO®-adapted elements and PCR product to create PCR template

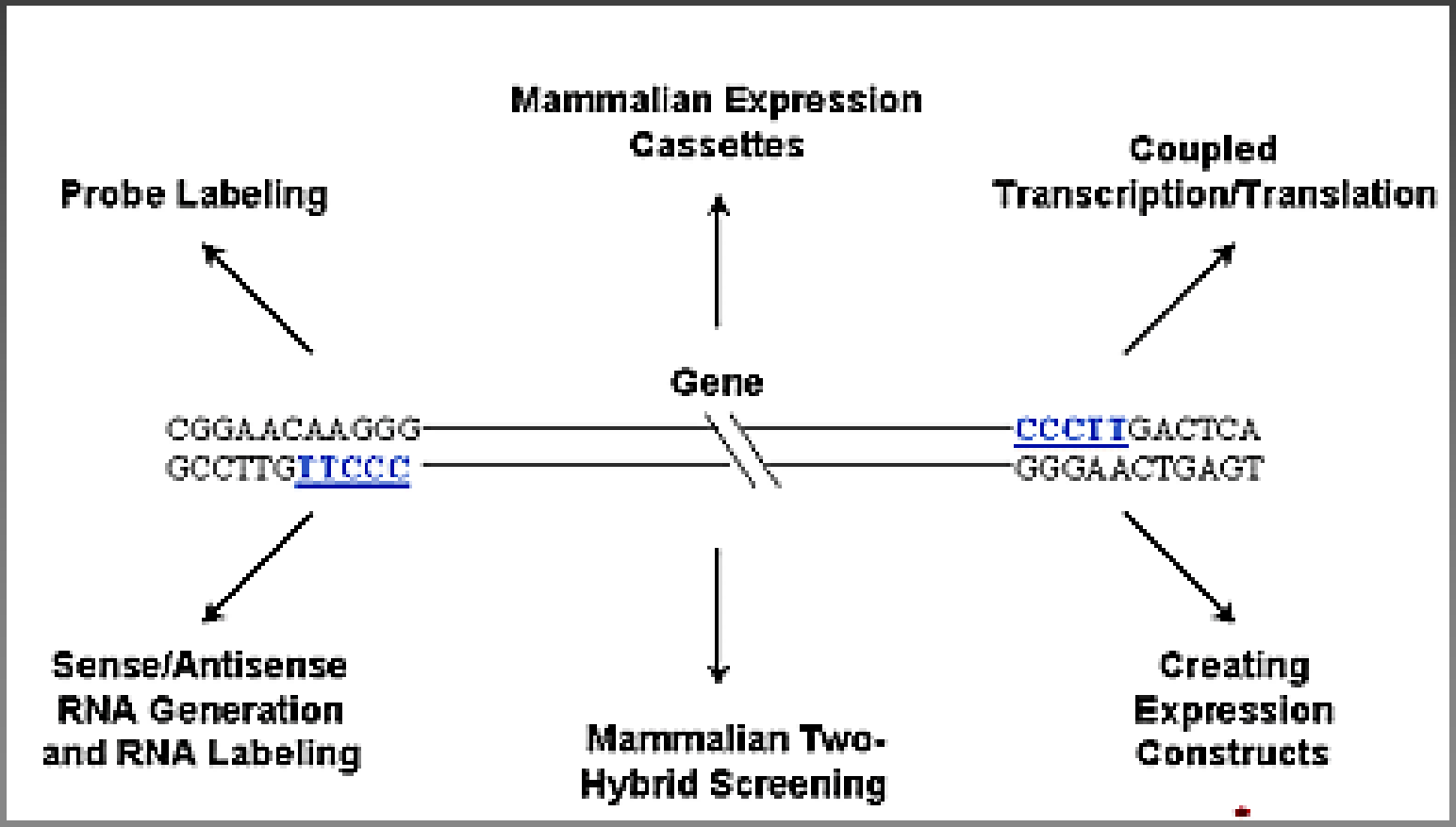


Paso 3

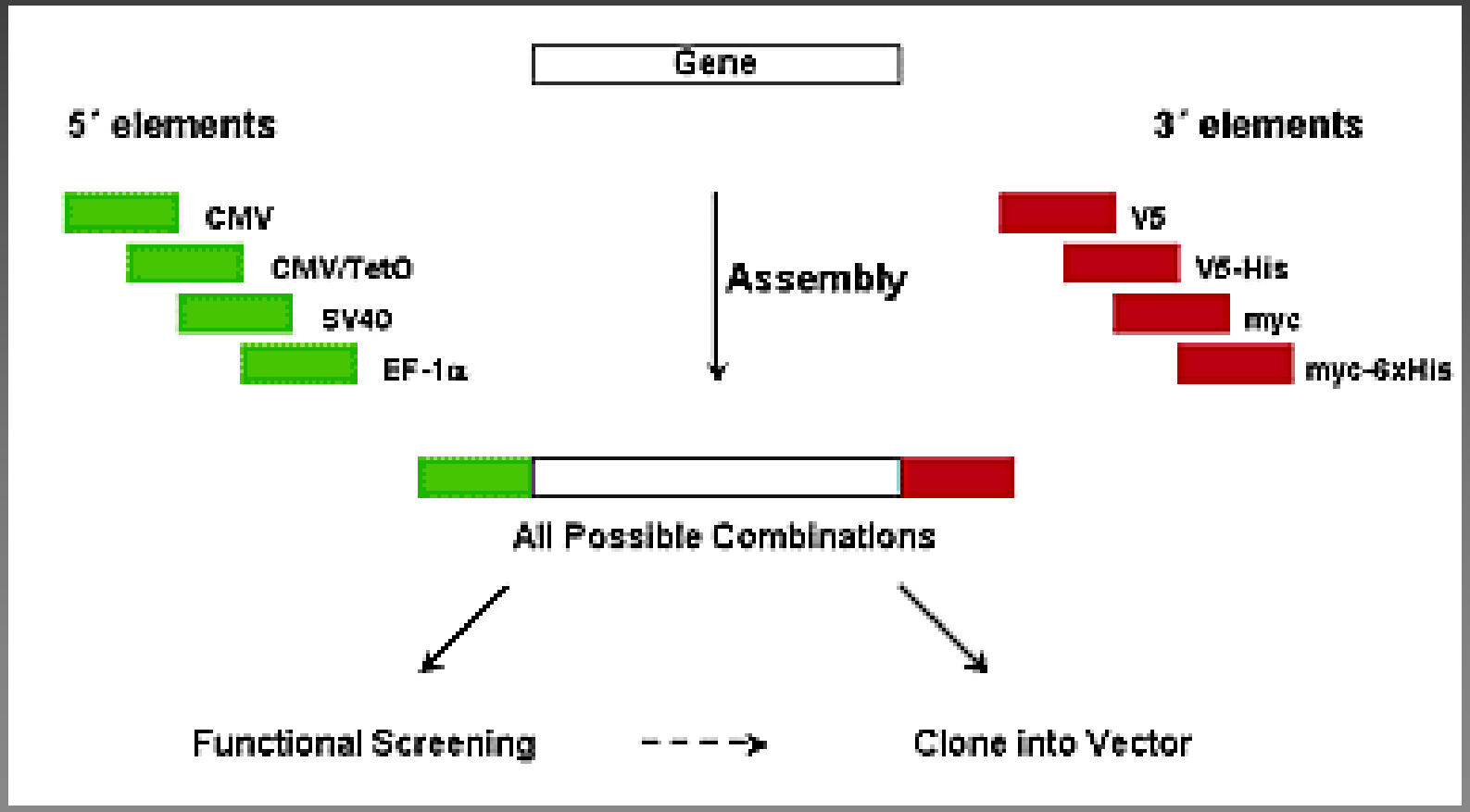
- PCR amplify template using element specific primers



Aplicaciones -TOPO



Flexibilidad



Gracias!!!