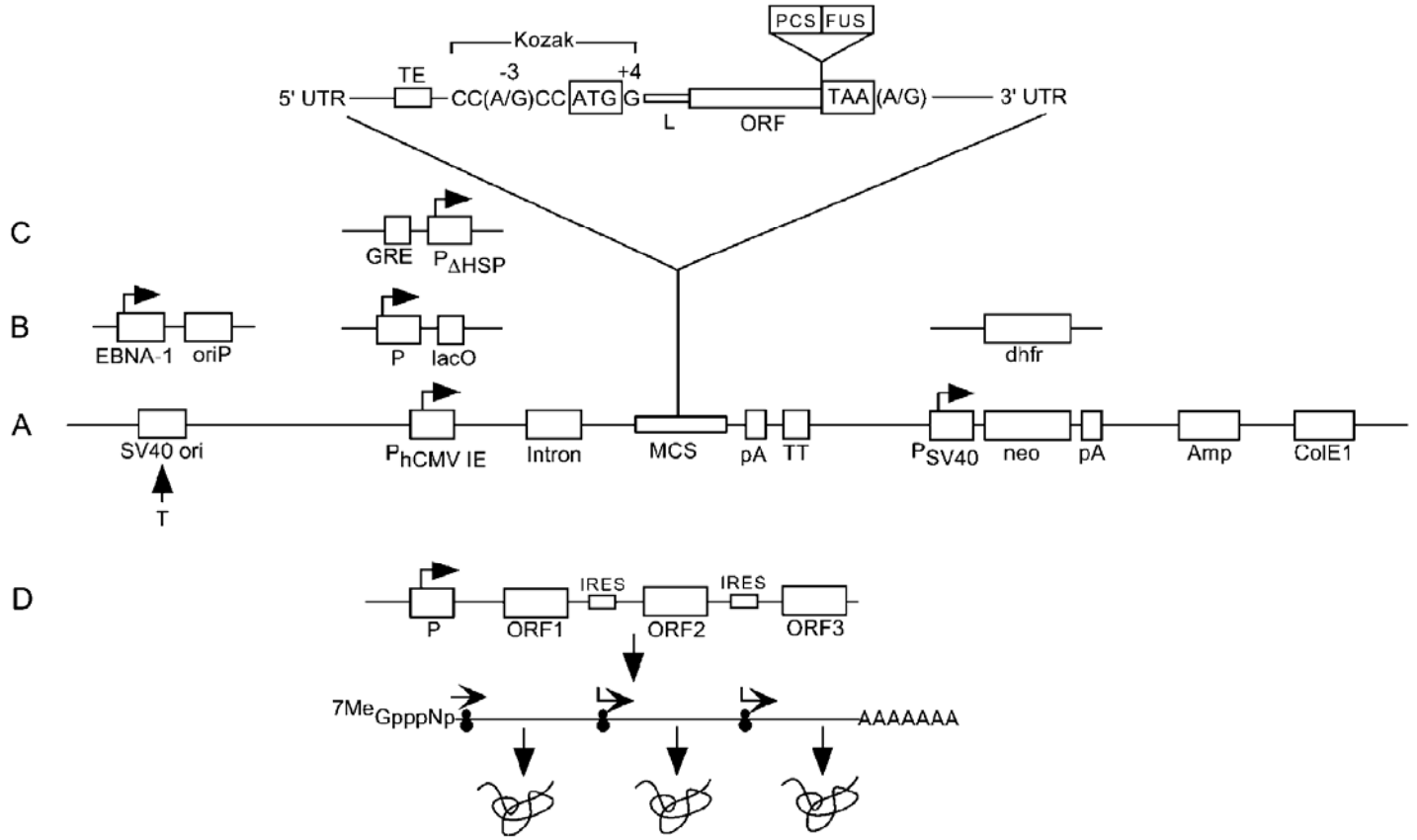
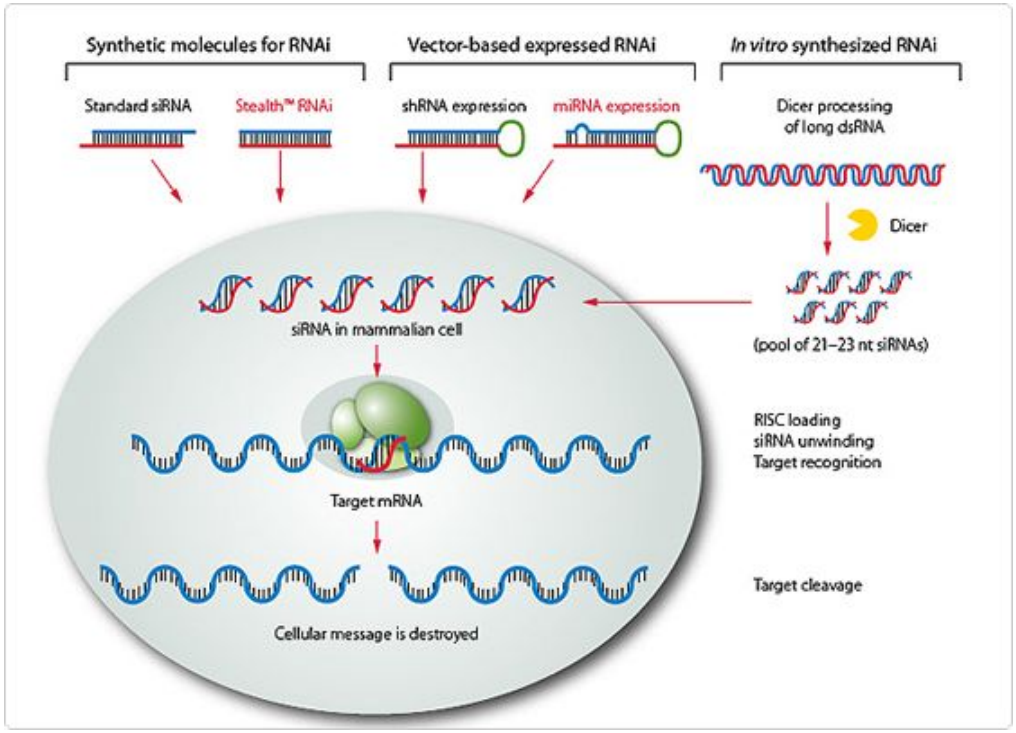
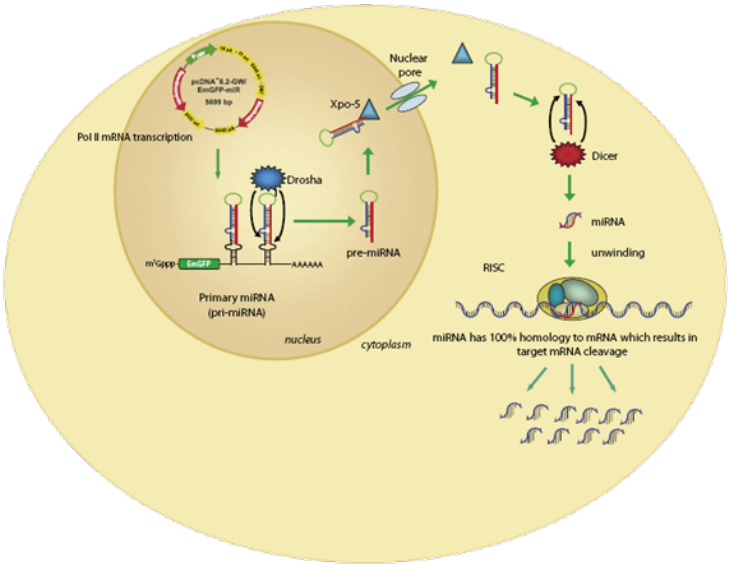
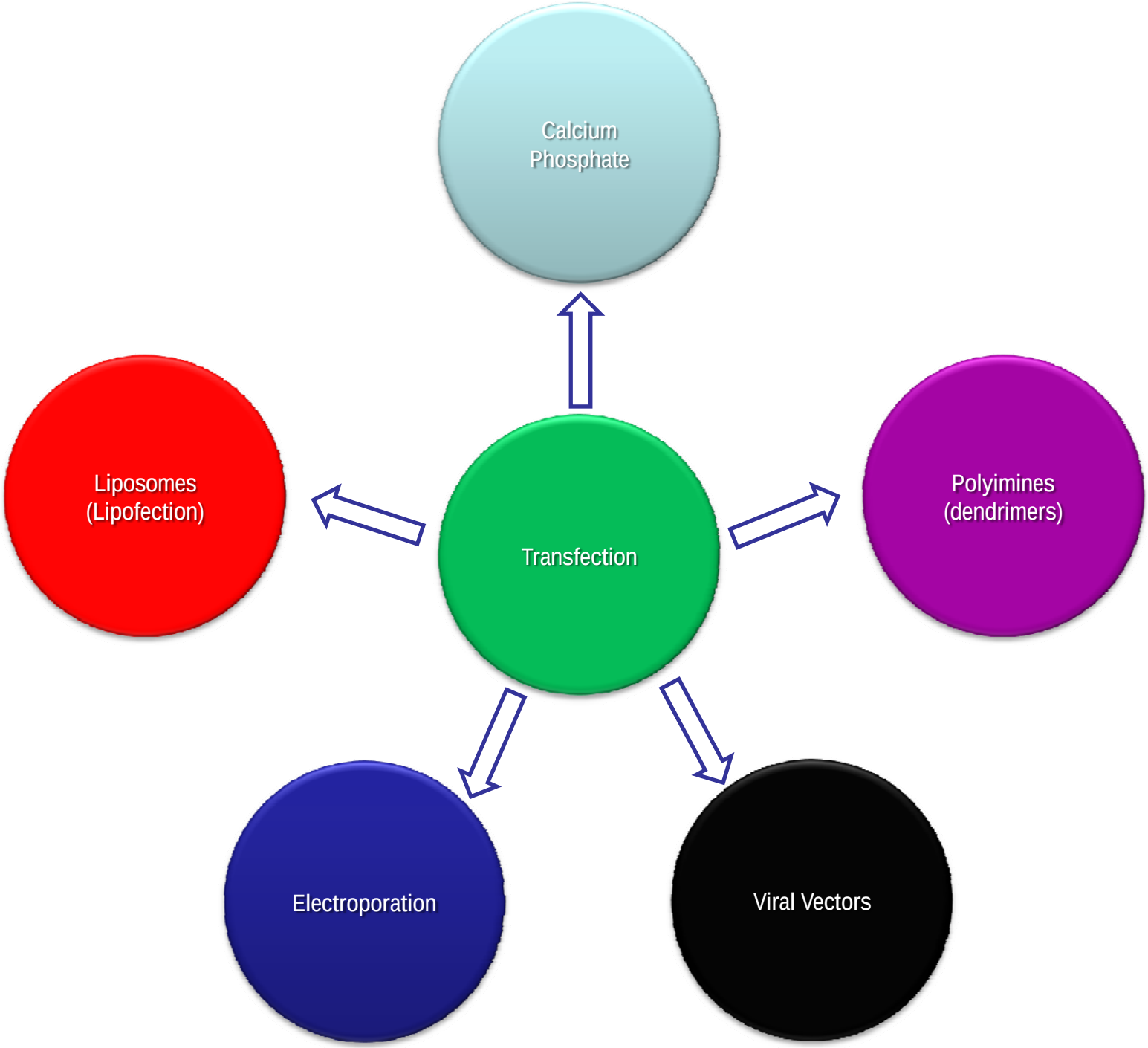
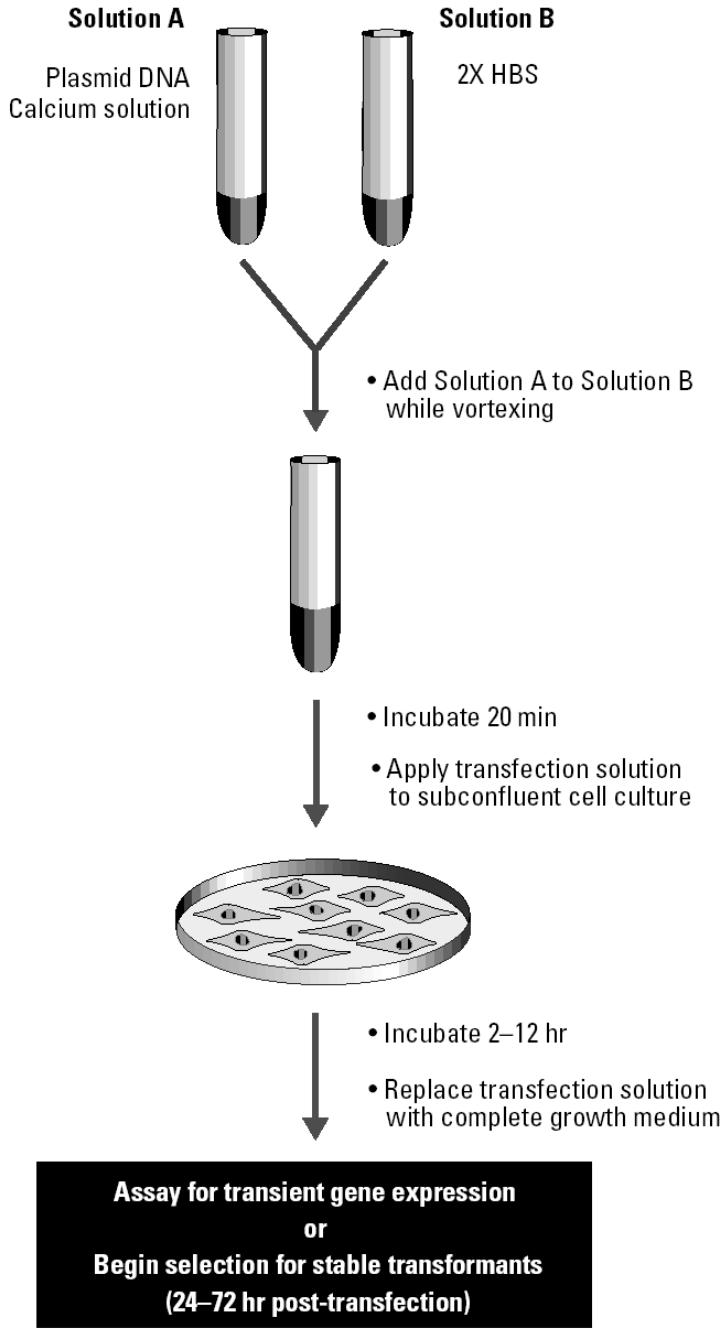


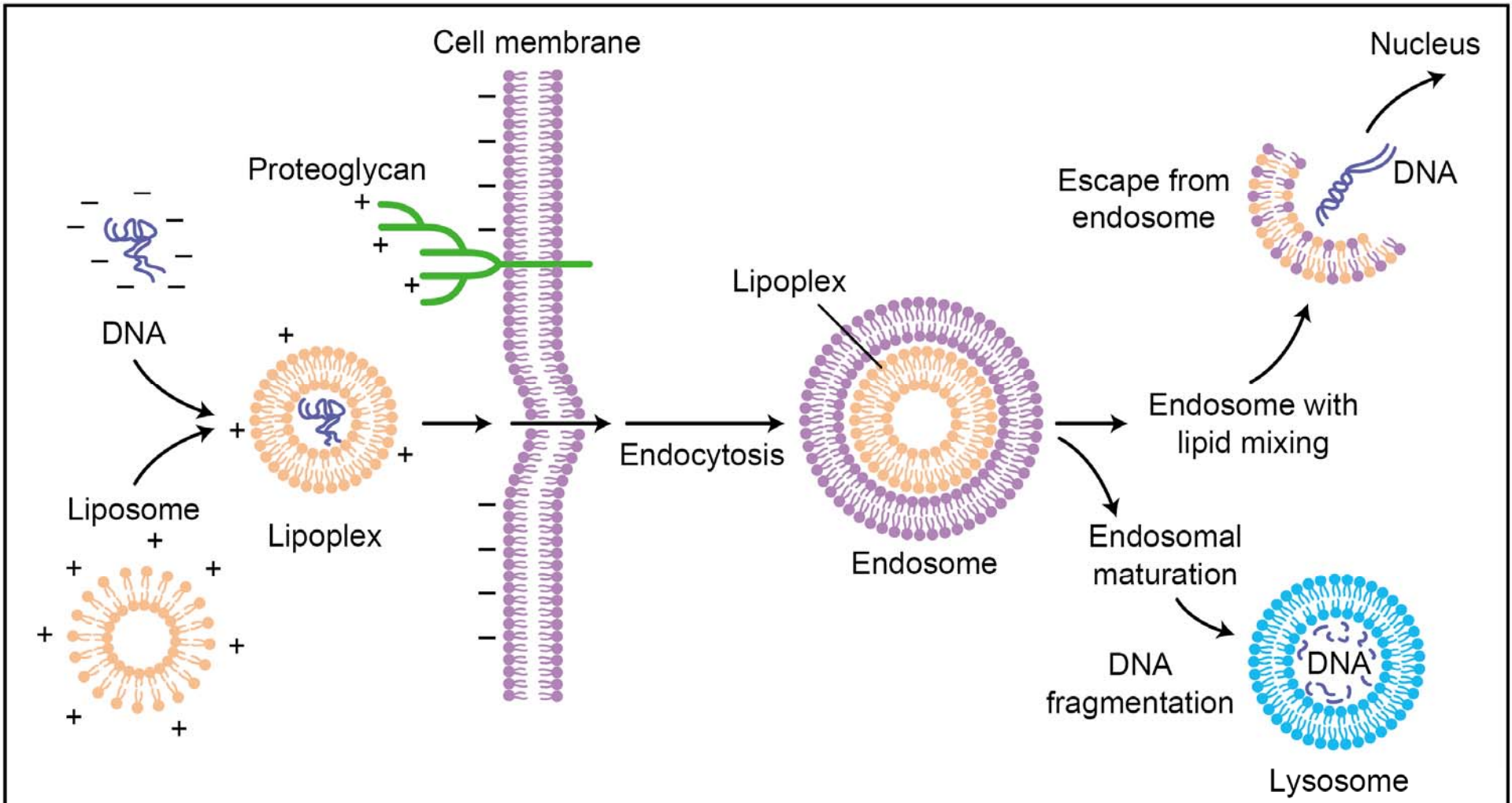
FIG. 1. Configuration of genetic elements in monocistronic (A) and polycistronic (D) expression vectors. Specific elements are shown for illustrative purposes and are not drawn to scale. The source, position, and combination of different components in the vector may vary in order to meet specific experimental requirements. SV40 ori is required for transient gene expression in COS cells. EBNA-1 and oriP facilitate high-copy episomal replication in primate and canine cell lines. The various promoter (P) elements allow constitutive (A) or inducible (B, C) expression. The optimal translational initiation sequence (Kozak) and termination codon followed by purines are shown. The ColE1 origin of replication and the ampicillin-resistance gene allow vector propagation in bacteria. The neomycin-resistance gene facilitates selection in mammalian cells, and the *dhfr* gene allows both selection and gene amplification. In a polycistronic vector (D) IRES elements allow multiple ORFs to be efficiently translated from a single transcript. See text for details. Amp, ampicillin resistance gene; ColE1, prokaryotic origin of replication; dhfr, dihydrofolate reductase; EBNA, Epstein-Barr virus nuclear antigen; FUS, fusion moiety; GRE, glucocorticoid response element; hCMV IE, human cytomegalovirus immediate early enhancer/promoter; HSP, heat shock protein; IRES, internal ribosome entry site; lacO, *lac* operator; L, leader (targeting sequence); MCS, multiple cloning site; neo, neomycin resistance gene; ORF, open reading frame; ori, origin of replication; oriP, Epstein-Barr virus origin of replication; P, promoter; pA, polyadenylation signal; PCS, protease cleavage site; T, SV40 large tumor (T) antigen; TE, translational enhancer; TT, transcription terminator; UTR, untranslated region.


Synthetic molecules for RNAi
Vector-based expressed RNAi
In vitro synthesized RNAi
Standard siRNA
Stealth™ RNAi
shRNA expression
miRNA expression
Dicer processing of long dsRNA
Dicer
siRNA in mammalian cell
(pool of 21–23 nt siRNAs)
RISC loading
siRNA unwinding
Target recognition
Target mRNA
Target cleavage
Cellular message is destroyed


Nuclear pore
Xpo-5
Pol II mRNA transcription
Drosha
pre-miRNA
Primary miRNA (pri-miRNA)
nucleus
cytoplasm
Dicer
miRNA
unwinding
RISC
miRNA has 100% homology to mRNA which results in target mRNA cleavage


Calcium Phosphate
Liposomes (Lipofection)
Transfection
Polyimines (dendrimers)
Electroporation
Viral Vectors


Solution A
Plasmid DNA
Calcium solution
Solution B
2X HBS
• Add Solution A to Solution B while vortexing
• Incubate 20 min
• Apply transfection solution to subconfluent cell culture
• Incubate 2–12 hr
• Replace transfection solution with complete growth medium
Assay for transient gene expression
or
Begin selection for stable transformants
(24–72 hr post-transfection)



Lipoplex-mediated transfection and endocytosis

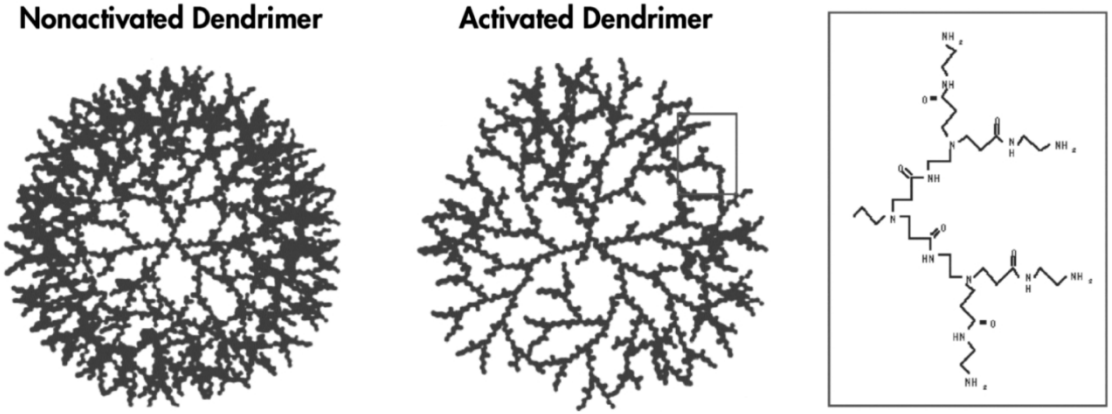


Fig. 4. Non-activated and activated PAMAM-dendrimers. Schematic diagram of a non-activated (left) and activated dendrimer (middle). The right panel shows a magnification of the dendrimer branches.

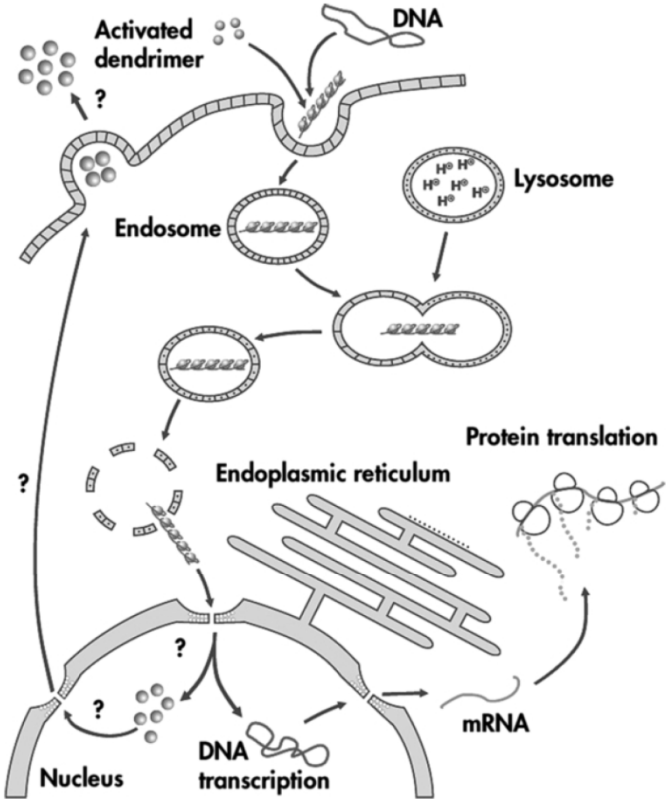
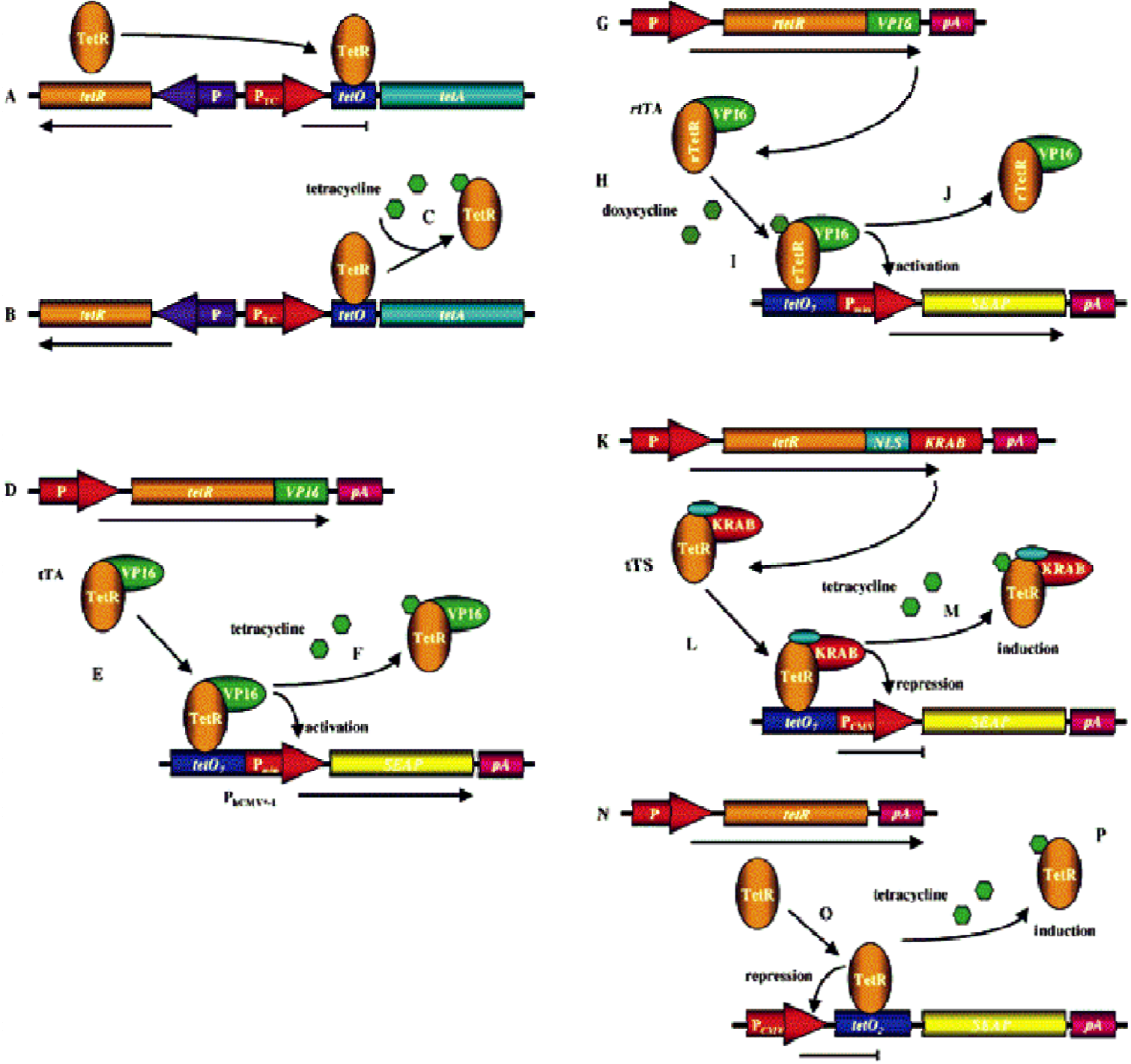
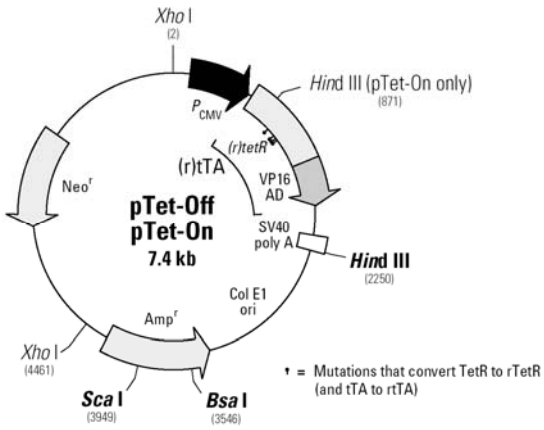
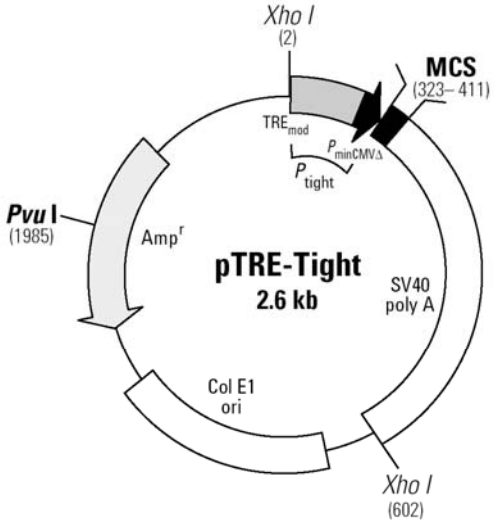


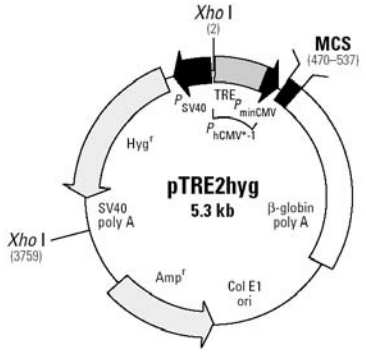
Fig. 1. Model of activated dendrimer-mediated DNA uptake. In the first step of the transfection process, the DNA–activated-dendrimer complex binds to the surface of the cell. The complex is then taken into the cell by endocytosis, and incorporated into the endosome of the cell. From the endosome the DNA is released into the cytosol. A small percentage of the released DNA reaches the nucleus, where it is transcribed into RNA. In the last step the RNA is transported back into the cytosol and then translated into protein. The exact pathway and metabolism of transfection reagents after release into the cytosol are still unclear.


A
TetR
tetR
P
P_TC
tetO
tetA
B
tetracycline
C
TetR
D
P
tetR
VP16
pA
tTA
E
F
activation
SEAP
P_hCMV-1
G
rtTA
rTetR
H
doxycycline
I
J
activation
K
NES
KRAB
tTS
L
M
induction
repression
N
O
P

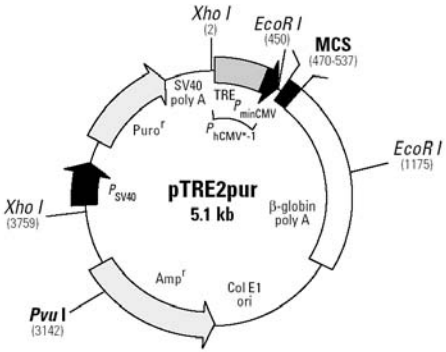




323
GAATTCGAGCTCGGTACCCGGGGATCCTCTAGTCAGCTGACGCGT
EcoR I Sac I Kpn I BamH I Pvu II Mlu I
Sma I
368
GCTAGCGCGGCCGCATCGATAAGCTTGTCGACGATATCTCTAGA
Nhe I Eag I Cla I Hind III Sal I EcoR V Xba I
Not I
Acc I

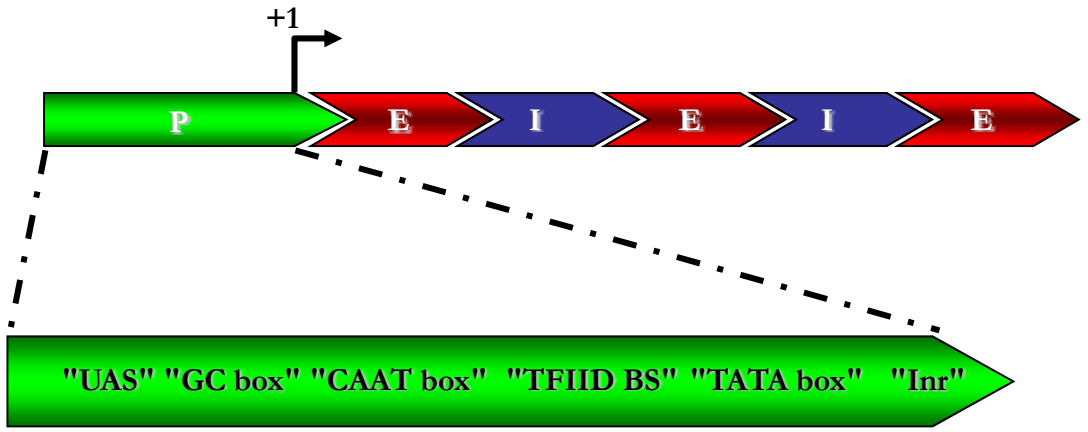


470 480 490 500 510
GGATCCTCTAGTCAGCTGACGCGTGCTAGCGCGGCCGCATCGAT
BamH I Pvu II Mlu I Nhe I Not I Cla I
514 520 530
AAGCTTGTCGACGATATCTCTAGA
Sal I Acc I EcoR V



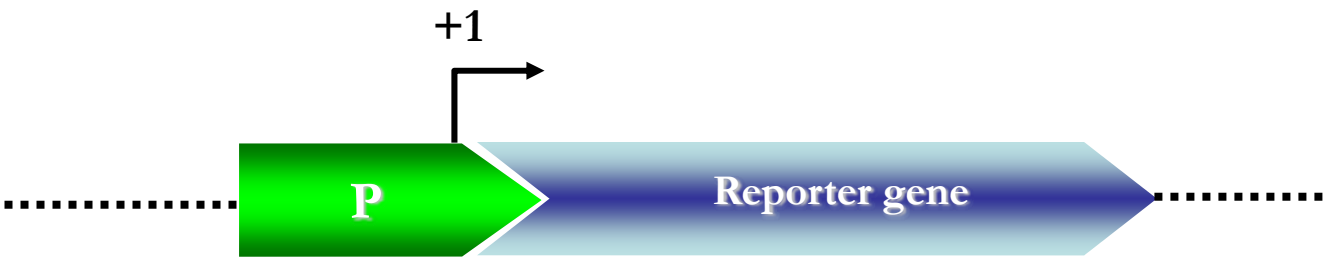
470 480 490 500 510
GGATCCTCTAGTCAGCTGACGCGTGCTAGCGCGGCCGCATCGAT
BamH I Pvu II Mlu I Nhe I Not I Cla I
514 520 530
AAGCTTGTCGACGATATCTCTAGA
EcoR V

Clonage et étude des régions promotrices d'un gène eucaryote



1. Clonage du cDNA
2. Clonage du gène
3. Comparaison séquence cDNA-gène, détérmination de la structure exon/intron.
4. Positionnement du nucléotide +1
5. Analyse in vivo de l'activité promotrice
6. Analyse des éléments régulateurs

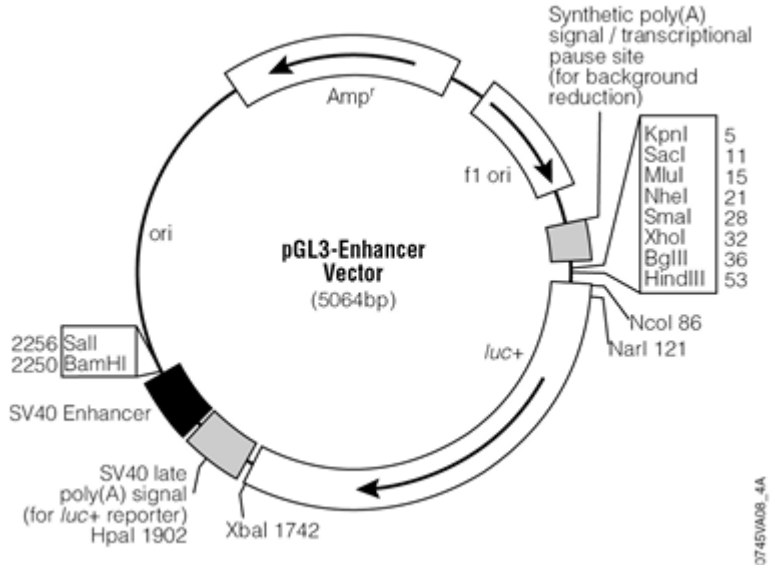
Reporter gene analysis



Compare expression with :

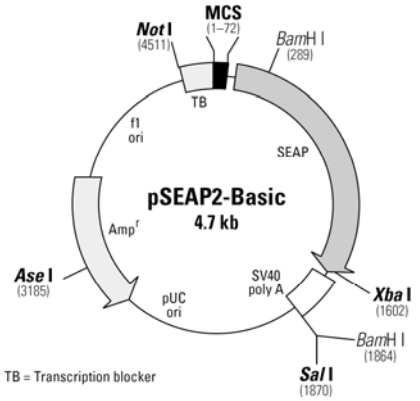
- **promotorless plasmid**
- **strong universal promoter driven expression**

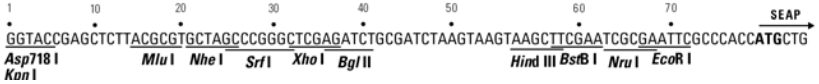
⇨Firefly Luciferase
⇨Renilla Luciferase
⇨CAT : Chloramphenicol acetyl transferase
⇨β-galactosidase
⇨Green fluorescent protein
⇨Secreted Alkaline phosphatase
Or any other protein which is NOT expressed and it is easy to detect

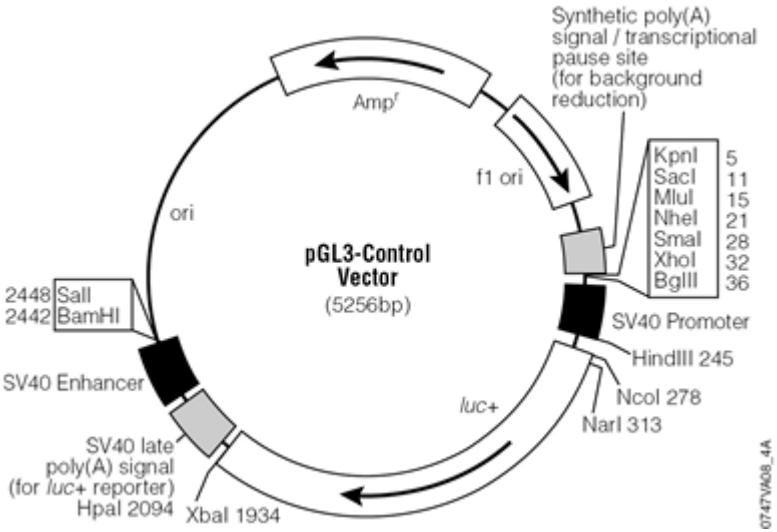


pSEAP2-Basic Vector Information PT3075-5

GenBank Accession #: U89937 Catalog #6049-1

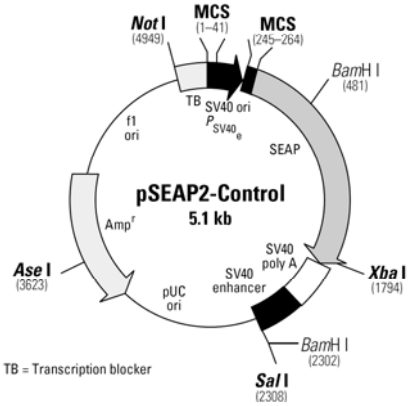


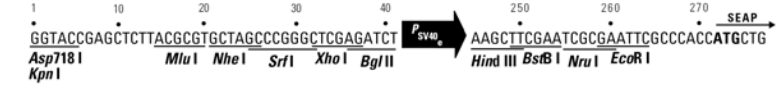




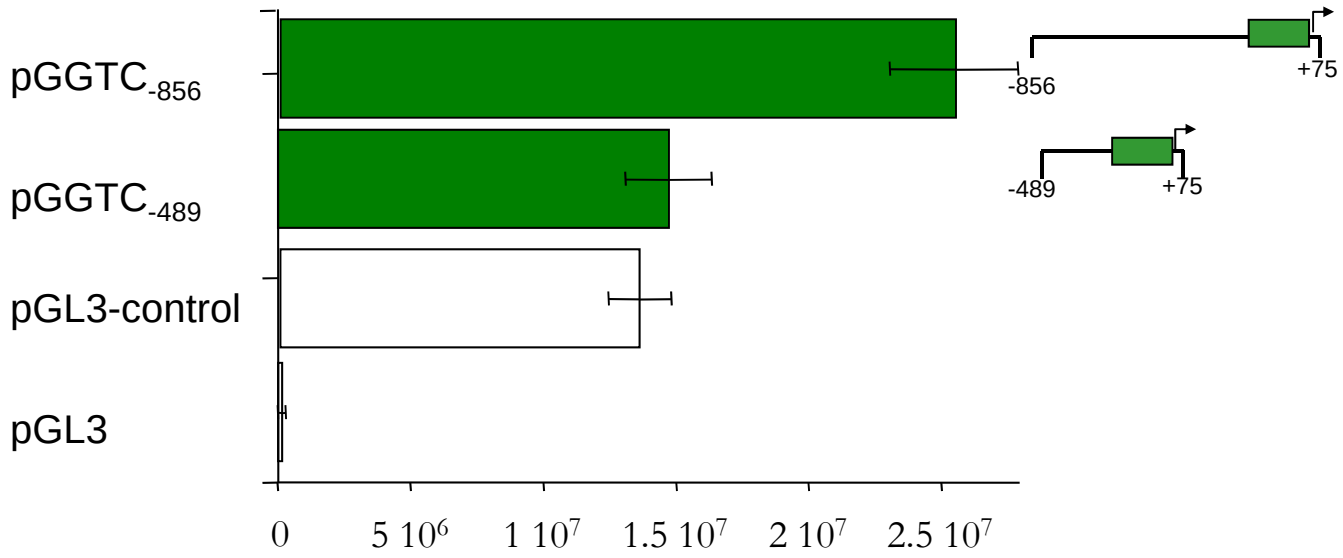
pSEAP2-Control Vector Information PT3074-5

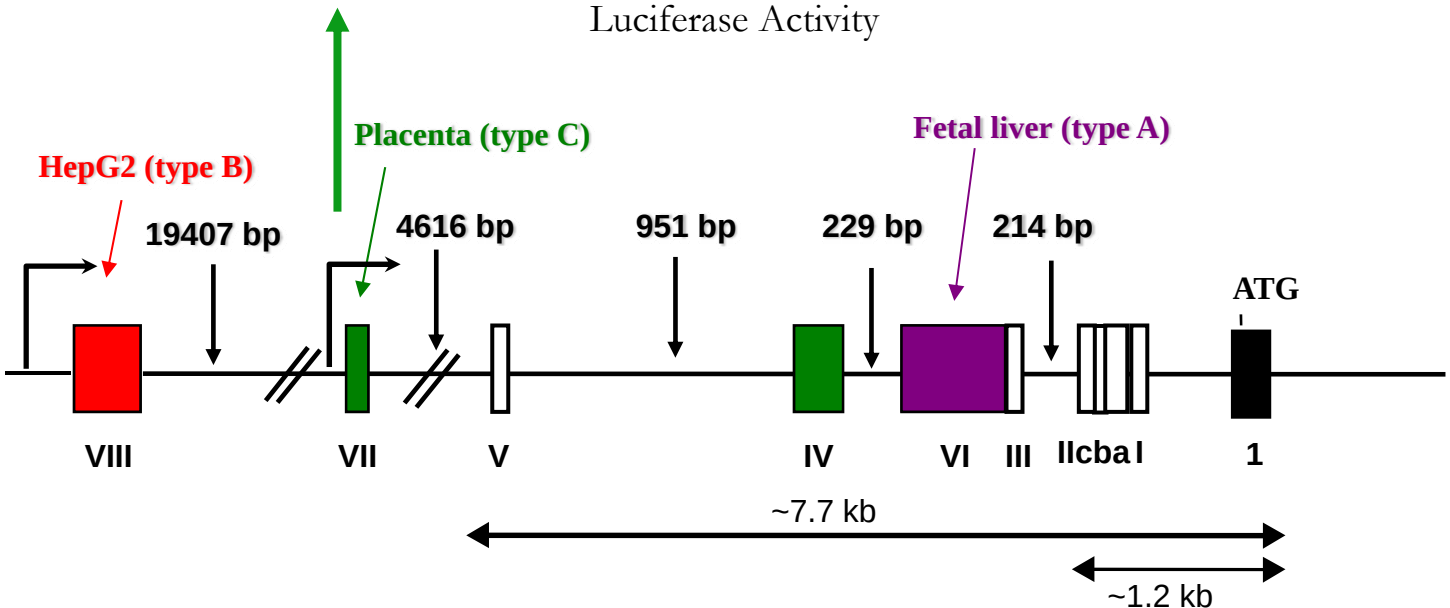
GenBank Accession #: U89938 Catalog #6052-1



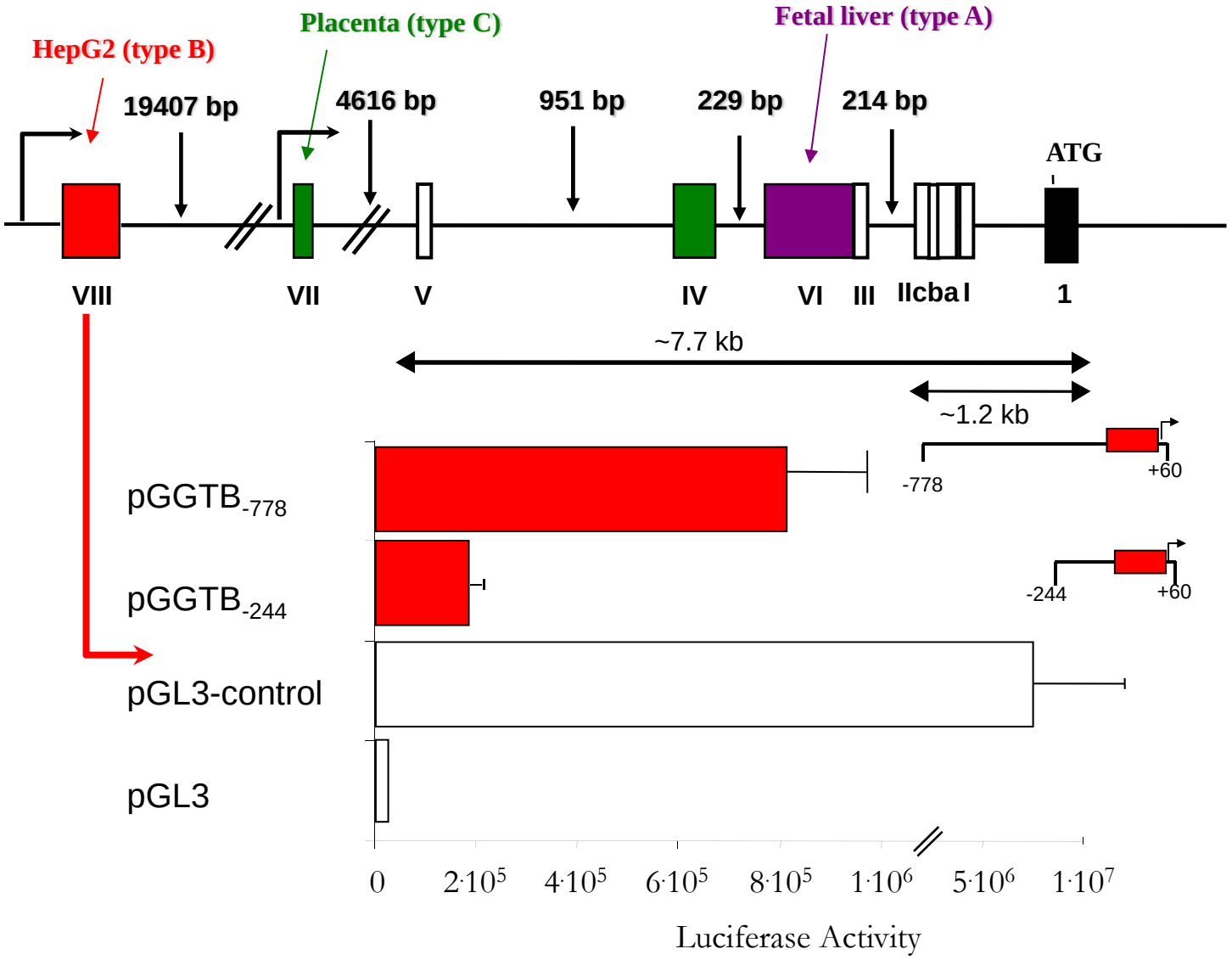


Structure of the 5' region of the human GGT gene and demonstration of the presence of multiple promoters. (*Eur J Biochem* (2001), **268,** 317-325)

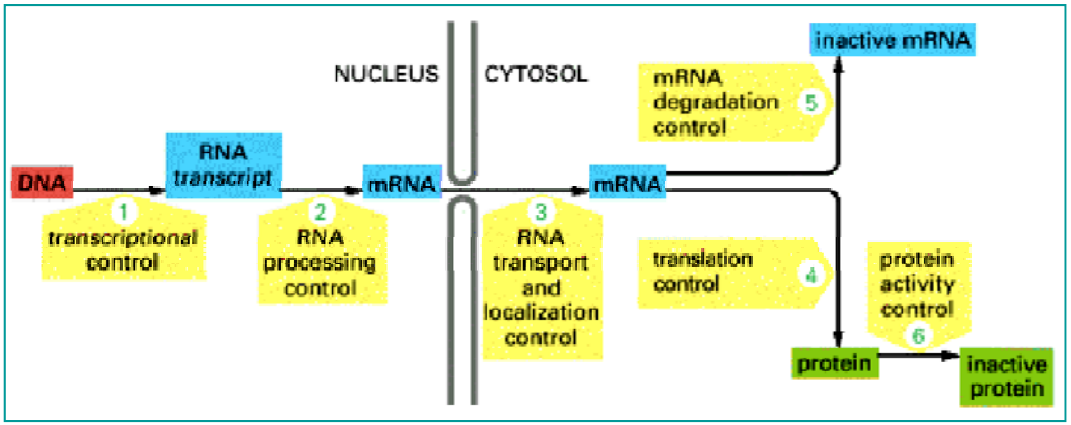




Structure of the 5' region of the human GGT gene and demonstration of the presence of multiple promoters. (*Eur J Biochem* (2001), **268,** 317-325)



Régulation de l'expression d'un gène



Animals or cells
Untreated control

Animals or cells
Treatment

Measure protein level (immuno, WB) or enzyme activity

Measure mRNA level (Northern, RT-PCR, RNase protection)

➢**Increase in enzyme activity only (activity ↗, RNA =) : cofactor, post translational modification**

↓

Probably no transcriptional activation

➢**Increase in enzyme activity and mRNA level**

↓

Increase in mRNA half-life and/or transcriptional activation

↓

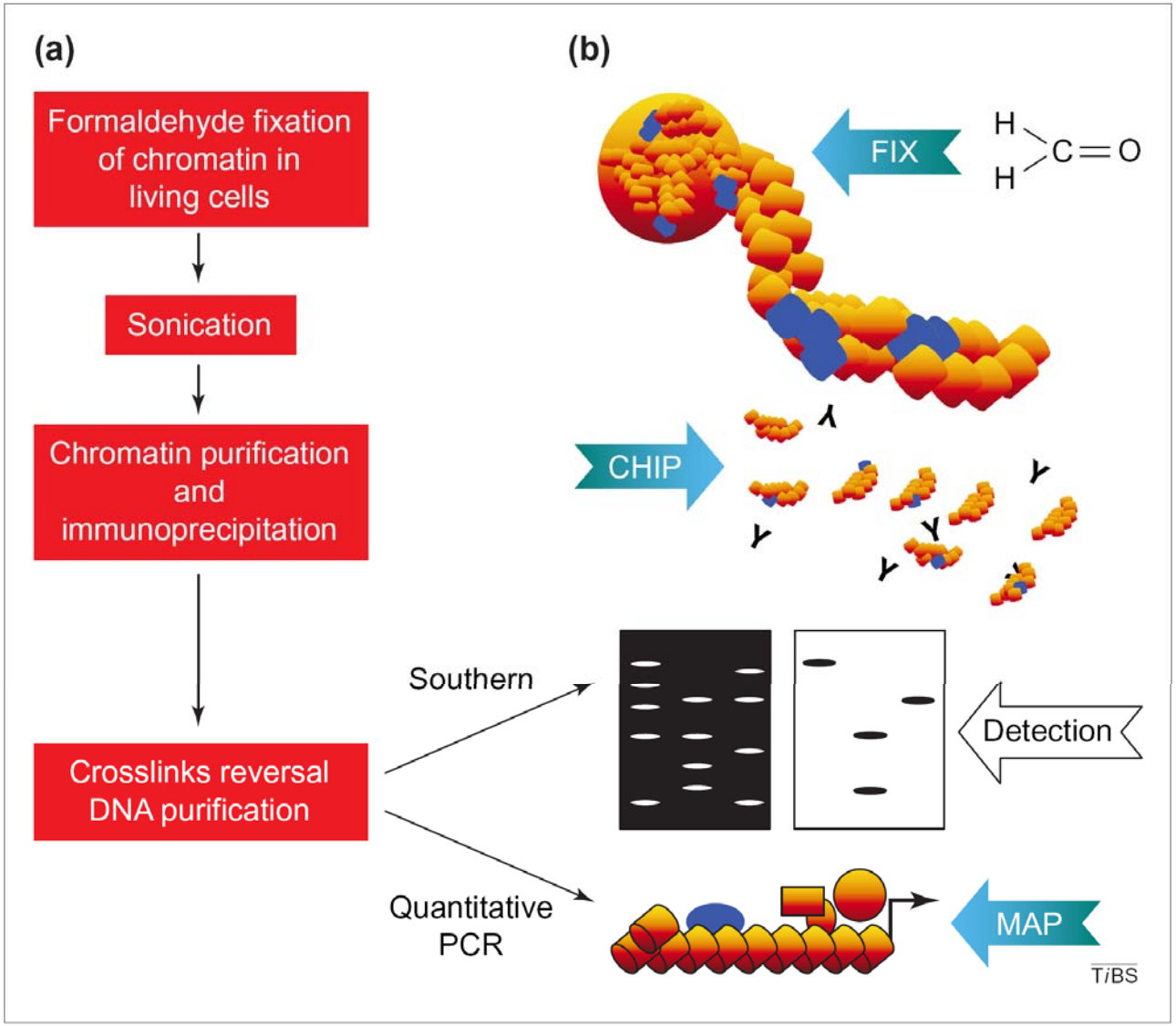
⇨Analyse by :

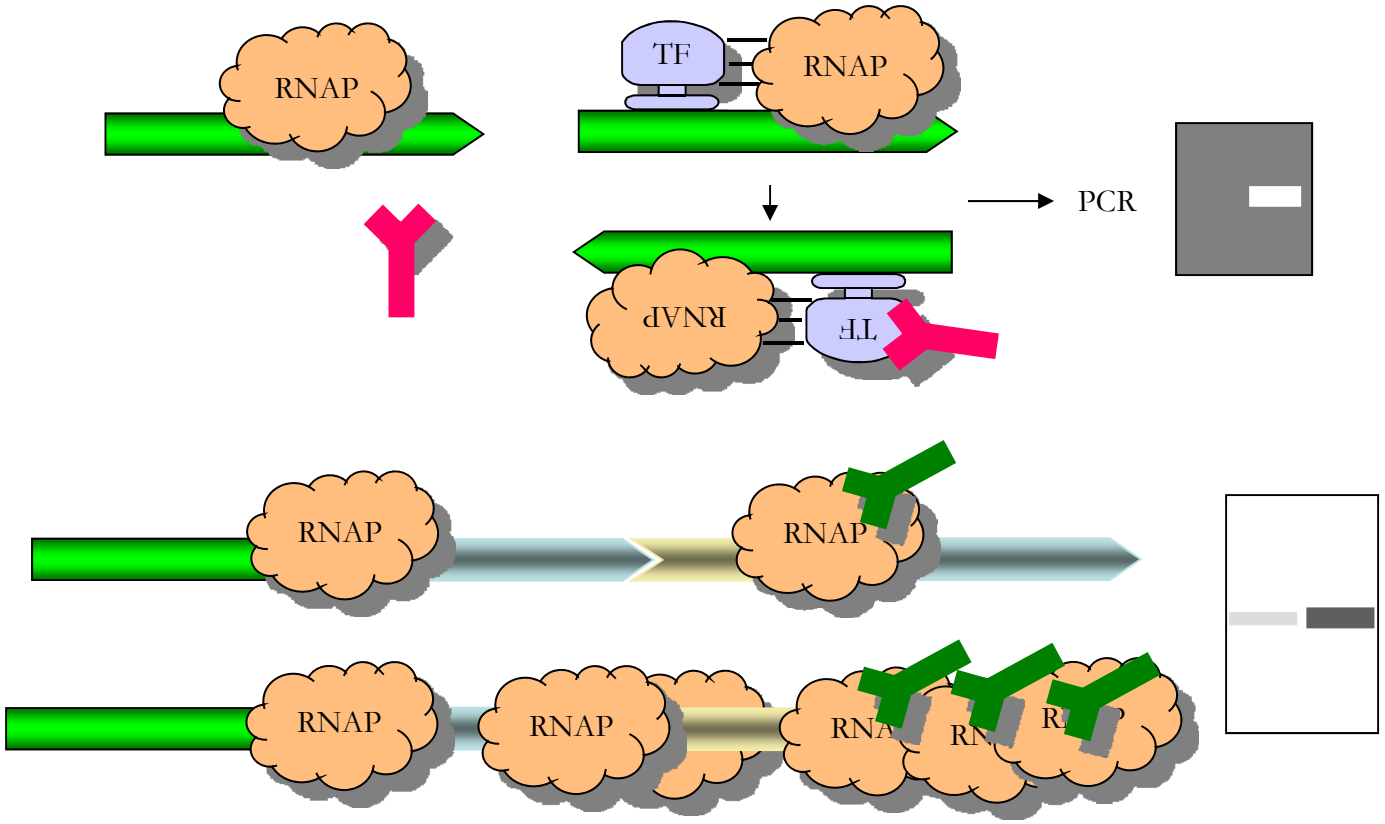
☑Nuclear Run on assay and/or
☑CHIP with RNAP immunoprecipitation } Confirm increase in transcription

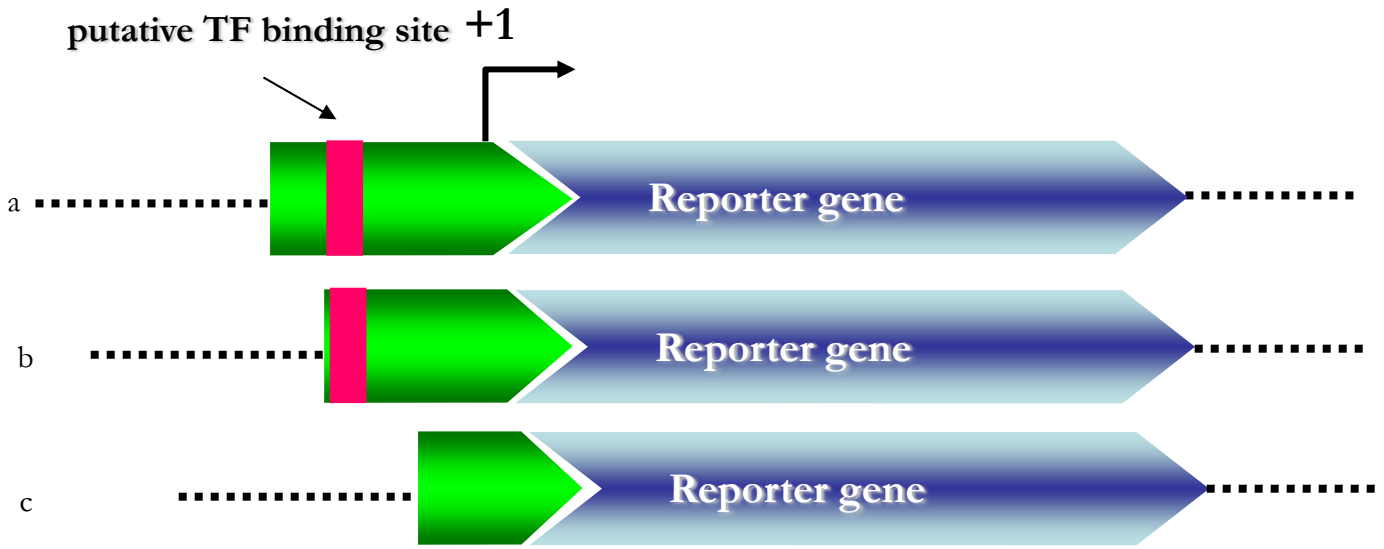
⇨Search sequence for transcription factor binding sites

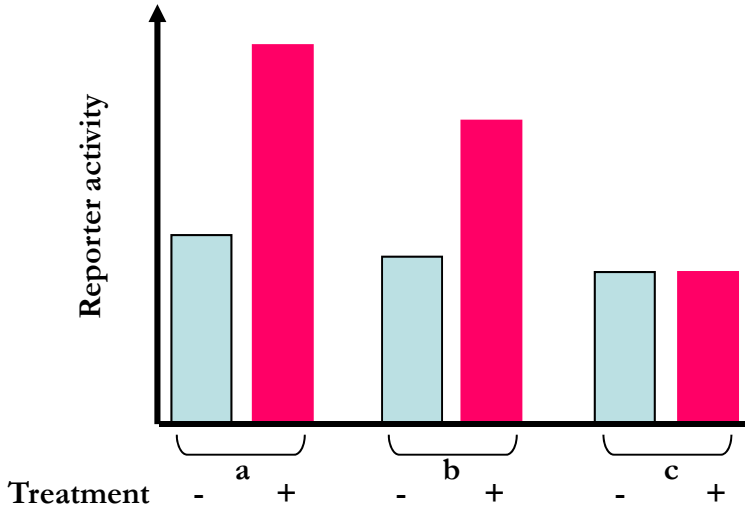
⇨Analyse for transcription factor binding and activation by :

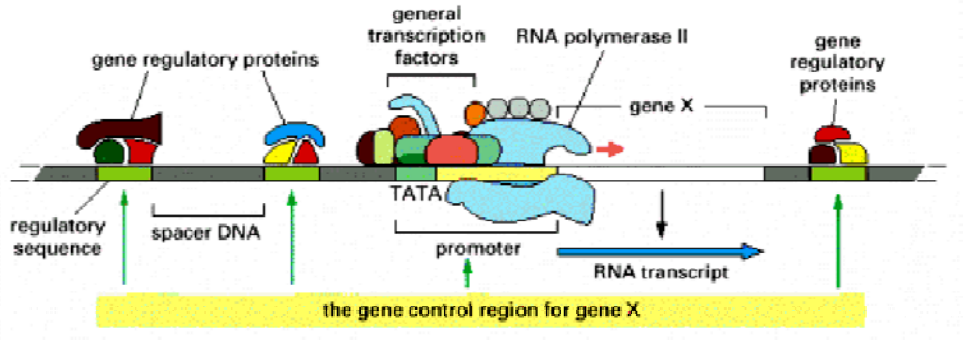
☑ EMSA (gel shift)
☑Transient transfection with reporter genes
☑ Inhibition of the transcription factor activation pathway

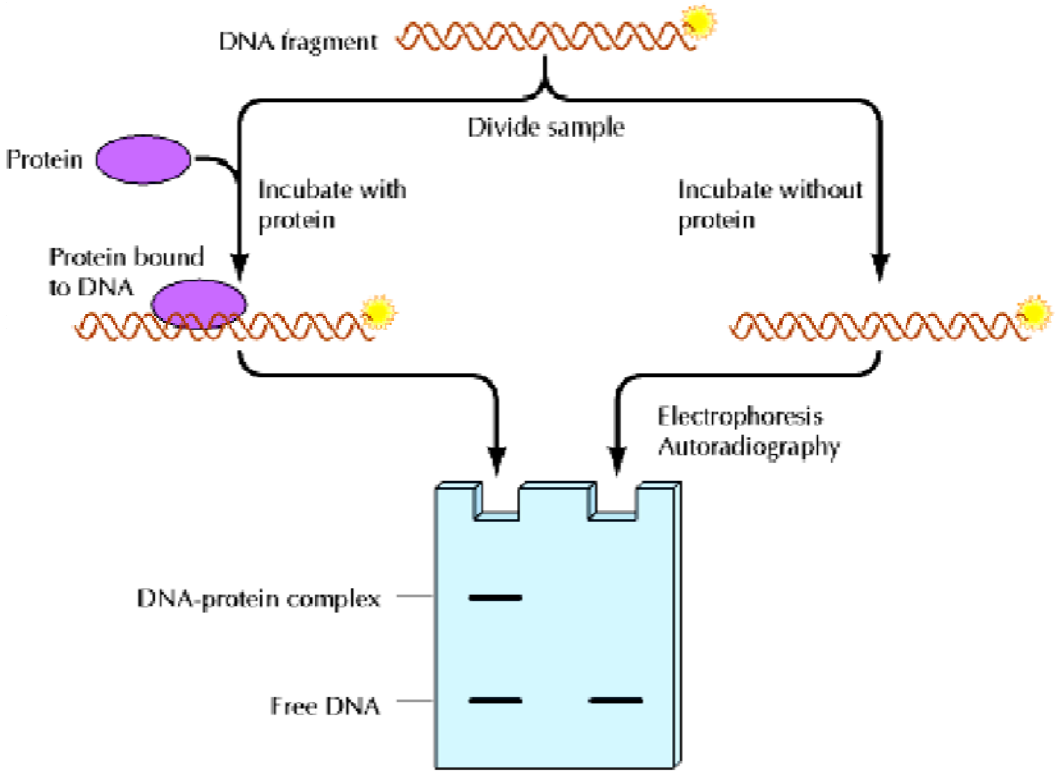

(a)
Formaldehyde fixation of chromatin in living cells
Sonication
Chromatin purification and immunoprecipitation
Crosslinks reversal DNA purification
(b)
FIX
H
H
C = O
CHIP
Southern
Detection
Quantitative PCR
MAP
TiBS


RNAP
TF
RNAP
PCR
RNAP
RNAP
RNAP
RNAP

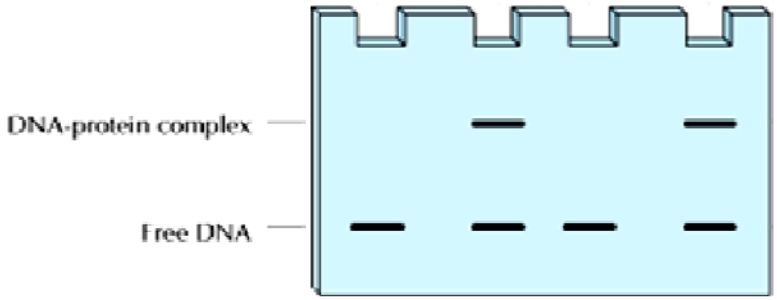

putative TF binding site
+1
a
Reporter gene
b
Reporter gene
c
Reporter gene


Reporter activity
a
b
c
Treatment
-
+
-
+
-
+

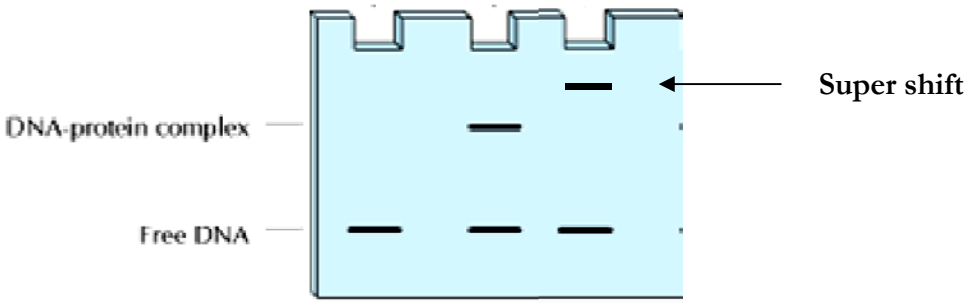

general transcription factors
RNA polymerase II
gene regulatory proteins
gene X
gene regulatory proteins
TATA
regulatory sequence
spacer DNA
promoter
RNA transcript
the gene control region for gene X

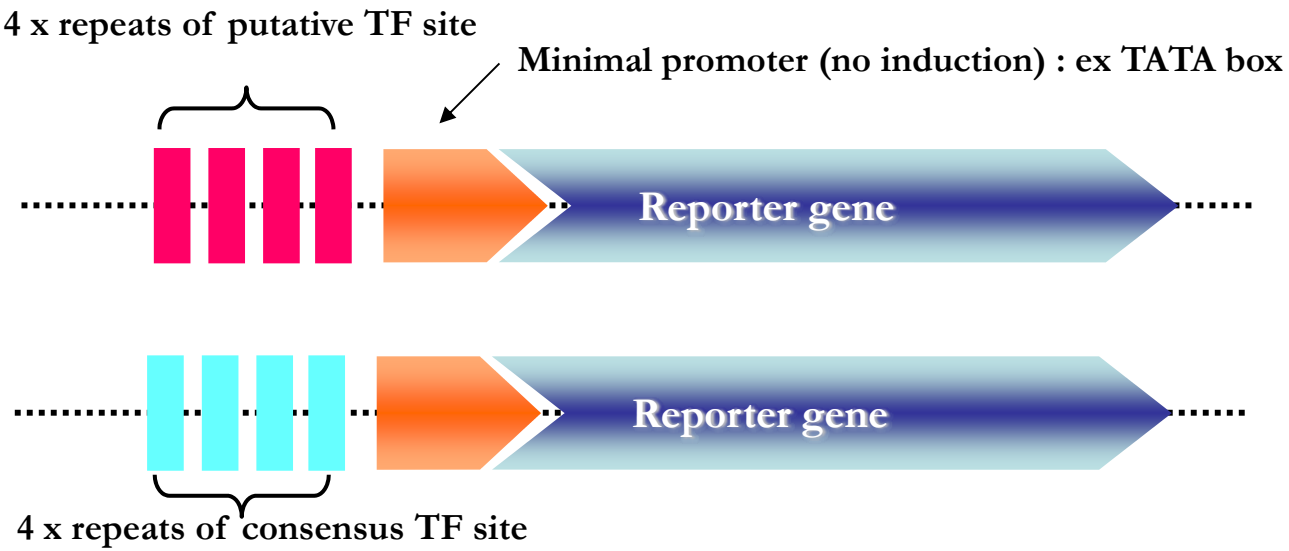

DNA fragment
Divide sample
Protein
Incubate with protein
Incubate without protein
Protein bound to DNA
Electrophoresis
Autoradiography
DNA-protein complex
Free DNA

+	+	+	+	**Sonde ^{32}P**
-	+	+	+	**Extrait nucléaire**
-	-	+	-	**Sonde non marquée (froide)**
-	-	-	+	**Oligonucléotide non compétiteur**

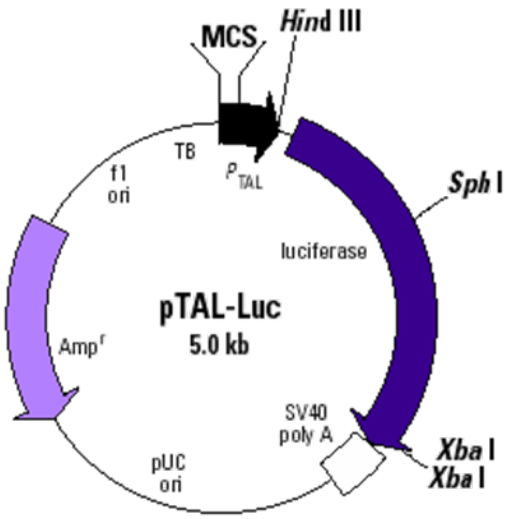


+	+	+	**Sonde ^{32}P**
-	+	+	**Extrait nucléaire**
-	-	+	**Ac anti-FT**

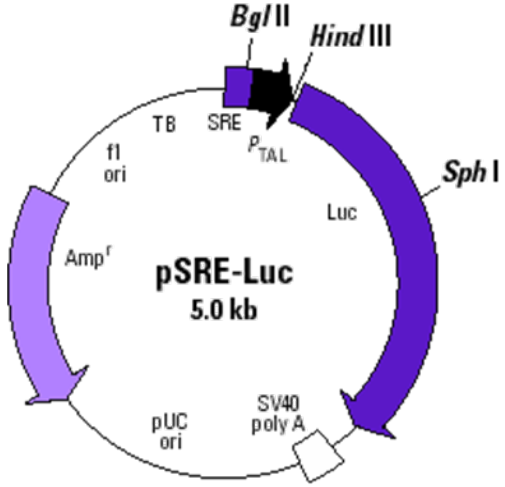




……GCTAGCGATG TCCATATTAG
GACATCGATG TCCGAATATG
GACATCGATG TCCATATTAG
GACATCAGAT…….

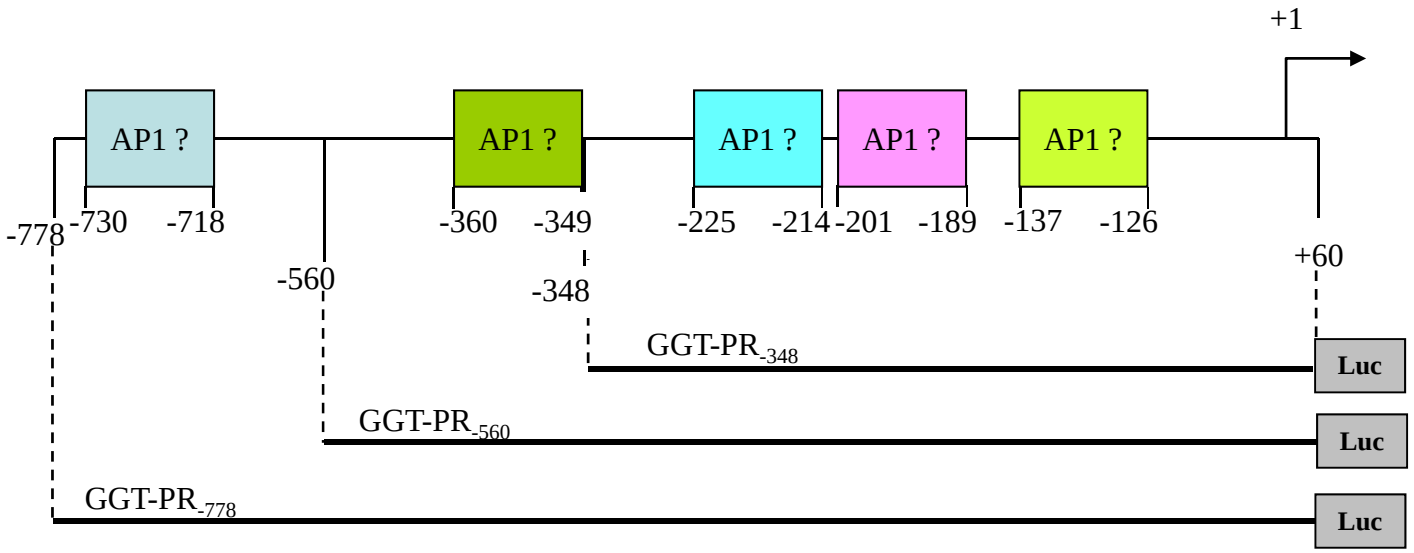


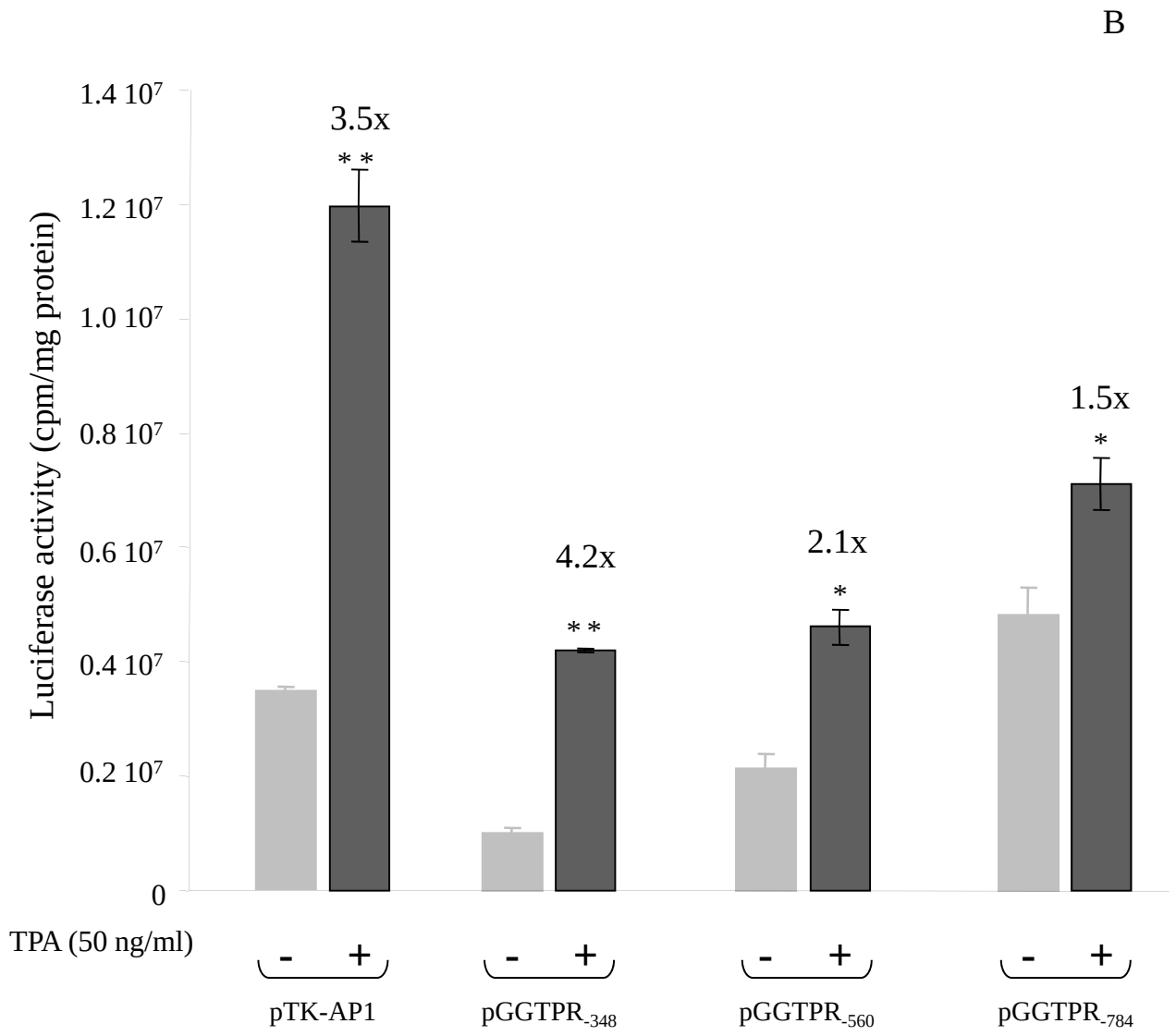
TB = Transcription blocker

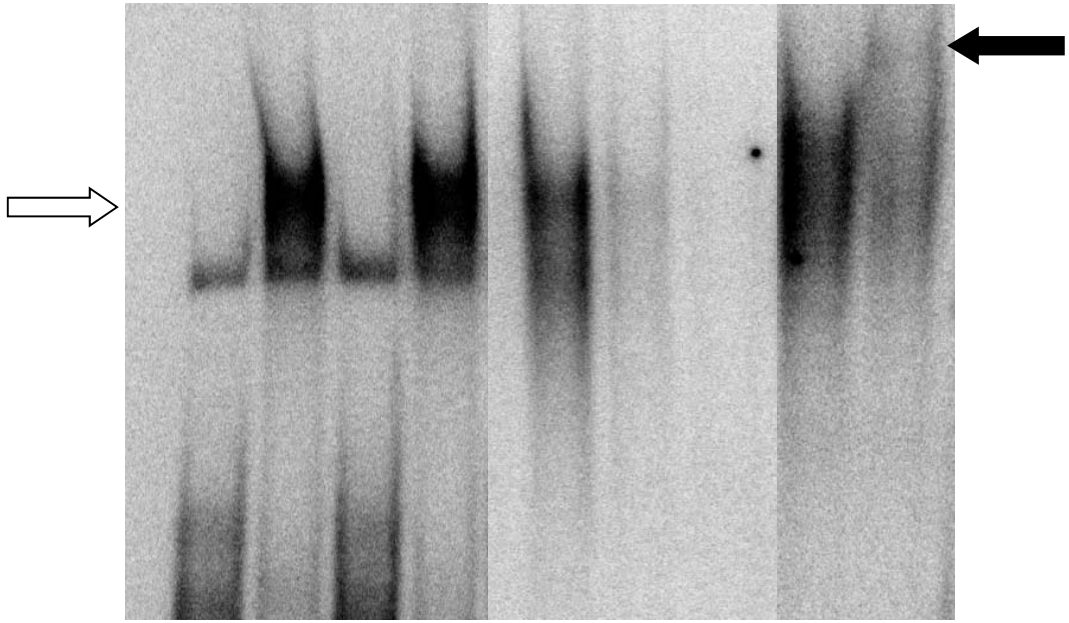


TB=Transcription Blocker

A


+1
AP1 ?
AP1 ?
AP1 ?
AP1 ?
AP1 ?
-778
-730
-718
-560
-360
-349
-348
-225
-214
-201
-189
-137
-126
+60
GGT-PR-348
GGT-PR-560
GGT-PR-778
Luc
Luc
Luc


B
Luciferase activity (cpm/mg protein)
1.4 10⁷
1.2 10⁷
1.0 10⁷
0.8 10⁷
0.6 10⁷
0.4 10⁷
0.2 10⁷
0
3.5x
* *
4.2x
* *
2.1x
*
1.5x
*
TPA (50 ng/ml)
- +
- +
- +
- +
pTK-AP1
pGGTPR-348
pGGTPR-560
pGGTPR-784

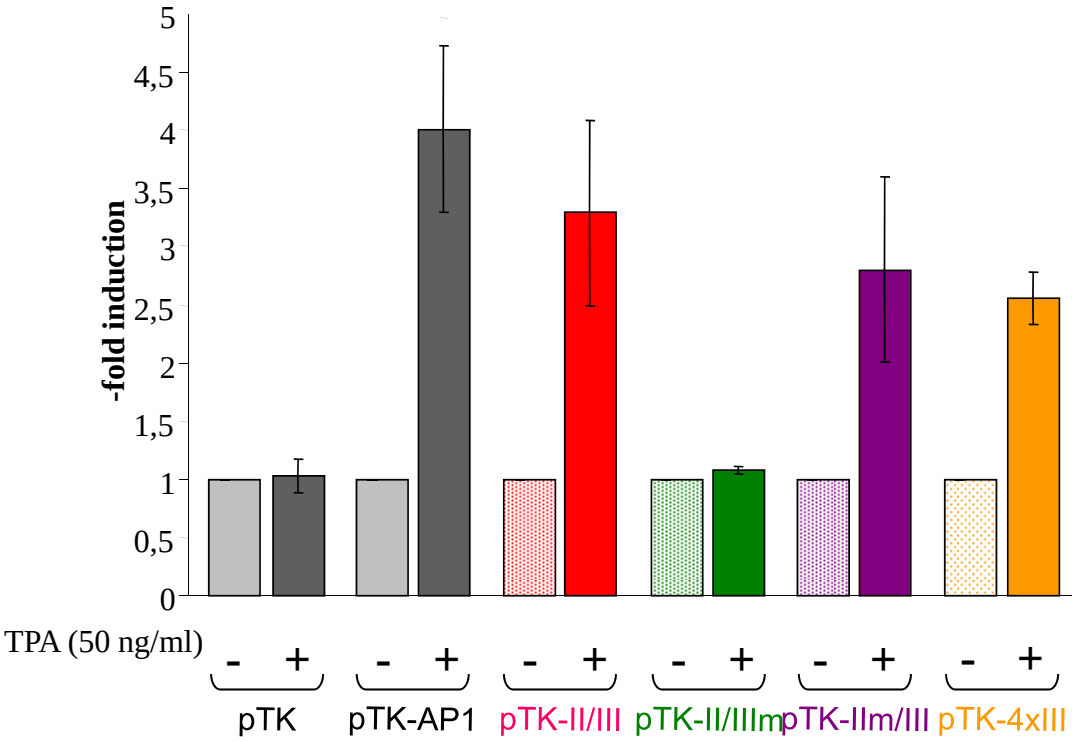


AP1-III	-	-	+	+	+	+	+	+	+
AP1-coll	+	+	-	-	-	-	-	-	-
TPA	-	+	-	+	+	+	+	-	-
competitor	-	-	-	-	▲	▲	▲	-	-
Anti-cJun	-	-	-	-	-	-	-	-	+

pTK-II/III 5’-TCTAGAACAGGCTCTGAGAGGACCACATCGCCAGCAAGTGACAGGCTGGGA-3’

pTK-II/IIIm 5’-TCTAGAACAGGCTCTGAGAGGACCACATCGCCAGCAA***GGATCC***GGCTGGGA-3’

pTK-IIm/III 5’-TCTAGAACAGGCTCT***GGATCC***ACCACATCGCCAGCAAGTGACAGGCTGGGA-3’



GST-pull down

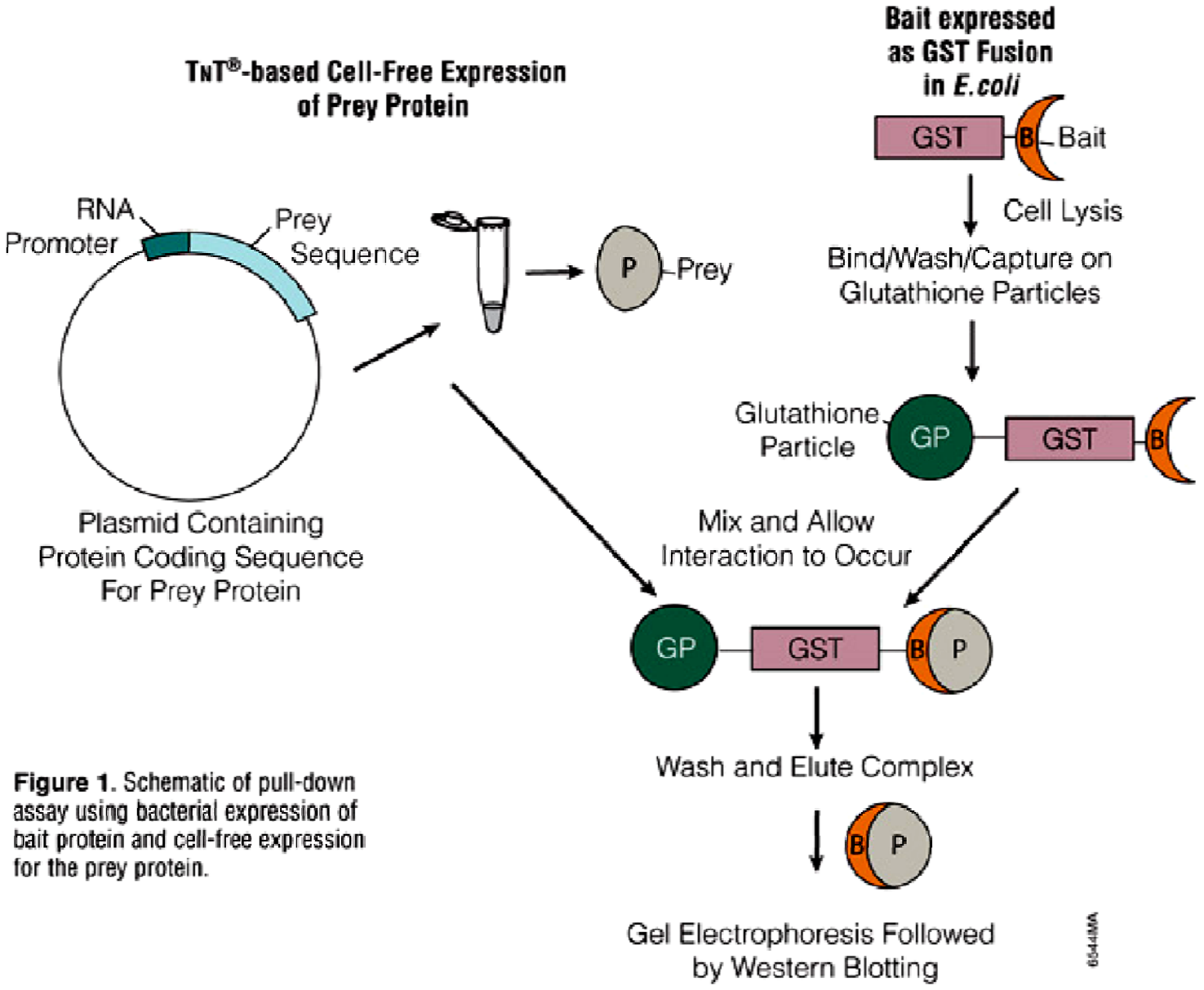
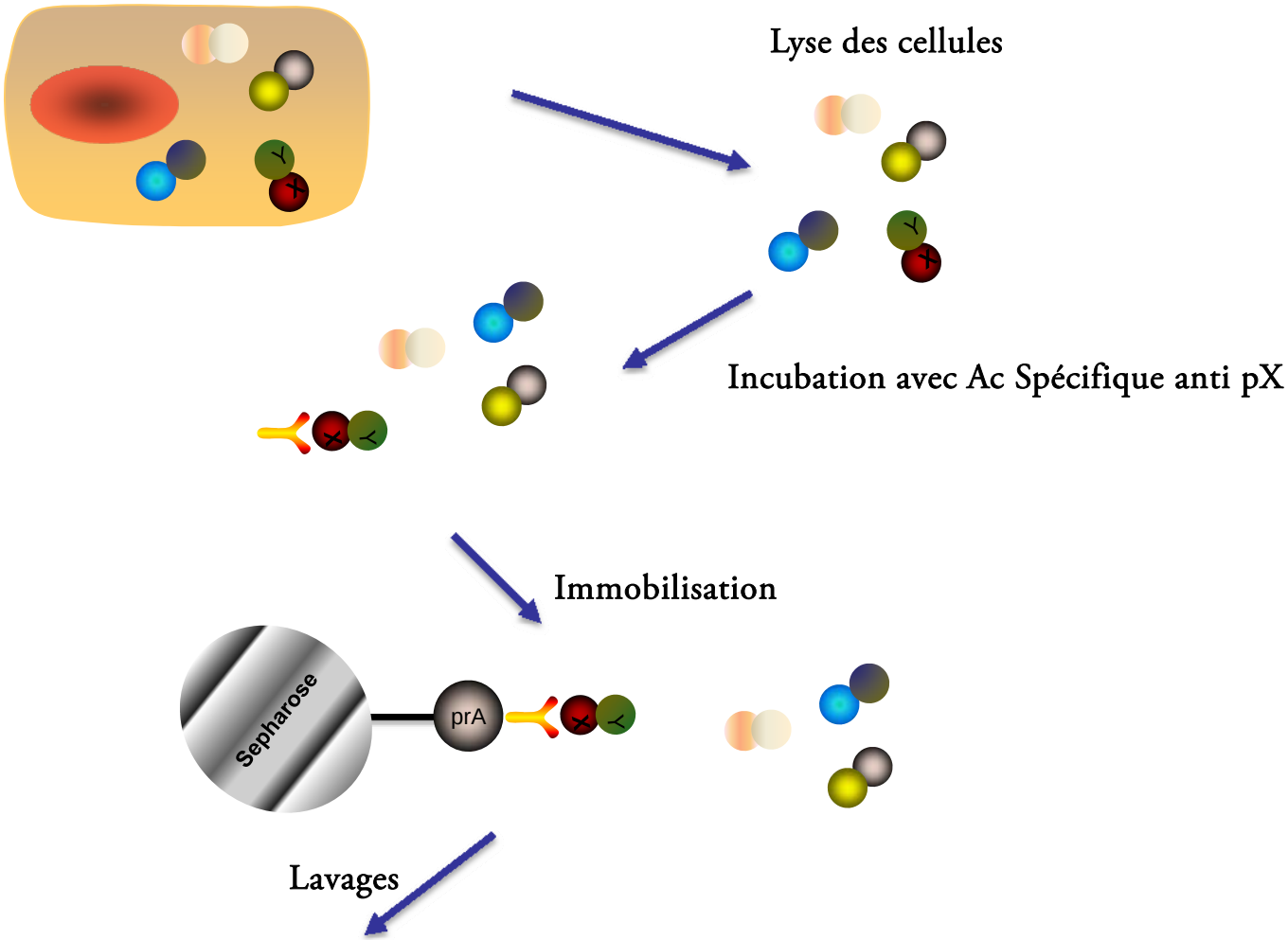
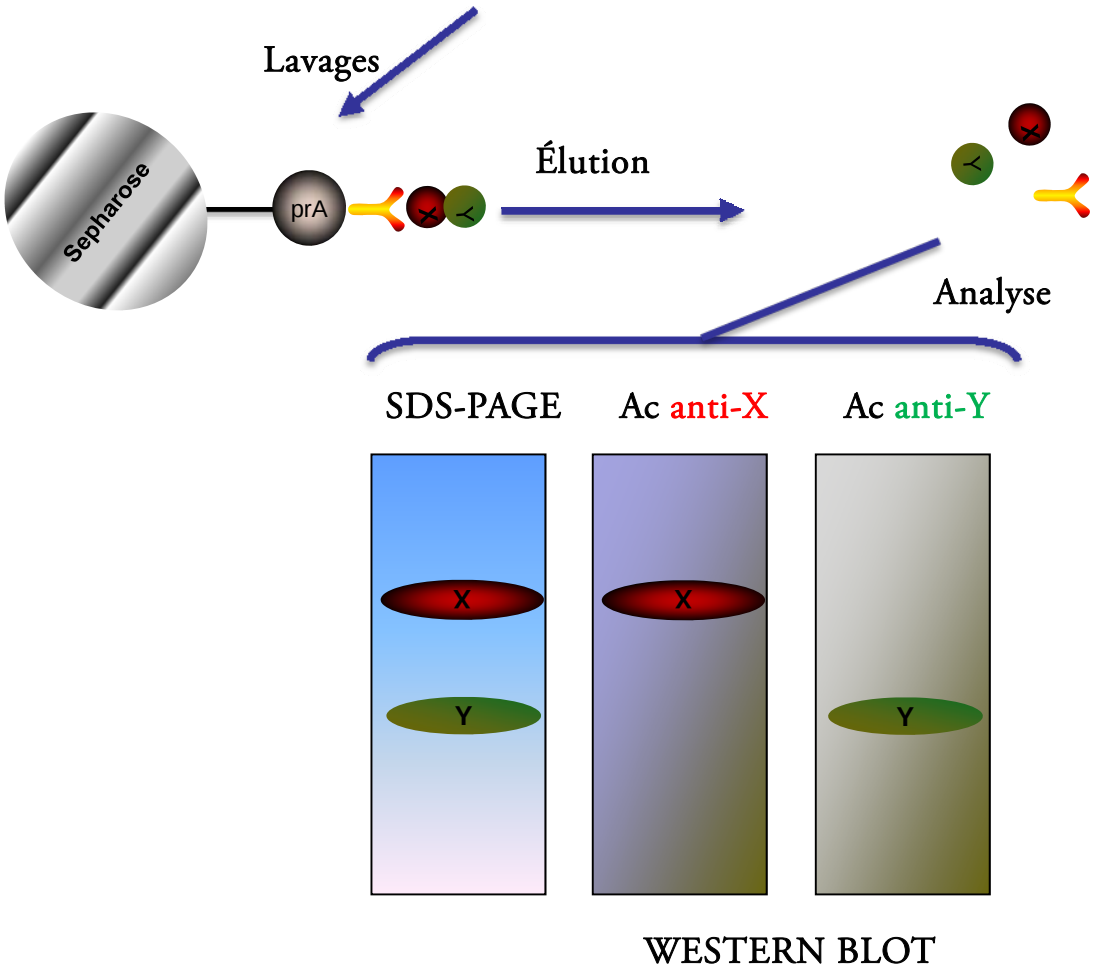


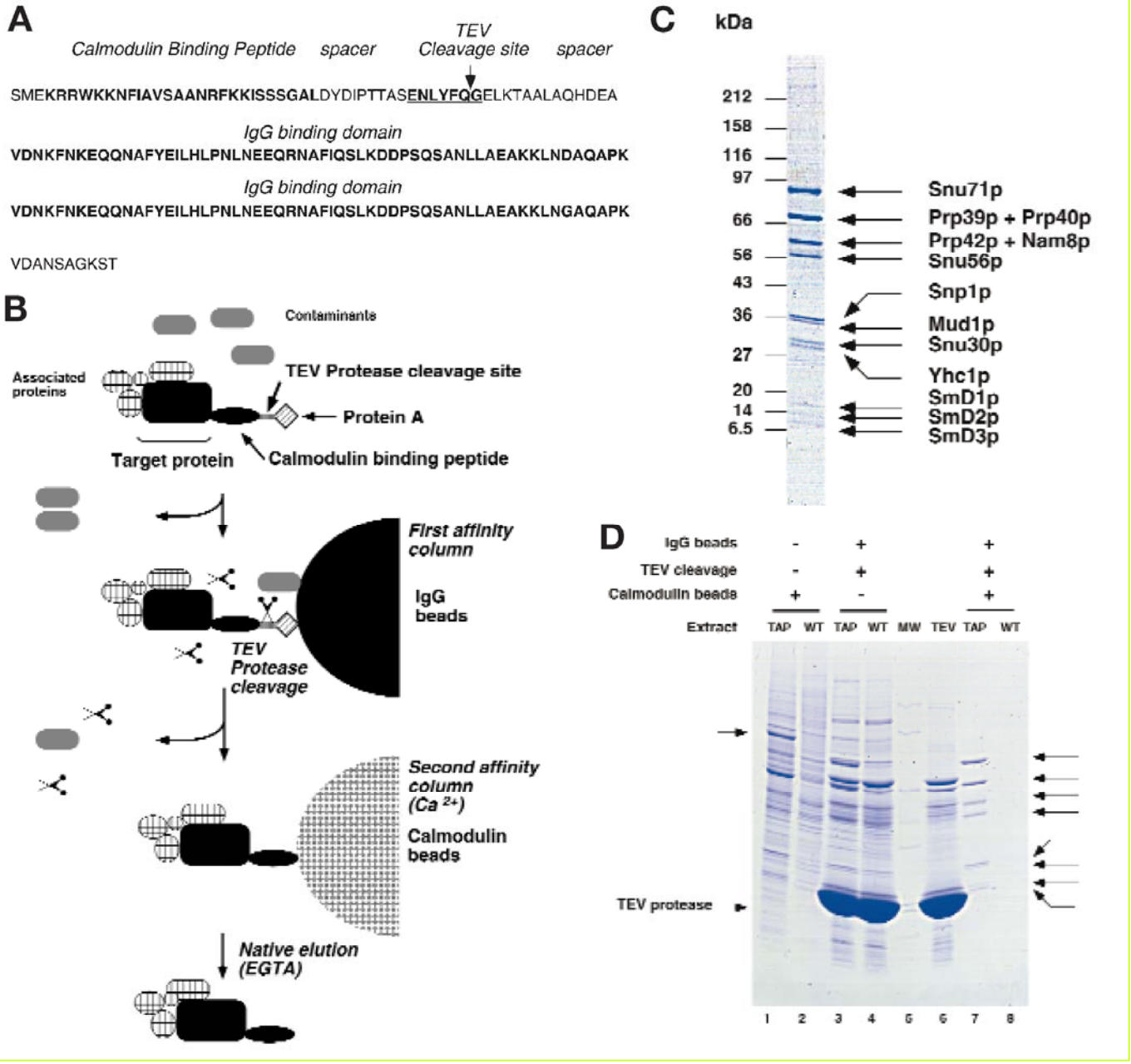
Figure 1. Schematic of pull-down assay using bacterial expression of bait protein and cell-free expression for the prey protein.

Co-immunoprécipitation

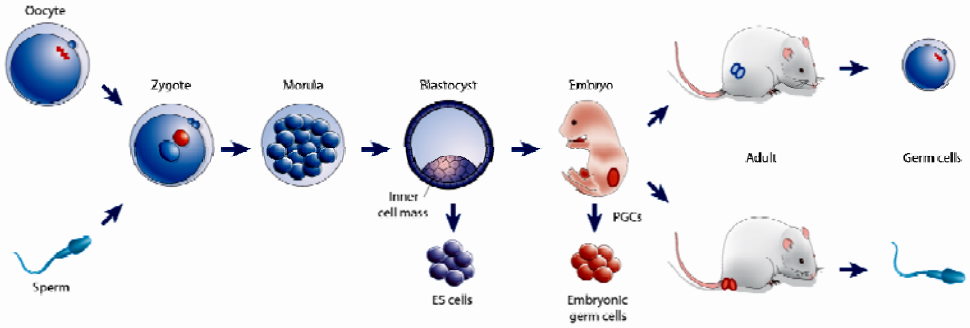


Co-immunoprécipitation

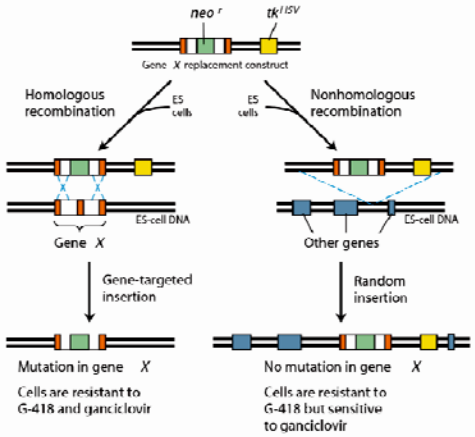




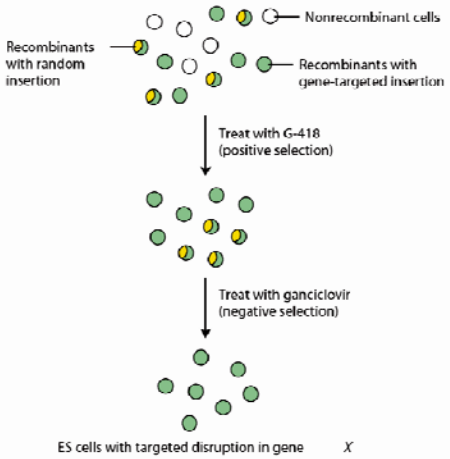
The TAP strategy: rationale and testing. (A) Sequence and structure of the TAP tag. The various domains constituting the TAP tag are indicated. (B) Overview of the TAP procedure. (C) Protein composition of TAP-purified U1 snRNP. Faster migrating ands potentially corresponding to the SmE, SmF, and/or SmG proteins were detected by silver staining but not analyzed. (D) Step-by-step analysis of the TAP strategy. Proteins present in the final TAP fraction (lanes 7 and 8), or present after each of the single affinity purification steps (lanes 1–4), were analyzed. Snu71-TAP (lanes 1, 3, and 7) or wild-type extracts (lanes 2, 4, and 8) were used. Lane 5: molecular weight marker. Lane 6: an amount of TEV protease identical to the amount used to elute proteins bound to IgG beads (lanes 2, 3, 7, and 8). Right arrows indicate the U1 snRNP-specific proteins including the tagged Snu71p after TEV cleavage; the arrow on the left indicates the Snu71p protein fused to the TAP tag before TEV cleavage



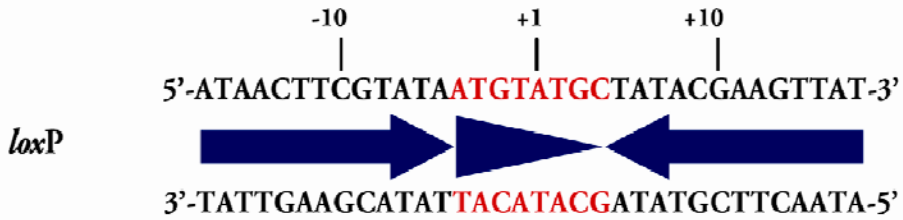
(a) Formation of ES cells carrying a knockout mutation

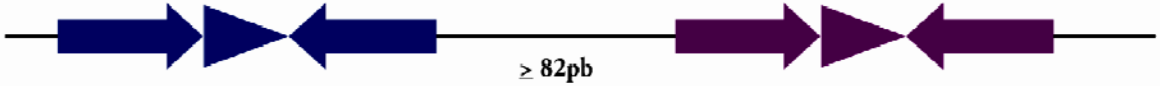


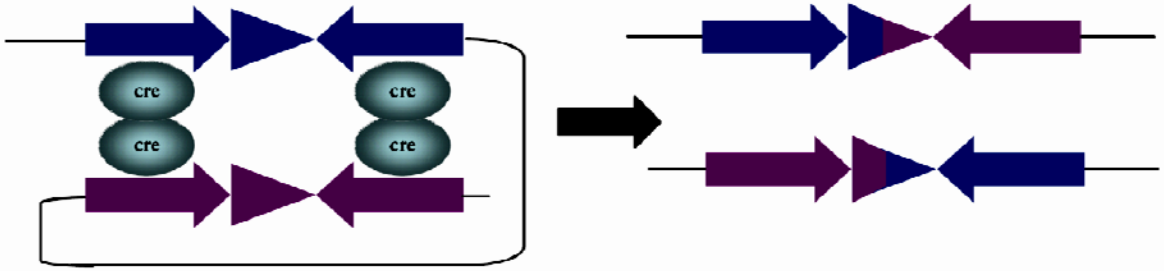
(b) Positive and negative selection of recombinant ES cells

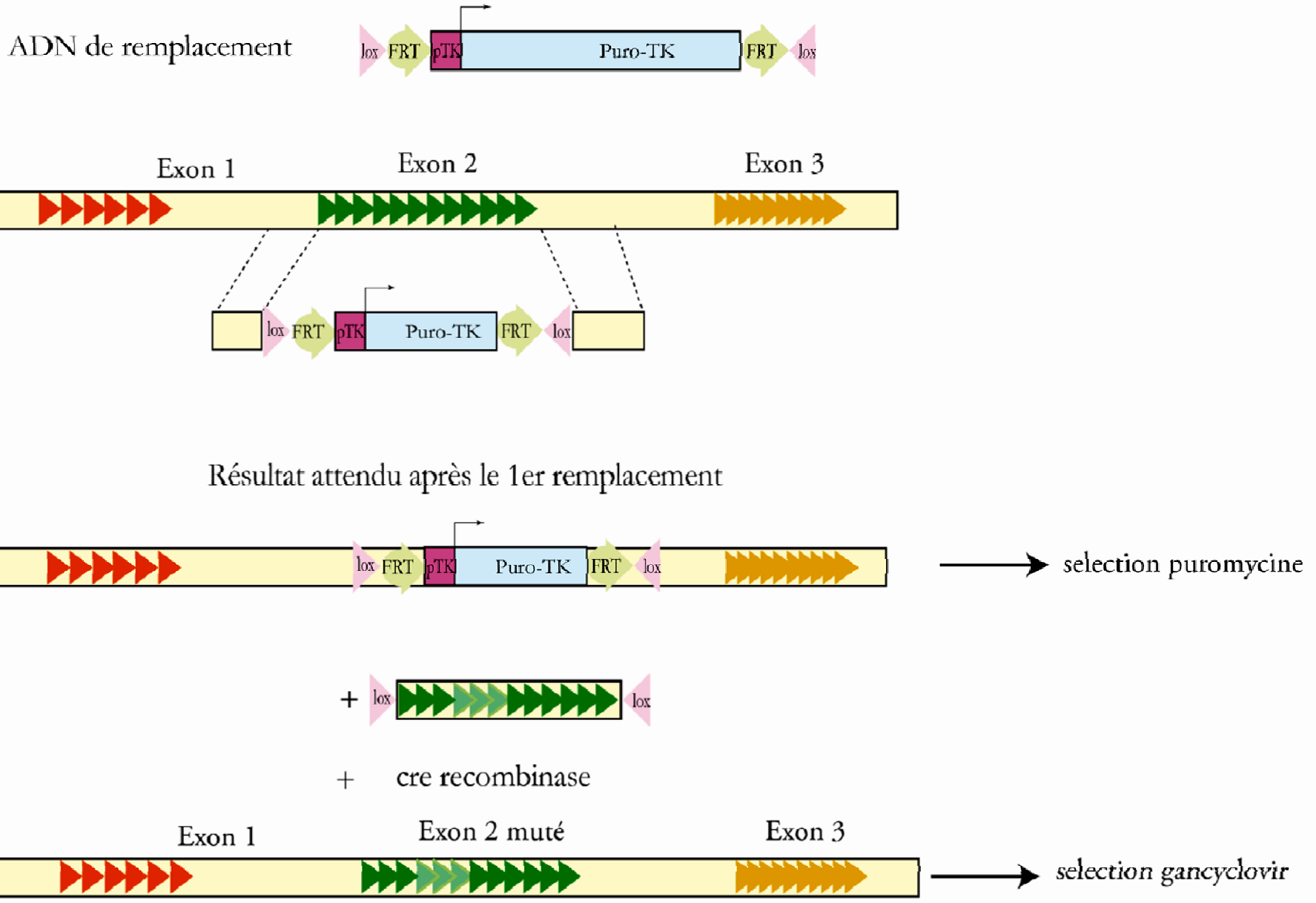


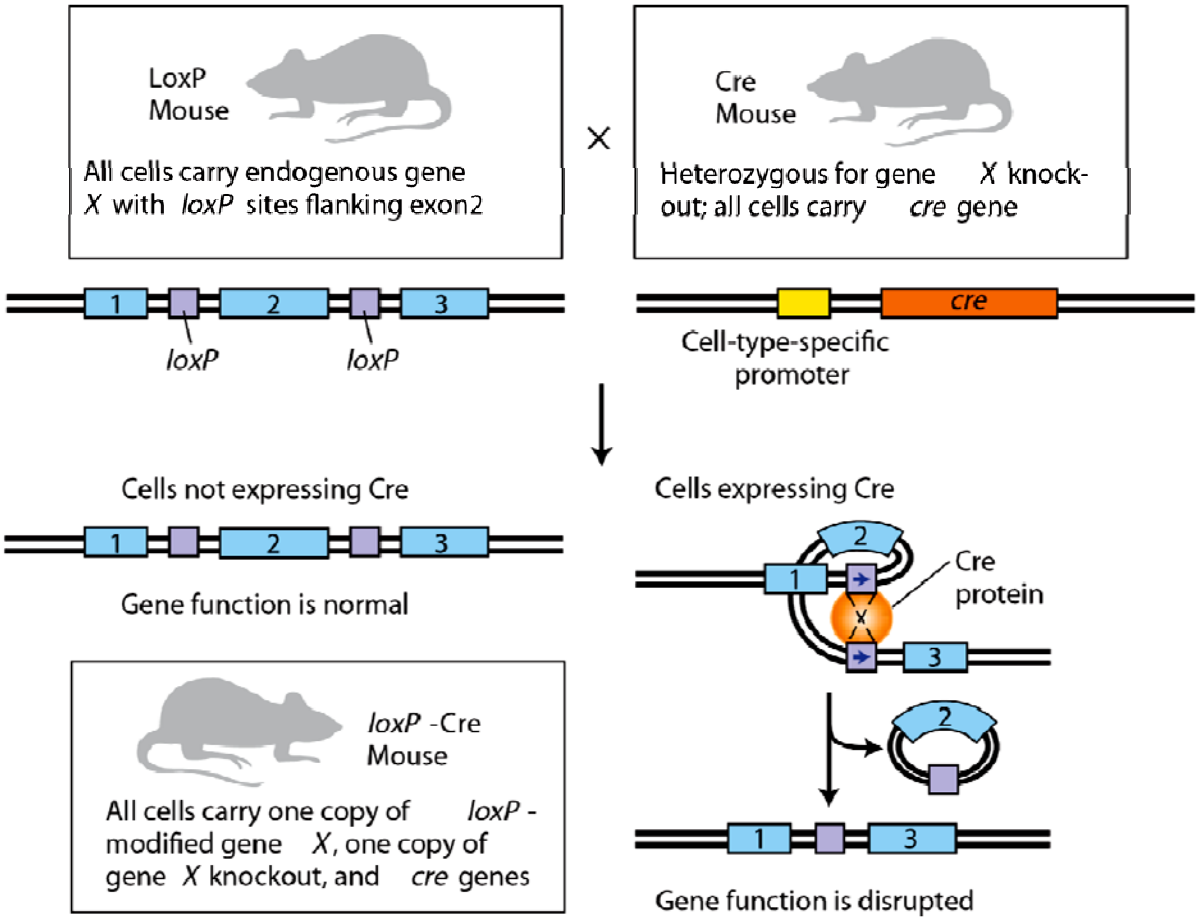
Séquence cible de la recombinase Cre

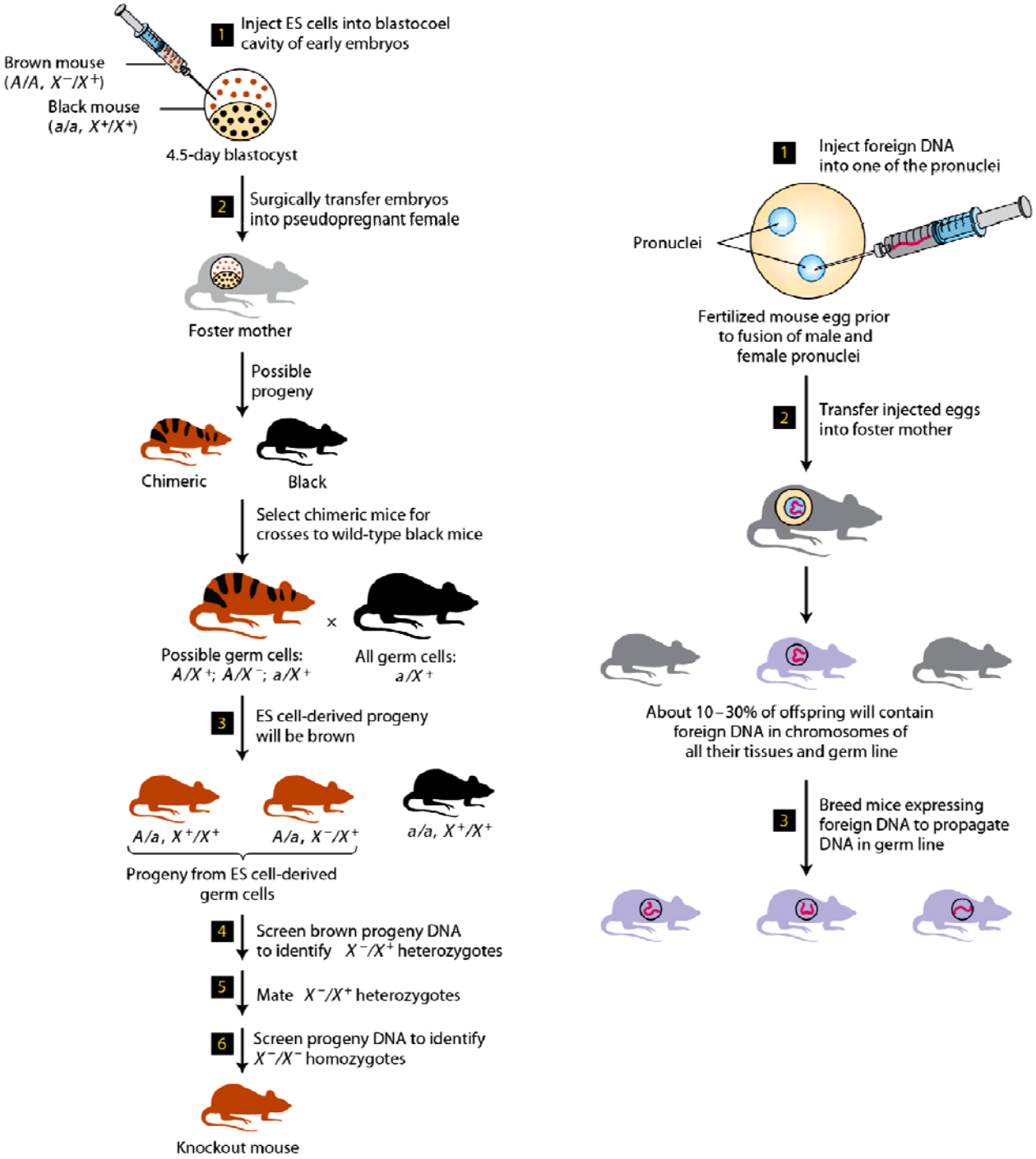







ADN de remplacement
lox
FRT
pTK
Puro-TK
FRT
lox
Exon 1
Exon 2
Exon 3
lox
FRT
pTK
Puro-TK
FRT
lox
Résultat attendu après le 1er remplacement
lox
FRT
pTK
Puro-TK
FRT
lox
selection puromycine
+
lox
lox
+
cre recombinase
Exon 1
Exon 2 muté
Exon 3
selection gancyclovir


LoxP
Mouse
All cells carry endogenous gene X with loxP sites flanking exon2
×
Cre
Mouse
Heterozygous for gene X knock-out; all cells carry cre gene
1
2
3
loxP
loxP
cre
Cell-type-specific promoter
Cells not expressing Cre
1
2
3
Gene function is normal
Cells expressing Cre
2
1
Cre protein
3
2
1
3
Gene function is disrupted
loxP -Cre
Mouse
All cells carry one copy of loxP -modified gene X, one copy of gene X knockout, and cre genes


1 Inject ES cells into blastocoel cavity of early embryos
Brown mouse (A/A, X⁻/X⁺)
Black mouse (a/a, X⁺/X⁺)
4.5-day blastocyst
2 Surgically transfer embryos into pseudopregnant female
Foster mother
Possible progeny
Chimeric
Black
Select chimeric mice for crosses to wild-type black mice
Possible germ cells: A/X⁺; A/X⁻; a/X⁺
×
All germ cells: a/X⁺
3 ES cell-derived progeny will be brown
A/a, X⁺/X⁺
A/a, X⁻/X⁺
a/a, X⁺/X⁺
Progeny from ES cell-derived germ cells
4 Screen brown progeny DNA to identify X⁻/X⁺ heterozygotes
5 Mate X⁻/X⁺ heterozygotes
6 Screen progeny DNA to identify X⁻/X⁻ homozygotes
Knockout mouse
1 Inject foreign DNA into one of the pronuclei
Pronuclei
Fertilized mouse egg prior to fusion of male and female pronuclei
2 Transfer injected eggs into foster mother
About 10–30% of offspring will contain foreign DNA in chromosomes of all their tissues and germ line
3 Breed mice expressing foreign DNA to propagate DNA in germ line