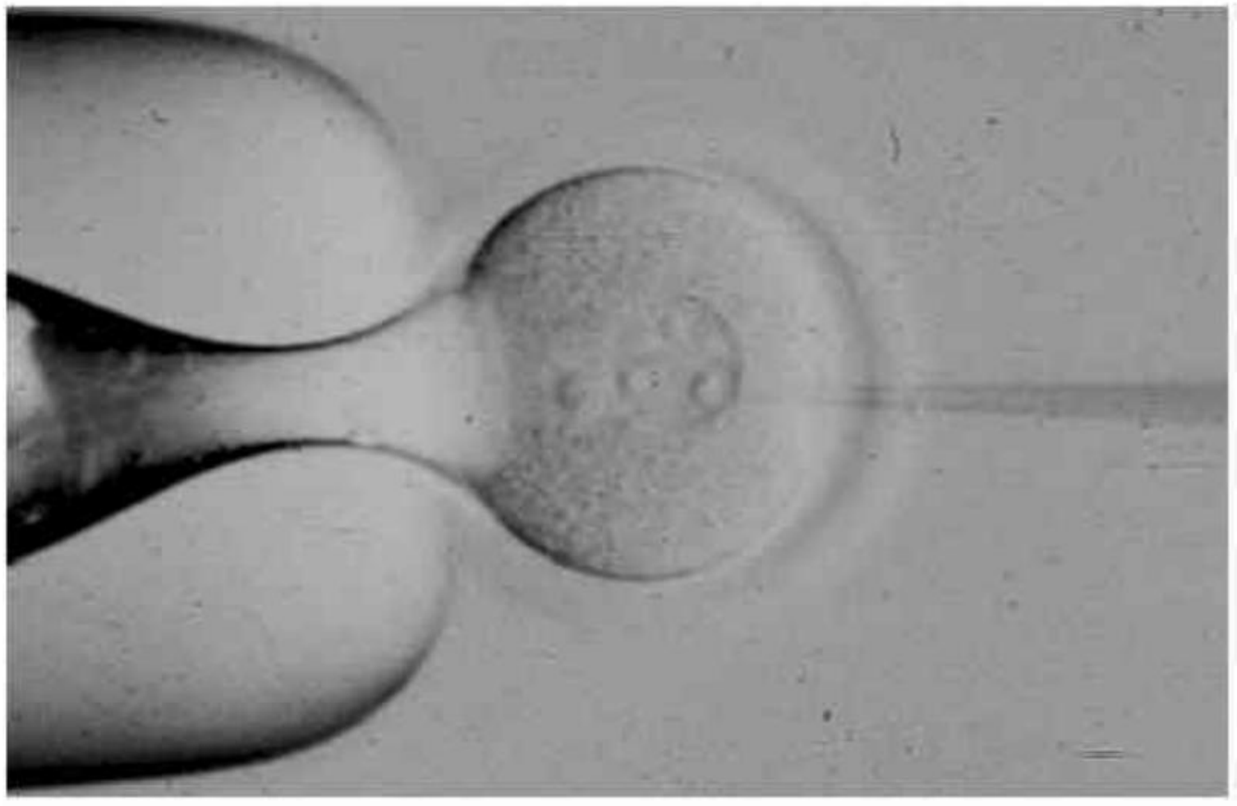
模式生物技术概论
小鼠遗传操作技术及其应用



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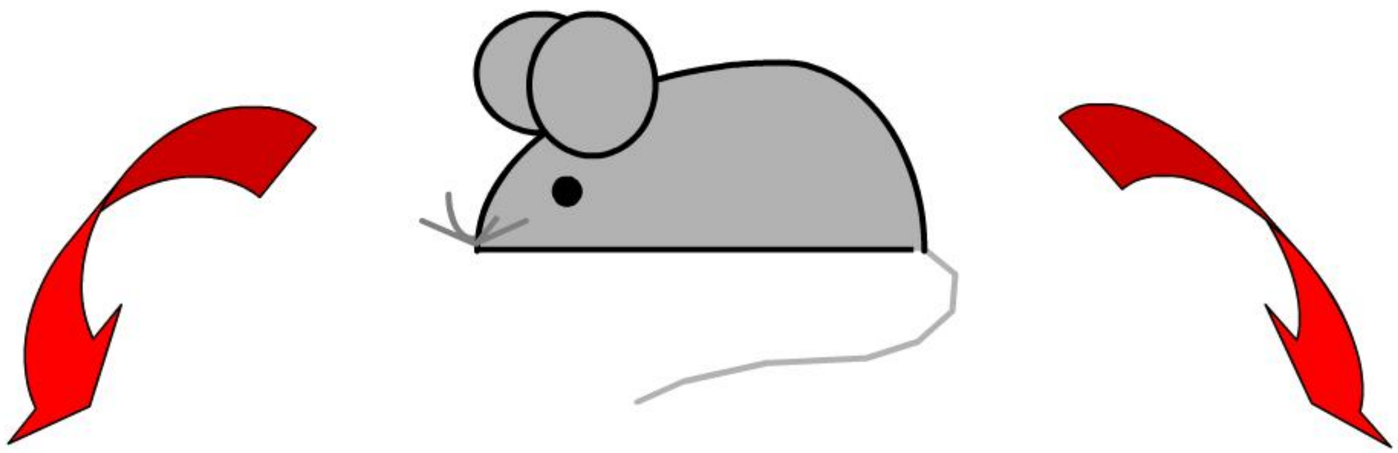
上海南方模式生物研究中心

小鼠遗传操作技术原理

目的基因



功能获得

功能缺失

转基因小鼠的几个里程碑工作

- 1974 Jaenisch and Mintz：将SV40病毒DNA注射小鼠囊胚，得到整合有SV40病毒的小鼠（Proc Natl Acad Sci U S A. 1974 Apr;71(4):1250-4.）
- 1976 Jaenisch：将逆转录病毒用于小鼠囊胚感染获得转基因小鼠，并能传代（Proc Natl Acad Sci U S A 1976 Apr;73(4):1260-4）
- 1980-81 Gordon et al., Brinster et al., Costantini et al.：DNA小鼠受精卵原核注射建立了转基因小鼠的方法（Science. 1981 Dec 11; 214(4526): 1244-6. Cell. 1981 Nov;27(1 Pt 2):223-31. Nature. 1981 Nov 5;294(5836):92-4.）
- 1982 Palmiter et al., 在小鼠中过度表达人生长激素基因，获得超级小鼠 （Nature. 1982 Dec 6;300(5893):611-5.）

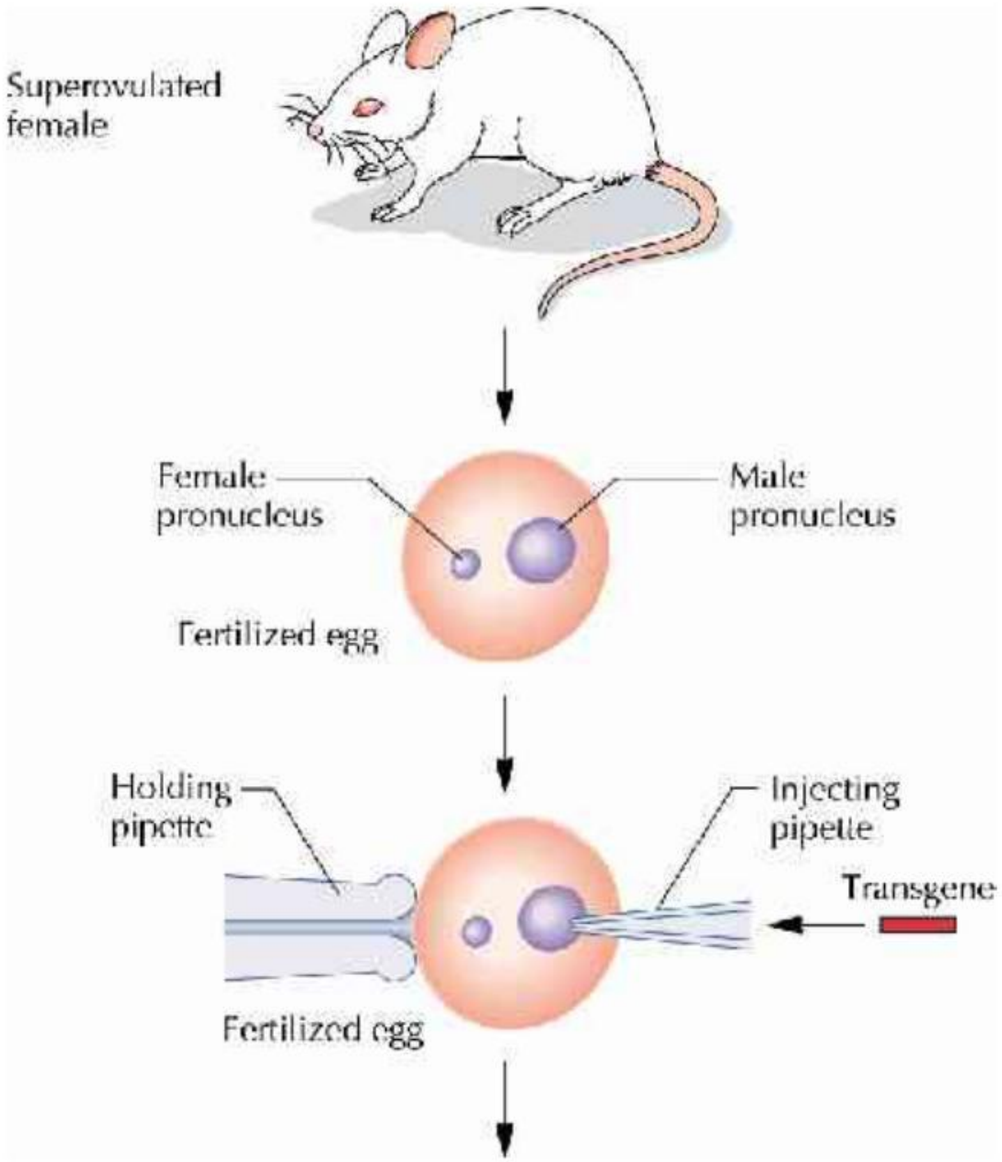
超级小鼠：人生长激素转基因小鼠

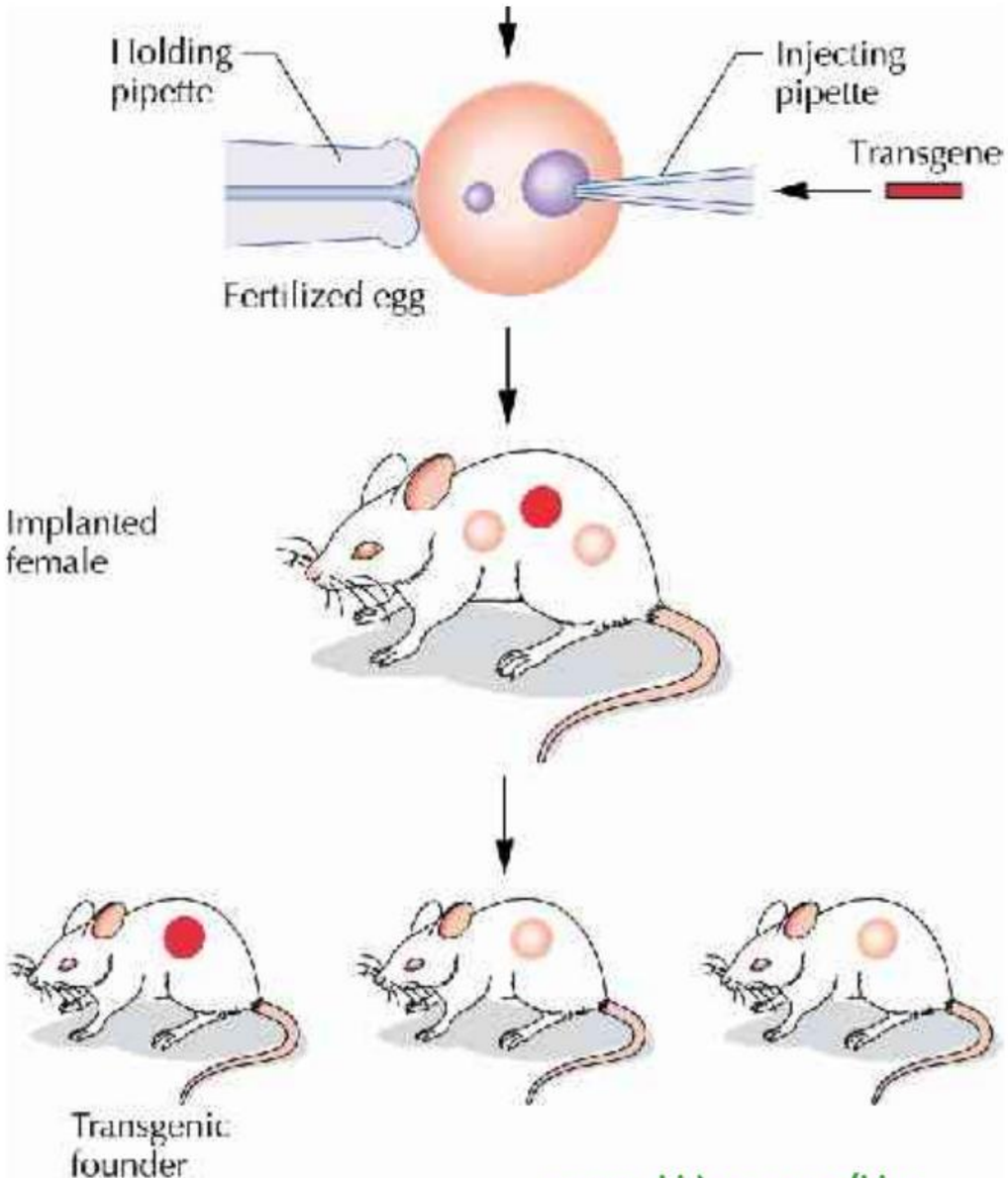
转基因小鼠技术

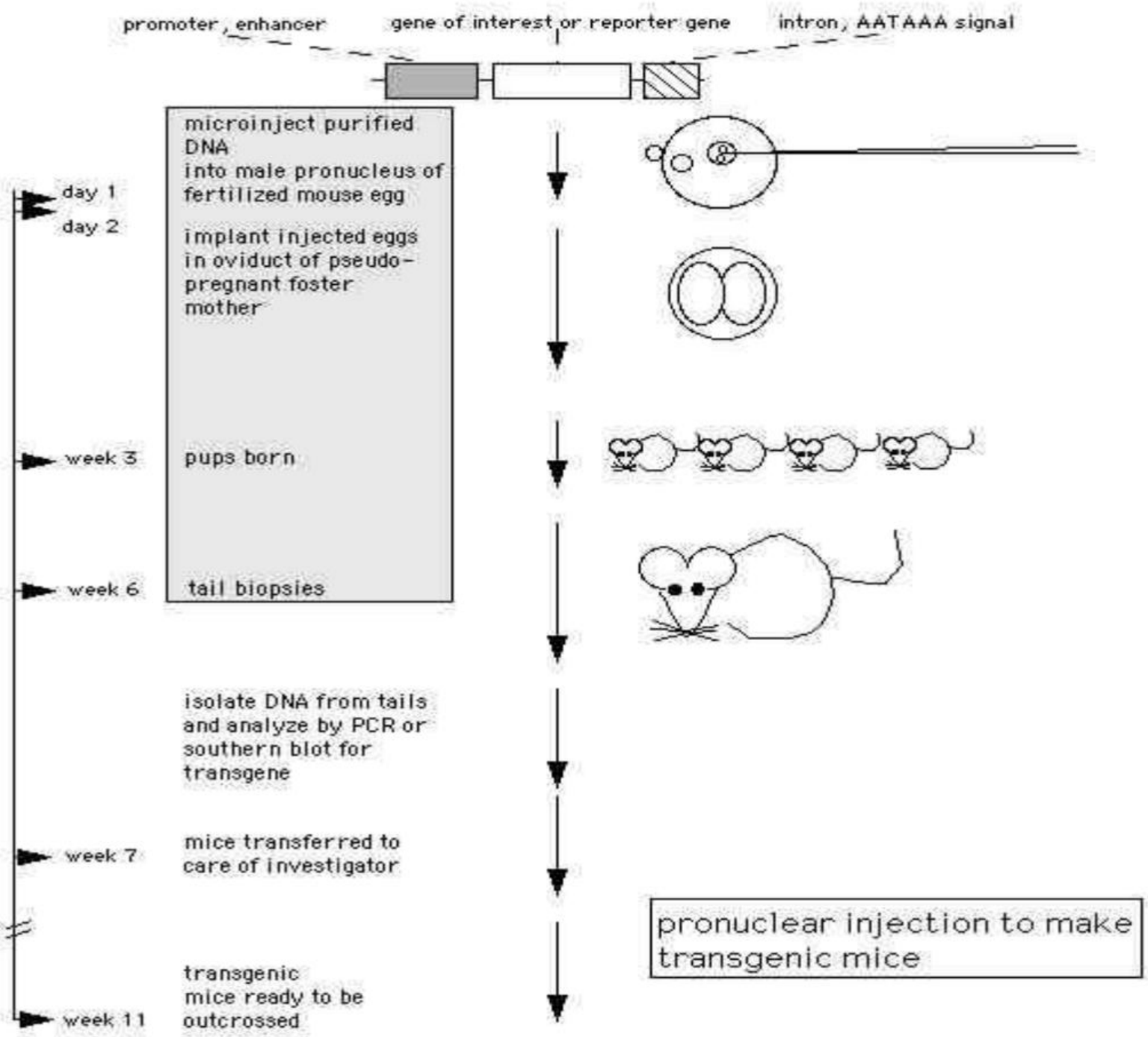
- 受精卵单细胞原核注射
- 精子介导的转基因技术
- 病毒介导的转基因技术（逆转录病毒、Lantivirus）
- 穿膜肽（核定位蛋白）介导的基因转移
- 转座酶介导的基因转移

Mouse Transgenesis

Microinjection Method






promoter, enhancer
gene of interest or reporter gene
intron, AATAAA signal
microinject purified DNA into male pronucleus of fertilized mouse egg
day 1
day 2
implant injected eggs in oviduct of pseudo-pregnant foster mother
week 3
pups born
week 6
tail biopsies
isolate DNA from tails and analyze by PCR or southern blot for transgene
week 7
mice transferred to care of investigator
transgenic mice ready to be outcrossed
week 11
pronuclear injection to make transgenic mice

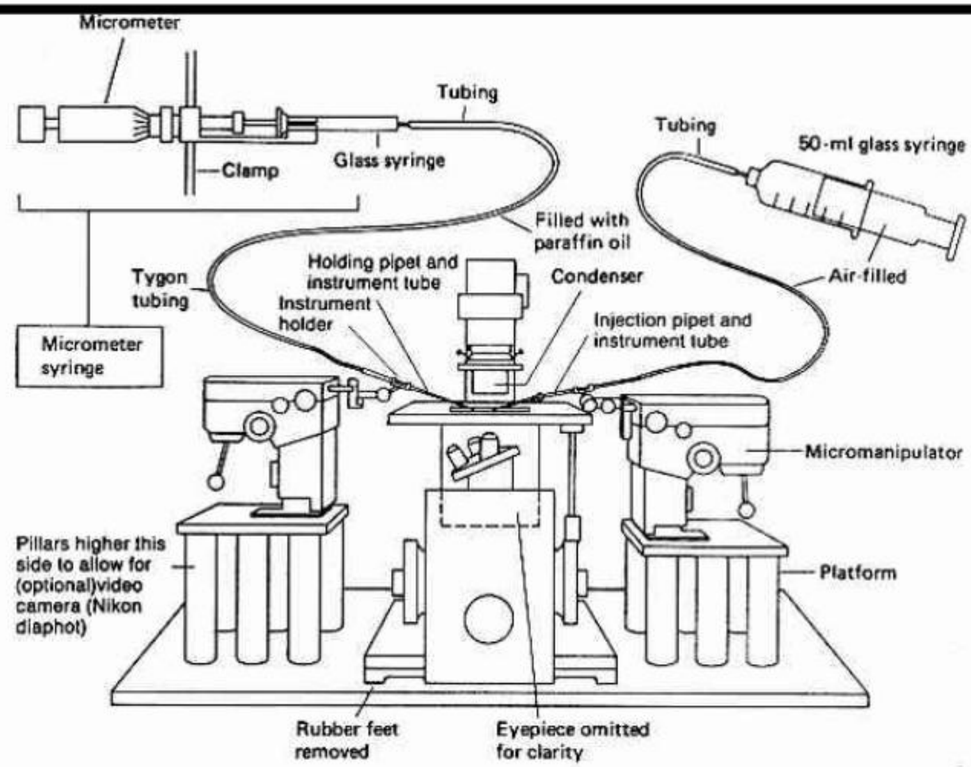


Figure 59 Arrangement of microscope, baseplate, and micromainipulators for the pronuclear injection method described in this manual. Details of the baseplate are given in Appendix 1, and sources of the apparatus are listed in this section.

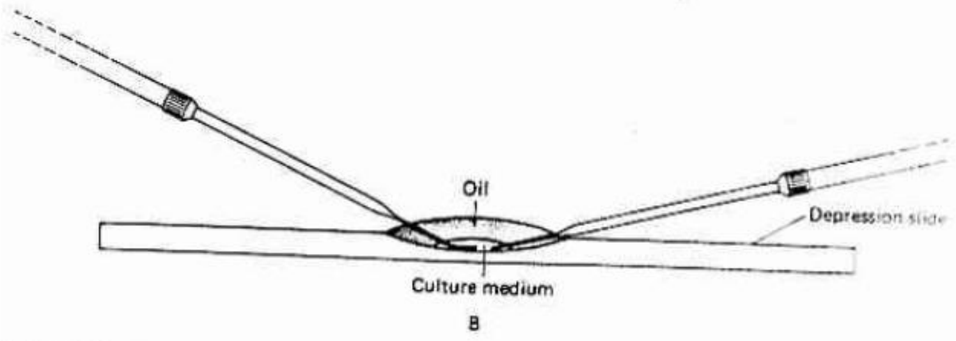
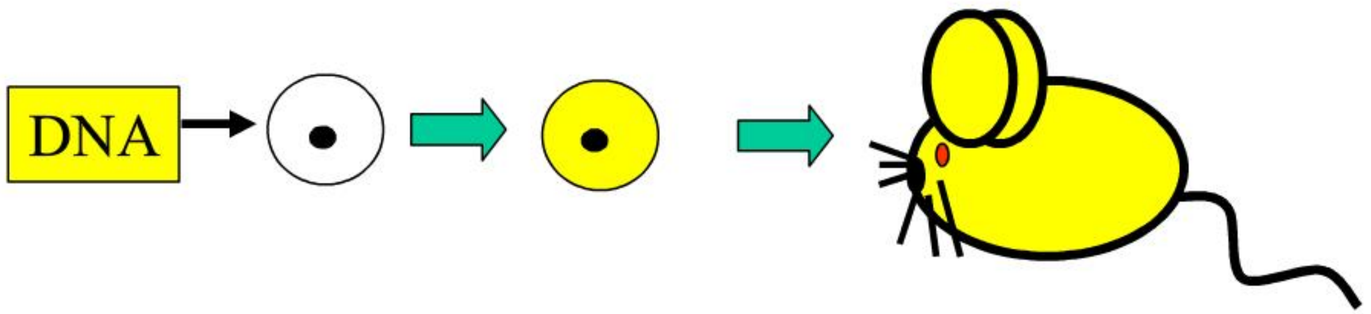
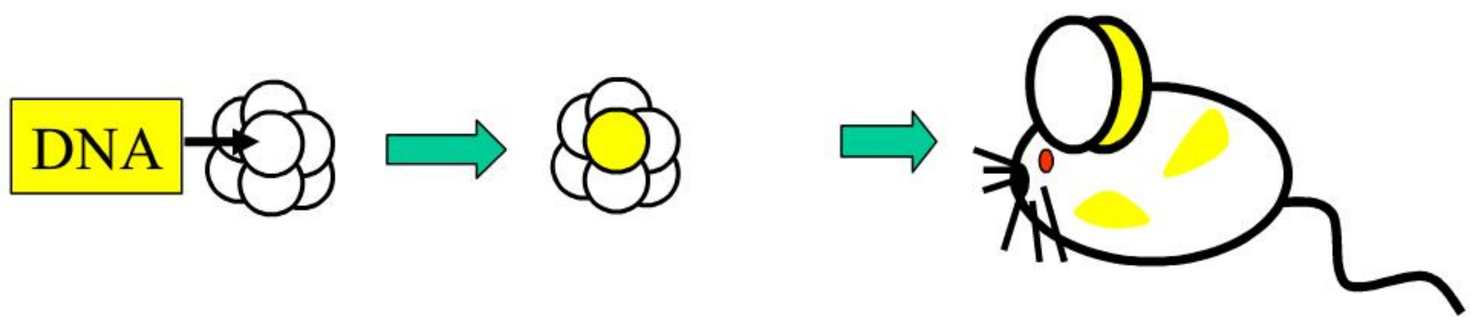


Figure 61 Depression slide injection chamber.

完全整合小鼠



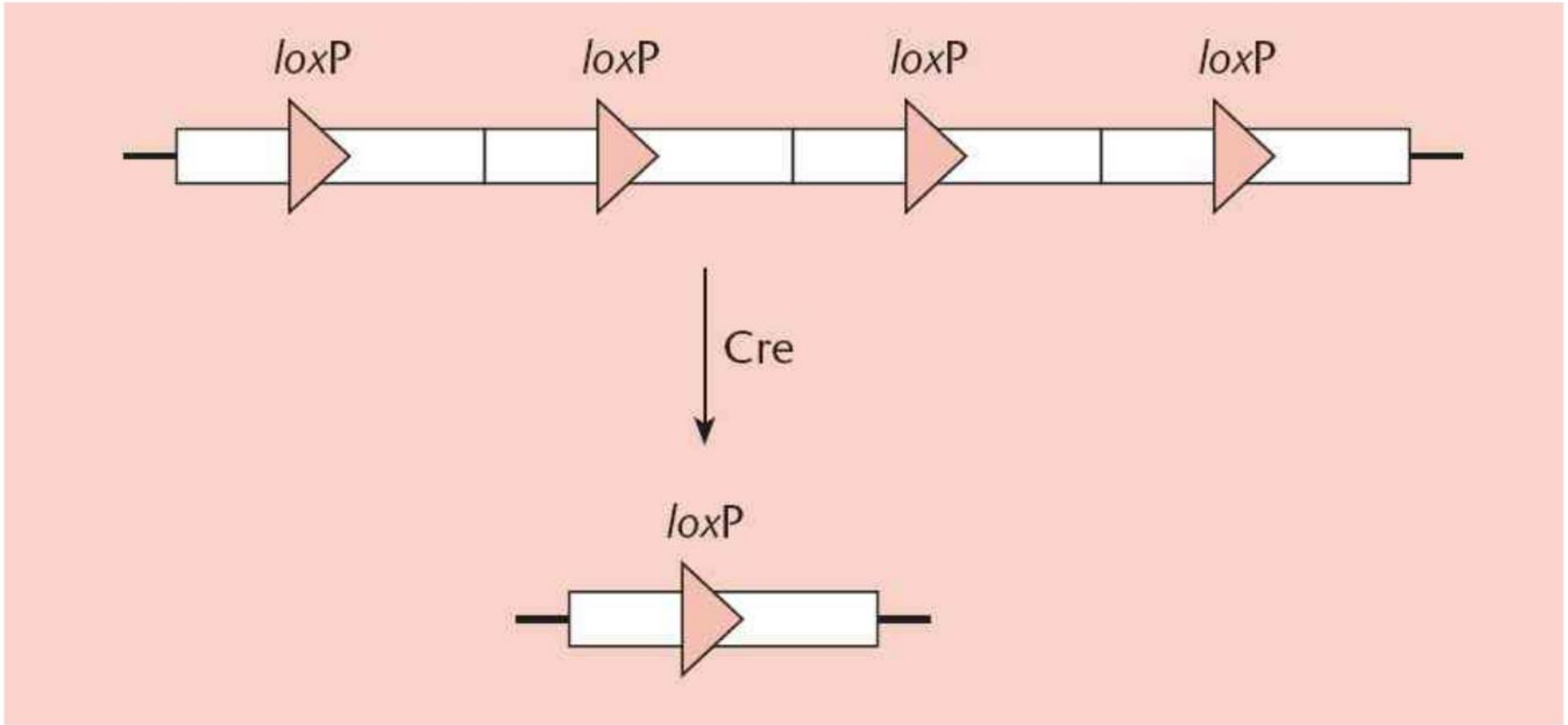
嵌合体小鼠



用受精卵单细胞原核注射导入基因的特点

- 随机插入
  - 单位点和多位点插入并存
  - 多拷贝串连
- 插入位点及整合方式对转入基因表达的影响
  - 插入位点影响
  - 插入序列引发的de novo甲基化
  - 同源性依赖的基因沉默
  - 使用大片段的DNA，如BAC等
  - 使用隔离子（Insulator如小鼠H19基因）来减少插入位点对转基因表达的影响
- 方法技术要求高，设备投入大

同源性依赖的基因沉默



转基因小鼠的繁育 I

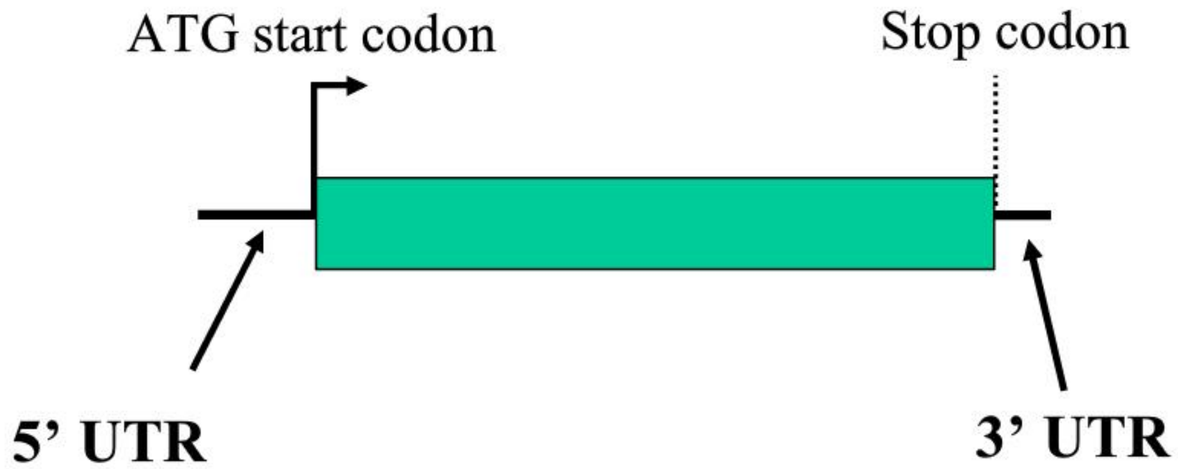
- 决定所需要的小鼠品系背景（ C57B6 ）
- 首建者分别和选定的野生型品系进行配种繁育
- 首建者小鼠之间不能进行相互交配

转基因小鼠繁育II

- 做好出生小鼠的标牌（出生日期，亲本信息）
- 小鼠编号（1-2周）
- 同时采集小鼠尾部组织或者耳轮组织
- 提取DNA，进行基因型鉴定（gene typing）
- 对阳性小鼠进行断奶、雌雄分笼

转基因载体构建 I

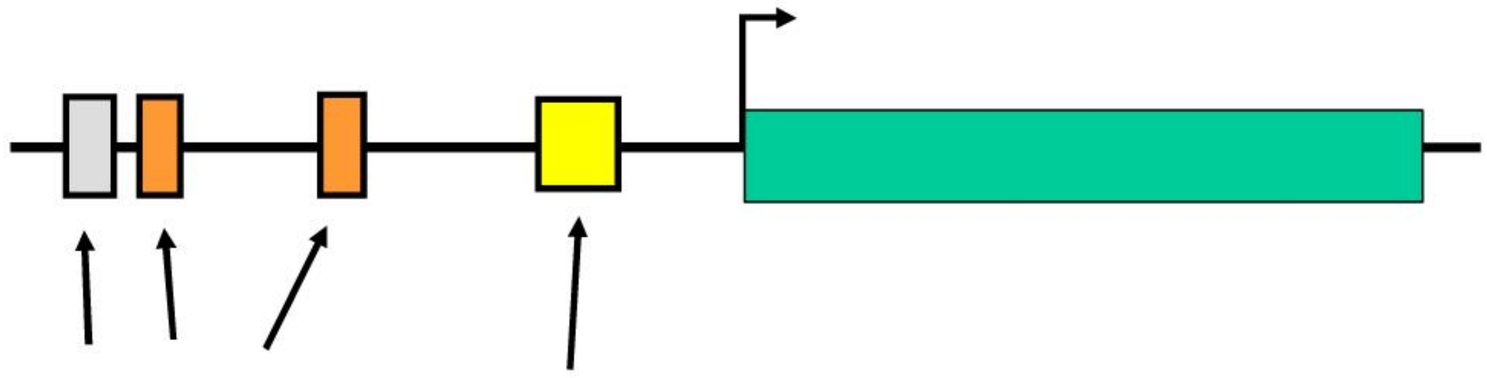
获得所需要表达蛋白的**cDNA**



转基因载体构建 II

获得所需要表达蛋白的cDNA

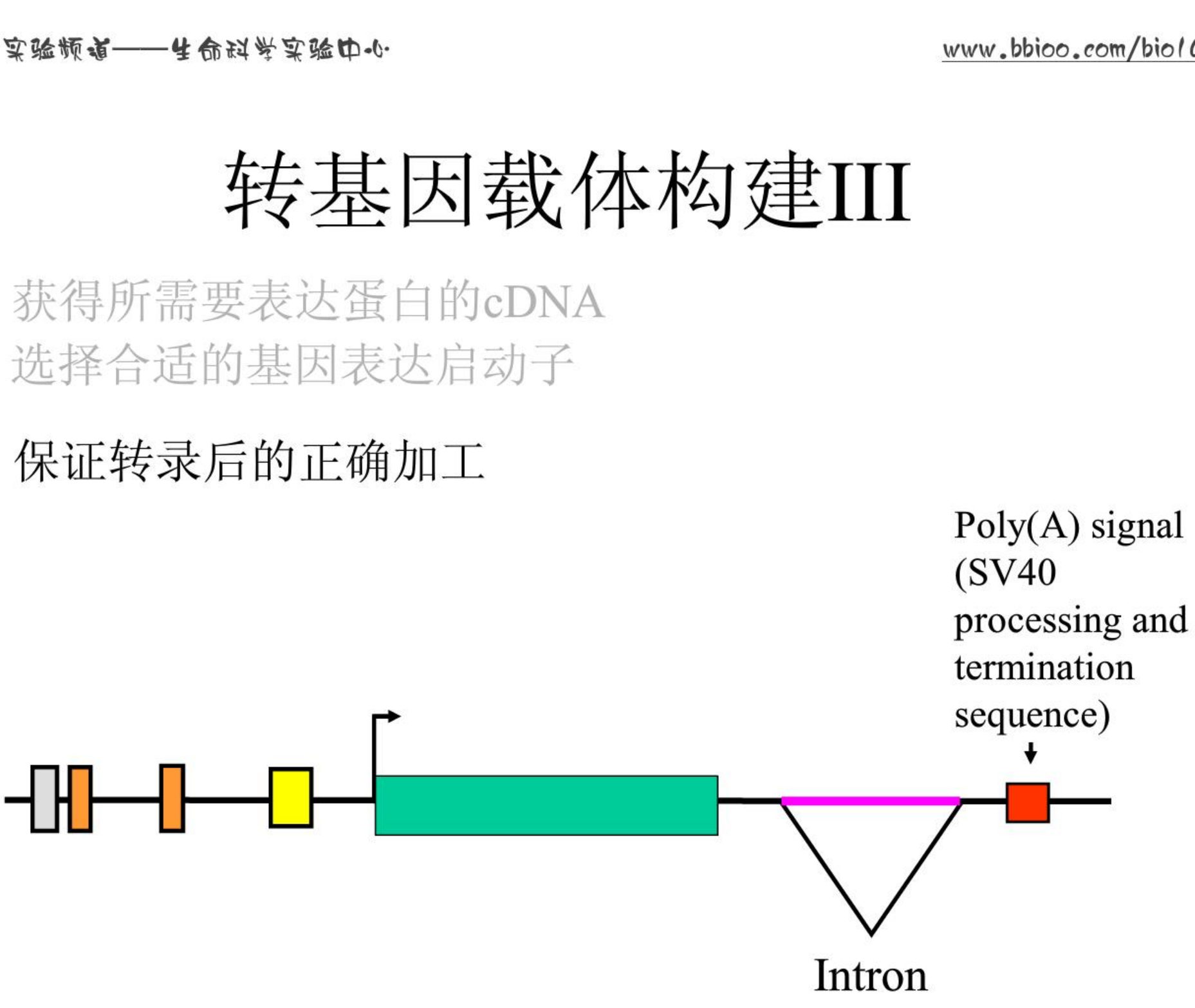
选择合适的基因表达启动子



转基因载体构建III

获得所需要表达蛋白的cDNA
选择合适的基因表达启动子

保证转录后的正确加工



Lentiviral 介导的转基因技术

- 可以用于小鼠ES细胞的转基因和胚胎（包括受精卵）的转基因

  优点：

  比原核注射效率高

  技术要求低

  局限：

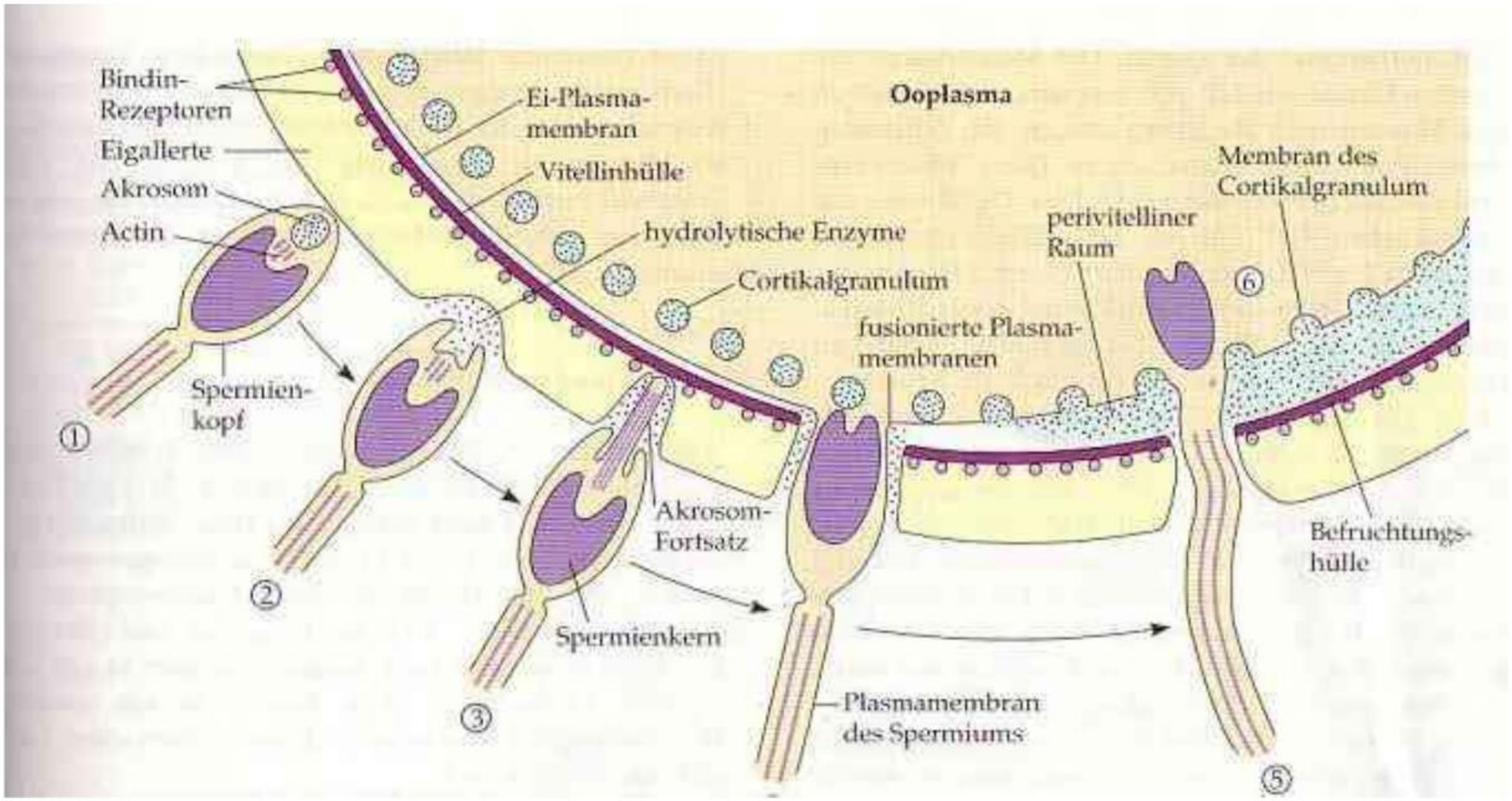
  最大插入容量 10kb

  需要制备病毒

  安全性

Injection into perivitelline space

- Injection of virus into the perivitelline space of single-cell mouse embryos



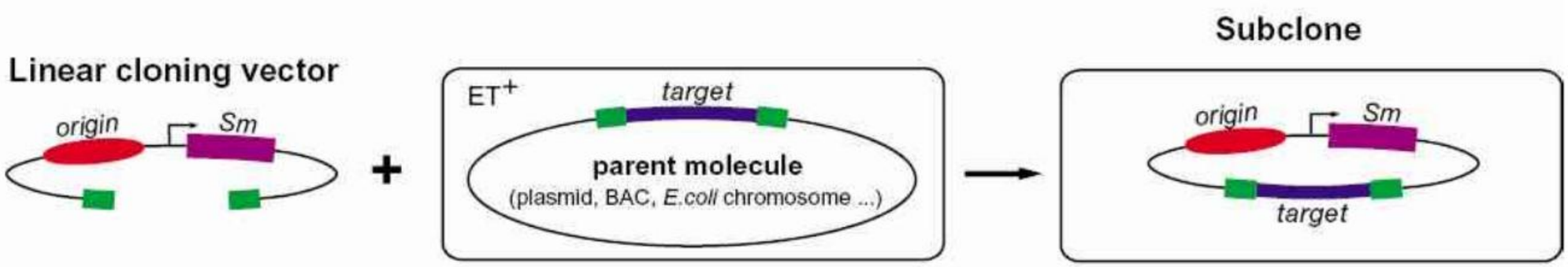
Results of Perivitelline Injection

- 82% of founder animals carried at least one copy of the integrated transgene
- 76% showed GFP fluorescence paws, tails, and face
- All GFP-positive animals carried an integrated provirus
- all animals with two or more copies of the provirus expressed the transgene at levels detectable by direct viewing of GFP fluorescence.

大片段DNA的转基因小鼠构建

- 需求
  - 基因或基因簇（组）功能研究
  - 调控序列的研究
- 技术特点
  - BAC和YAC （100 和 200 kb或者更大）
  - 载体构建方法采用同源重组；ET克隆
  - 整合位点对基因表达的影响减少
  - 基因表达的强度和整合的拷贝数正相关
  - 大片段DNA的粘度和易受剪切破坏给完整片段的整合带来困难

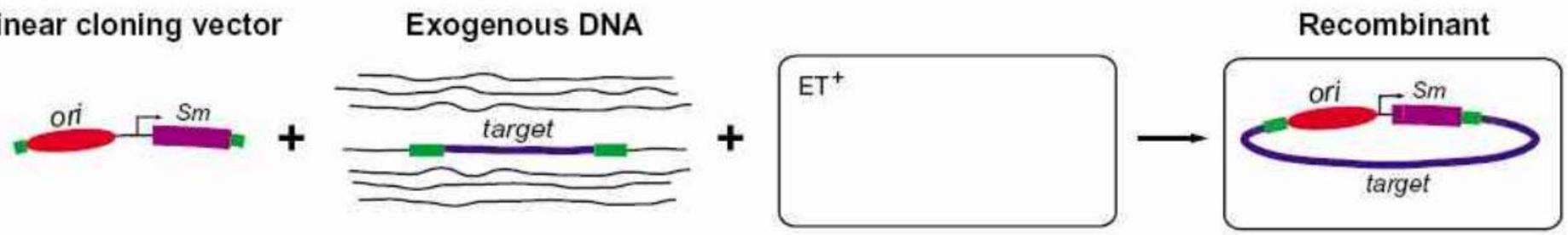
A



B

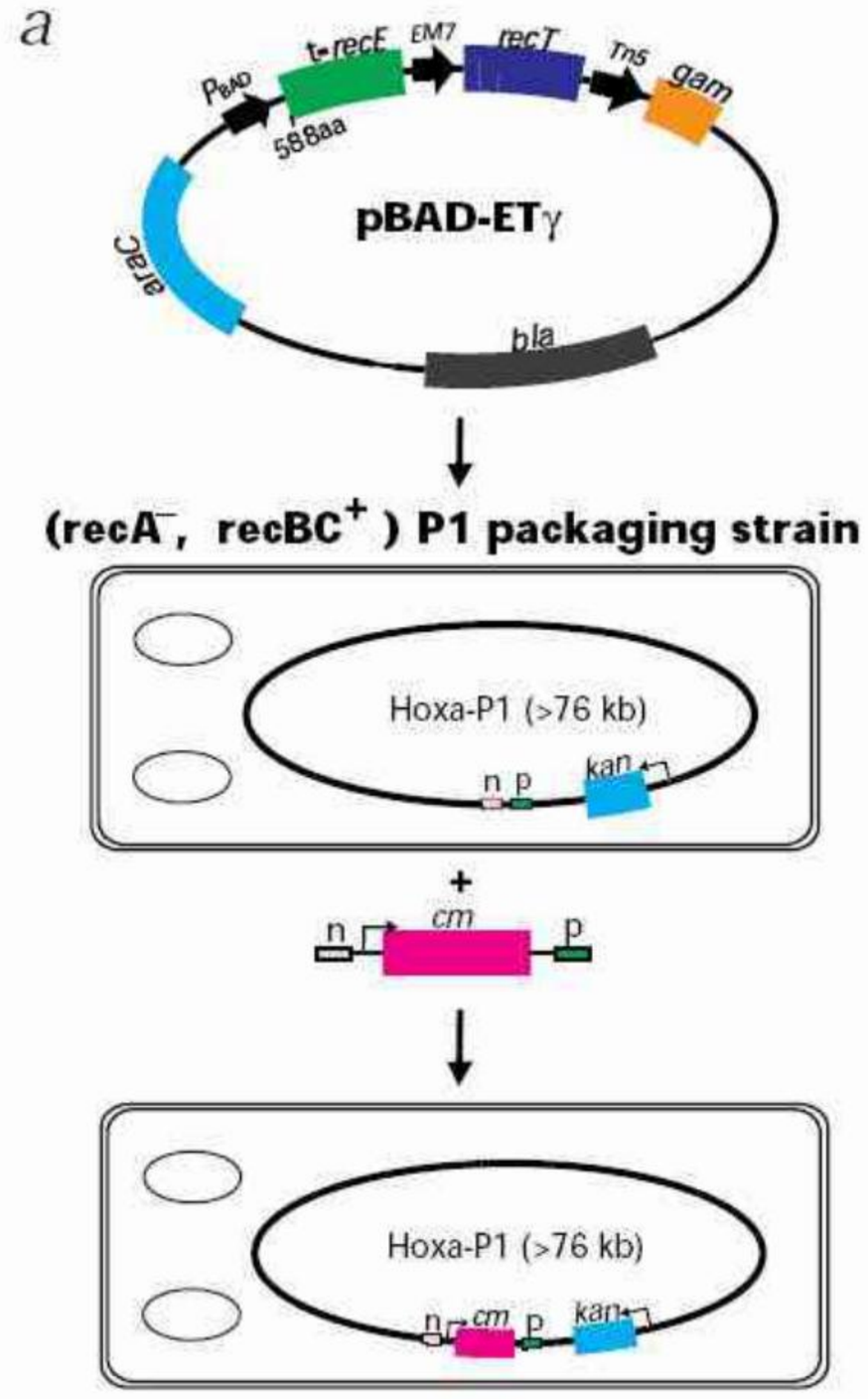
Example	Linear cloning vector	ET expression	*E. coli* host	Target	Parent molecule	Total colonies	Percentage correct (out of 18)
1	p15A+*Cm*	Endogenous RecE/Rec*T*	JC9604	2.3 kb, endogenous *lacZ* gene	*E. coli* chromosome	290	100
2	p15A+*Cm*	pBAD-αβγ	HB101	2.3 kb, endogenous *lacZ* gene	*E. coli* chromosome	370	94
3	p15A+*Km*	pBAD-recET	JC5519	0.8 kb, gentamicin gene	pFastBac1 (high-copy plasmid)	>3,000	100
4	p15A+*Km*	pBAD-αβγ	HS996	28 kb, intron3 of *mAf*4	BAC	160	83
5	p15A+*Amp*	pR6K116/BAD /αβγ	HS996	19kb, exons 2-3 of *mAf*4	BAC	>2,000	83

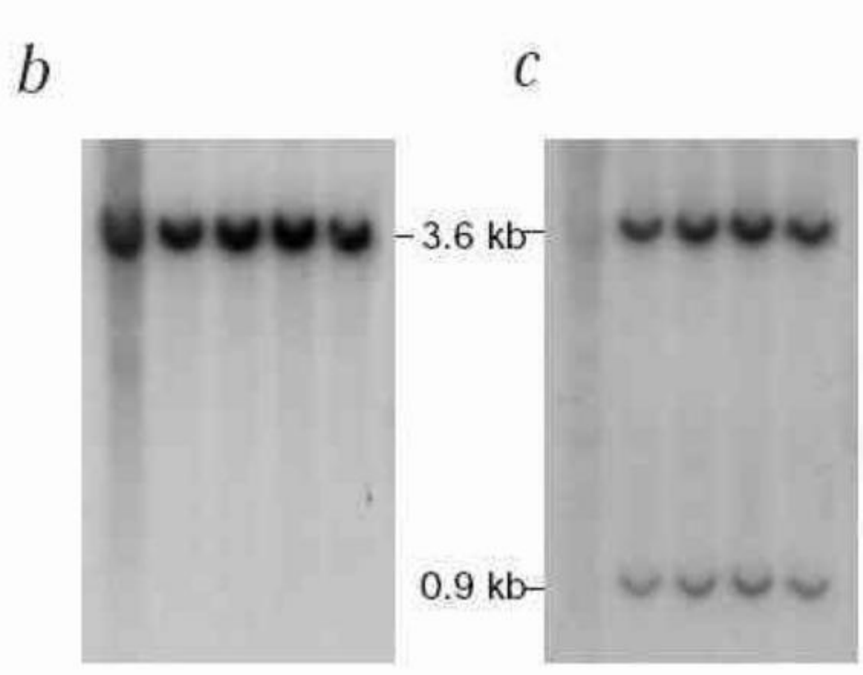
A



B

Example	Linear cloning vector	Target	Exogenous DNA source	ET expression	Total colonies	Percentage correct (out of 18)
1	*ColE1+Km*	4.5 kb, *lacI+lacZ* gene	*E. coli* chromosome DNA	YZ2000	174	100
2	*p15A+Km*	1.1 kb, *bla* gene in chromosome	Yeast genomic DNA	YZ2000 +pBAD/recT	58	33
3	*ColE1+Cm*	1.0 kb, *neo* gene in chromosome	Mouse ES cell genomic DNA	YZ2000+ pR6K116/ BAD/recT	32	17


a
PBAD
t-recE
588aa
EM7
recT
Tns
gam
araC
pBAD-ETγ
bla
(recA⁻, recBC⁺) P1 packaging strain
Hoxa-P1 (>76 kb)
n
p
kan
+
cm
n
p
Hoxa-P1 (>76 kb)
n
cm
p
kan


b
c
3.6 kb
0.9 kb

可诱导的基因表达方法

- 利用内源性可诱导的启动子:
  - 热休克蛋白，hsp70 基因
  - 金属硫蛋白基因
- 重组型的可诱导启动子
  - LacI；tet-on or tet-off；蜕皮激素；化合物诱导的二聚化（CID）
- 蛋白水平的诱导
  - ER受体融合蛋白/Tamoxifen
- 重组酶系统

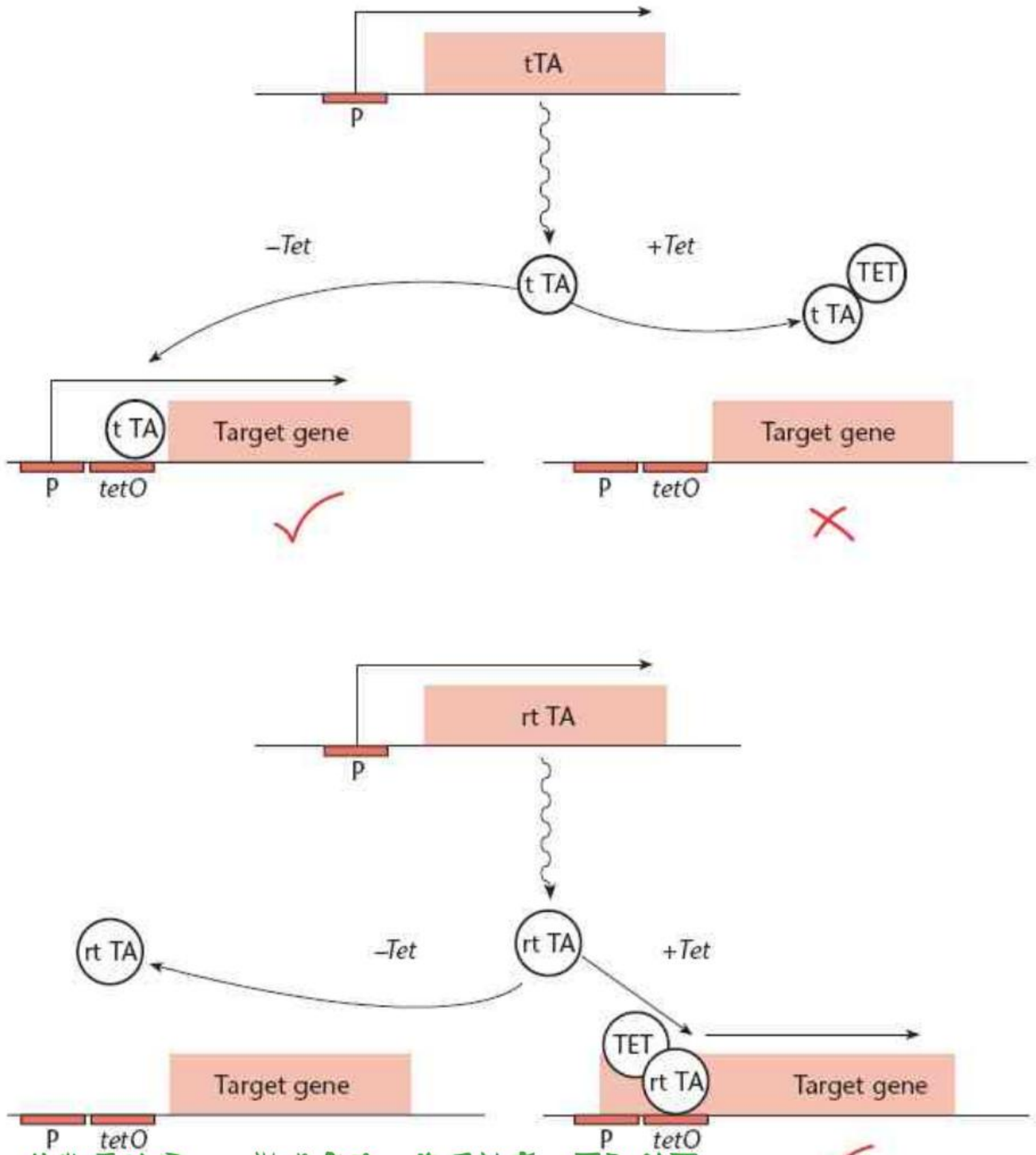
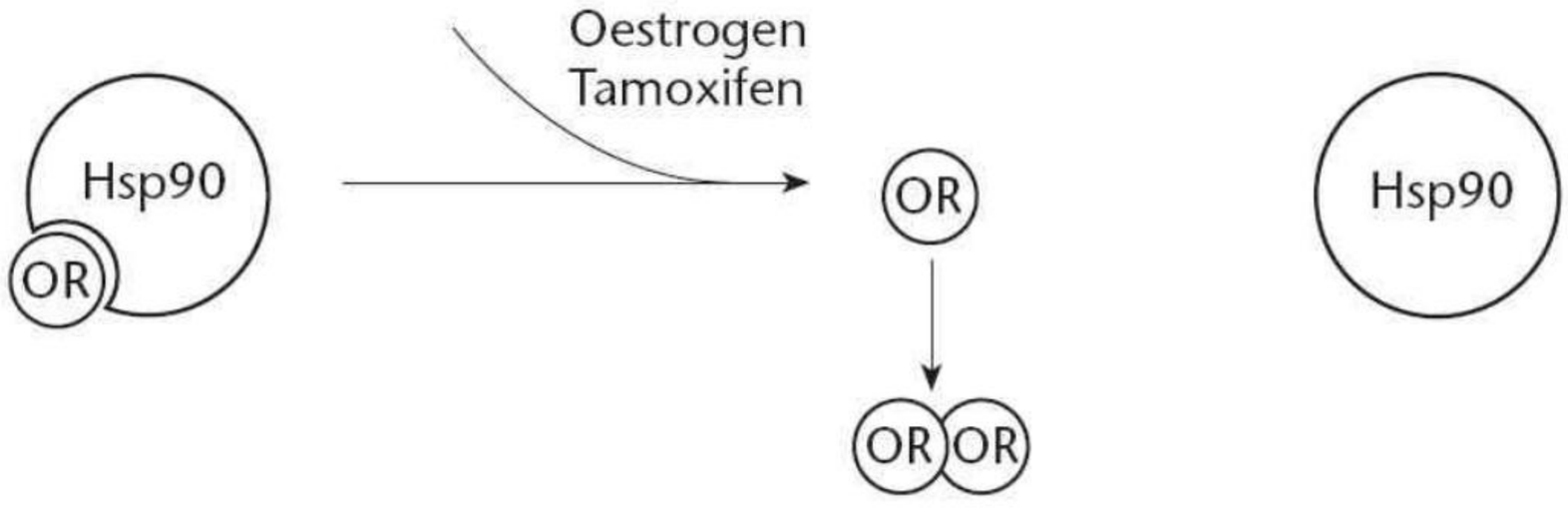
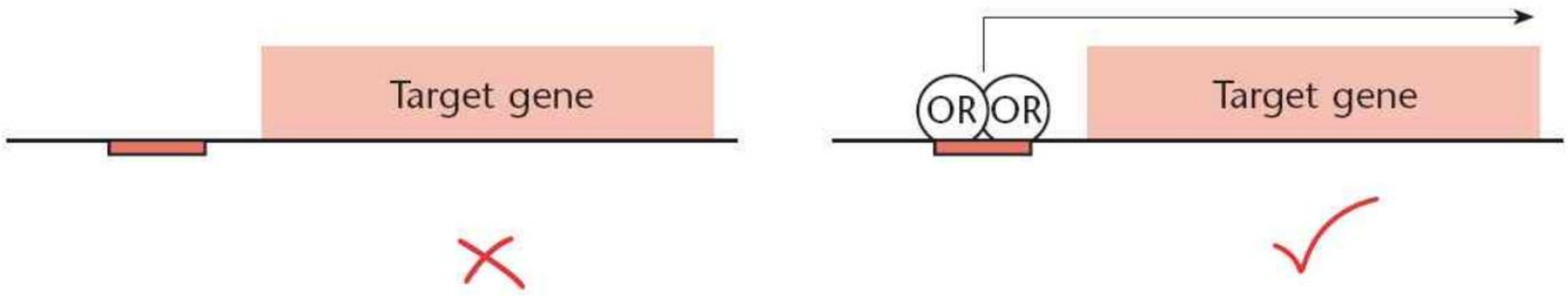


Fig. 13.2 The *tet* transactivator system (tTA).

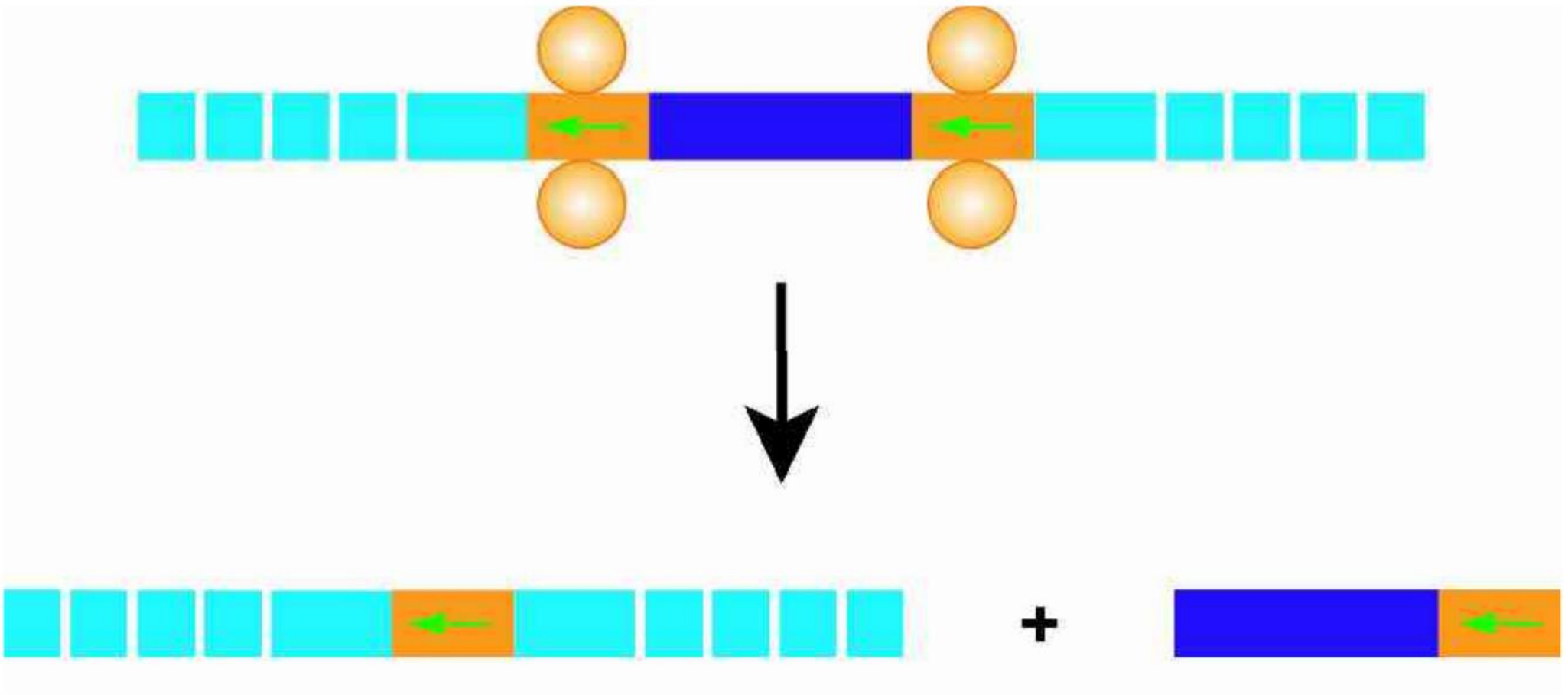

Oestrogen
Tamoxifen
Hsp90
OR
OR
OR
Hsp90


Target gene
OR
OR
Target gene

Principles of Cre-Mediated Recombination

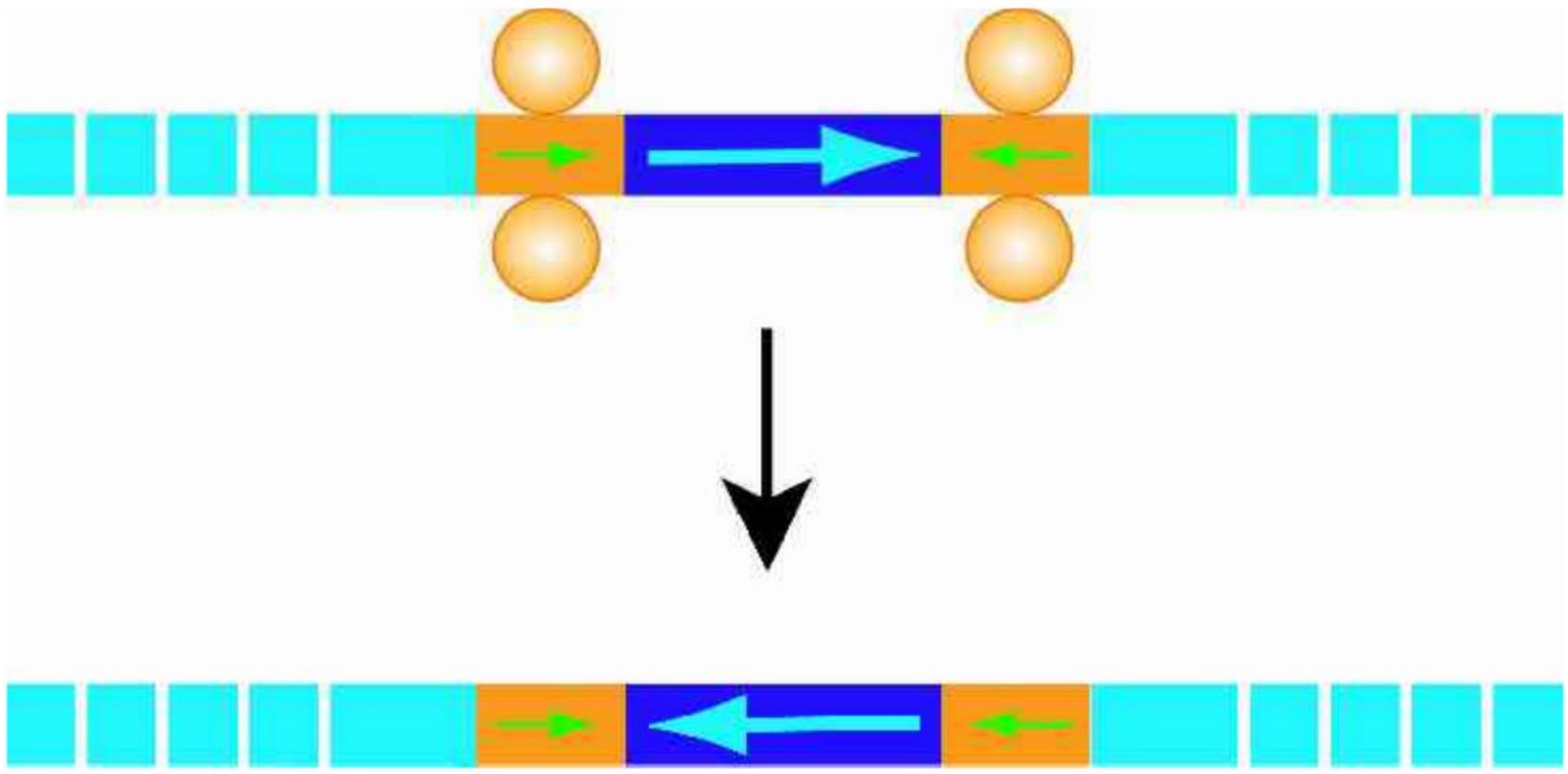
- Cre is the 38 kDa product of the *cre* gene of bacteriophage P1
- It belongs to the Int family of DNA recombinases, but unlike other family members, Cre requires no accessory factors
- Cre recognizes a 34 bp site on the bacteriophase genome called *LoxP*, catalyzing reciprocal conservative DNA recombination between *LoxP* pairs
- *LoxP* consists of two 13 bp inverted repeats separated by an 8 bp asymmetric core region
- This core region is responsible for the directionality of the *LoxP* site
- Two molecules of Cre bind to each *LoxP* site, so that [theoretically] 4 molecules of Cre are required for each recombination event

Cre-Mediated Recombination - tandem repeats



Result: Excision of intervening sequence (with retention of 1 LoxP site)

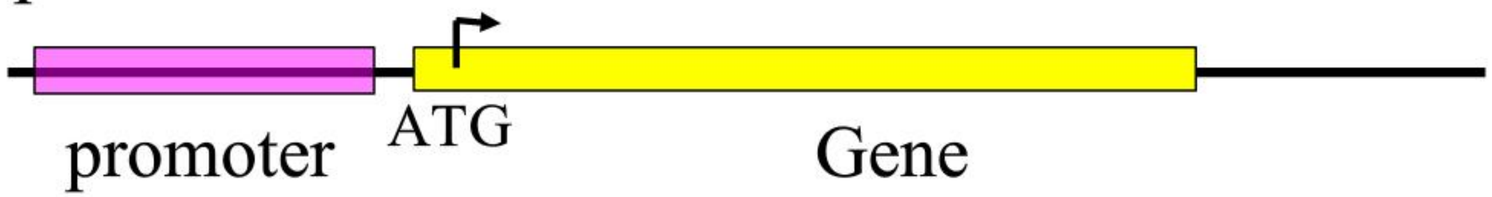
Cre-Mediated Recombination - inverted repeats

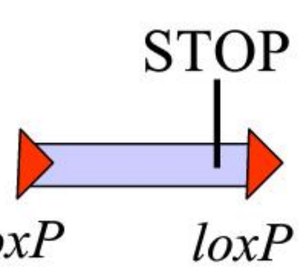


Result: Inversion of intervening sequence

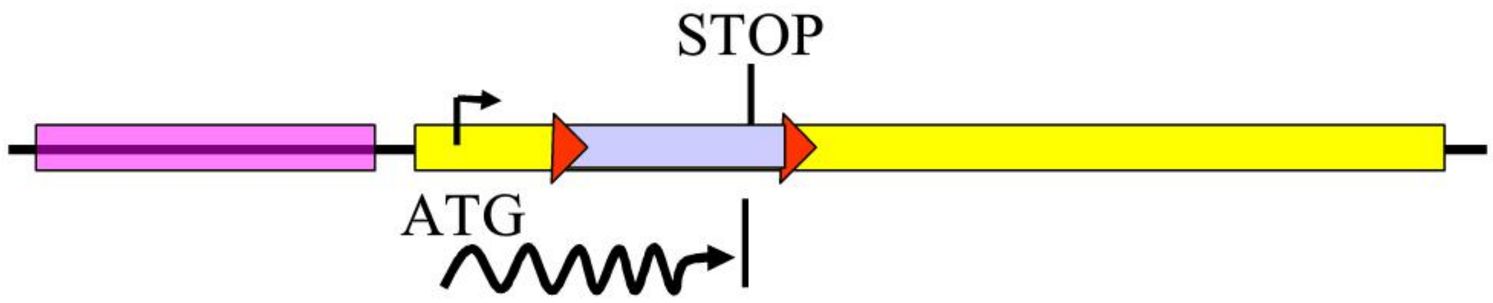
Expression of Cre can be used to turn on a transgene in temporally regulated manner.

Transgene: want to express the gene of interest (in yellow) off the promoter of interest (in purple), but not until late in development.

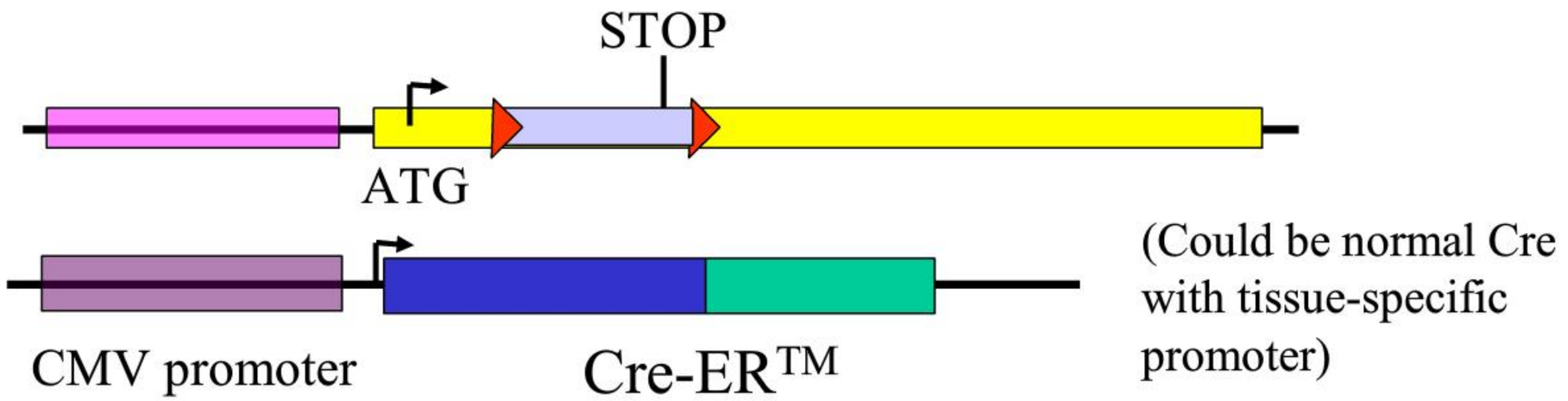




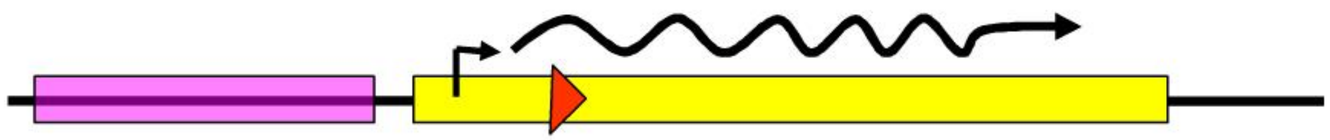
Clone a short bit of floxed sequence near the front of the construct such that when the transgene turns on it produces only a short bit of nonsense.



Breed the mice carrying your transgene with another transgenic line expressing endogenous Cre-ER^{TM}.



Upon injection of tamoxifen at required stage of development, Cre is free to recombine *loxP* sites and remove translation STOP codon.



This allows transgene to produce full length mRNA.
NB. Have to check all still in frame AND hope retained loxP site does not affect function of transgene

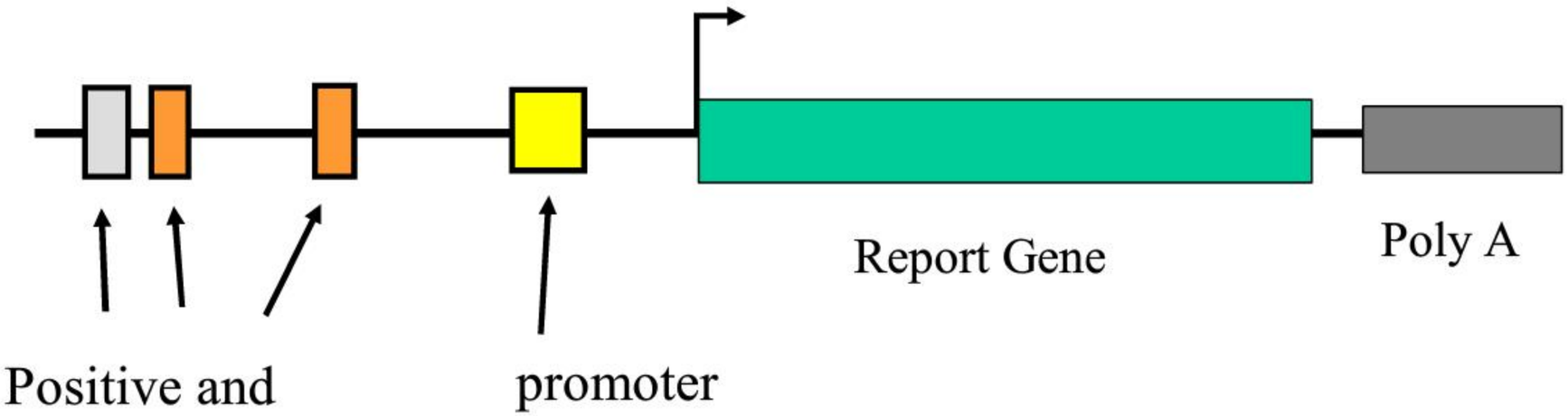
基因调控序列的研究及报告基因

- 启动子；增强子；负调控元件 等等
- 常用的报告基因
  - E.coli β–半乳糖苷酶 (lac Z)
    - 底物:ONPG;MUG;X-gal
  - E.coli 葡糖苷酸酶 (gusA)
    - 底物:X-gluc
  - 荧光素酶(luc)
    - 底物:luciferin
  - 绿色荧光蛋白(GFP)等

报告基因载体构建

选择合适的报告基因

连接在所需要研究的启动子序列下游



神经前体细胞特性性启动子的研究
(neurogenin 1)

抑制基因表达的方法

- 反义核酸
- 核酶
- RNAi
- miRNA
- 显性负作用（Dominant Negative）
- 共抑制现象(cosuppression)
- 单链抗体

Conditional Expression of an antisense mRNA of the mineralocorticoid receptor (MR) in cardiomyocytes

Cardiomyocyte specific expression of tTA → dox-dependent expression of MR antisense mRNA

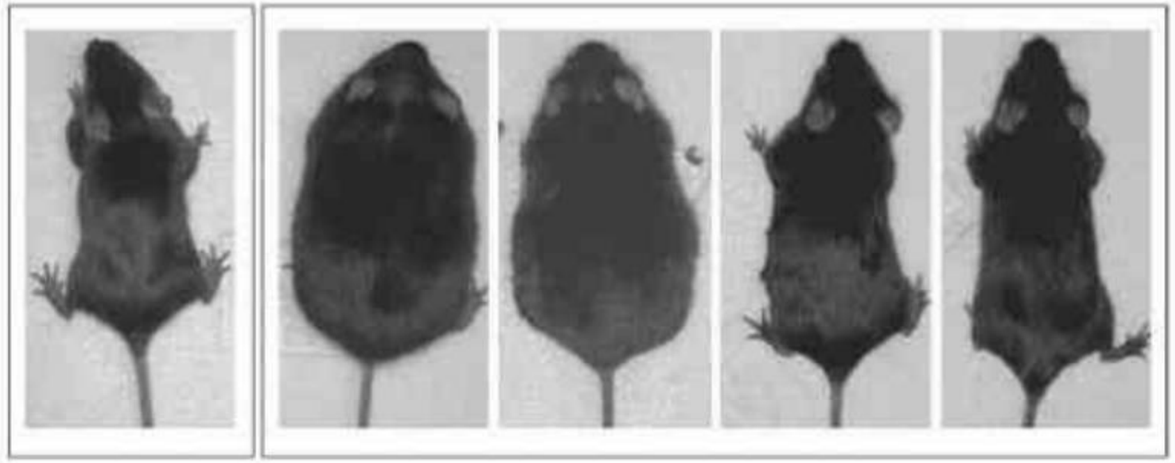
Without dox treatment

Control: normal

Transgenic: Severe heart disease, rapid weight gain, edema

control | **Doxycycline treatment**

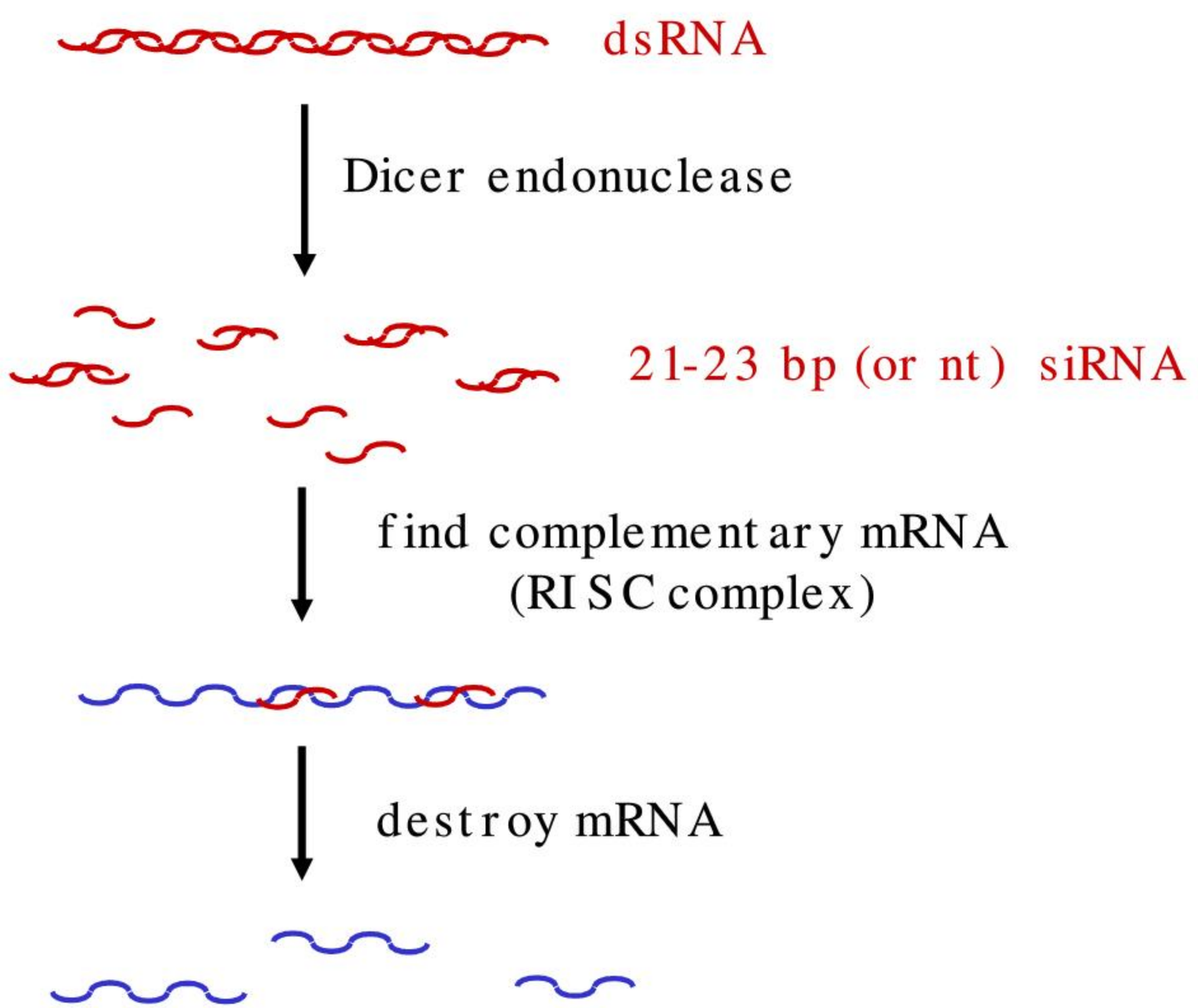
Day 0 | **Day 0** | **Day 4** | **Day 8** | **Day 10**



Reversibility of edema

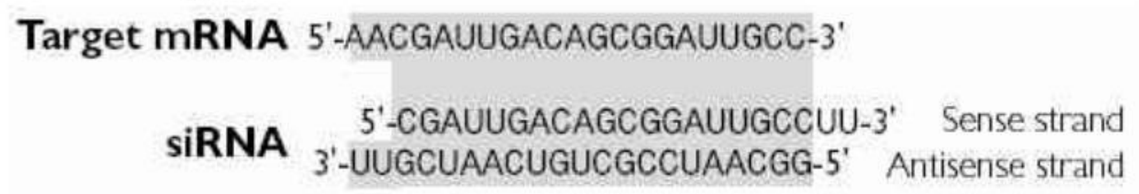
Beggah *et. al.* (2002) *PNAS* **99:**7160

RNA Interference



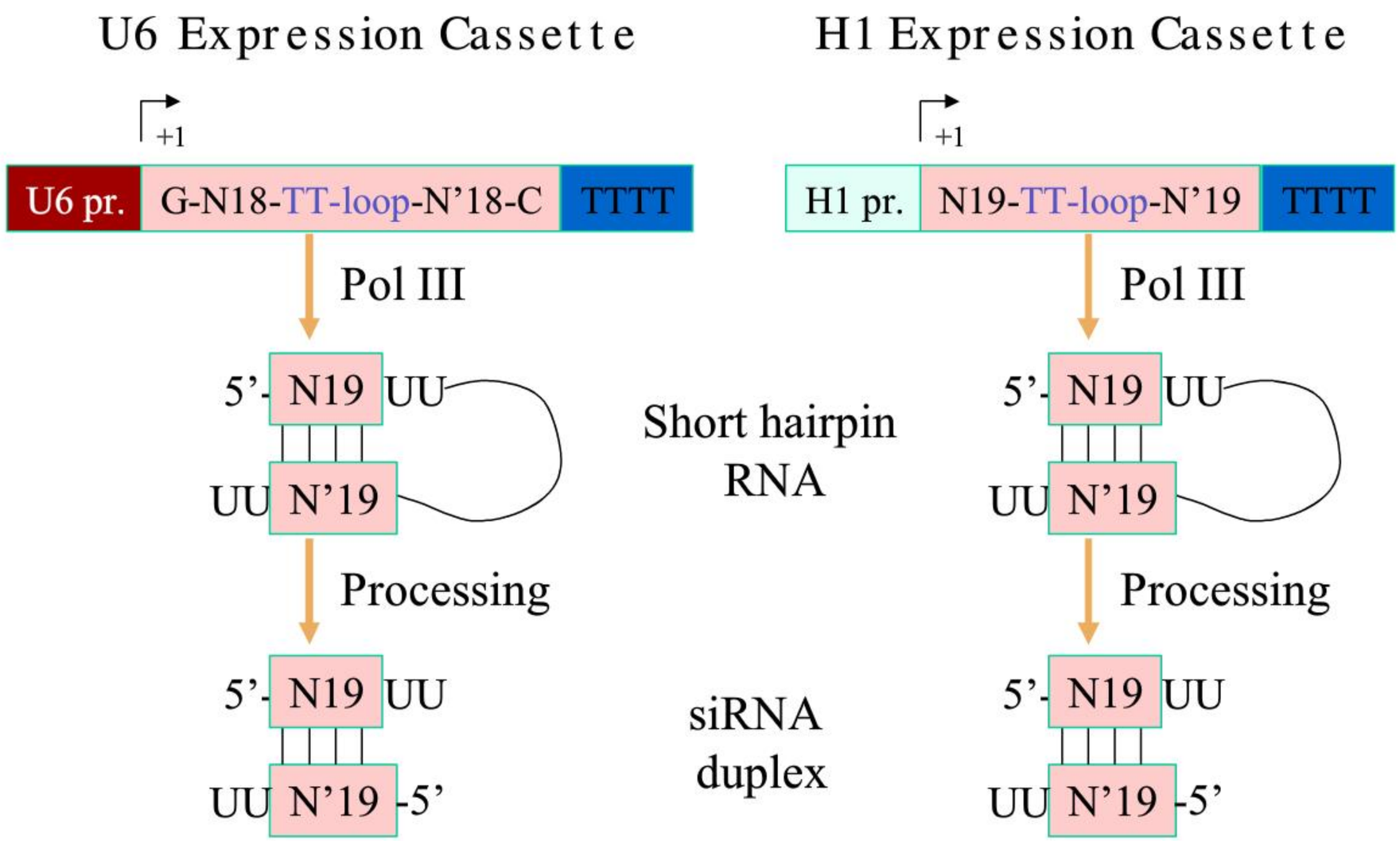
siRNA design

- 21-23nt
- 2-nt 3' overhangs (UU overhangs)
- G/C content: 30-50%.
- No basepair mismatch

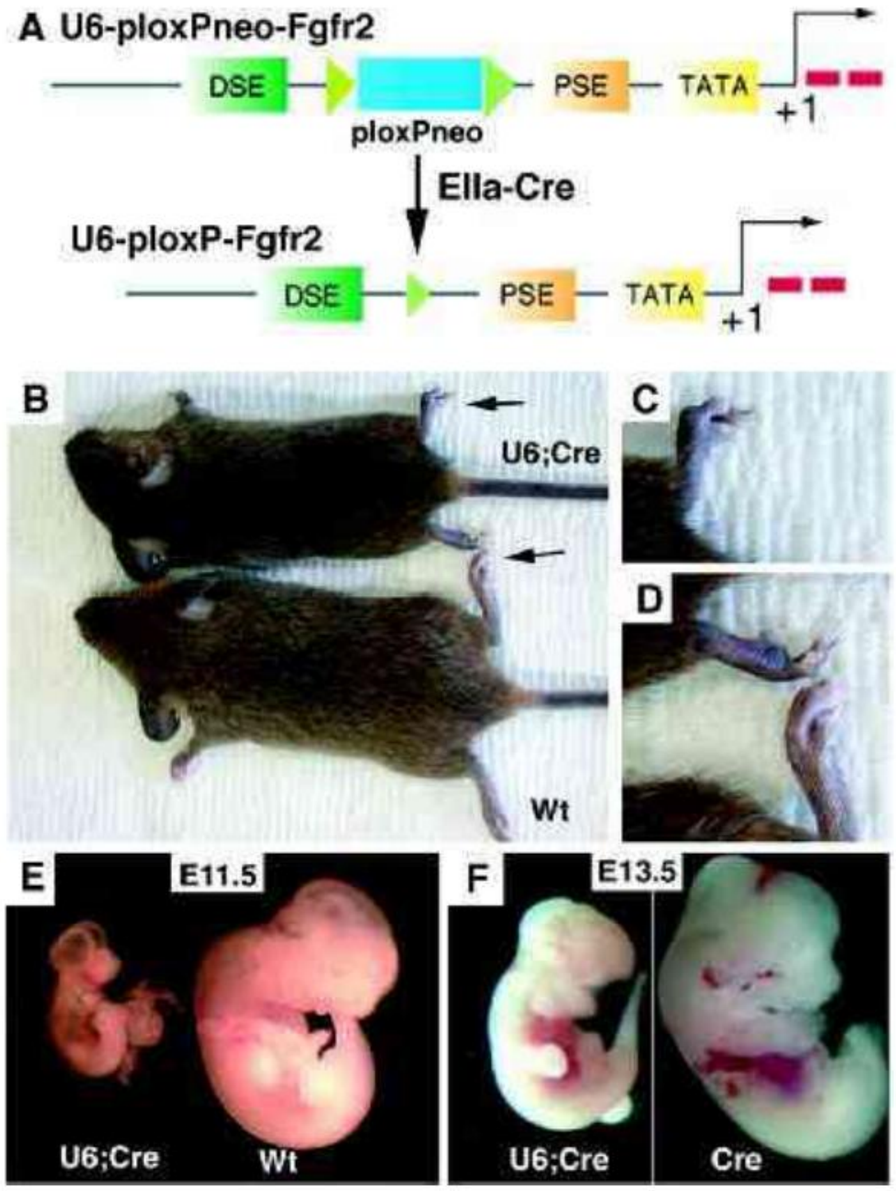


- BLAST : eliminate any target sequences with significant homology to other coding sequences.
- design and test 3–4 siRNA sequences
- http://www.ambion.com/techlib/misc/siRNA_finder.html

Expression of siRNAs from pol III promoters



Expression of U6-Fgfr2 results in embryonic lethality



Coumoul, X. et al. Nucl. Acids Res. 2005 33:e102; doi:10.1093/nar/gni100

Nucleic Acids Research

小鼠基因剔除技术（knockout）

- 小鼠胚胎干细胞
  - 体外传代
  - 发育全能性（特别是能发育为生殖细胞）
- DNA同源重组技术
- 大规模小鼠基因突变技术
  - 基因捕获技术（随机方法）
  - 转座子插入突变技术
  - 化学诱变技术（ENU）

小鼠基因剔除技术的里程碑工作

- 1981Evans and Kaufman: （Nature. 1981 Jul 9;292(5819):154-6, Proc Natl Acad Sci U S A. 1981 Dec;78(12):7634-8）建立了ES细胞
- 1986－1987 Robertson et al，Hooper et al, Kuehn et al,：对ES细胞进行遗传操作，并获得ES细胞来源的小鼠个体（Nature. 1986 Oct 2-8;323(6087):445-8. Nature 1987 Mar 19-25;326(6110):292-5, Nature. 1987 Mar 19-25;326(6110):295-8.）
- 1988 Thomas et al., Doetschman T.et al.在ES细胞中建立基于同源重组的基因打靶技术(Cell. 1987 Nov 6;51(3):503-12.; Nature. 1987 Dec 10-16;330(6148):576-8.)
- 1989 Jaenisch et al: 建立*b*2-microglobulin-deficient基因剔除小鼠(Nature. 1989 Nov 23;342(6248):435-8.)
- 1994 Gu et al, 将Cre-LoxP系统用于ES细胞的打靶，建立条件性基因剔除技术（Science. 1994 . 265:103. ）

The Nobel Prize in Physiology or Medicine 2007

"for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells"

Photo: Tim Roberts/PR Newswire, © HHMI

Photo: The Press Association Limited

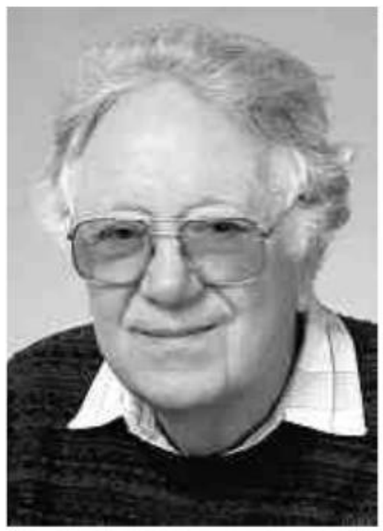


Photo: Scanpix/Dan Sears

Mario R. Capecchi

1/3 of the prize

USA

University of Utah; Howard Hughes
Medical Institute
Salt Lake City, UT, USA

b. 1937
(in Italy)

Sir Martin J. Evans

1/3 of the prize

United Kingdom

Cardiff University
Cardiff, United Kingdom

b. 1941

Oliver Smithies

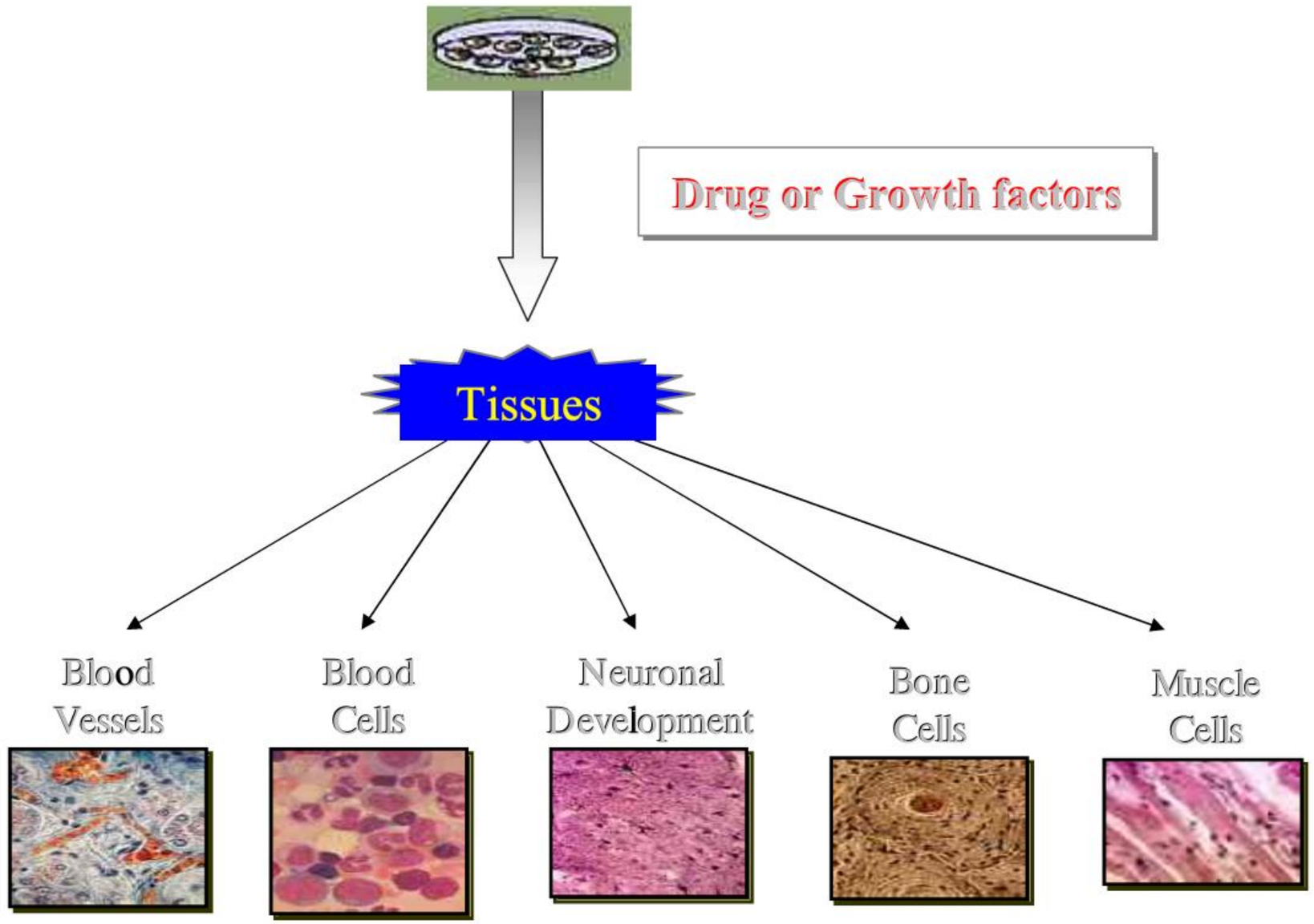
1/3 of the prize

USA

University of North Carolina at
Chapel Hill
Chapel Hill, NC, USA

b. 1925
(in United Kingdom)

ES Cells Differentiation



Generating ES Cells

Blastocyst 3.5 days

~30 cells

Inner Cell Mass (128 cell stage)

1-totipotent

2-tissue culture

3-Transfectable

4-Selection

5- Differentiation In vitro

Harvest blastocysts on day 3.5 by flushing from the uterine lumem with M2 medium

↓

Plate out blastocysts in individual 10 mm dishes on mitotically inactive MEF or STO feeder cells to provide LIF and other factors

↓

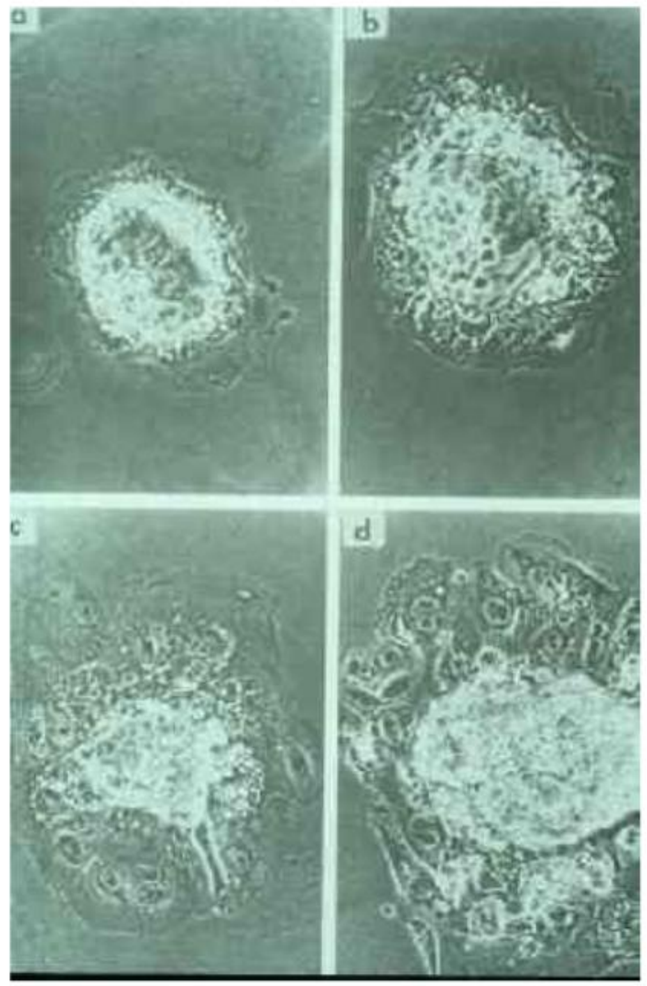
After 1-2 days, blastocysts hatch (A) and attach to the dish by migration of the trophectoderm (TE), while the inner cell mass (ICM) grows (B)

↓

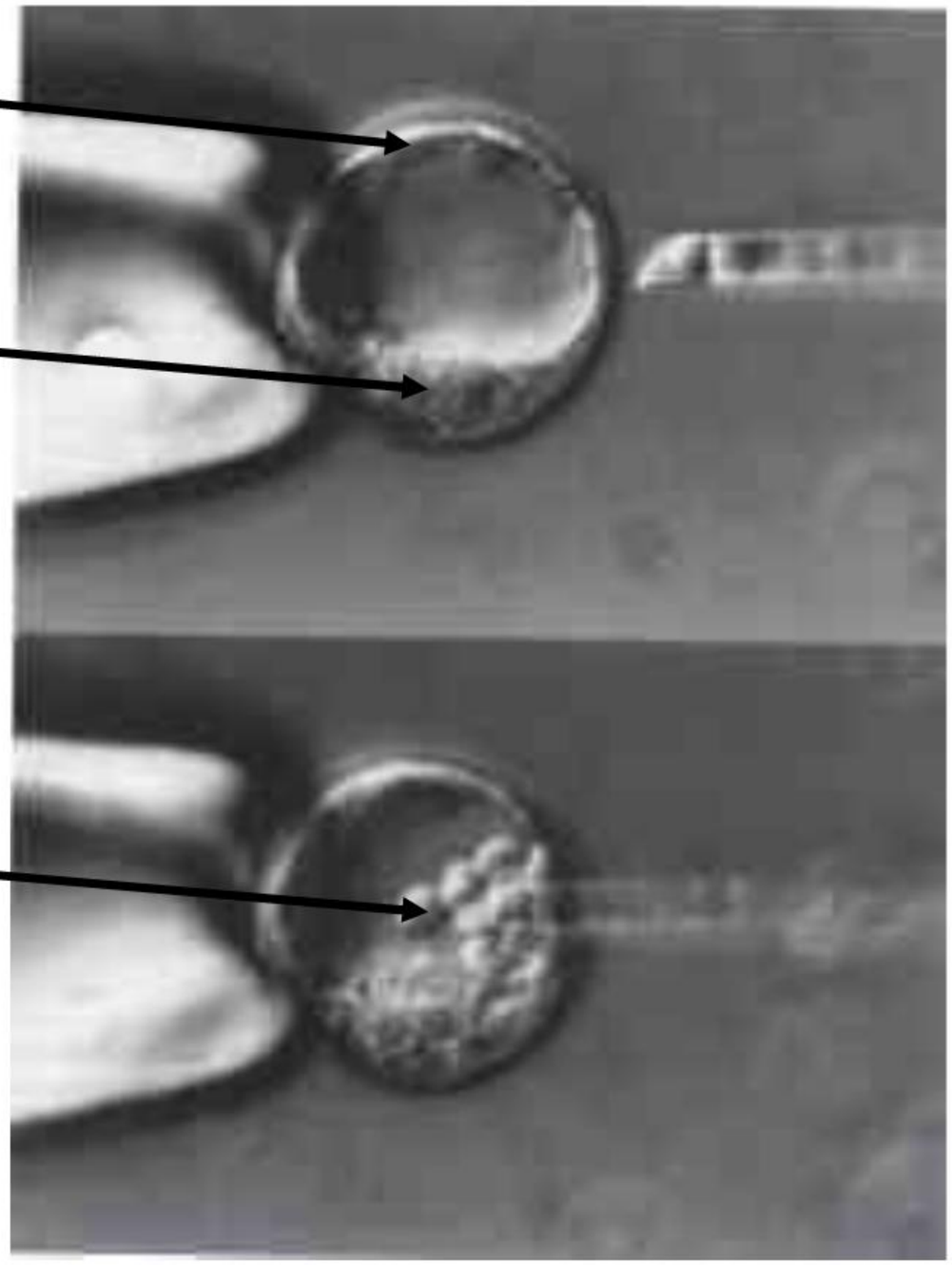
After about 96-120 hours in culture (C, D), the ICM can be dislodged from the TE layer, washed and transplanted to microdrops of medium containing trypsin to disperse the clumps

↓

Transfer disaggregated contents to a fresh feeder cell tissue culture well and inspect daily for signs of differentiation. Primary cell colonies are clearly visible as distinct clumps

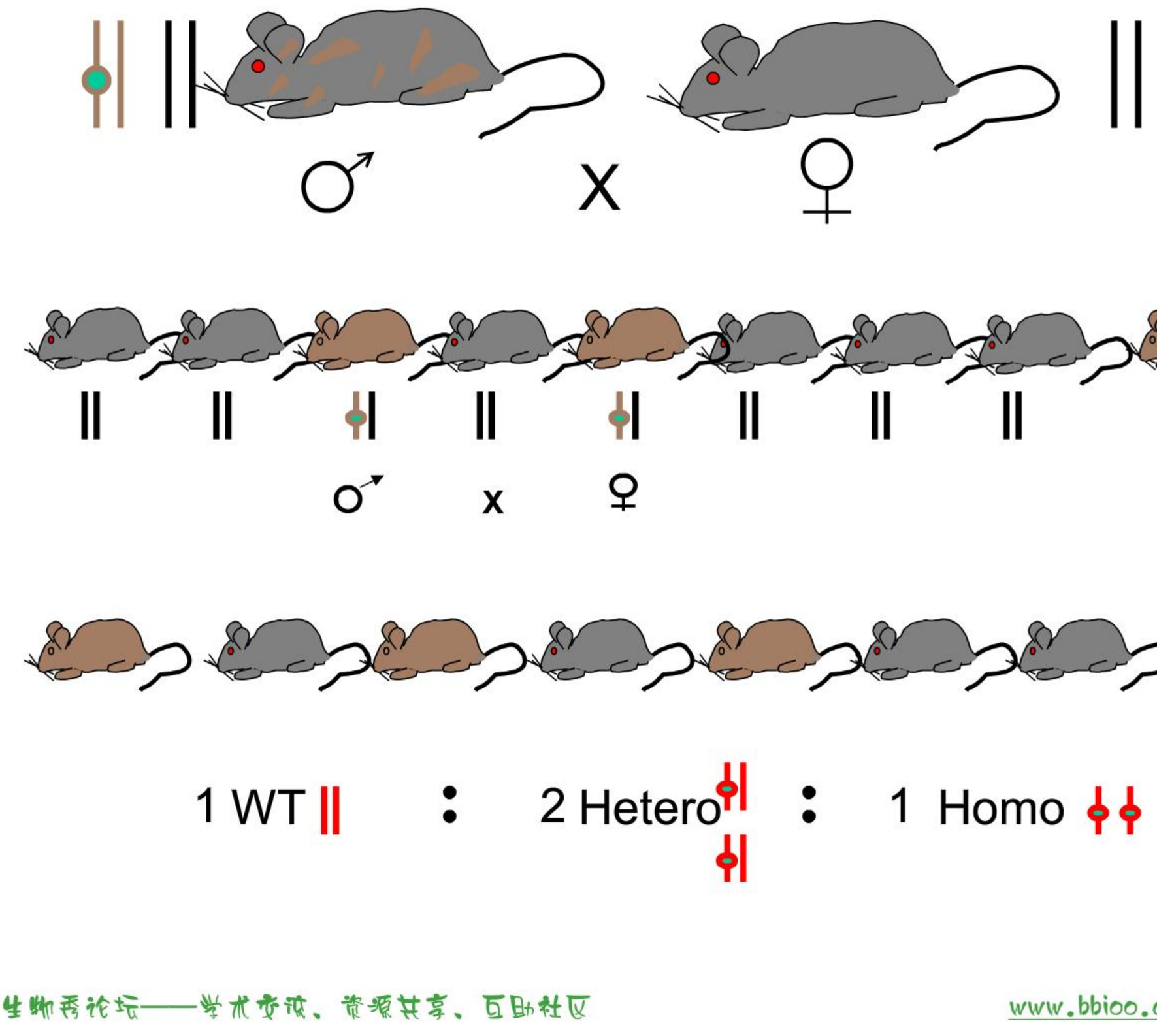


Normal
C57BL/6
Blastocyst
(black)
ICM

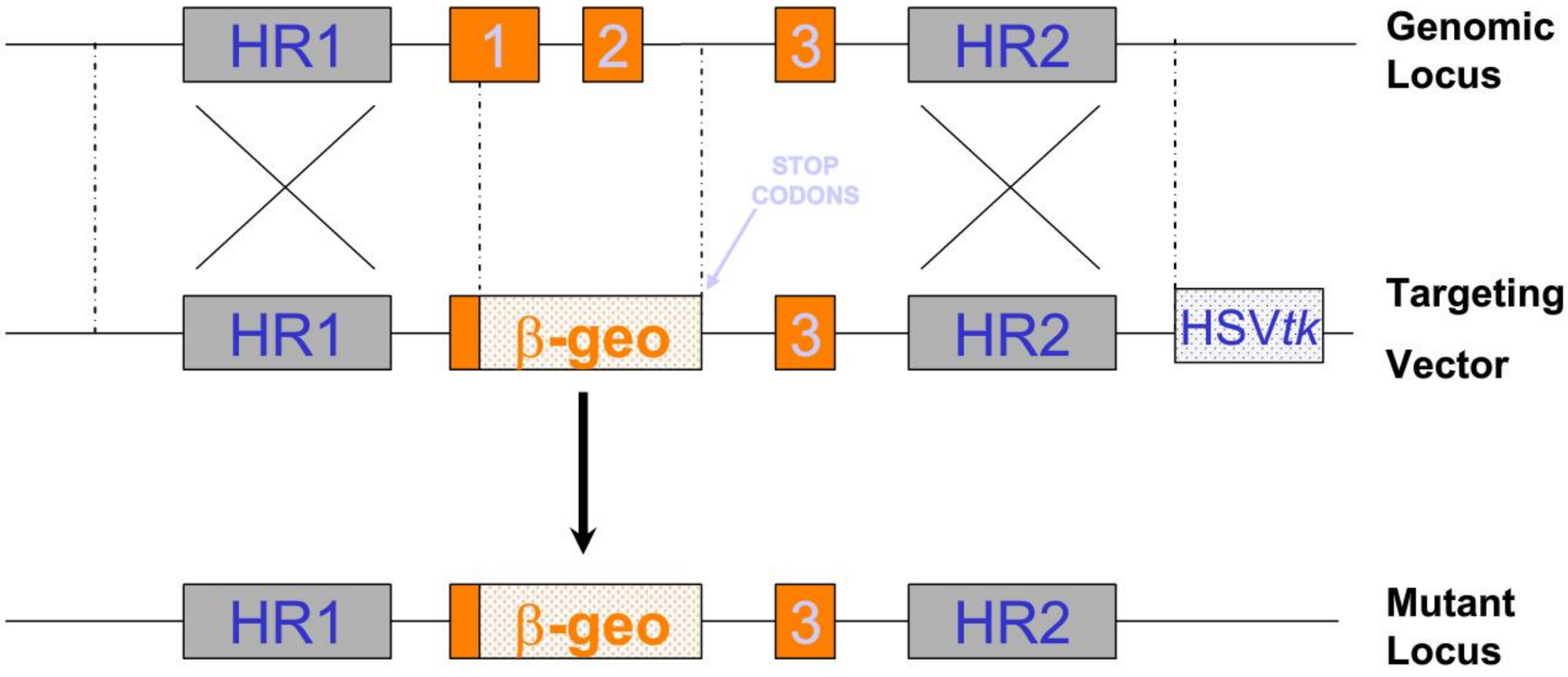


agouti black

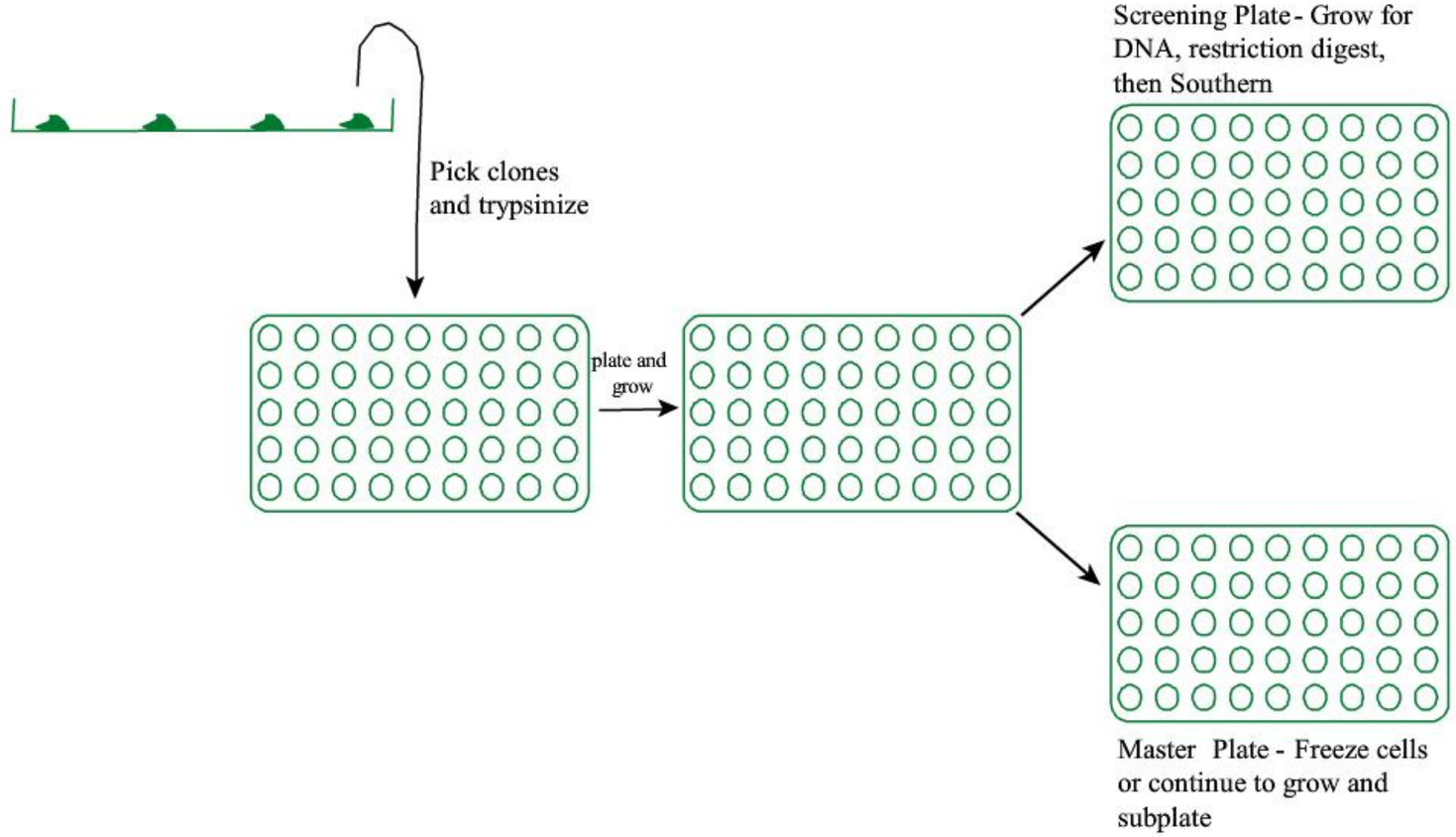
ES cells
129/SvJ
(agouti)


♂
X
♀
♂
x
♀
1 WT
:
2 Hetero
:
1 Homo

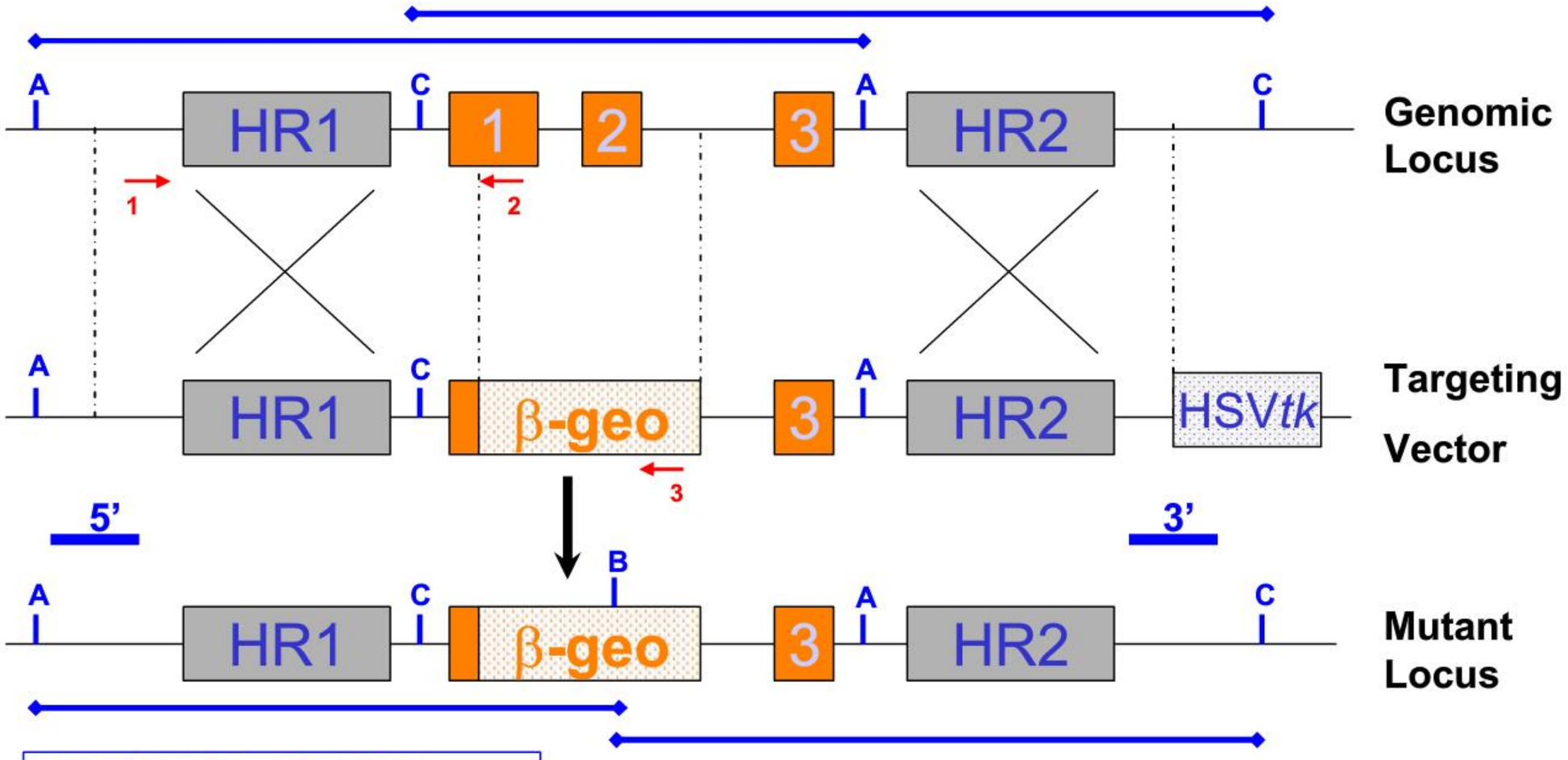
Positive-Negative Selection



Screening ES Cell Clones



Strategies for Screening



SOUTHERN BLOT SCREENING:

A-A, C-C = both WT and MT, but different sizes, when probed with 5' and 3' probes, respectively

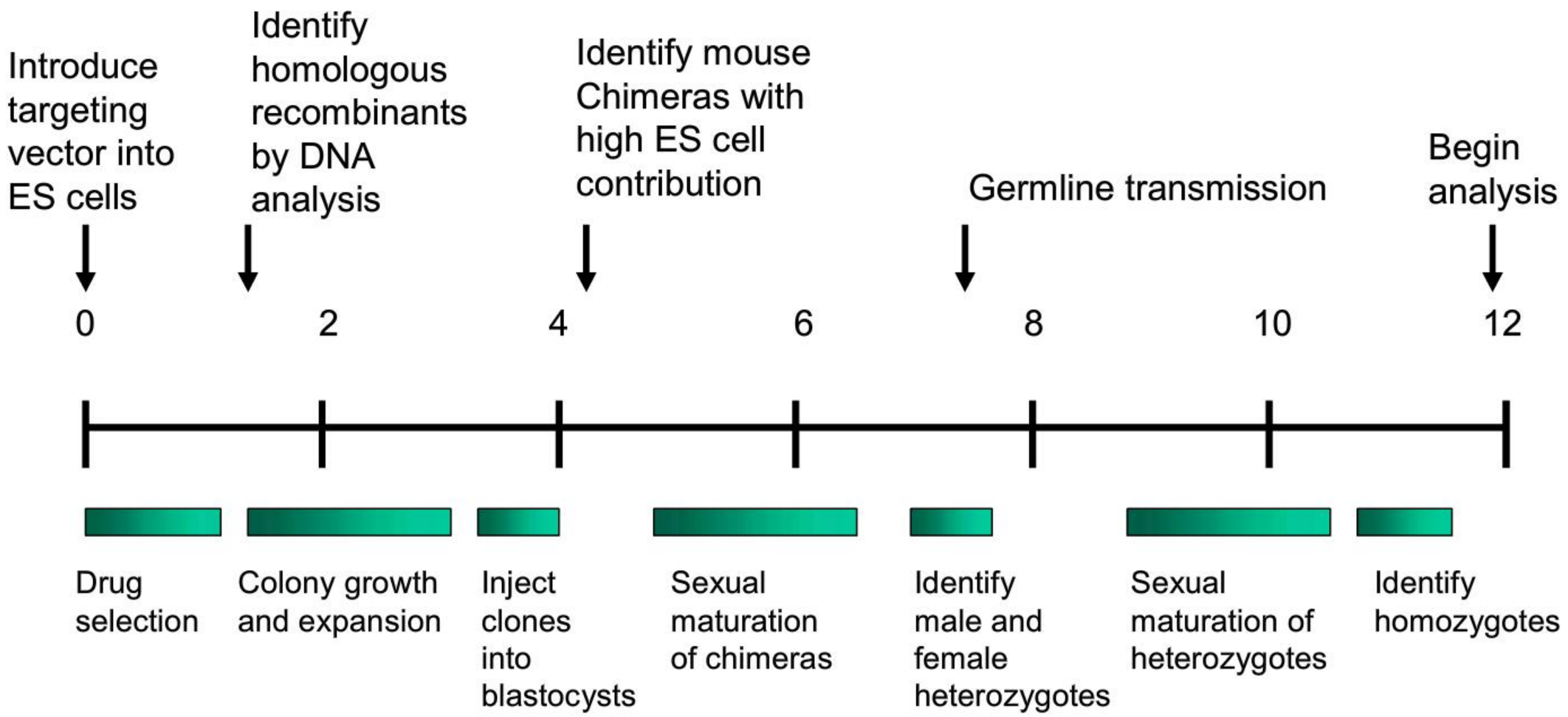
A-B, B-C = Specific to MT when probed with the same probes

PCR SCREENING:

1-2 = WT locus

1-3 = MT locus

Timeline: generation of ES cell-derived mice



Low Homologous Recombination Possibility

Typically:

Homologous recombination accounts for < 2% of all integration events.

1 in 10 million cells will undergo homologous recombination.

1 in 200 cells that come through positive-negative selection will turn out to be correctly targeted.

Factors influencing targeting efficiency

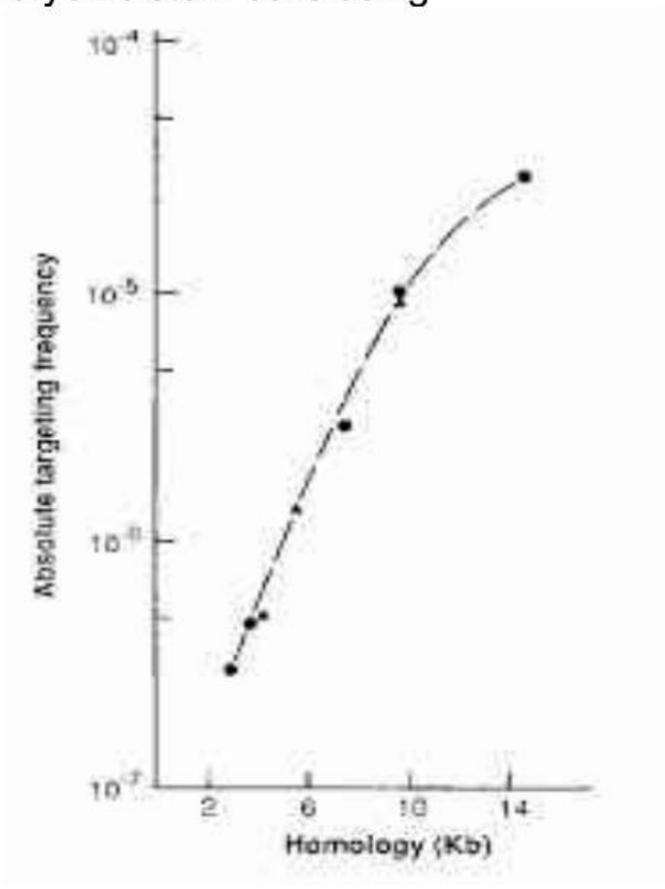
- **isogenic DNA (perfect homology)**
  ____10-25 fold

  van Deursen J, Wieringa B. Targeting of the creatine kinase M gene in embryonic stem cells using isogenic and nonisogenic vectors. Nucleic Acids Res. 20:3815-20, 1992.

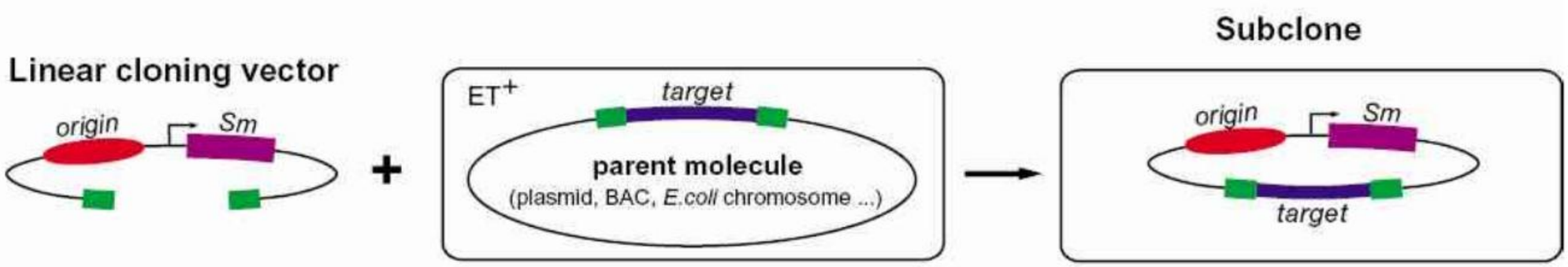
- **size of region of homology**
  ____exponential relationship

  Capecchi MR. Altering the genome by homologous recombination. Science. 244:1288-9, 1989

- **robust screen!**
  Positive controls

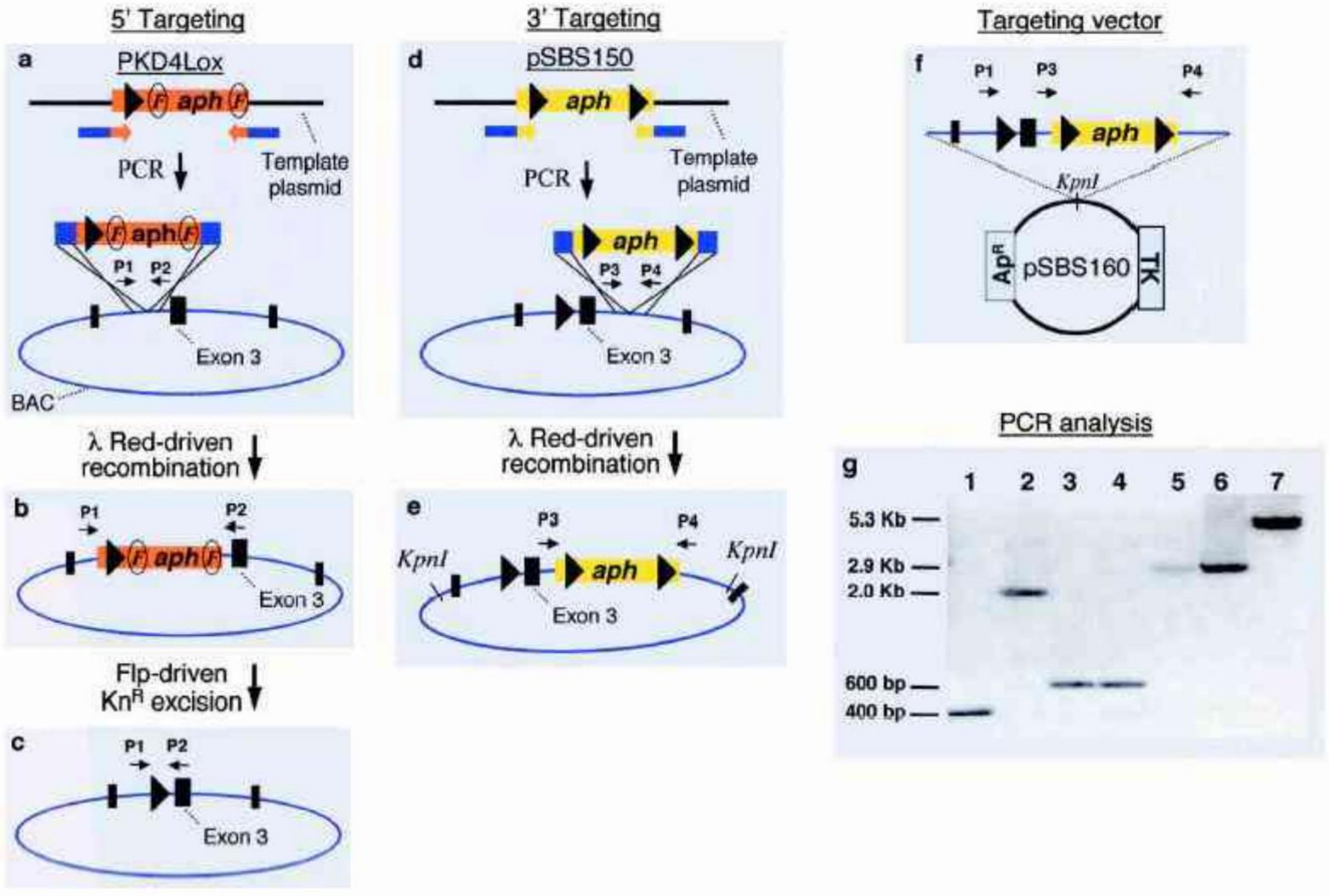


A



B

Example	Linear cloning vector	ET expression	*E. coli* host	Target	Parent molecule	Total colonies	Percentage correct (out of 18)
1	p15A+*Cm*	Endogenous RecE/Rec*T*	JC9604	2.3 kb, endogenous *lacZ* gene	*E. coli* chromosome	290	100
2	p15A+*Cm*	pBAD-αβγ	HB101	2.3 kb, endogenous *lacZ* gene	*E. coli* chromosome	370	94
3	p15A+*Km*	pBAD-recET	JC5519	0.8 kb, gentamicin gene	pFastBac1 (high-copy plasmid)	>3,000	100
4	p15A+*Km*	pBAD-αβγ	HS996	28 kb, intron3 of *mAf*4	BAC	160	83
5	p15A+*Amp*	pR6K116/BAD /αβγ	HS996	19kb, exons 2-3 of *mAf*4	BAC	>2,000	83


5' Targeting
a
PKD4Lox
aph
PCR
Template plasmid
P1
P2
Exon 3
BAC
λ Red-driven recombination
b
P1
P2
aph
Exon 3
Flp-driven
Kn^R excision
c
P1
P2
Exon 3
3' Targeting
d
pSBS150
aph
PCR
Template plasmid
P3
P4
Exon 3
λ Red-driven recombination
e
P3
P4
KpnI
aph
KpnI
Exon 3
Targeting vector
f
P1
P3
P4
aph
KpnI
Ap^R
pSBS160
TK
PCR analysis
g
1 2 3 4 5 6 7
5.3 Kb
2.9 Kb
2.0 Kb
600 bp
400 bp

基因打靶技术的局限性

- 胚胎致死
- 所有细胞都改变
- 功能代偿
- 冗余
- 单元与系统的关系

条件性打靶技术
conditional knockout

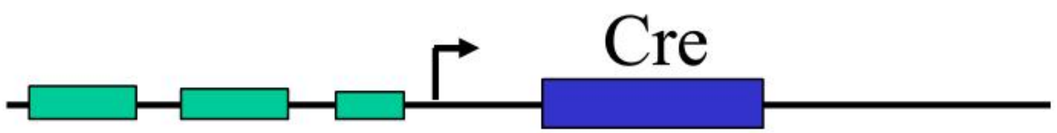
- Cre/loxP recombinase
- FLP/FRT recombinase

Principles

Need to create and maintain two strains (lines) of mice

1) A line of mice in which *loxP* sites have been inserted around the gene of interest.

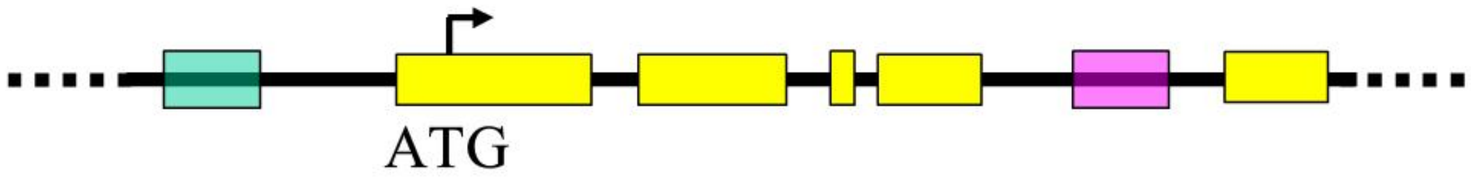
2) A line of mice that express Cre recombinase from a tissue-specific promoter (e.g. directs Cre expression to pancreas only).



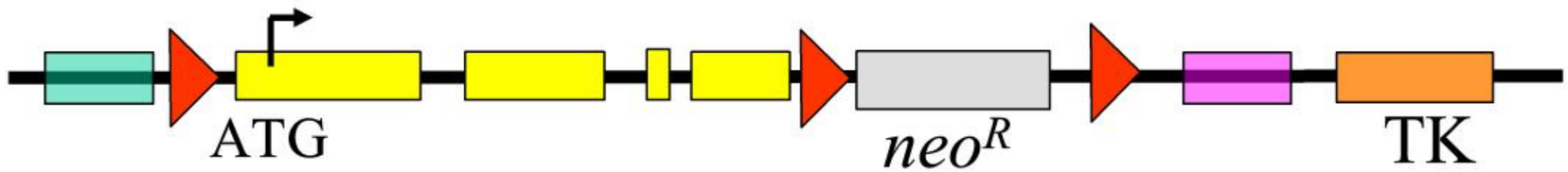
AND BREED THE TWO TOGETHER - gene is knocked out only in tissues where Cre is expressed.

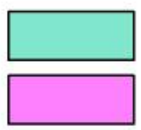
Conditional targeting by homologous recombination in ES cells

Endogenous gene



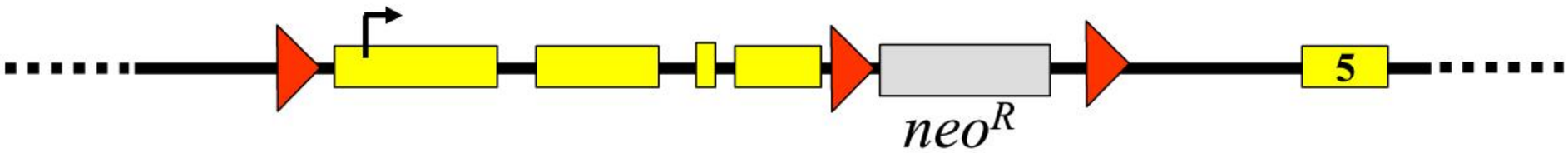
Targeting vector



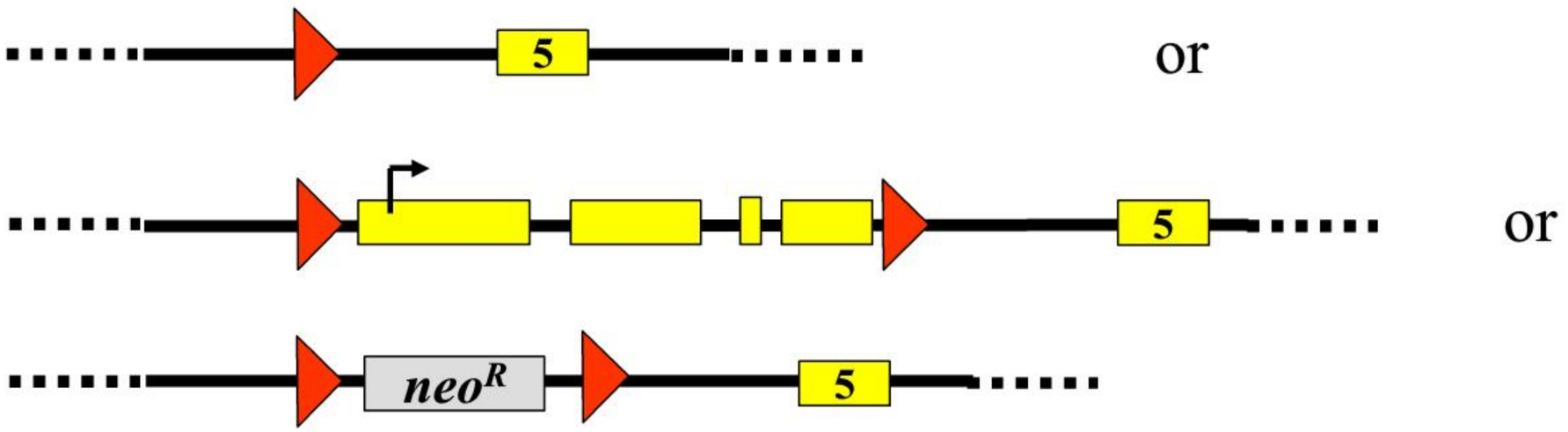


= regions of homologous DNA sequence

Electroporate target vector into ES cells and perform positive-negative selection, PCR and southern blotting to isolate clones that have integrated correctly, so that genomic DNA of ES cells =

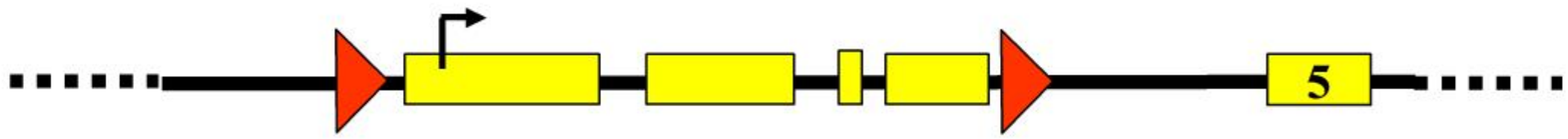


Transiently transfect these ES cells with constitutive Cre construct, creating cells that are...



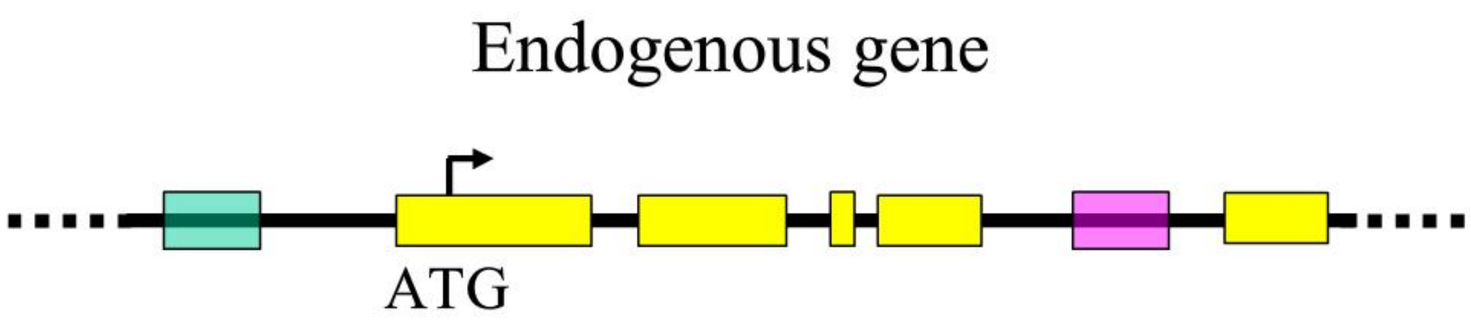
Select and isolate clones of cells that are now G418-sensitive

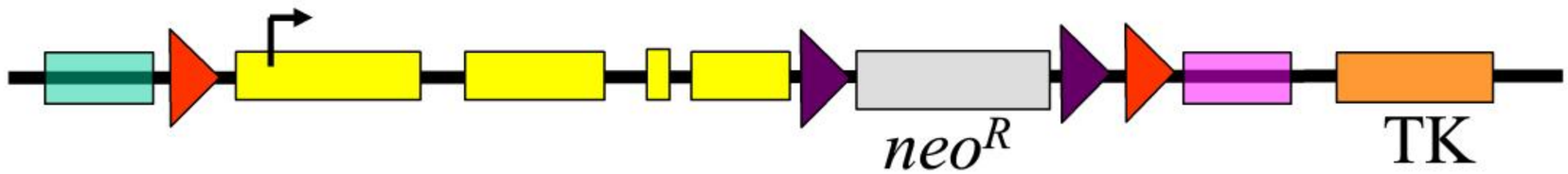
ES cells that whose genomic DNA is modified as above can be used to create a conventional knockout.



ES cells containing the ‘floxed’ allele form the basis of the tissue- specific knockout. Inject into blastocysts to get chimeras, breed from chimeras to get heterozygotes and breed heterozygotes together to get homozygous floxed mice. These can be bred with the appropriate Cre transgenic line.

Alternative Floxing strategy - avoids extra ES cell step

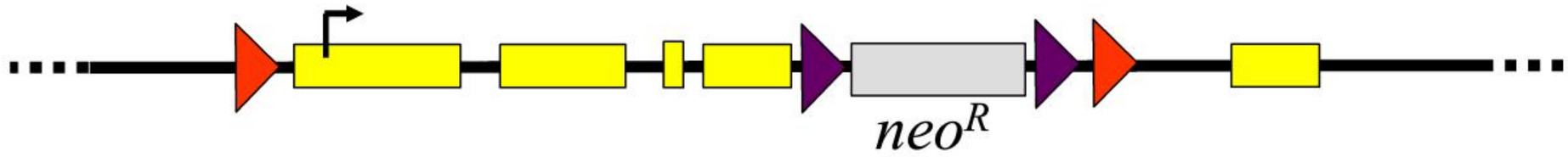




loxP site

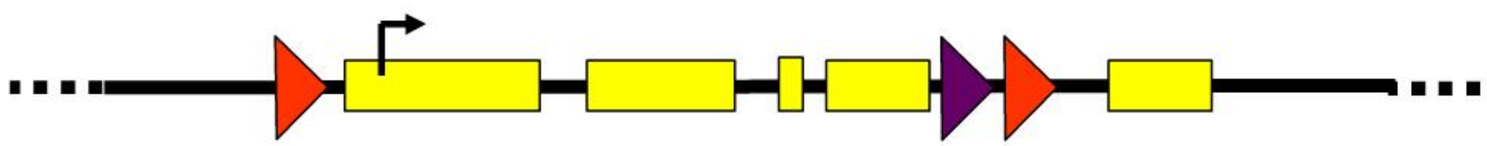
FRT site

Electroporate targeting vector into ES cells and perform selection as before



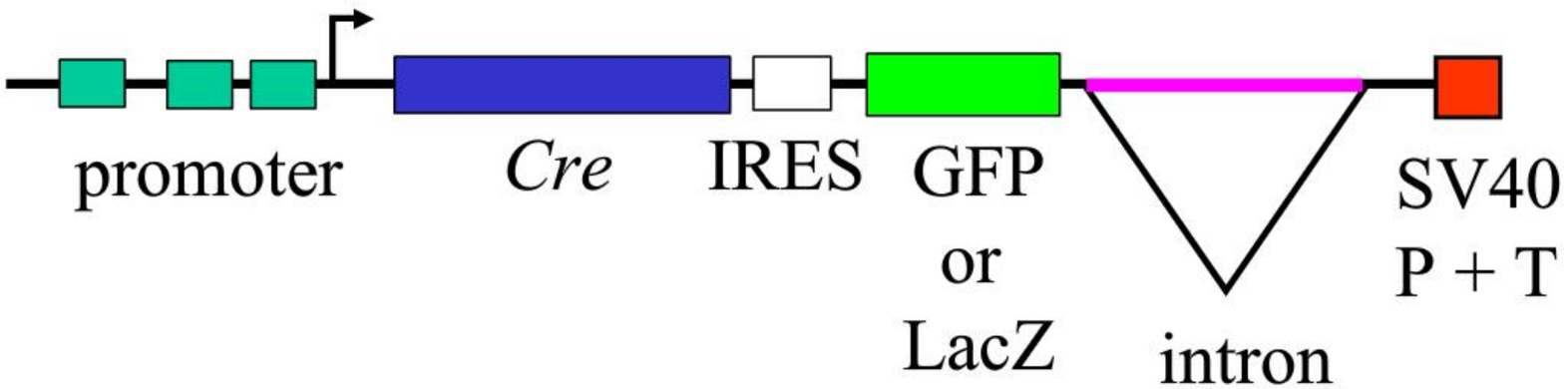
Inject into blastocysts, create chimeras and breed these to get heterozygotes as before.

Breed these mice with a line that constitutively expresses Flp recombinase from the β-actin promoter.



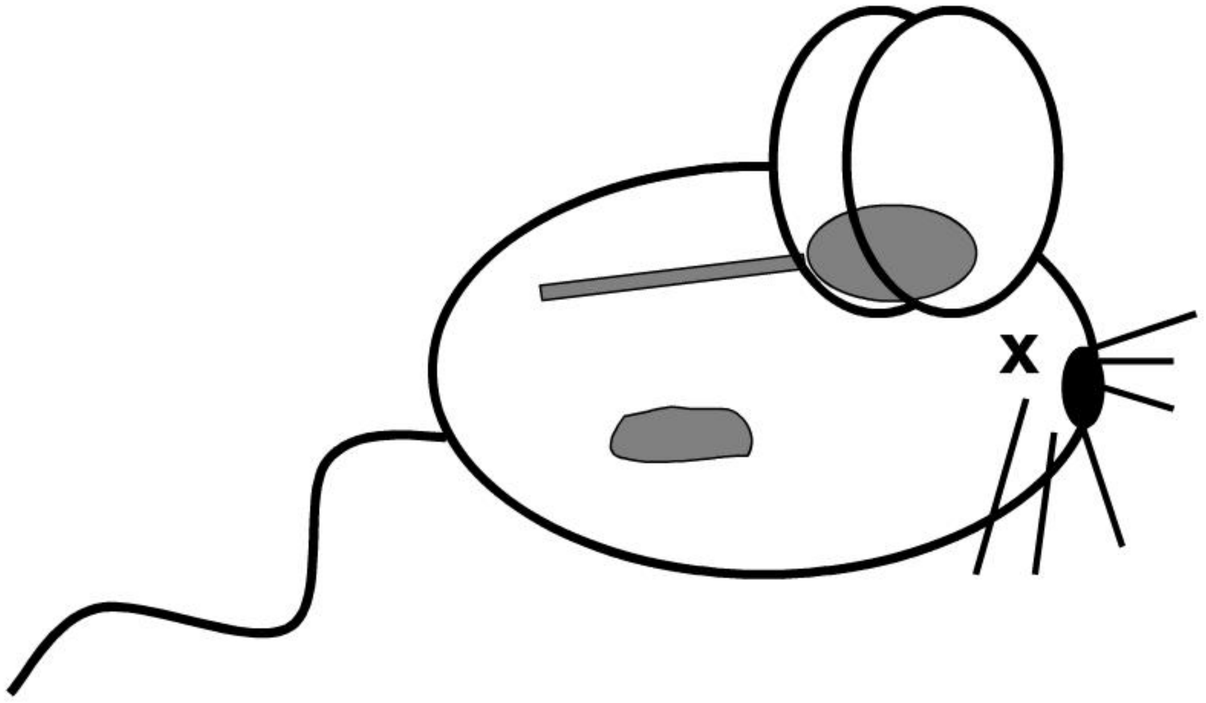
This removes the *neo*^{R} cassette and gives the floxed allele

A typical Cre construct

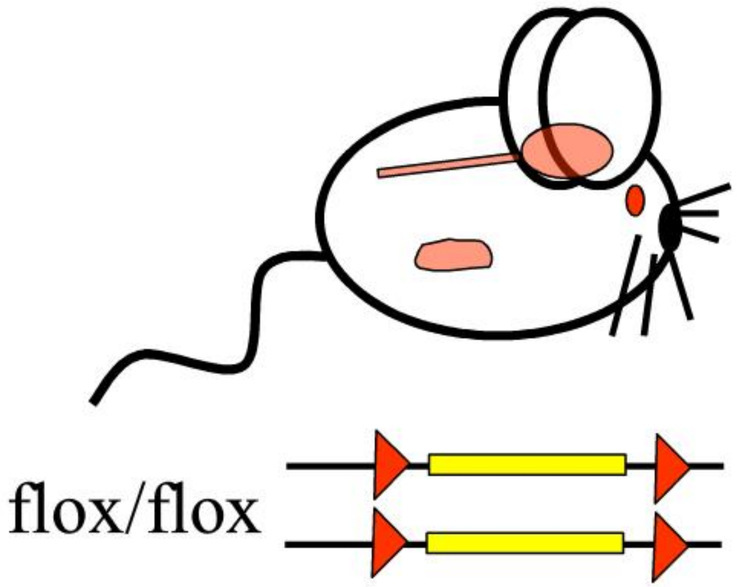


IRES - an internal ribosome entry site.

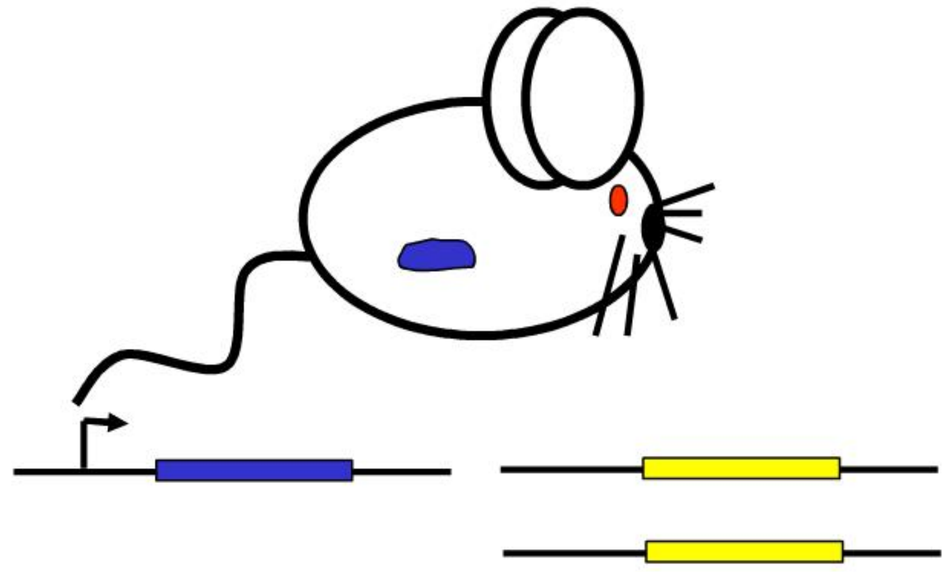
Resumée: knock out gene in all tissues - mouse dies



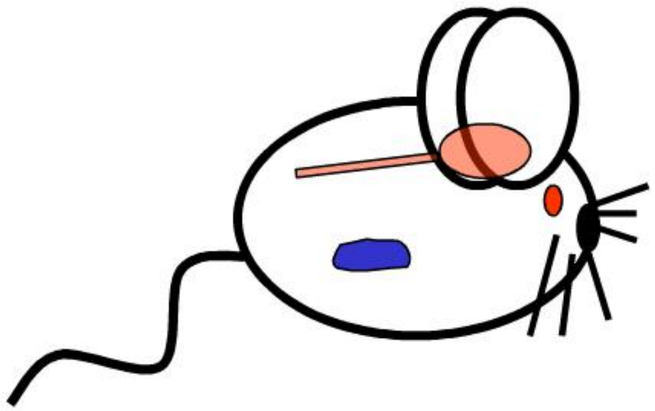
Create floxed mice

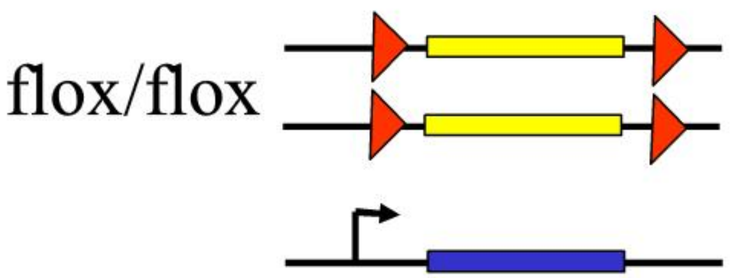


Create hemizygous Cre mice



Cross mice for a couple of generations to get Cre+ flox/flox homozygotes



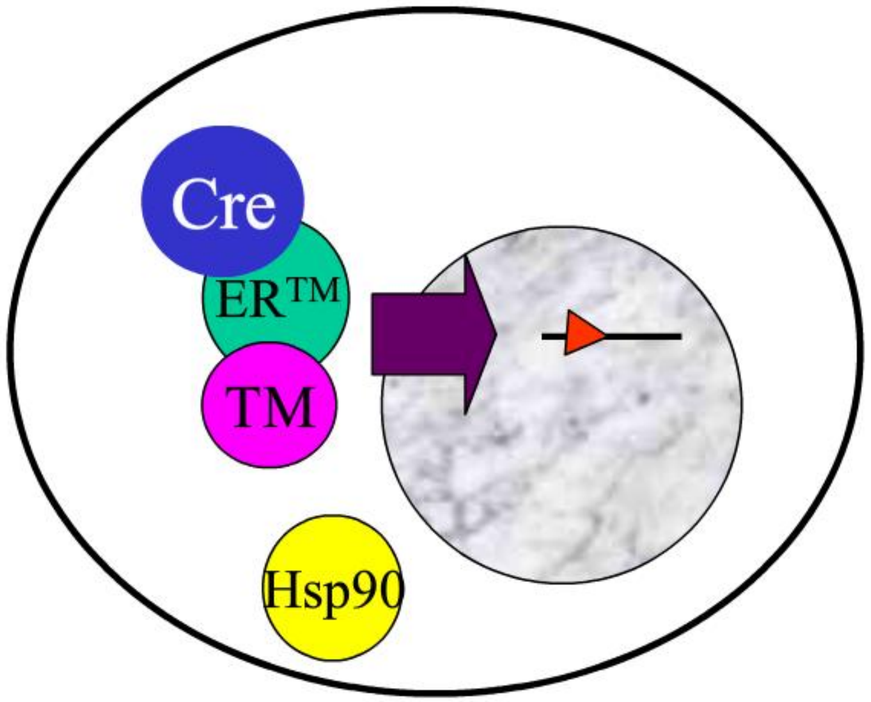


Temporal induction of Cre expression: 2

Tamoxifen-inducible system

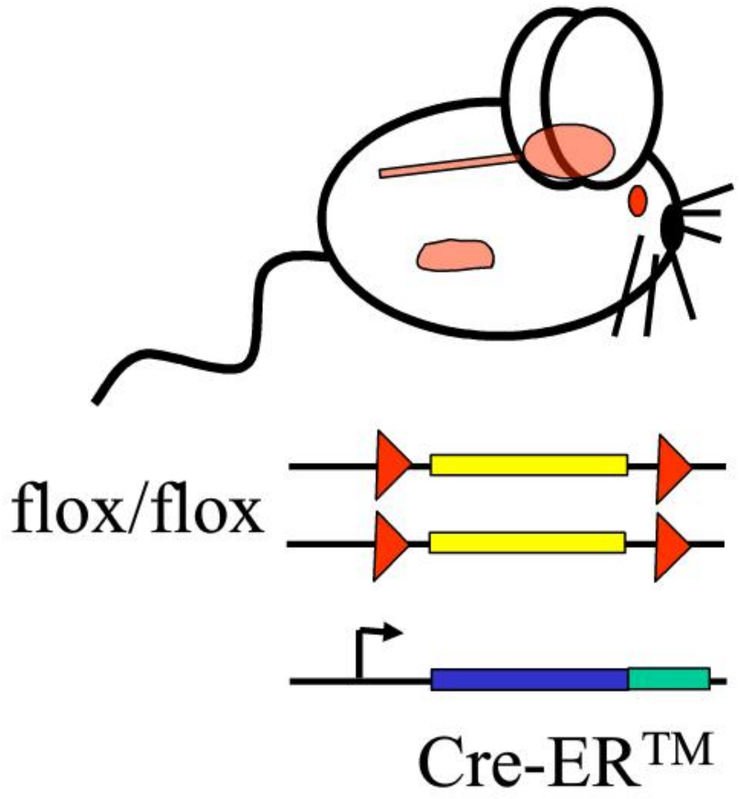
Uses a fusion protein combining activity of Cre and a mutant form of the ligand binding domain of the estrogen receptor (ER^{TM})

Cre-ER^{TM} does not bind estrogen, but does bind 4-OH-tamoxifen



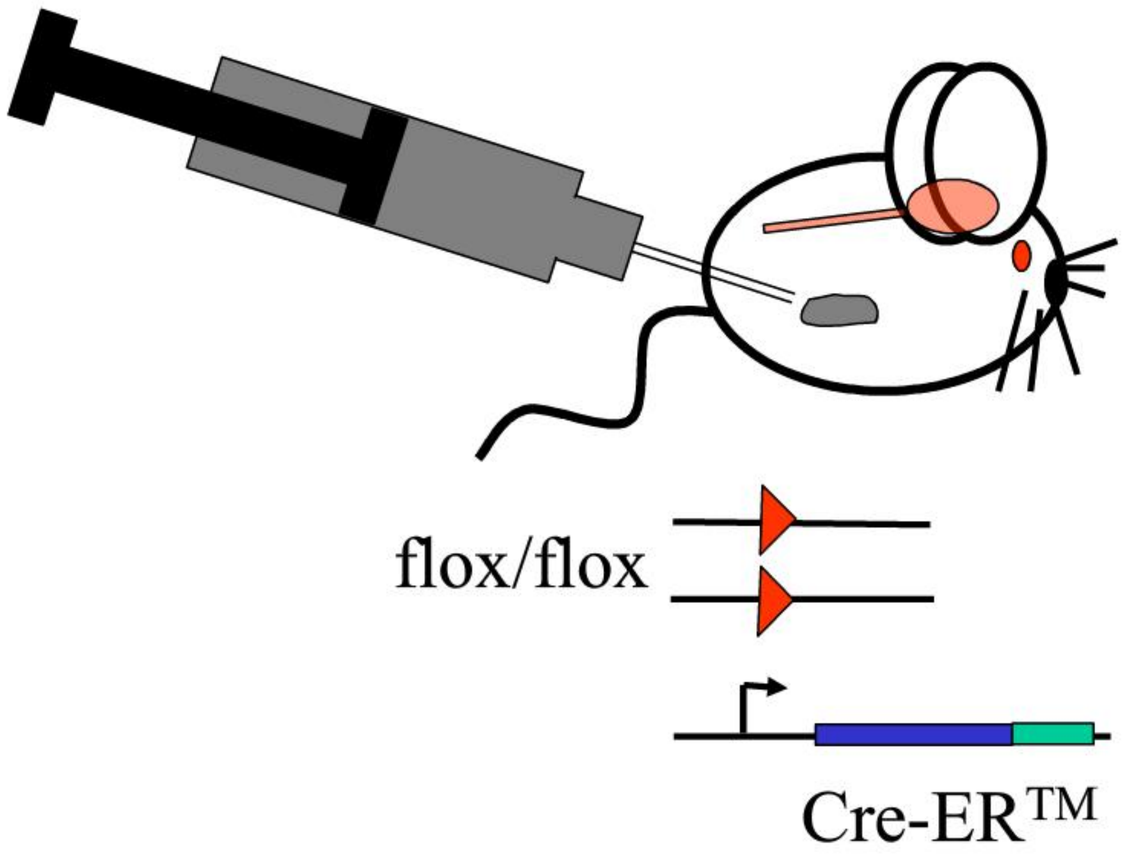
Upon addition of tamoxifen, Cre-ER^{TM} is released from Hsp90, allowing access to the nucleus and Cre-mediated recombination

Temporal induction of Cre expression: 2



Create floxed/Cre double transgenics as before

Cre is being expressed in tissue of interest (e.g. pancreas), but is sequestered in cytoplasm and inactive

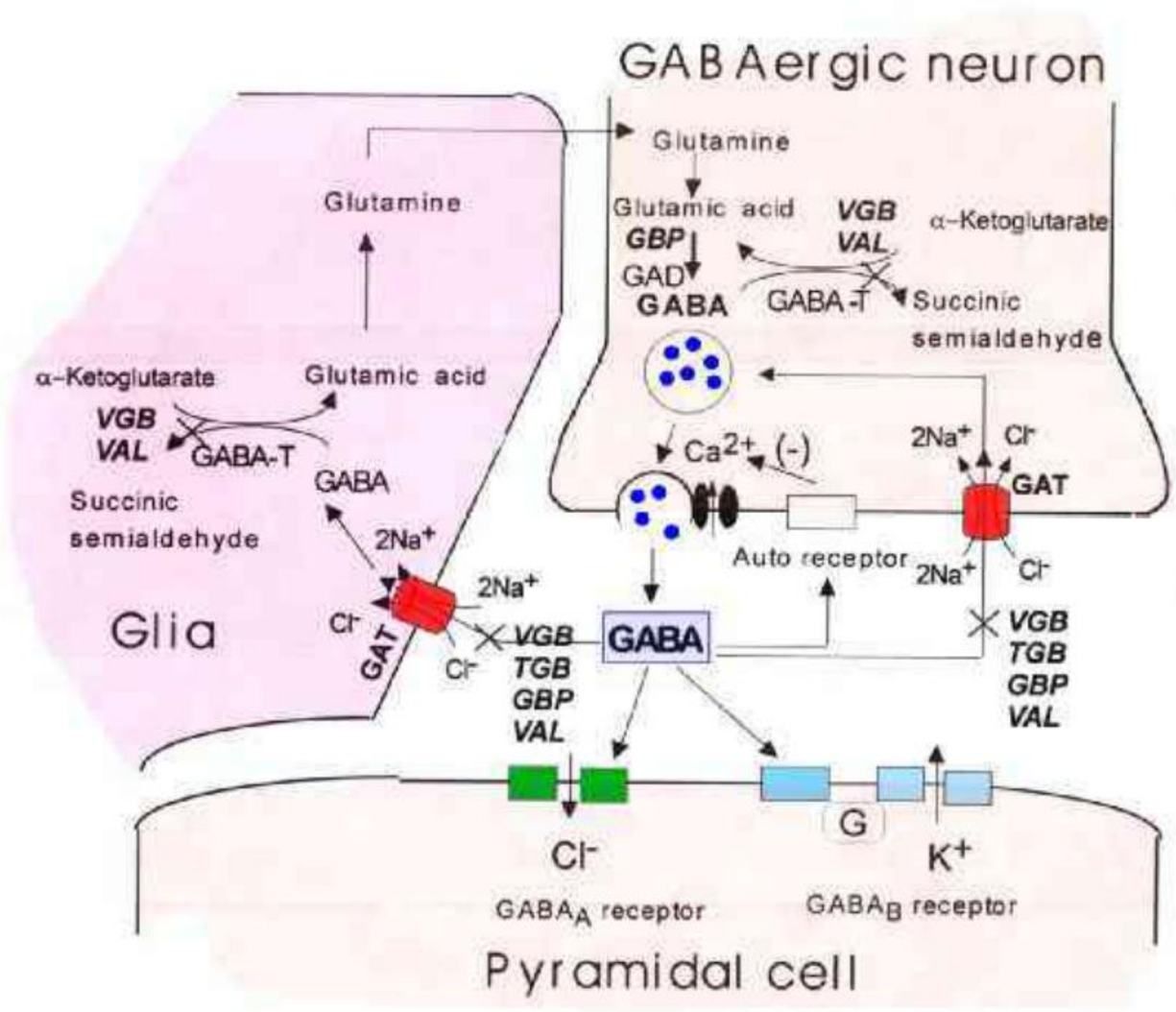


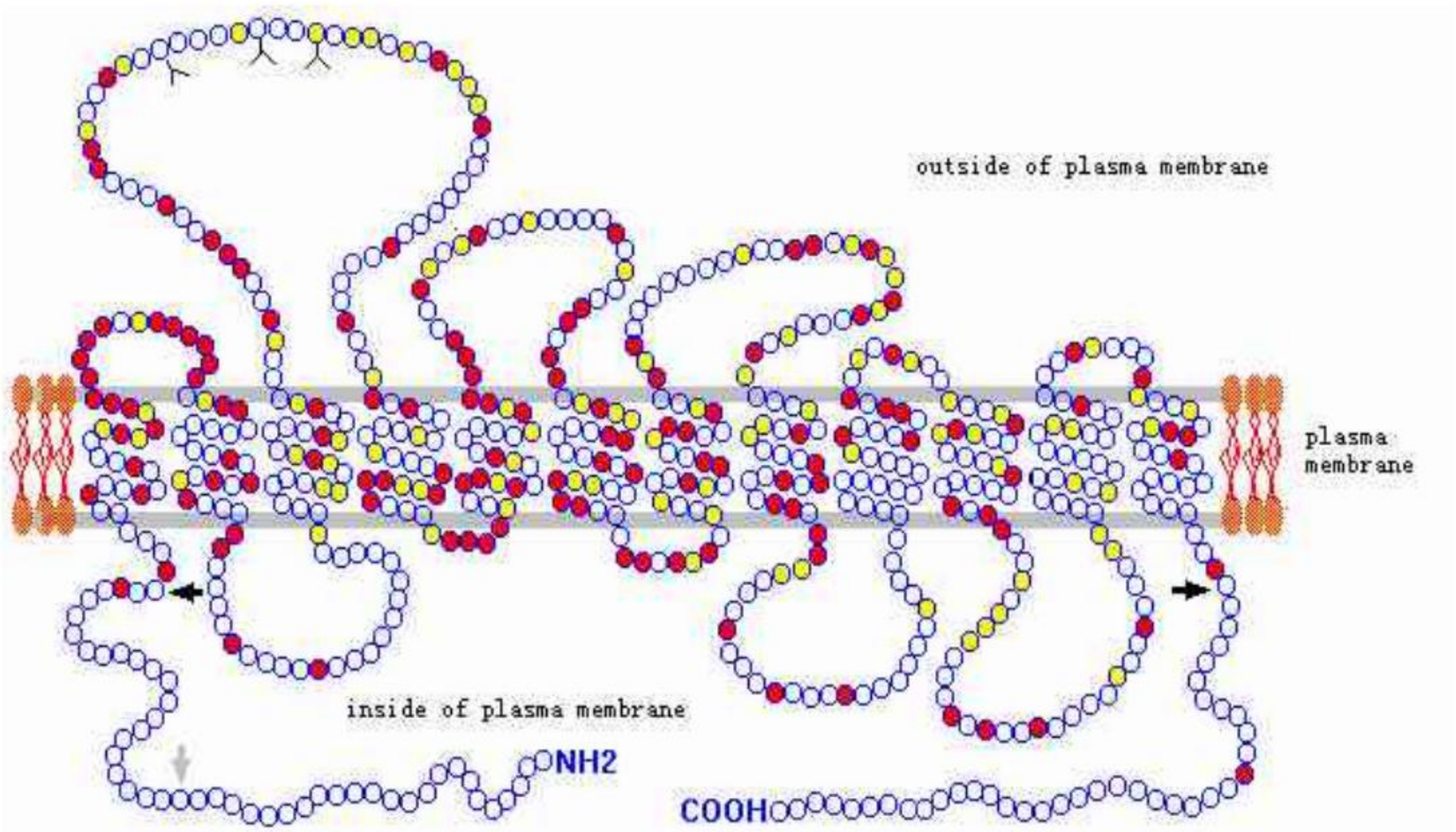
Inject with tamoxifen

Cre is released into nucleus, leading to knockout of gene only in tissues that are exposed to tamoxifen and where Cre is being expressed.

GABA Transporter (GAT1) and its function in vivo

GABAergic synaptic transmission

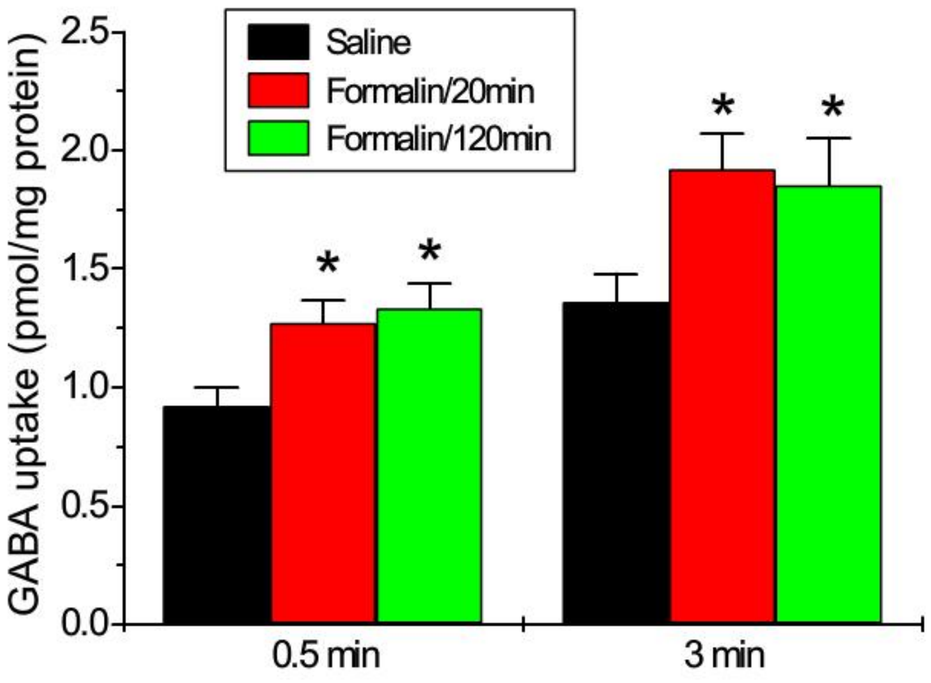


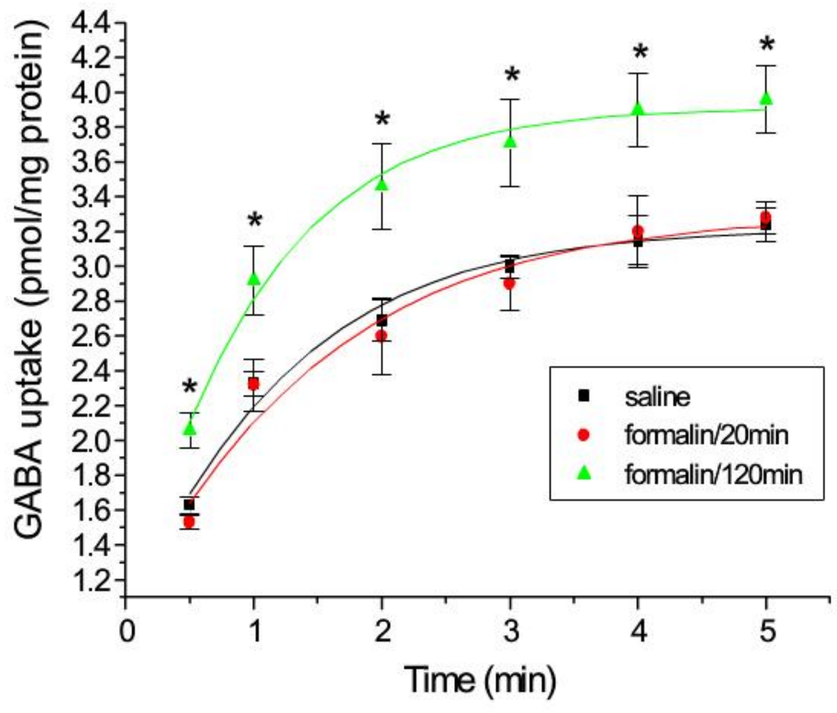


Modle of the transmembrane topology of the GABA transporter subtype A (gat-1). Y: indicate the potential glycosylation sites; The putative kinase C phosphorylation sites in N- and C-terminal regions are marked by arrows. Amino acid residues which conserved between four subtypes of GABA transporters are presented by yellow and red solid dots, the red solid dots denote amino acid residues also conserved in rat DAT, human NET, rat 5-HTT, and canine BGT-1 transporter.

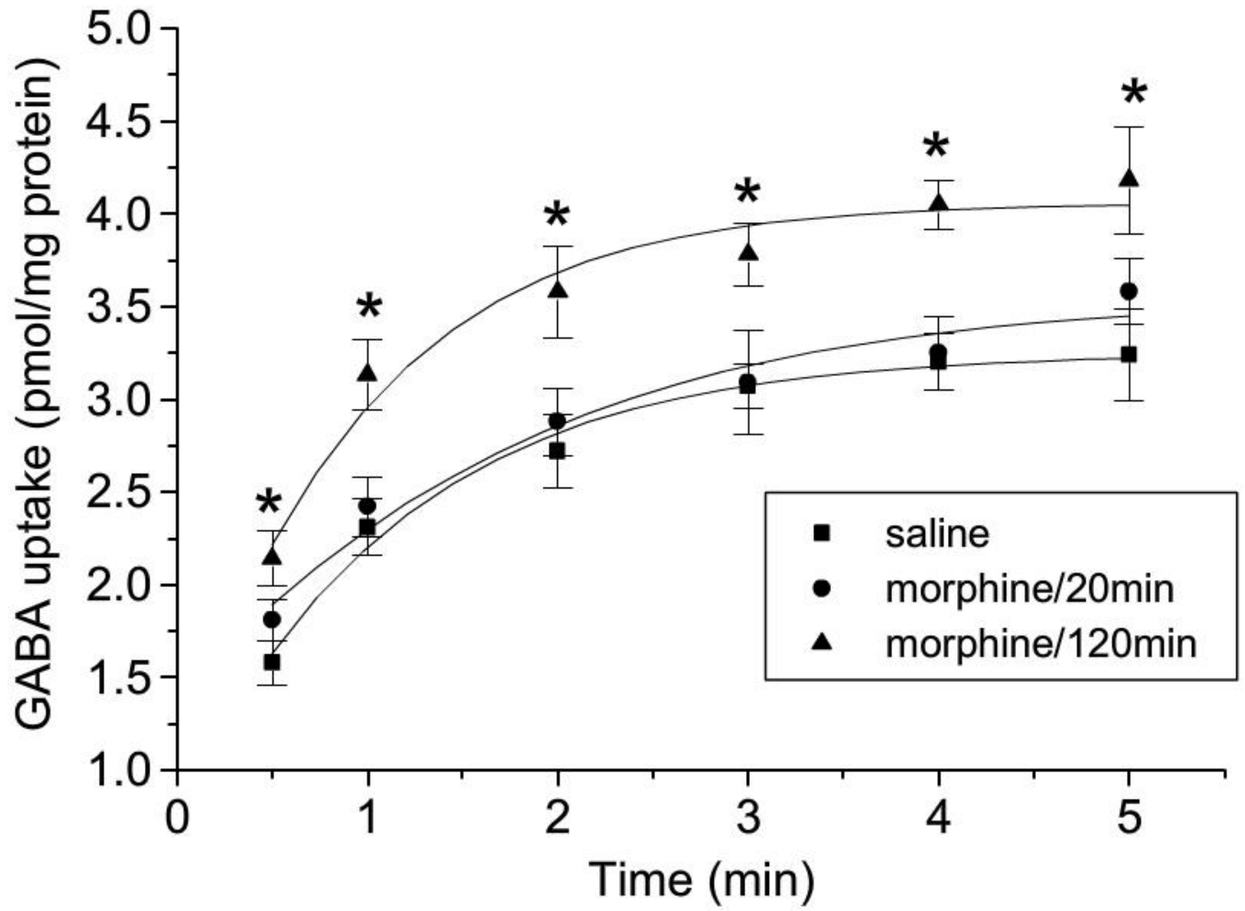
Activity of GAT1 is very sensitive to the outside stimulus

Up-regulation of GABA uptake activity in Spinal Cord and Brain after Formalin Injection

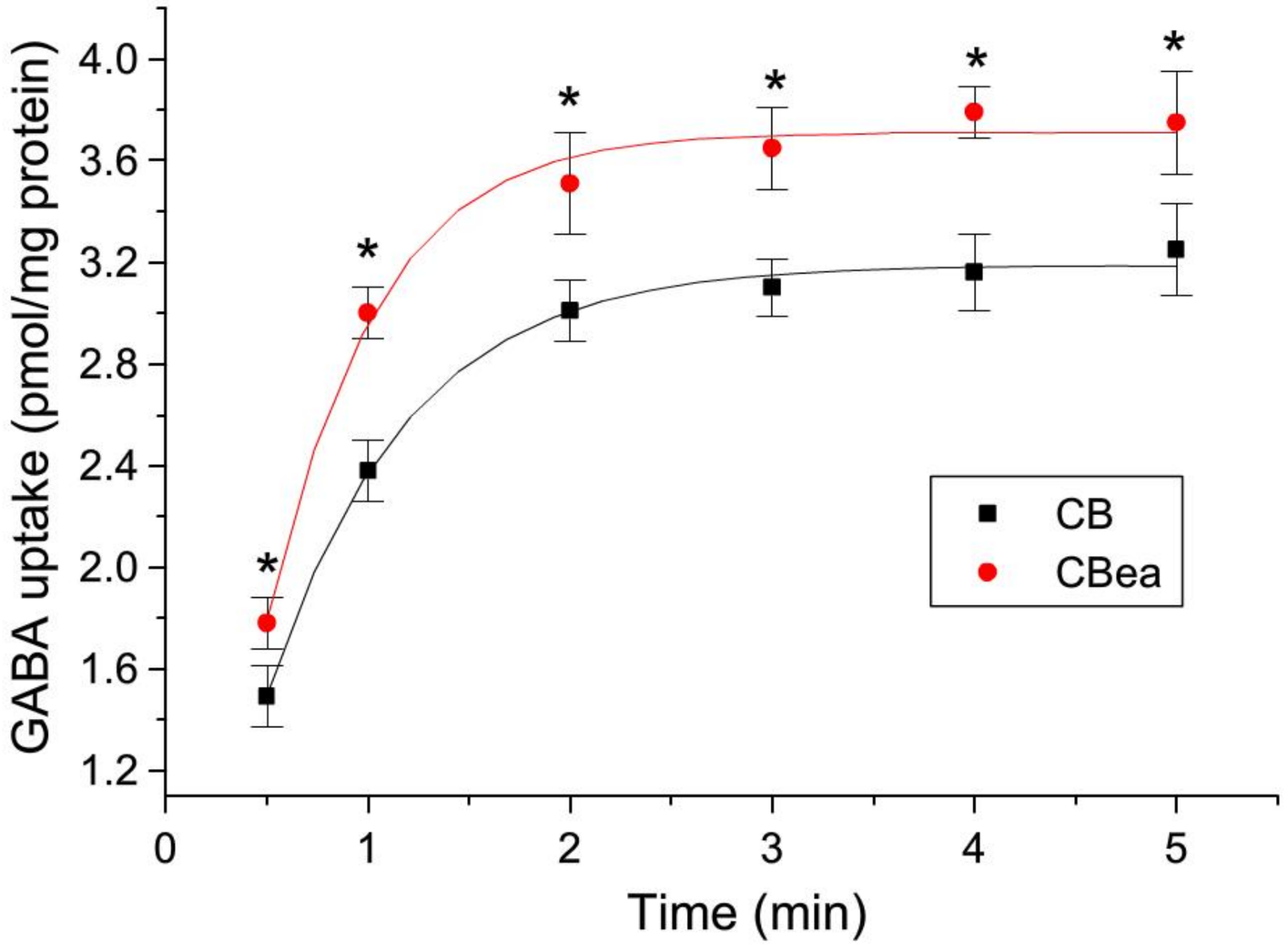




Up-regulation of GABA uptake activity in mouse brain after morphine consumption

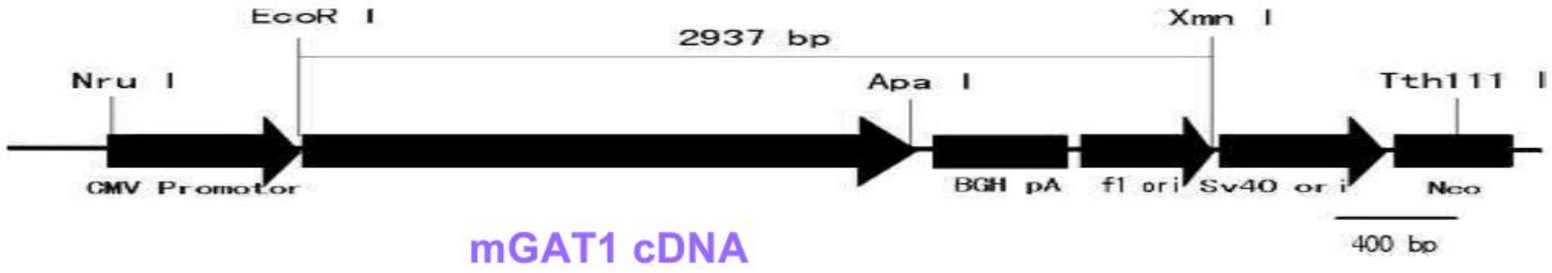


Up-regulation of GABA uptake activity after acute ethanol administration

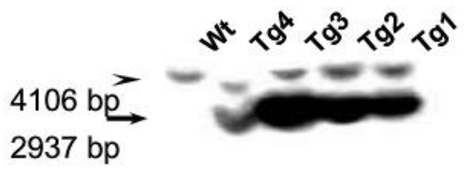


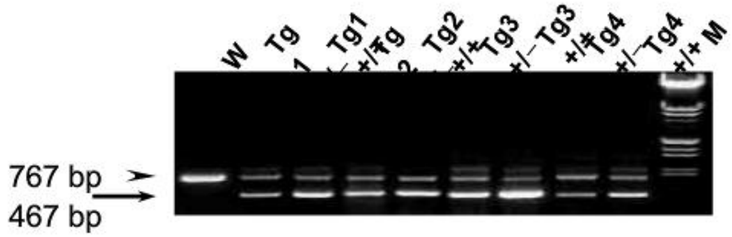
Overexpression or gene knockout of GAT1 in mice

pcDNA3-GAT1

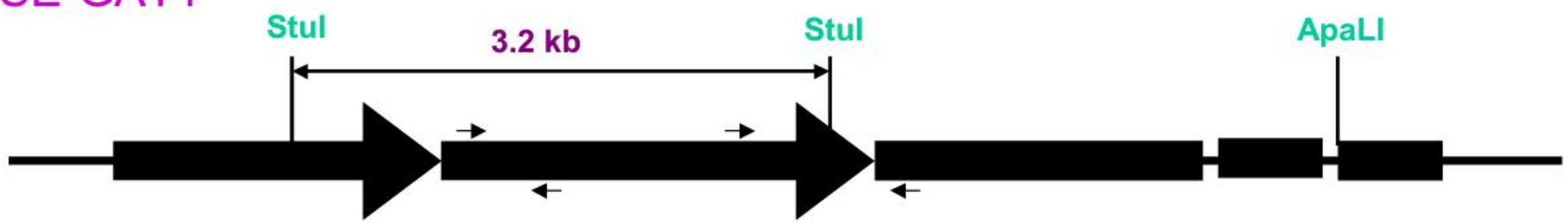


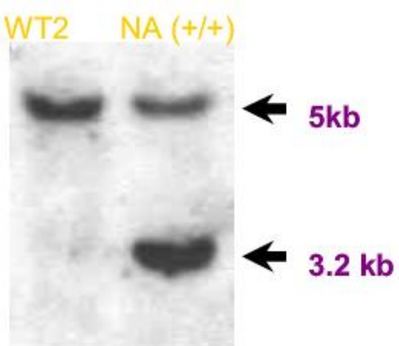
mGAT1 cDNA

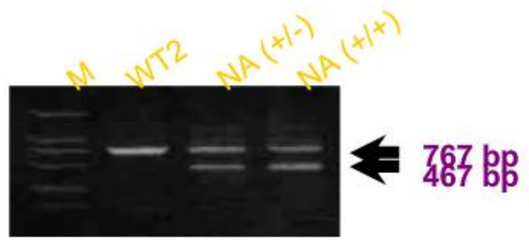




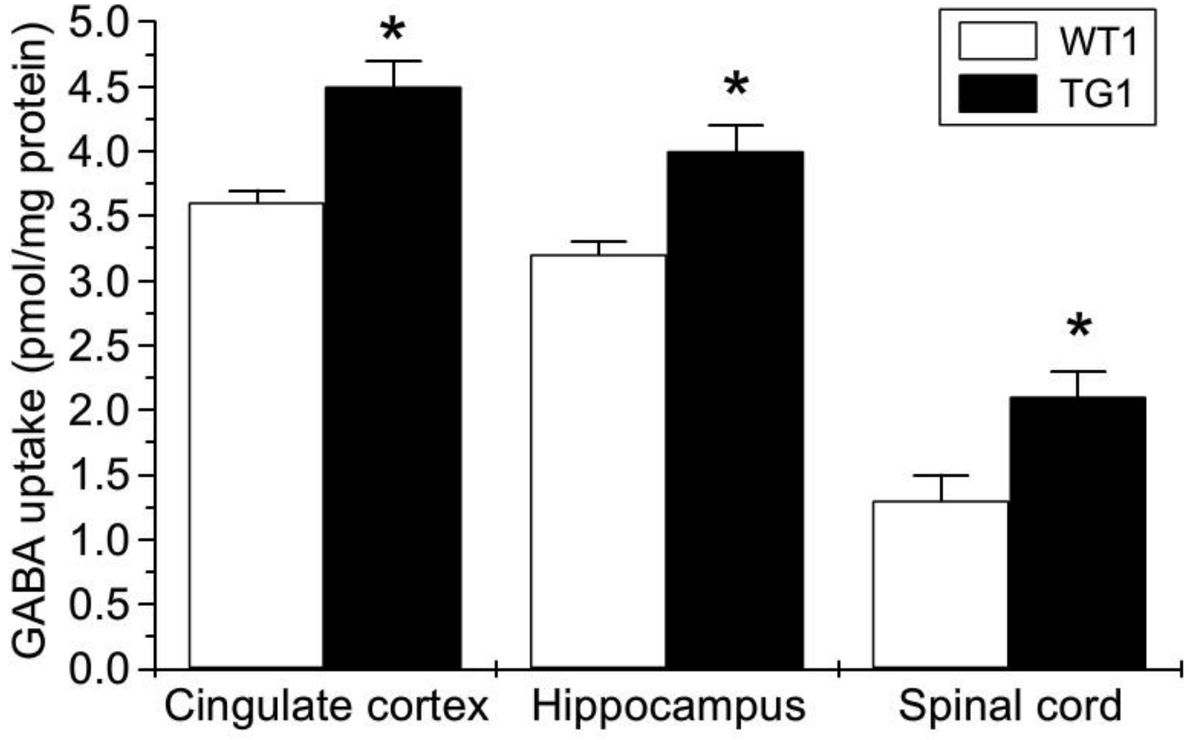
pNSE-GAT1

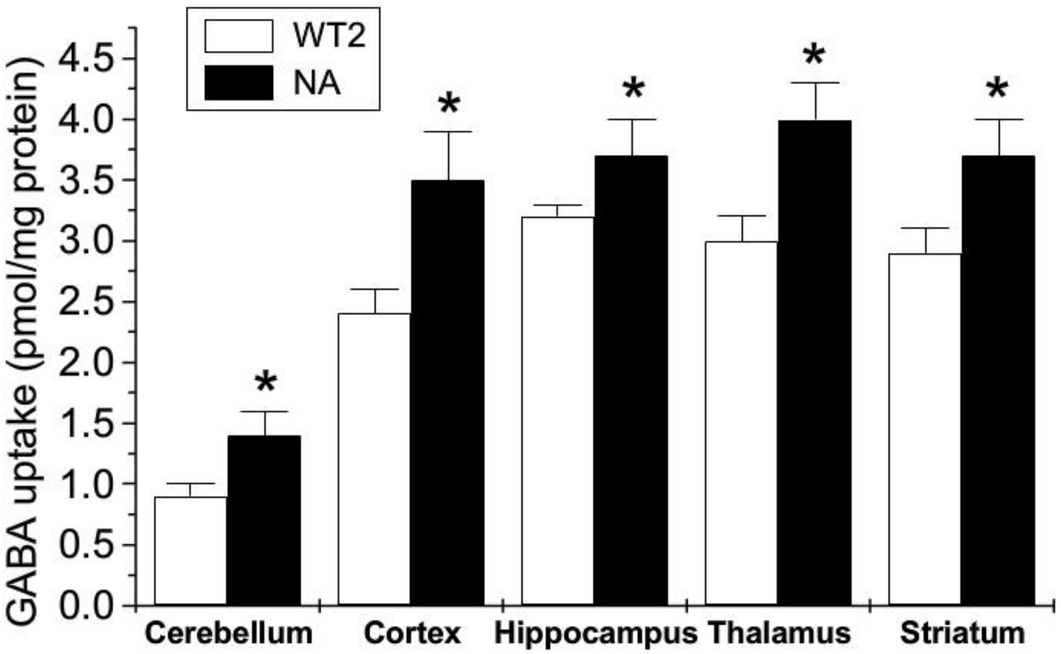




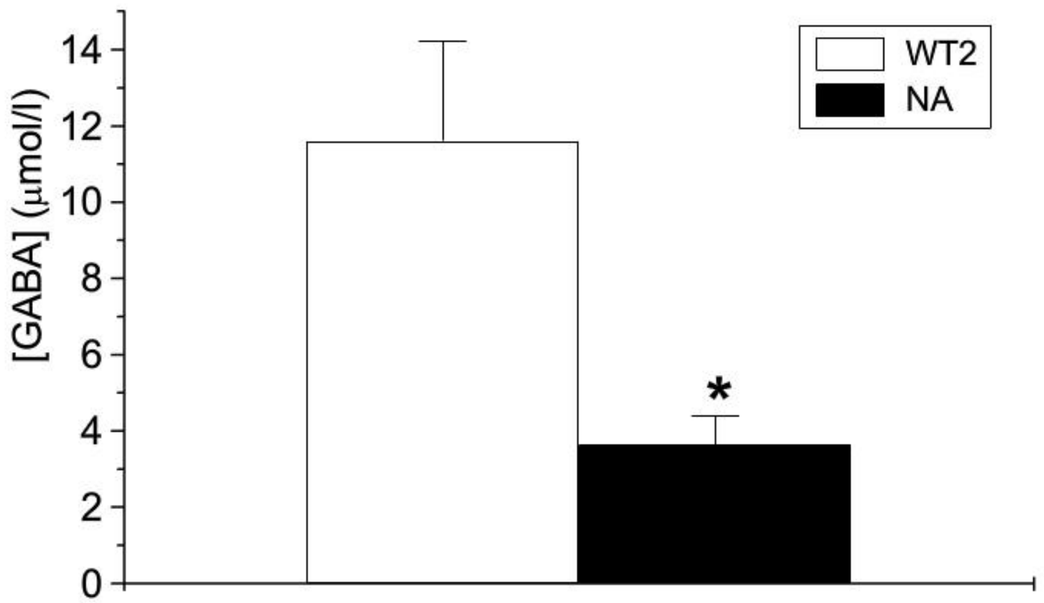


GABA uptake in the pain-relative regions of TG1 mice



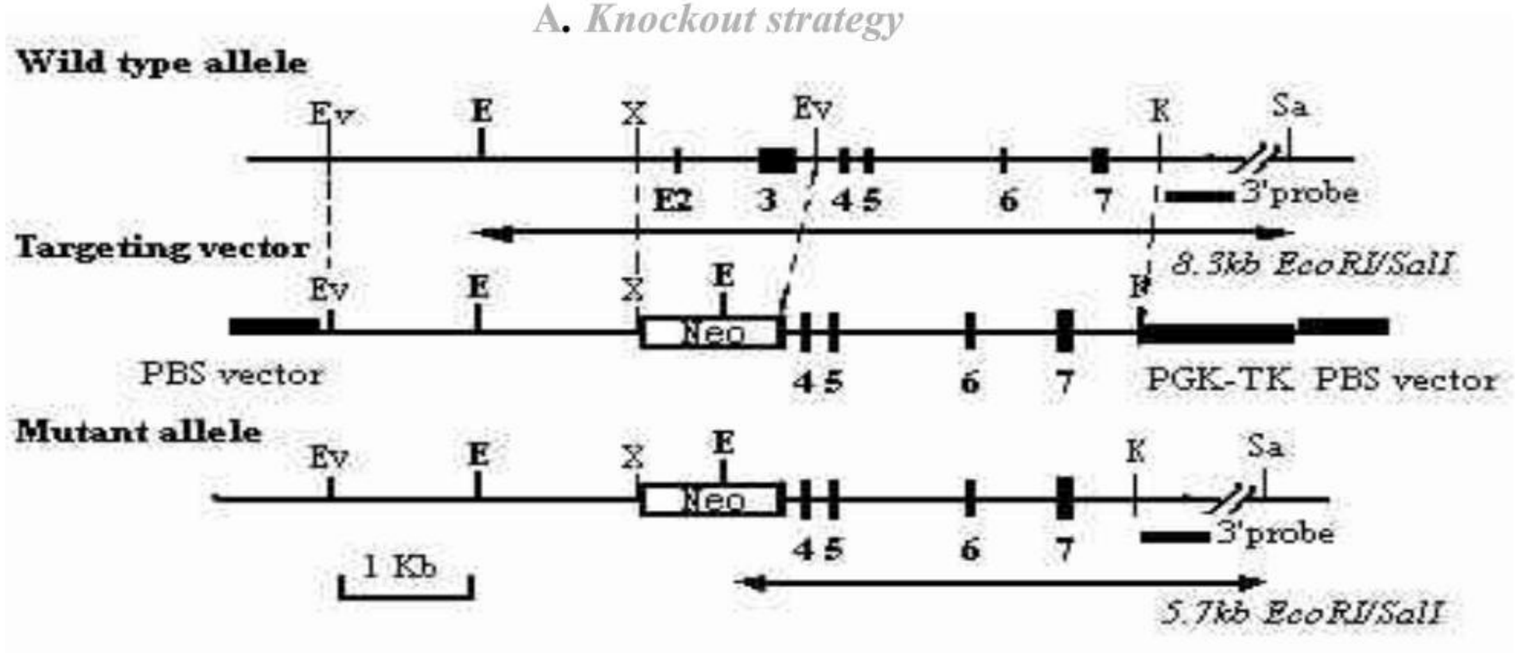


GABA uptake in mouse brain and GABA level in the hippocampus

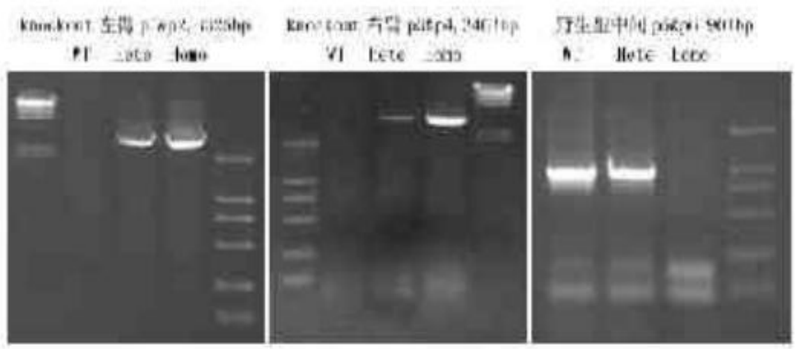


Generation of GAT1 knockout mice by homologous recombination

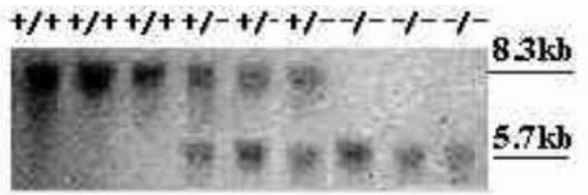
A. *Knockout strategy*



B. *Genotyping of three strains by PCR*

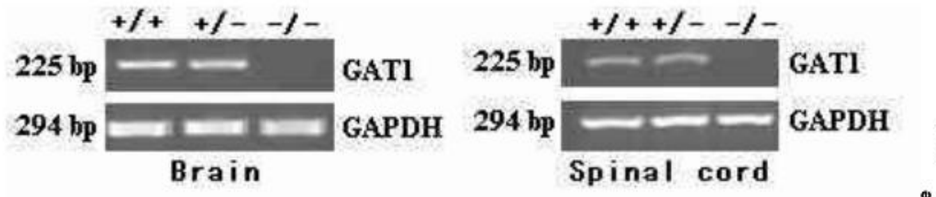


C. *Genotyping of three strains by Southern-blot analysis*

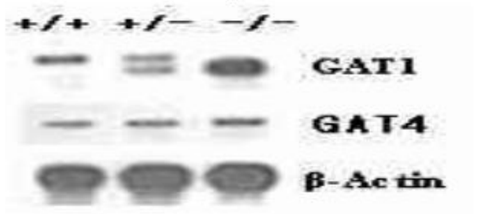


Detection of GAT1 expression in three genotypes

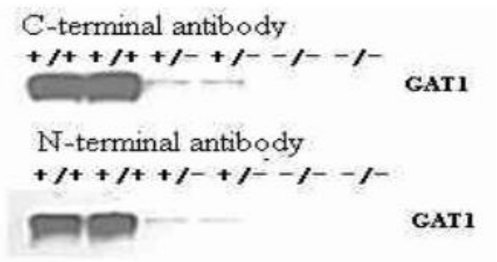
A ***.RT-PCR analysis of GAT1 expression in brain and spinal cord***



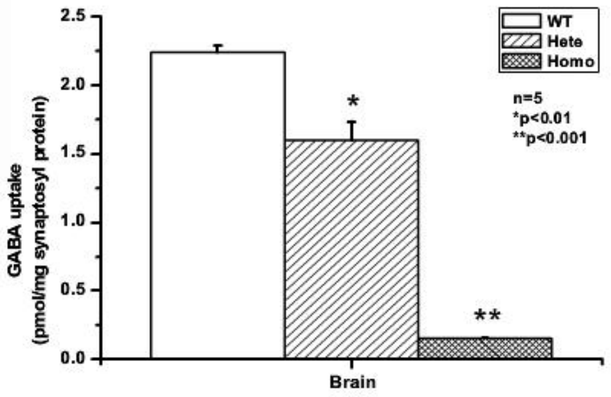
B. ***Northern blot analysis of mGAT1 and mGAT4 expression in brain***

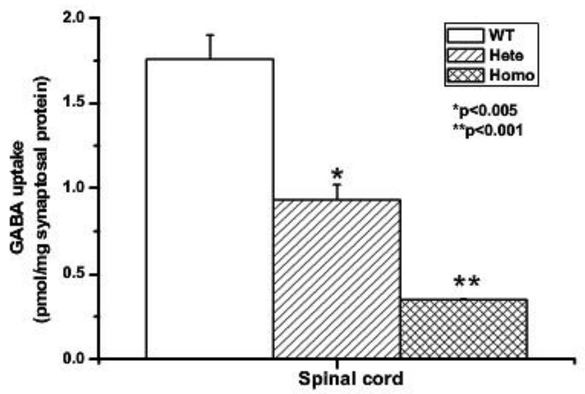


C..***Western blot analysis of GAT1 expression in brain***

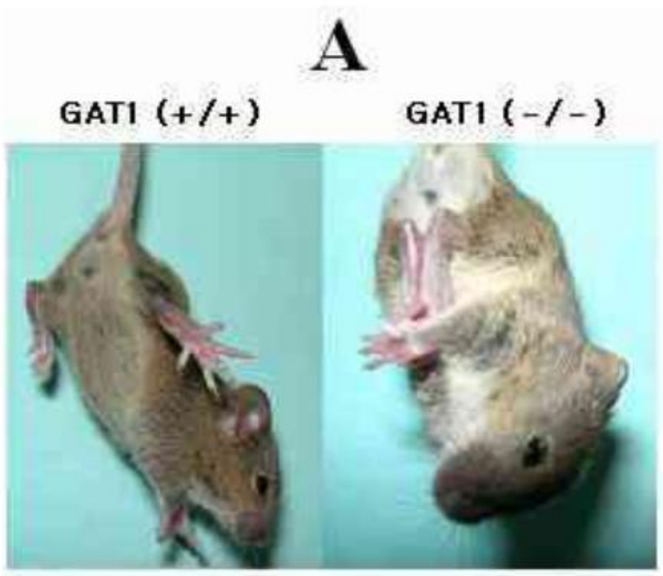


D. ***GABA uptake assay analysis of GAT1 function in brain and spinal cord***

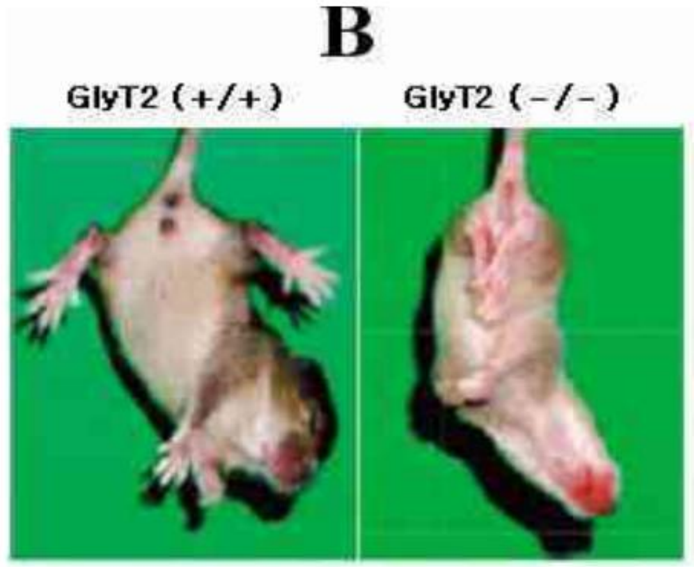




Obvious phenotype :appearance of spontaneous strong tremor in GAT1(-/-) mice



A. GAT1(-/-) mice show strong spontaneous tremor , ataxia, and nervousness

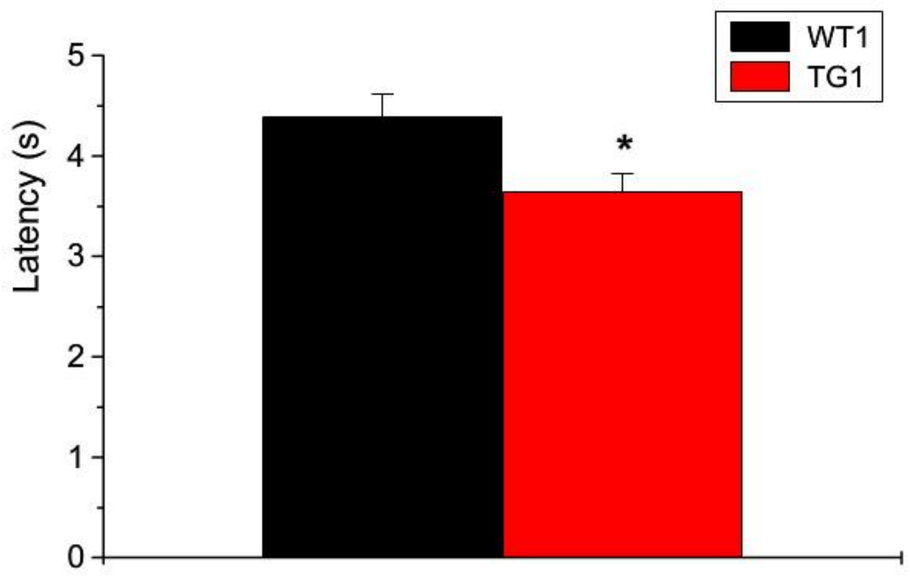


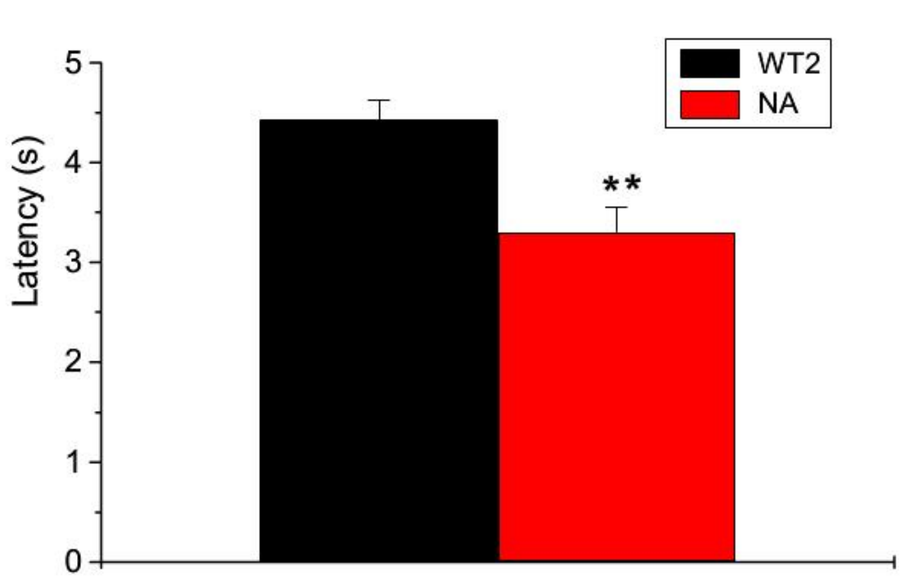
B. GlyT2(-/-) mice show strong spontaneous tremor
(Jesus et al. Neuron, 2003, 40:797-806)

*Hind feet clasping" phenotype in both GlyT2−/− mice and GAT1(−/−) mice

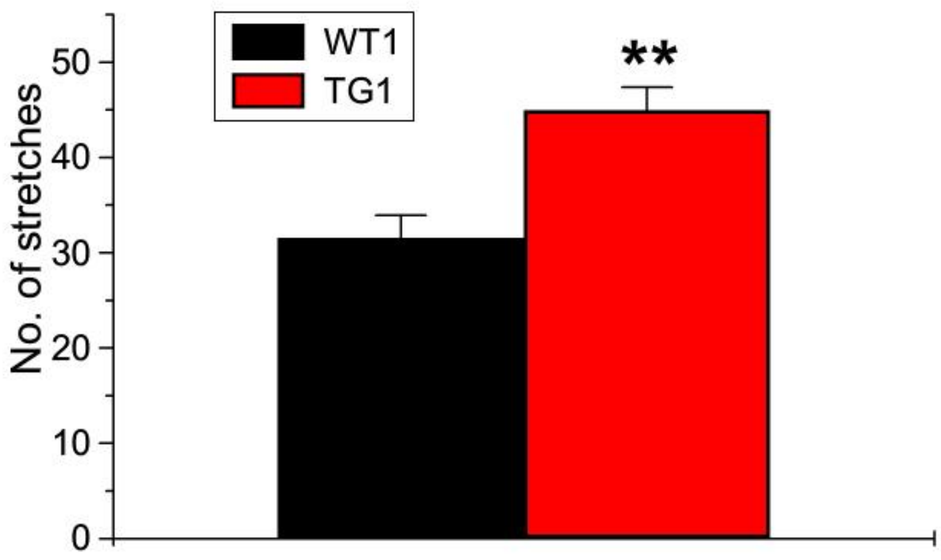
Function of GAT1 in Nociceptive Response

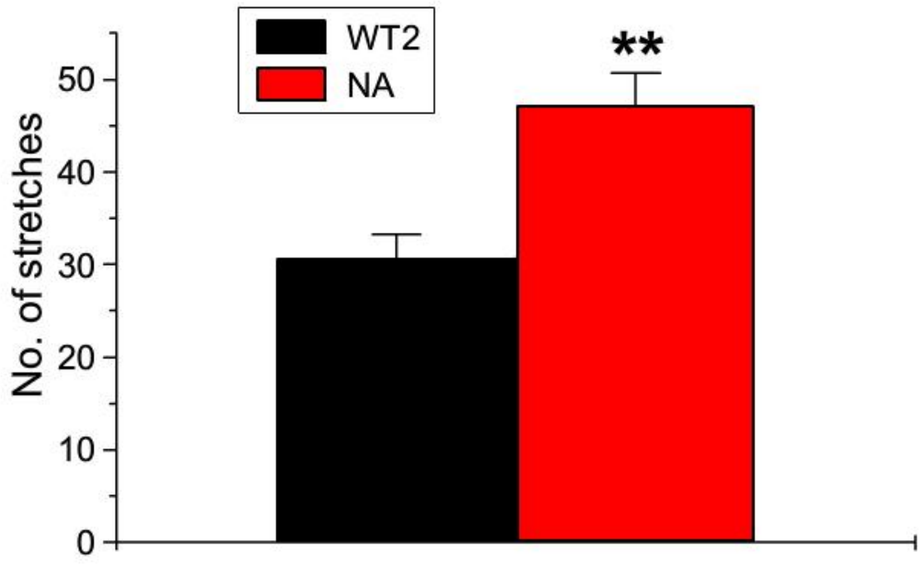
Hyperalgesic effect in GAT1 overexpressing mice—tail flick



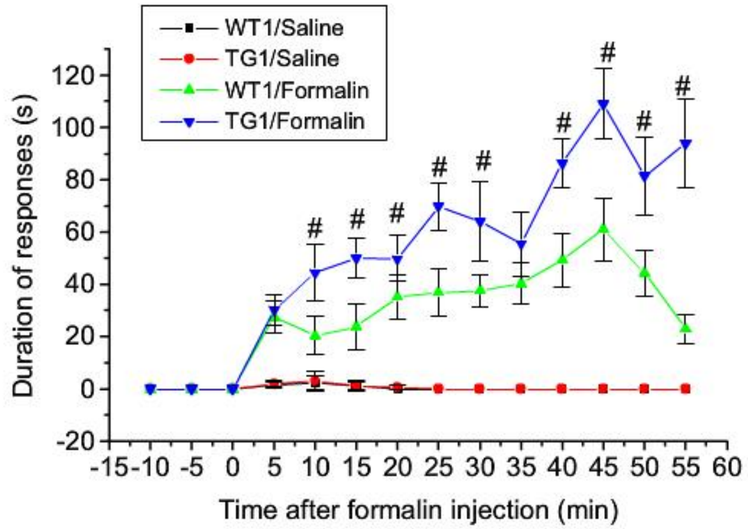


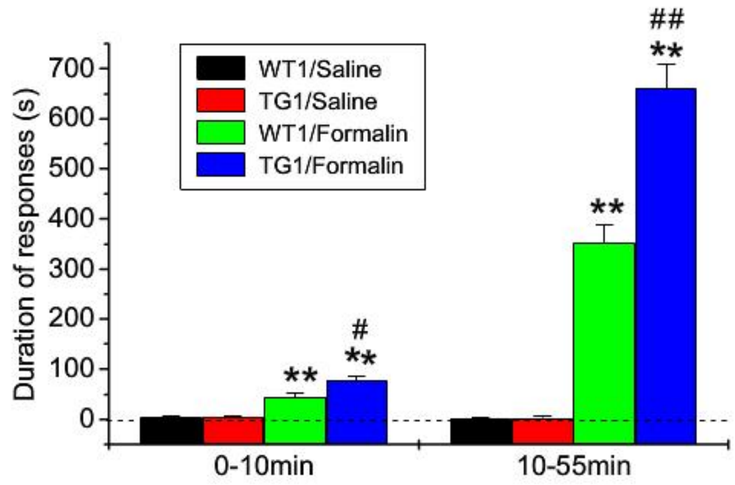
Hyperalgesic effect in GAT1 overexpressing mice—visceral pain

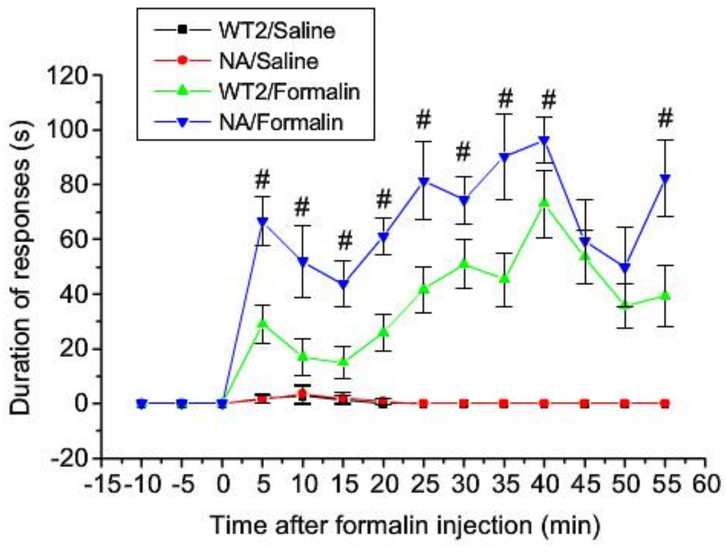


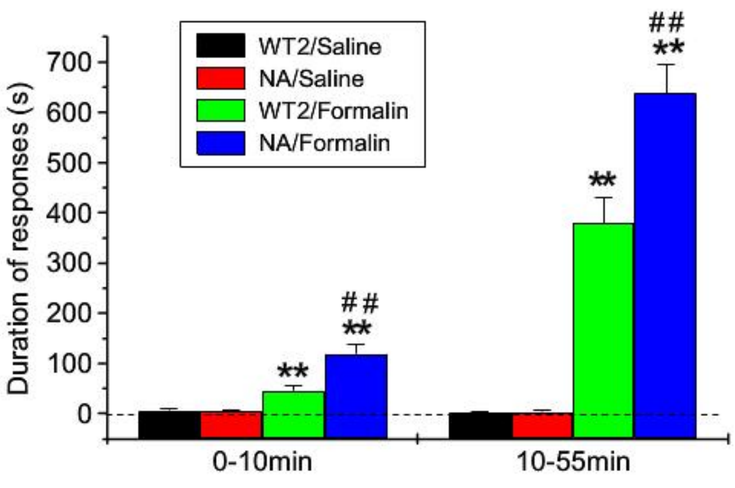


Hyperalgesic effect in GAT1 overexpressing mice—inflammatory pain



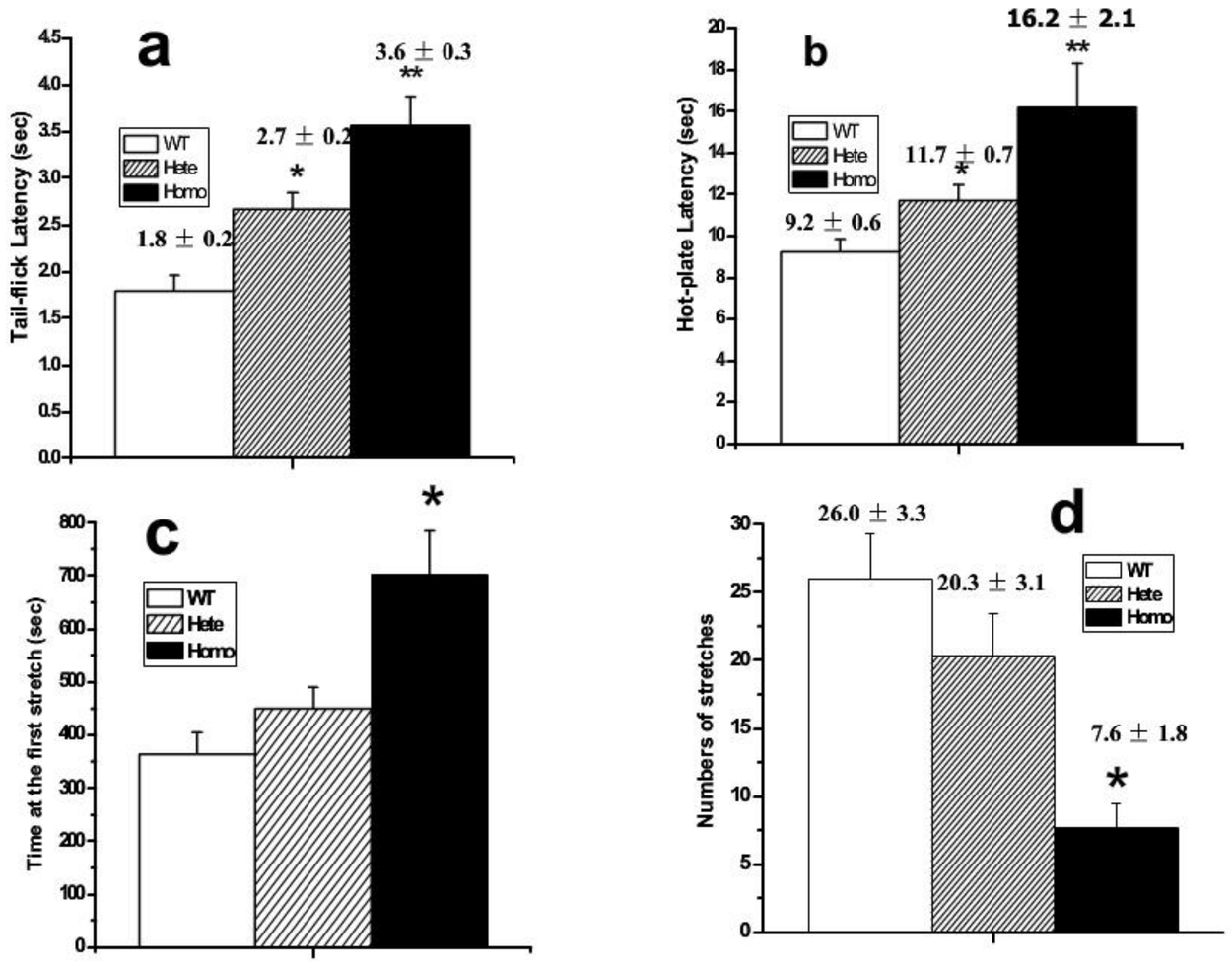






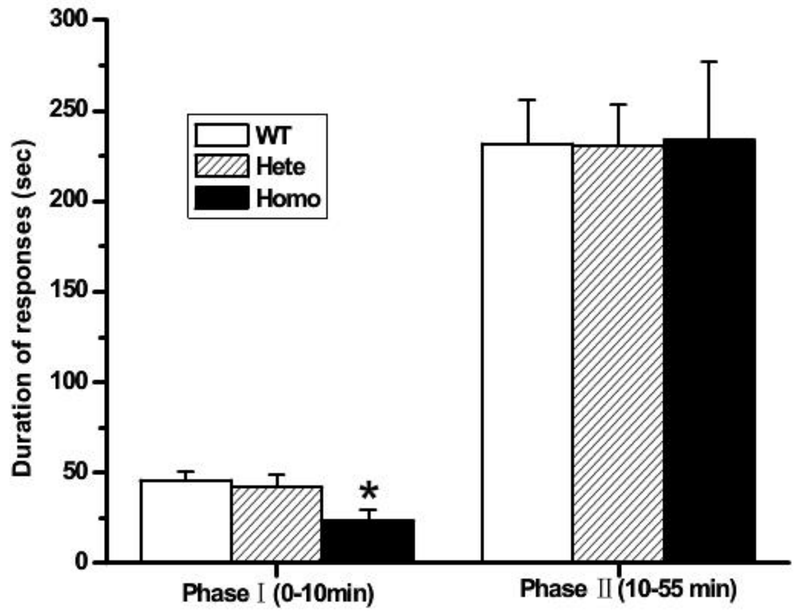
Antinociceptive effects in GAT1-deficient mice

(1). Acute thermal and visceral nociceptive responses



Antinociceptive effects in GAT1-deficient mice

(2). The Formalin Test

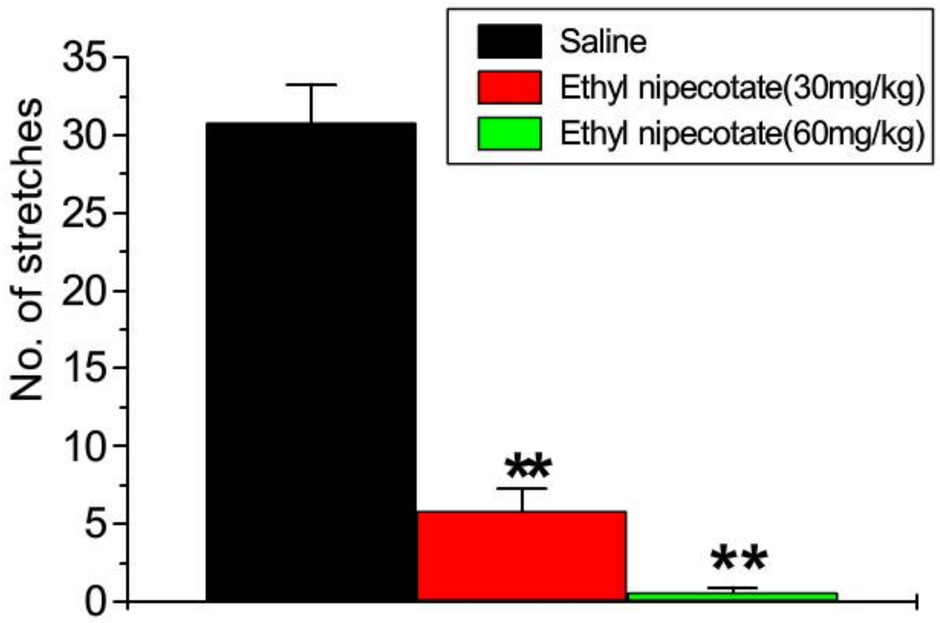


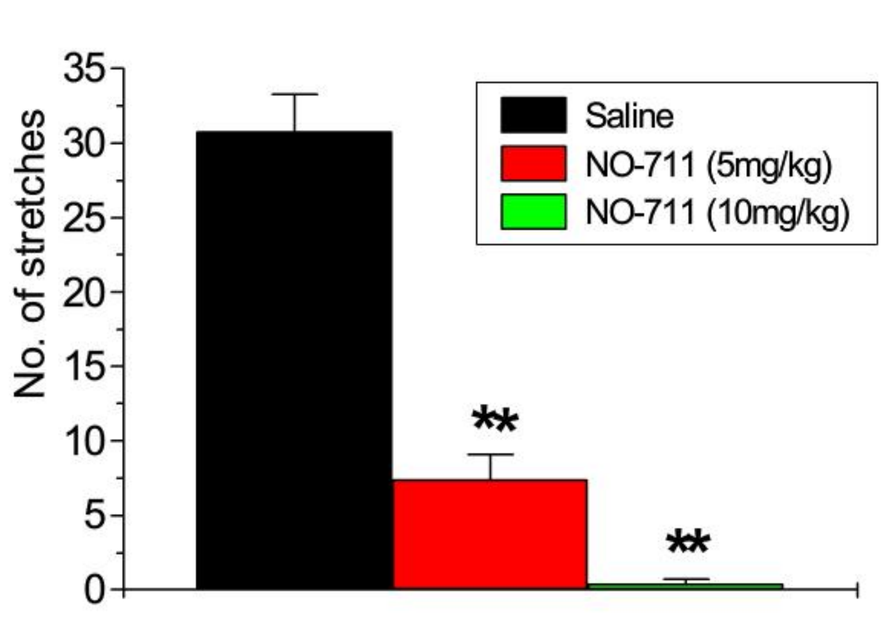
#GAT1(-/-) mice show decreased nociceptive reponses during the acute phase（Phase I，0-10 min), but not in the late phase(10-45min).

The duration of lifting, shaking, licking and biting the injected paw in the formalin test were recorded

Analgesic Effect of GAT1 antagonists (II)

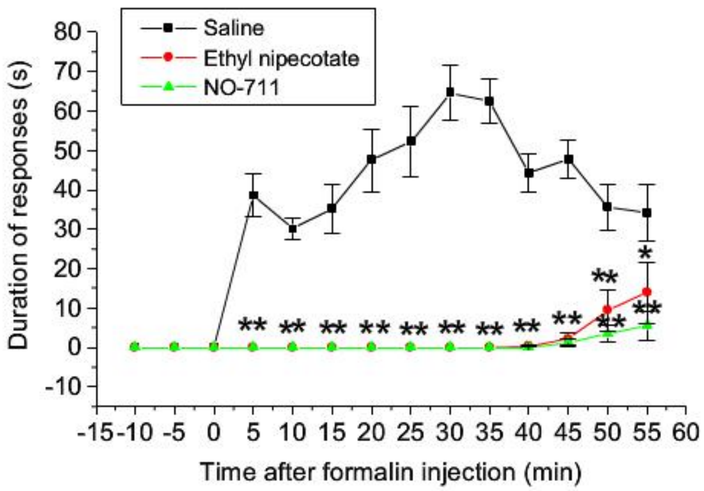
—visceral pain

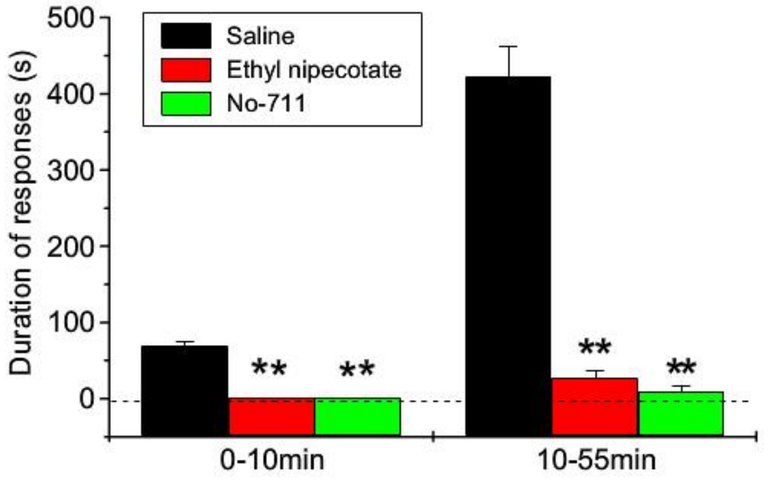


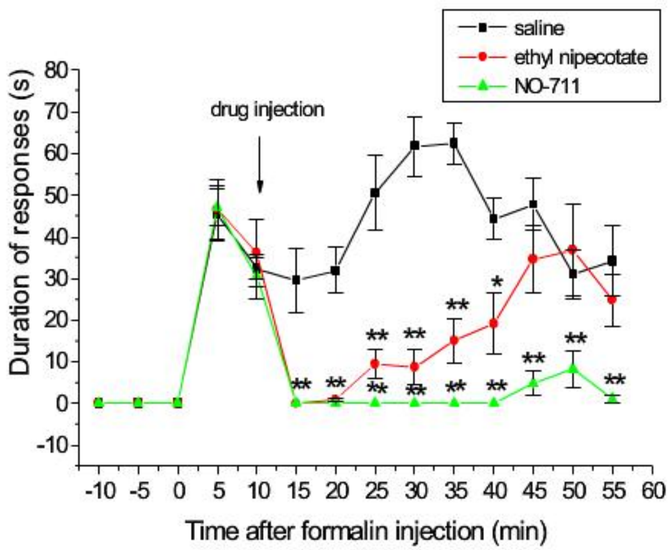


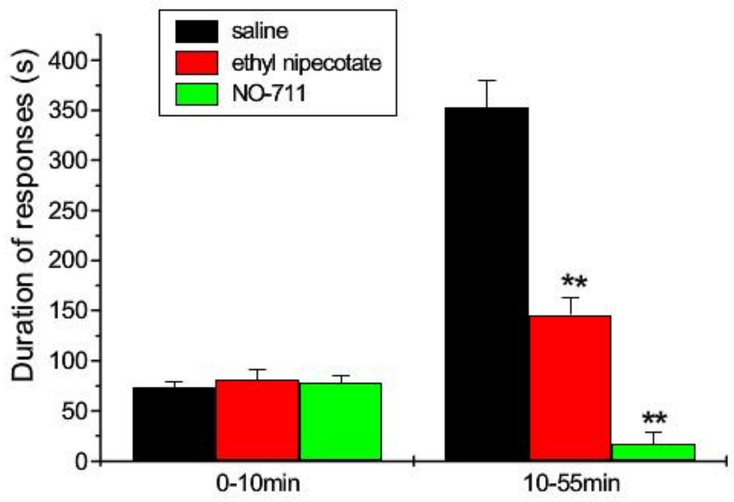
Analgesic Effect of GAT1 antagonists (III)

—inflammatory pain



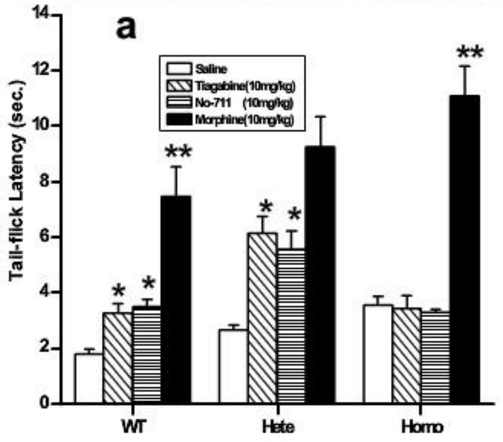


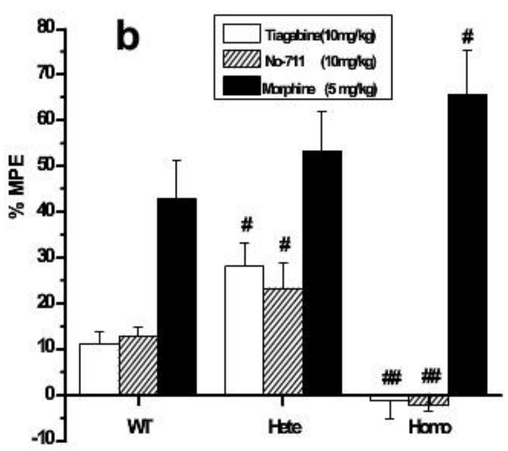




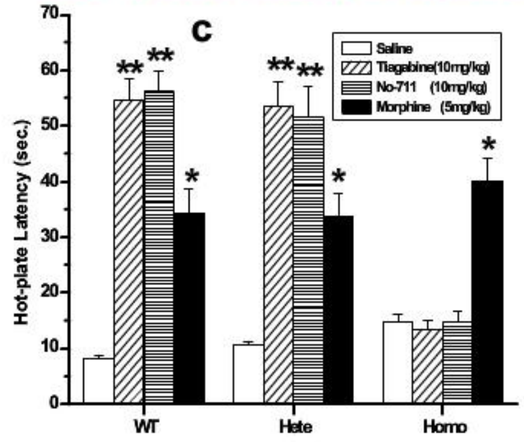
Analgesic effects of GAT1 selective inhibitors and morphine on wt and GAT1 KO mice

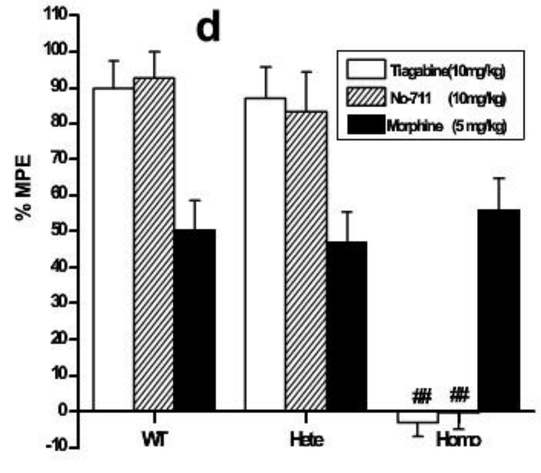
Tail-flick test(a and b)



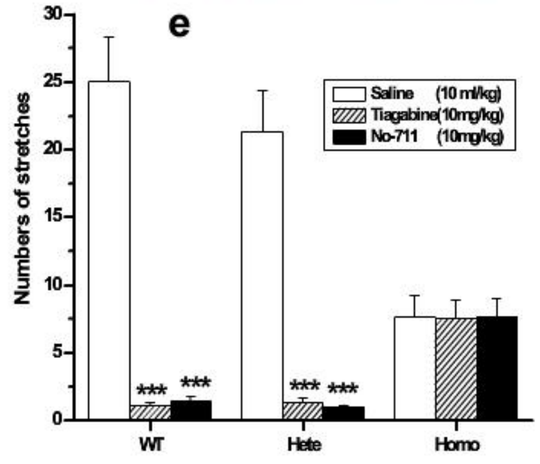


Hot-plate test(c and d)

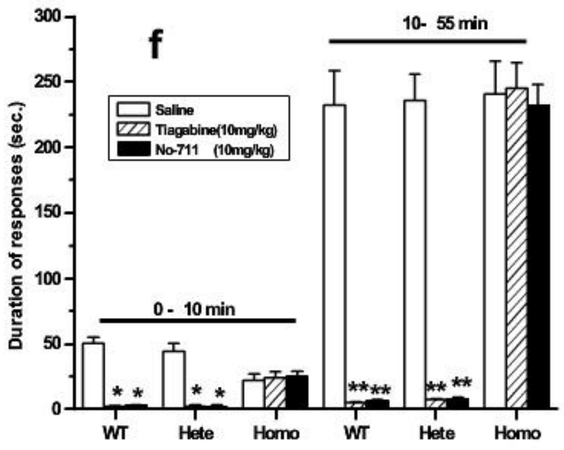




e. Acetic acid test

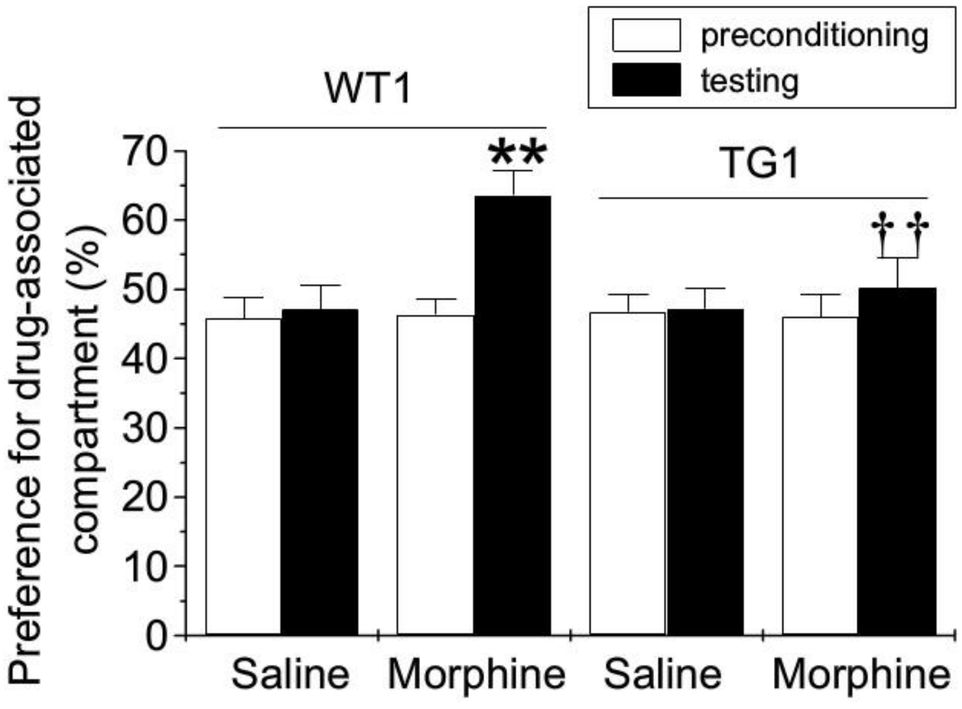


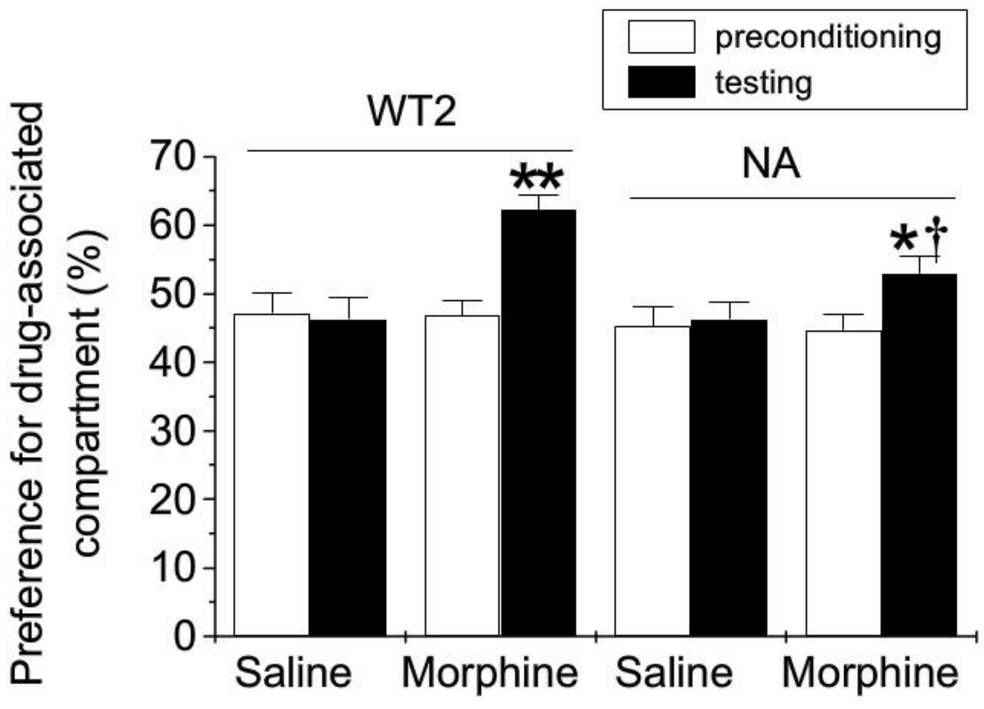
f. Formalin test

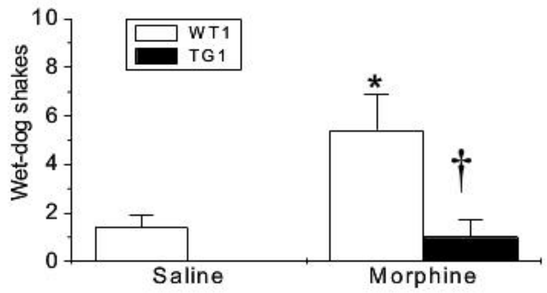


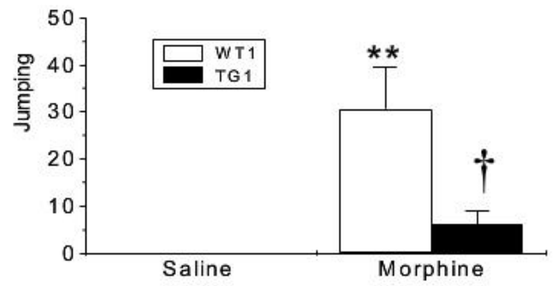
Function of GAT1 in addiction

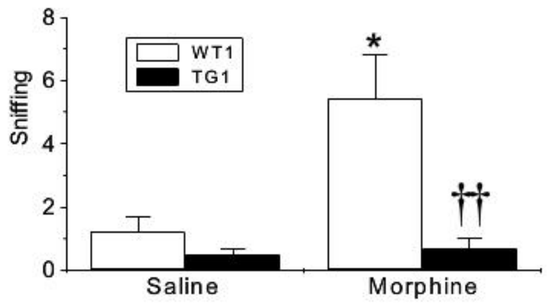
Decreased morphine-induced place preference (CPP) in GAT1 overexpressing mice



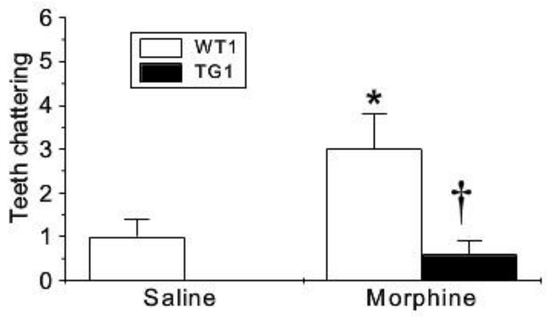


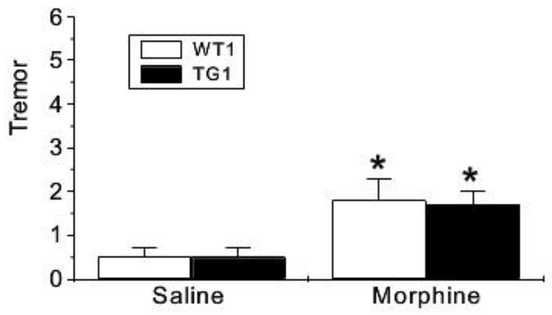


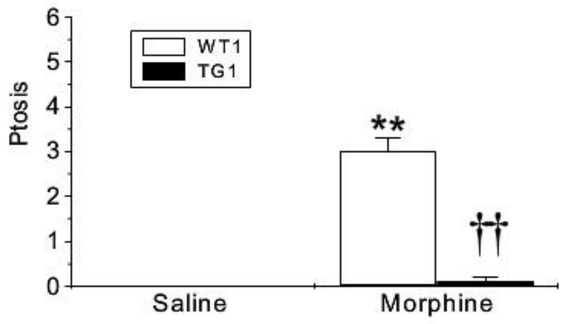


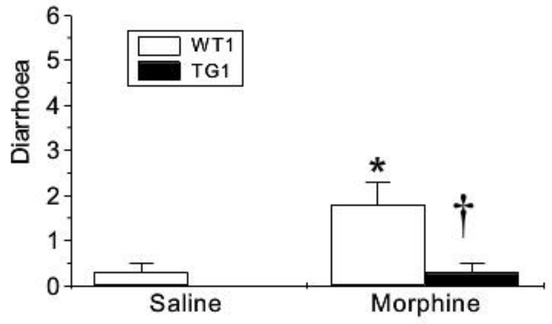


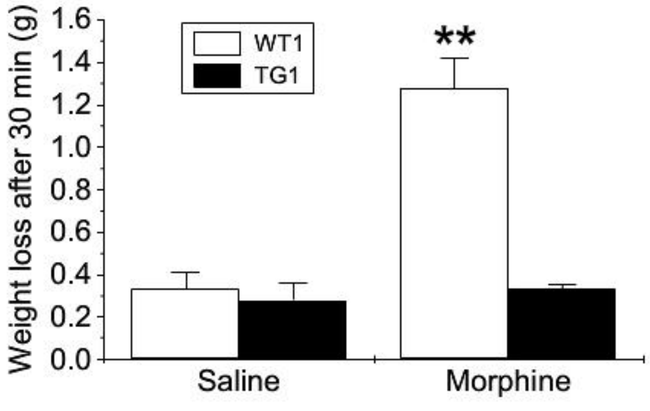
Naloxone-precipitated morphine withdrawal syndrome in TG1 mice

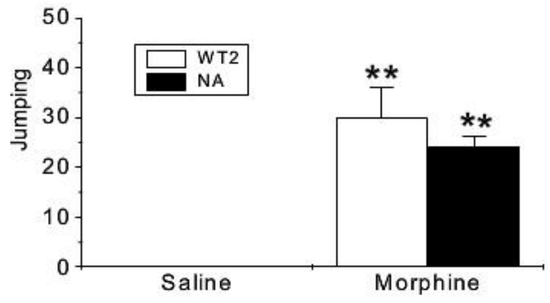


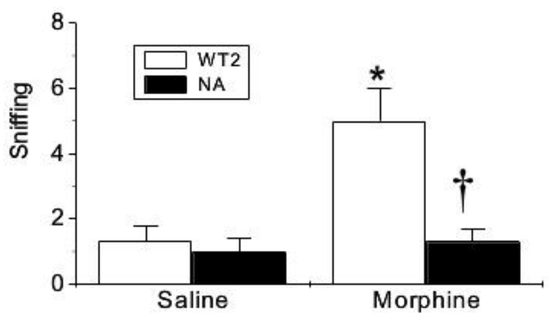


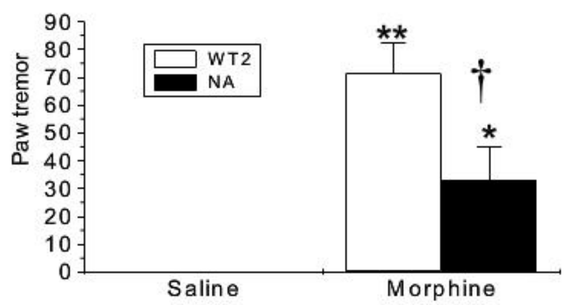




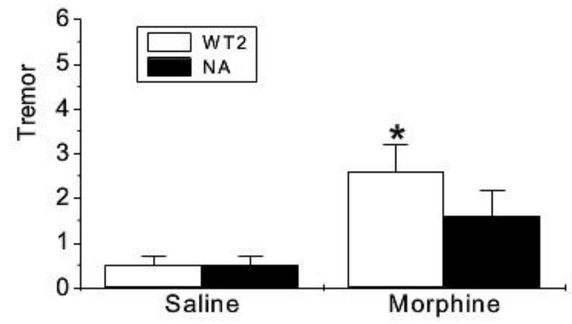


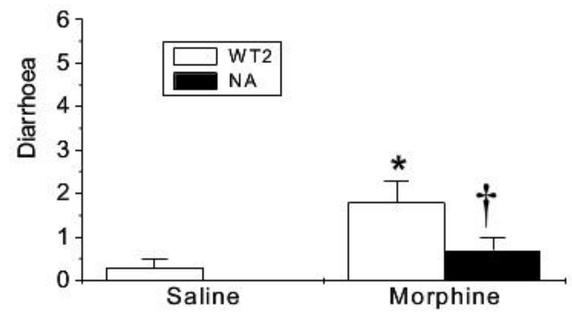


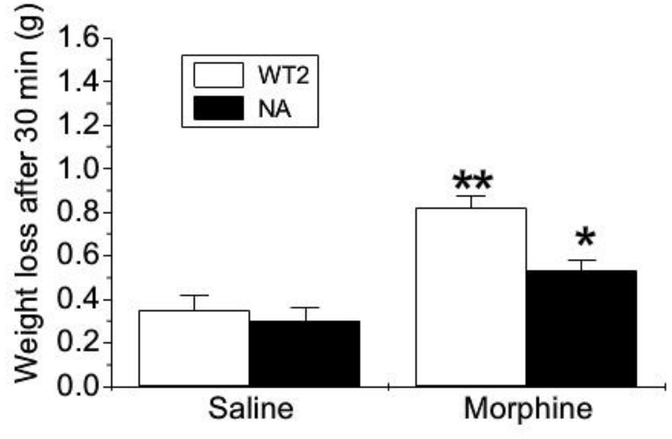




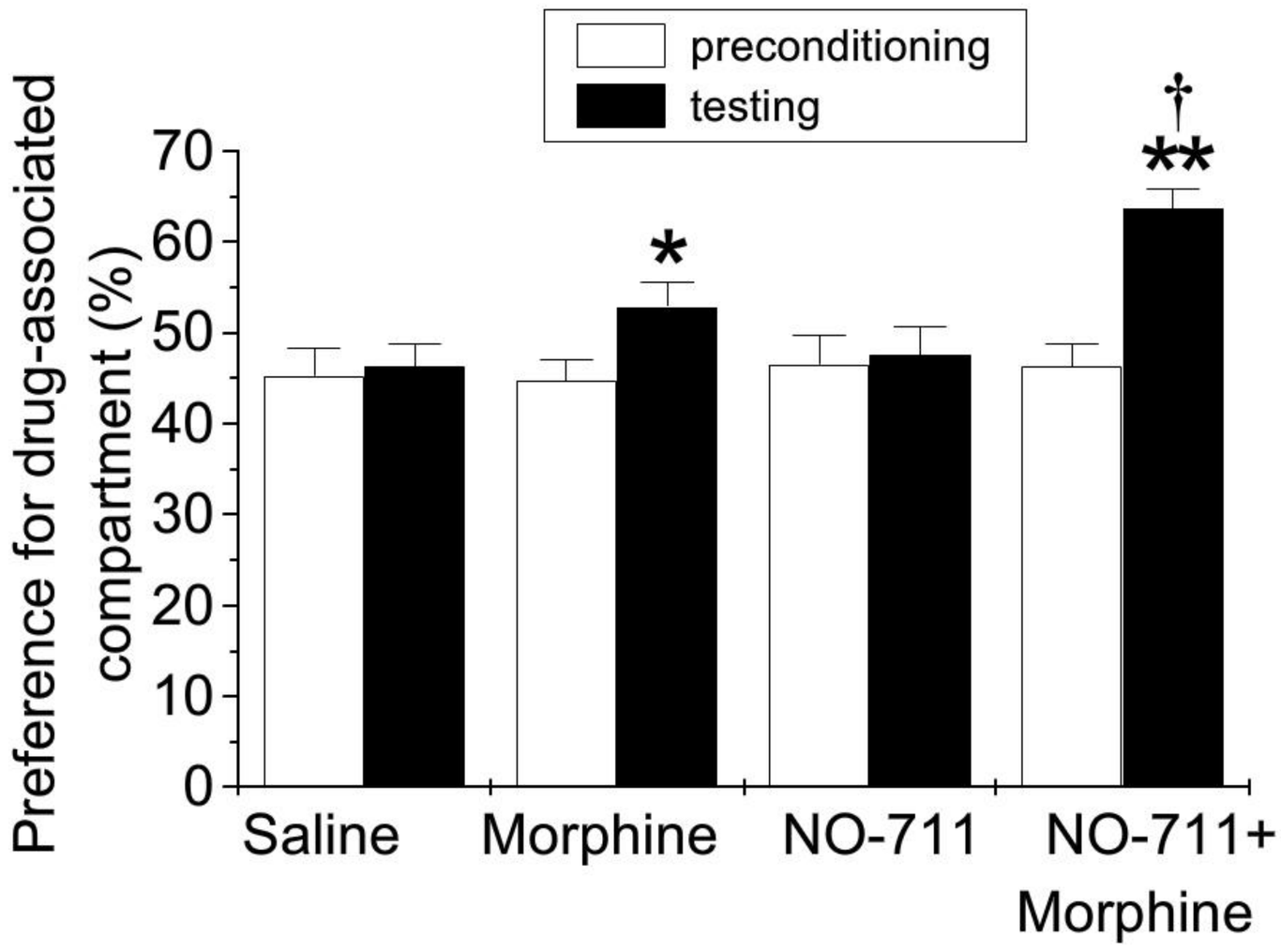
Naloxone-precipitated morphine withdrawal syndrome in NA mice



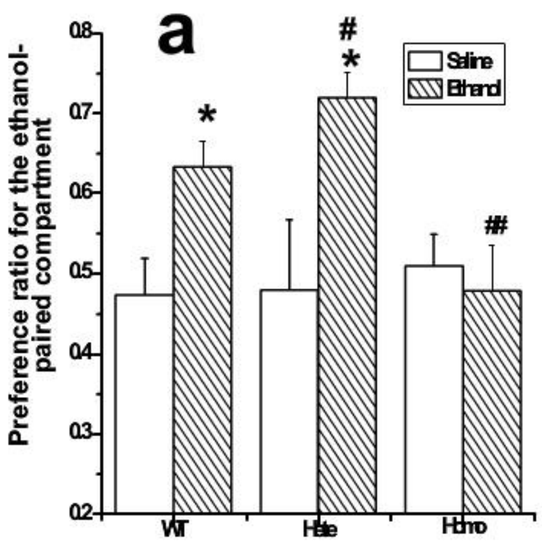


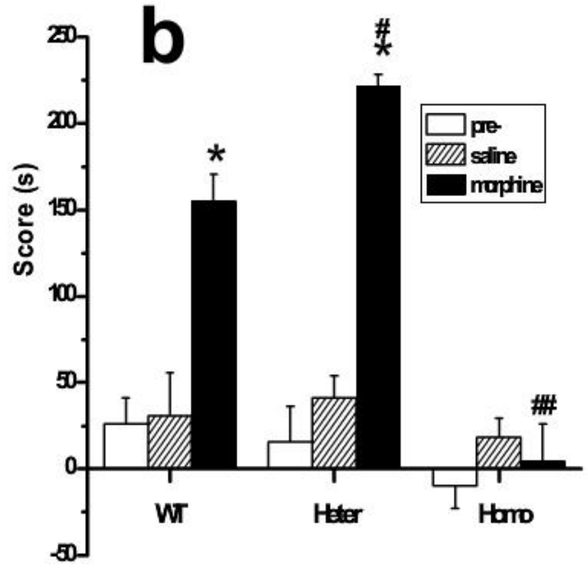


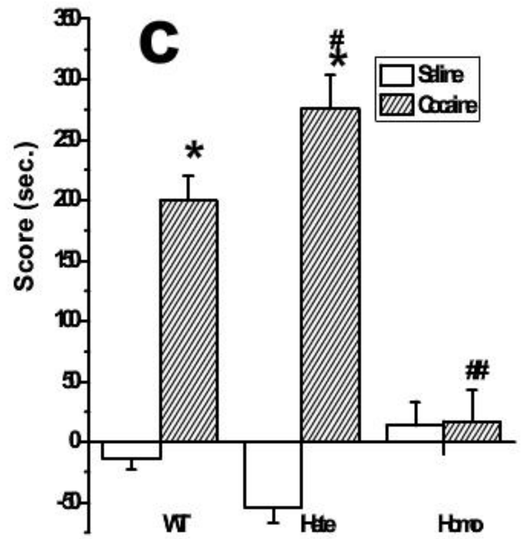
Recovery from decreased rewarding effects in NA mice after NO-711 treatment



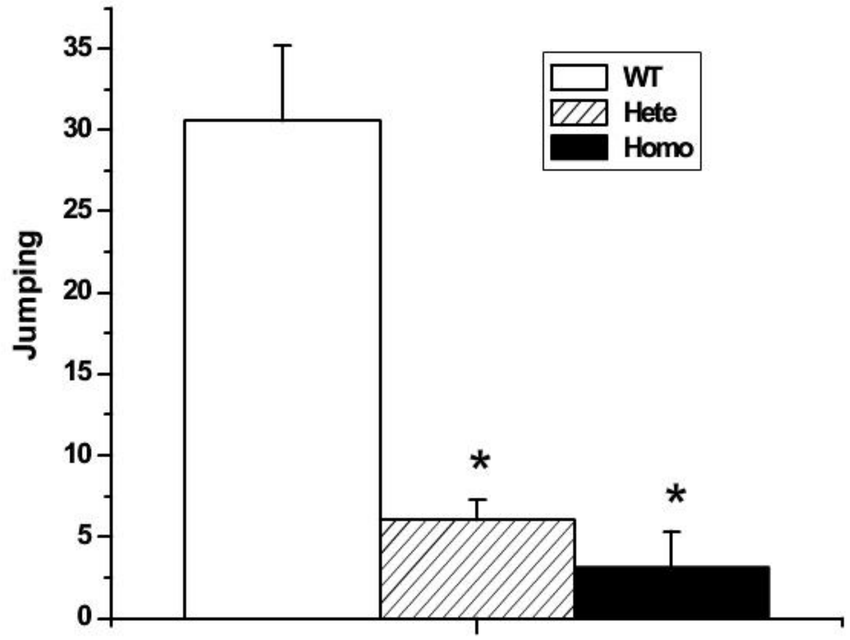
GAT1-deficient mice and the rewarding effects of drugs(ethanol, morphine, and cocaine)







Analysis of naloxone-precipiated morphine withdrawal syndrome



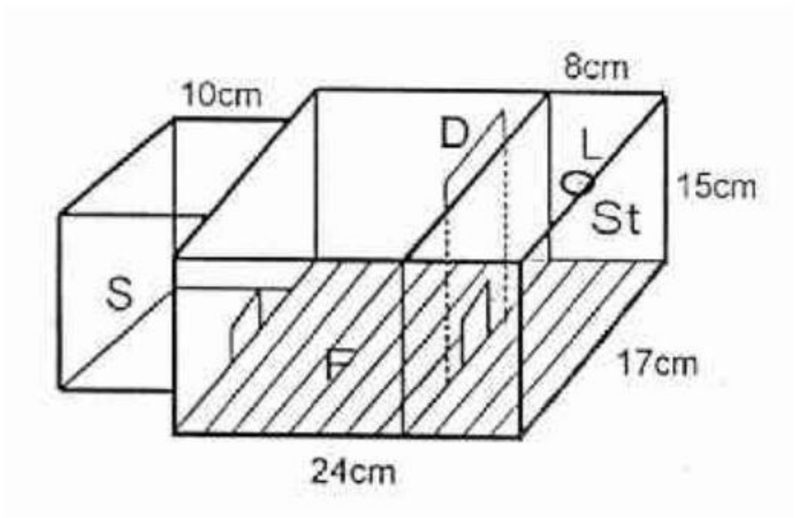
Jumping times: 30.6±4.6, 6±1.3 and 3.1±2.2 for wild-type, heterozygous and homozygous mice, respectively)

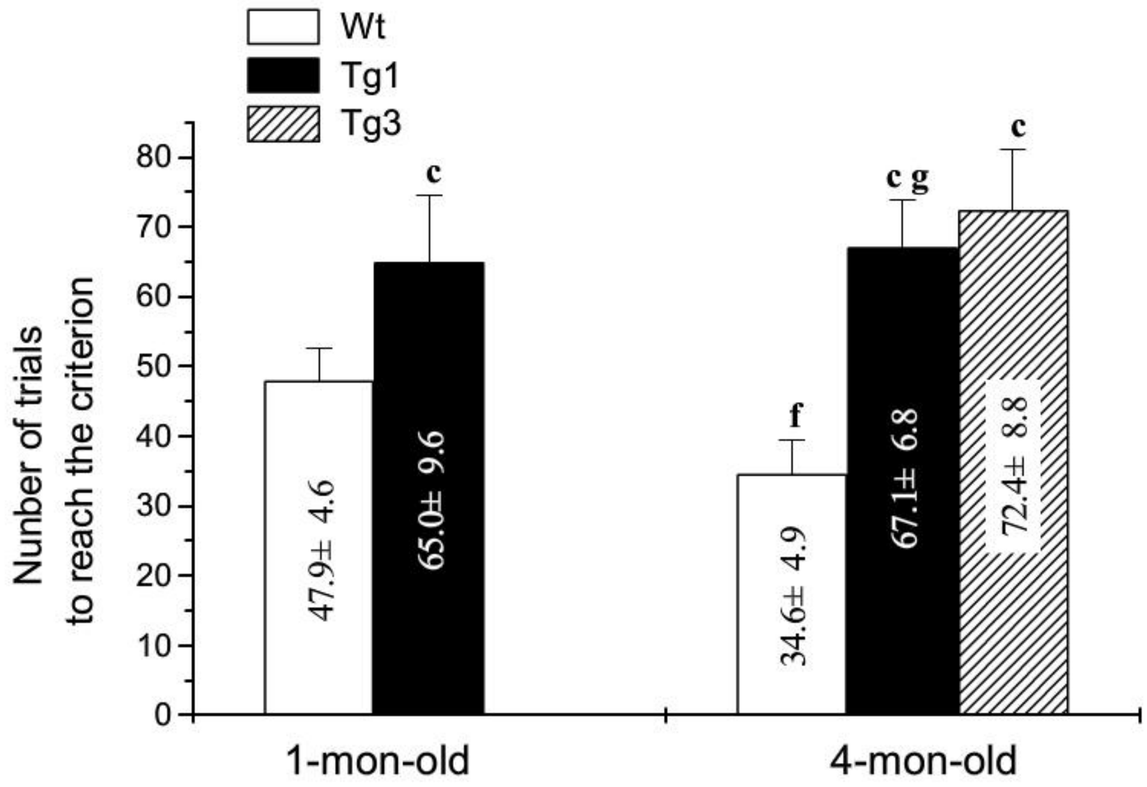
There is no difference in other abstinence somatic signs (e.g. wet-dog shakes, teeth chattering, tremor, sniffing, paw tremor, Ptosis, and diarrhea)

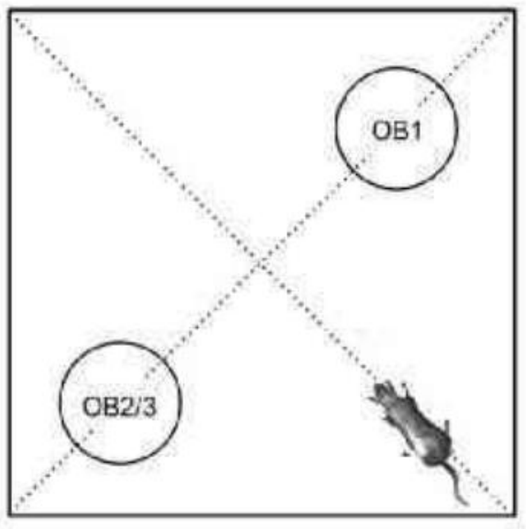
Function of GAT1 in Learning and Memory

Associative learning

Conditioned avoidance task

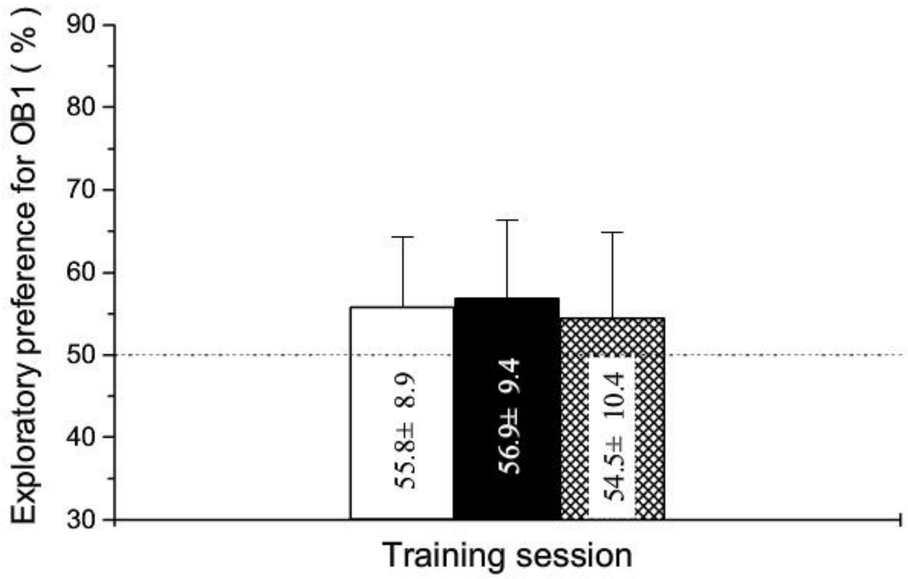






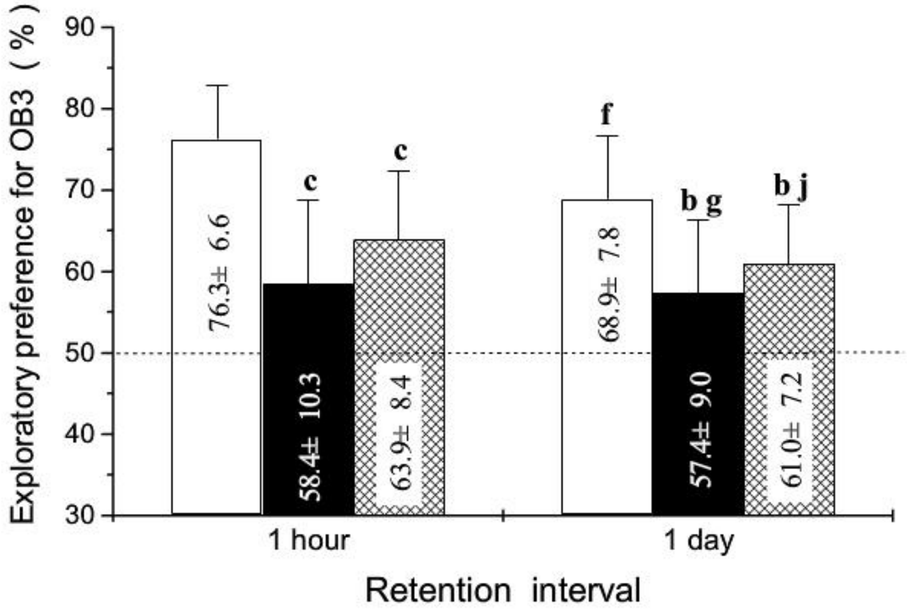
Visual recognition memory

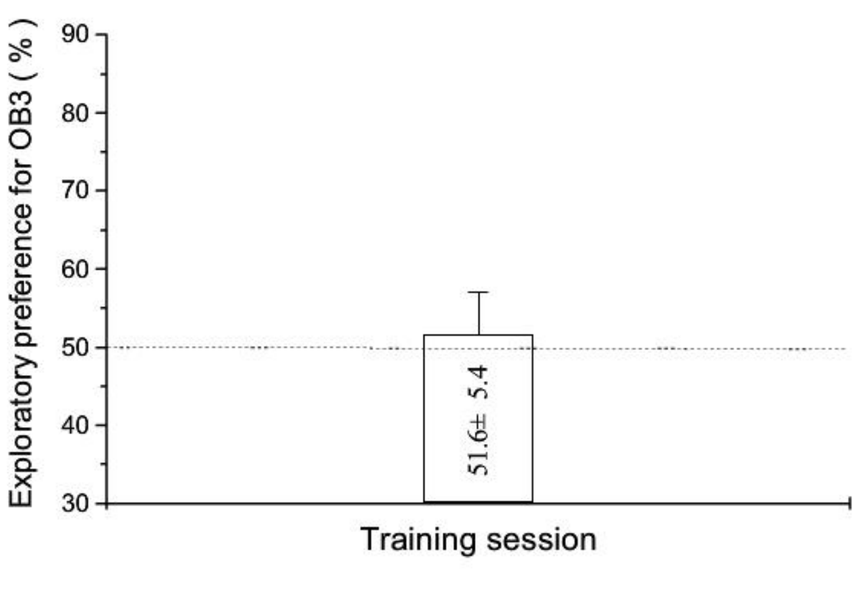
Novel-object recognition task



Visual recognition memory

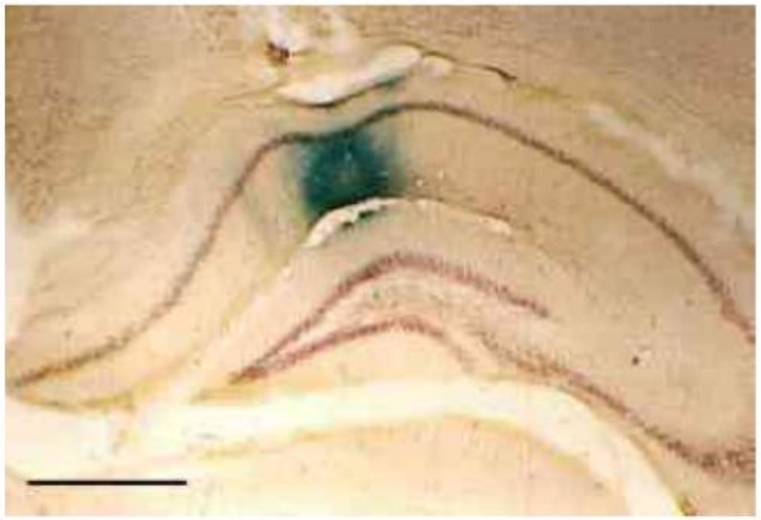
Memory retention

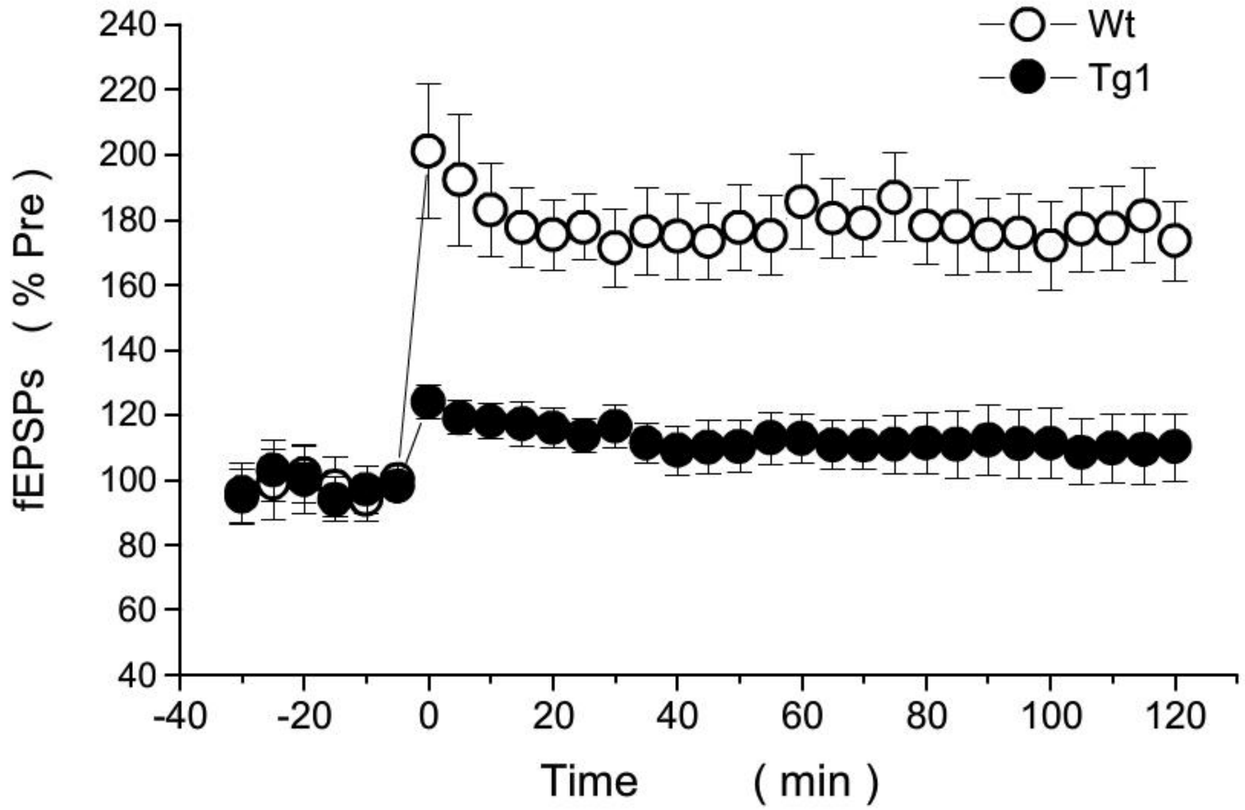




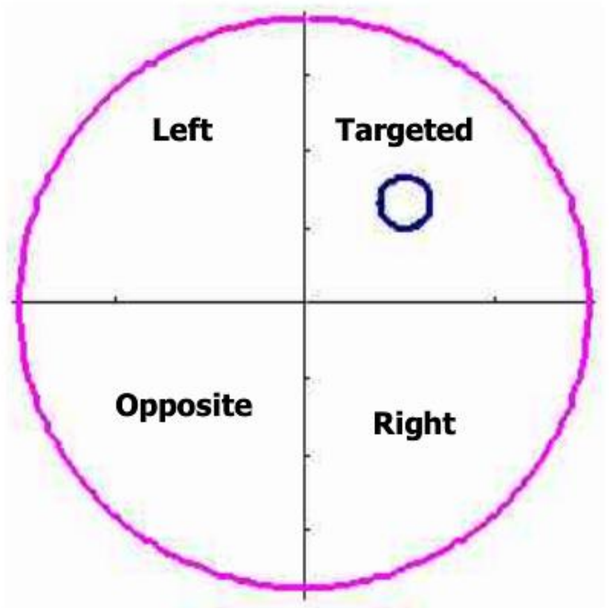
Synapse plasticity

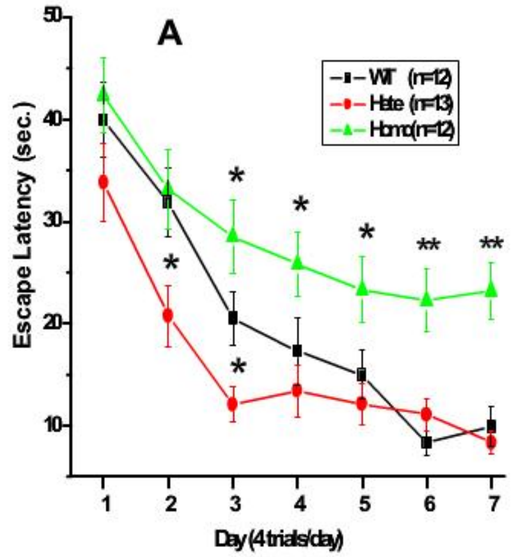
Long-term potentiation

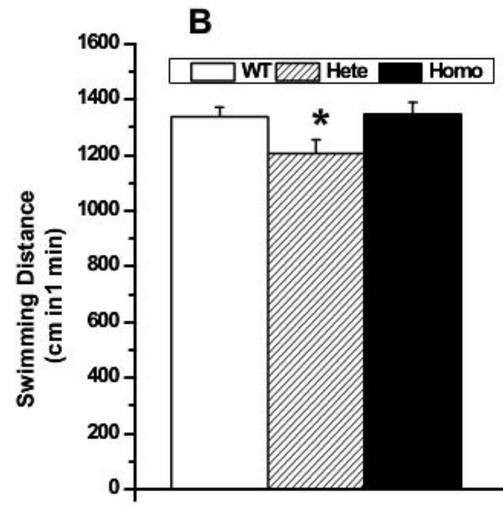


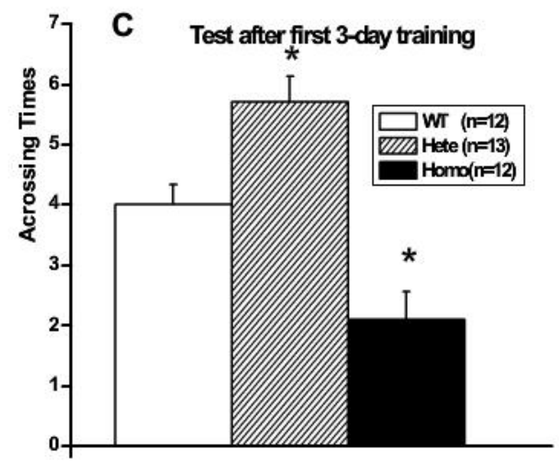


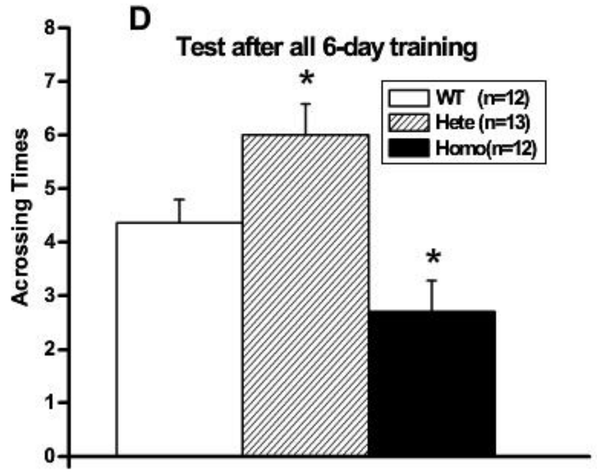
Effect of GAT1 dysfunction on the hidden-platform Morris water maze test (I)





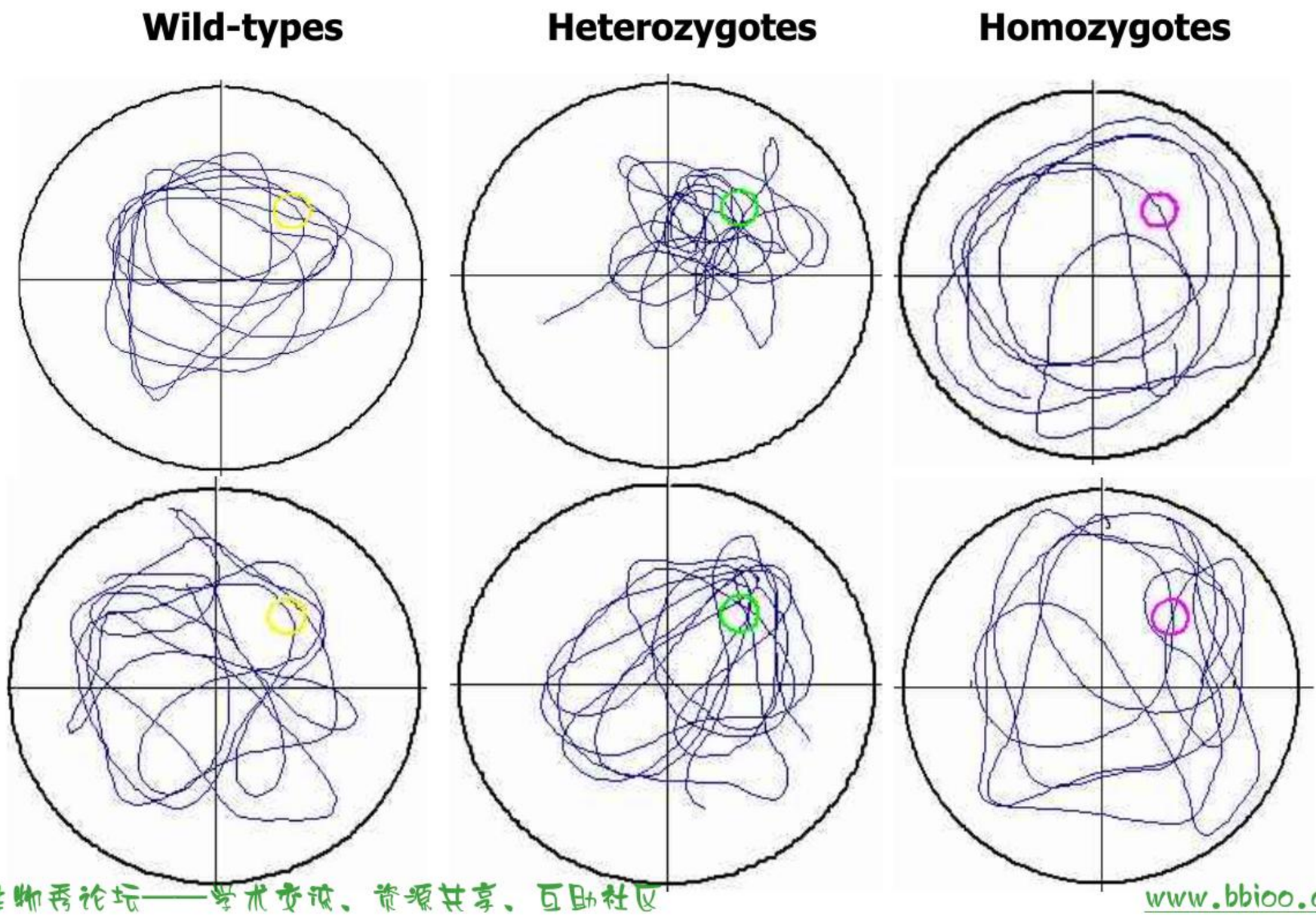




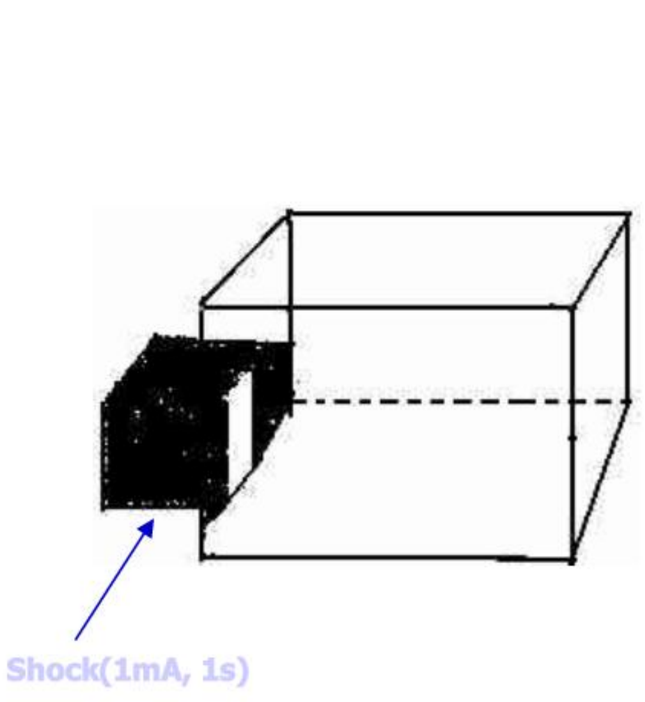


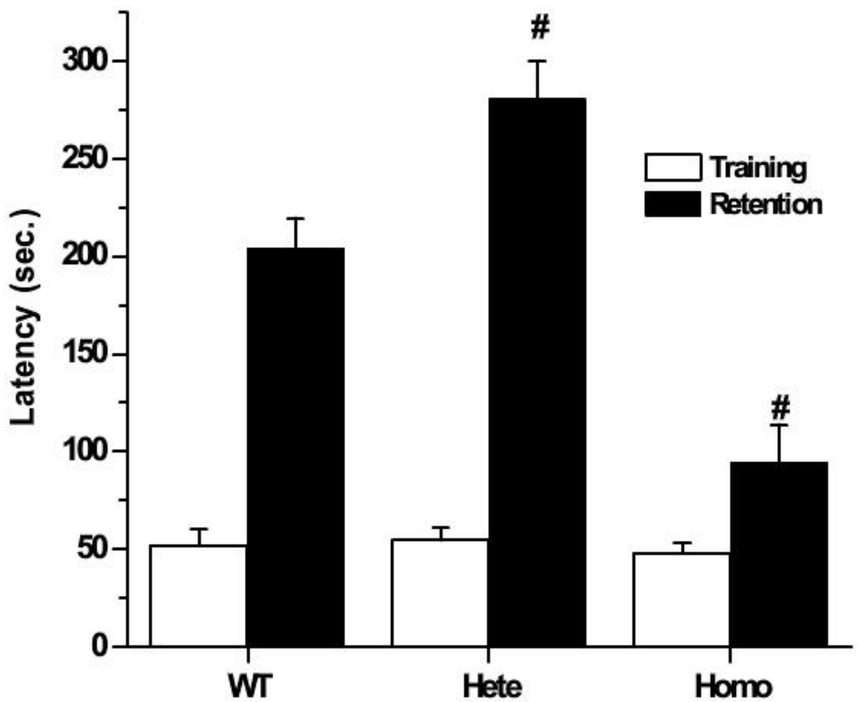
In b: Distance traveled 1336.5 ± 35.5 (+/+), 1206.4±49.7(+/-) and 1349.0±41.1 cm(-/-)

The navigation routes during the last transfer test in the Morris water maze test

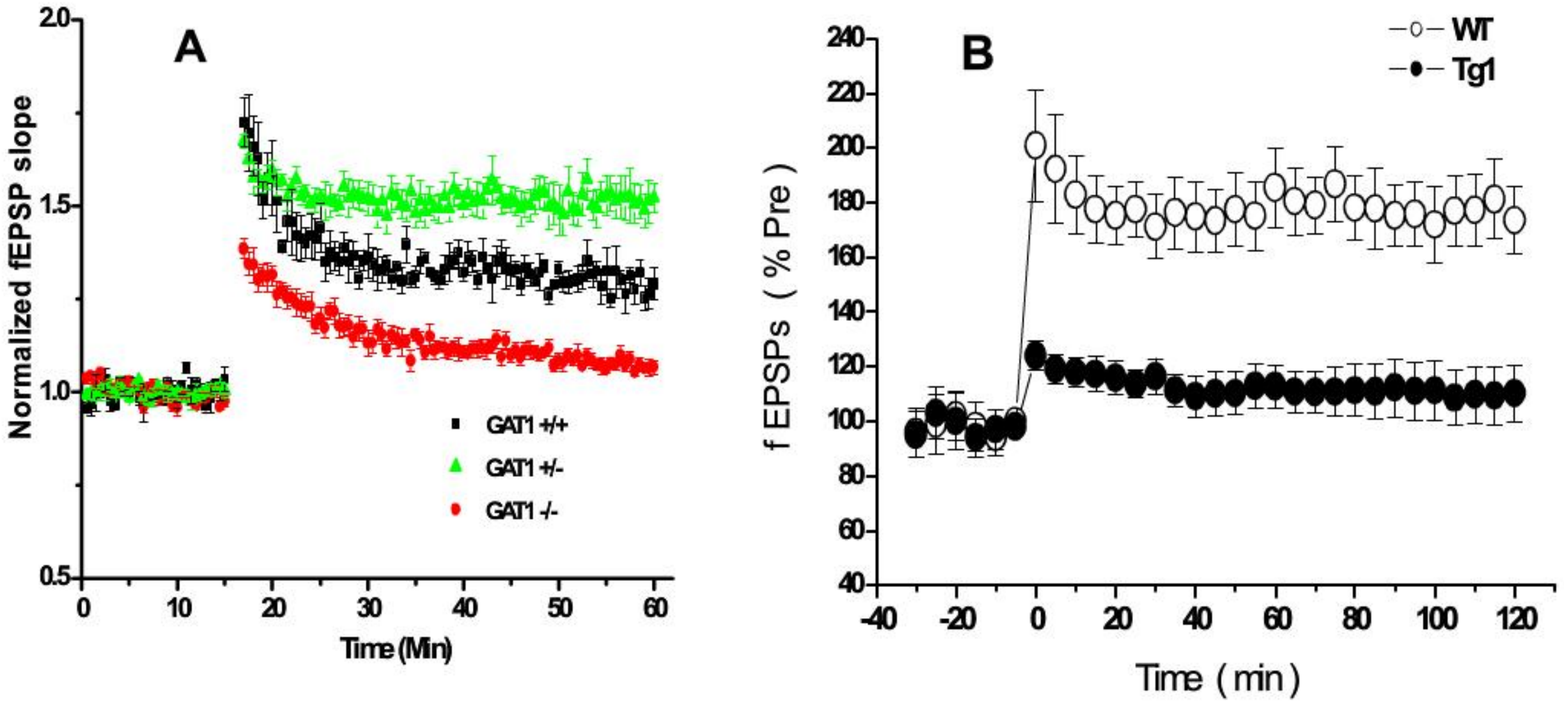


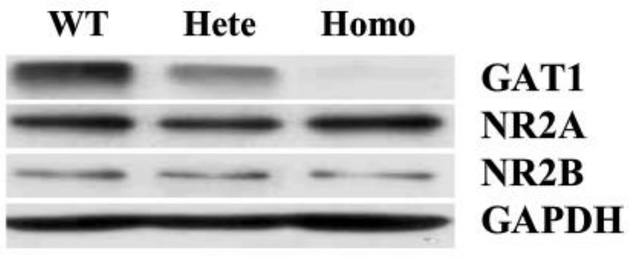
Effect of GAT1 dysfunction on passive avoidance learning



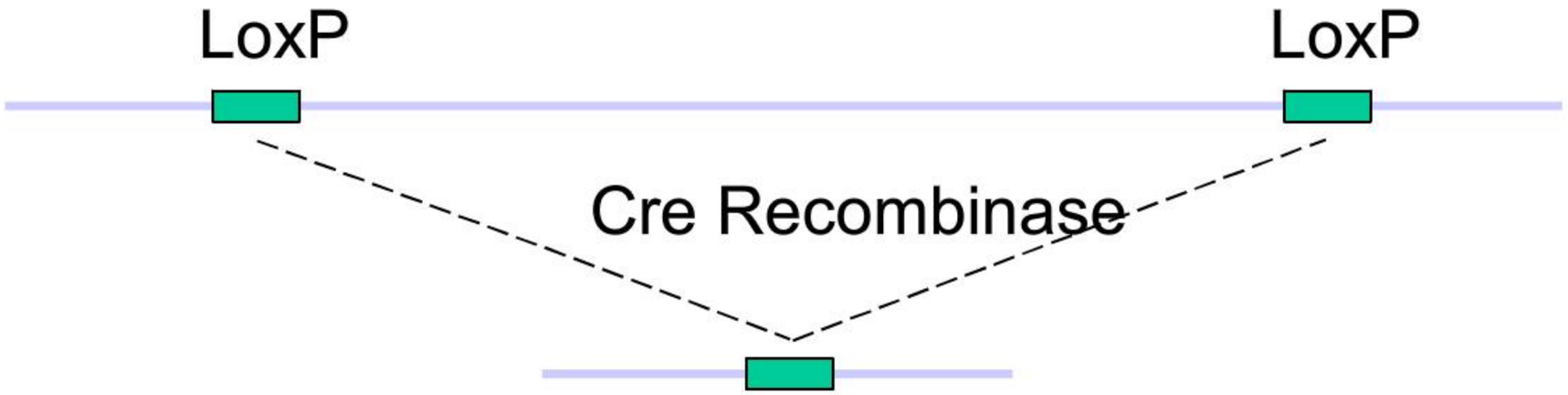


Altered long-term potentiation (LTP) in the hippocampal CA1 field in the GAT1-deficient mice and the transgenic mice



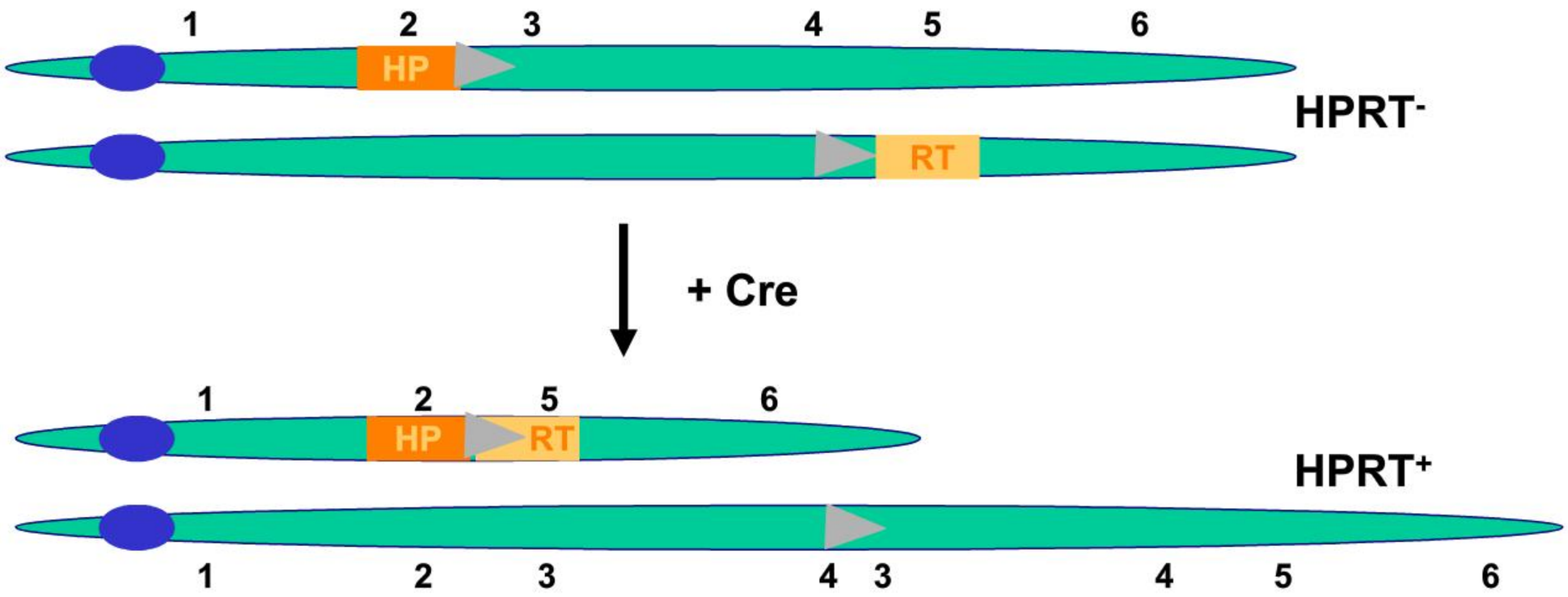


Chromosome Engineering in Mice



- Engineer genetic reagents genome-wide
  - Deletions
  - Inversions
  - Duplications
- Track deletions with coat color transgene

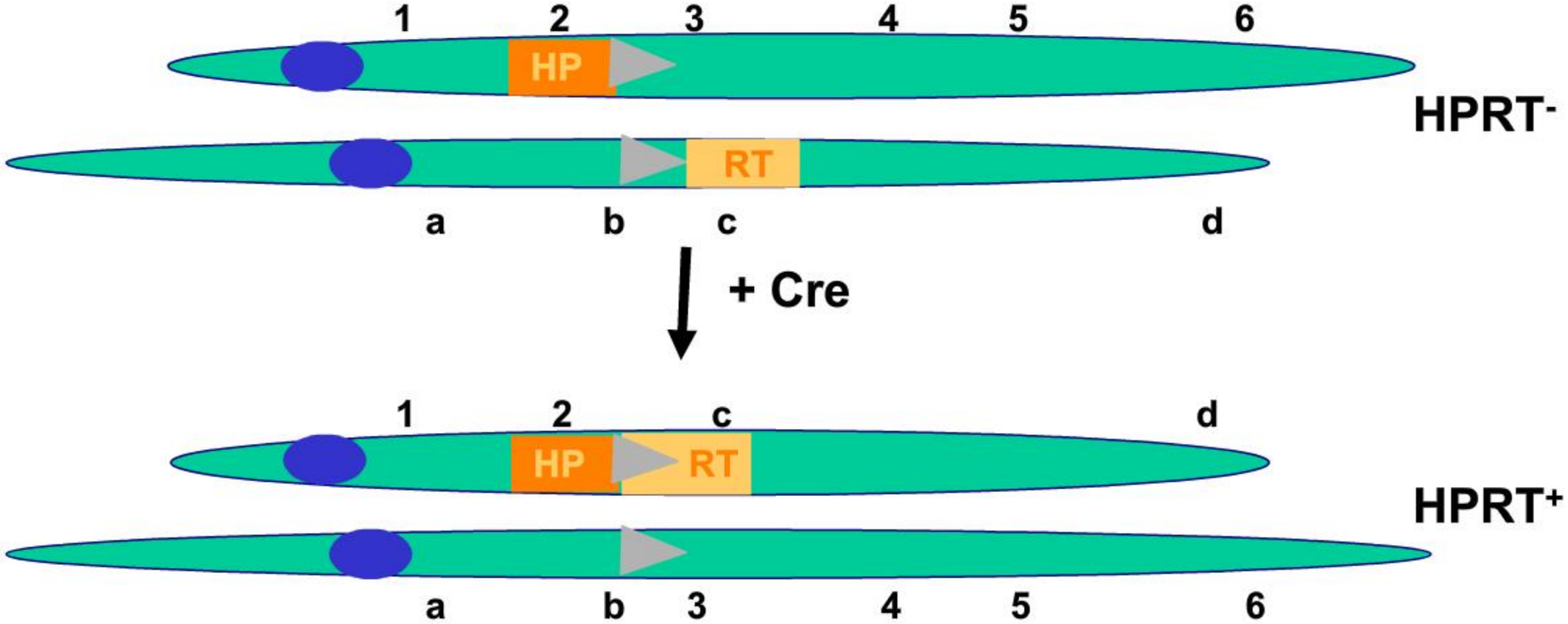
Chromosomal Alterations with Cre 2



HAT selection survival

(1 deletion and 1 duplication)

Chromosomal Alterations with Cre 3



HAT selection survival

(reciprocal translocation)

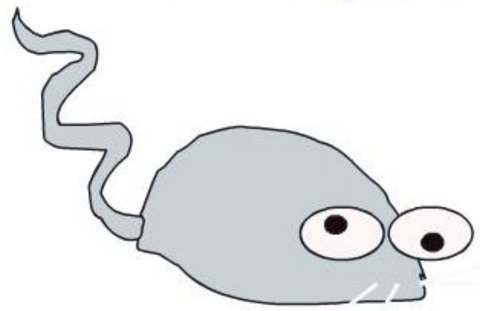
Gene Traps

Gene driven

Phenotypes

Phenotype driven

ENU



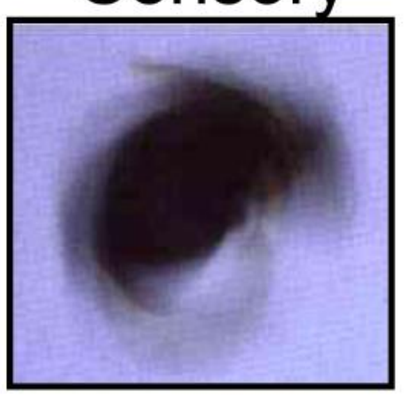
Human Disease Models

Immunity & Hematopoiesis

Cancer & Development

Cardiovascular
Endocrine
Metabolism

Neurological & Sensory

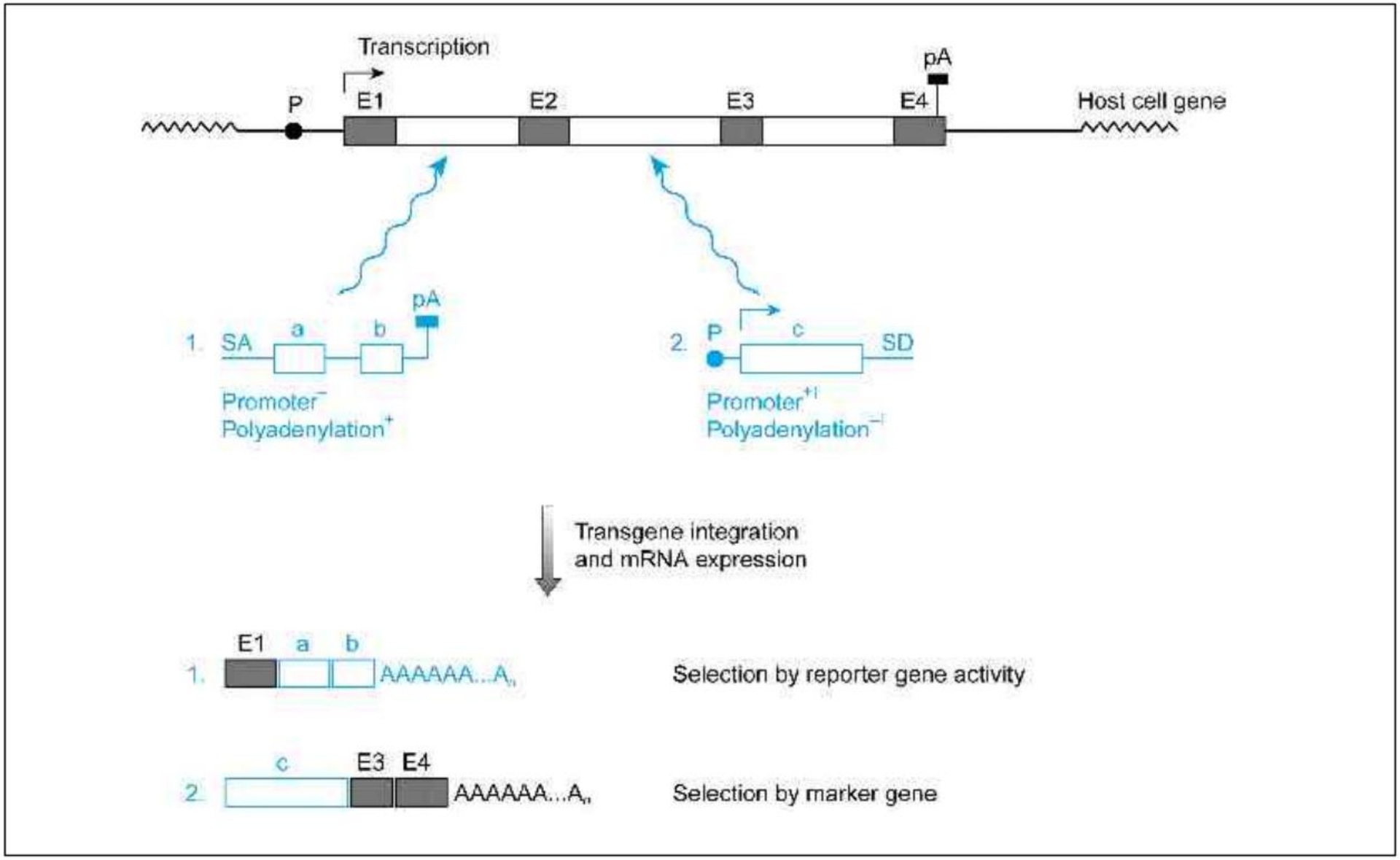


Musculoskeletal
Dental

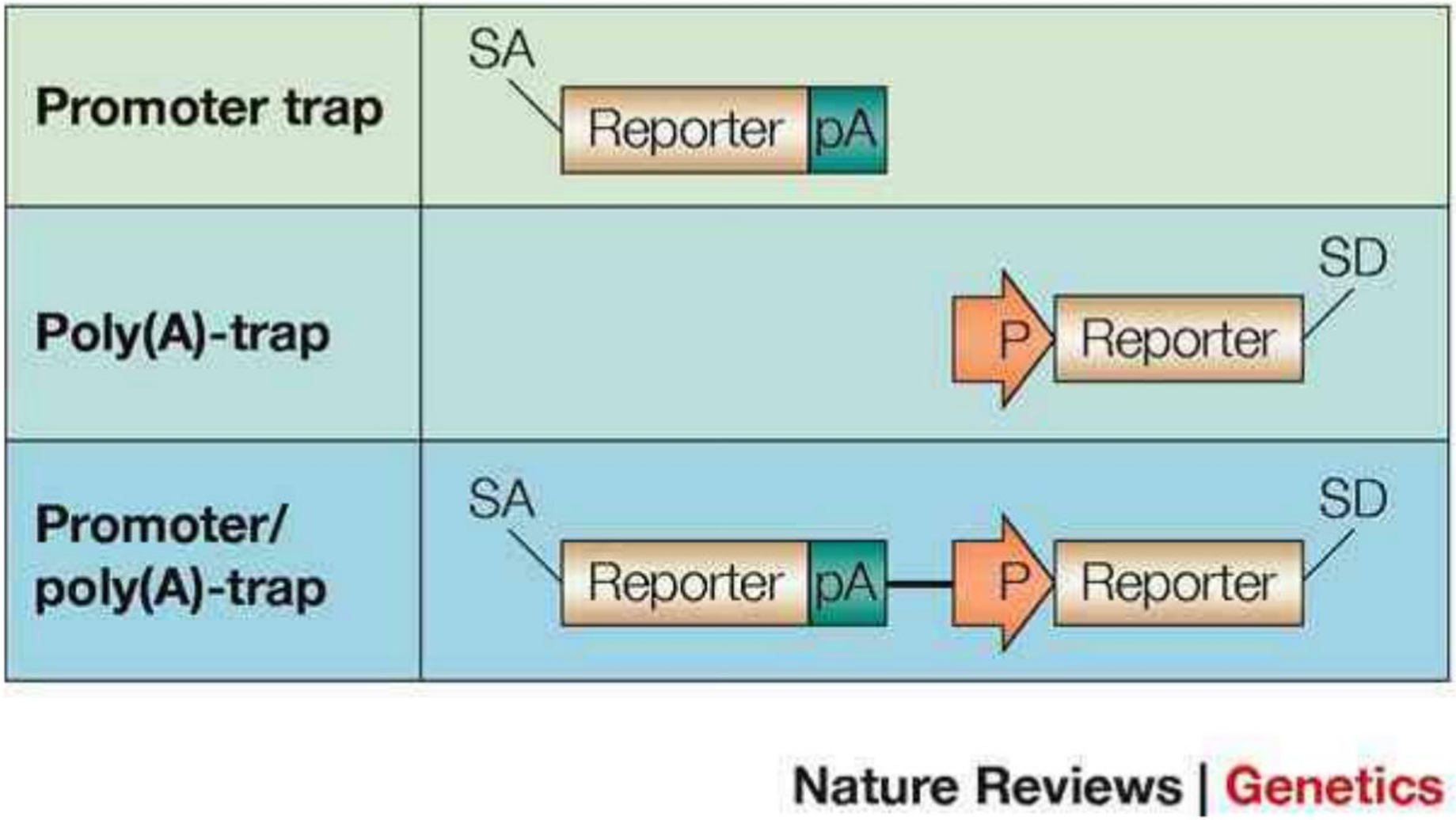
GENE TRAPPING USING EXPRESSION-DEFECTIVE TRANSGENES

1. promoter-less reporter gene for gene trapping

2. pA-less selection marker for polyA-addition trapping



P, promoter; E1-E4, exons1-4; pA, polyadenylation signal; SA, splice acceptor sequence, SD, splice donor sequence;



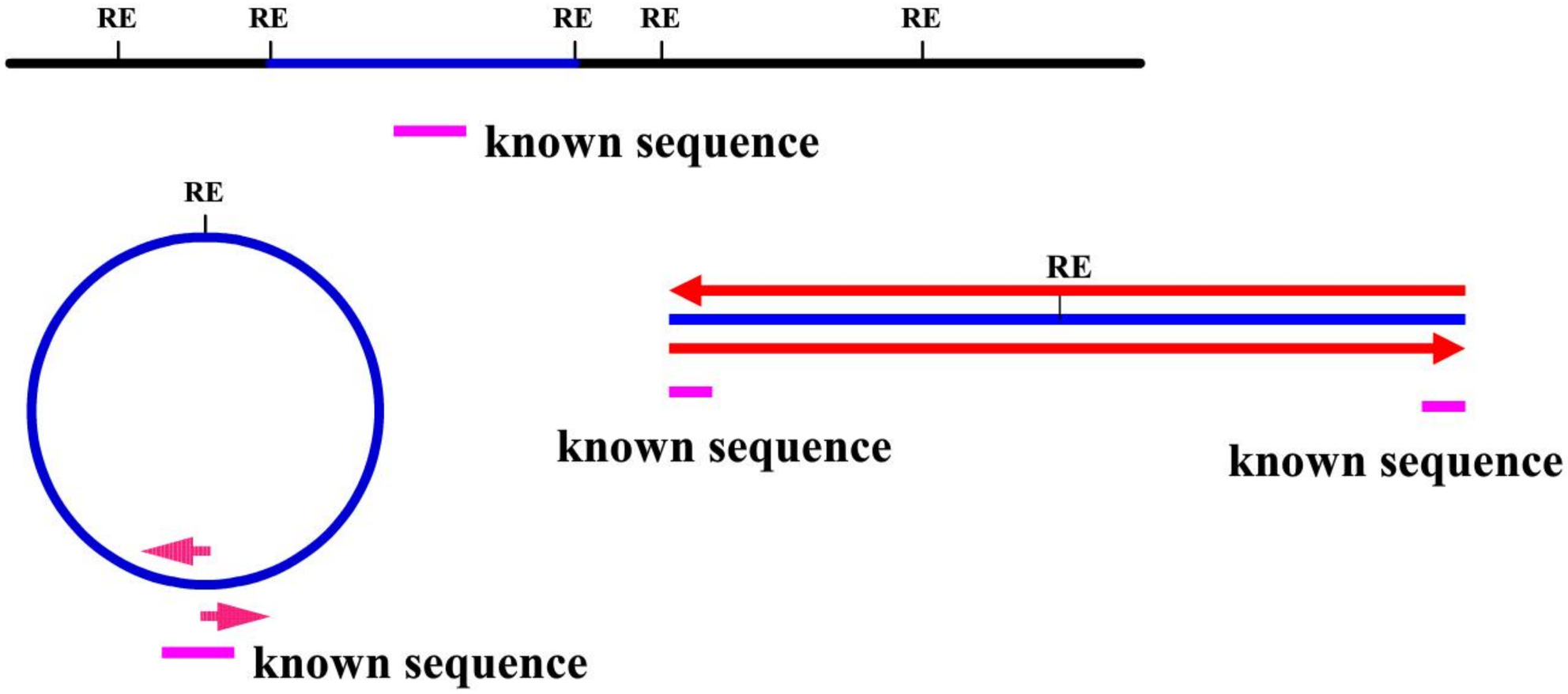
INSERTIONAL MUTAGENESIS IN MICE: NEW PERSPECTIVES AND TOOLS.
Nature Reviews Genetics 6, 568-580 (2005)

Inverse PCR

Used to obtain (clone) sequence adjacent to ONE known sequence
Standard PCR requires 2 regions of known sequence

Digest target DNA with restriction enzyme and ligate into circles

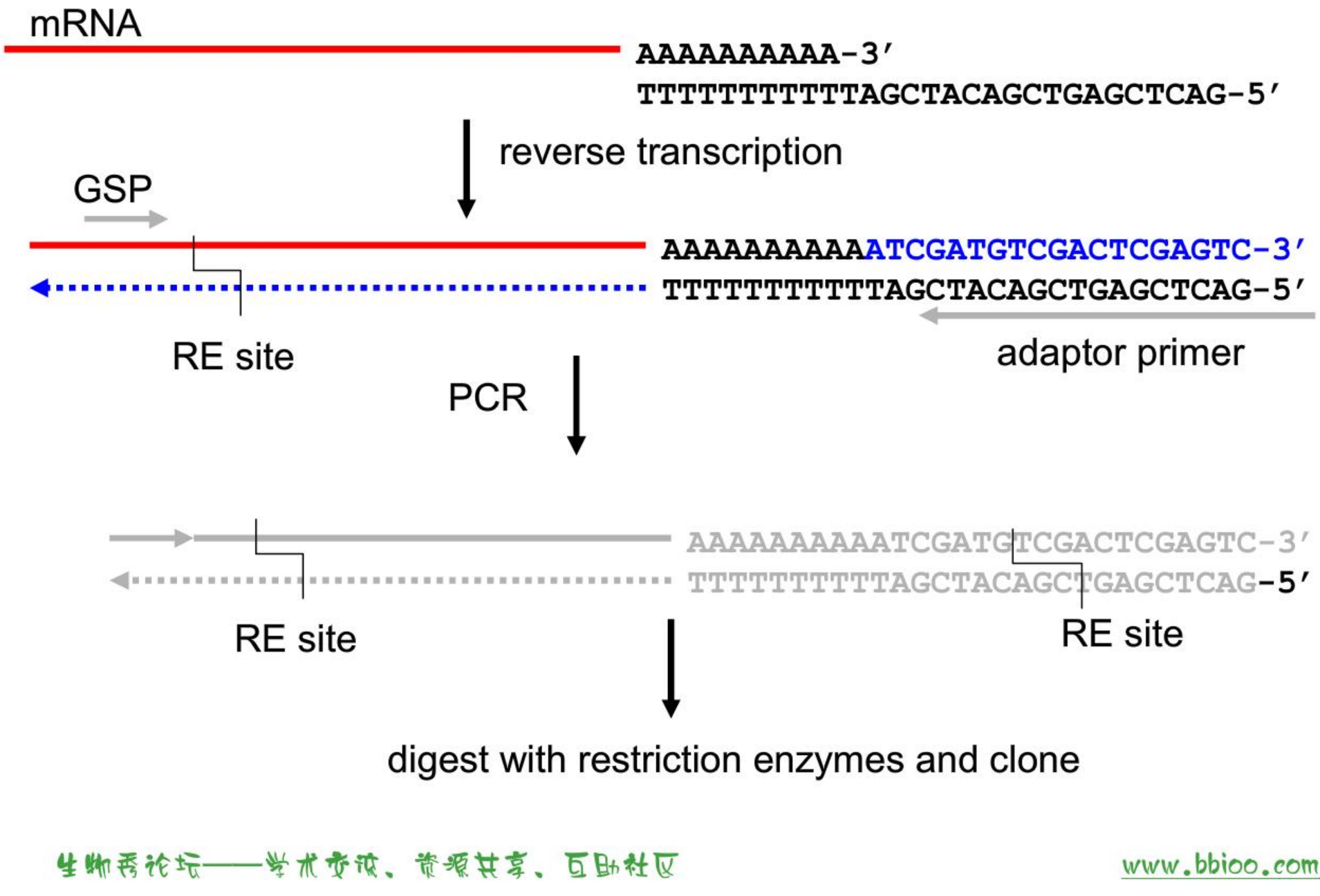
Use 2 outward pointing primers to amplify around the circle towards each other



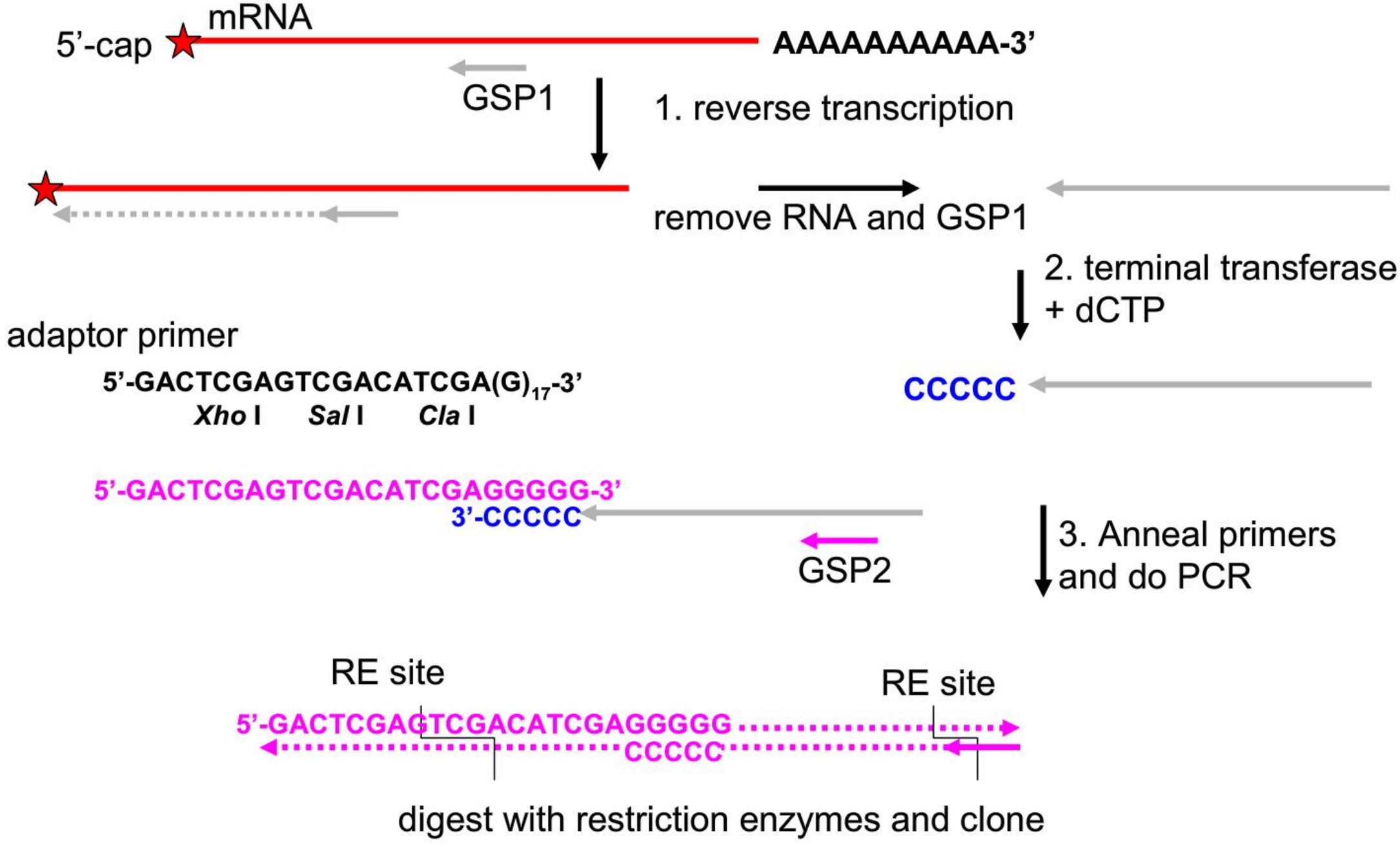
3′-RACE

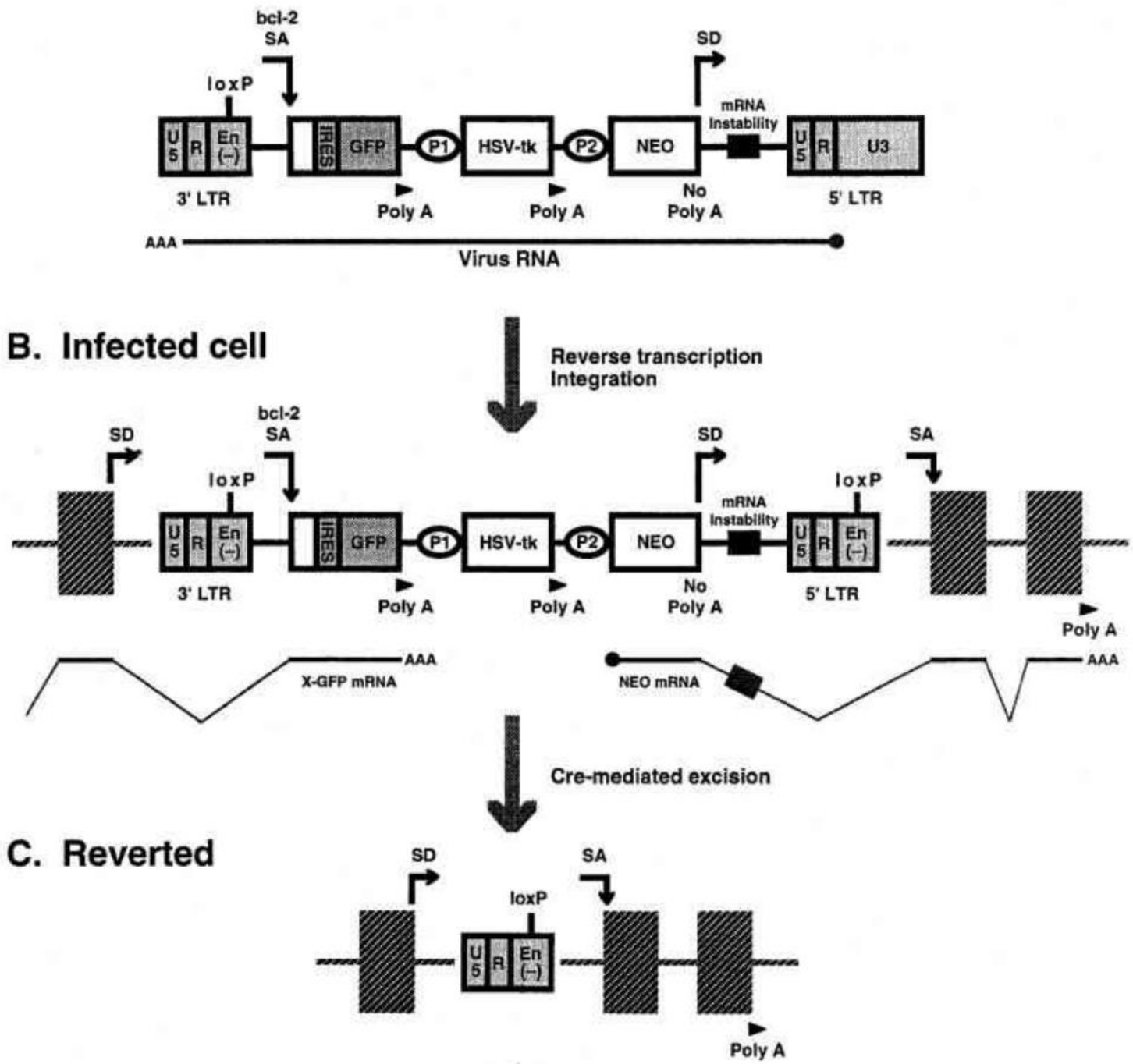
5’-GACTCGAGTCGACATCGA$(T)_{17}$-3’
Xho I *Sal* I *Cla* I

Oligo(dT) adaptor primer



5′-RACE

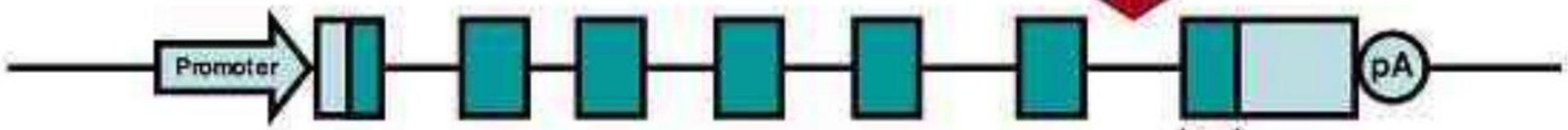

5’-cap
mRNA
AAAAAAAAAA-3’
GSP1
1. reverse transcription
remove RNA and GSP1
2. terminal transferase
+ dCTP
adaptor primer
5’-GACTCGAGTCGACATCGA(G)17-3’
Xho I Sal I Cla I
CCCCC
5’-GACTCGAGTCGACATCGAGGGGG-3’
3’-CCCCC
GSP2
3. Anneal primers
and do PCR
RE site
RE site
5’-GACTCGAGTCGACATCGAGGGGG
CCCCC
digest with restriction enzymes and clone


A. Packaging cell line
bcl-2
SA
loxP
SD
mRNA
Instability
U5
R
En
(-)
IRES
GFP
P1
HSV-tk
P2
NEO
U5
R
U3
3' LTR
Poly A
Poly A
No
Poly A
5' LTR
AAA
Virus RNA
B. Infected cell
Reverse transcription
Integration
SD
bcl-2
SA
SD
SA
loxP
loxP
mRNA
Instability
3' LTR
Poly A
Poly A
No
Poly A
5' LTR
Poly A
AAA
X-GFP mRNA
NEO mRNA
AAA
Cre-mediated excision
C. Reverted
SD
SA
loxP
Poly A

Poly-A trap

Strongly biased integration site

92 %

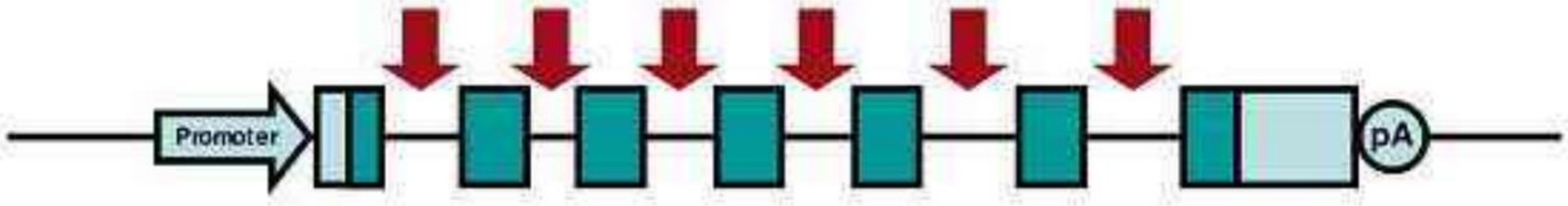


Small
C-terminal
deletion

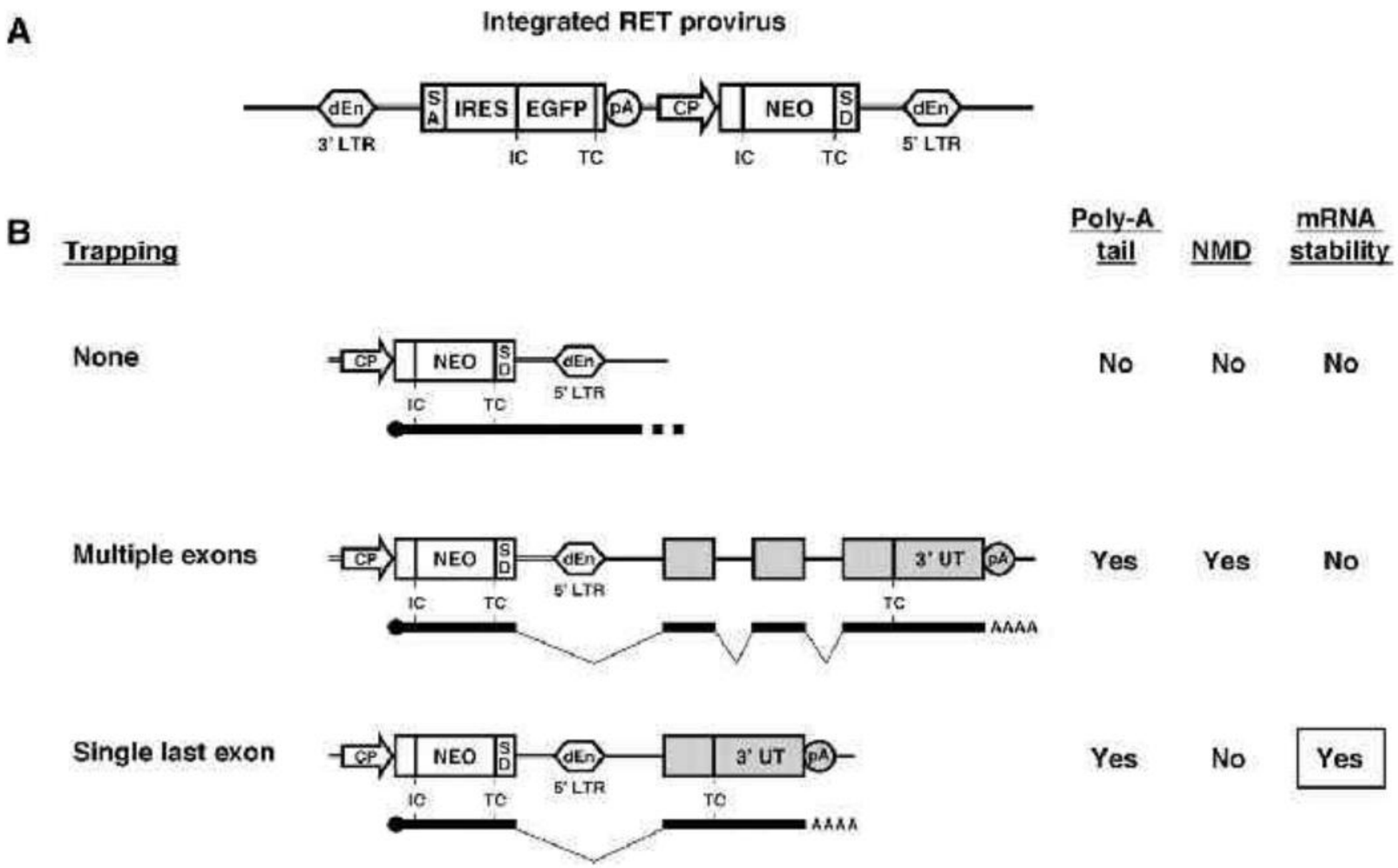
UPATrap

Suppression of NMD

Randomly distributed integration site

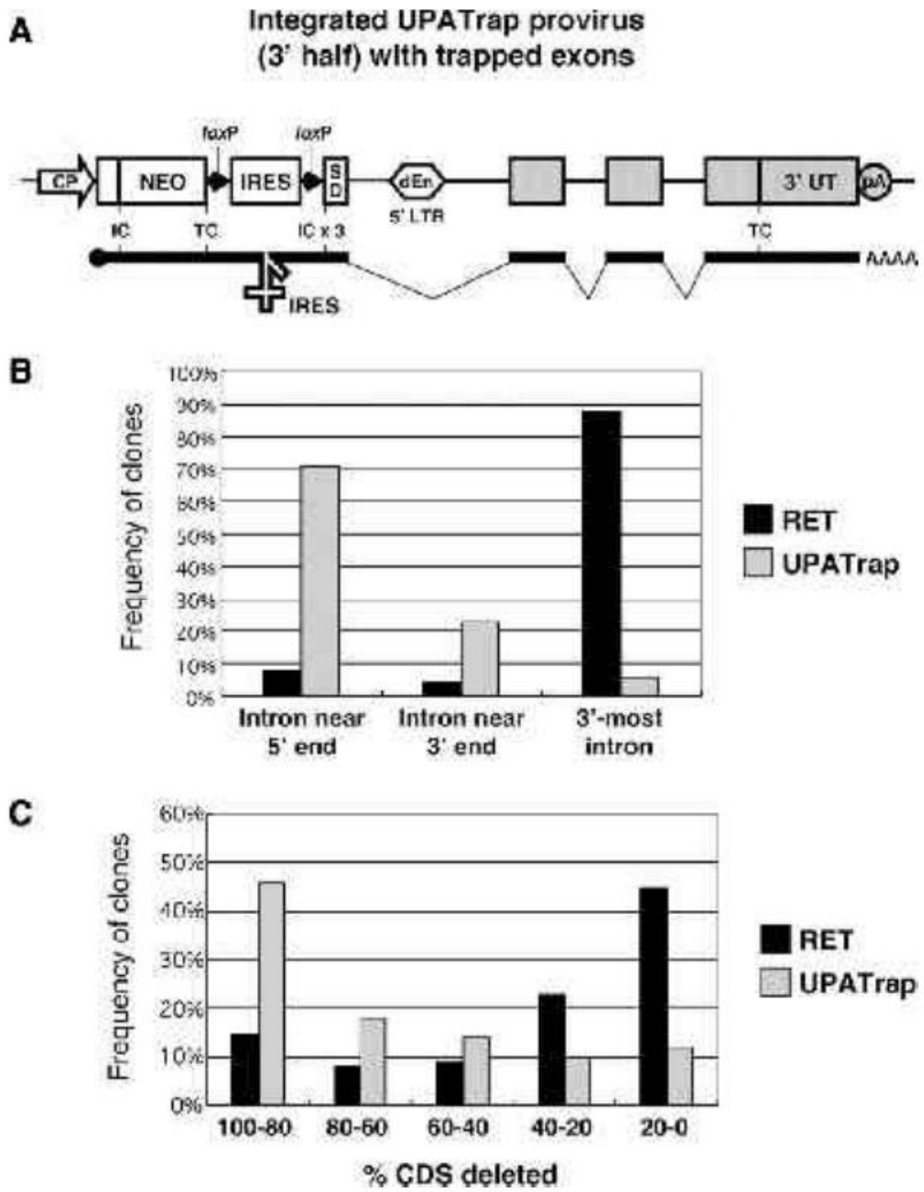


A model showing a biased selection of the vector-integration sites in poly-A trapping



Shigeoka, T. et al. Nucl. Acids Res. 2005 33:e20; doi:10.1093/nar/gni022

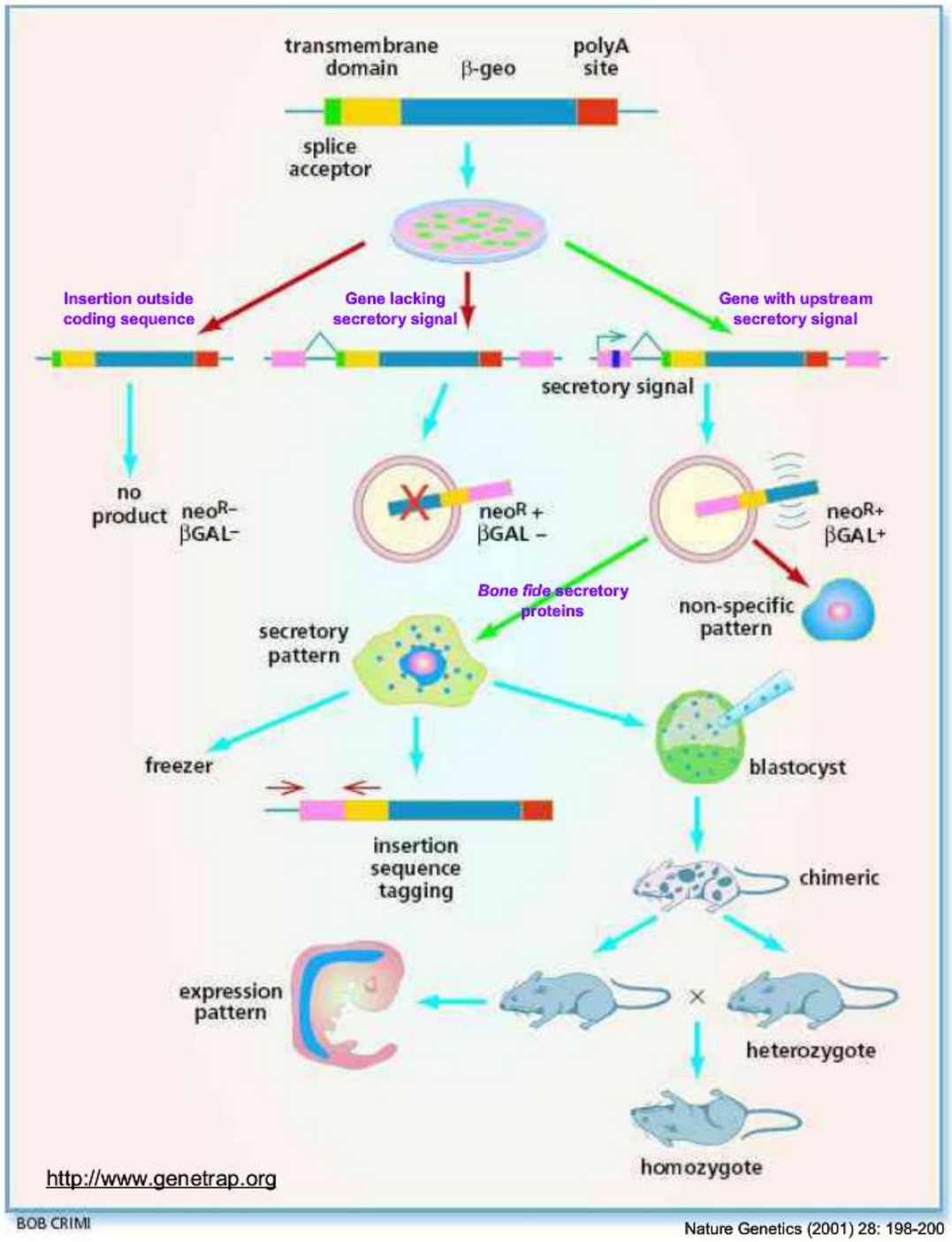
Unbiased gene trapping using the UPATrap vector



Shigeoka, T. et al. Nucl. Acids Res. 2005 33:e20; doi:10.1093/nar/gni022

Nucleic Acids Research

Secretory Gene Traps



MOLECULAR AND CELLULAR BIOLOGY, Dec. 2003, p. 9189–9207 Vol. 23, No. 24

Characterization of *Sleeping Beauty* Transposition and Its Application to Genetic Screening in Mice

Kyoji Horie,1,2 Kosuke Yusa,2 Kojiro Yae,2,3 Junko Odajima,4 Sylvia E. J. Fischer,5 Vincent W. Keng,2,6 Tomoko Hayakawa,2 Sumi Mizuno,2,6 Gen Kondoh,2 Takashi Ijiri,7 Yoichi Matsuda,7,8 Ronald H. A. Plasterk,5 and Junji Takeda1,2,6*

*Collaborative Research Center for Advanced Science and Technology,*1 *Department of Social and Environmental Medicine,*2

*Department of Hematology and Oncology,*4 *and Japan Science and Technology Corporation,*6 *Graduate School of Medicine,*

Osaka University, Suita, Osaka 565-0871, Division of Gene Function in Animals, Nara Institute of Science and Technology,

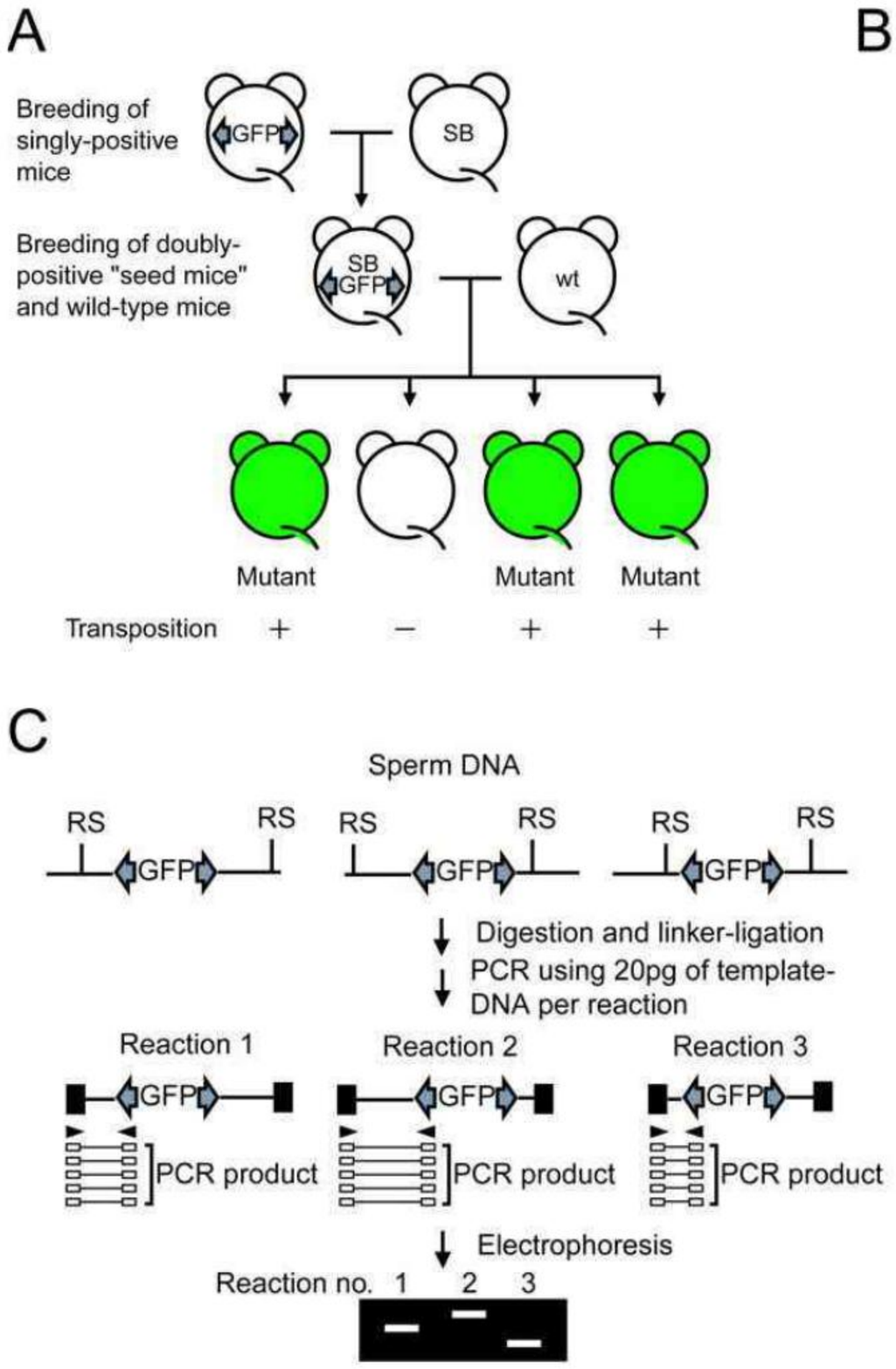
*Ikoma, Nara 630-0101,*3 *and Laboratory of Cytogenetics, Division of Bioscience, Graduate School of Environmental*

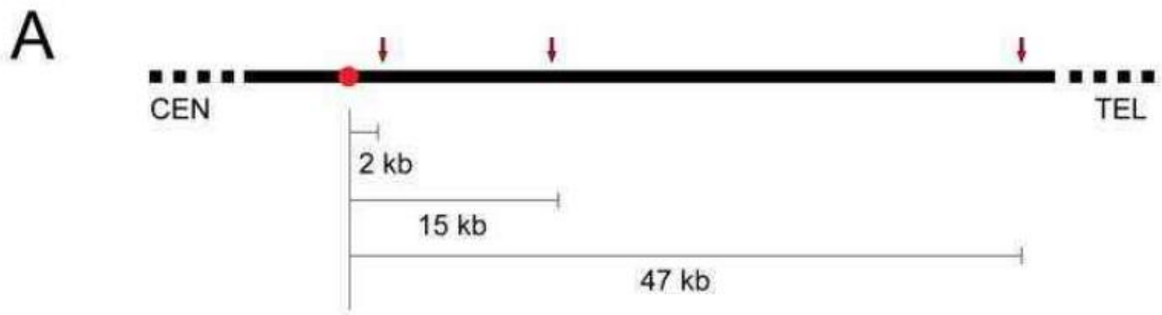
*Earth Science,*7 *and Laboratory of Animal Cytogenetics, Center for Advanced Science and Technology,*8

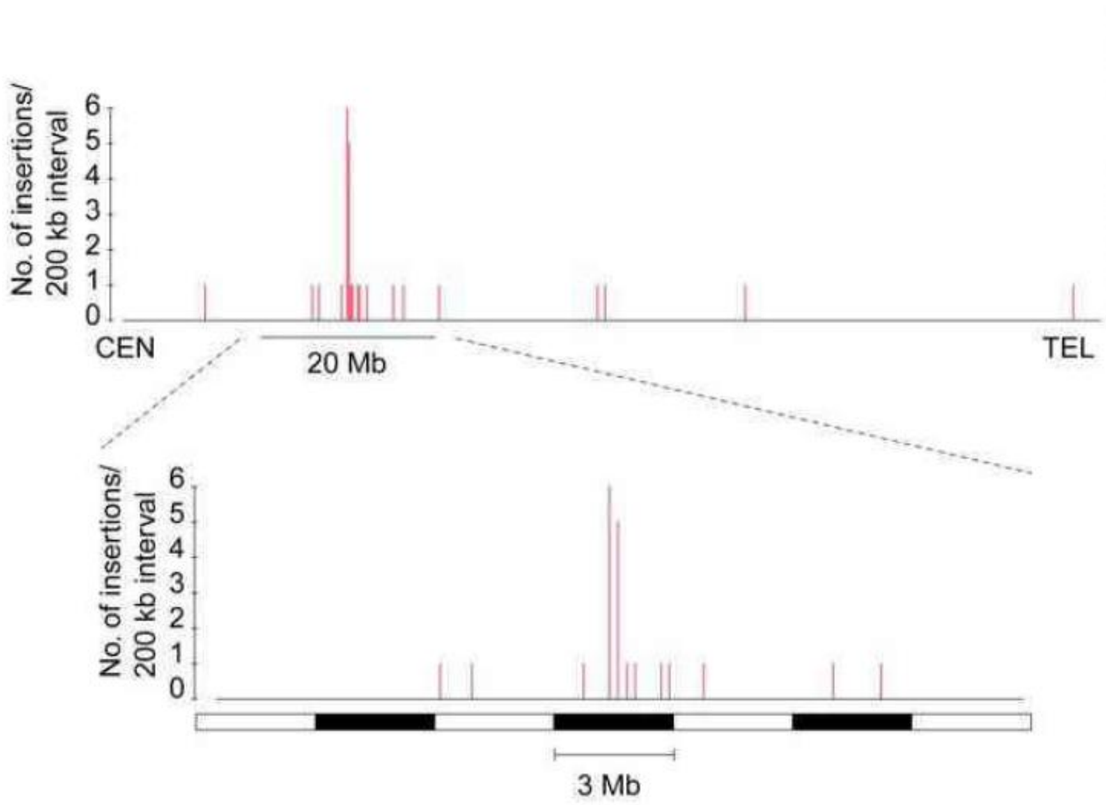
Hokkaido University, Kita-ku, Sapporo 060-0810, Japan, and Hubrecht Laboratory, Center

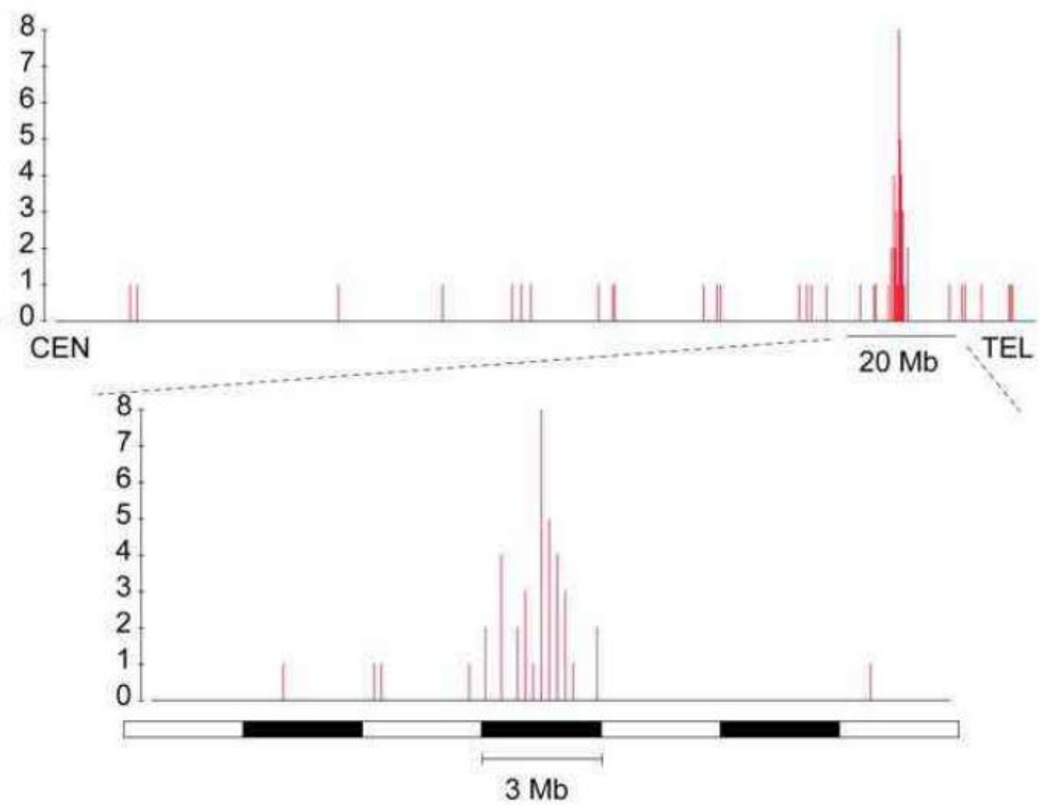
*for Biomedical Genetics, 3584 CT Utrecht, The Netherlands*5

Received 7 July 2003/Returned for modification 22 August 2003/Accepted 17 September 2003



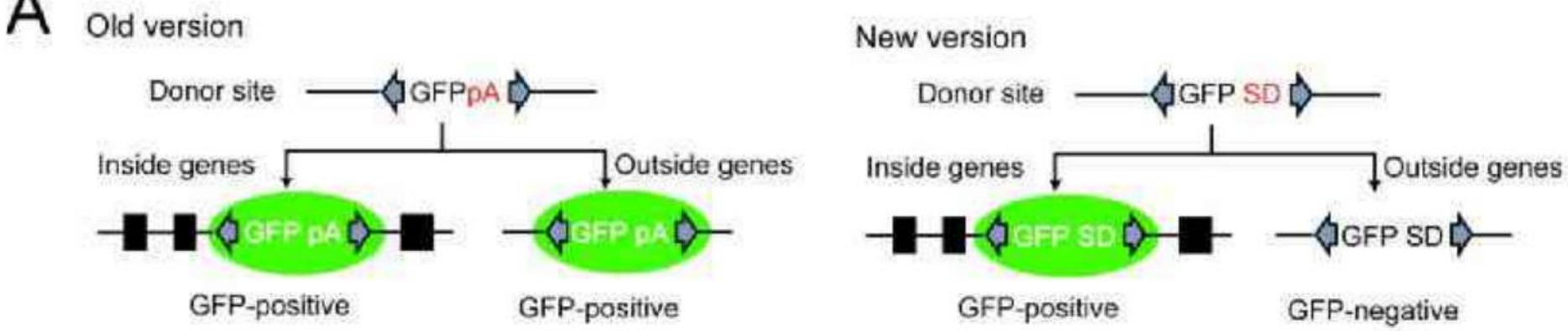


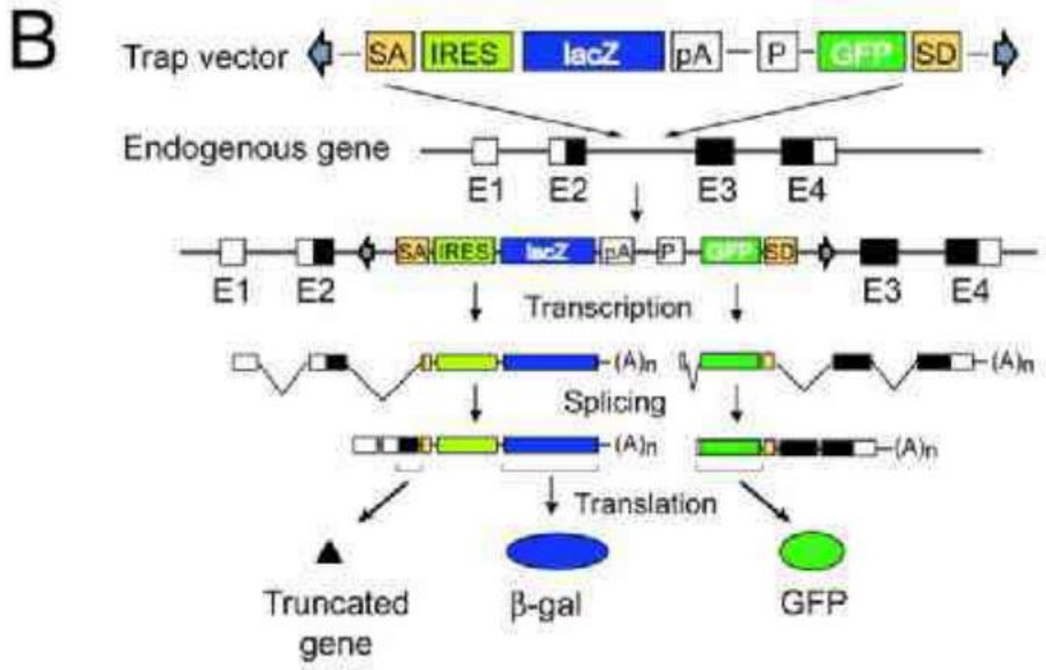


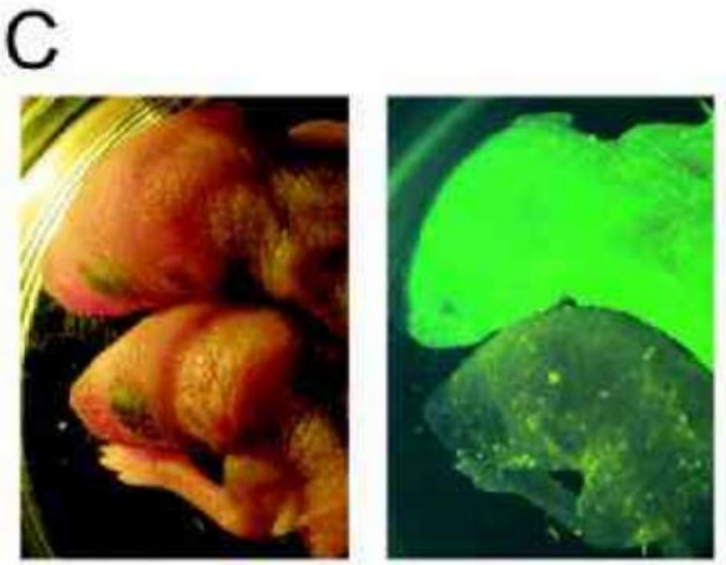


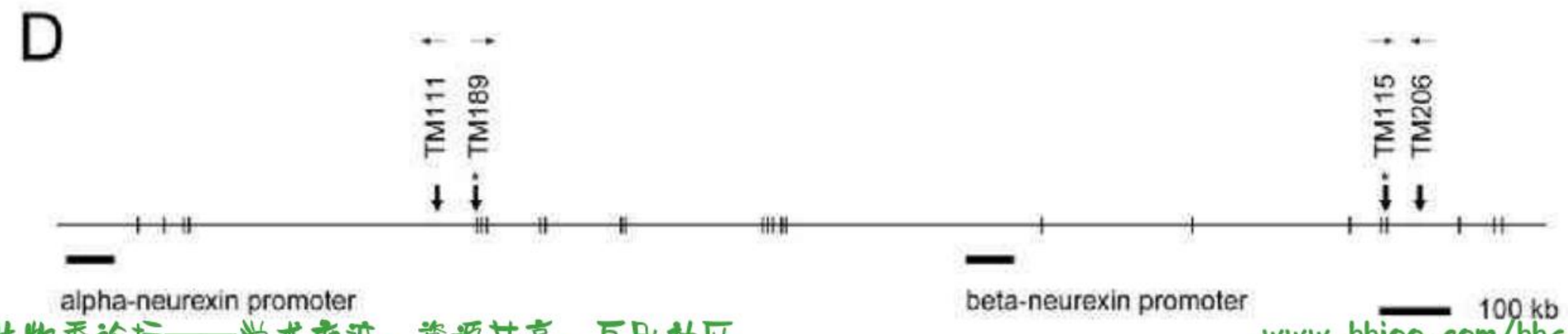
Other chromosomes 9 hits

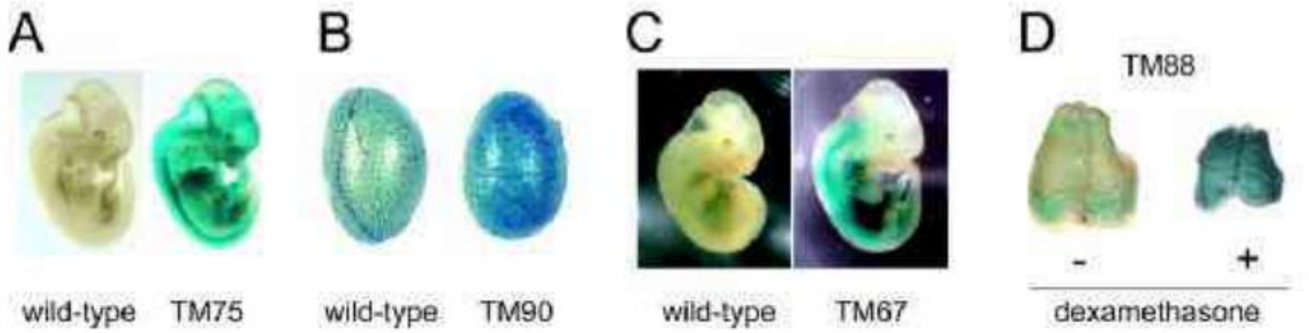
Other chromosomes 21 hits


A
Old version
Donor site
GFPpA
Inside genes
Outside genes
GFP pA
GFP pA
GFP-positive
GFP-positive
New version
Donor site
GFP SD
Inside genes
Outside genes
GFP SD
GFP SD
GFP-positive
GFP-negative


B
Trap vector
SA
IRES
lacZ
pA
P
GFP
SD
Endogenous gene
E1
E2
E3
E4
Transcription
Splicing
Translation
Truncated gene
β-gal
GFP


C


D
TM111
TM189
TM115
TM206
alpha-neurexin promoter
beta-neurexin promoter
100 kb


A
B
C
D
TM88
wild-type
TM75
wild-type
TM90
wild-type
TM67
-
+
dexamethasone
E
TM67
SA
IRES
lacZ
pA
P
SD
p2
p1
endogenous
p3
10 kb
trapped
wt
+/-
-/-
0.5 kb
endogenous
0.5 kb
trapped
F
TM88
SA
IRES
lacZ
pA
P
SD
p2
p1
endogenous
p3
1 kb
trapped
wt
+/-
-/-
0.5 kb
endogenous
0.5 kb
trapped
G
Day 1
Day 2
Day 3
wild-type
TM117
homozygous

nature Vol 436|14 July 2005|doi:10.1038/nature03681

LETTERS

Cancer gene discovery in solid tumours using transposon-based somatic mutagenesis in the mouse

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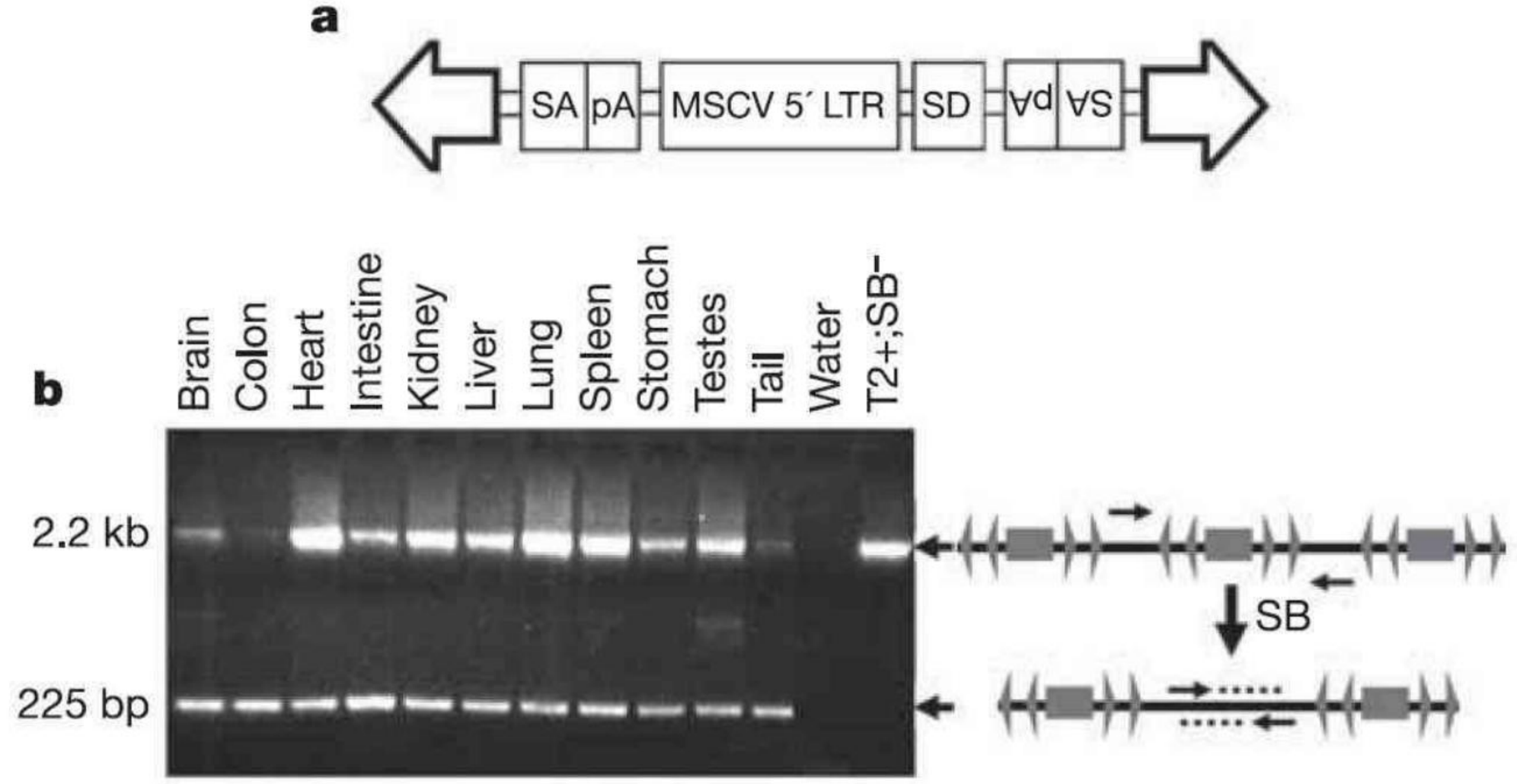


Figure 1 | Vector design and somatic transposition. **a**, The T2/Onc

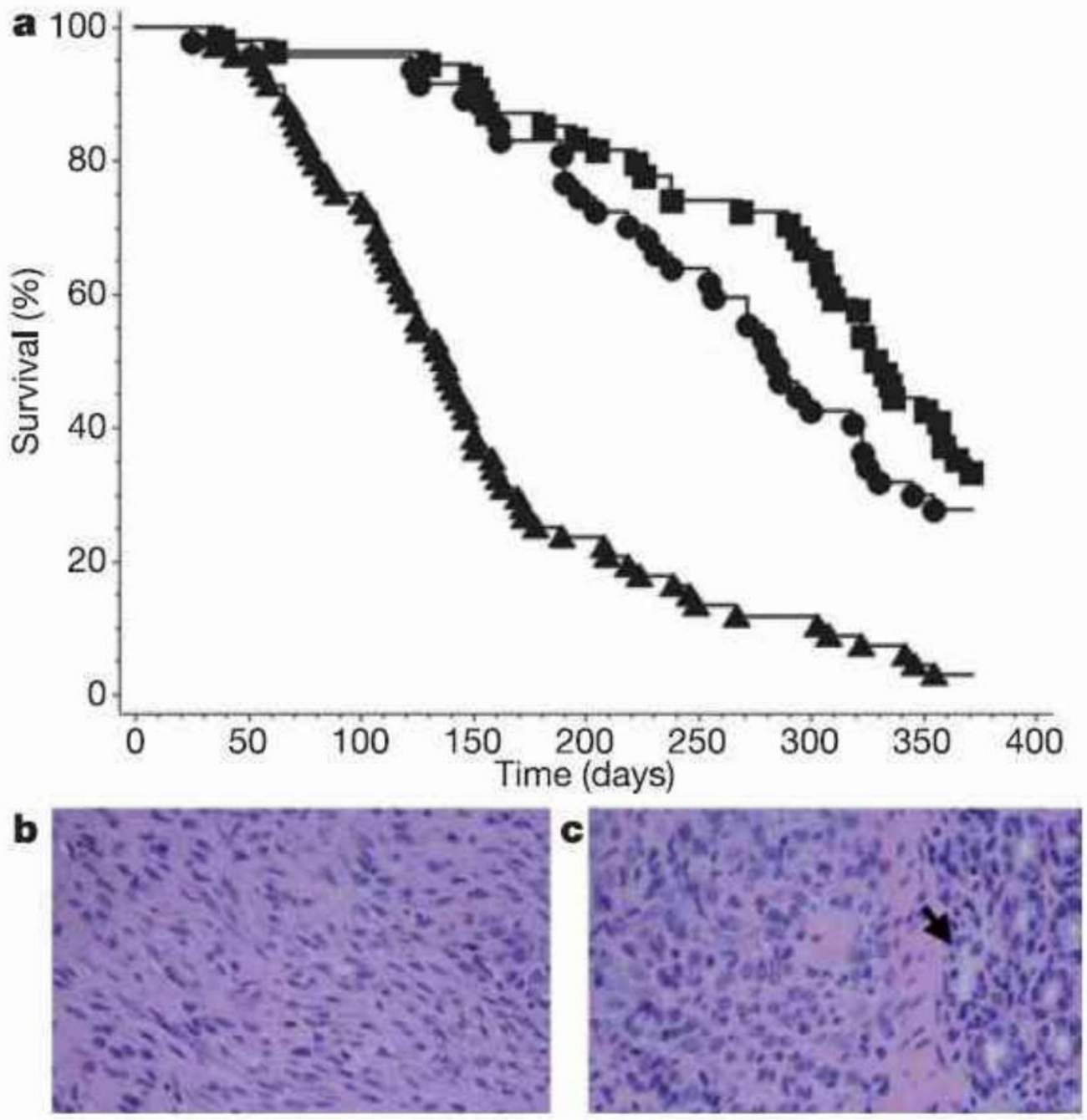


Figure 2 | *Arf*$^{-/-}$;T2/Onc;CAGGS-SB10 mice have a shorter tumour latency than singly transgenic controls. **a**, The Kaplan–Meier survival

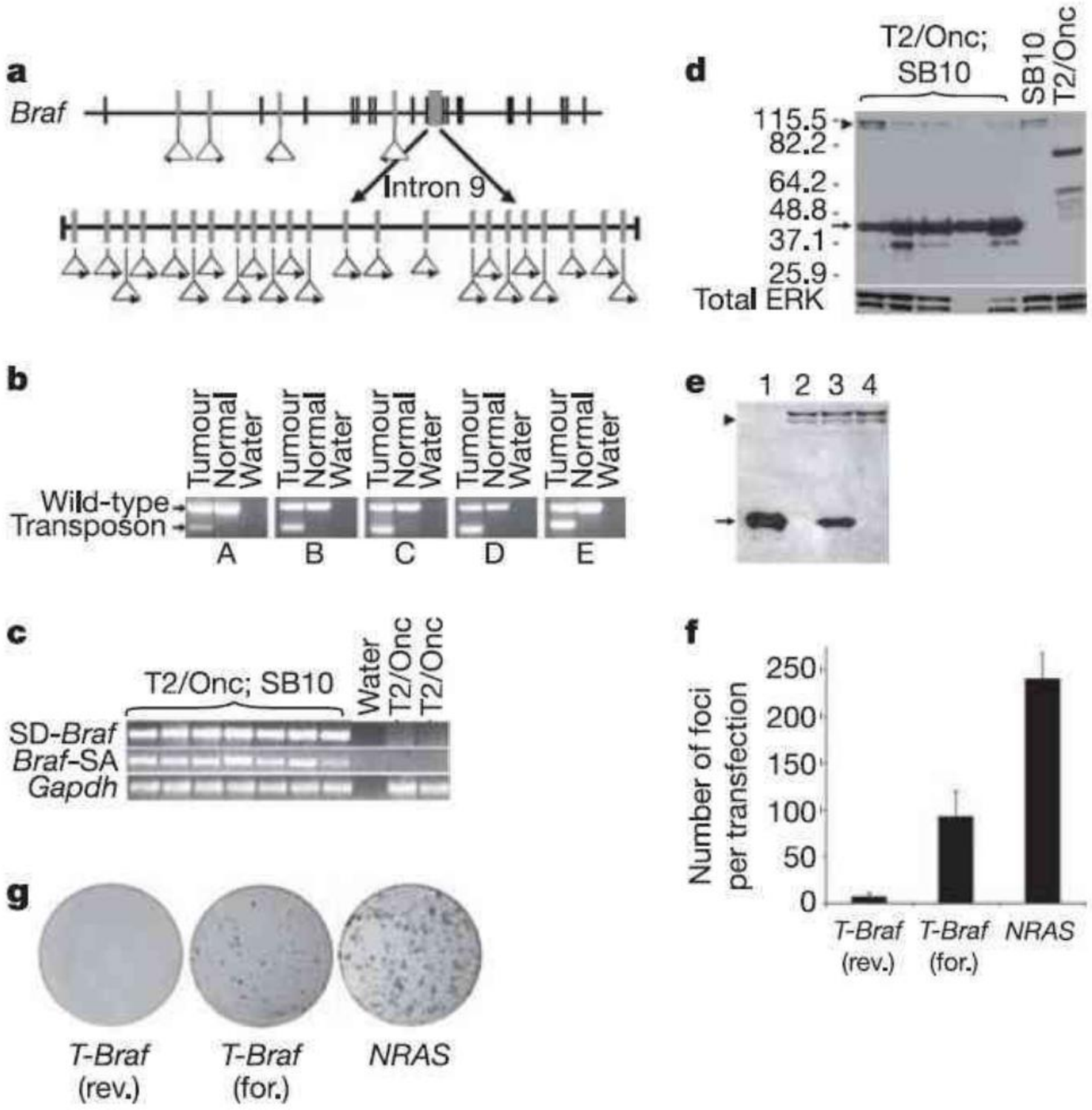
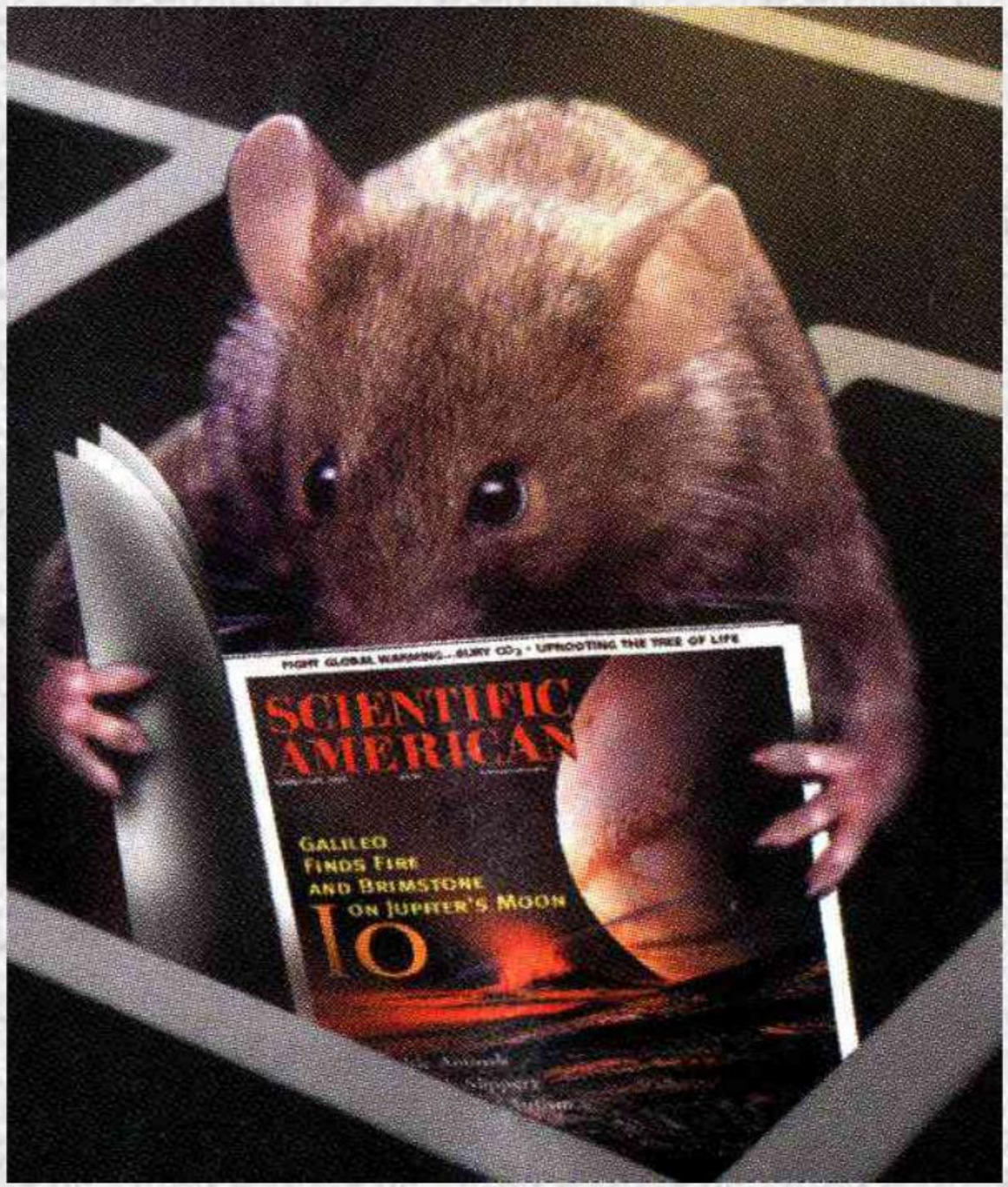


Figure 3 | Activation of *Braf* by T2/Onc insertion. **a**, Position and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