Jiří K a ň k a

Embryonální kmenové buňky savců

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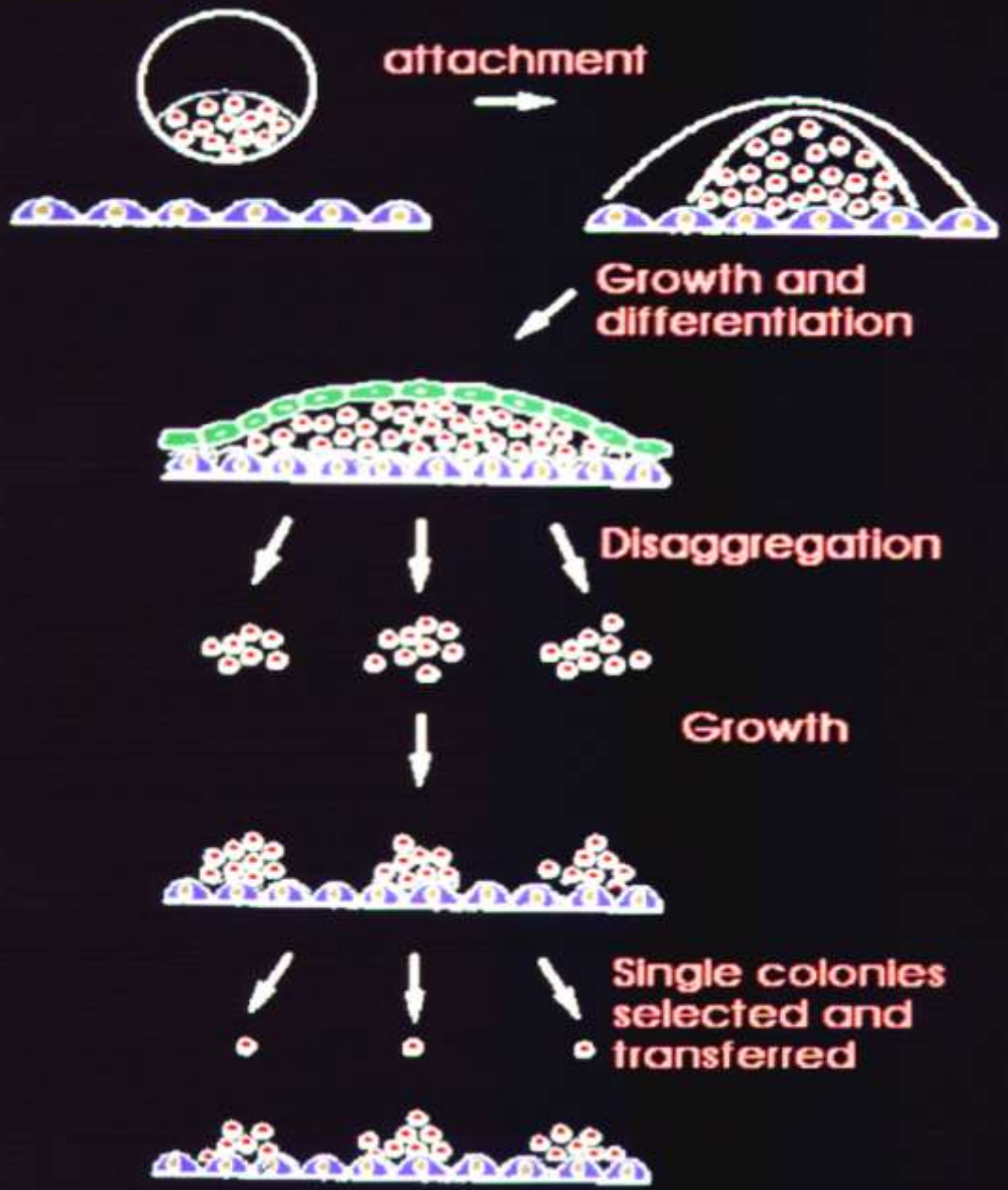
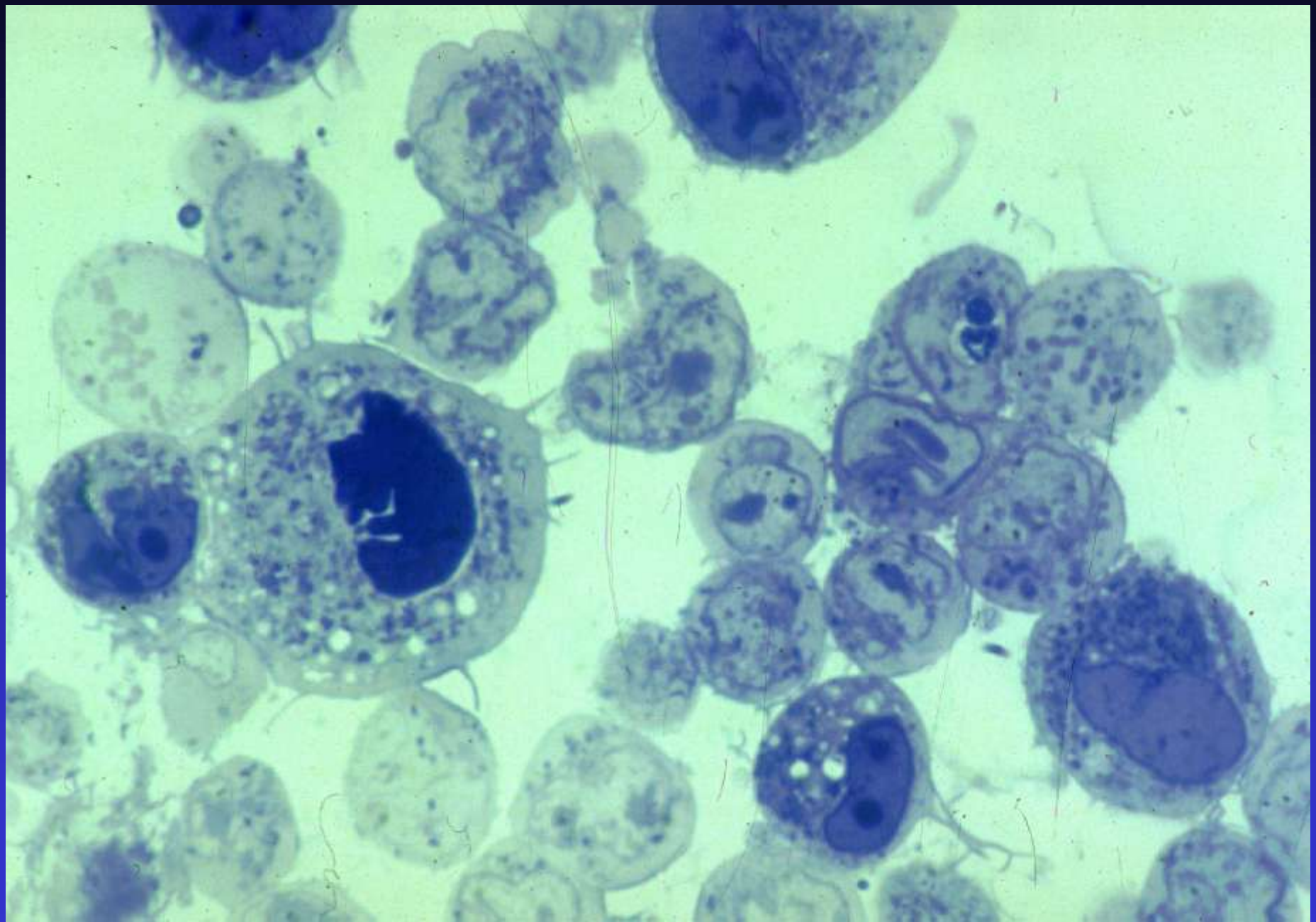
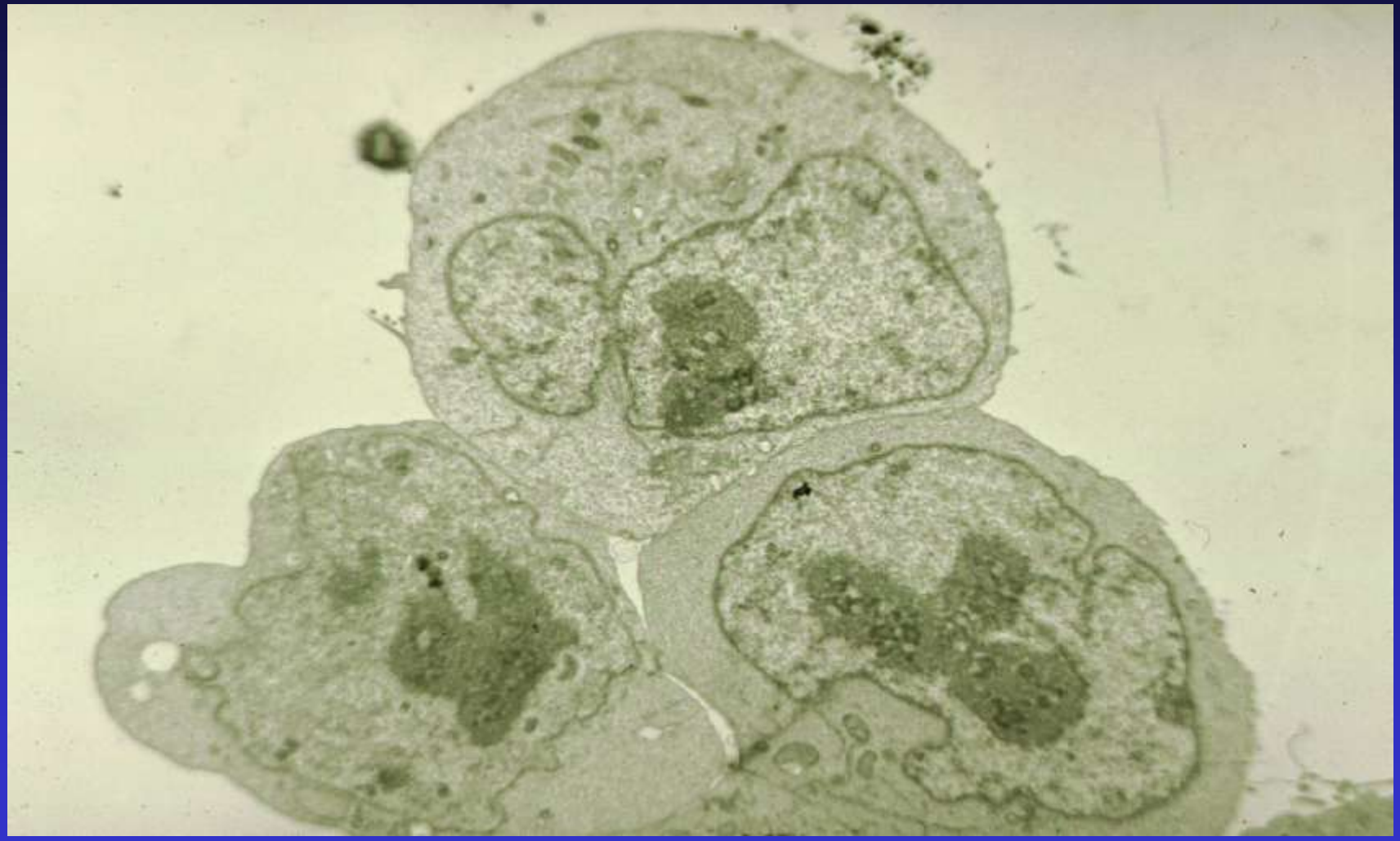
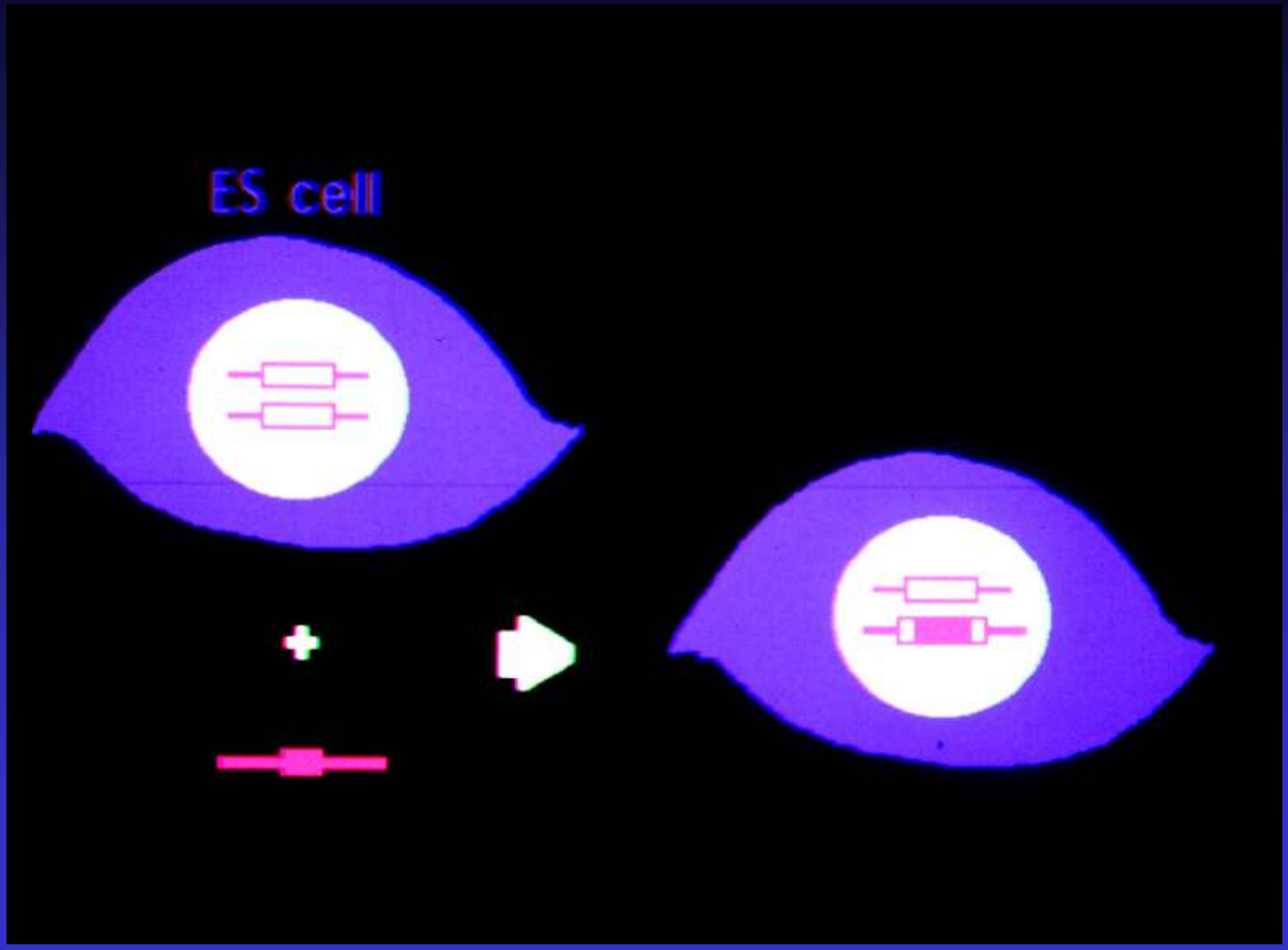
Isolation of embryo-derived stem cells








ES cell

EMBRYONIC STEM CELLS

STEVENS (1959, 1964) TERATOCARCINOMA
PIERCE (1960) EC
KLEINSMITH, PIERCE (1964) EC
STEWART, MINTZ (1981)

EVANS, KAUFMAN (1981) ES
MARTIN (1981)
SMITH, HOOPER (1987) - FEEDER CELLS, LIF
FOLGER, THOMAS, CAPECCHI (1982) - HOMOLOGOUS RECOMBINATION
THOMSON (1989) - GENE TARGETING, GERM LINE
SIMS, FIRST (1993) - CLONING, FARM ANIMAL
STEWART et al. (1994), MATSUI (1992) – GERM CELLS
J. GEARHART´S GROUP (SHAMBLOTT et al., 1998) – HUMAN EG
UNIV. OF WISCONSIN (THOMSON et al., 1998) – HUMAN ES

POUŽITÍ EMBRYONÁLNÍCH KMENOVÝCH BUNĚK

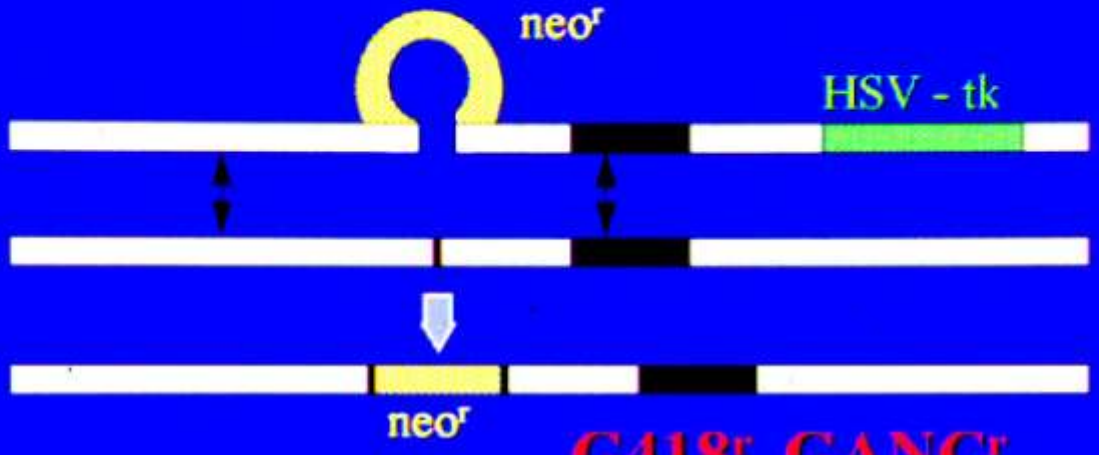
GENE TARGETING EXP.
SUBTLE MUTATION (HIT AND RUN)
CRE RECOMBINASE

GENE TRAP
ENHARCER TRAP
CLONING

POSITIVE - NEGATIVE SELECTION

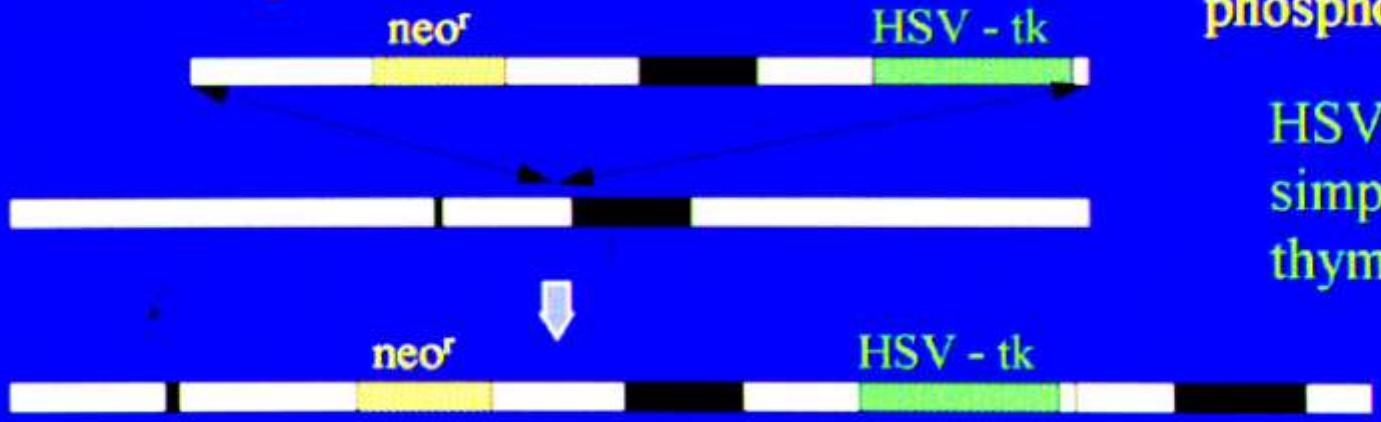
A/ Gene Targeting

M. Capecchi,
K. Thomas



G418^r, GANC^r

B/ Random Integration

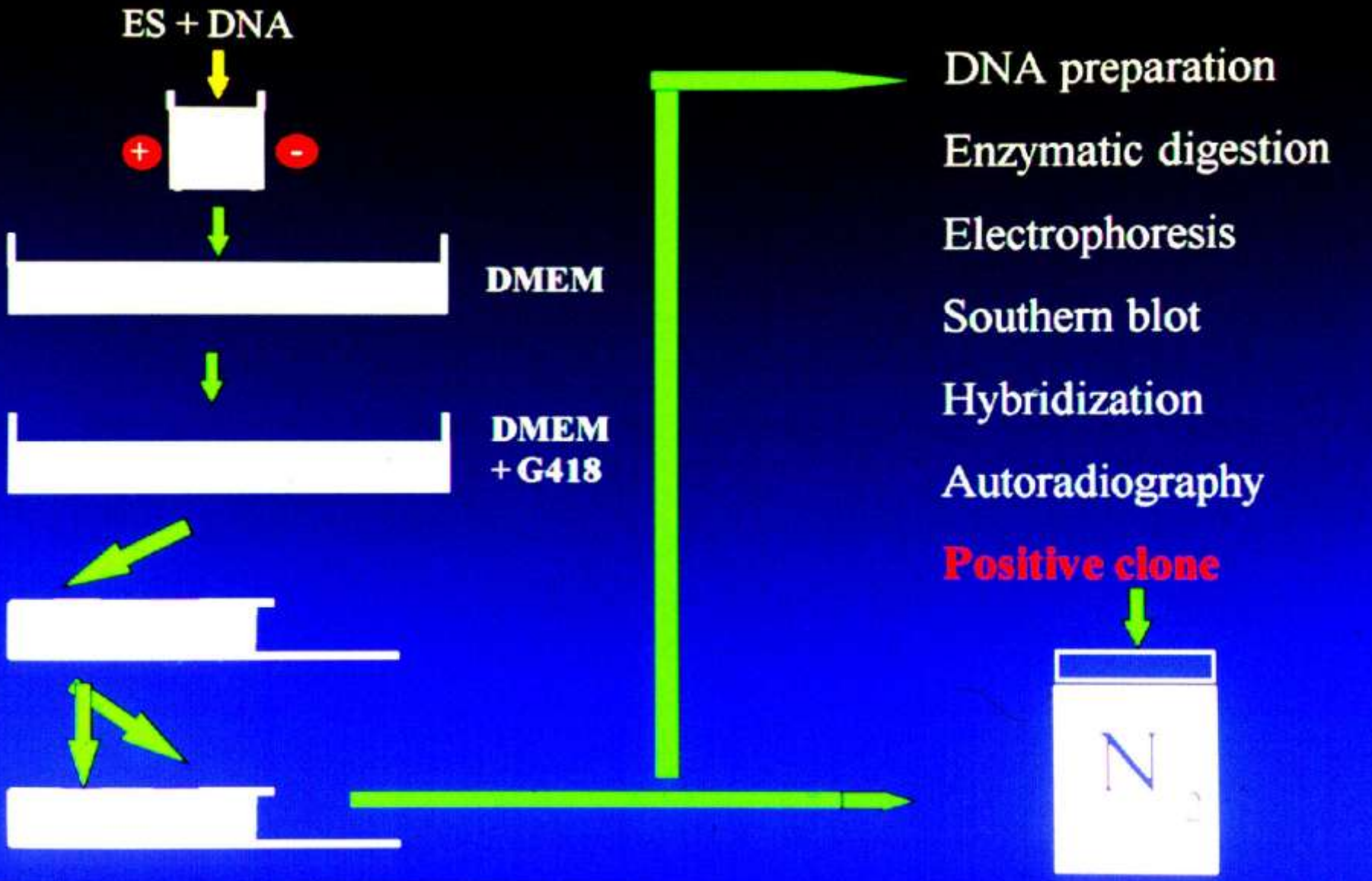


G418^r, GANC^s

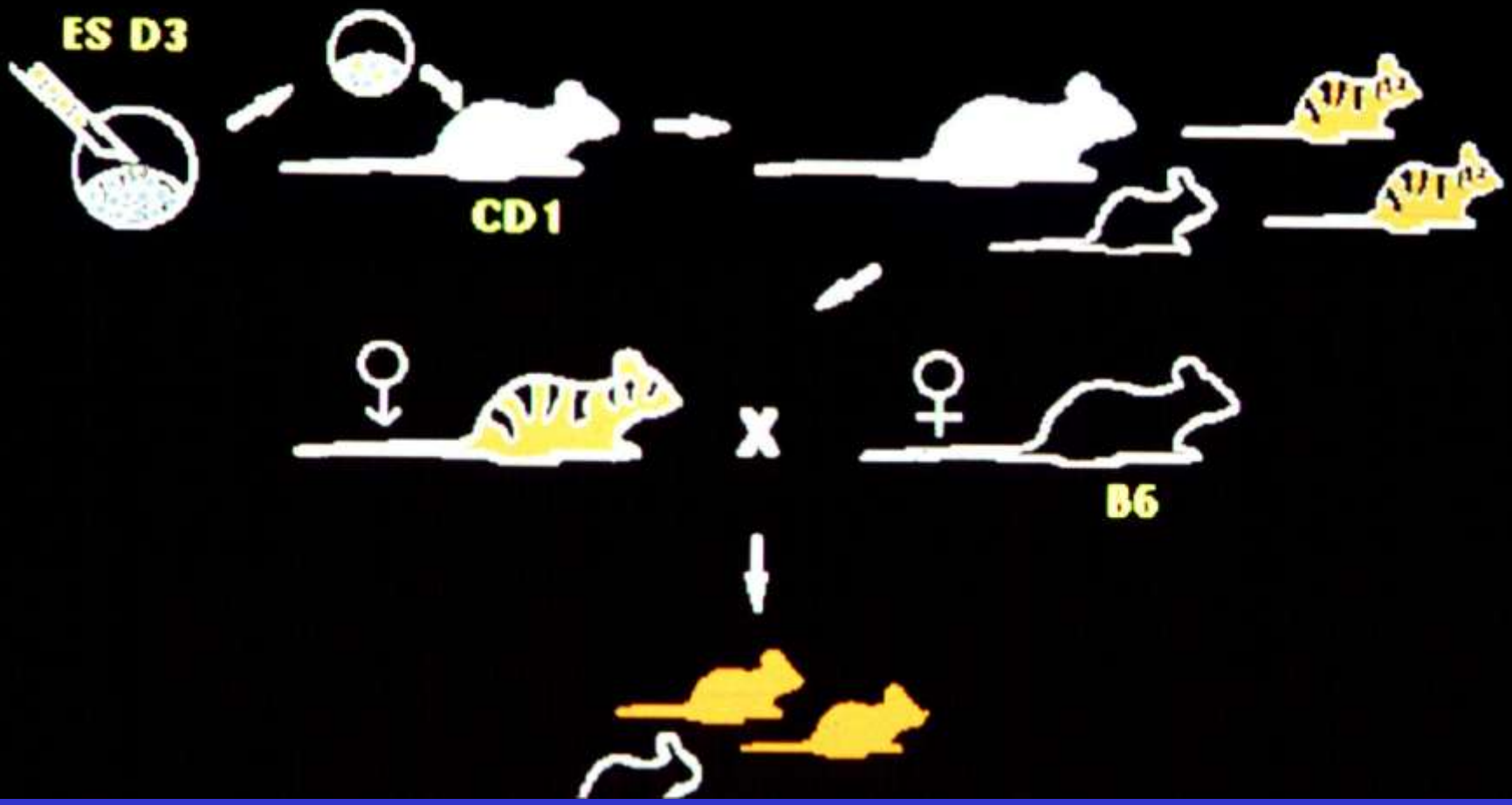
neo - neomycin-phosphotransferase

HSV-tk - Herpes simplex virus thymidine kinase

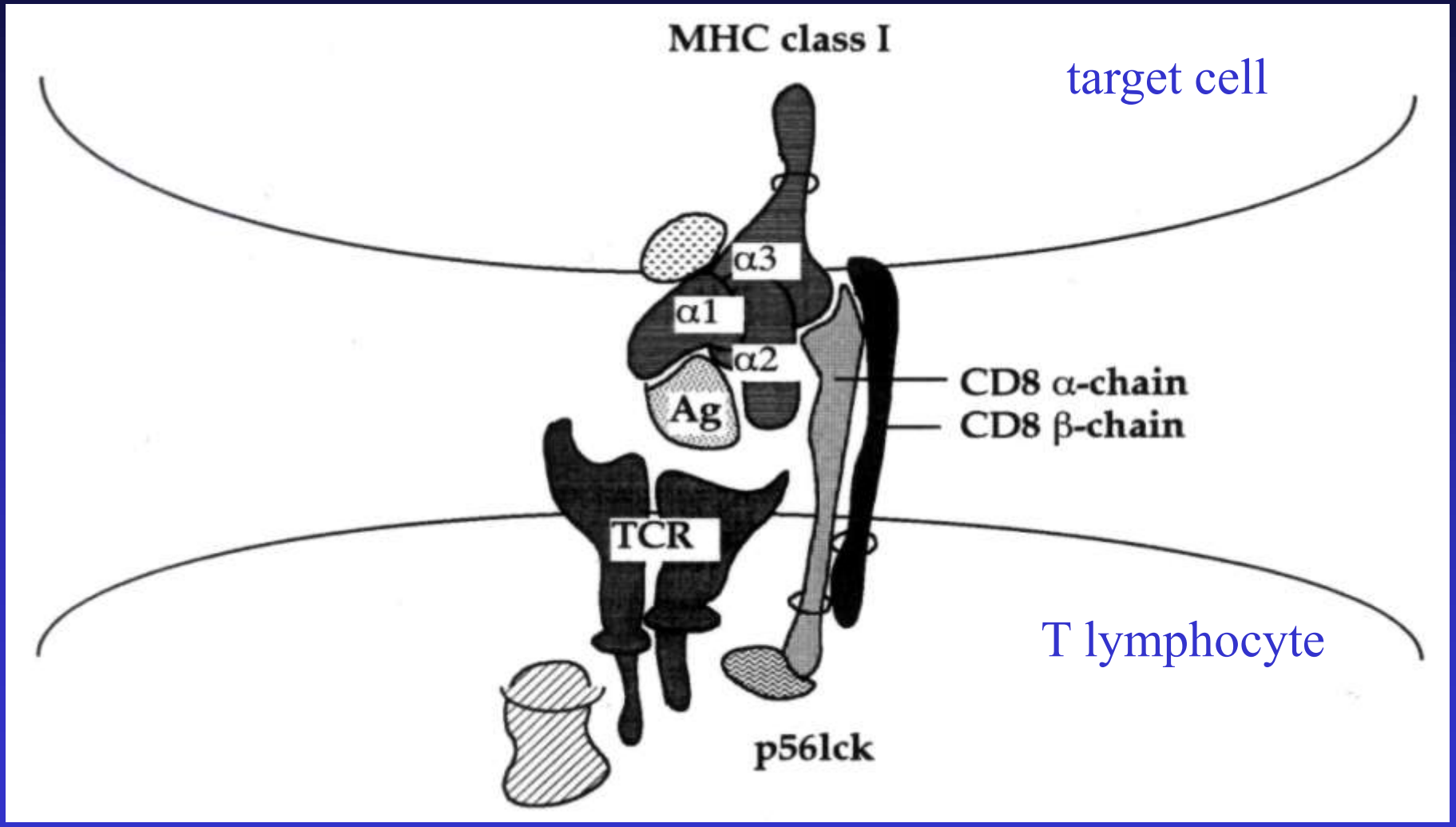
General selection strategy for homologous recombination

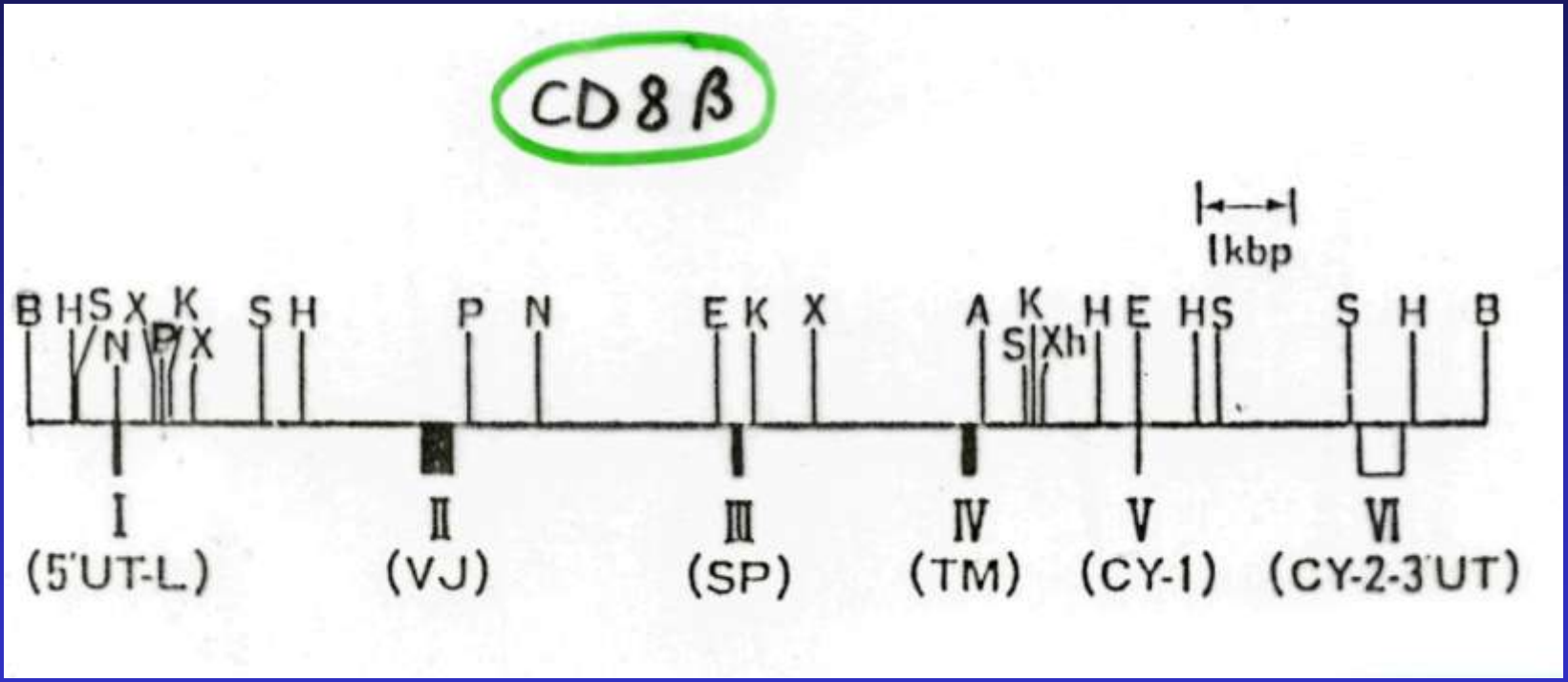


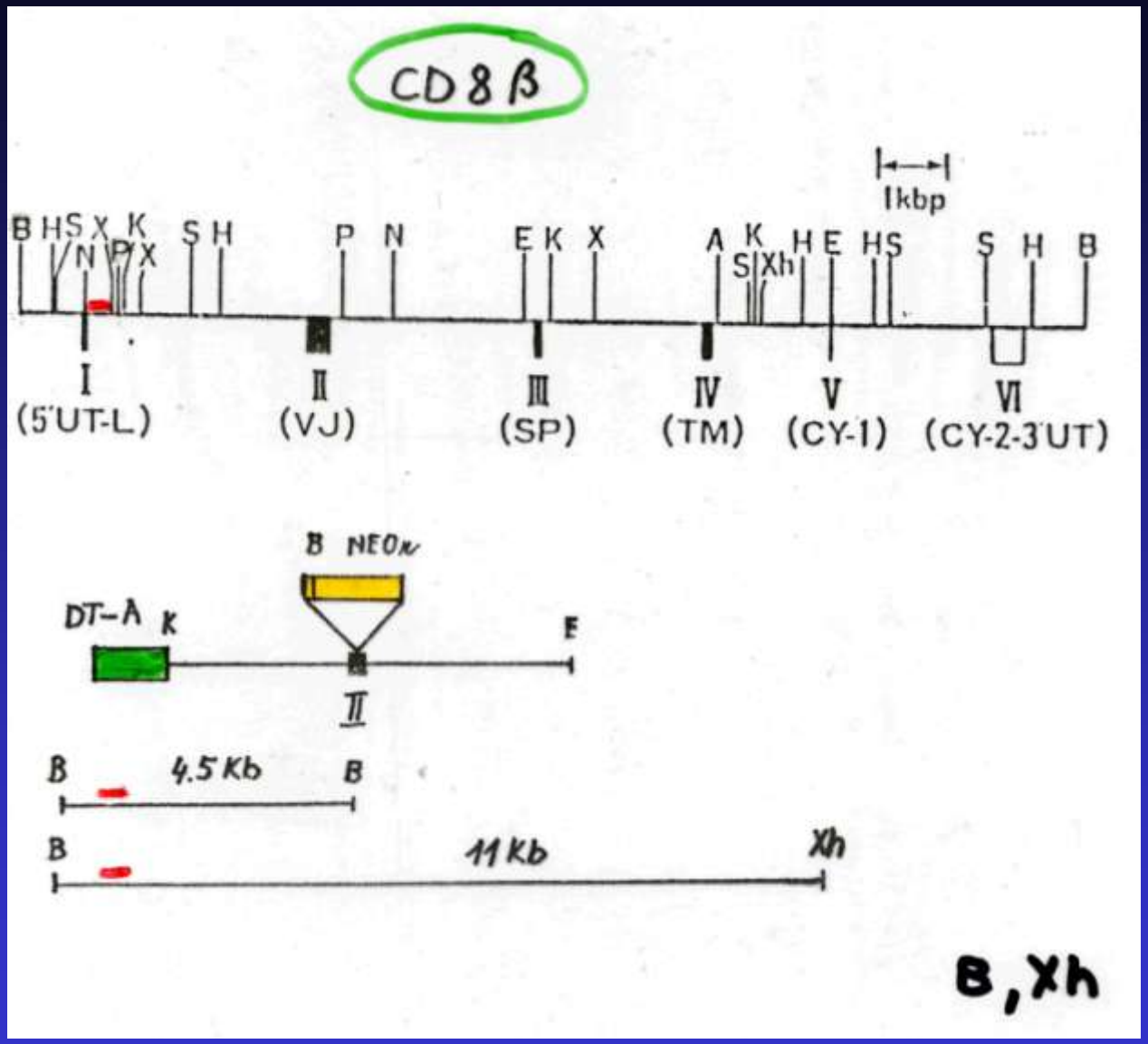
Generation of mouse germ-line chimeras

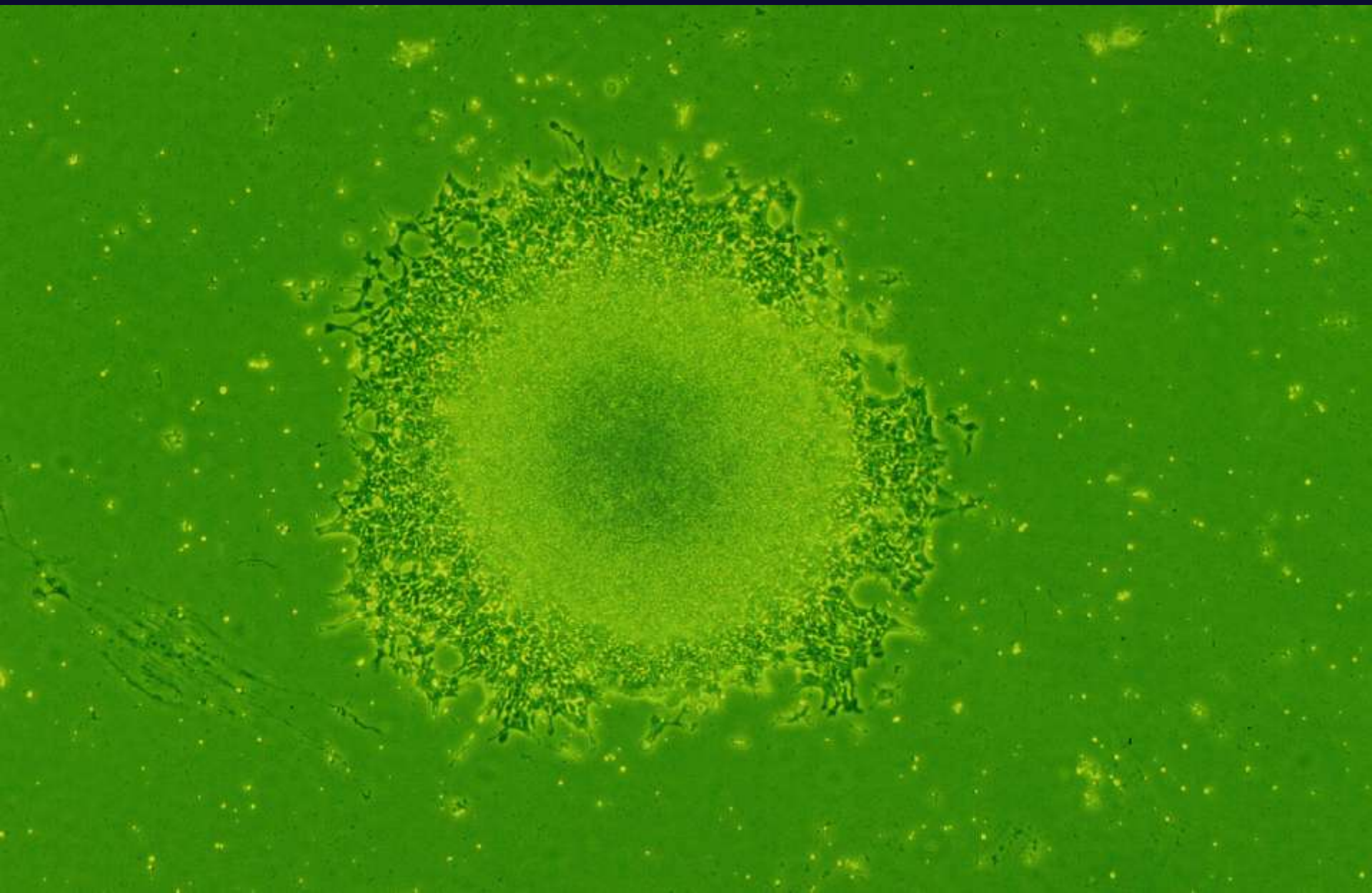


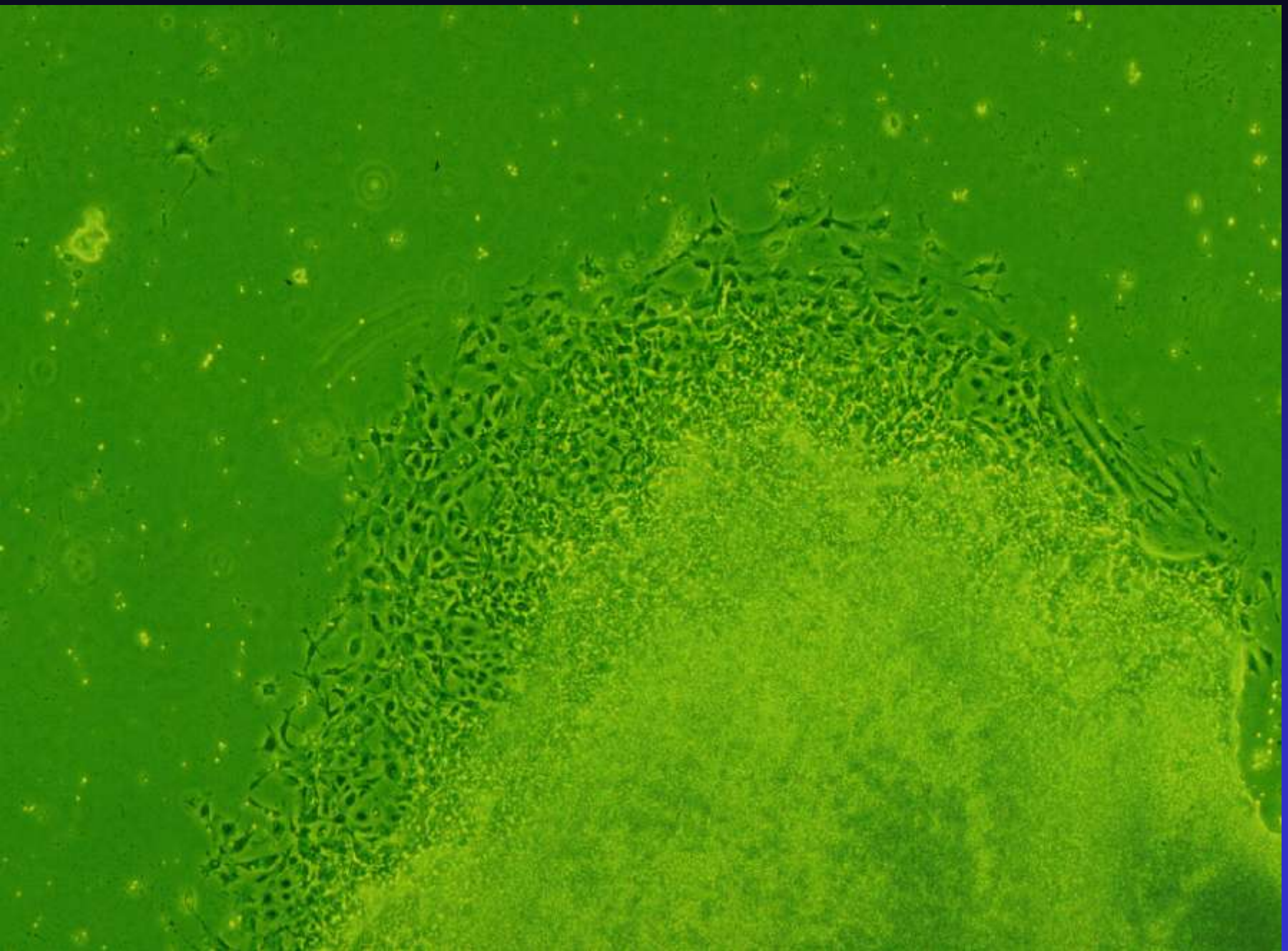
Gene targeting of CD8 beta

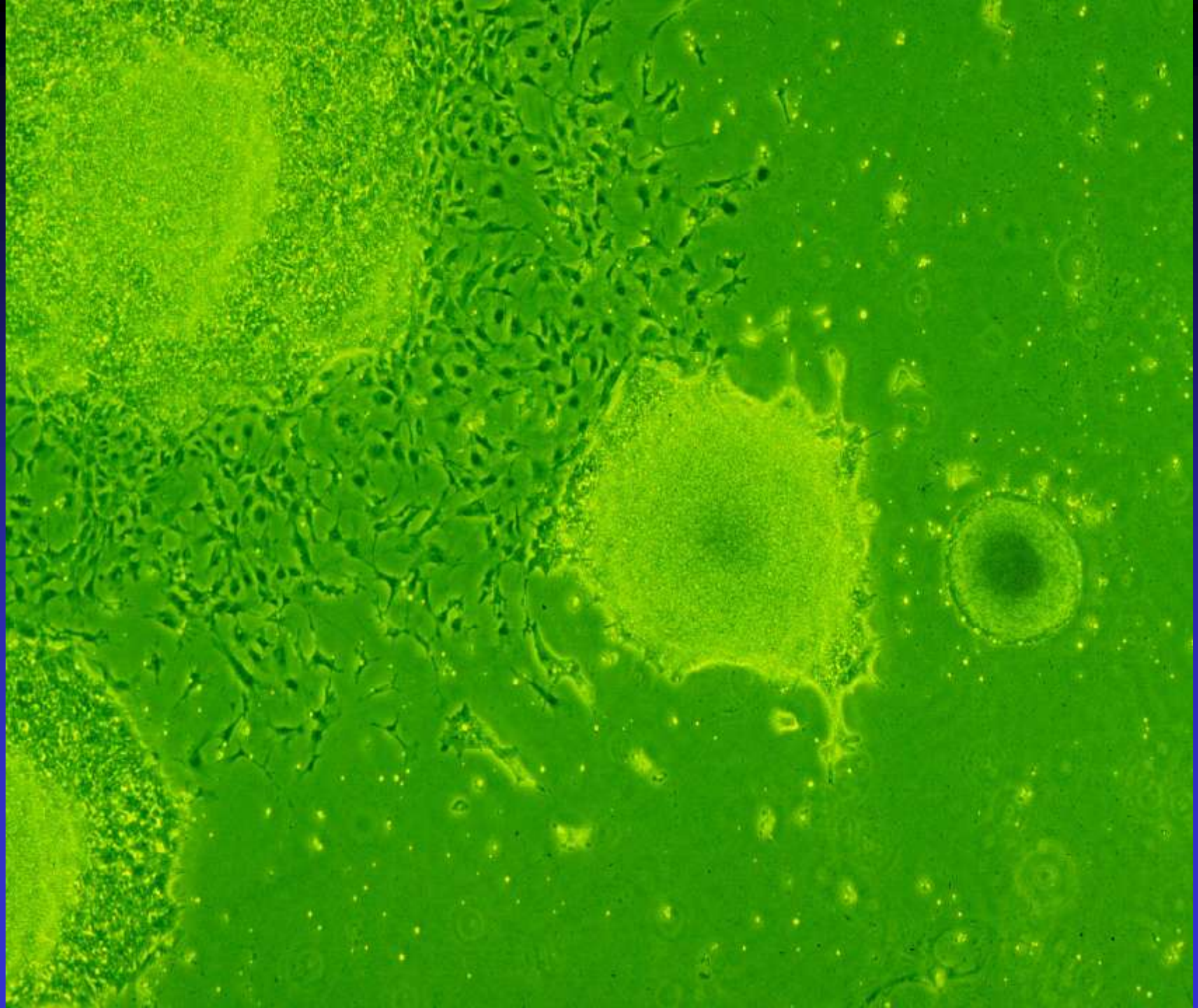



CD8β
1kbp
B H S X K S H P N E K X A K H E H S S H B
N P X S Xh
I
(5'UT-L)
II
(VJ)
III
(SP)
IV
(TM)
V
(CY-1)
VI
(CY-2-3'UT)

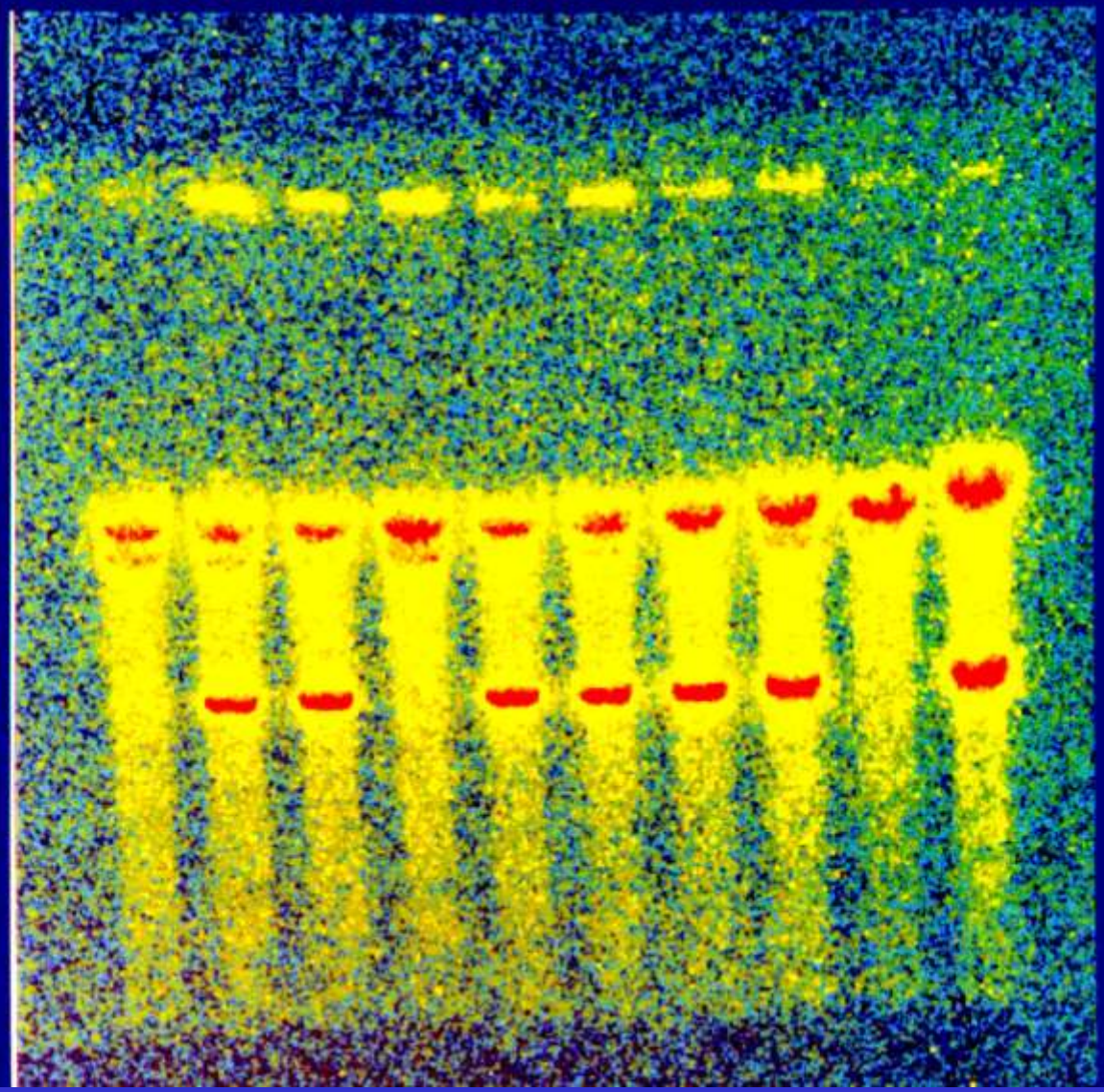

CD8β
1kbp
B H S X K S H P N E K X A K H E H S S H B
N P X S Xh
I
(5'UT-L)
II
(VJ)
III
(SP)
IV
(TM)
V
(CY-1)
VI
(CY-2-3'UT)
B NEOr
DT-A K E
II
B 4.5 Kb B
B 11 Kb Xh
B, Xh



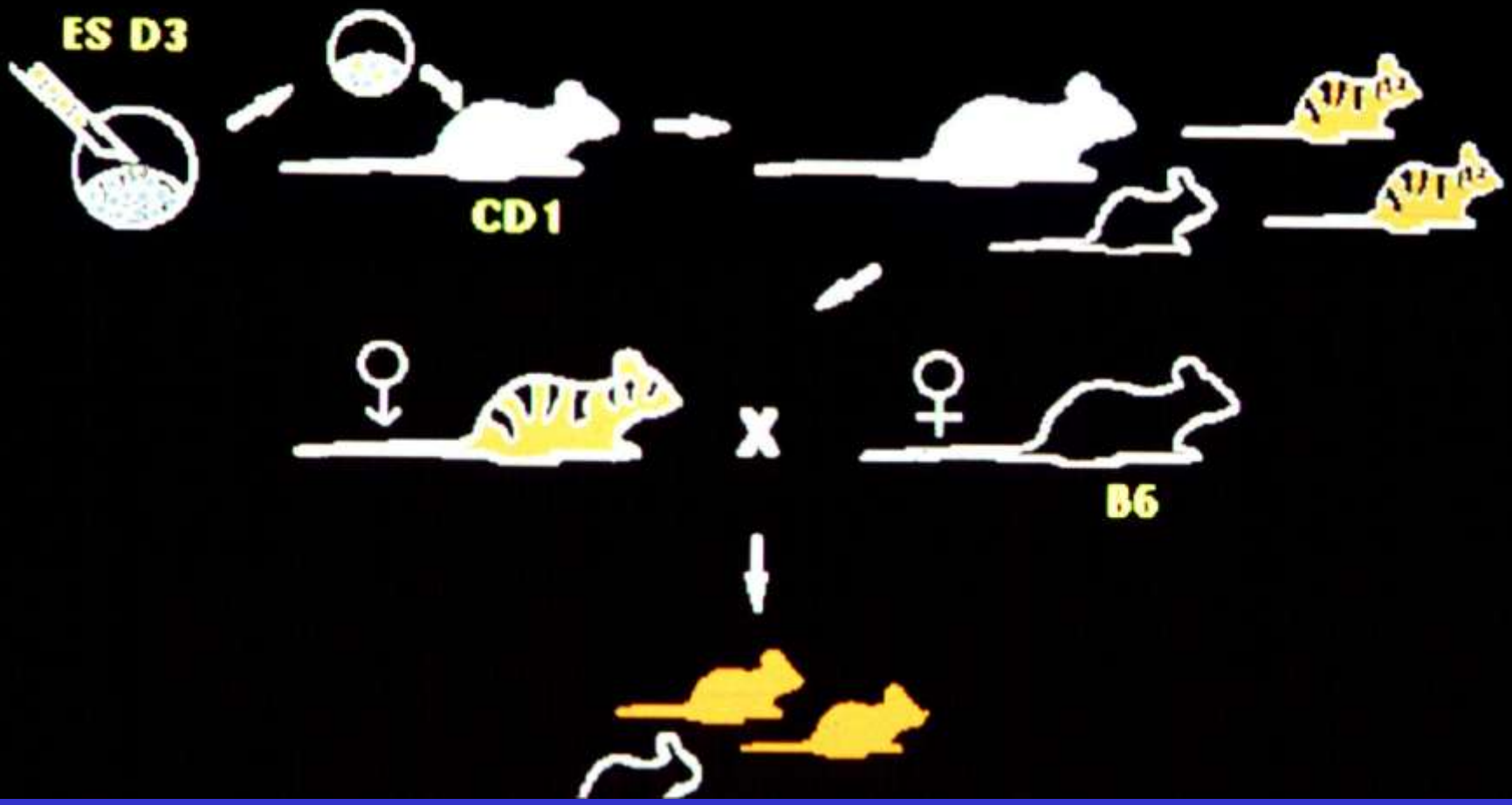


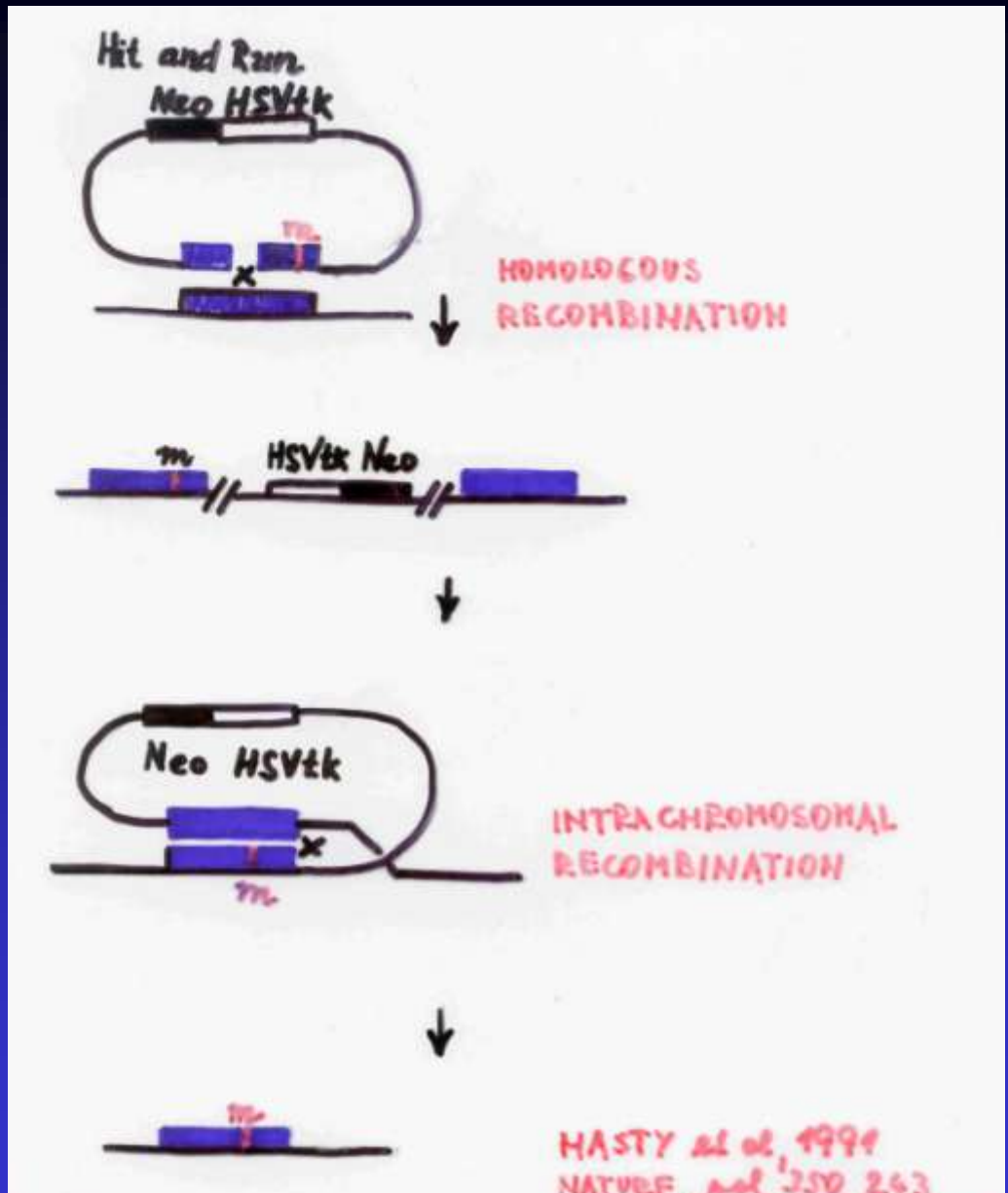


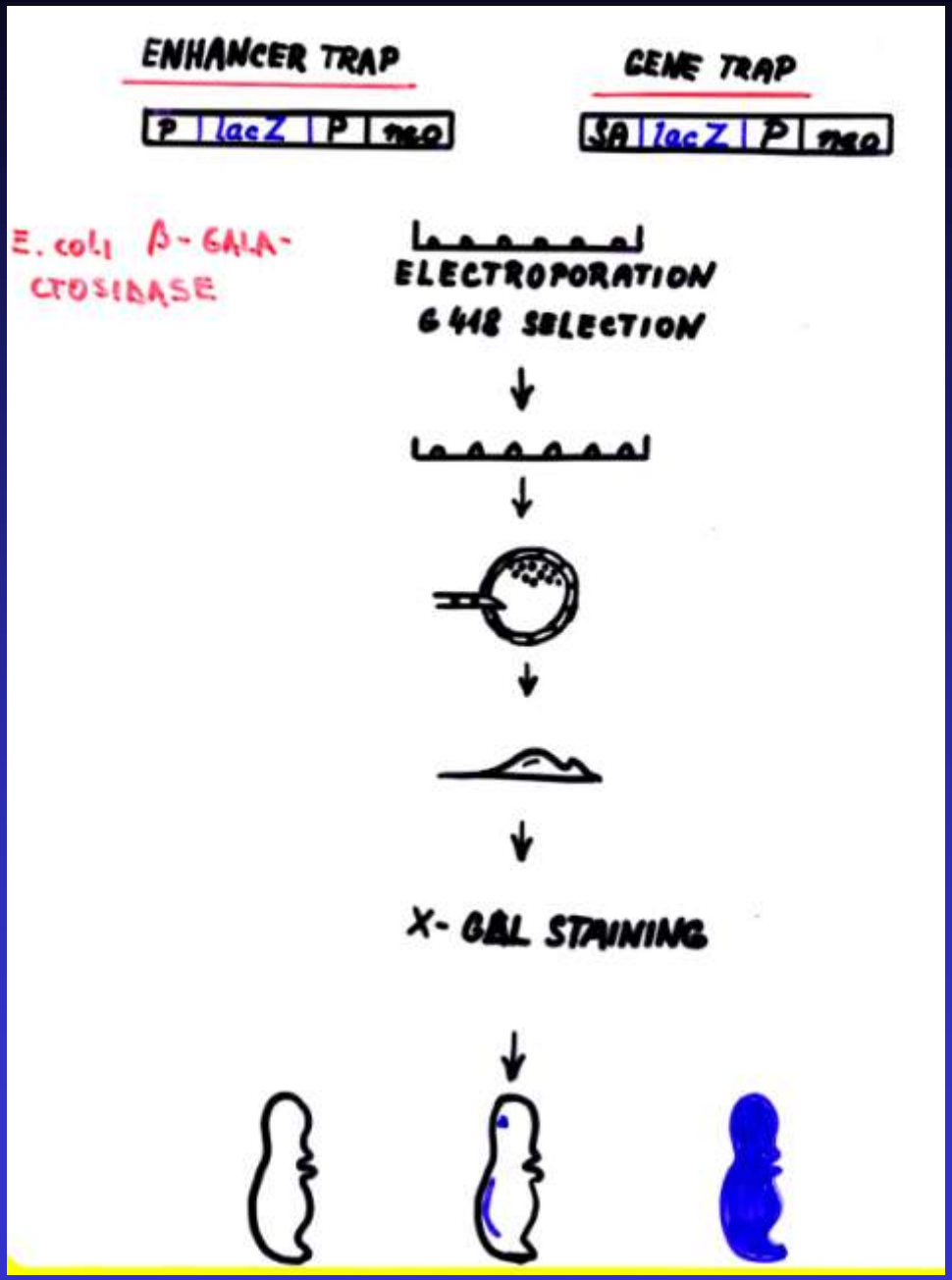
Southern blot analysis of ES E14 cell clones

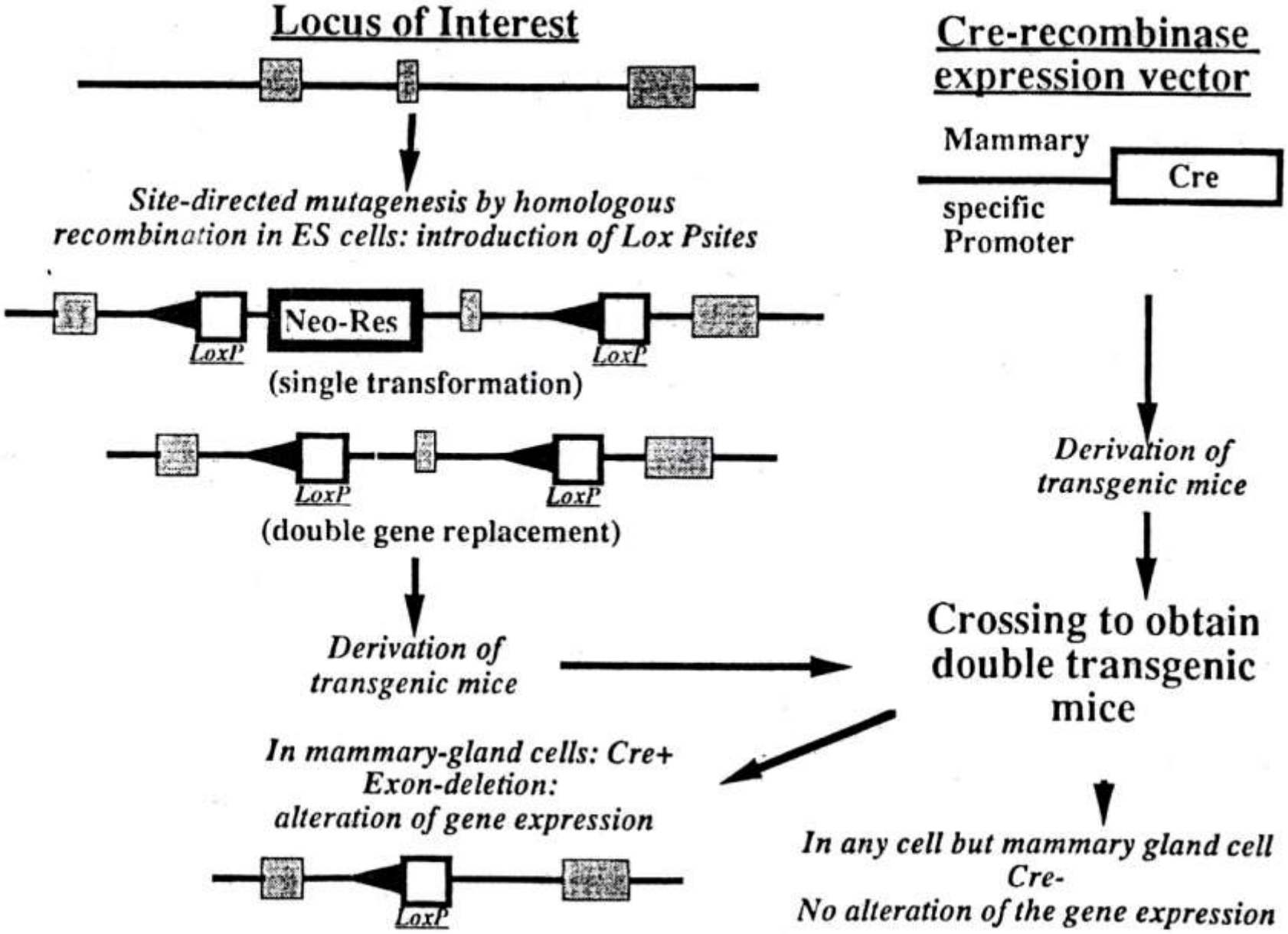


Generation of mouse germ-line chimeras



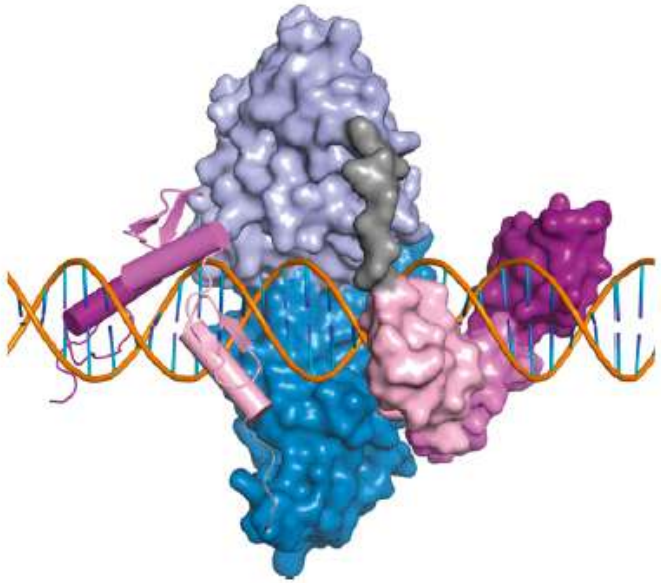

Hit and Run
Neo HSVtk
HOMOLOGOUS
RECOMBINATION
HSVtk Neo
Neo HSVtk
INTRACHROMOSOMAL
RECOMBINATION
HASTY et al, 1991
NATURE, vol 350, 243
HPRT - HYPOXANTHINE PHOSPHORIBOSYLTRANSFERASE
HOX - 2.6

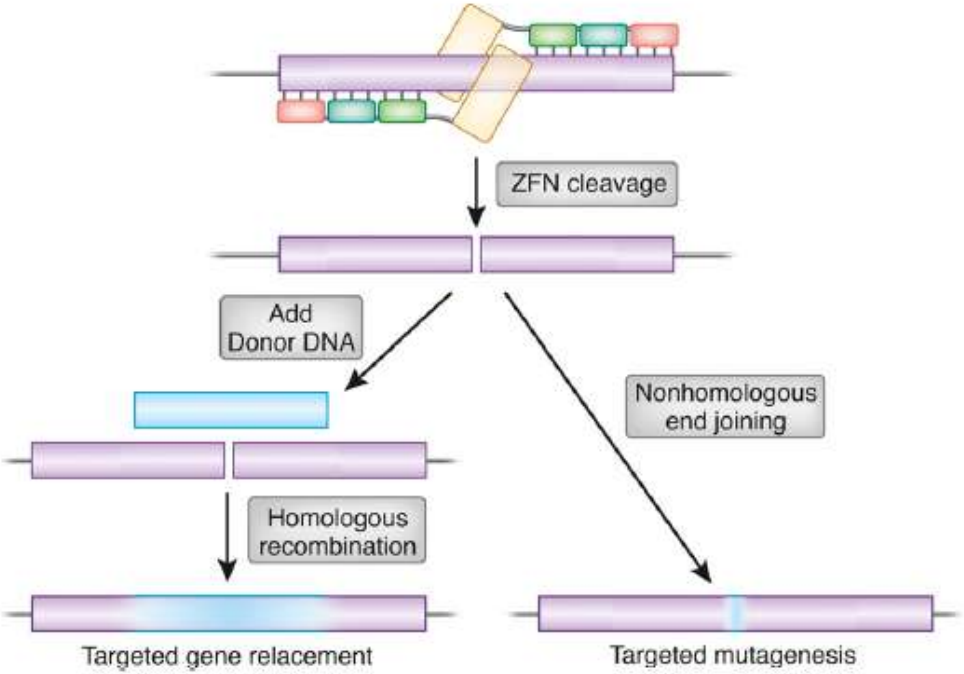

ENHANCER TRAP
P
lacZ
P
neo
GENE TRAP
SA
lacZ
P
neo
E. coli β-GALACTOSIDASE
ELECTROPORATION
G418 SELECTION
X-GAL STAINING


Locus of Interest
Site-directed mutagenesis by homologous recombination in ES cells: introduction of Lox Psites
Neo-Res
LoxP
LoxP
(single transformation)
LoxP
LoxP
(double gene replacement)
Derivation of transgenic mice
In mammary-gland cells: Cre+
Exon-deletion:
alteration of gene expression
LoxP
Cre-recombinase expression vector
Mammary
specific
Promoter
Cre
Derivation of transgenic mice
Crossing to obtain double transgenic mice
In any cell but mammary gland cell
Cre-
No alteration of the gene expression

ZFNs – Zinc finger nukleásy – DNA binding domain + Fok I endonuclease monomer

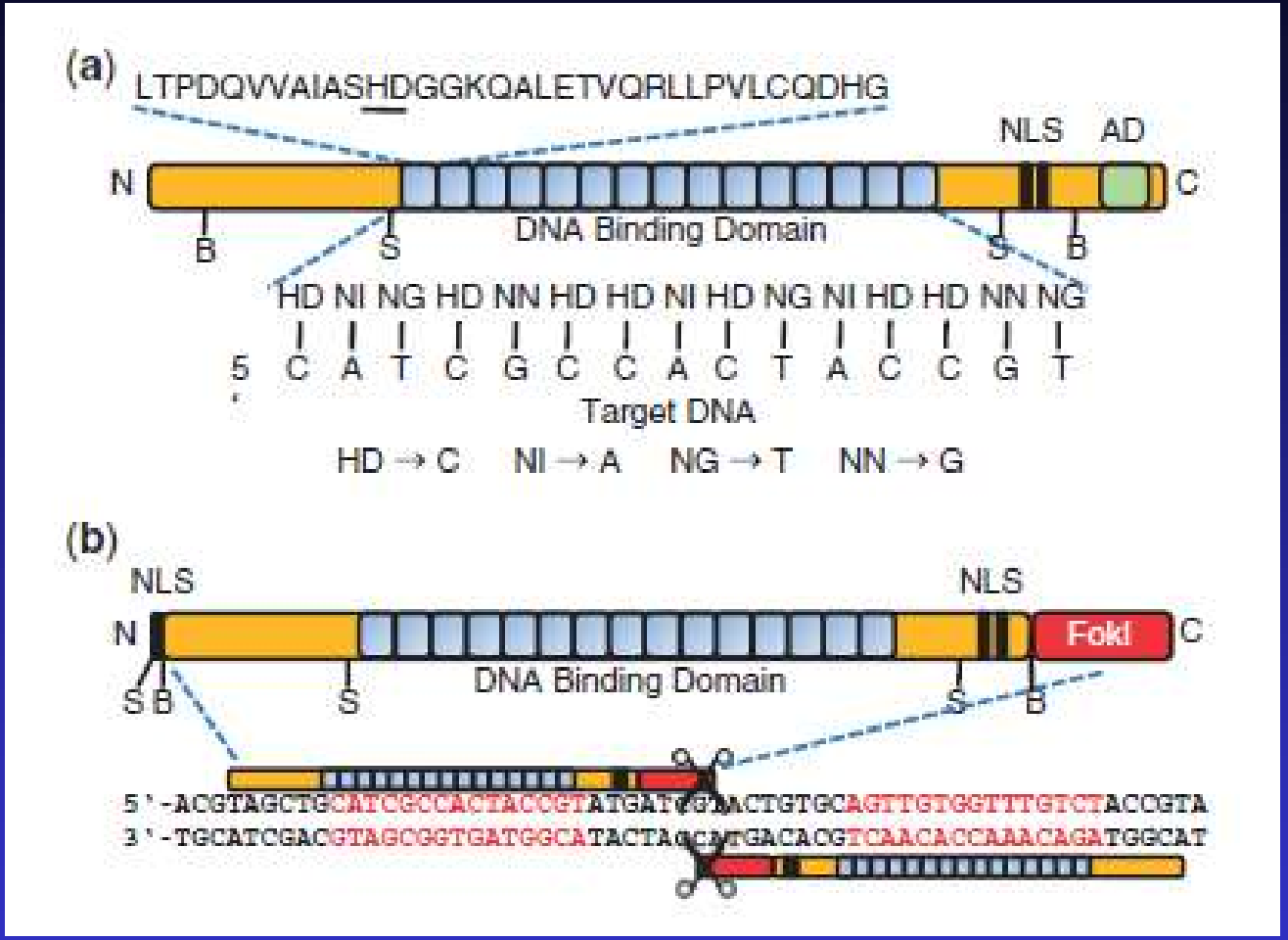
Double-strand break – oprava pomocí non-homologous end-joining, drobné inzerce nebo delece (indels)





Caroll D., Genetics, 188: 773-782, 2011

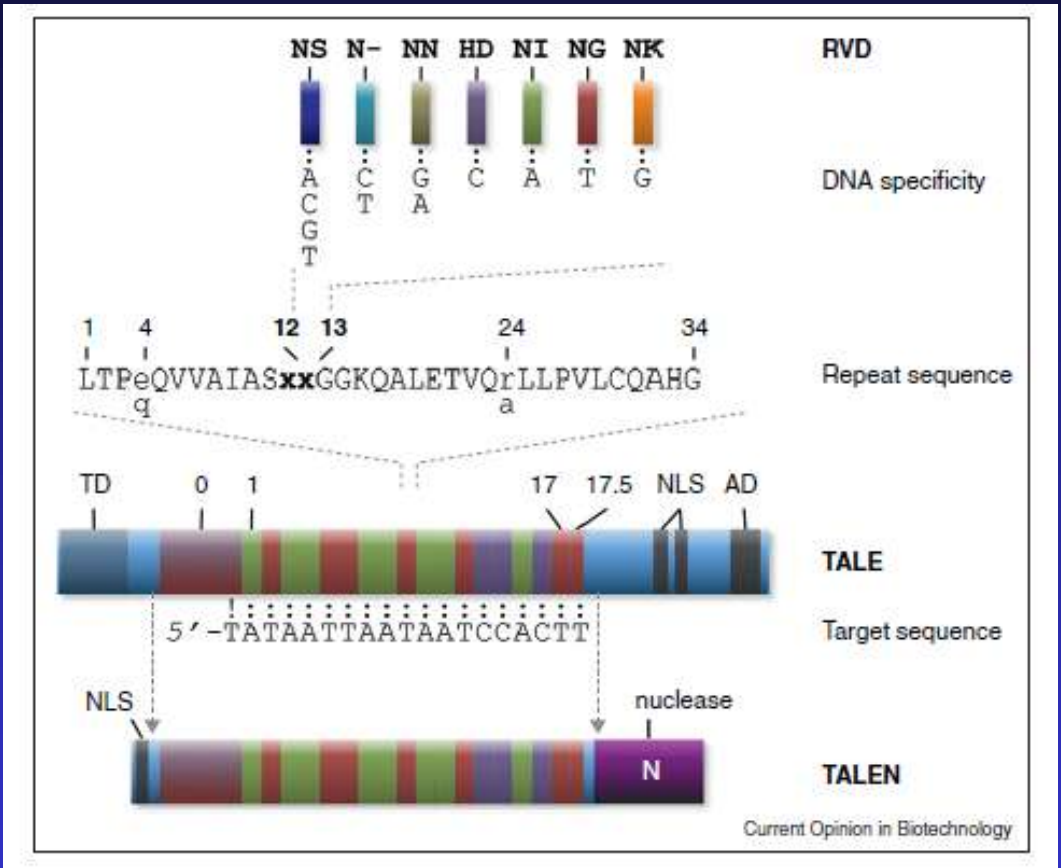
TALENs – transcription aktivátor-like effector nucleases, TALE-based nucleases

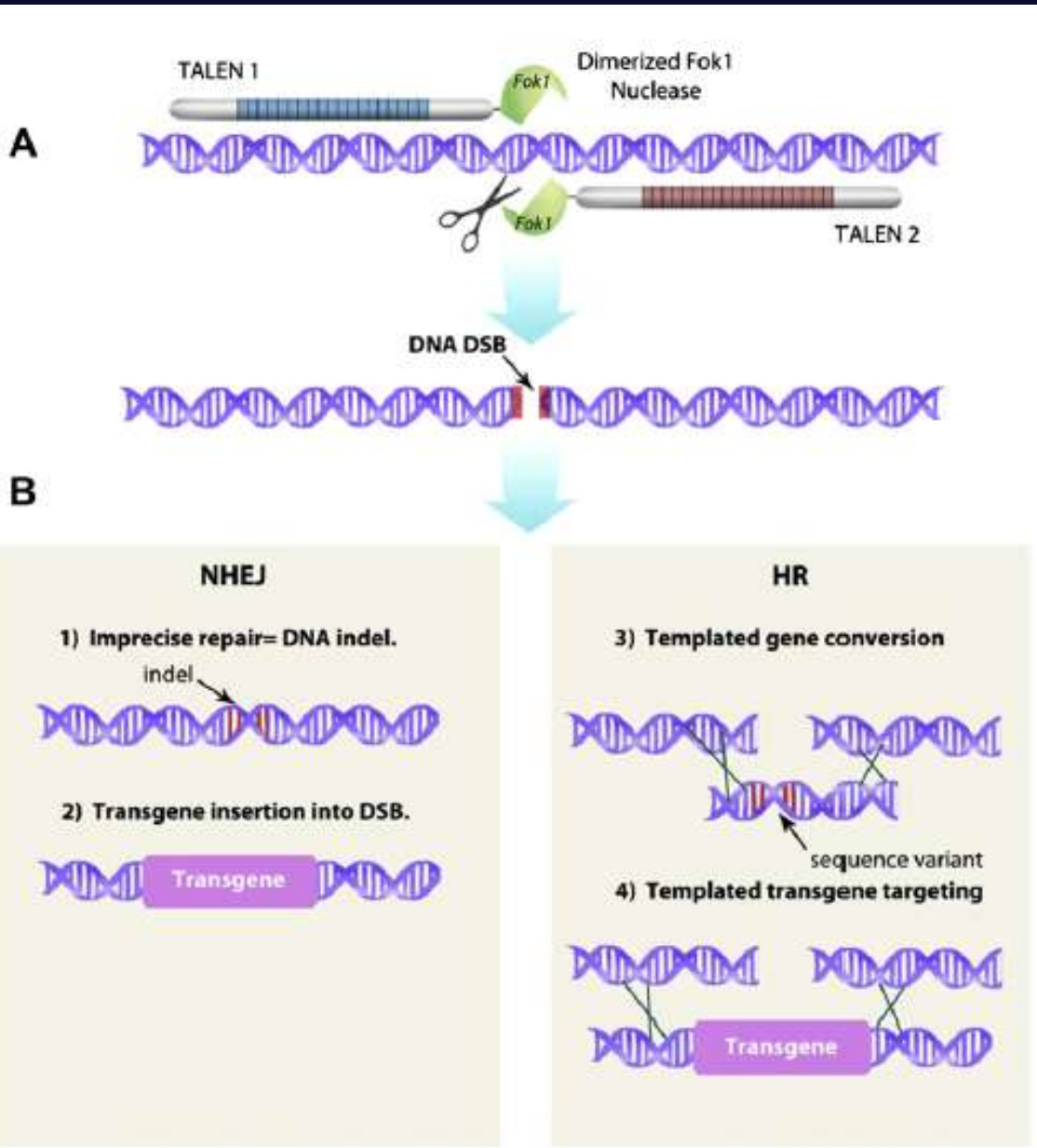


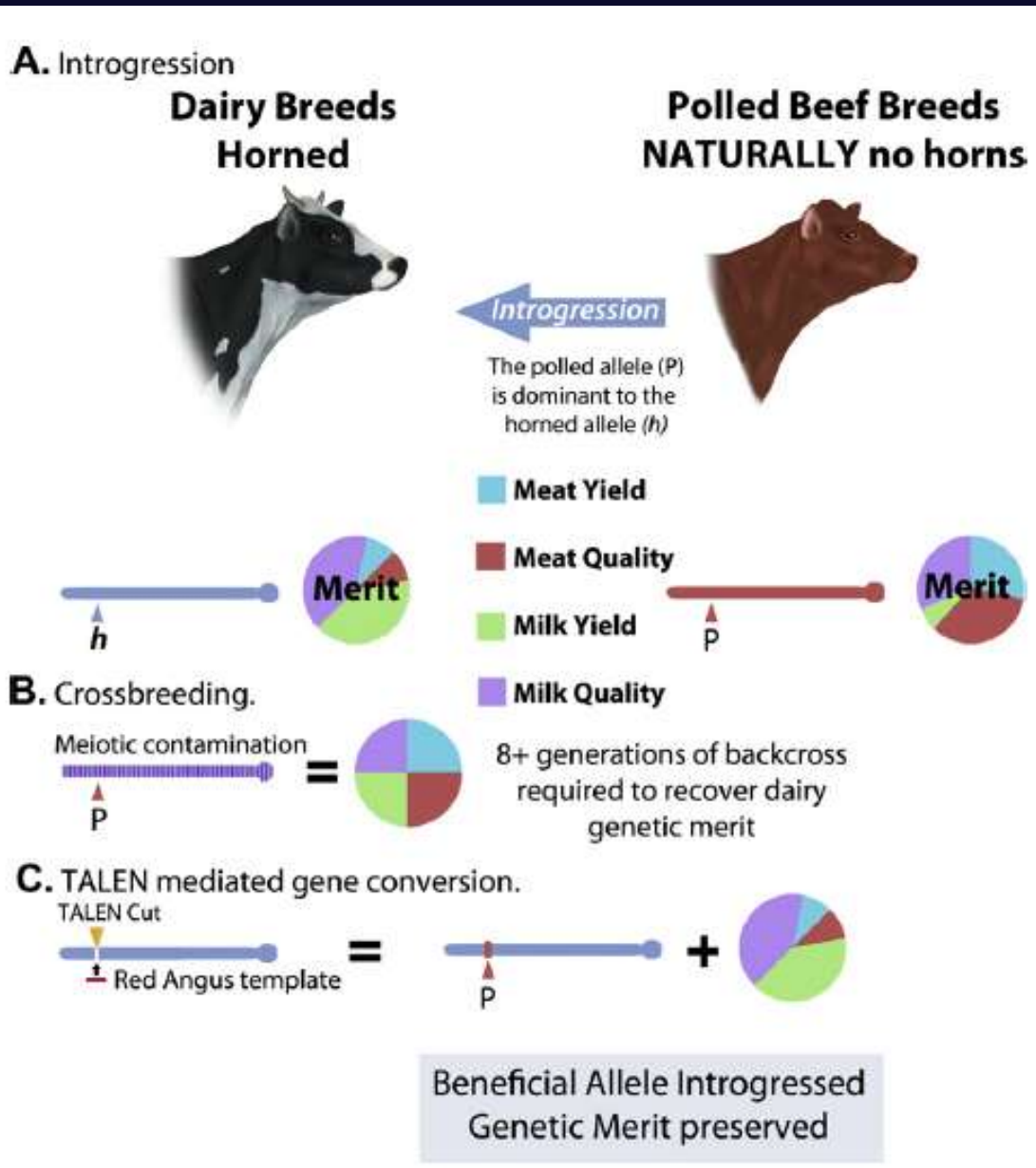
TALE – bakteriální proteiny, pathogen Xanthomonas – injikace proteinů do infikovaných rostlinných buněk
Rozpoznání cílové DNA v hostitelském genomu, aktivace exprese genů, nezbytných pro multiplikaci pathogenu

DNA binding domain – skládá se z tandemu 15,5 – 19,5 single repeats, každý se skládá z 34 vysoce konzervovaných zbytků

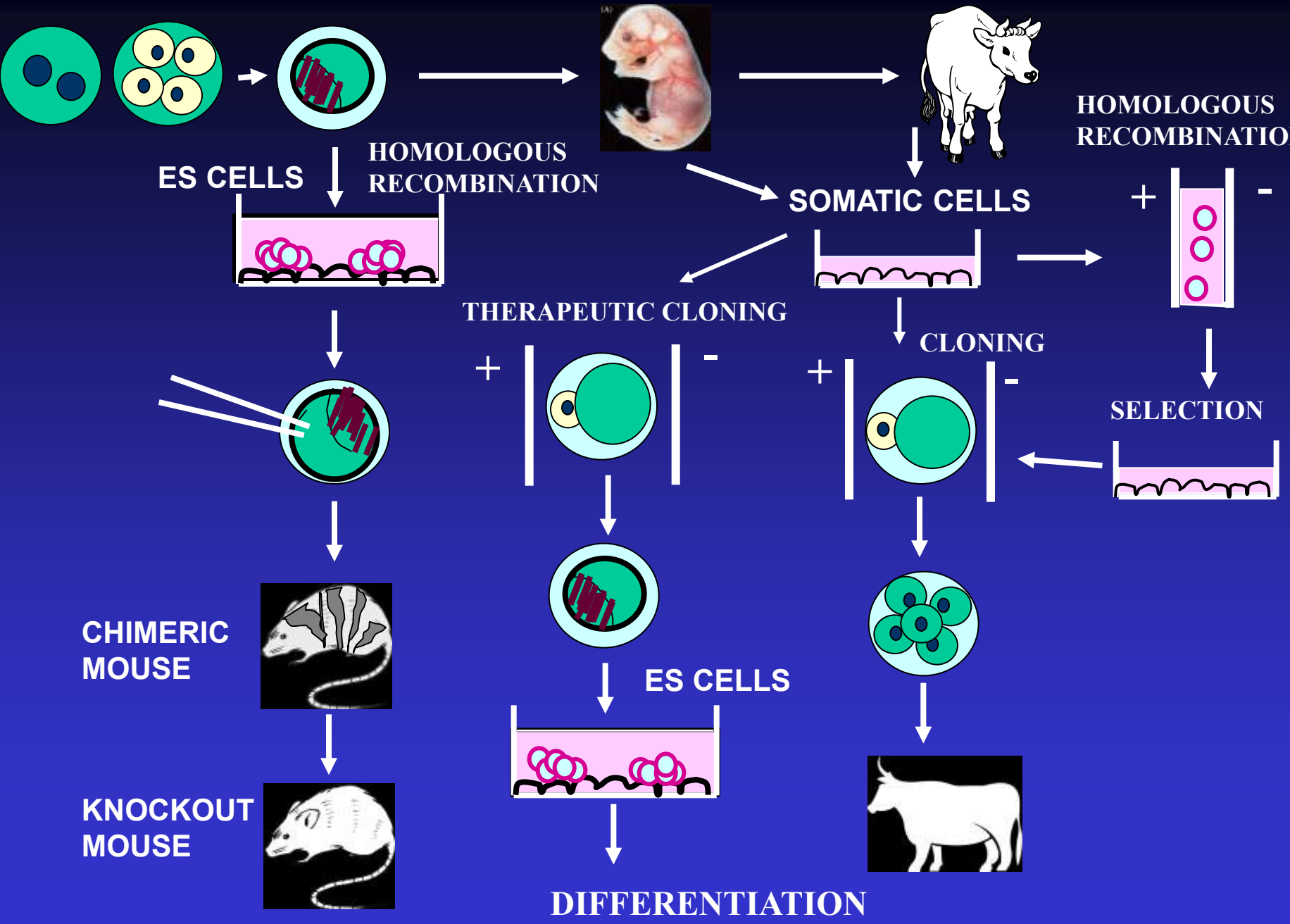
Carlson D.F. et al., www.pnas.org/cgi/doi/10.1073/pnas.1211446109


NS N- NN HD NI NG NK
RVD
A C G T
C T
G A
C
A
T
G
DNA specificity
1 4 12 13 24 34
LTPeQVVAIASxxGGKQALETVQrLLPVLCQAHG
q
a
Repeat sequence
TD 0 1 17 17.5 NLS AD
TALE
5′-TATAATTAATAATCCACTT
Target sequence
NLS
nuclease
N
TALEN
Current Opinion in Biotechnology

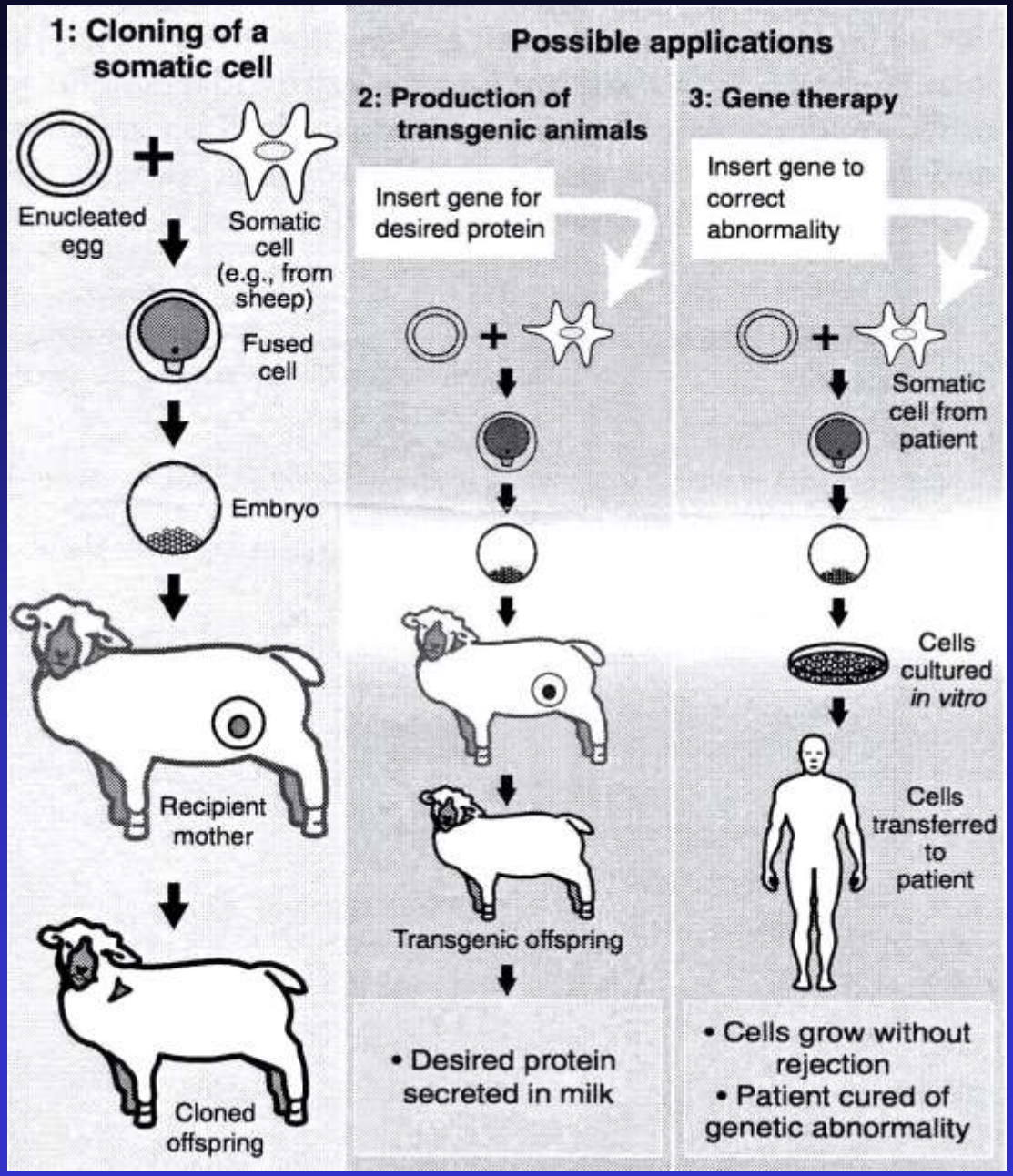

A
TALEN 1
Fok1
Dimerized Fok1
Nuclease
Fok1
TALEN 2
DNA DSB
B
NHEJ
1) Imprecise repair= DNA indel.
indel
2) Transgene insertion into DSB.
Transgene
HR
3) Templated gene conversion
sequence variant
4) Templated transgene targeting
Transgene


A. Introgression
Dairy Breeds
Horned
Polled Beef Breeds
NATURALLY no horns
Introgression
The polled allele (P) is dominant to the horned allele (h)
Meat Yield
Meat Quality
Milk Yield
Milk Quality
h
Merit
P
Merit
B. Crossbreeding.
Meiotic contamination
P
=
8+ generations of backcross required to recover dairy genetic merit
C. TALEN mediated gene conversion.
TALEN Cut
Red Angus template
=
P
+
Beneficial Allele Introgressed
Genetic Merit preserved

PRE-IMPLANTATION EMBRYO



CLONING - POTENTIAL BENEFITS



Trounson, A. ; MJA 167:568-569 ; 1997