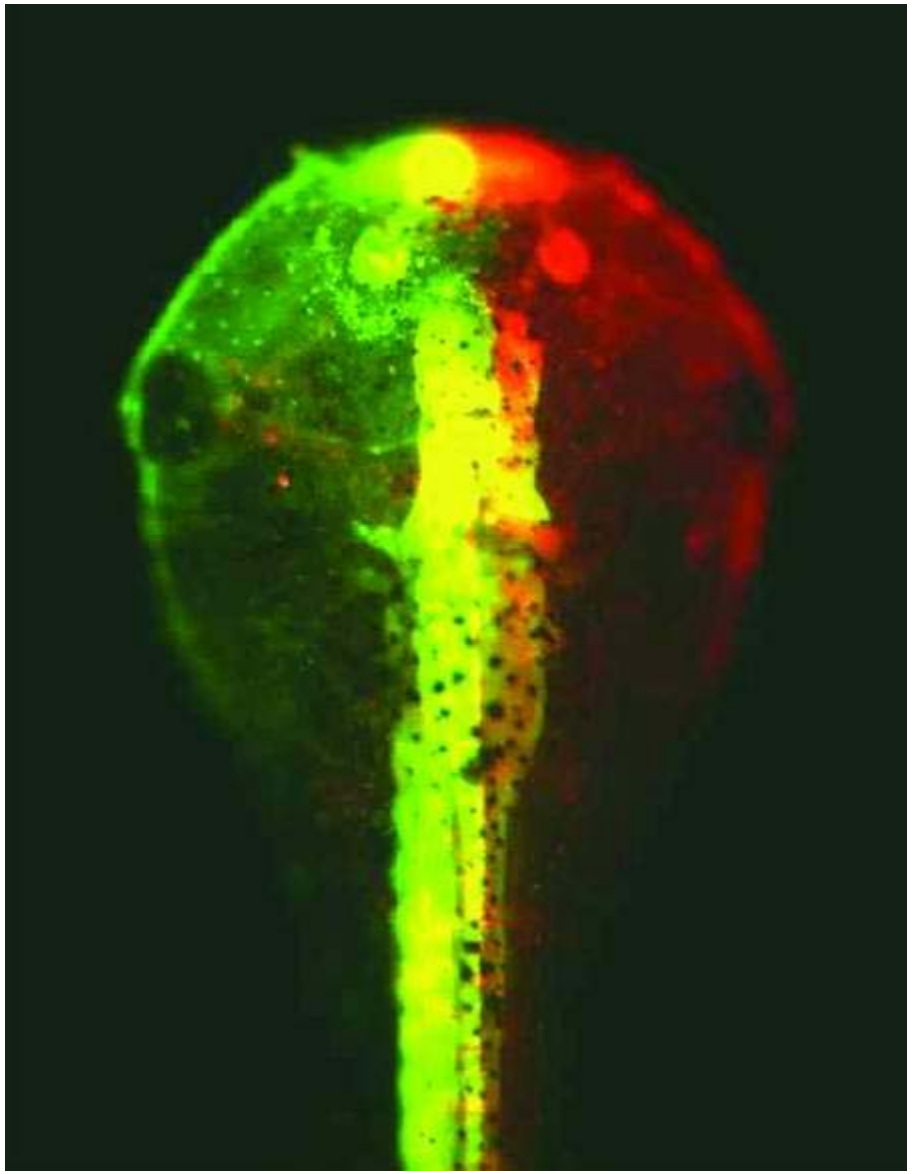
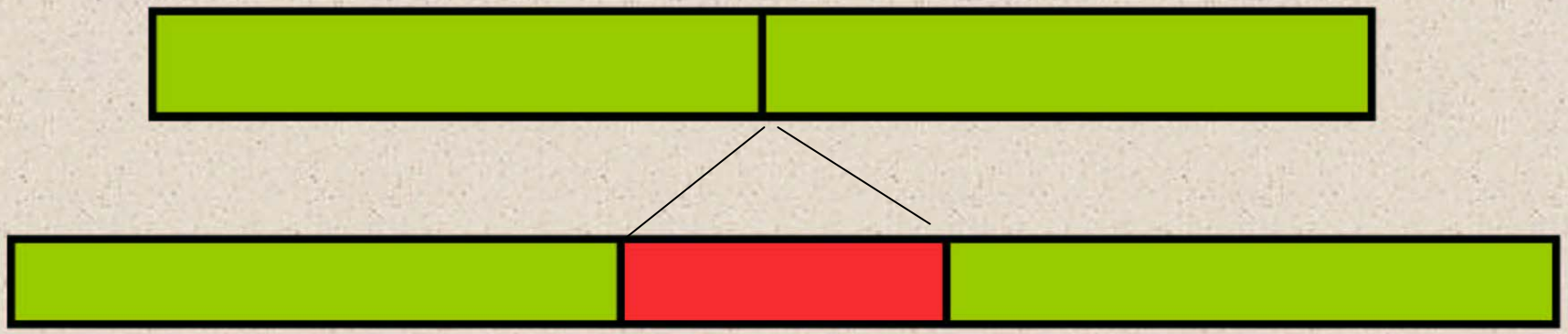
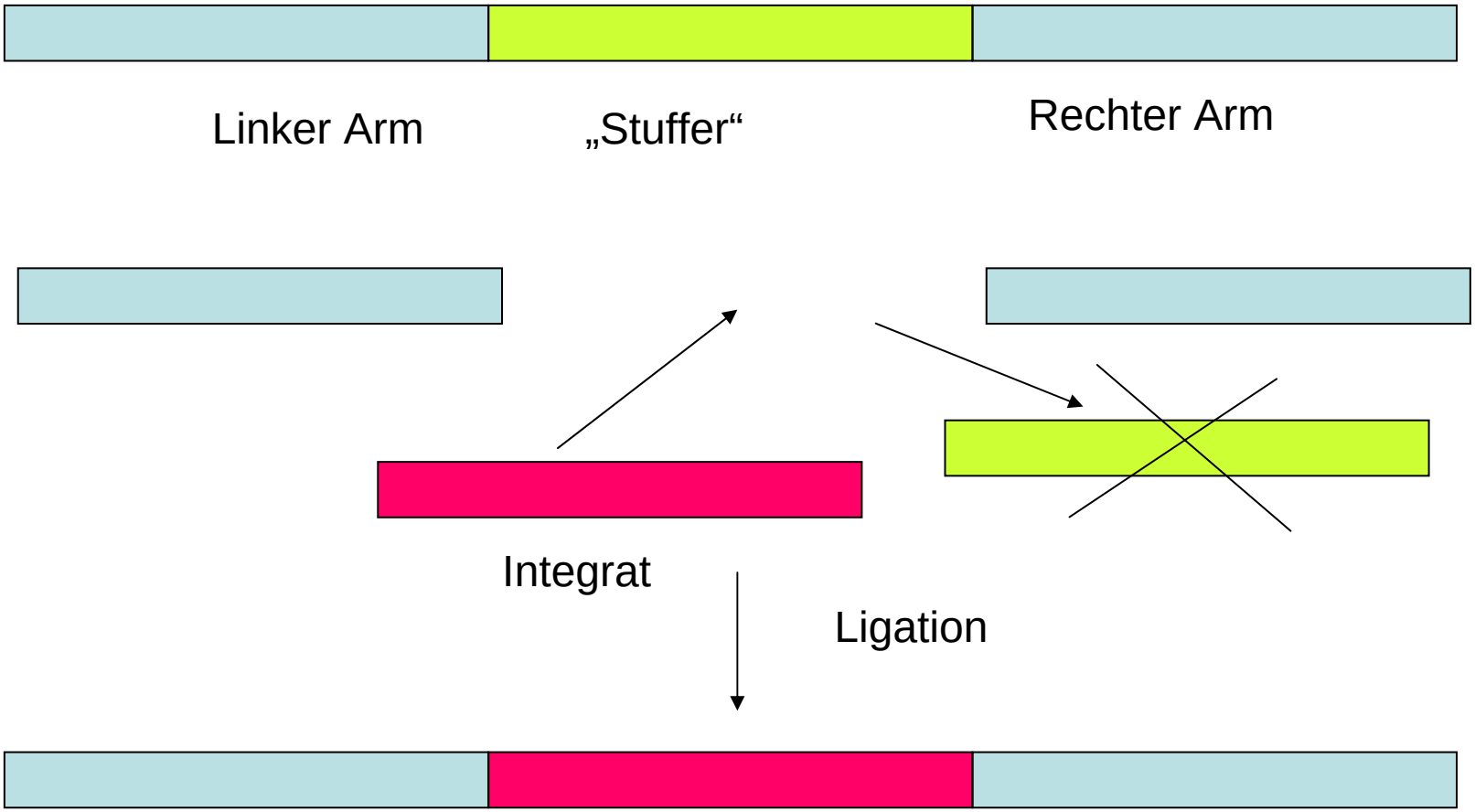


Bakteriophage λ

Integrationsvektoren



Substitutionsvektoren



Lambda gt10/11

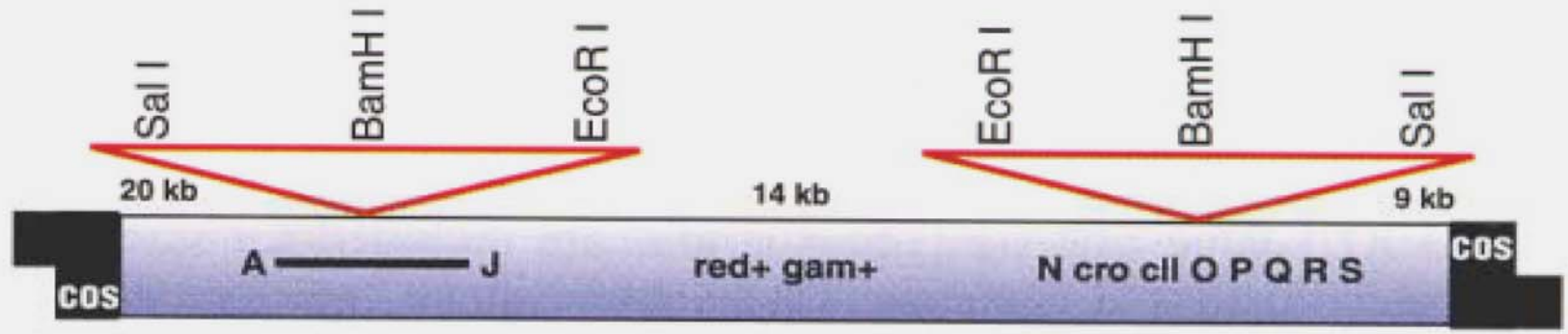
The Lambda gt11 Vector

- Predigested with *EcoR* I
- For construction of cDNA libraries

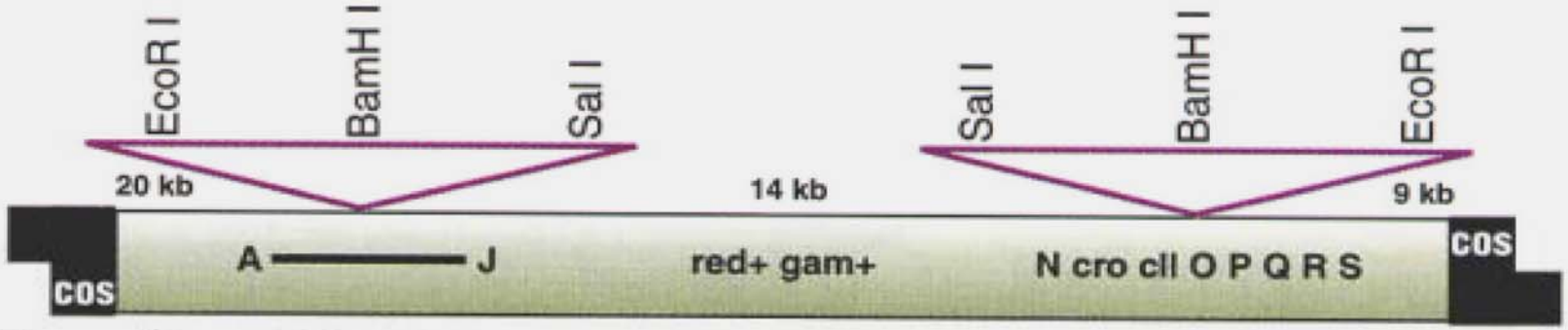
Map of Lambda gt11 Vector

CLONING CAPACITY Capacity of 7.2 kb

Lambda EMBL3/4



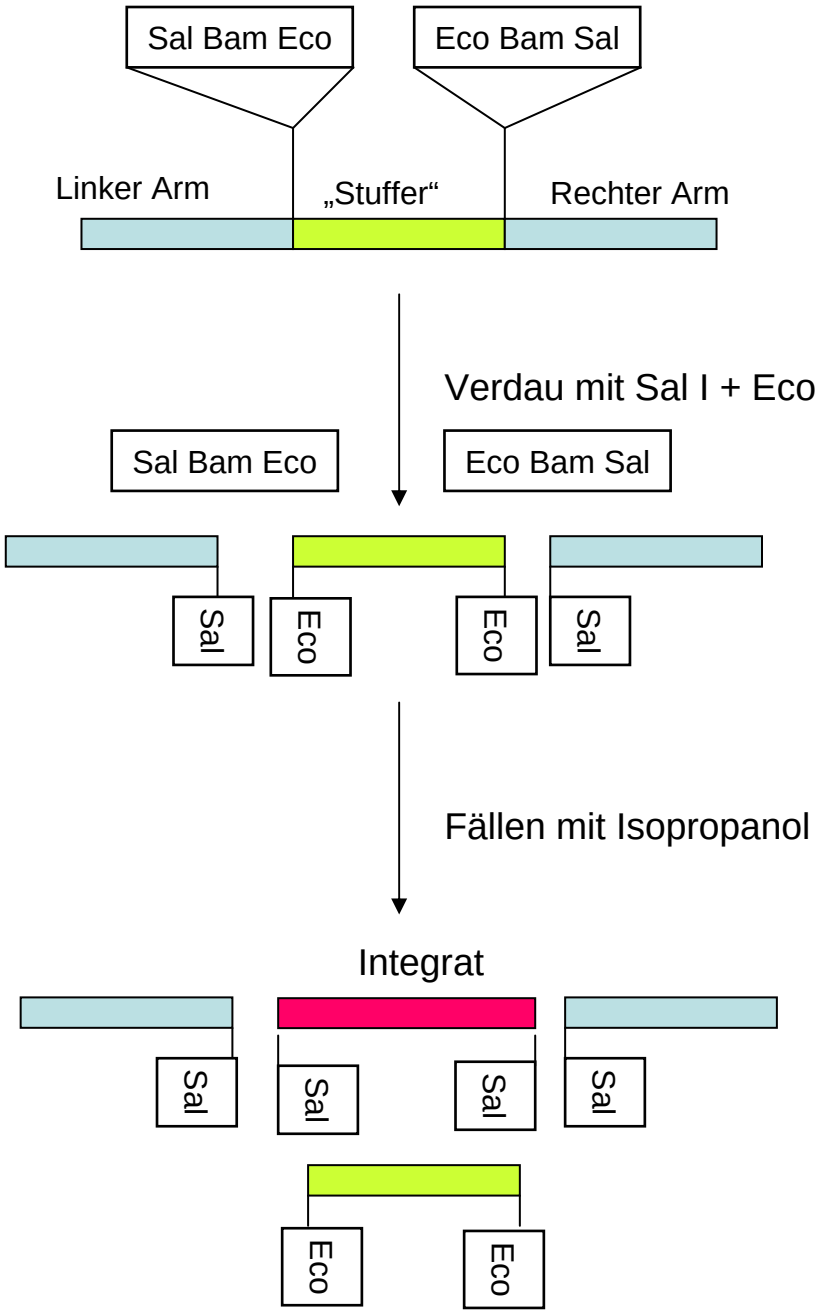
Map of Lambda EMBL3



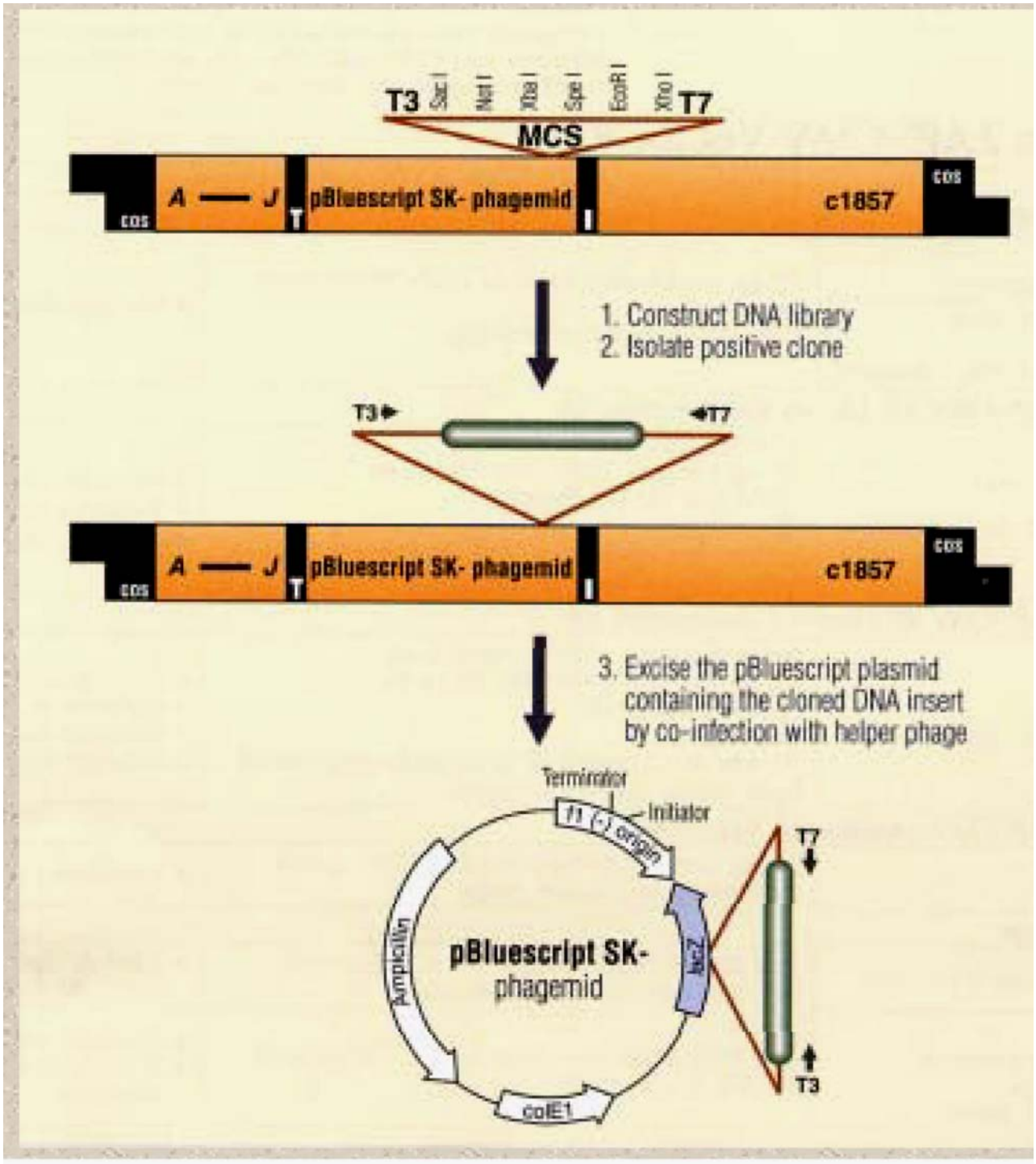
Map of Lambda EMBL4

Lambda EMBL3/4

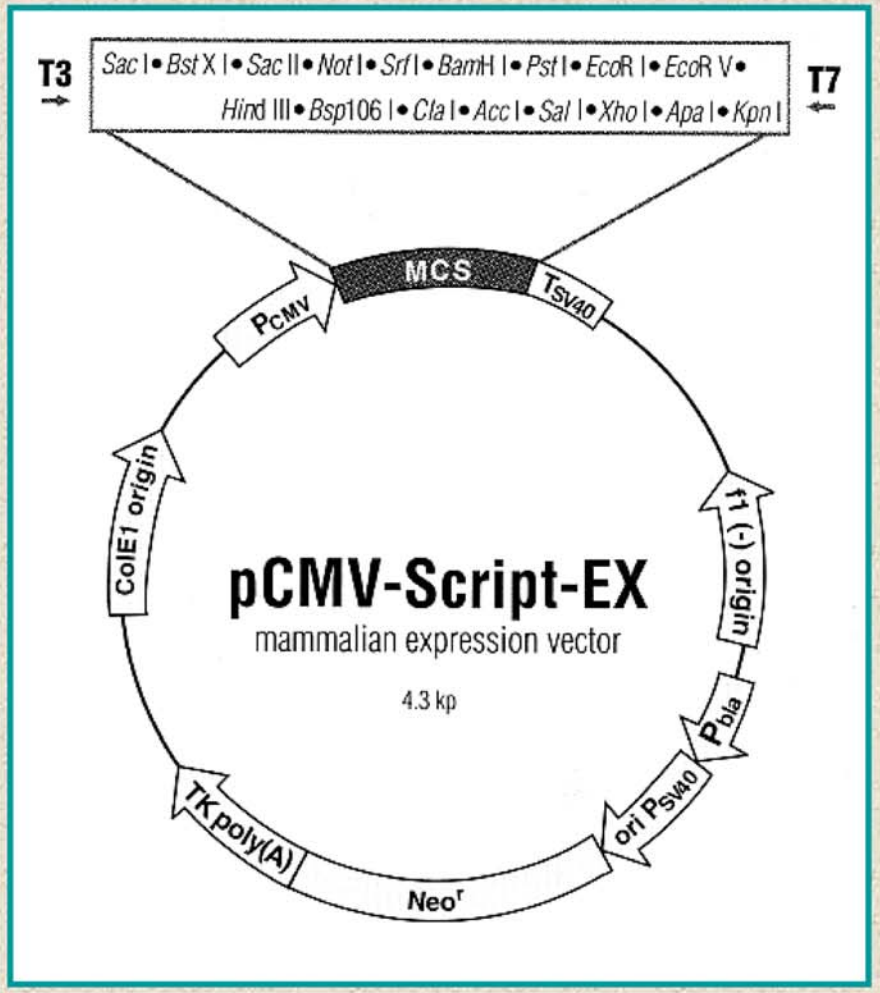
Durch Doppelverdau mit SalI und EcoRI erhalten die Arme andere Enden als das zentrale Fragment („stuffer“), so dass eine Religation des Vektors sehr unwahrscheinlich ist, insbesondere wenn die Linker durch Fällung aus dem Gemisch entfernt werden

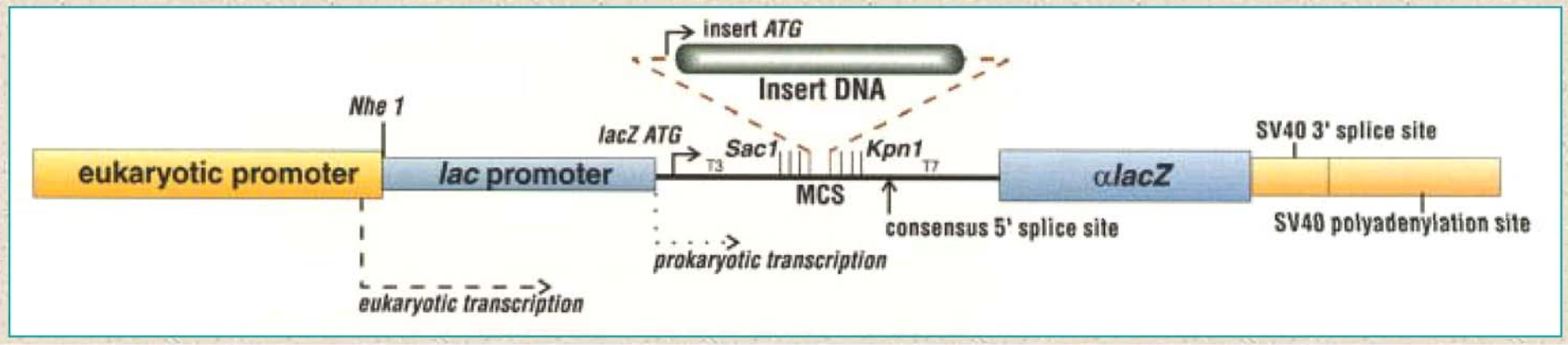


Lambda Zap:
Moderne Vektoren
Mit Phagen und Plasmid-
eigenschaften

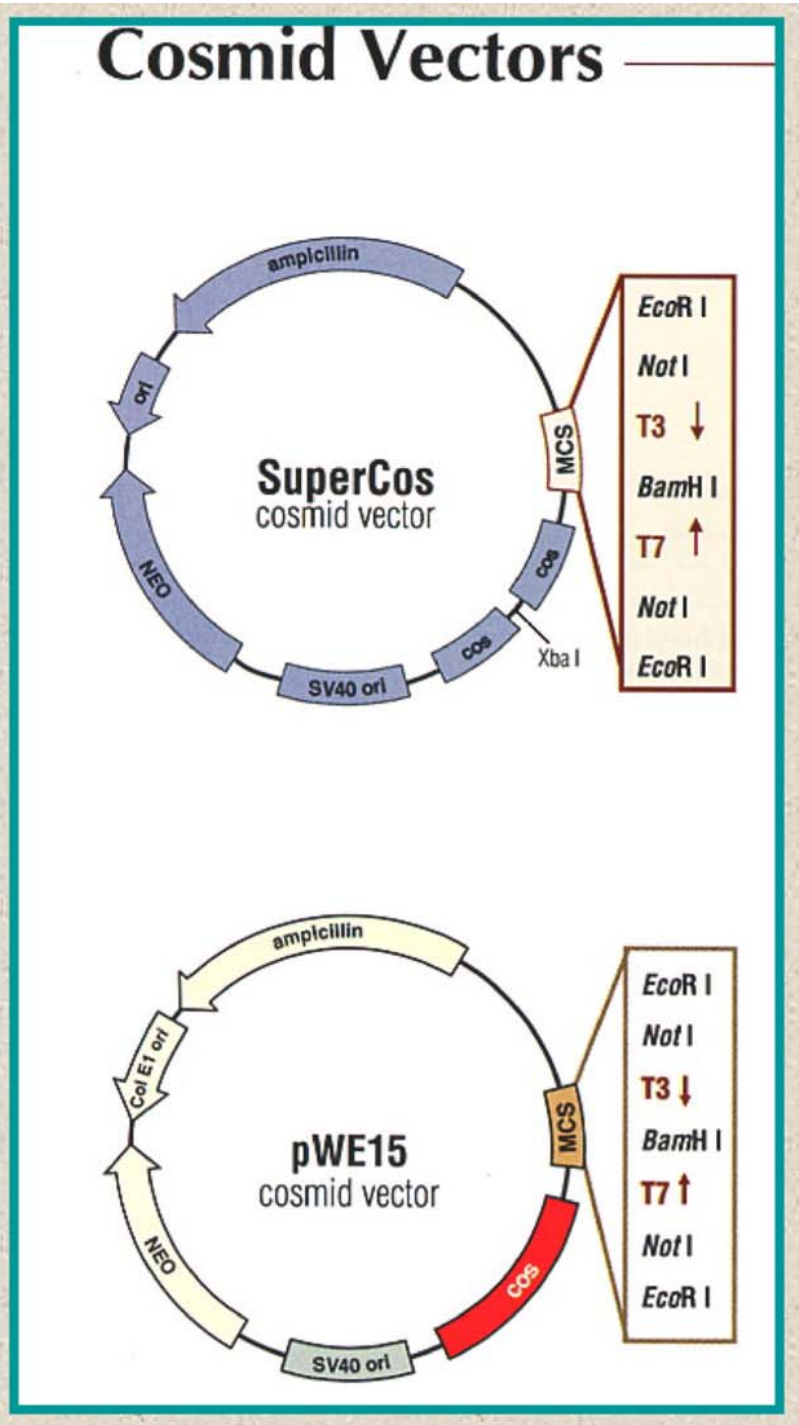


Lambda - „shuttle“ Expressions vektoren

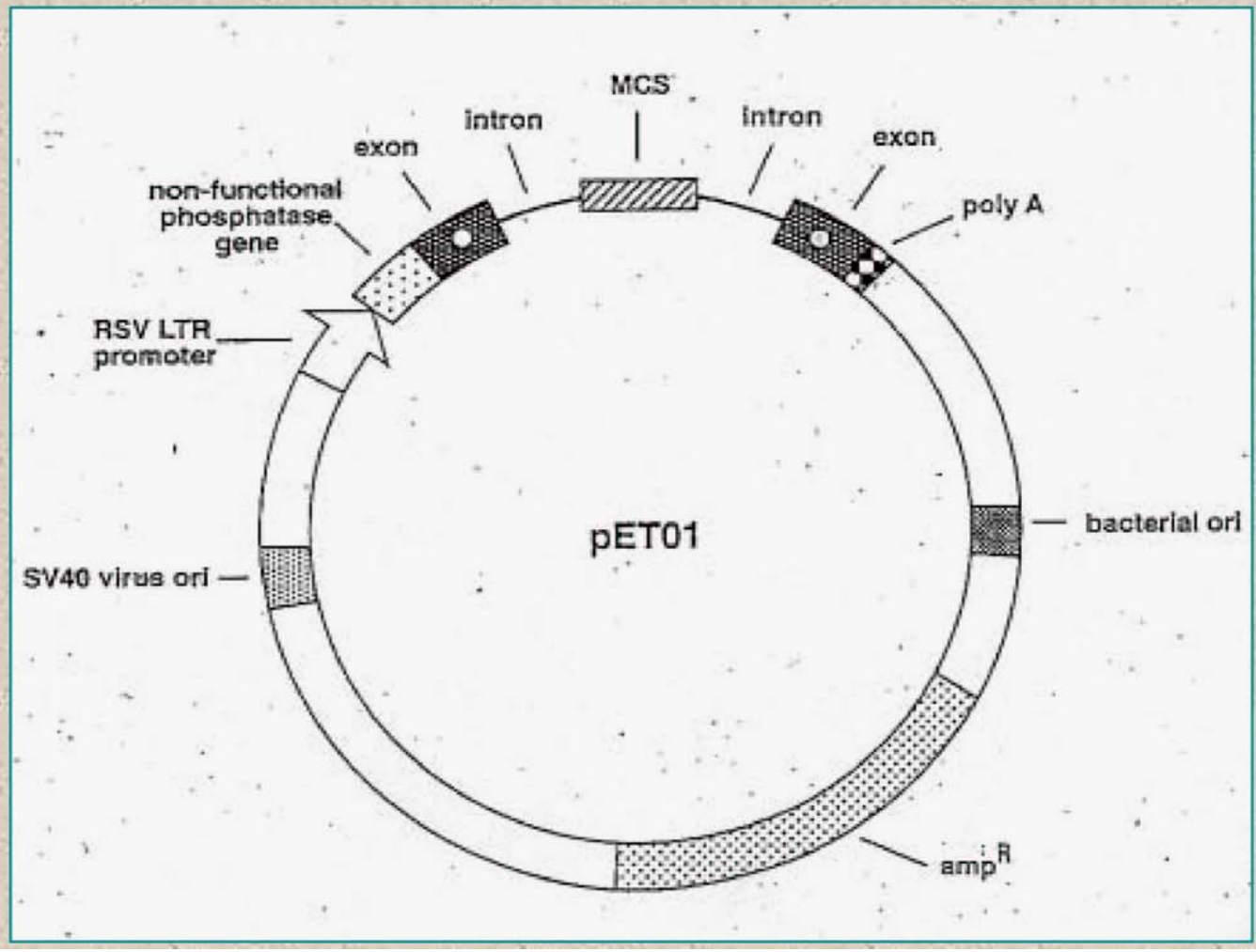




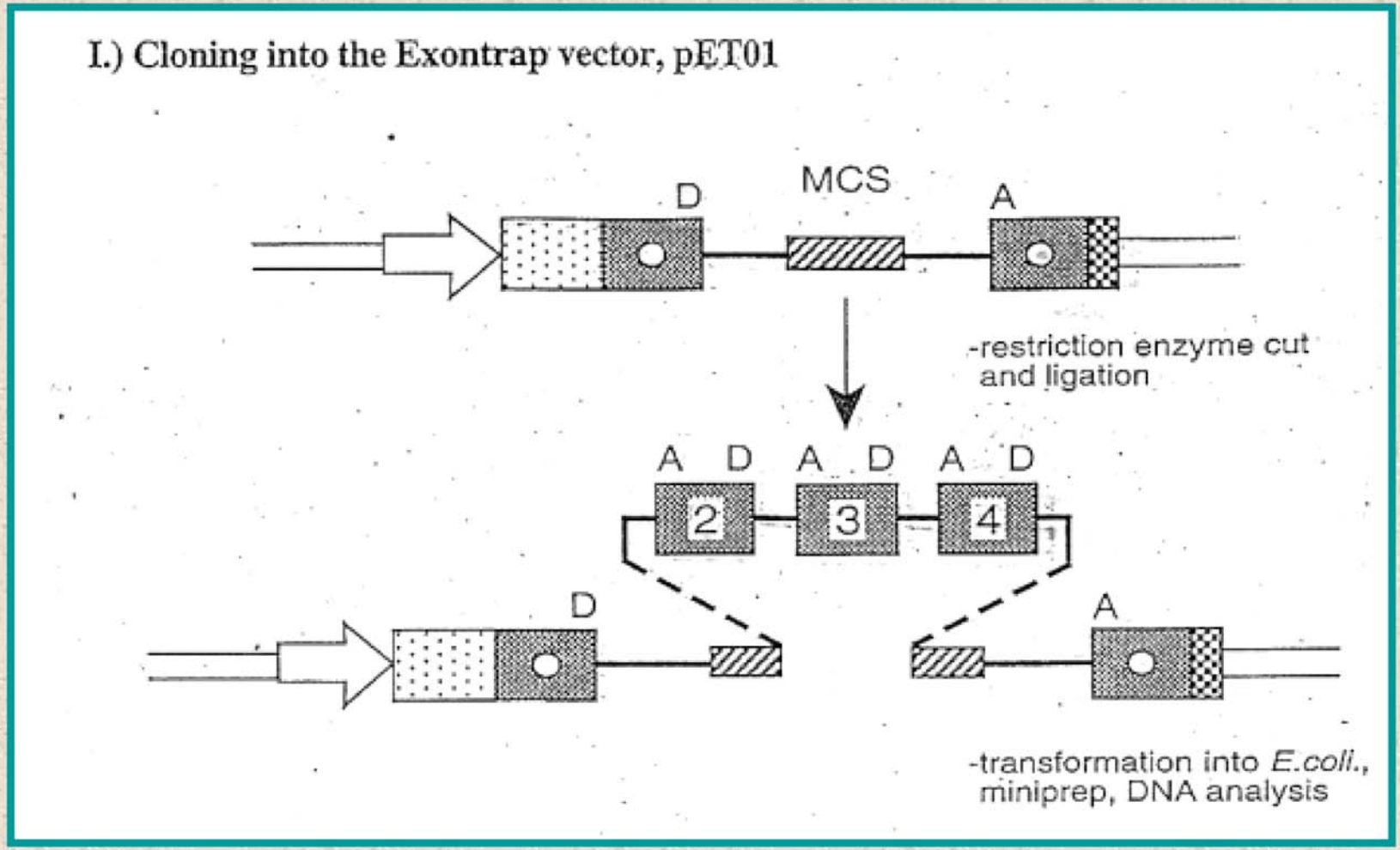
Cosmide,
Plasmide, die wie Bakteriophagen in die Zellen eingeschleust werden



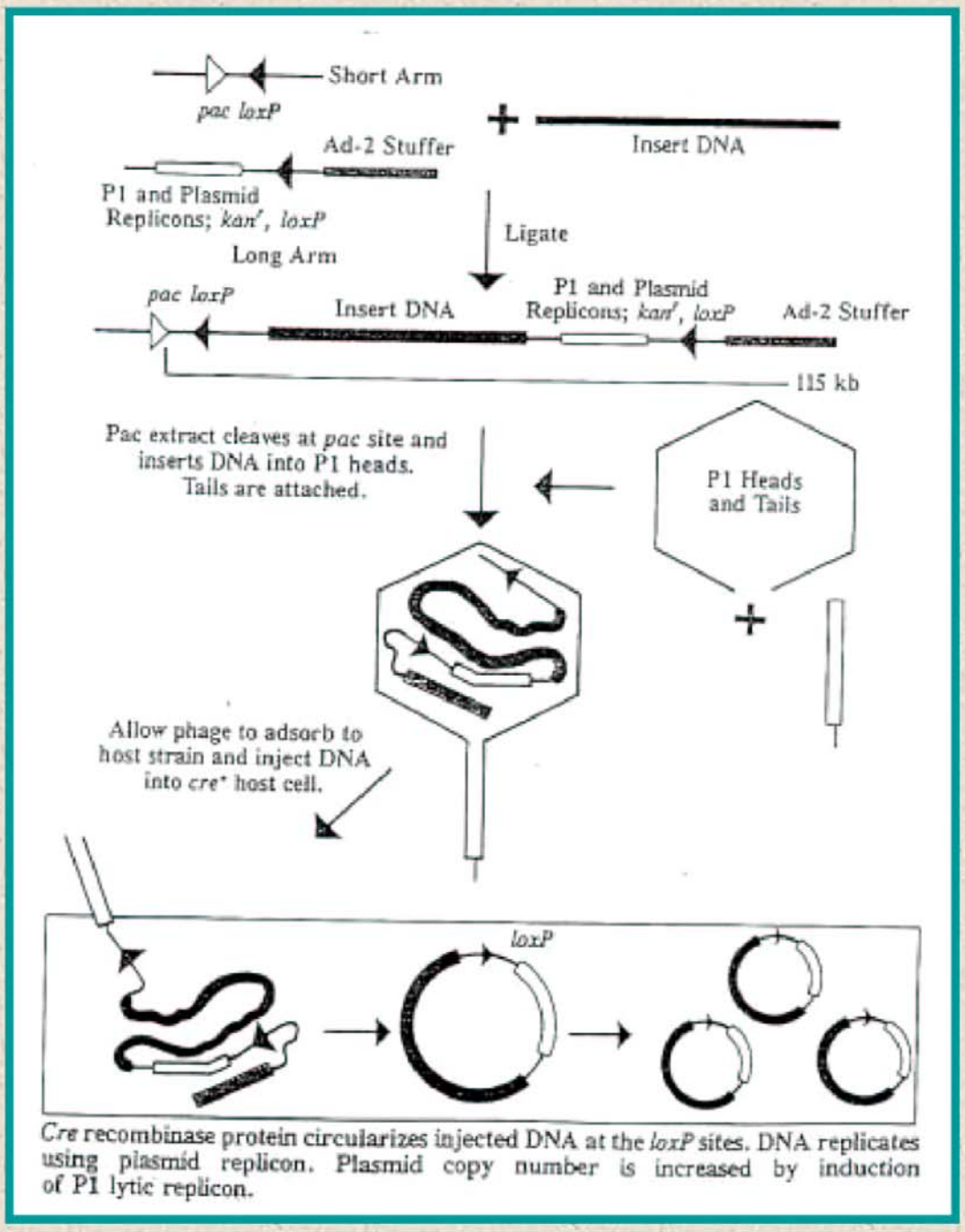
Spezialvektoren für „Exontrapping“



Spezialvektoren für „Exontrapping“

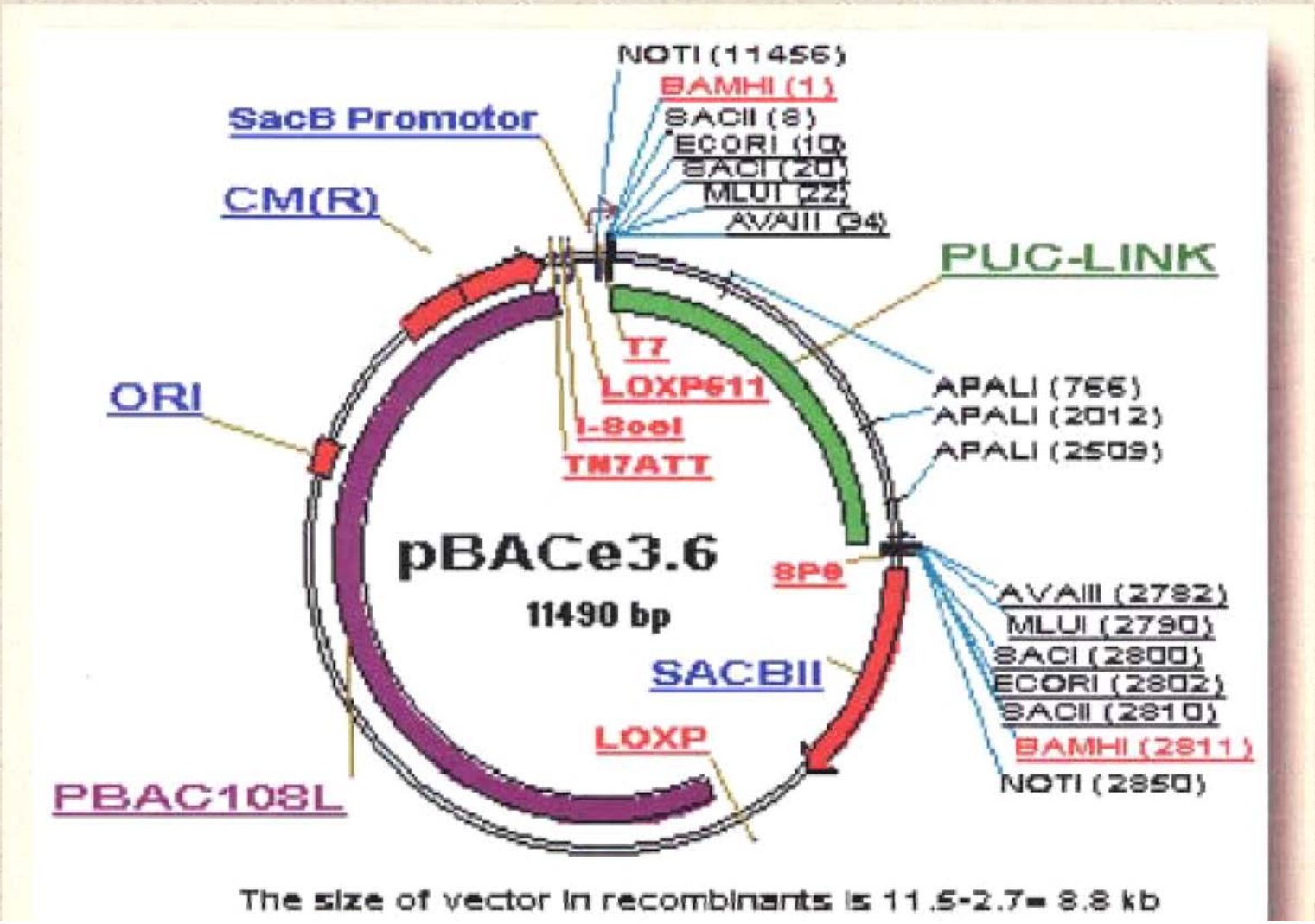


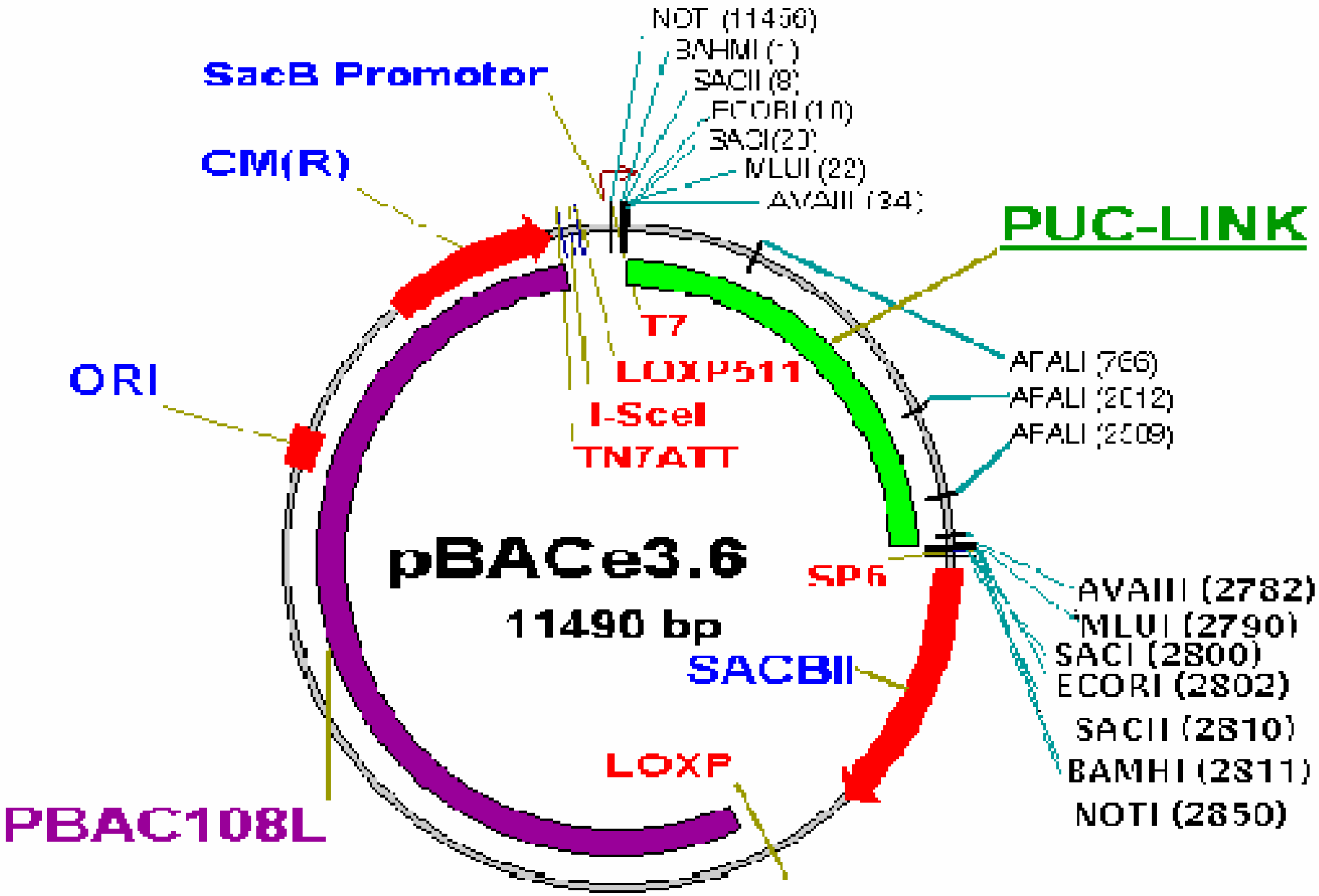
P1-Phage als Vektor: PAC: P1 artificial chromosome

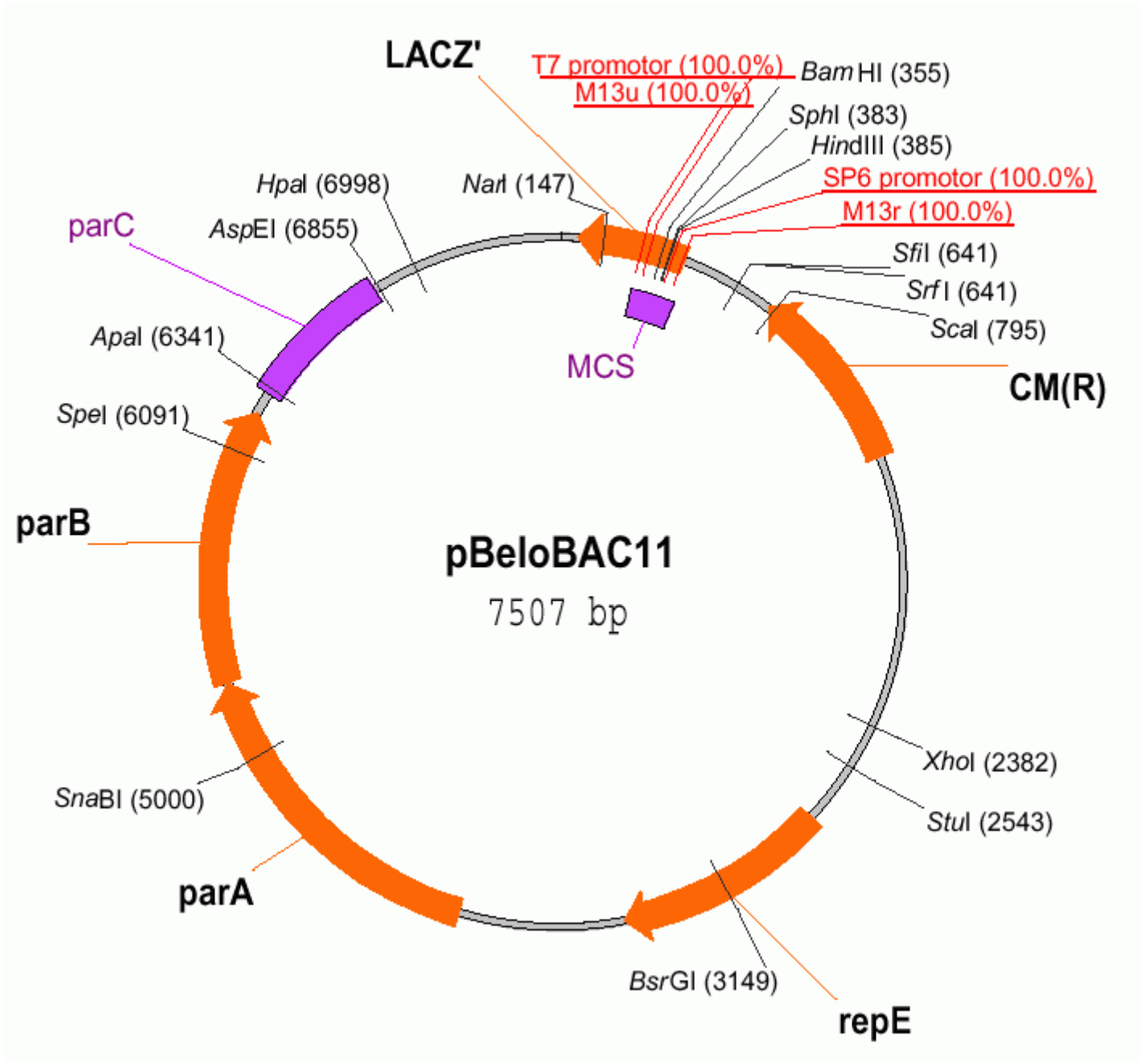


Cre recombinase protein circularizes injected DNA at the *loxP* sites. DNA replicates using plasmid replicon. Plasmid copy number is increased by induction of P1 lytic replicon.

BAC-Vektoren
„bacterial artificial chromosomes“




NOT (11456)
BAHMI (1)
SACII (8)
ECORI (10)
SACI (20)
MLUI (22)
AVAIII (34)
SacB Promotor
CM(R)
PUC-LINK
T7
LOXP511
I-SceI
TN7ATT
ORI
AFALI (766)
AFALI (2012)
AFALI (2509)
pBACe3.6
11490 bp
SP6
AVAIII (2782)
MLUI (2790)
SACI (2800)
ECORI (2802)
SACII (2810)
BAMHI (2811)
NOTI (2850)
SACBII
LOXP
PBAC108L


LACZ'
T7 promotor (100.0%)
M13u (100.0%)
BamHI (355)
SphI (383)
HindIII (385)
SP6 promotor (100.0%)
M13r (100.0%)
SfiI (641)
SrfI (641)
ScaI (795)
CM(R)
XhoI (2382)
StuI (2543)
repE
BsrGI (3149)
parA
SnaBI (5000)
parB
SpeI (6091)
ApaI (6341)
parC
AspEI (6855)
HpaI (6998)
NarI (147)
MCS
pBeloBAC11
7507 bp

Künstliche Chromosomen für Eukaryoten

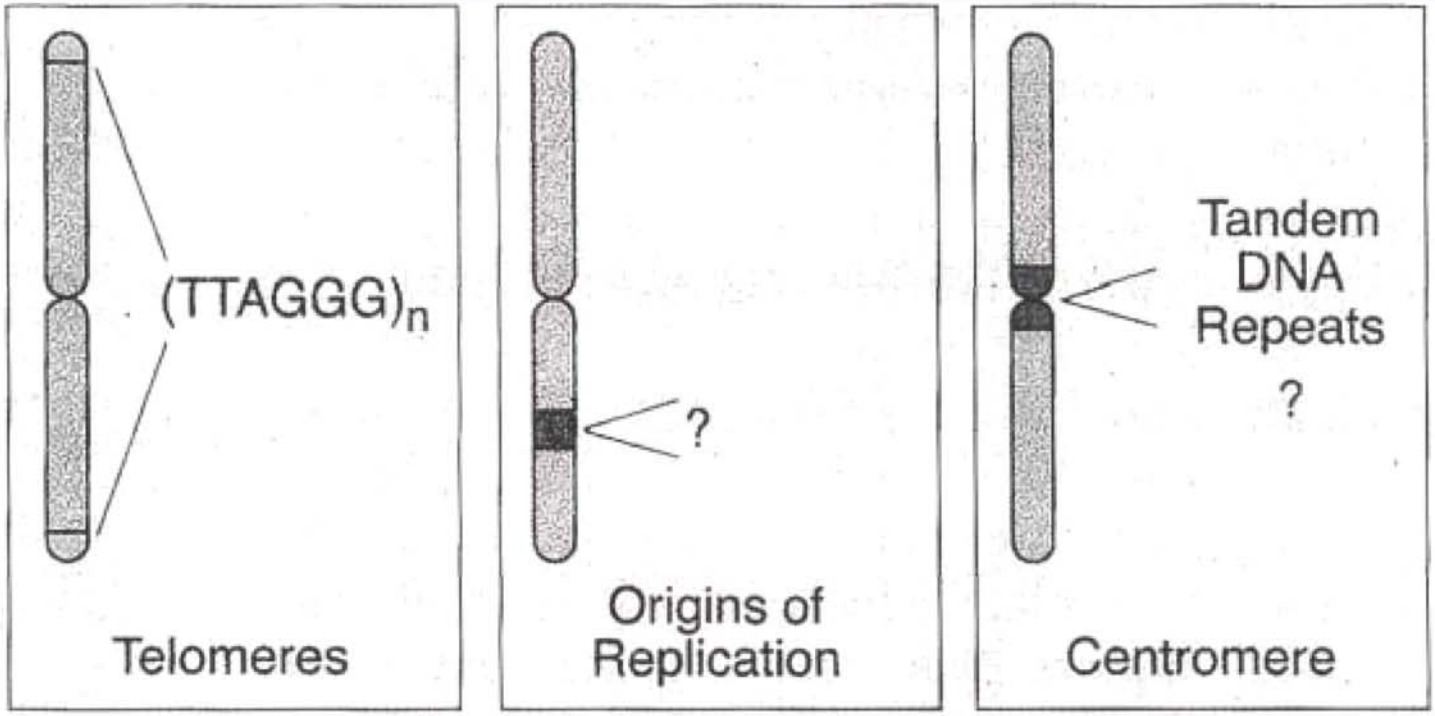
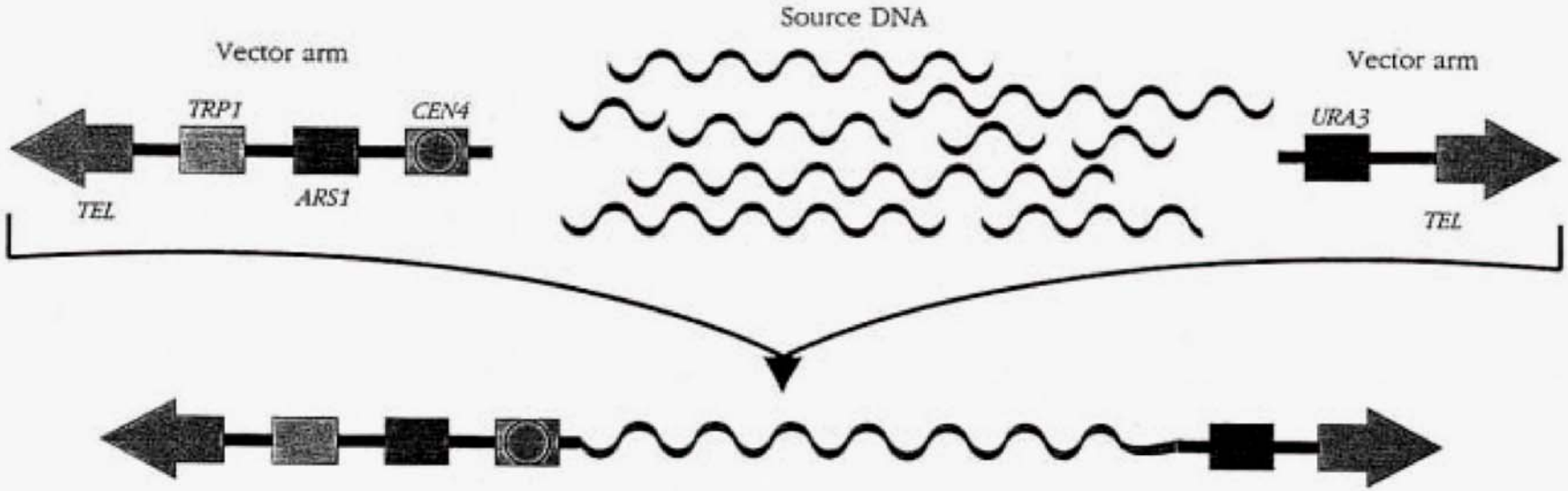


Fig. 1 Basic functional elements of linear chromosomes.

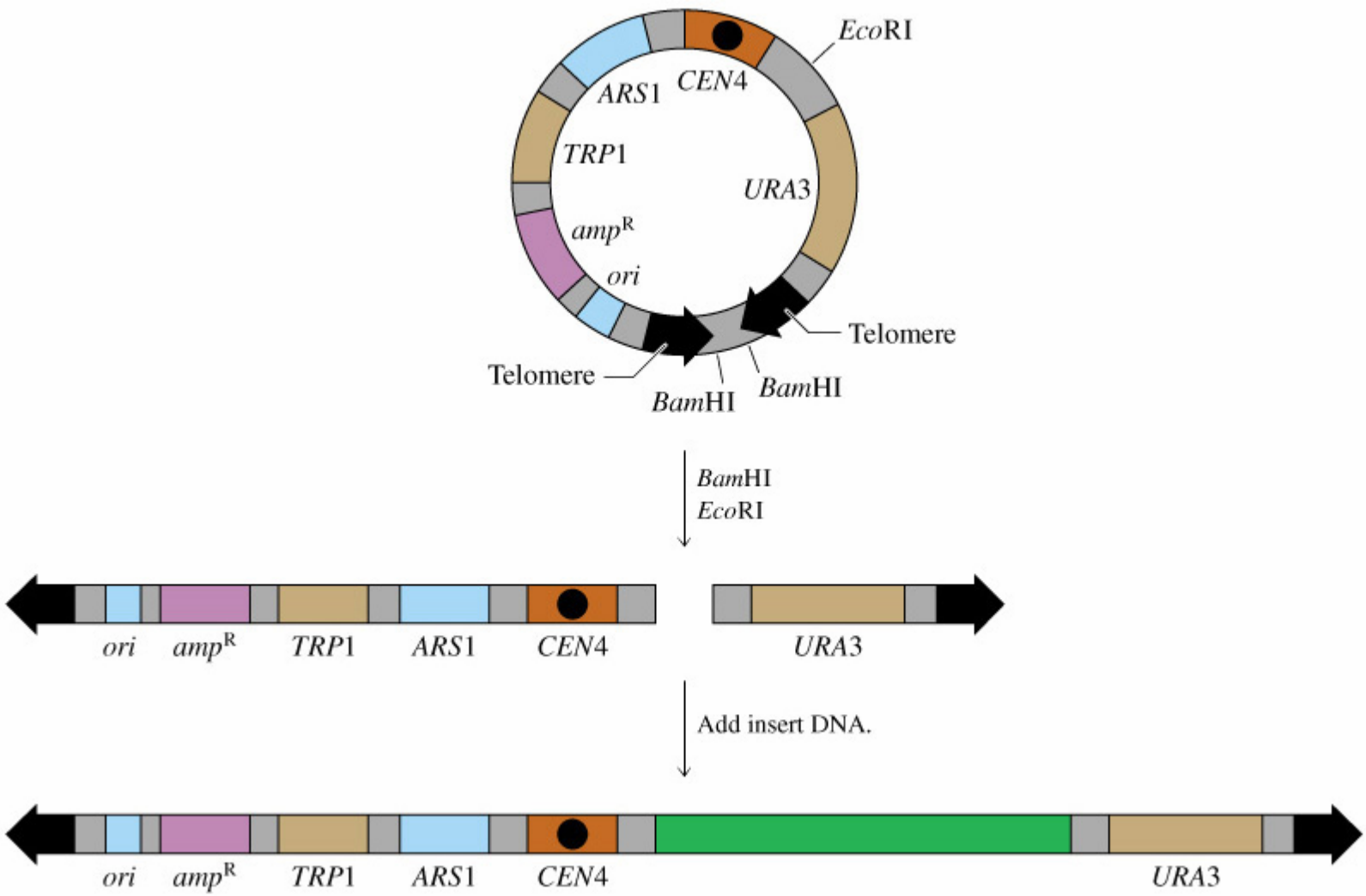
aus: Rosenfeld, M.: Human artificial chromosomes get real; Nature Genetics 15(4), pp 333-335

YACs = Yeast Artificial Chromosomes

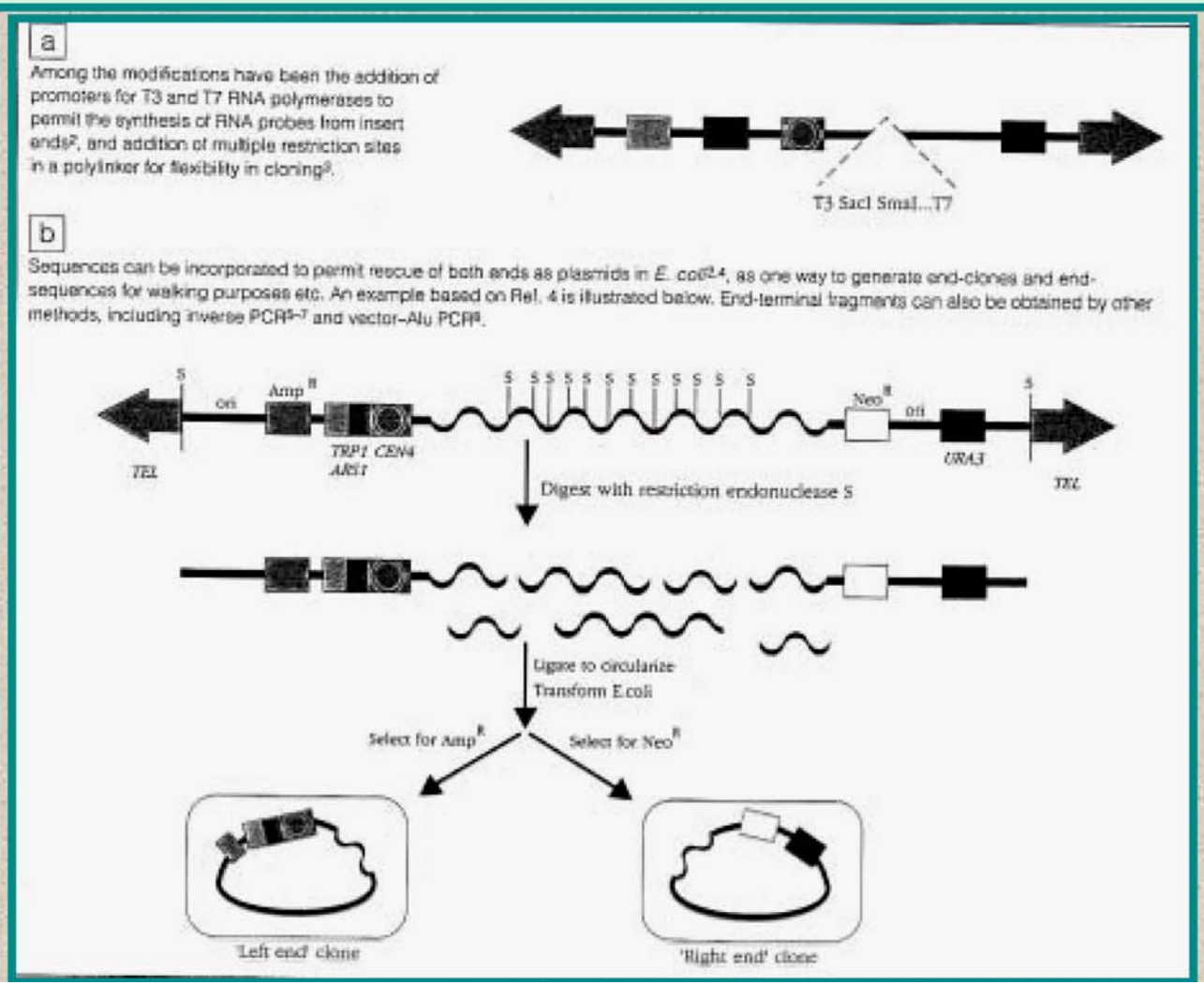


Large inserts of DNA are ligated between vector arms to form a YAC (from Ref. 1). The arms end in telomere sequences and contain centromeres (*CEN*), replication origins (*ARS*) and selectable markers (here, *TRP1* and *URA3*) to stabilize the YACs in yeast. A source DNA fragment is ligated between two arms to form a YAC. The original vectors[1] also contained a suppressor tRNA (which, because it was split by the insert, allowed YAC colonies to be recognized) and pBR322 sequences to facilitate mapping and recovery of one end of the insert as a plasmid in *E. coli* (see Panel 2b). Note that this and other diagrams here are not to scale: the arms total about 10 kb, the insert hundreds of kb.

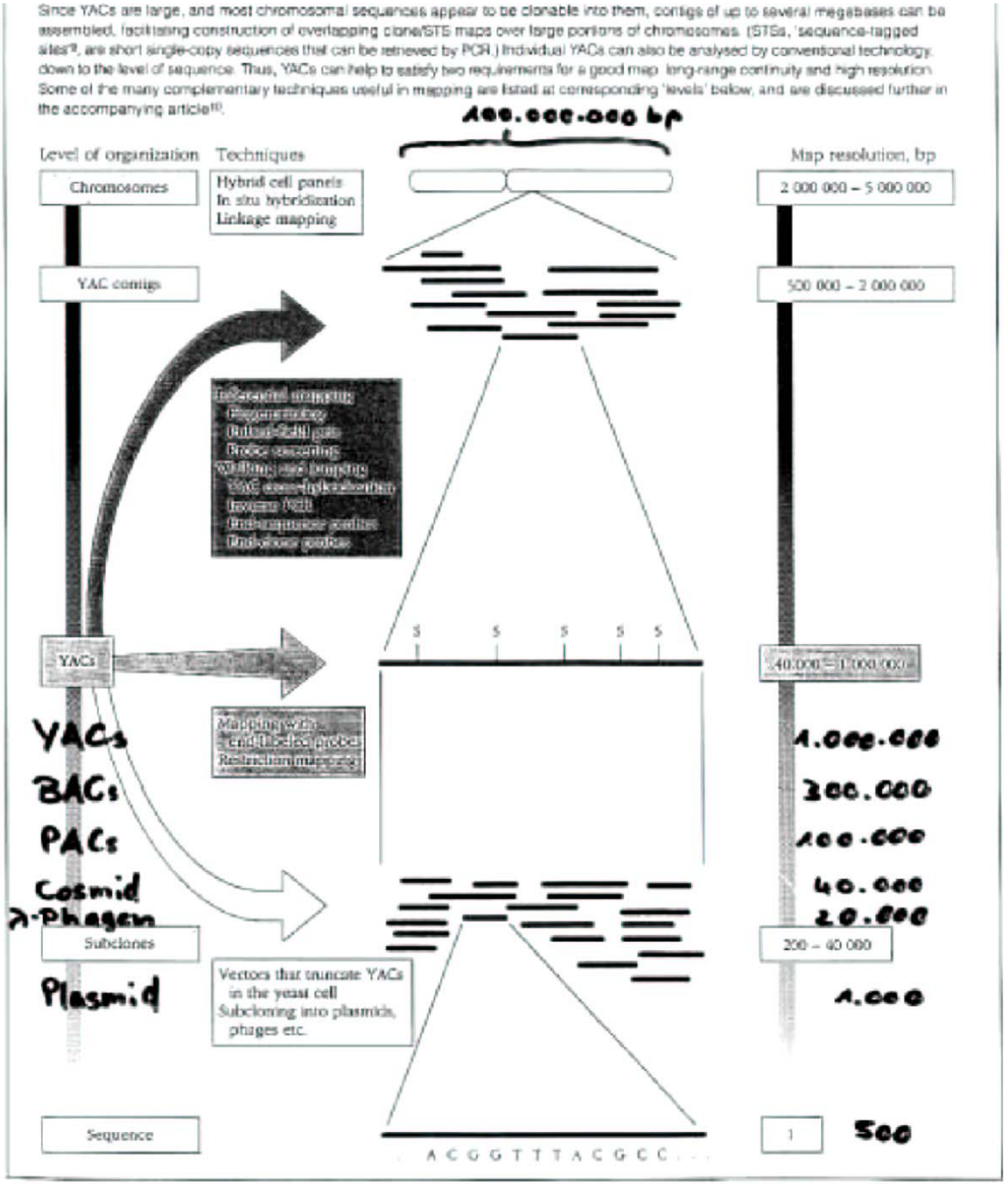
YACs = Yeast Artificial Chromosomes



Mit YACs kann man schnell die Endpunkte großer DNA-Fragmente klonieren

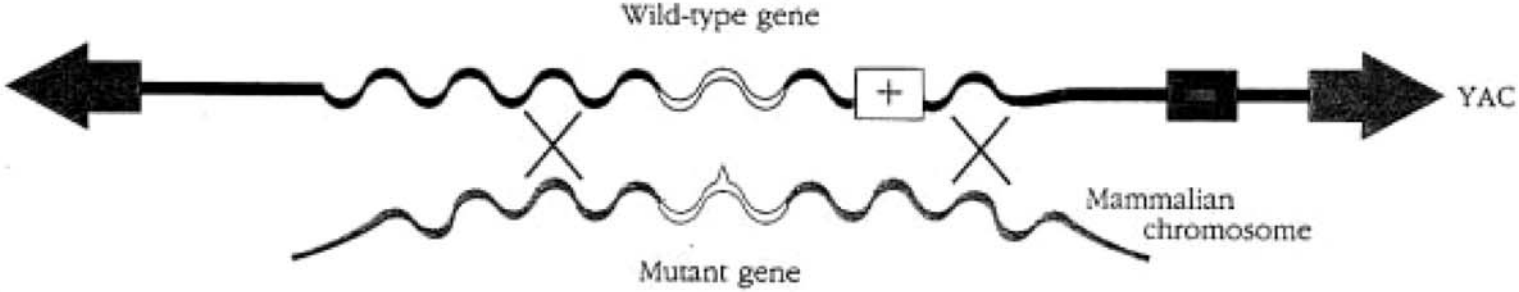


YACs sind besonders für große DNA Fragmente geeignet



YACs können für homologe Rekombination verwendet werden

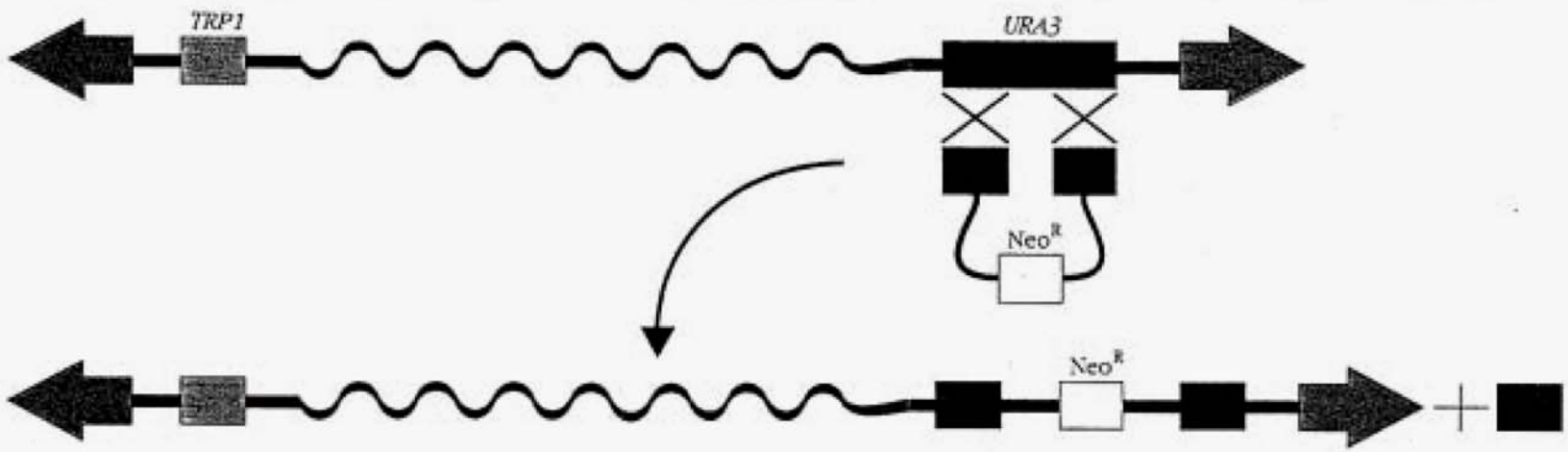
6 GENE THERAPY/HOMOLOGOUS RECOMBINATION



In principle, YACs could be used to provide an unaffected homologue to cells carrying a mutant gene associated with a disease. Repair of the mutant allele by homologous recombination remains a distant possibility, but might be favored by the large size of the homologous region and possibly by selectable markers, positive in the insert DNA (+) and negative in the vector arm (-) (see accompanying article[10] and Ref. 15).

YACs können für eukaryotische Expression eingesetzt werden

5 GENE EXPRESSION STUDIES



YACs can be large enough to contain genes longer than 200 kb, intact and in their normal context. When introduced into mammalian cells, for example by spheroplast fusion they can be expressed into products seen after 1–2 days (transient expression) or after selection of cloned stable transformants. YAC vectors may be designed so that they contain markers selectable in mammalian cells (e.g. the Neo^R gene conferring G418 resistance), or existing YACs may be fitted with such markers by homologous recombination: in the example shown above, a DNA fragment carrying the Neo^R gene and portions of the *URA3* gene recombines with an existing YAC, yielding a new YAC that has a split *URA3* gene and has gained Neo^R (adapted from B. Eliceiri *et al.*, work in progress).

Vektoren, die fremde DNA in die Chromosomen der Keimzellen einschleusen können, gibt es nur wenige

Transposons und **Retroviren** sind genetische Elemente, die sich gezielt in die chromosomale DNA der Wirtszelle einschleichen können

P-Elemente sind aktive Transposons bei Drosophila melanogaster und verursachen „Hybrid-dysgenesis“

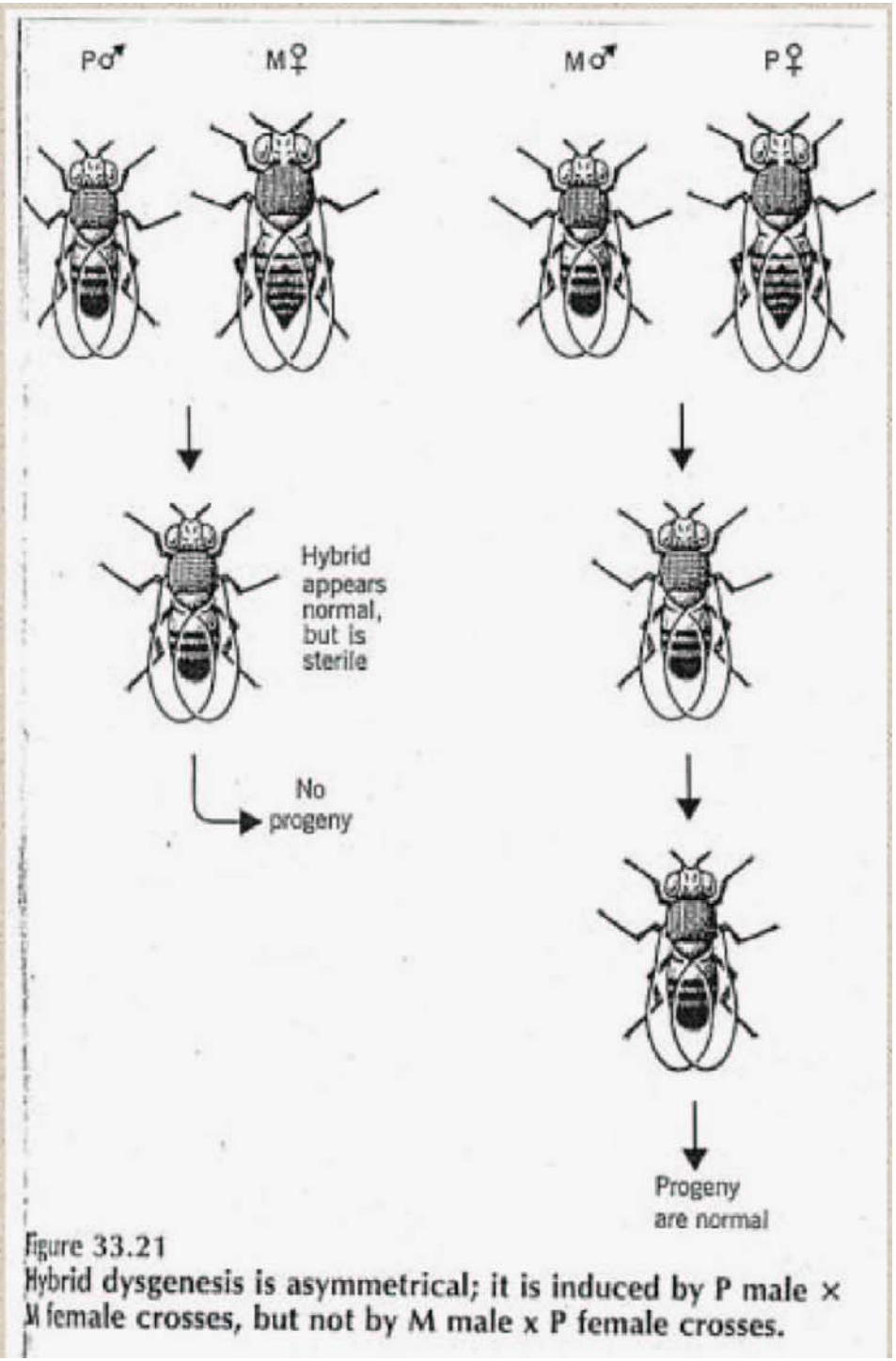


Figure 33.21
Hybrid dysgenesis is asymmetrical; it is induced by P male × M female crosses, but not by M male x P female crosses.