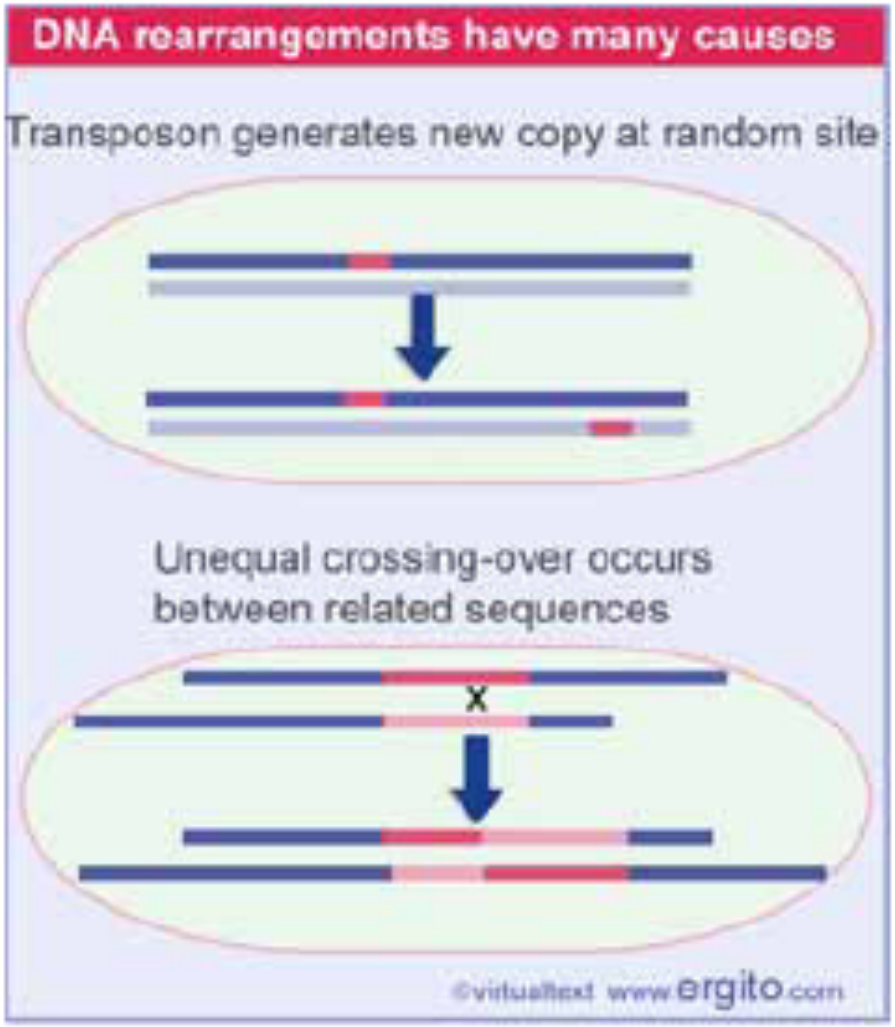
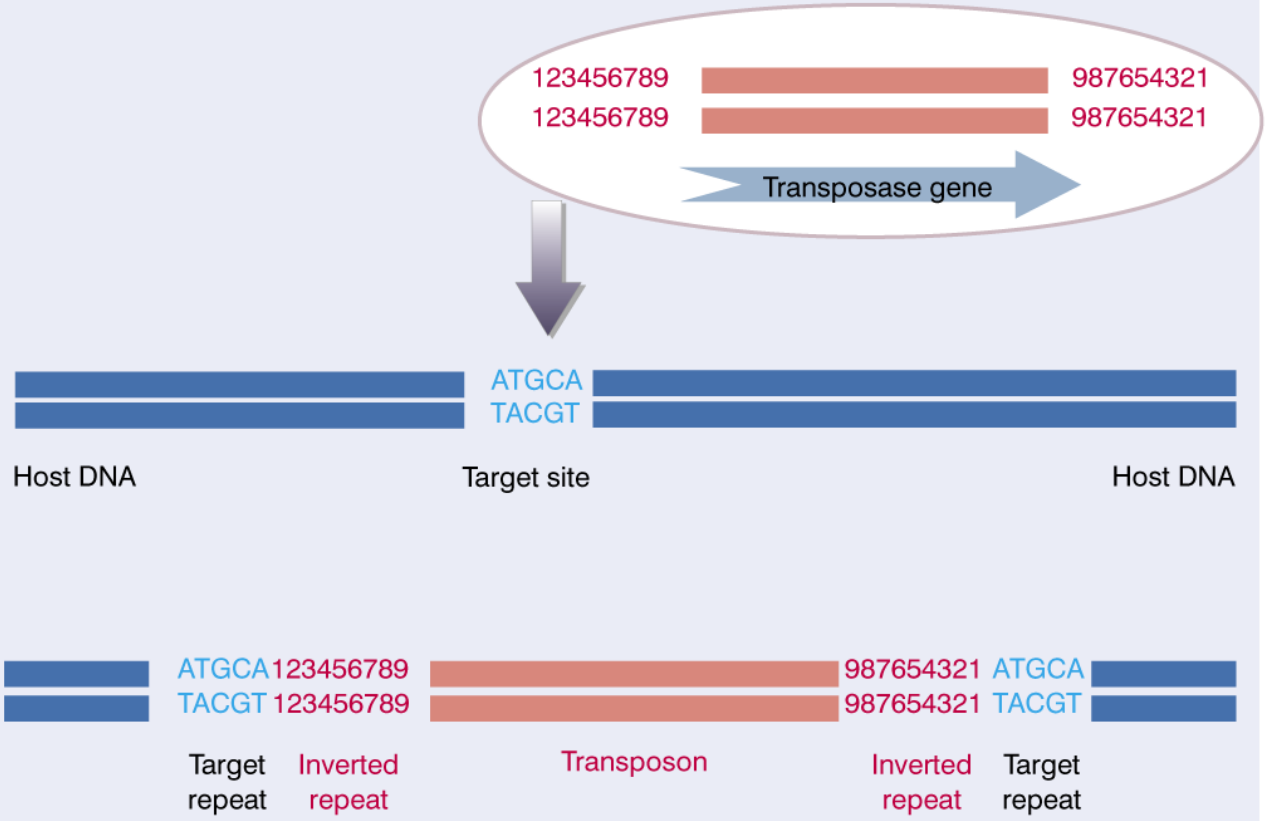


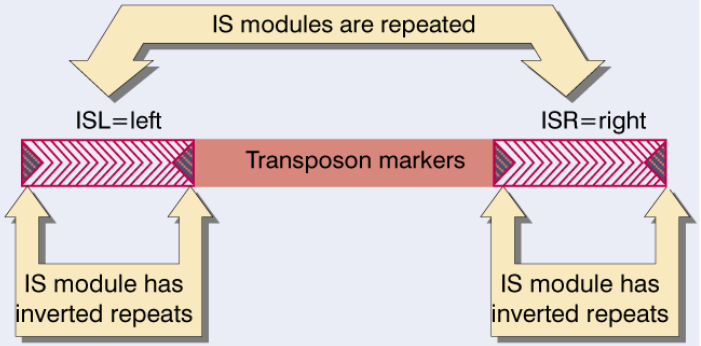
Figure 16.1 A major cause of sequence change within a genome is the movement of a transposon to a new site. This may have direct consequences on gene expression. Unequal crossing-over between related sequences causes rearrangements. Copies of transposons can provide targets for such events.

Figure 15.1 Overview: transposons have inverted terminal repeats and generate direct repeats of flanking DNA at the target site. In this example, the target is a 5 bp sequence. The ends of the transposon consist of inverted repeats of 9 bp, where the numbers 1 through 9 indicate a sequence of base pairs.

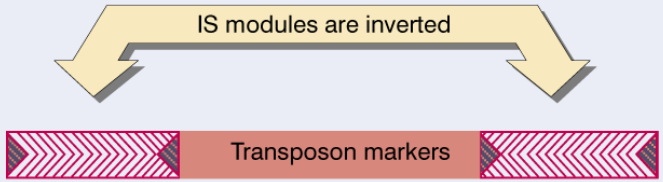


			Overall length	Target selection
IS1	9 bp	23 bp	768 bp	random
IS2	5 bp	41 bp	1327 bp	hotspots
IS4	11-13 bp	18 bp	1428 bp	$AAAN_{20}TTT$
IS5	4 bp	16 bp	1195 bp	hotspots
IS10R	9 bp	22 bp	1329 bp	NGCTNAGCN
IS50R	9 bp	9 bp	1531 bp	hotspots
IS903	9 bp	18 bp	1057 bp	random

Figure 15.2 A composite transposon has a central region carrying markers (such as drug resistance) flanked by IS modules. The modules have short inverted terminal repeats. If the modules themselves are in inverted orientation (as drawn), the short inverted terminal repeats at the ends of the transposon are identical.



Example			
Tn9	IS1	*cam*^{R}	IS modules identical both functional



Transposon	Left end	Markers	Right end
Tn903	IS903	*kan*^{R}	both IS ends functional
Tn10	IS10L nonfunctional	*tet*^{R}	IS10R functional
Tn5	IS50L nonfunctional	*kan*^{R}	IS50R functional

Figure 15.3 Two IS10 modules create a composite transposon that can mobilize any region of DNA that lies between them. When Tn10 is part of a small circular molecule, the IS10 repeats can transpose either side of the circle.

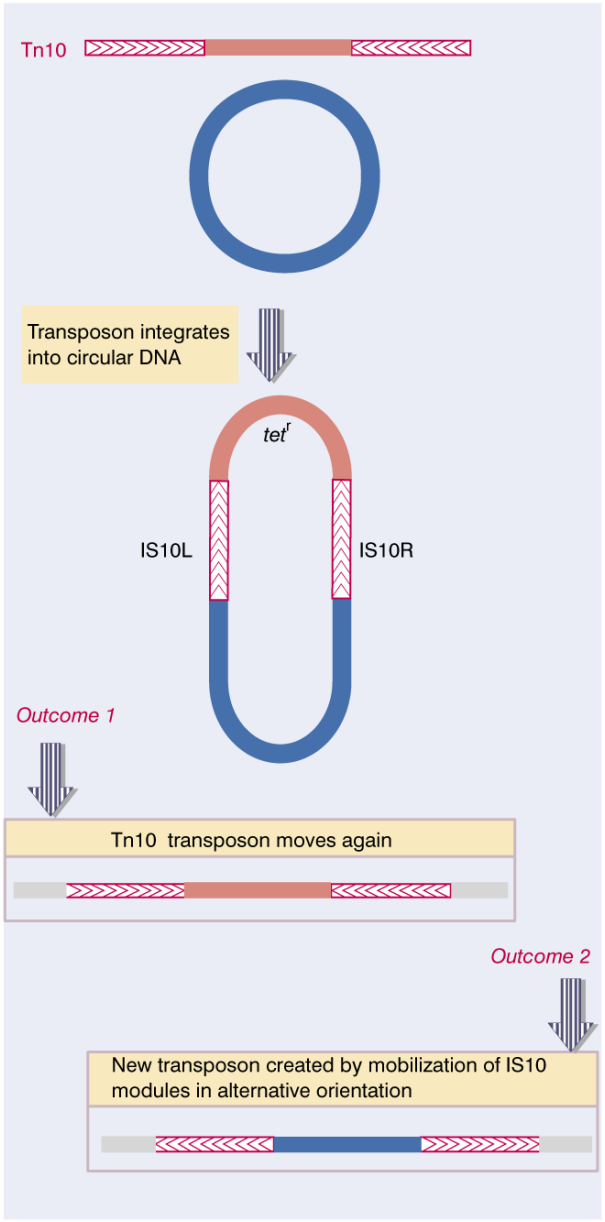


Figure 15.4 The direct repeats of target DNA flanking a transposon are generated by the introduction of staggered cuts whose protruding ends are linked to the transposon.

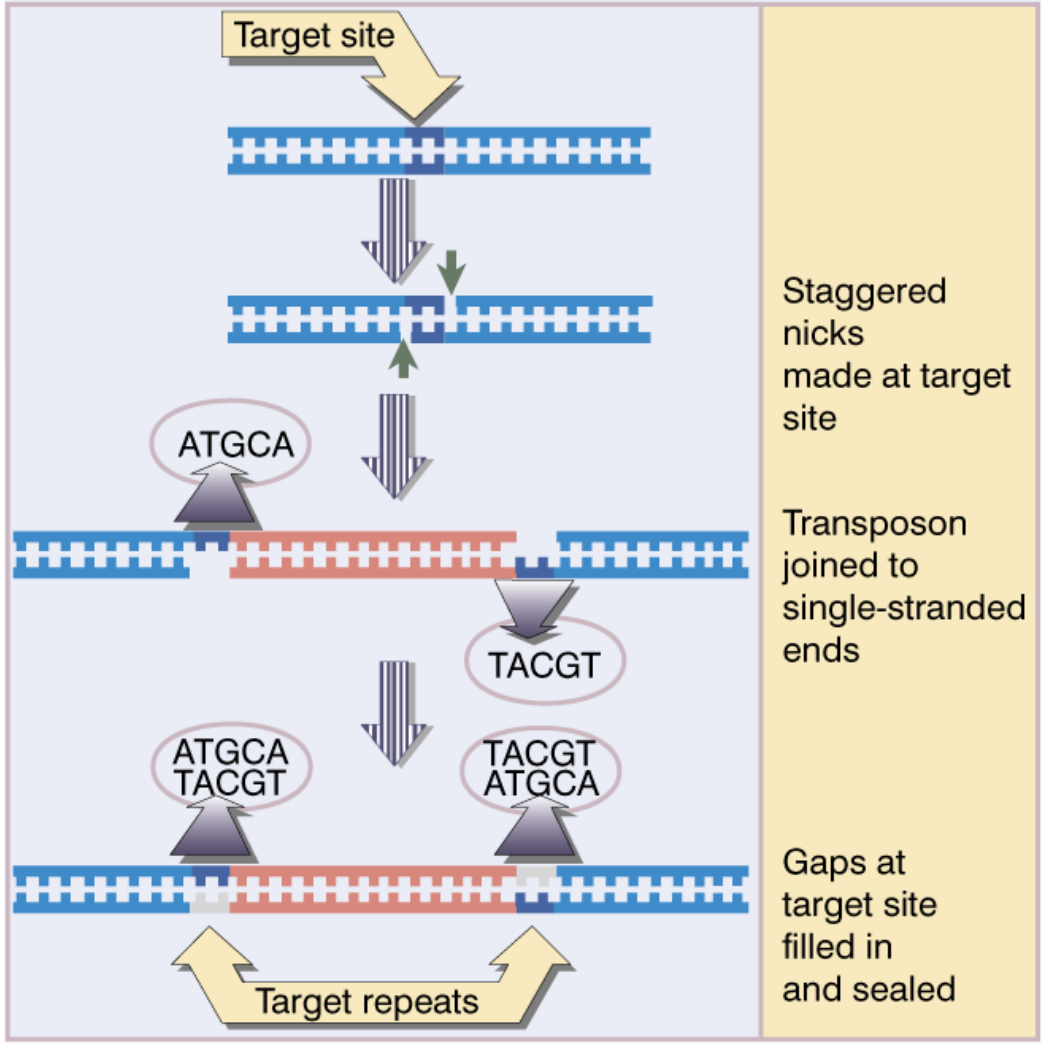


Figure 15.5 Replicative transposition creates a copy of the transposon, which inserts at a recipient site. The donor site remains unchanged, so both donor and recipient have a copy of the transposon.

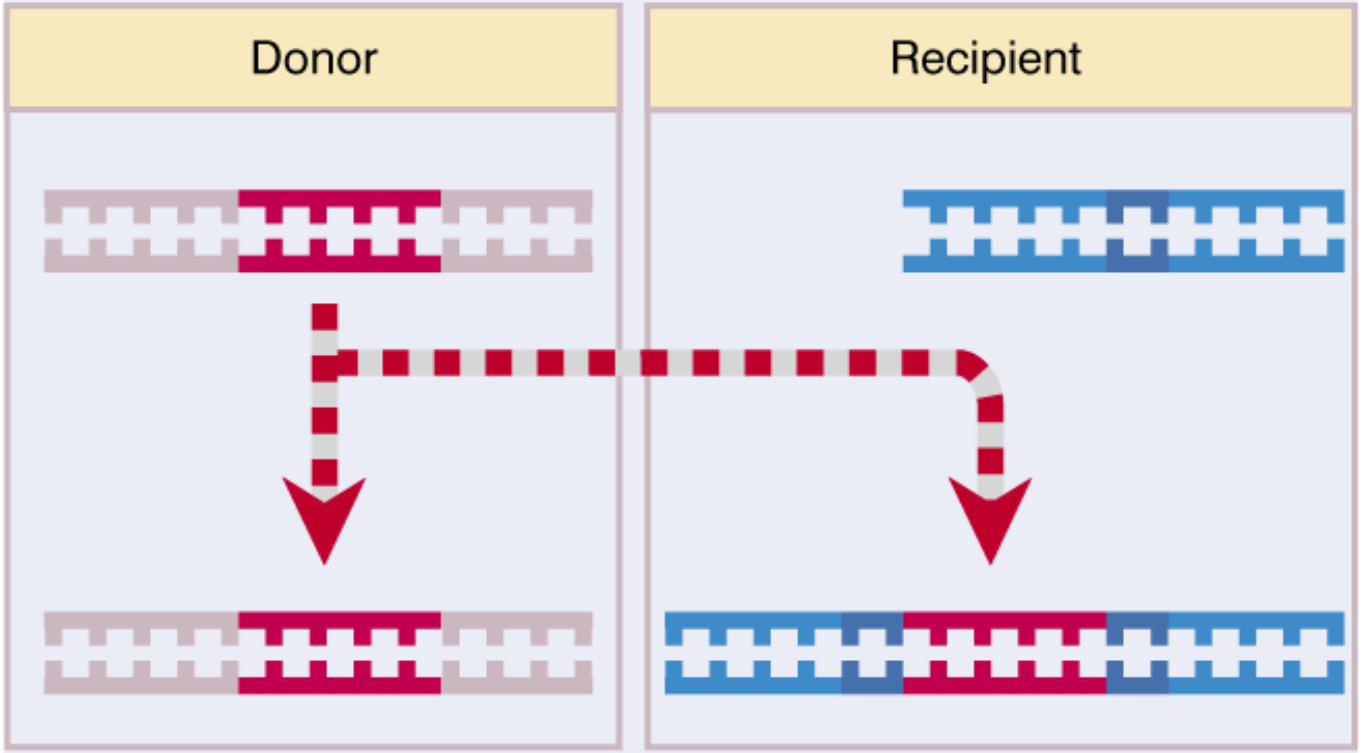


Figure 15.6 Nonreplicative transposition allows a transposon to move as a physical entity from a donor to a recipient site. This leaves a break at the donor site, which is lethal unless it can be repaired.

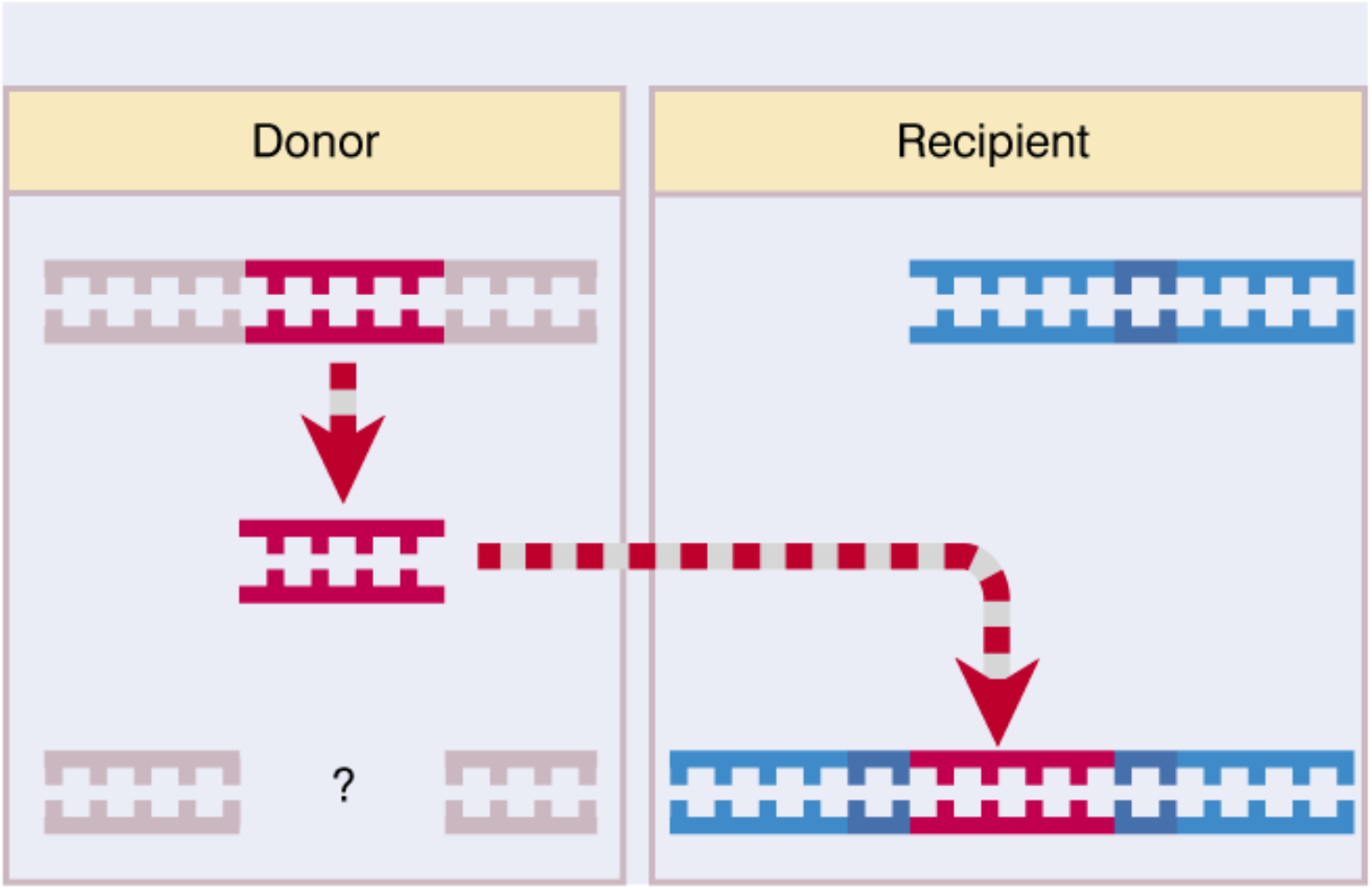


Figure 15.7 Conservative transposition involves direct movement with no loss of nucleotide bonds; compare with lambda integration and excision.

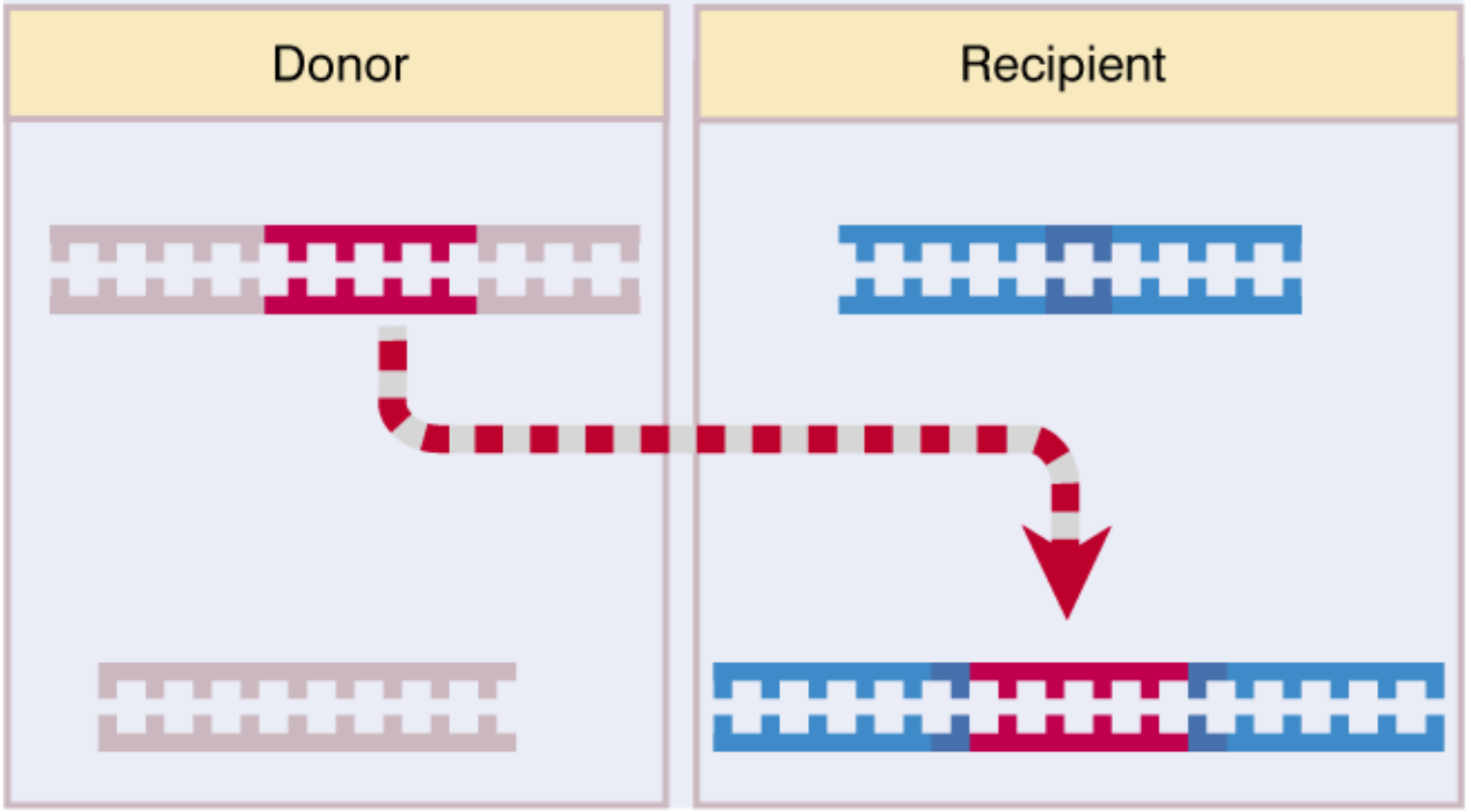


Figure 15.8 Reciprocal recombination between direct repeats excises the material between them; each product of recombination has one copy of the direct repeat.

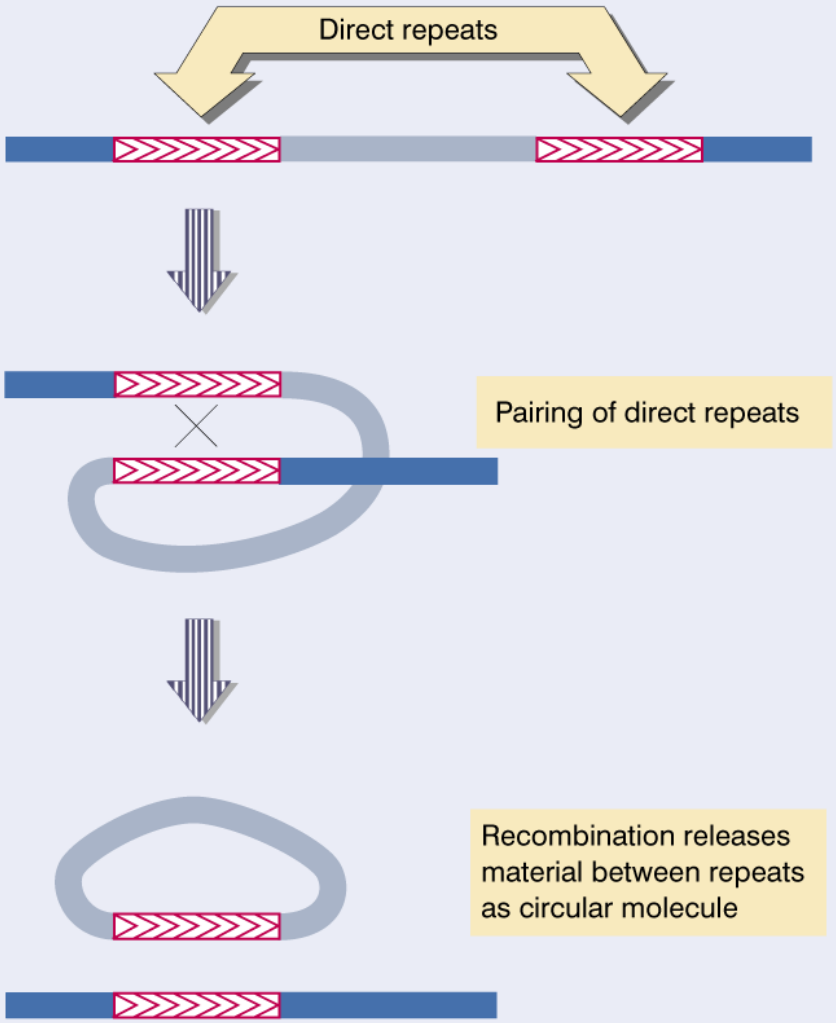


Figure 15.9 Reciprocal recombination between inverted repeats inverts the region between them.

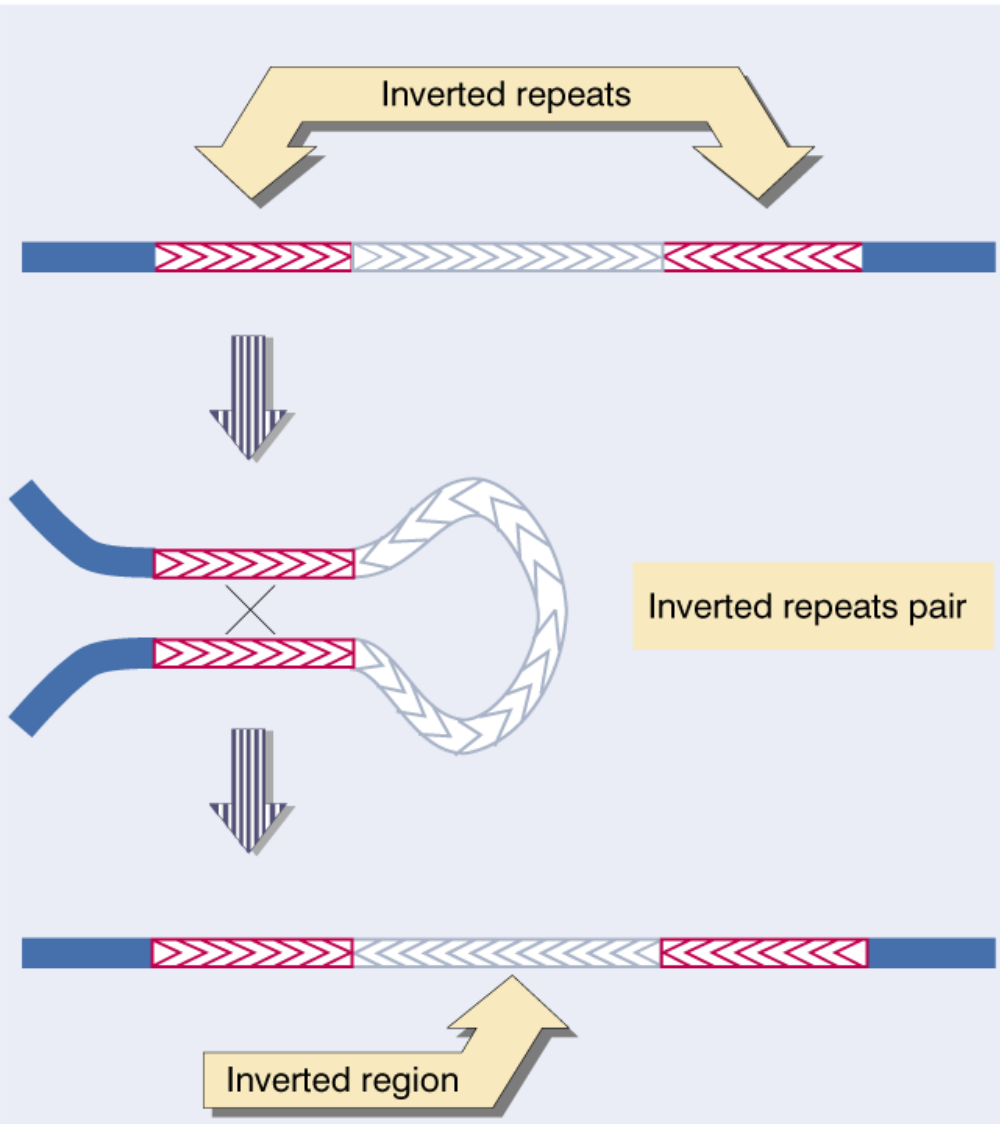


Figure 15.10 Transposition is initiated by nicking the transposon ends and target site and joining the nicked ends into a strand transfer complex.

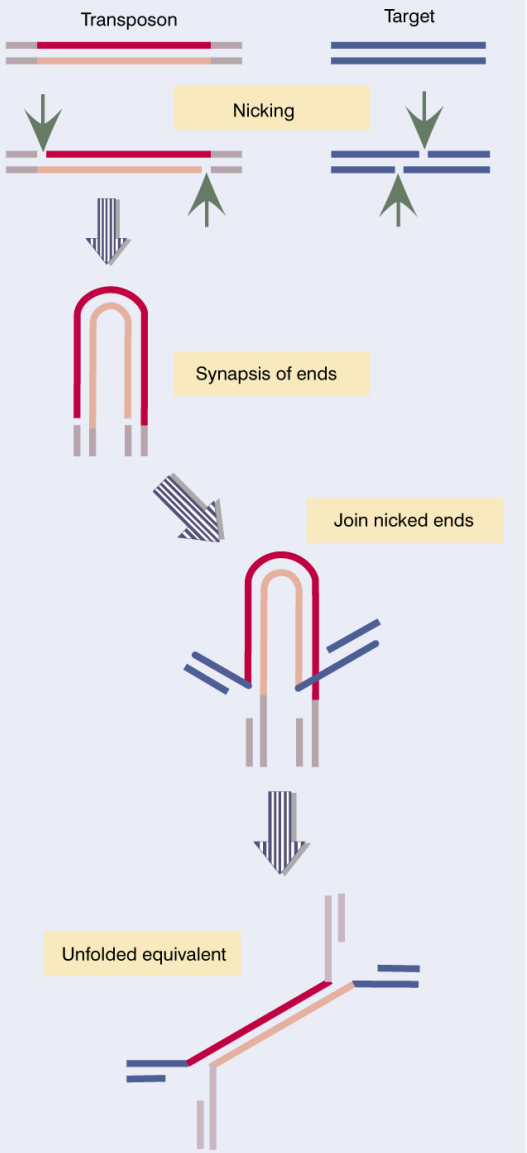


Figure 15.12 Transposition may fuse a donor and recipient replicon into a cointegrate. Resolution releases two replicons, each containing a copy of the transposon.

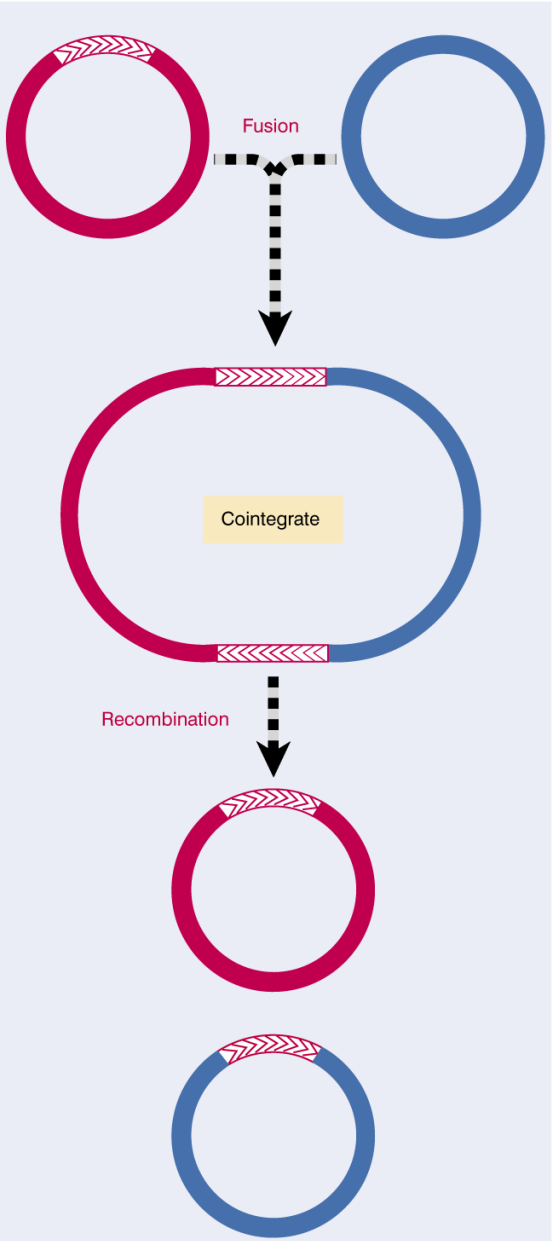


Figure 15.13 Mu transposition generates a crossover structure, which is converted by replication into a cointegrate.

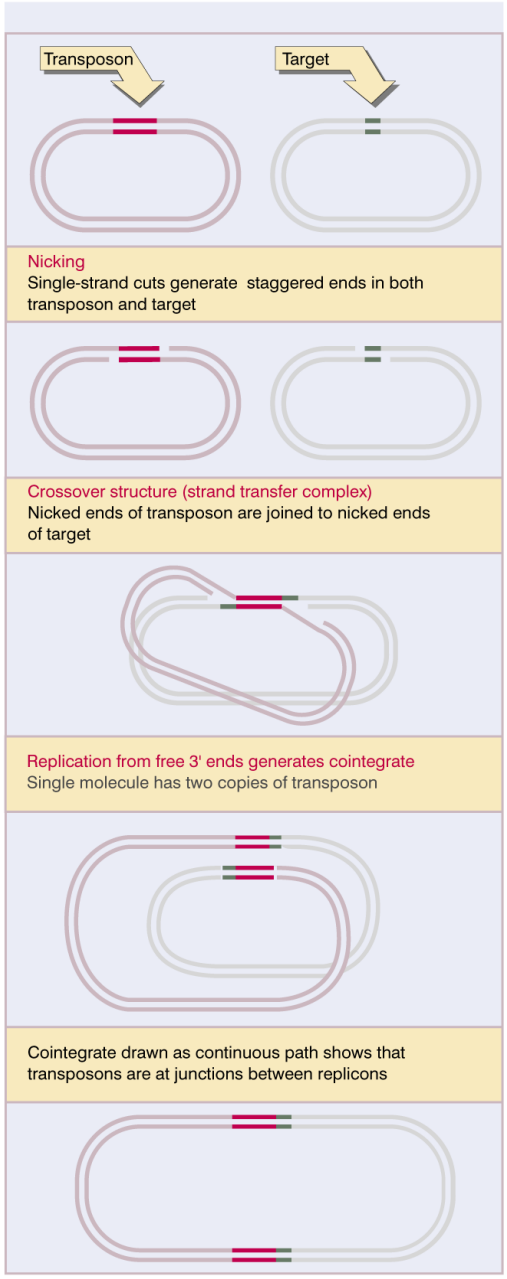


Figure 15.14 Nonreplicative transposition results when a crossover structure is released by nicking. This inserts the transposon into the target DNA, flanked by the direct repeats of the target, and the donor is left with a double-strand break.

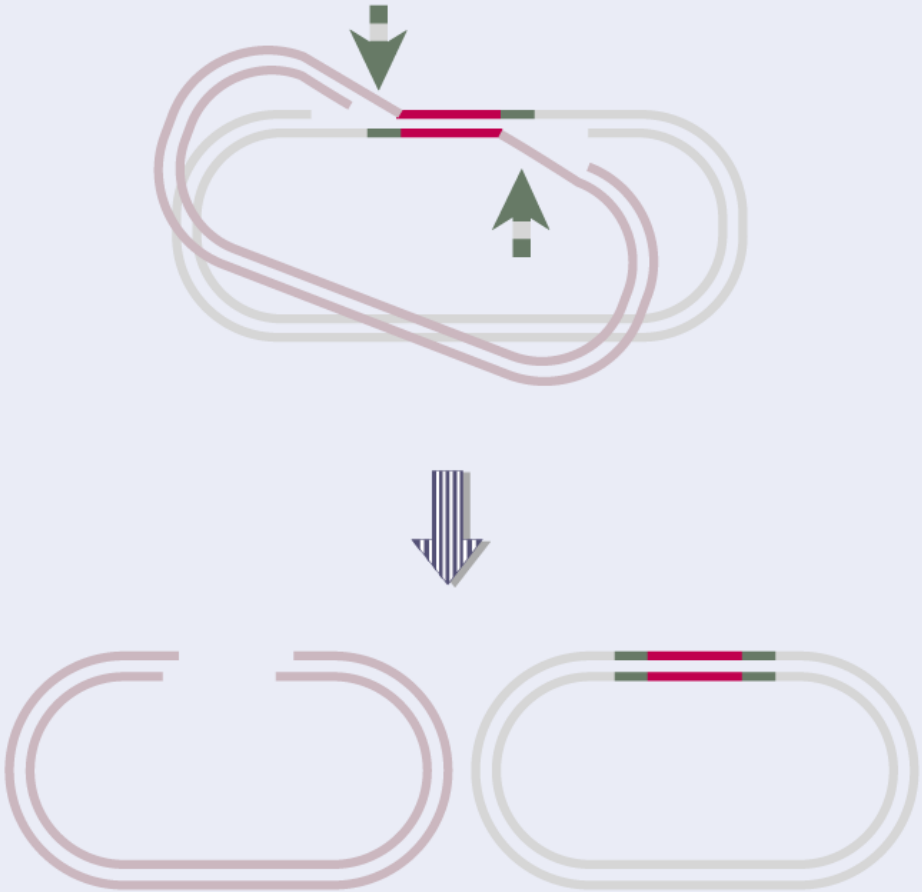


Figure 15.15 Both strands of Tn10 are cleaved sequentially, and then the transposon is joined to the nicked target site.

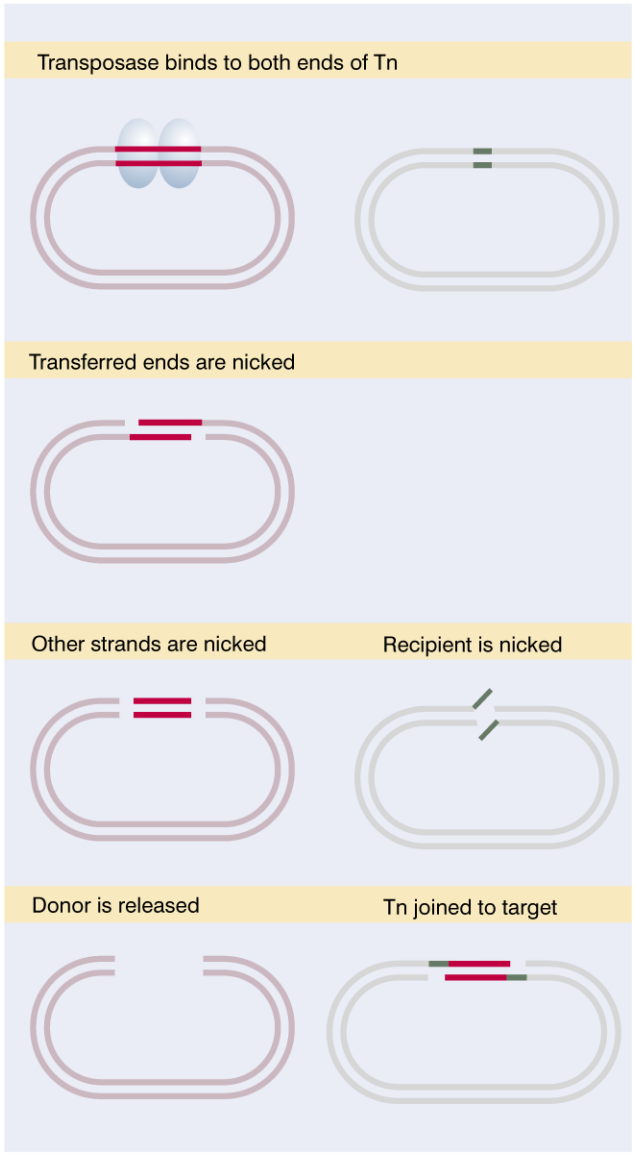
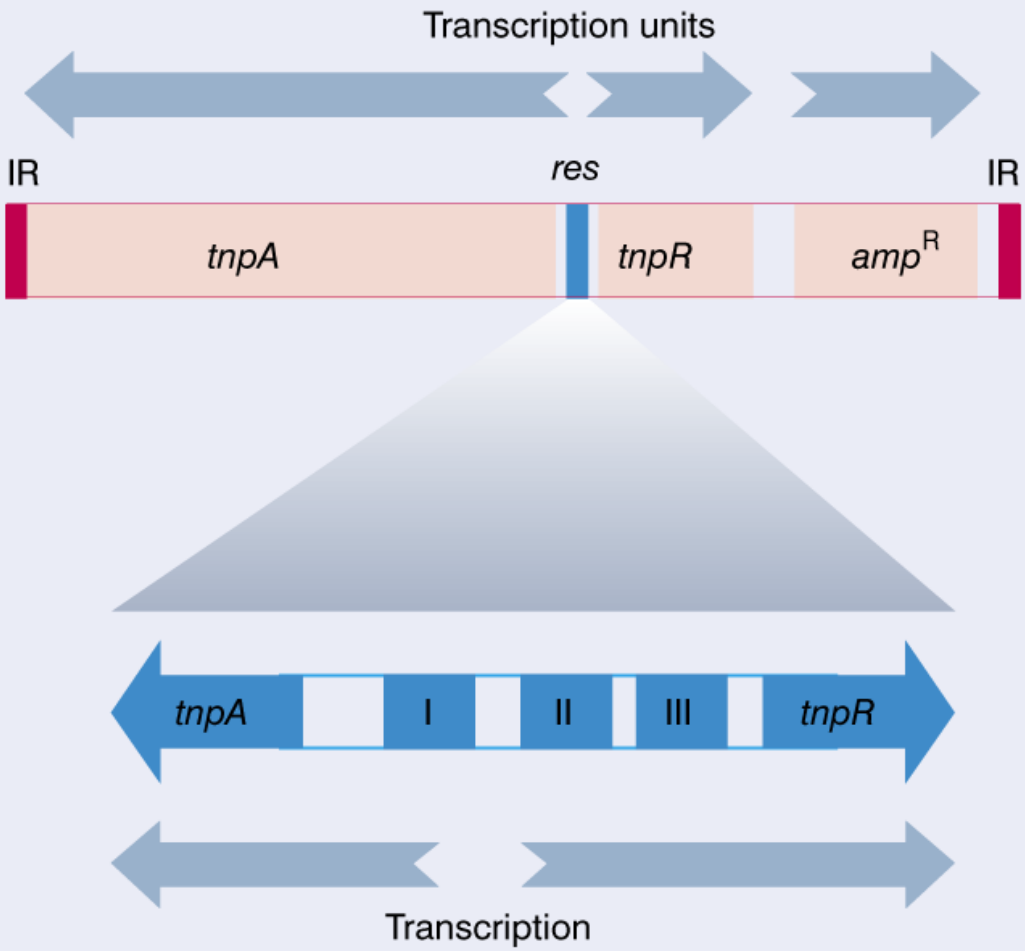


Figure 15.16 Transposons of the TnA family have inverted terminal repeats, an internal *res* site, and three known genes.



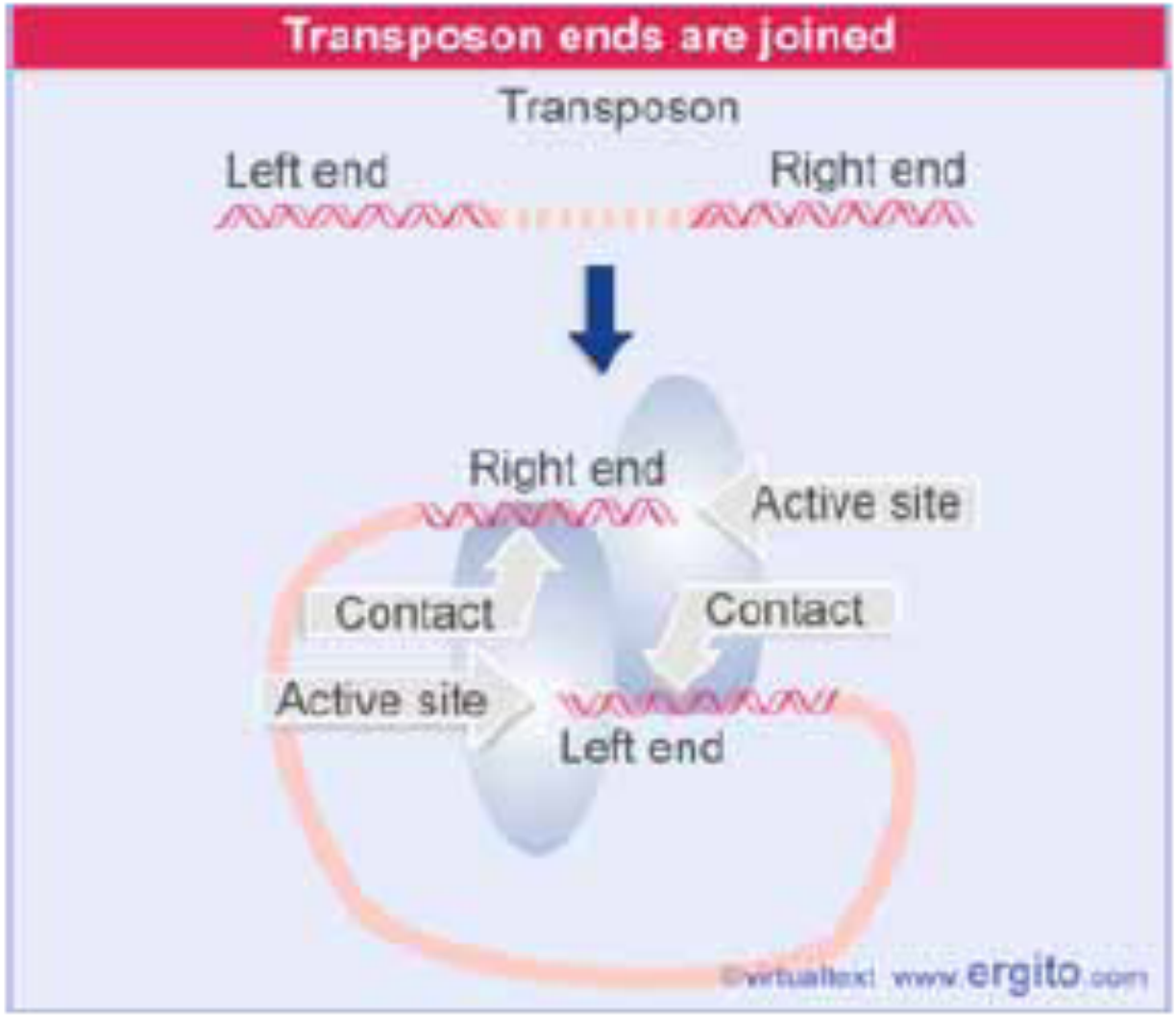


Figure 16.18 Each subunit of the Tn5 transposase has one end of the transposon located in its active site and also makes contact at a different site with the other end of the transposon.

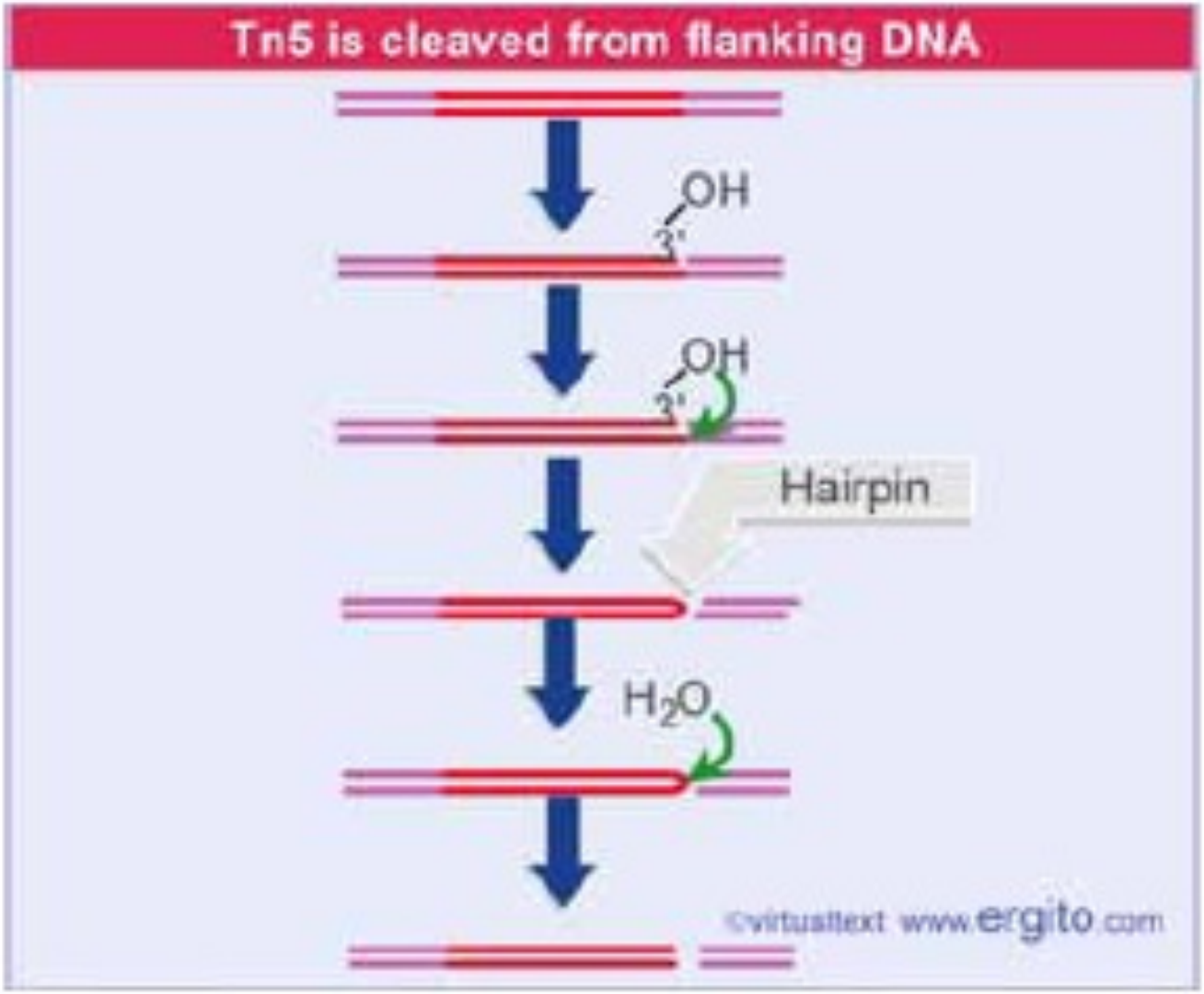


Figure 16.17 Cleavage of Tn5 from flanking DNA involves nicking, interstrand reaction, and hairpin cleavage.

Figure 15.17 Two promoters in opposite orientation lie near the outside boundary of IS10R. The strong promoter P_{OUT} sponsors transcription toward the flanking host DNA. The weaker promoter P_{IN} causes transcription of an RNA that extends the length of IS10R and is translated into the transposase.

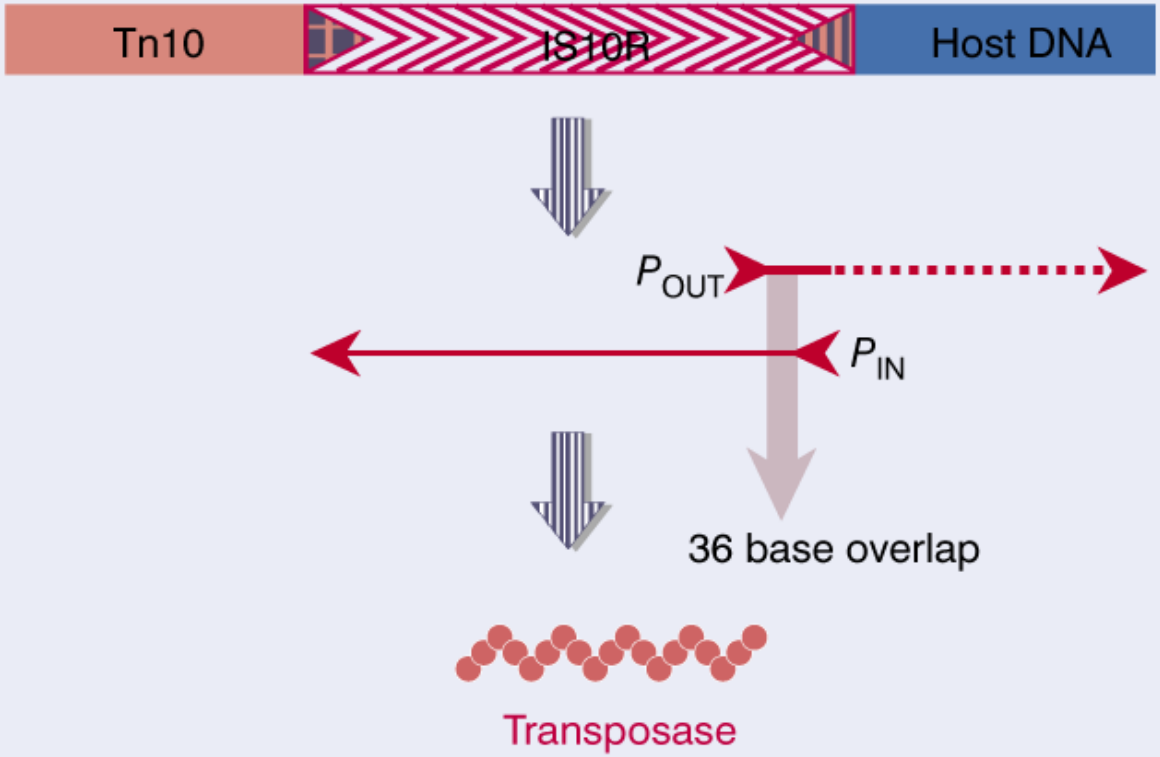
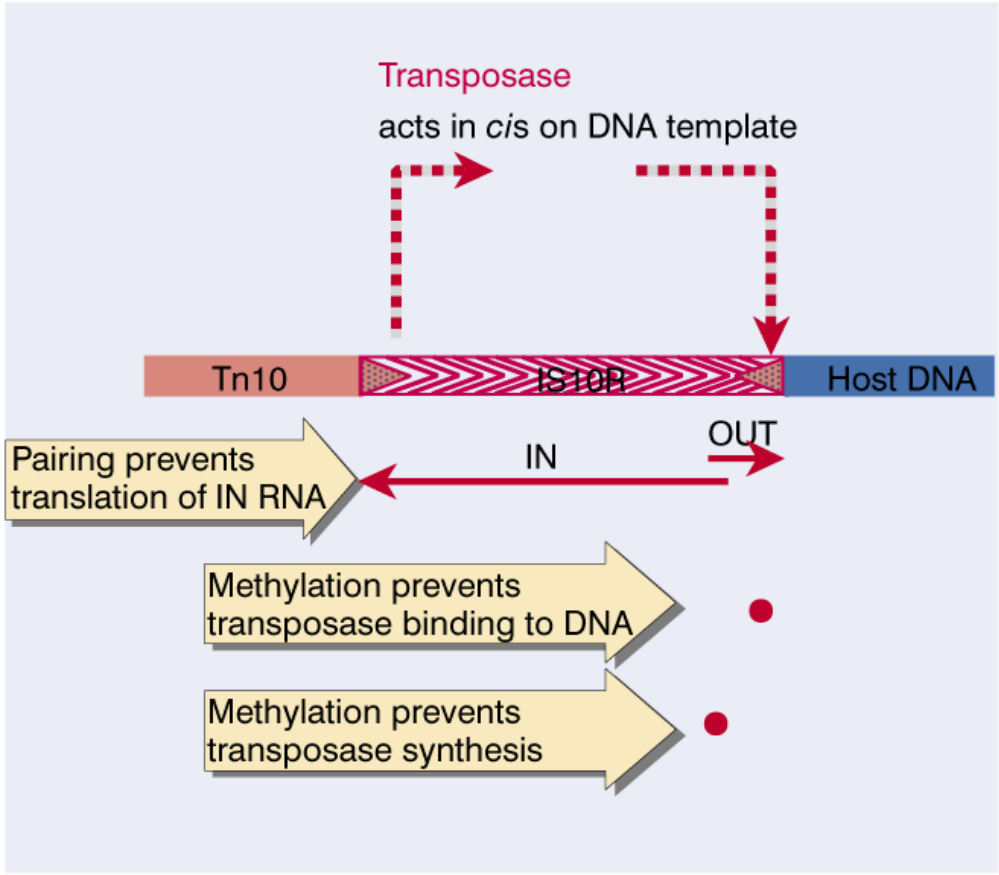


Figure 15.18 Several mechanisms restrain the frequency of Tn10 transposition, by affecting either the synthesis or function of transposase protein. Transposition of an individual transposon is restricted by methylation to occur only after replication. In multicopy situations, *cis*-preference restricts the choice of target, and OUT/IN RNA pairing inhibits synthesis of transposase.



Mutagénesis por transposición.
Uso de λ::Tn5

Preparar stock λ::Tn5 el cual tiene mutaciones en los genes *O* y *P* (esenciales para la replicación). Estas mutaciones (ambar) son suprimidas en huéspedes supresores.

Infectar (baja MOI) la cepa a mutagenizar (Su^0).
Plaquear en placas selectivas con Km para seleccionar los eventos de transposición.

Seleccionar mutantes (si se buscan mutantes en el cromosoma)

Si se busca mutantes en genes codificados en plasmidios, preparar DNA plasmidial a partir de las colonias Km^R.
Transformar con esta preparación de plasmidio y seleccionar por resistencia a kanamicina