

# Clonaggio Molecolare

**DNA da inserire:inserto**

- 1) DNA genomico
- 2) DNA sintetico
- 3) DNA da PCR
- 4) cDNA

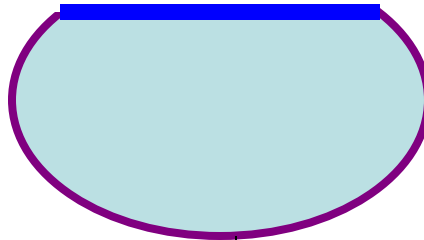
**Digestione con enzimi  
di restrizione**

**DNA del vettore**

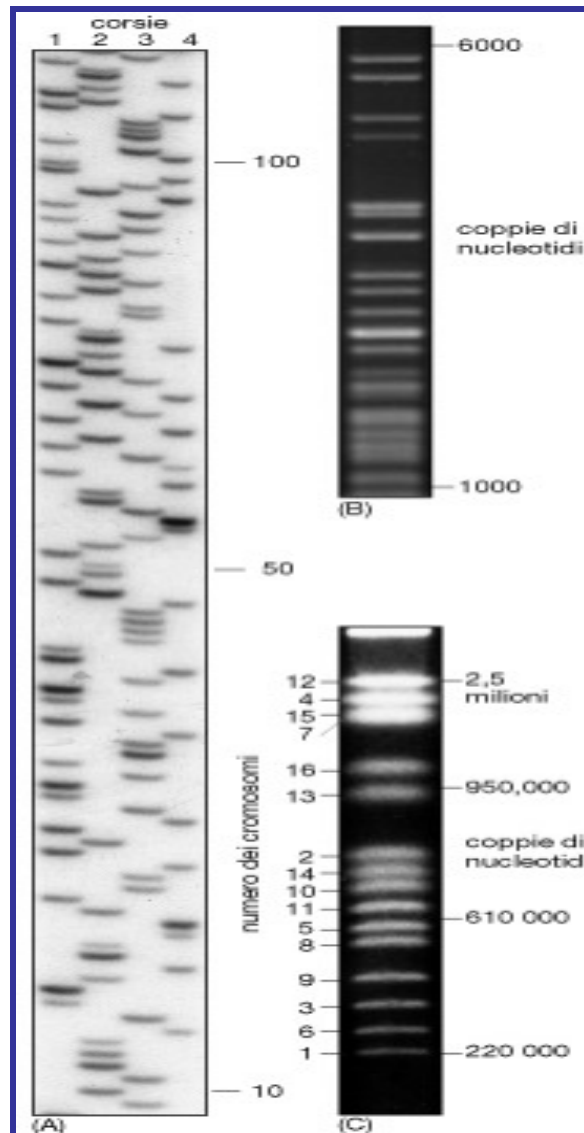
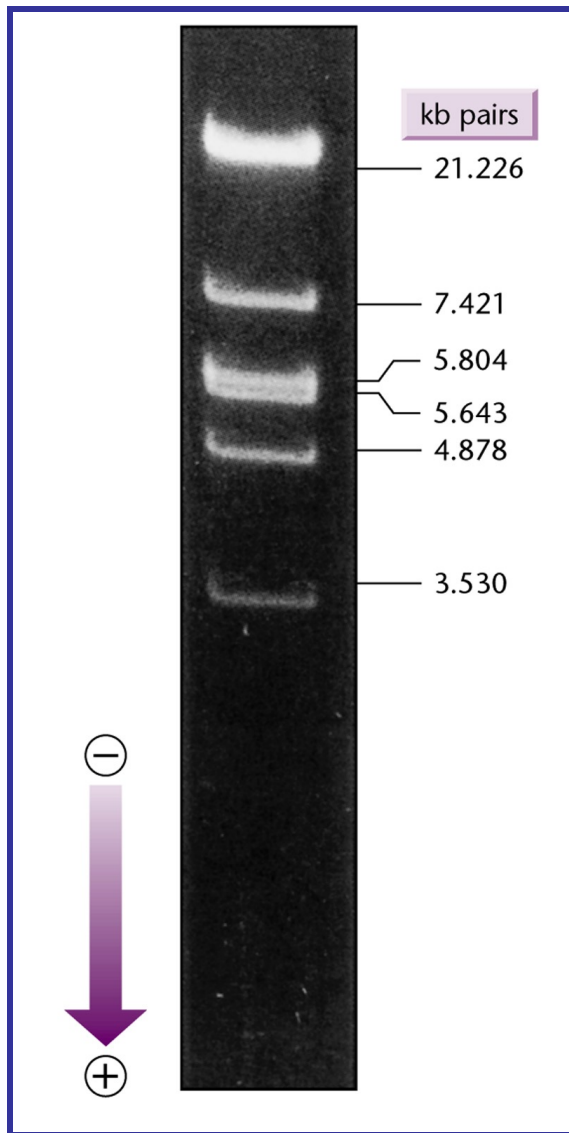
**Ligasi**

**Trasformazione delle cellule di E.coli**

**Selezione dei trasformanti**

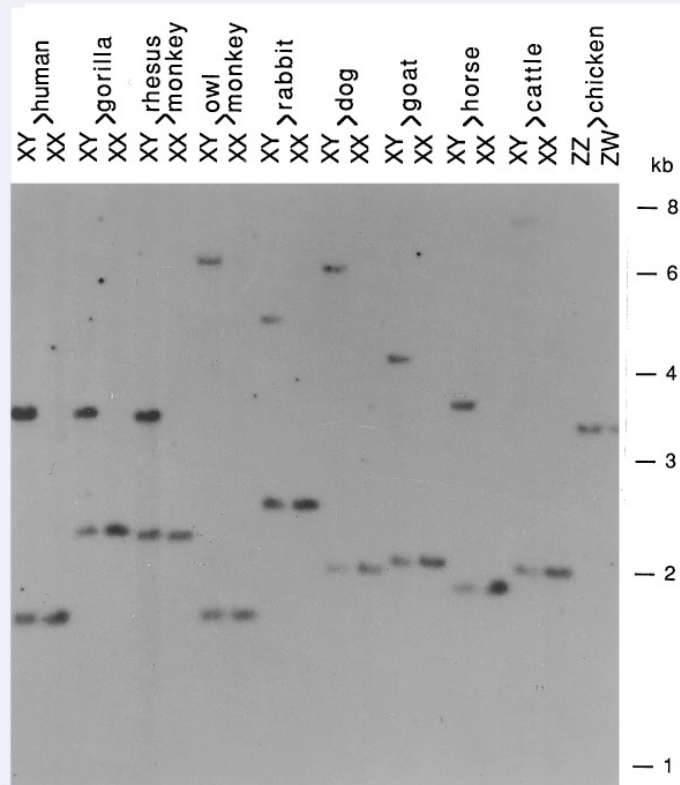


# Separazione del DNA



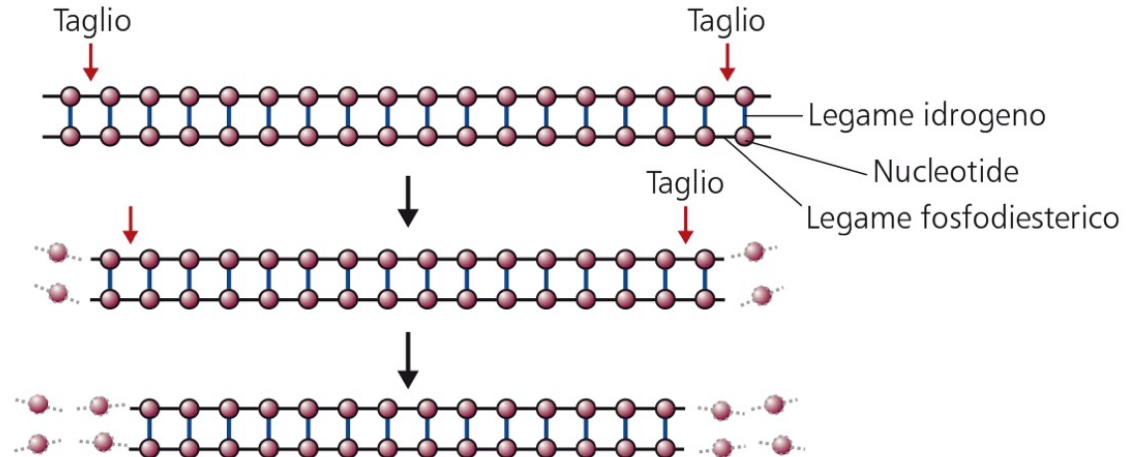
# DNA genomico

**Figure 2.17** A zoo blot with a probe from the human Y chromosomal gene *zfy* identifies cross-hybridizing fragments on the sex chromosomes of other mammals and birds. There is one reacting fragment on the Y chromosome and another on the X chromosome. Data kindly provided by David Page.

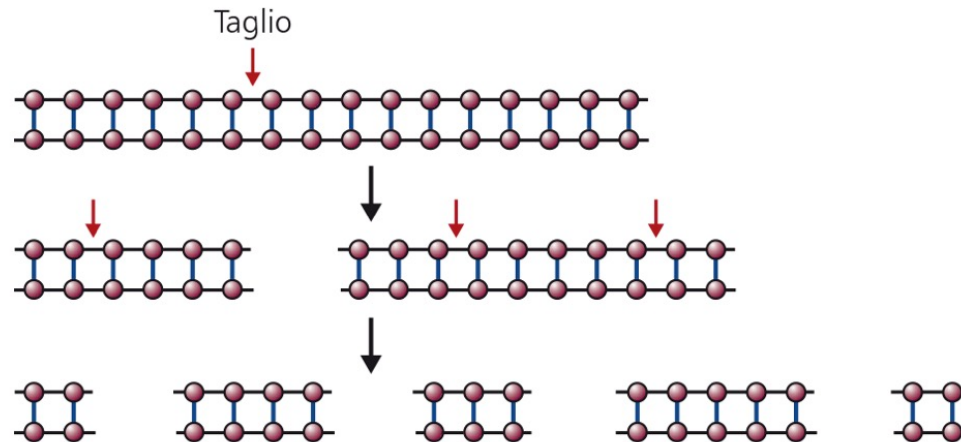


# Manipolazione del DNA - Nucleasi

(a) Esonucleasi

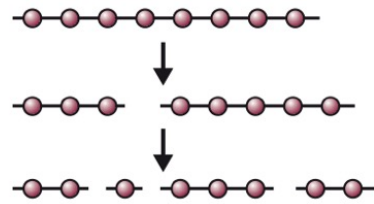


(b) Endonucleasi

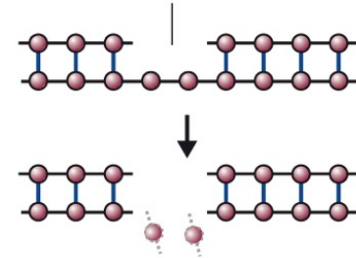


# Manipolazione del DNA - endoNucleasi

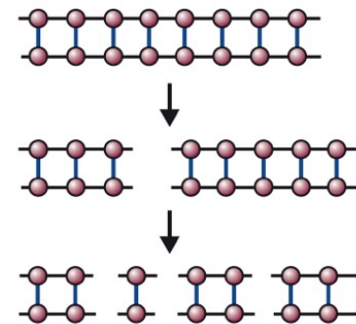
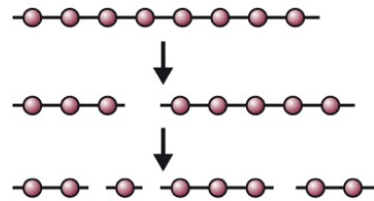
(a) Nucleasi S1



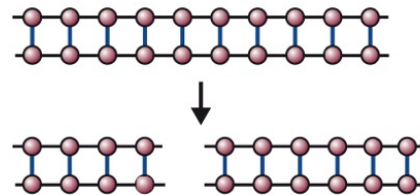
Interruzione a singolo filamento



(b) DNasi I

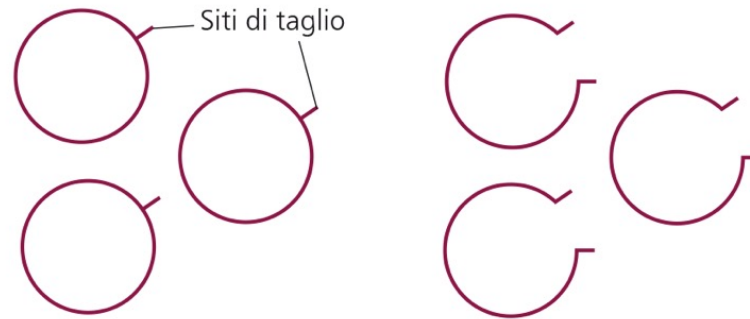


(c) Endonucleasi di restrizione



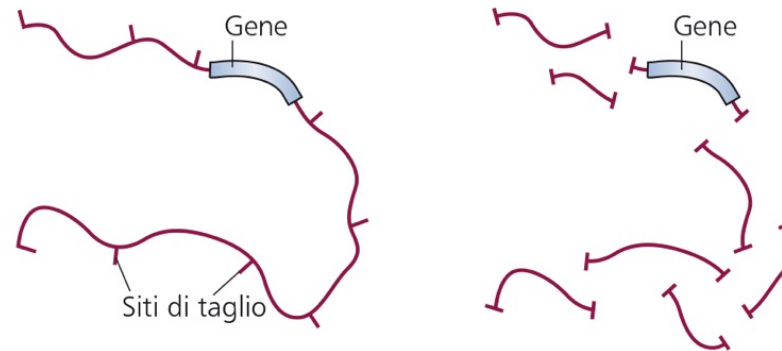
# Manipolazione del DNA - Enzimi di restrizione

(a) Molecole del vettore



Ogni molecola del vettore deve essere tagliata una sola volta e sempre nella stessa posizione

(b) Molecola di DNA contenente il gene da clonare

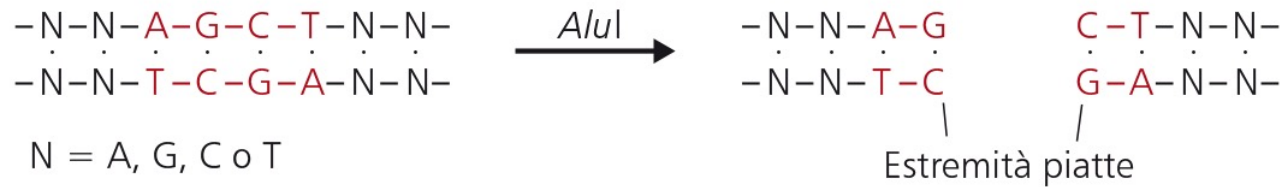


Grande molecola di DNA

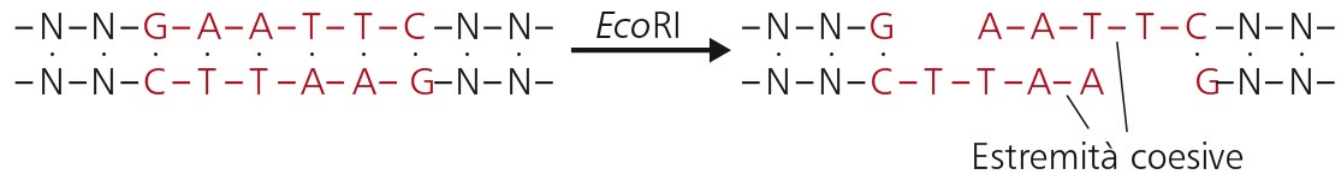
Frammenti di DNA sufficientemente piccoli da poter essere clonati

# Manipolazione del DNA - Enzimi di restrizione

(a) Produzione di estremità piatte



(b) Produzione di estremità coesive



(c) Estremità coesive identiche prodotte da endonucleasi di restrizione differenti



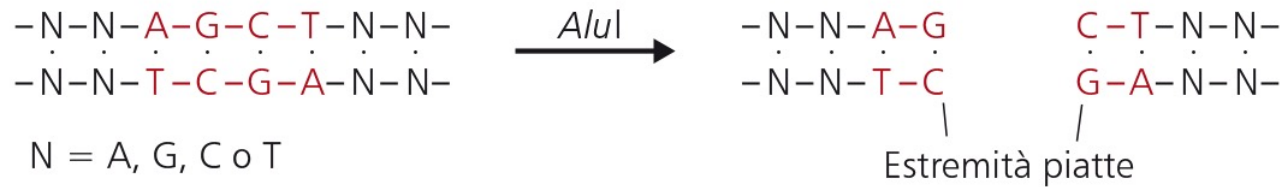
# Manipolazione del DNA - Enzimi di restrizione

Enzyme	Recognition site	Type of cut end
<i>EcoRI</i>	G <sup>↓</sup> A—A—T—T—C C—T—T—A—A <sup>↑</sup> G	5'-phosphate extension
<i>BamHI</i>	G <sup>↓</sup> G—A—T—C—C C—C—T—A—G <sup>↑</sup> G	5'-phosphate extension
<i>PstI</i>	C—T—G—C—A <sup>↓</sup> G G <sup>↑</sup> A— — — — — T—C	3'-hydroxyl extension
<i>Sau3AI</i>	<sup>↓</sup> G—A—T—C C—T—A—G <sup>↑</sup>	5'-phosphate extension
<i>PvuII</i>	C—A—G <sup>↓</sup> C—T—G G—T—C <sup>↑</sup> G—A—C	Blunt end
<i>HpaI</i>	G—T—T <sup>↓</sup> A—A—C C—A—A <sup>↑</sup> T—T—G	Blunt end
<i>HaeIII</i>	G—G <sup>↓</sup> C—C C—C <sup>↑</sup> G—G	Blunt end
<i>NotI</i>	G <sup>↓</sup> C—G—G—C—C—G—C C—G—C—C—G—G—C <sup>↑</sup> G	5'-phosphate extension

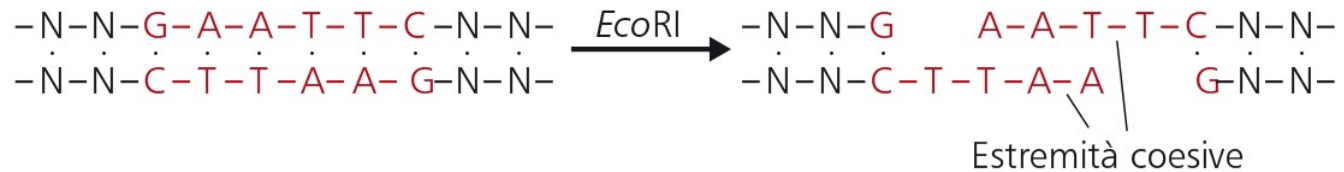


# Manipolazione del DNA - Enzimi di restrizione

(a) Produzione di estremità piatte



(b) Produzione di estremità coesive

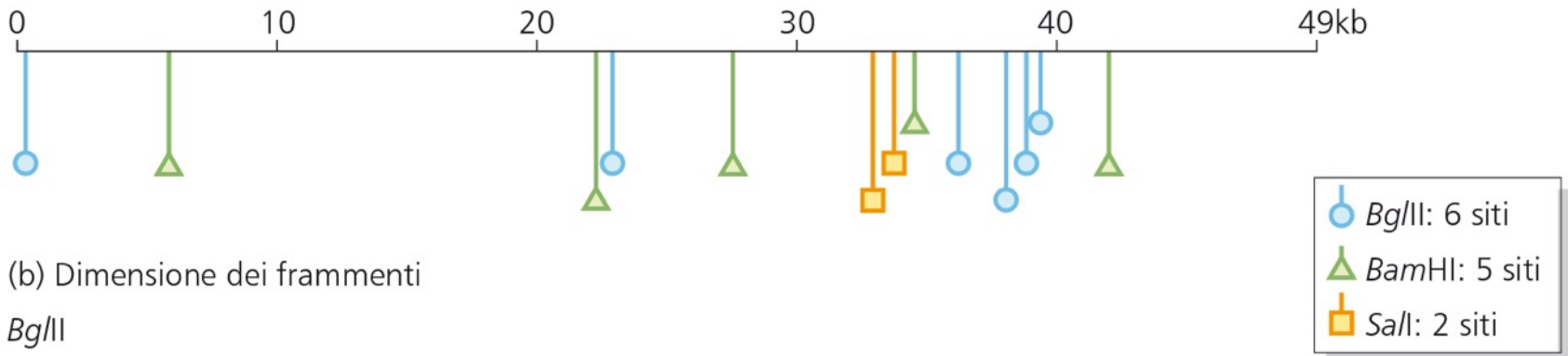


(c) Estremità coesive identiche prodotte da endonucleasi di restrizione differenti



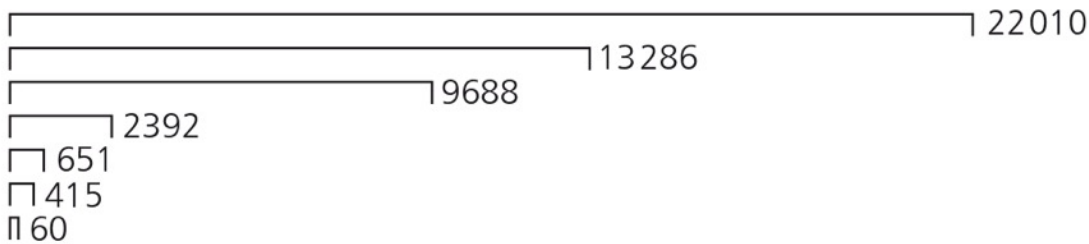
# Manipolazione del DNA - Enzimi di restrizione

(a) Siti di restrizione sul DNA di  $\lambda$

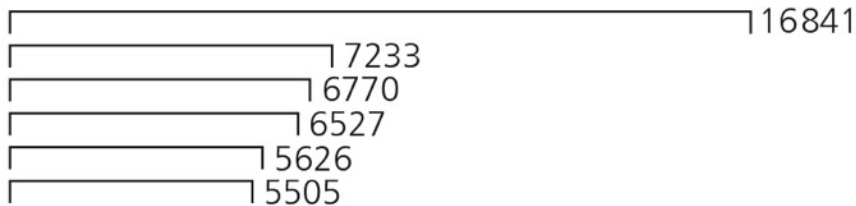


(b) Dimensione dei frammenti

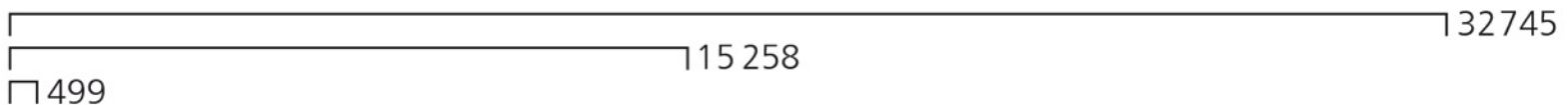
*Bgl*II



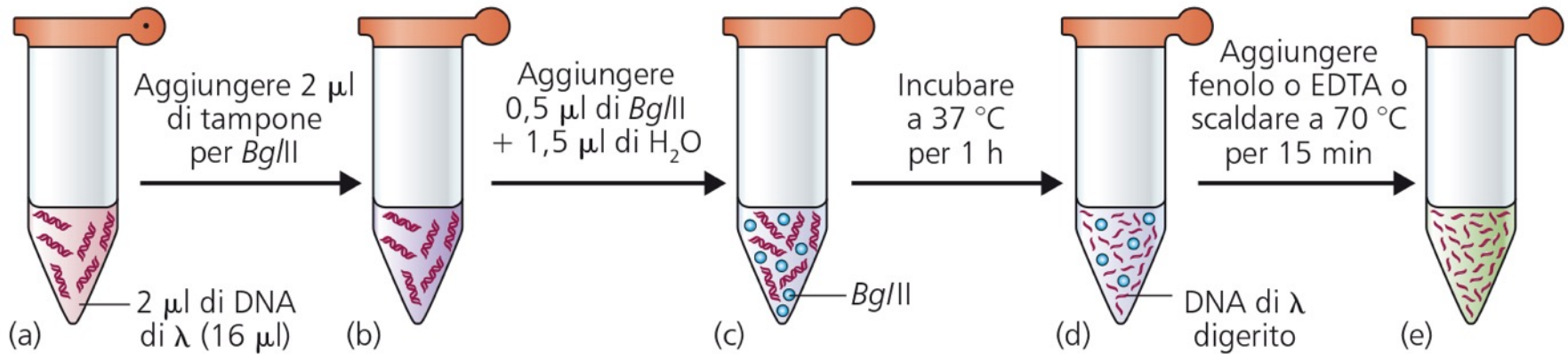
*Bam*HI



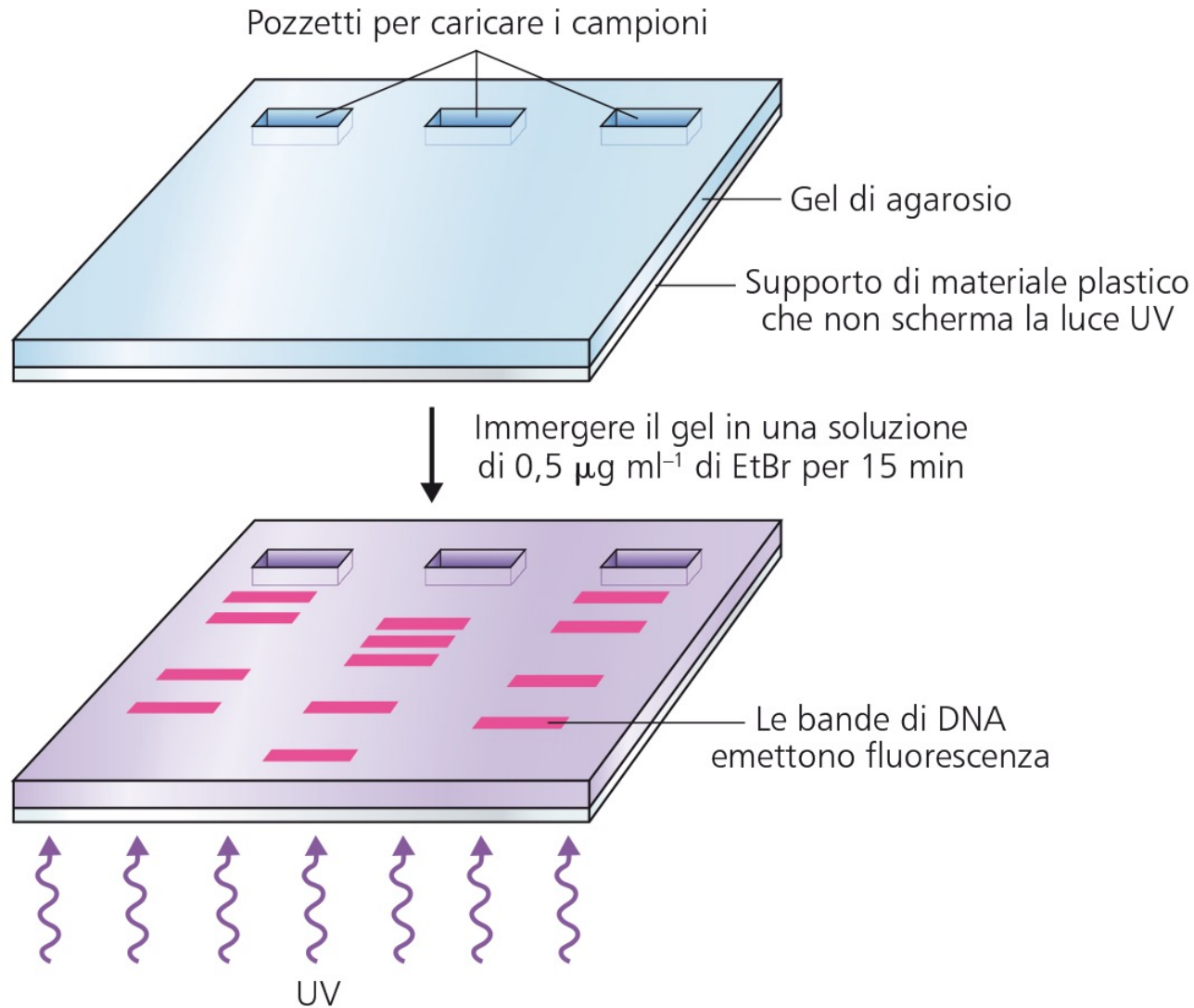
*Sa*II



# Manipolazione del DNA - Enzimi di restrizione



# Manipolazione del DNA - Enzimi di restrizione



# DNA genomico

## Dimensioni del DNA genomico (Kb)

E.coli	4.0	X	$10^3$
S.cerevisiae	1.3	X	$10^4$
A.thaliana	1.3	X	$10^5$
Z.mais	1.5	X	$10^7$
D.melanogaster	1.8	X	$10^7$
Topo	2.3	X	$10^9$
Uomo	2.8	X	$10^9$
X.lavis	3.0	X	$10^9$

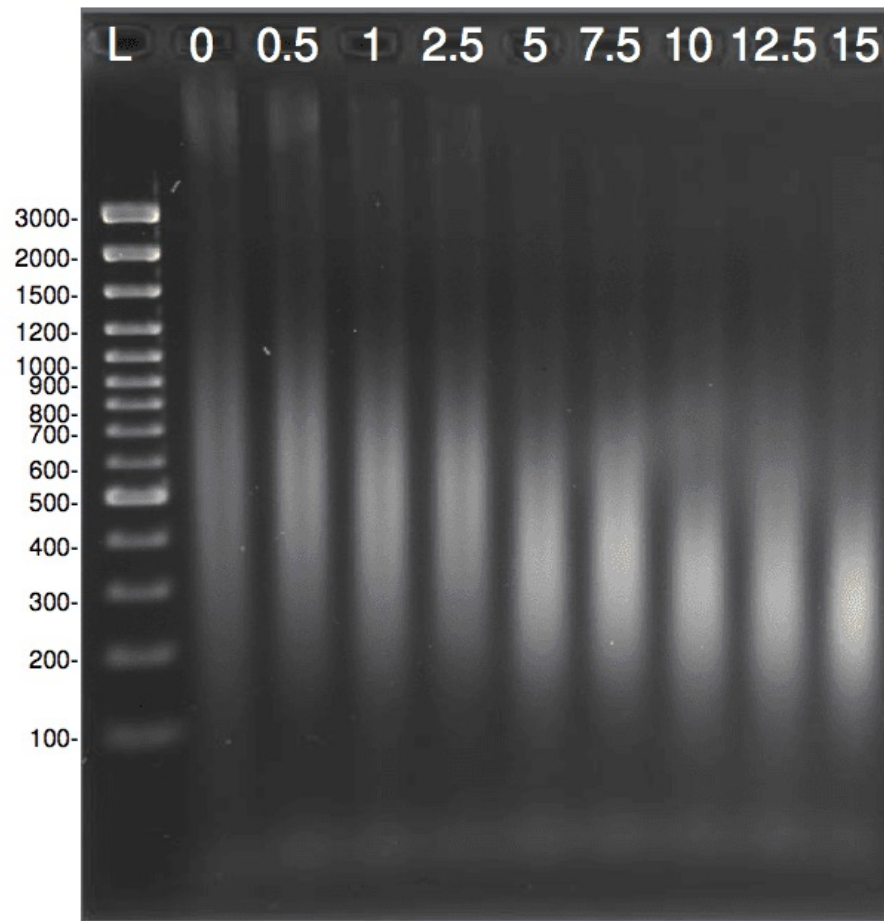
# DNA genomico

Enzyme	Sequence	E.coli	A.thaliana	C.elegans	Drosophila	H.sapiens
Apa I	GGGCC	15000	25000	40000	6000	2000
Avr II	CCTAGG	150000	15000	20000	20000	8000
BamH I	GGATCC	5000	6000	9000	4000	5000
Dra I	TTTAAA	2000	2000	1000	1000	2000

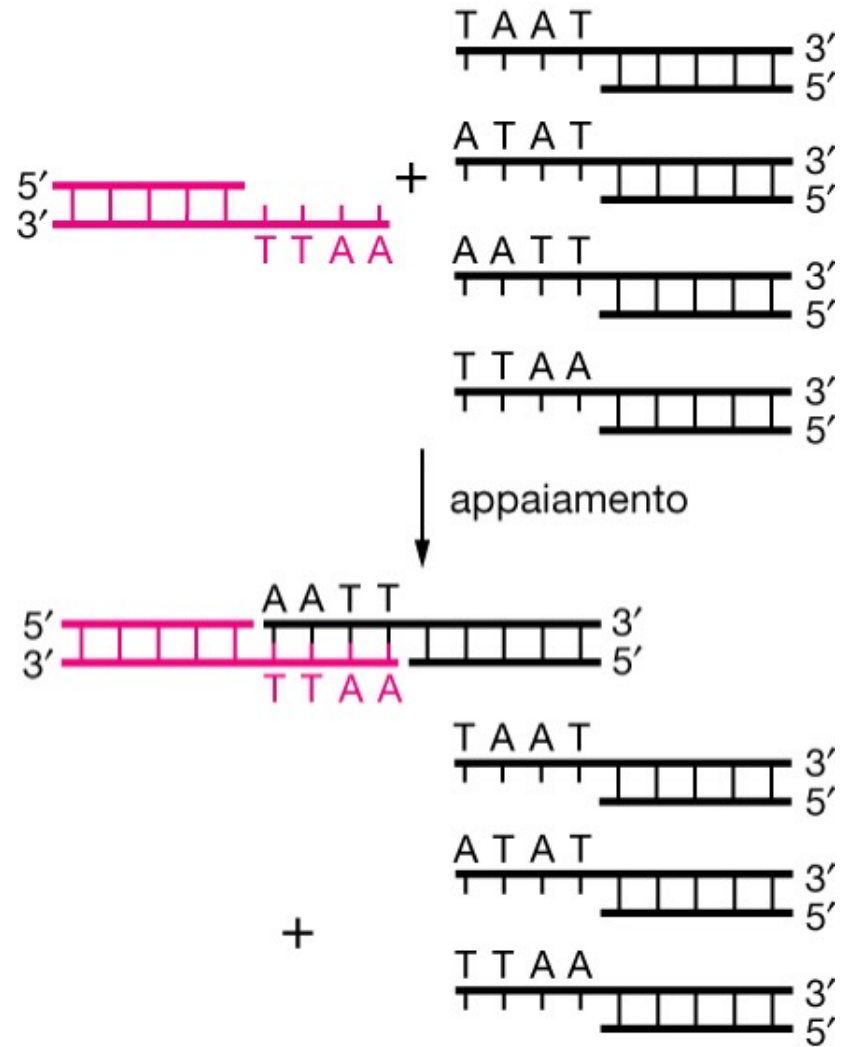
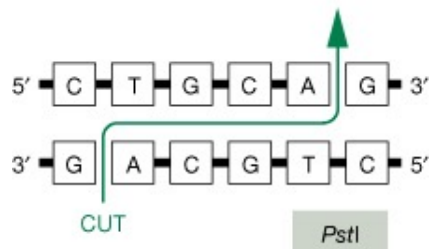
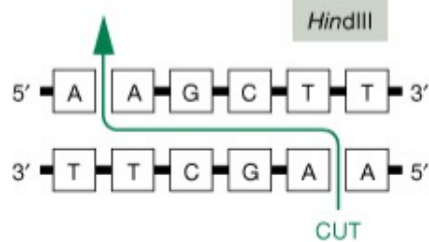
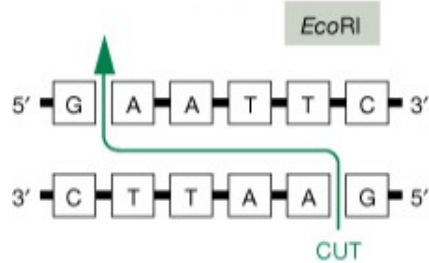
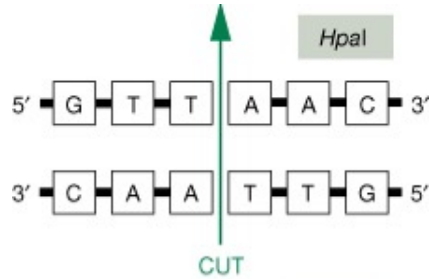
Dimensione media dei frammenti dopo restrizione

# DNA genomico

sonication time [min]



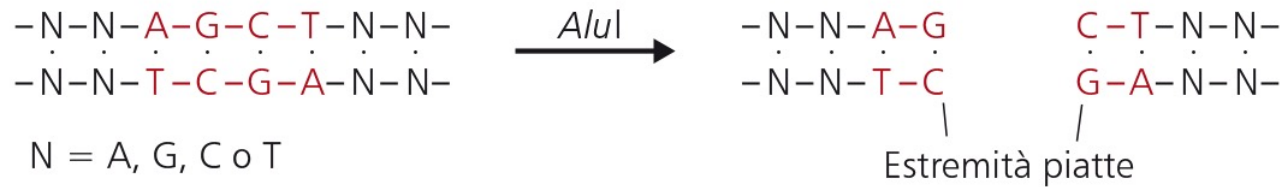
# Enzimi di Restrizione



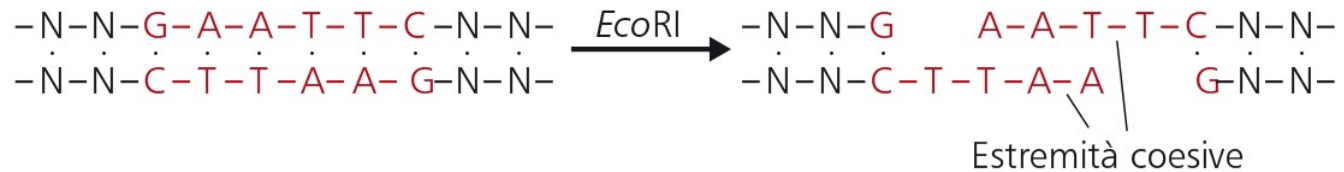


# Manipolazione del DNA - Enzimi di restrizione

(a) Produzione di estremità piatte



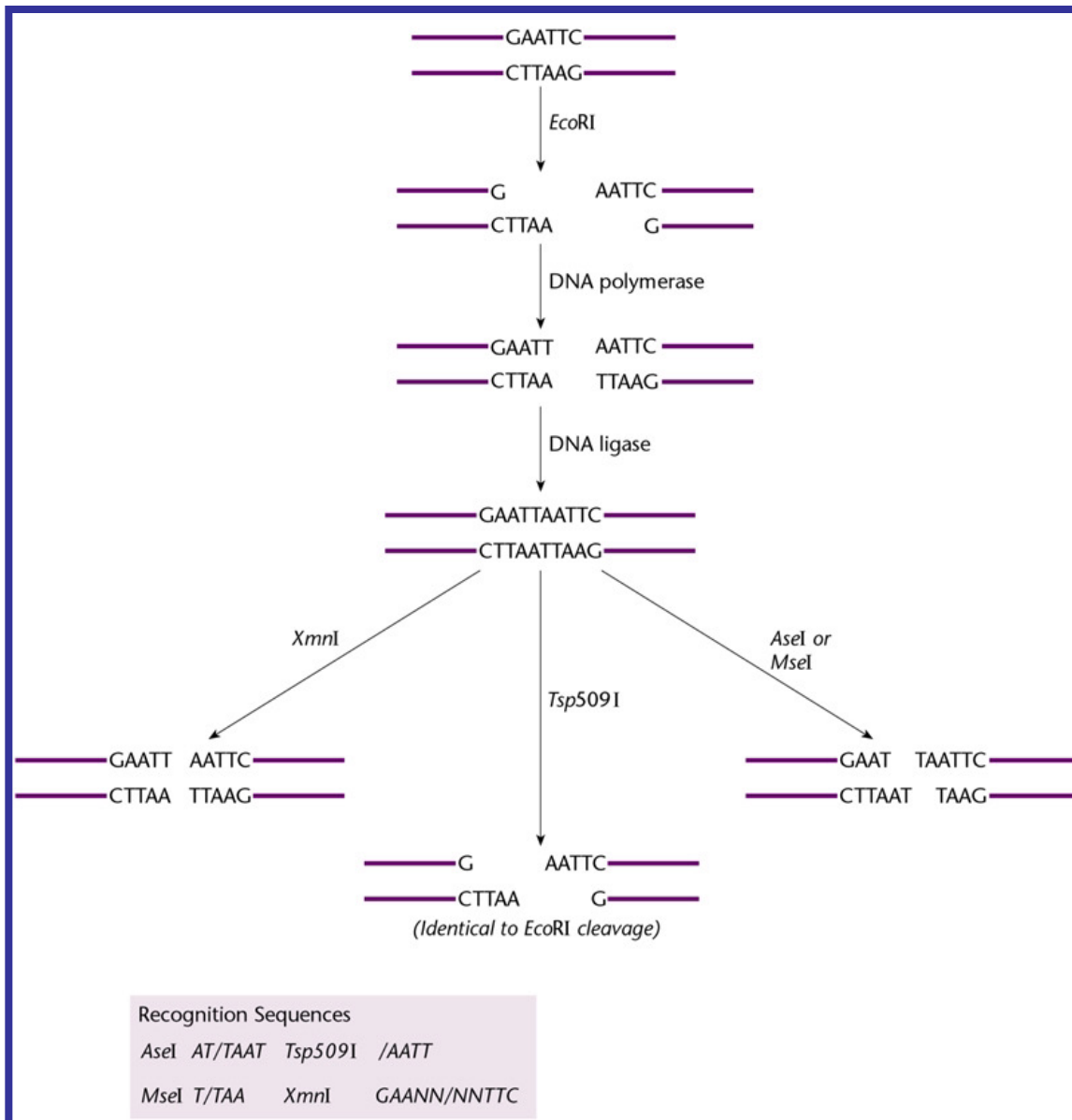
(b) Produzione di estremità coesive



(c) Estremità coesive identiche prodotte da endonucleasi di restrizione differenti



# Enzimi di Restrizione



# Clonaggio Molecolare

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- 4) cDNA

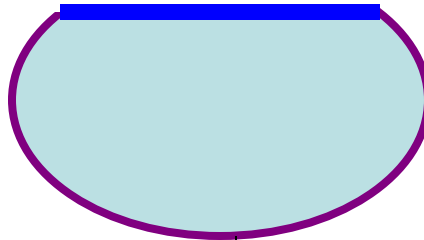
**Digestione con enzimi  
di restrizione**

**DNA del vettore**

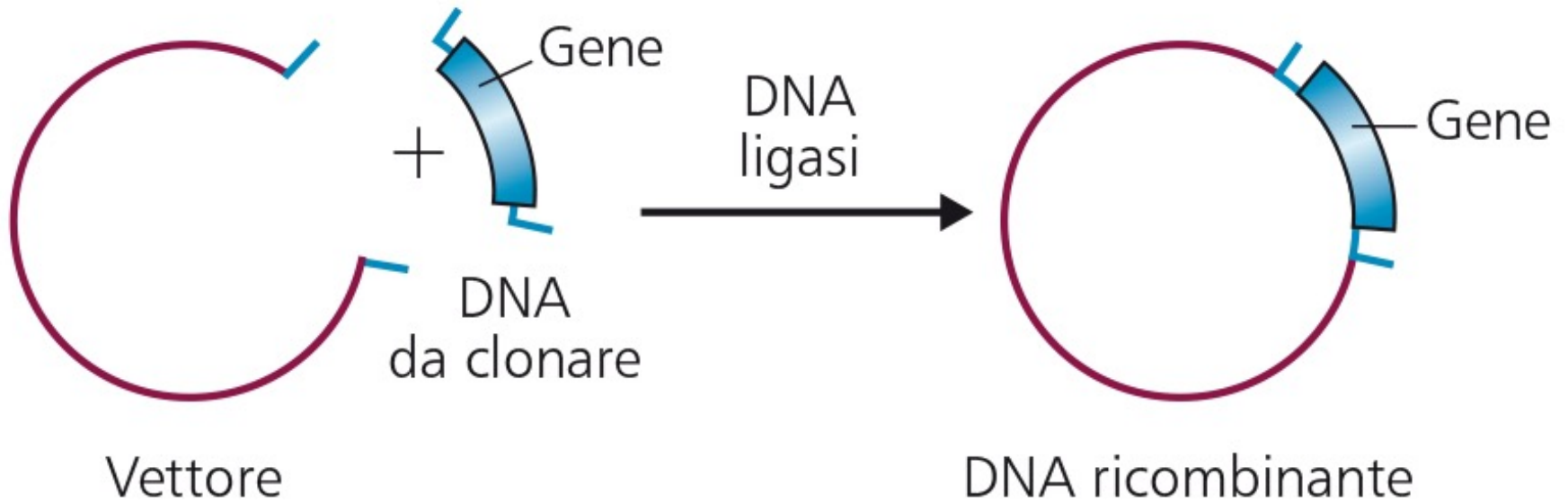
**Ligasi**

**Trasformazione delle cellule di E.coli**

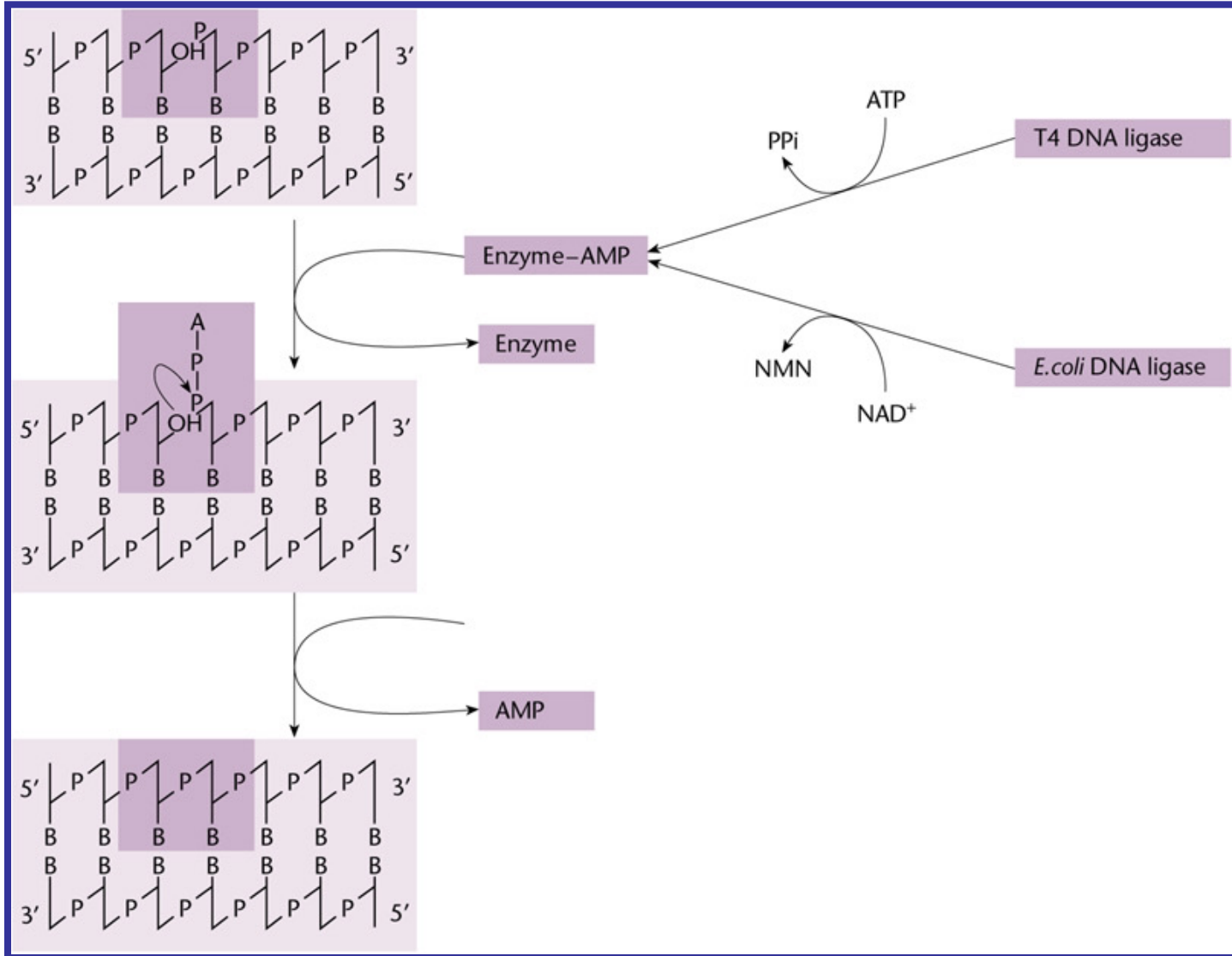
**Selezione dei trasformanti**



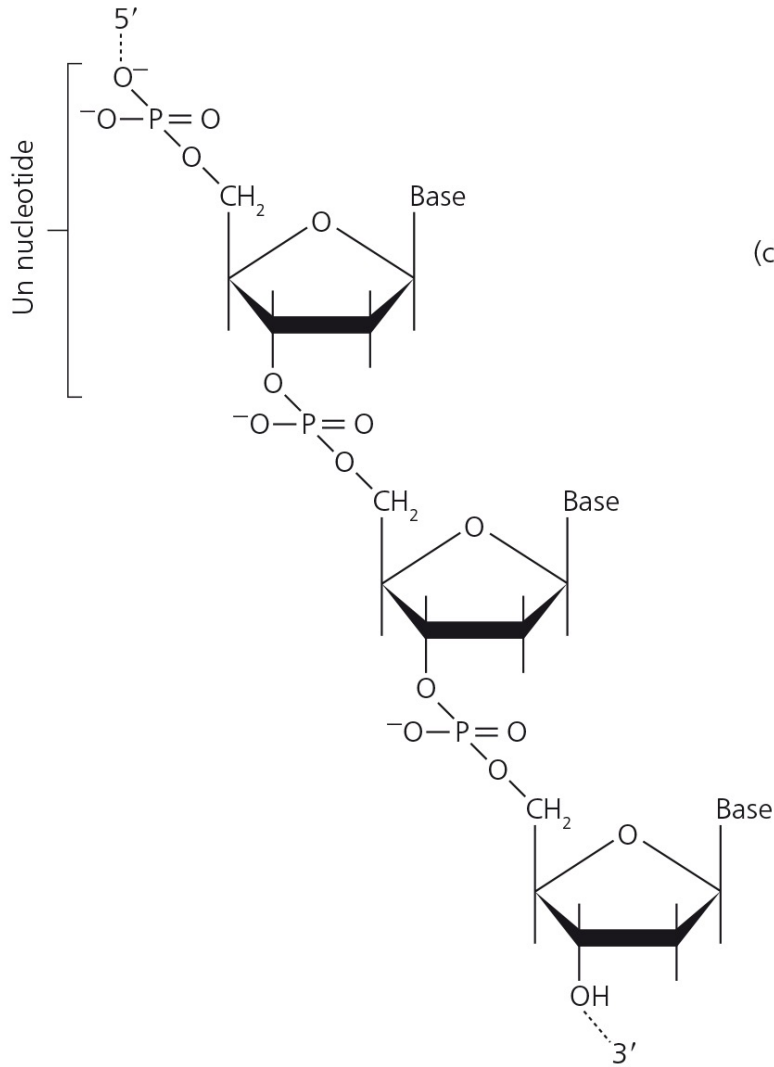
# DNA ligasi



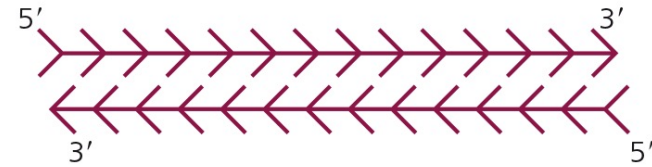
# DNA ligasi



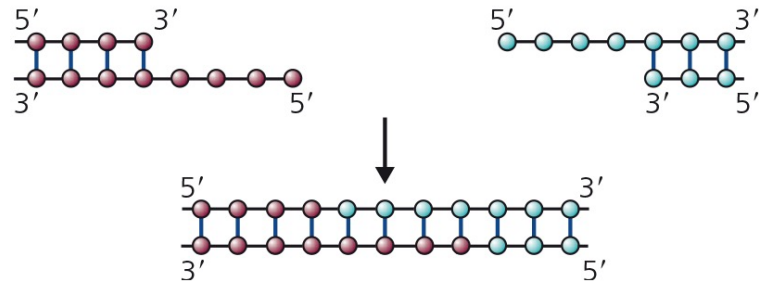
(a) Struttura di un polinucleotide che mostra le differenze chimiche tra estremità 5'-P e 3'-OH



(b) Nella doppia elica i filamenti di nucleotidi sono antiparalleli



(c) La ligazione avviene tra le estremità 5'-P e 3'-OH



# DNA ligasi

Dna Ligasi:

T4 Dna Ligasi - Agisce su qualsiasi estremità'

E.Coli Ligasi - Agisce su estremità' coesive

# DNA ricombinante: enzimi di modificazione

**Fosfatasi Alcalina** -  $5'G_{OH}$        $5'G_{OH}$   
 $3'CTTAA_p$        $3'CTTAA_{OH}$

**Polinucleotide chinasi** -  $5'G_{OH}$        $5'G_{OH}$   
 $3'CTTAA_{OH}$        $3'CTTAA_p$

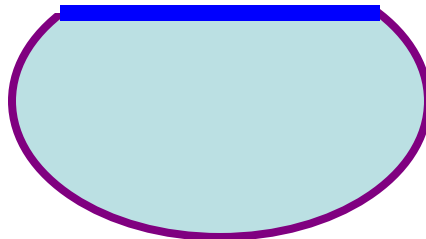
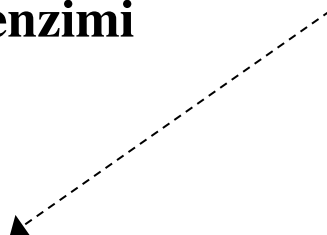
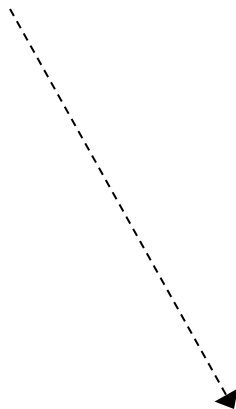
## DNA da inserire:

- 1) DNA genomico
- 2) DNA sintetico
- 3) DNA da PCR
- 4) cDNA

Digestione con enzimi  
di restrizione

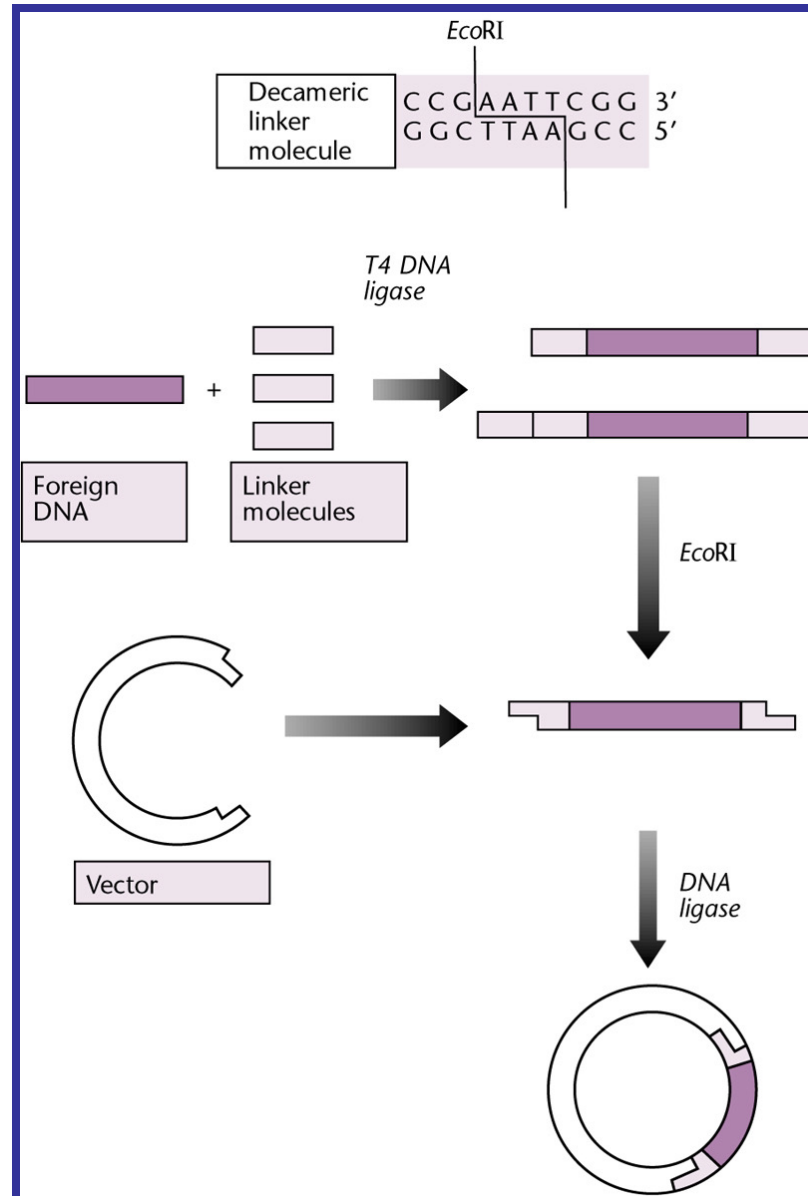
DNA del vettore

Ligasi

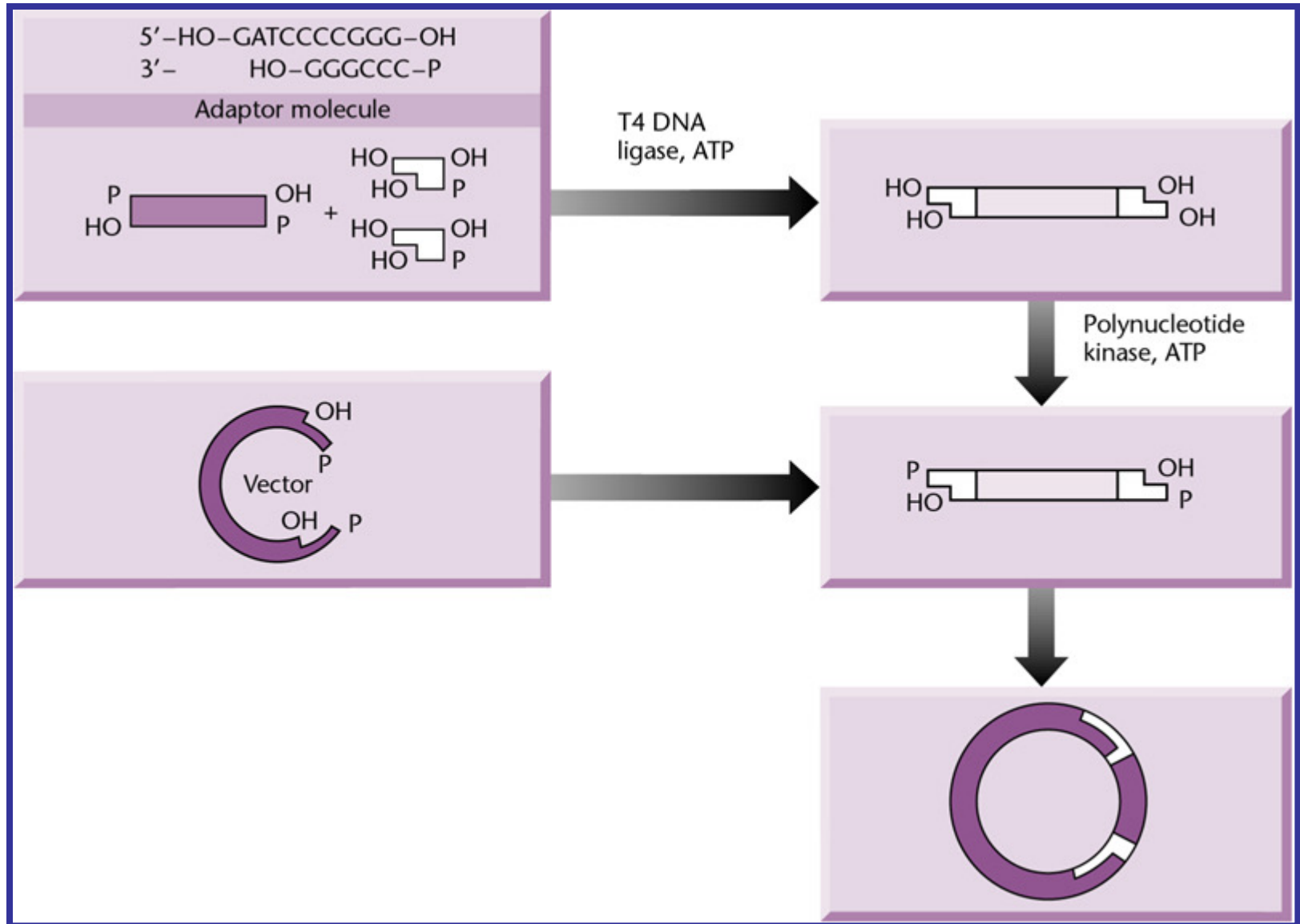




# DNA ricombinante: linkers e adattatori

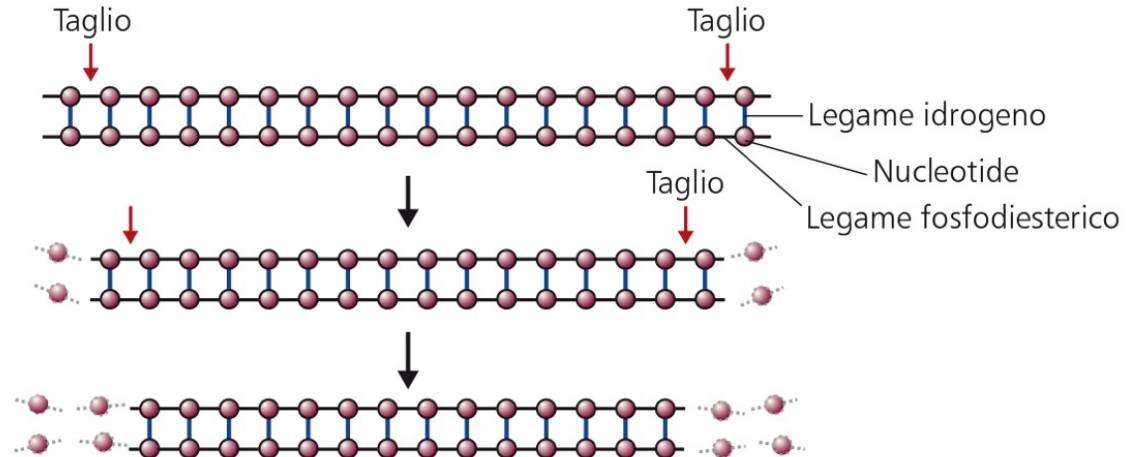


# DNA ricombinante: linkers e adattatori

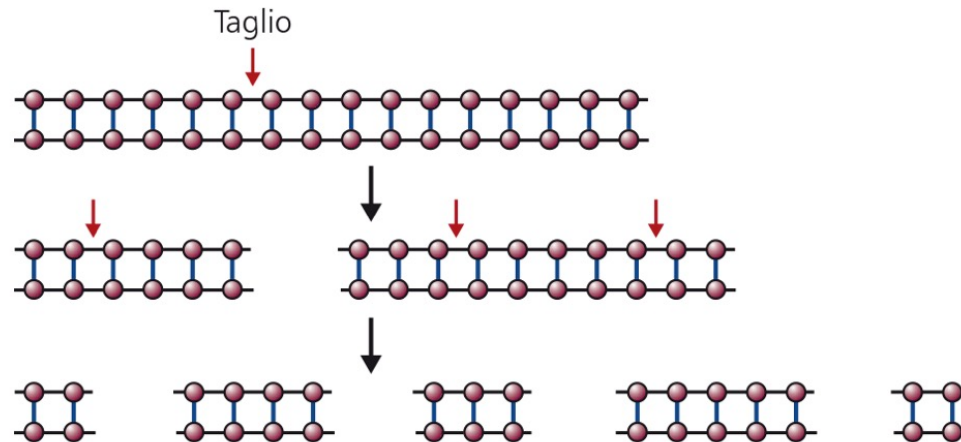


# Manipolazione del DNA - Nucleasi

(a) Esonucleasi



(b) Endonucleasi



# DNA ricombinante: enzimi di modificazione

## Dna nucleasi:

**Esonucleasi di  $\lambda$  - Agisce in dir. 5' -> 3'**

**5'GAATT<sub>OH</sub>  
3'CTTAA<sub>p</sub>**

**5'GAATT<sub>OH</sub>  
3'C<sub>p</sub>**

**Esonucleasi III - Agisce in dir. 3' -> 5'**

**5'GAATT<sub>OH</sub>  
3'CTTAA<sub>p</sub>**

**5'G<sub>OH</sub>  
3'CTTAA<sub>p</sub>**

**Esonucleasi Bal31 - Agisce in entrambe le direzioni**

**5'GAATT<sub>OH</sub>  
3'CTTAA<sub>p</sub>**

**5' pAA<sub>OH</sub>  
3'OH<sub>TTp</sub>**

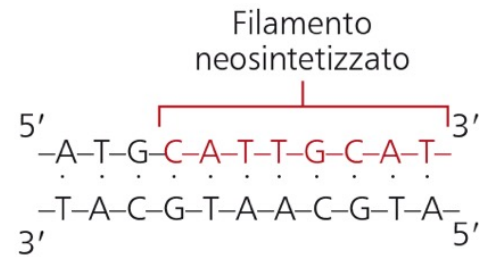
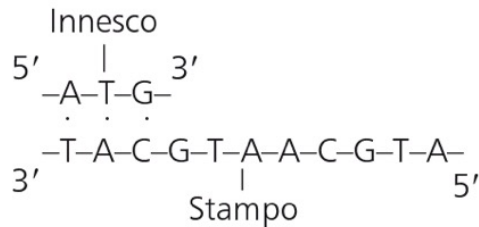
**Endonucleasi S1 - Agisce sul DNA a singolo filamento**

**5'GA<sub>OH</sub>  
3'CTTAA<sub>p</sub>**

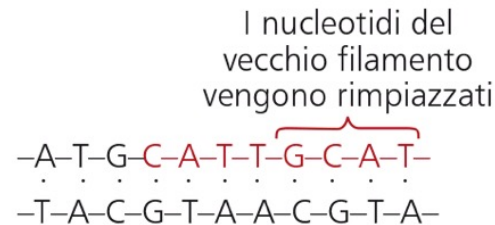
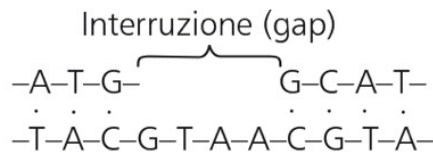
**5'GA<sub>OH</sub>  
3'CT<sub>p</sub>**

# Manipolazione del DNA - Polimerasi

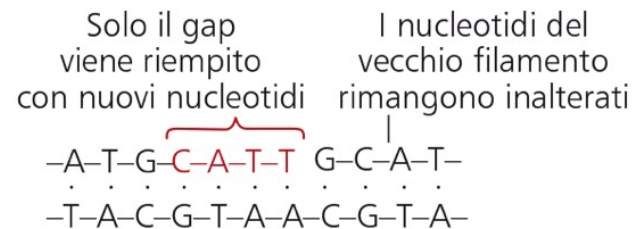
(a) La reazione fondamentale



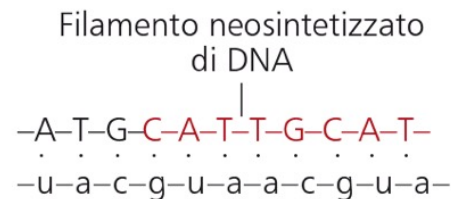
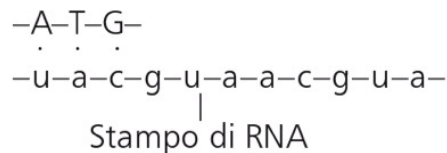
(b) DNA polimerasi I



(c) Il frammento di Klenow



(d) Trascrittasi inversa



# DNA ricombinante: enzimi di modificazione

**Fosfatasi Alcalina** -  $5'G_{OH}$        $5'G_{OH}$   
 $3'CTTAAp$        $3'CTTAA_{OH}$

**Polinucleotide chinasi** -  $5'G_{OH}$        $5'G_{OH}$   
 $3'CTTAA_{OH}$        $3'CTTAAp$

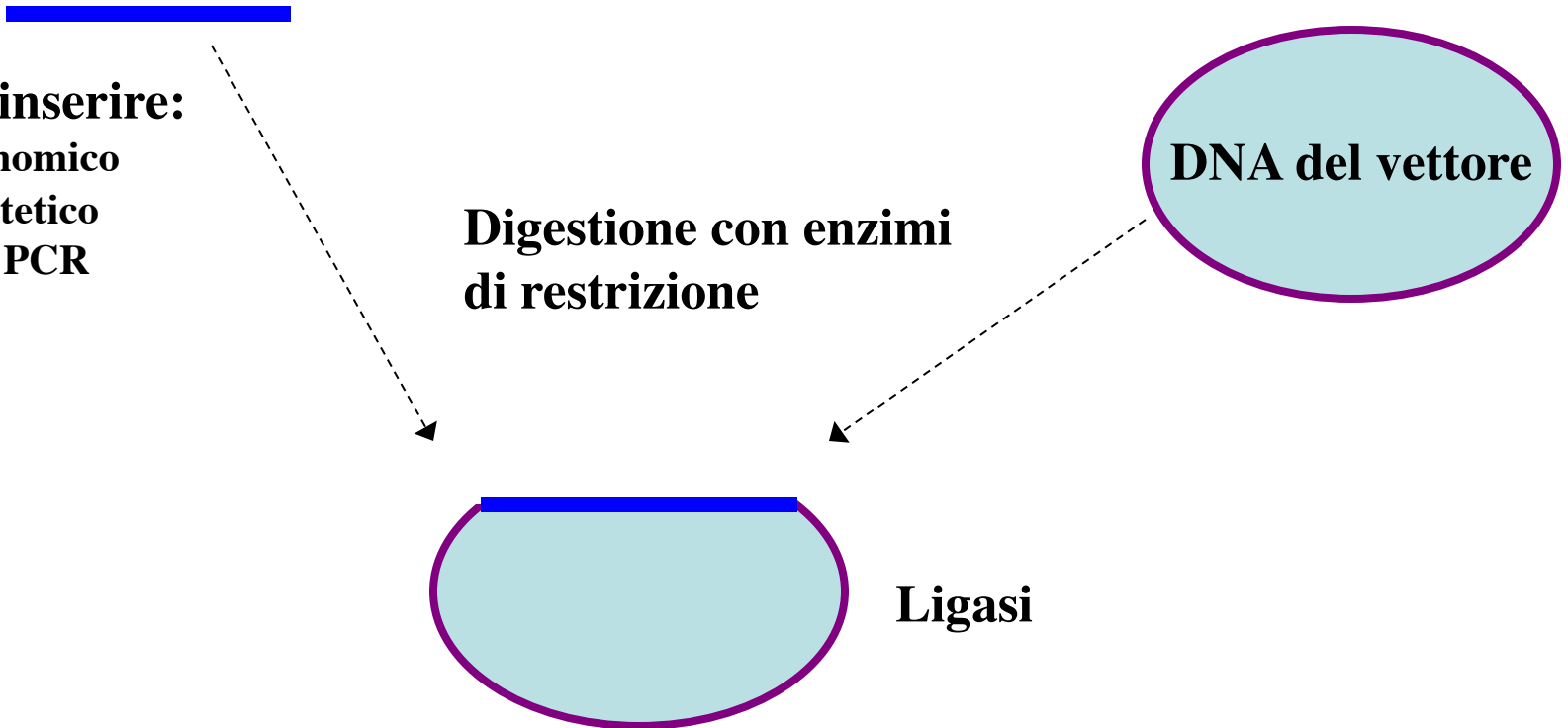
## DNA da inserire:

- 1) DNA genomico
- 2) DNA sintetico
- 3) DNA da PCR
- 4) cDNA

**Digestione con enzimi di restrizione**

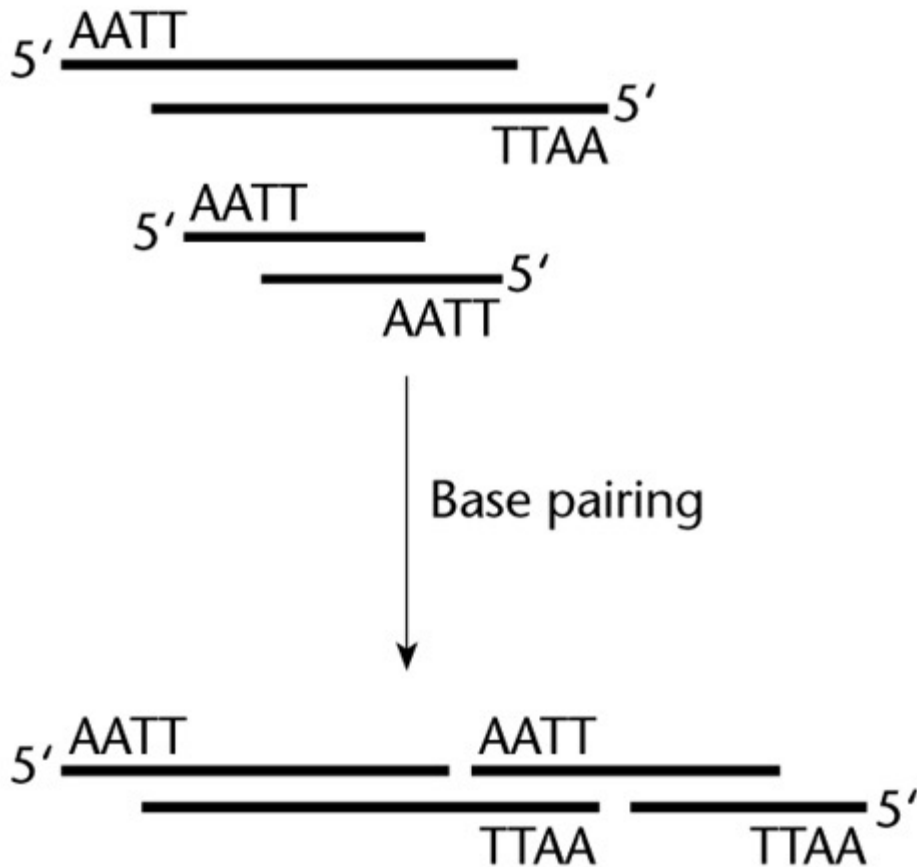
**DNA del vettore**

**Ligasi**

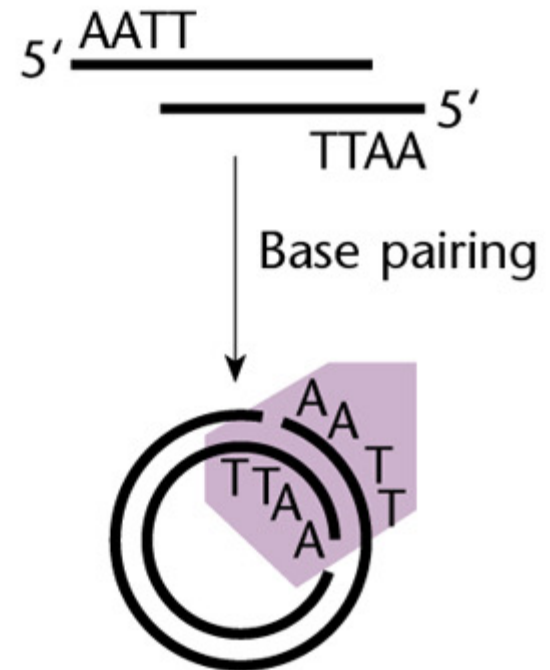


# Enzimi di Restrizione

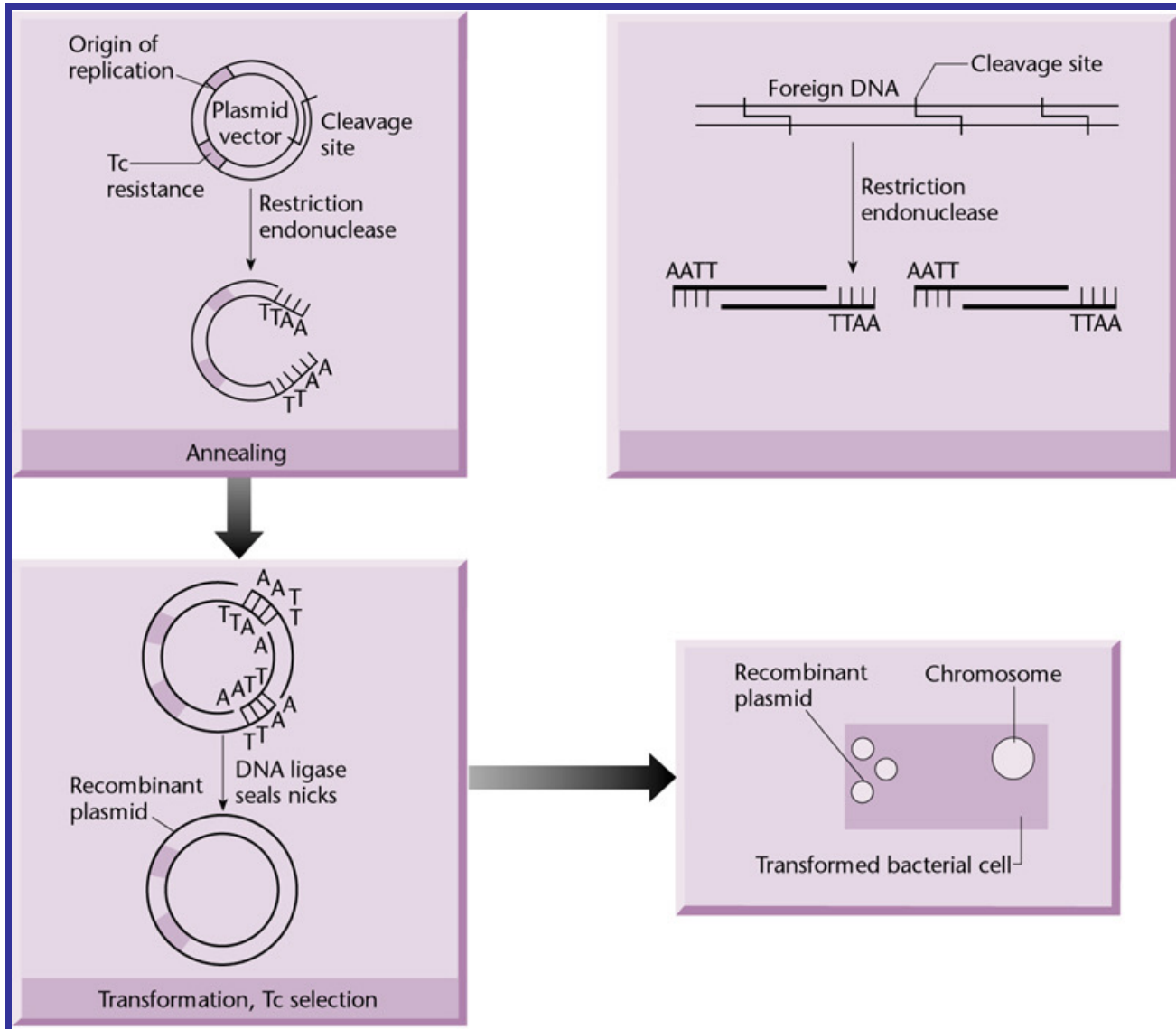
Intermolecular association



Intramolecular association

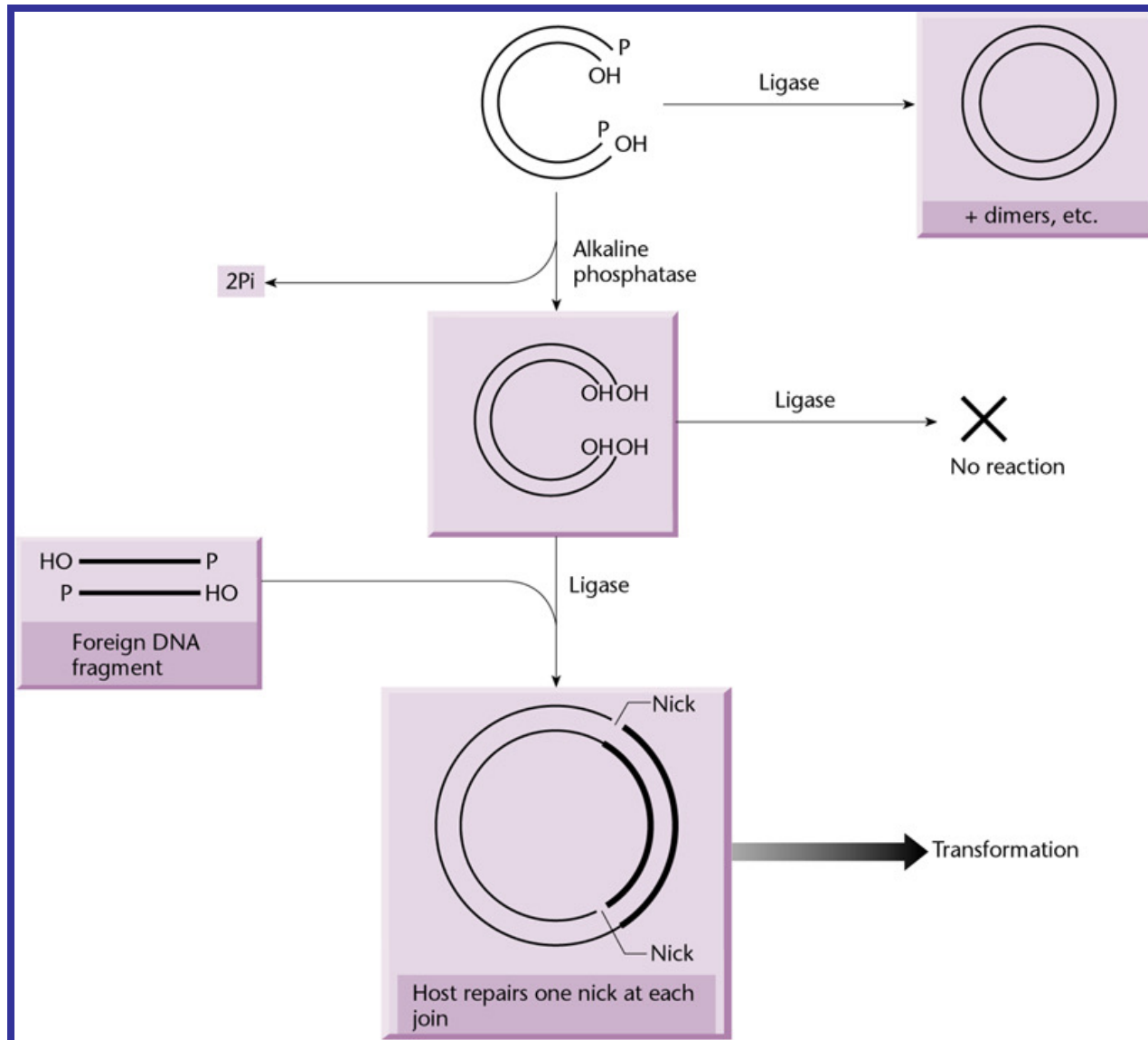


# DNA ligasi

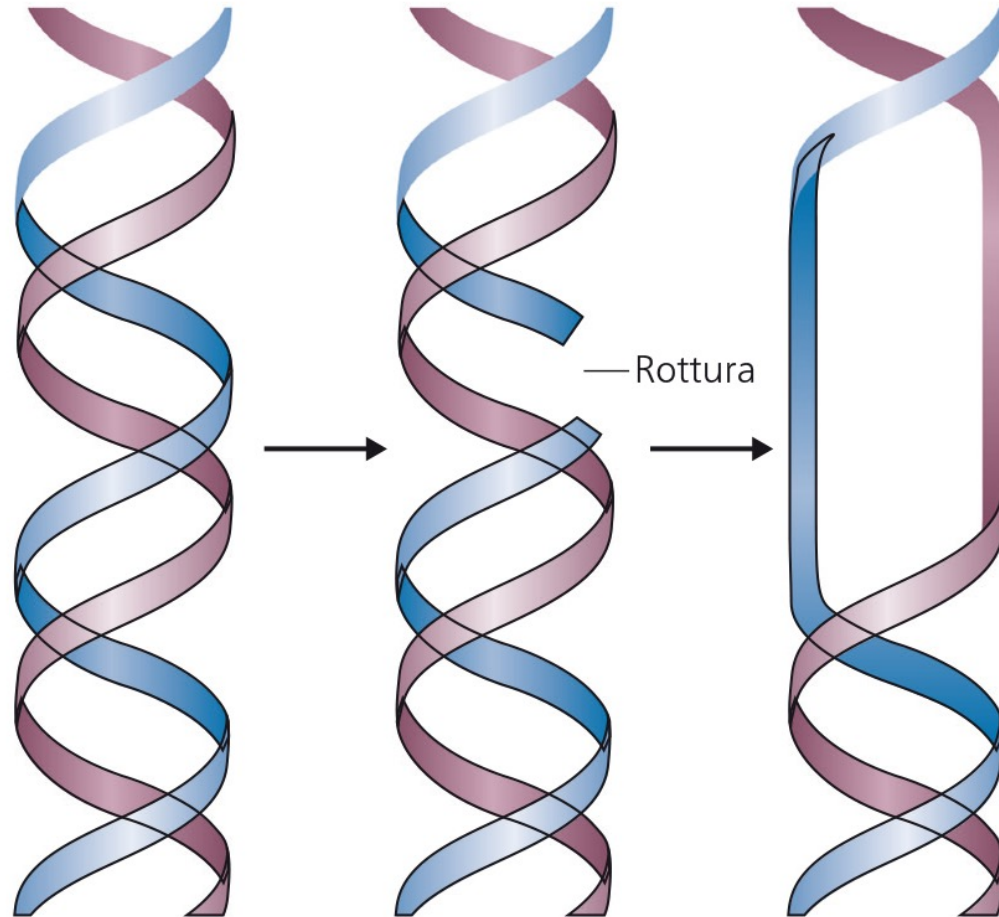




# Clonaggio Molecolare: Fosfatasi alcalina

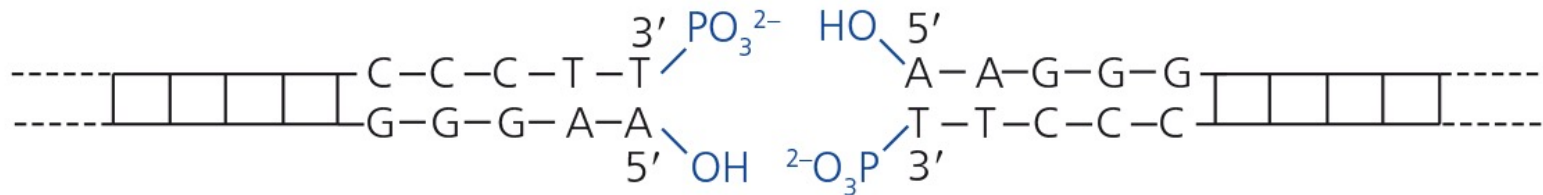


# Clonaggio Molecolare: DNA Topoisomerasi



# Clonaggio Molecolare: DNA Topoisomerasi

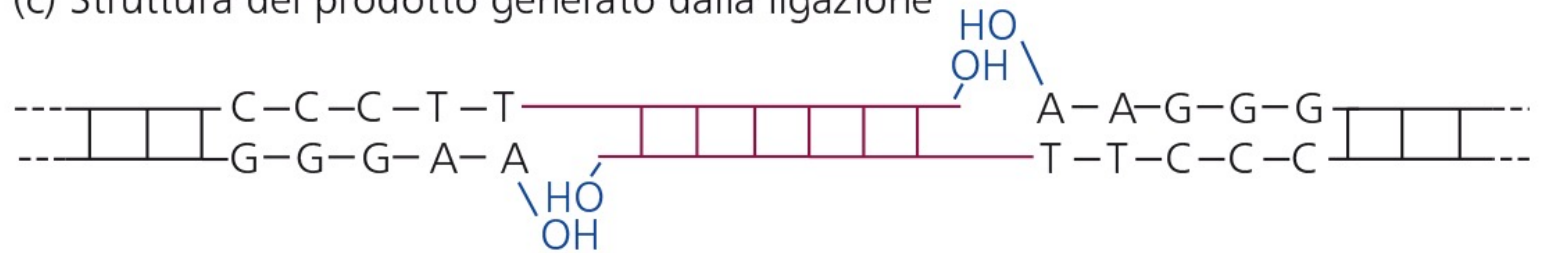
(a) Terminazioni del vettore risultanti dal taglio della topoisomerasi



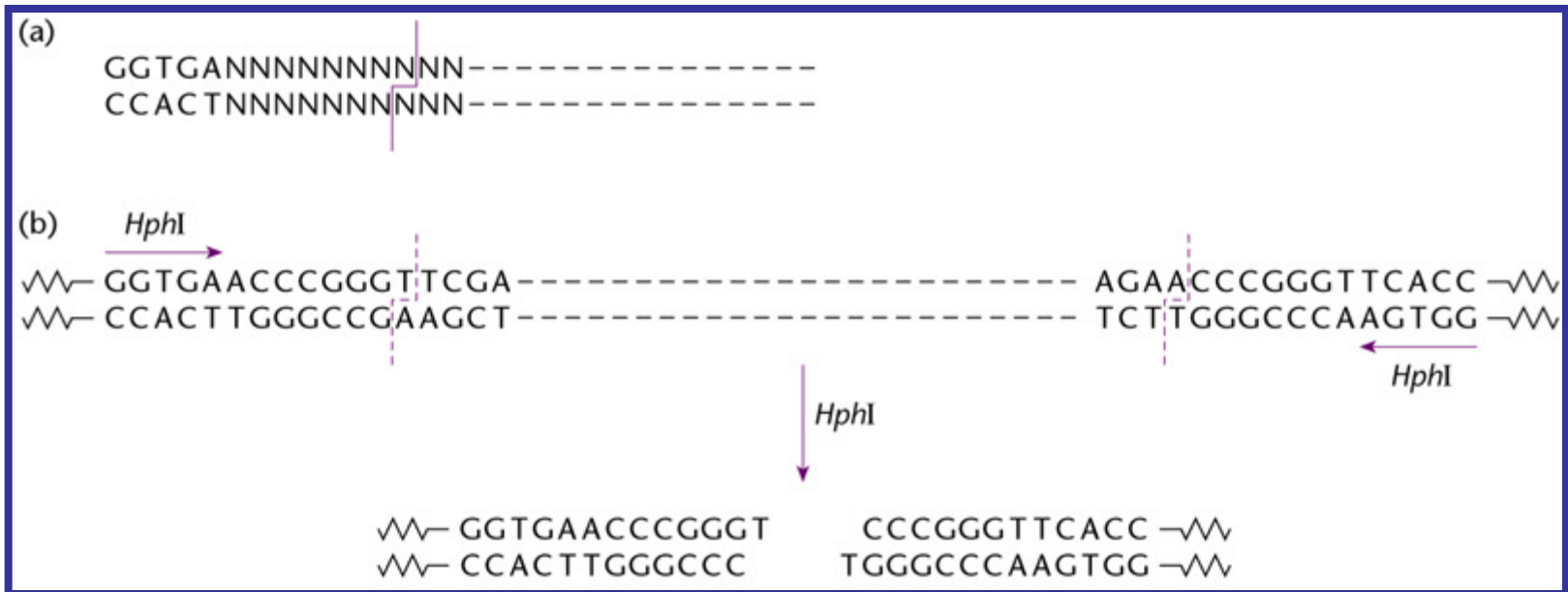
(b) Rimozione dei fosfati dalle terminazioni della molecola da clonare



(c) Struttura del prodotto generato dalla ligazione



# DNA ricombinante: PCR, TA cloning



# Clonaggio Molecolare

