



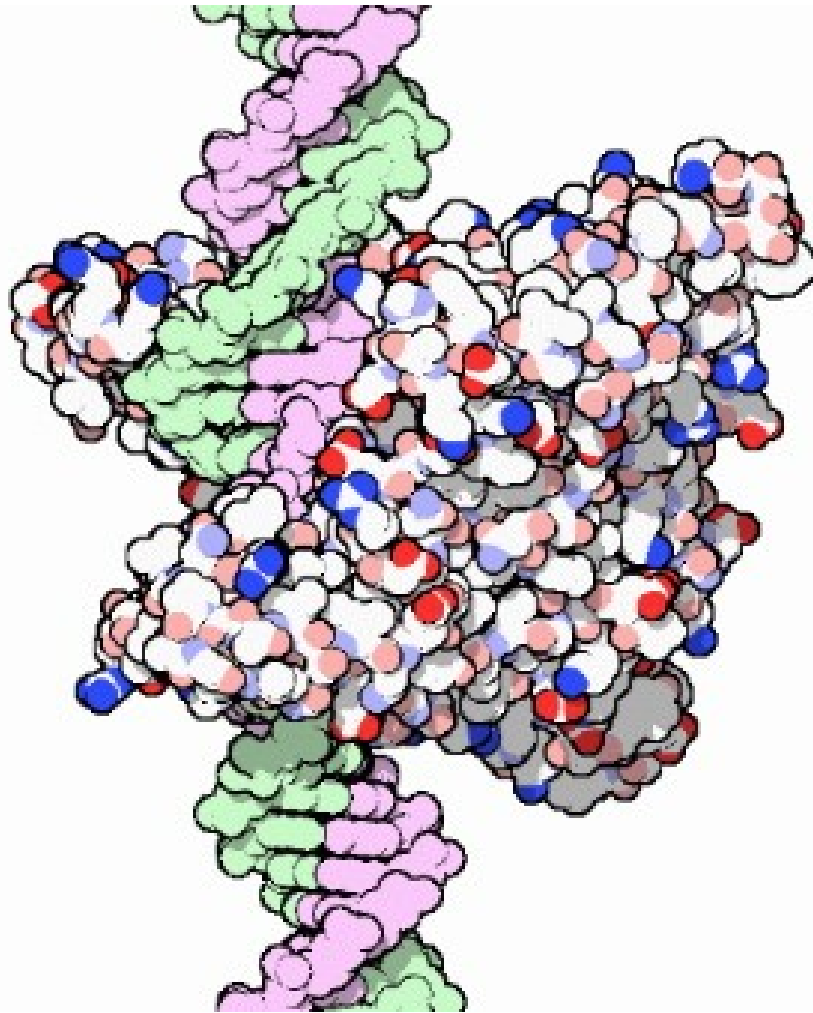
# **GENETICS AND MOLECULAR BIOLOGY FOR ENVIRONMENTAL ANALYSIS**

## **MOLECULAR ECOLOGY LESSON 4: DNA MODIFYING ENZYMES**

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# THE DISCOVERY OF RESTRICTION ENZYMES

- For their 1970 discovery of **restriction endonucleases** (often called by the shorter name **restriction enzymes**) Werner Arber, Hamilton Smith, and Daniel Nathans received the 1978 Nobel Prize for Physiology or Medicine.



# THE PHYSIOLOGICAL ACTIVITY

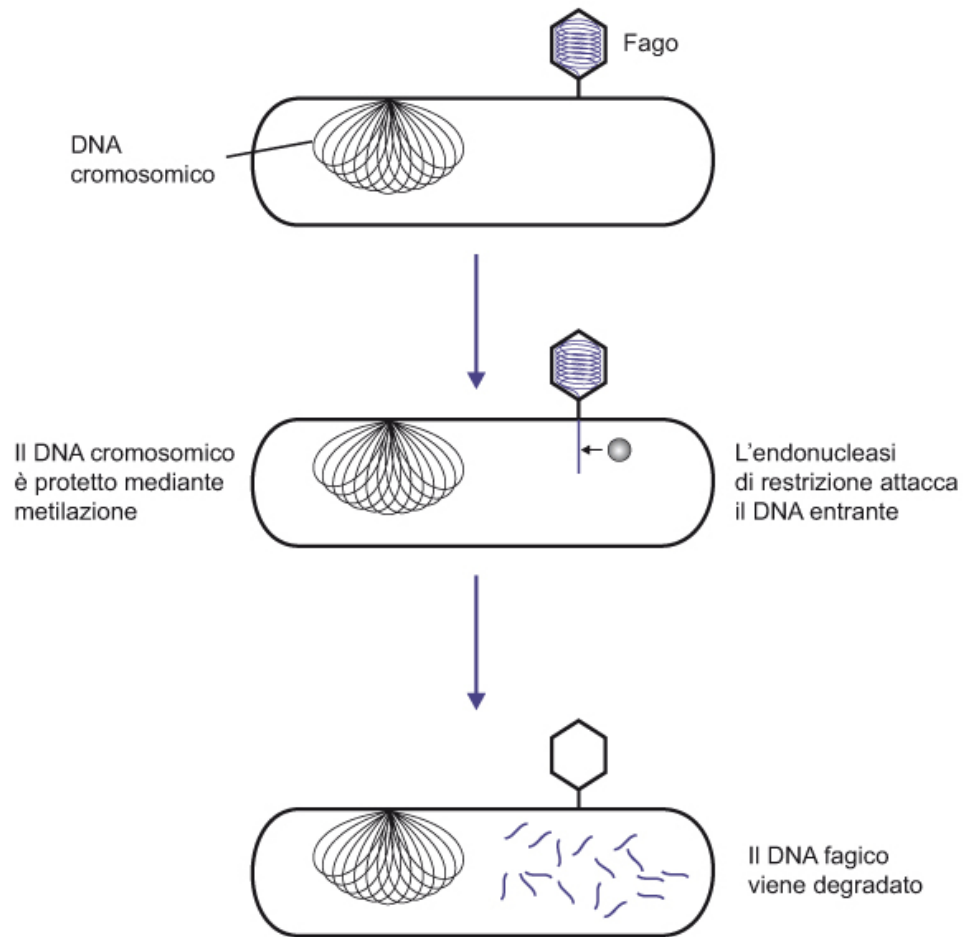
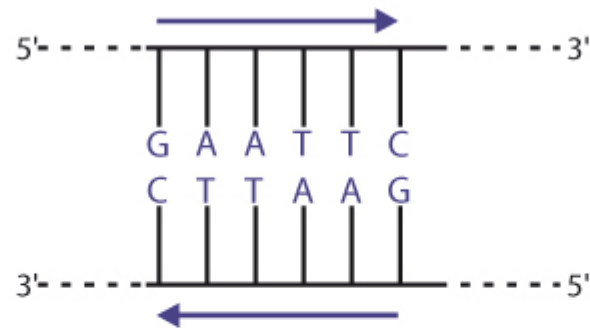


Figura 2.8 La restrizione del batteriofago.



# CUT AND ...



**Figura 2.9** Leggere una sequenza palindromica. Il filamento superiore, letto da sinistra verso destra ( $5' \rightarrow 3'$ ), è uguale al filamento inferiore letto  $5' \rightarrow 3'$  (da destra verso sinistra).



# ...PASTE

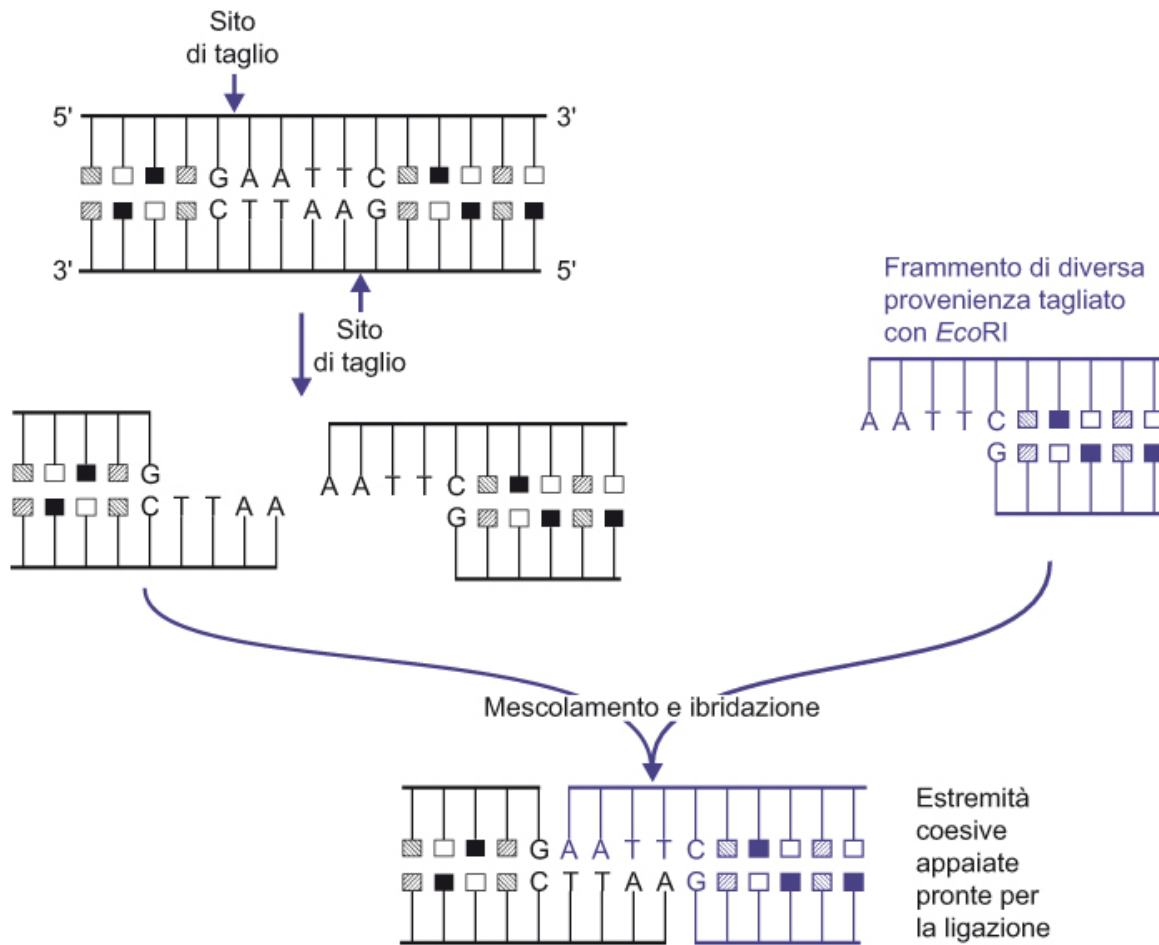


Figura 2.10 Estremità coesive generate da *EcoRI*.



# ...PASTE

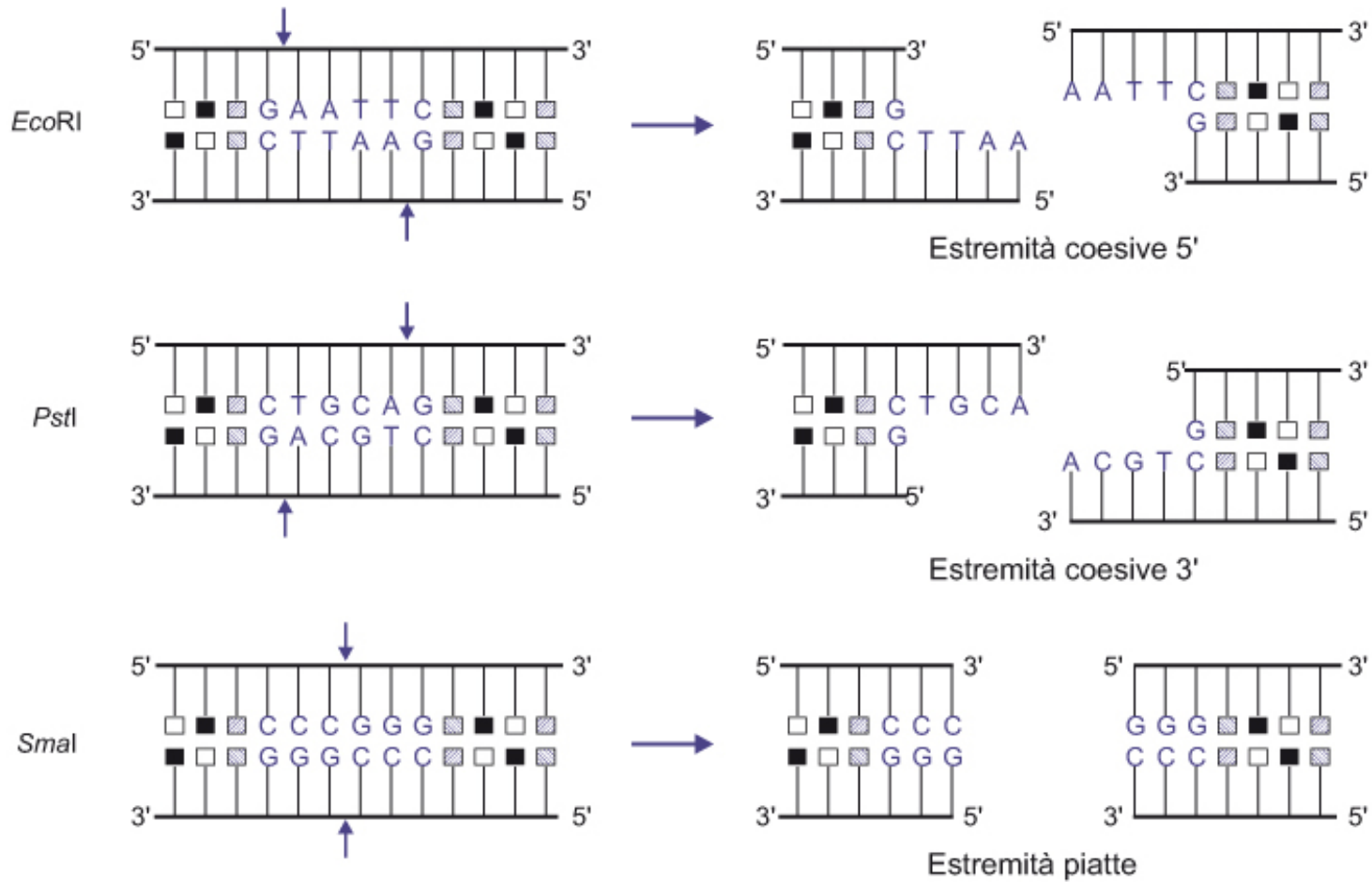
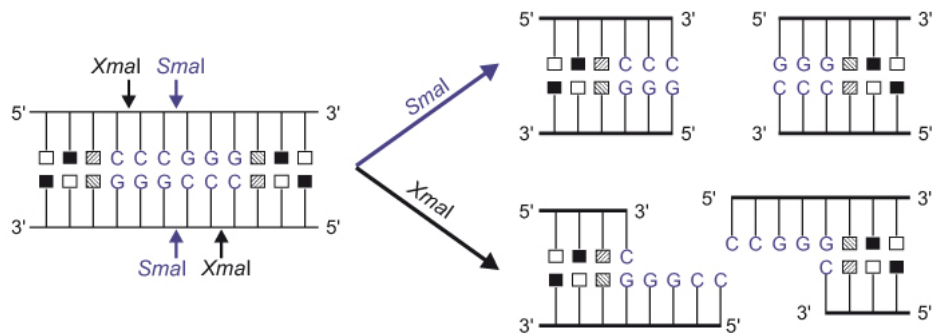
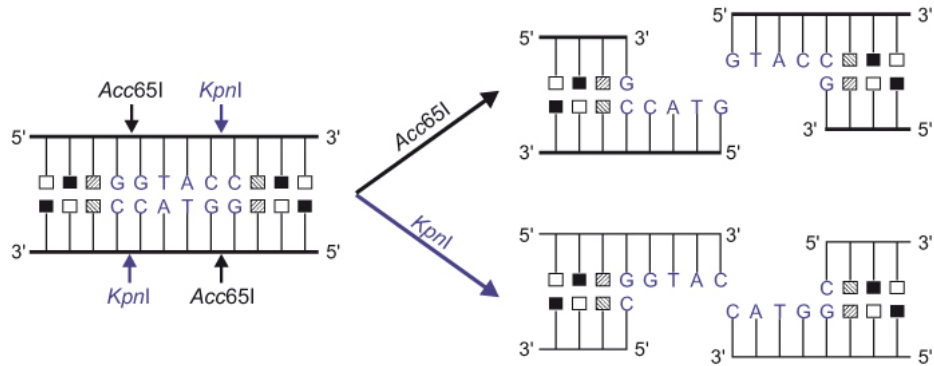


Figura 2.11 Estremità dei frammenti di restrizione.



# ISOSCHIZOMERS NEOSCHIZOMERS



# DNA LIGASE

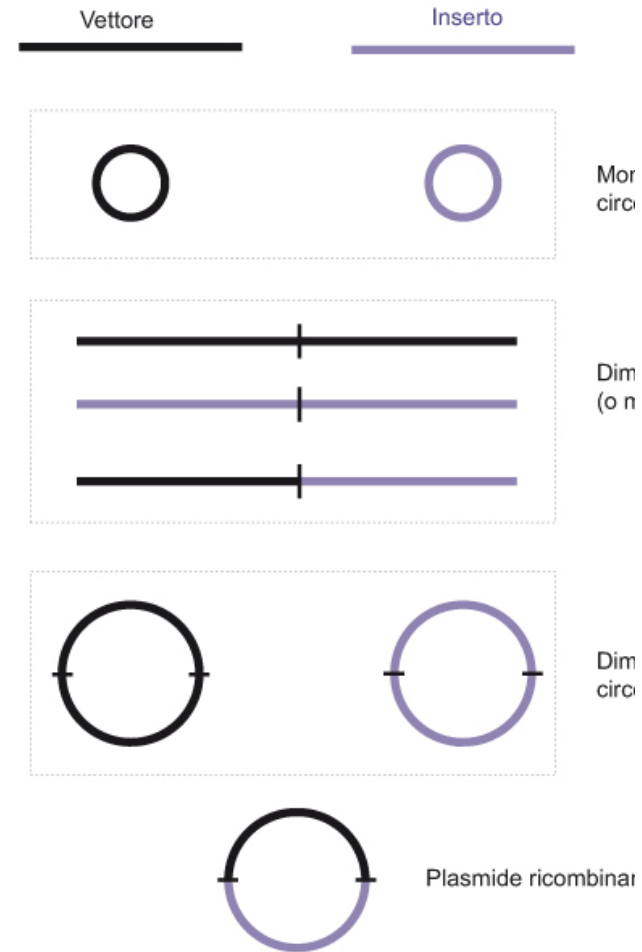
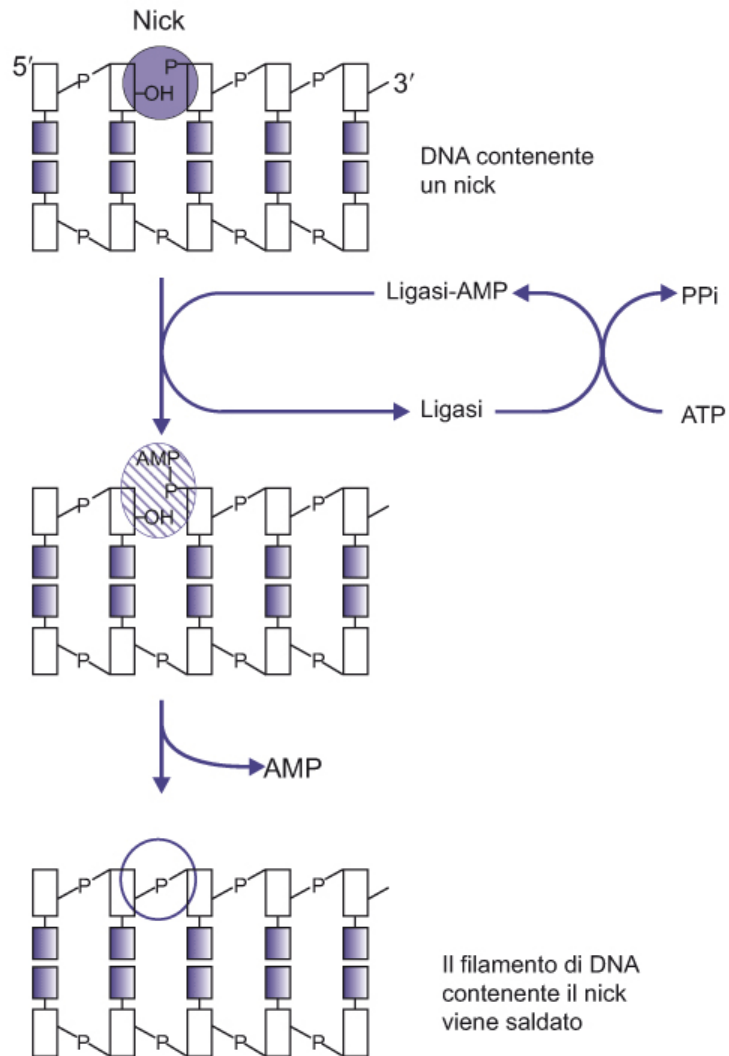


Figura 2.13 Attività della T4 DNA ligasi.





# ADAPTORS/LINKERS

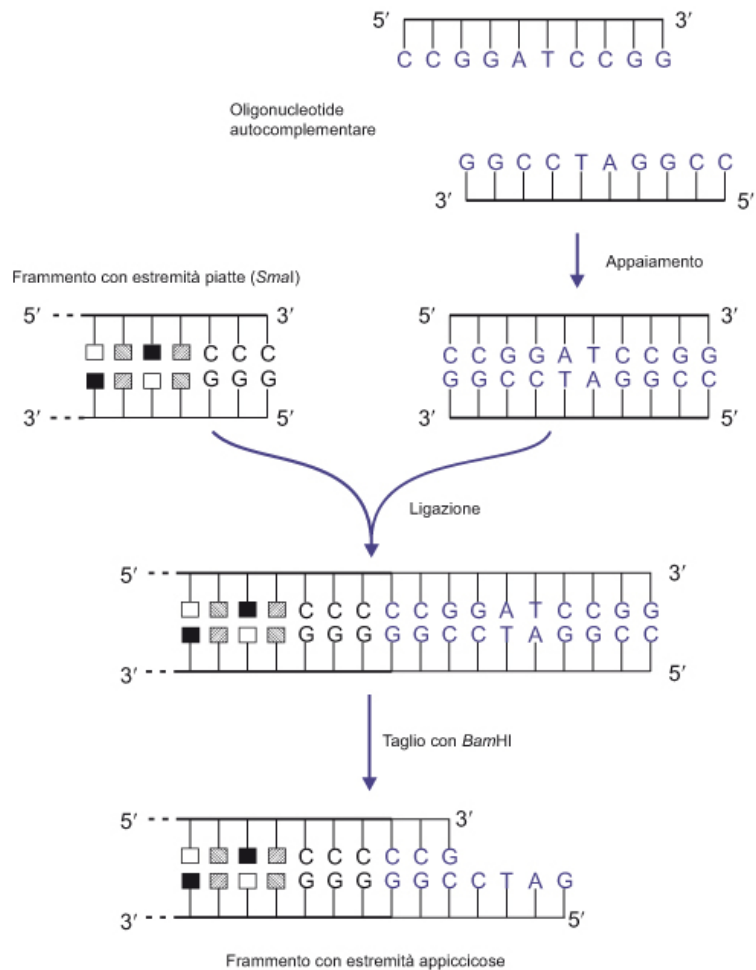


Figura 2.17 Linker.



# OTHER ENZYMES

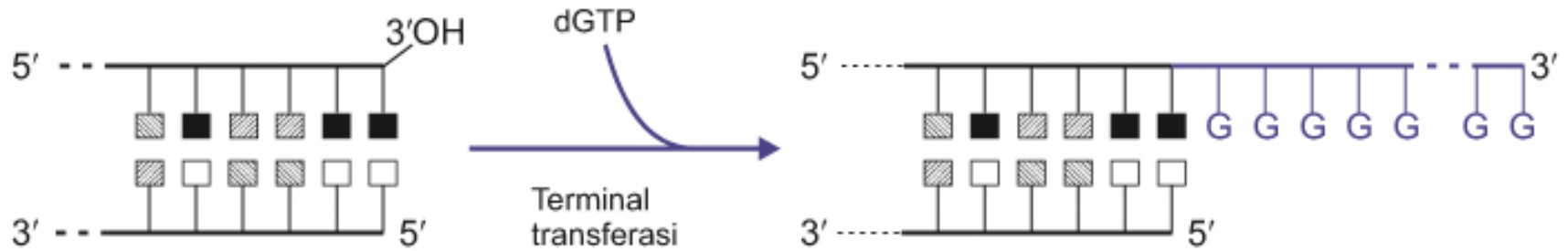
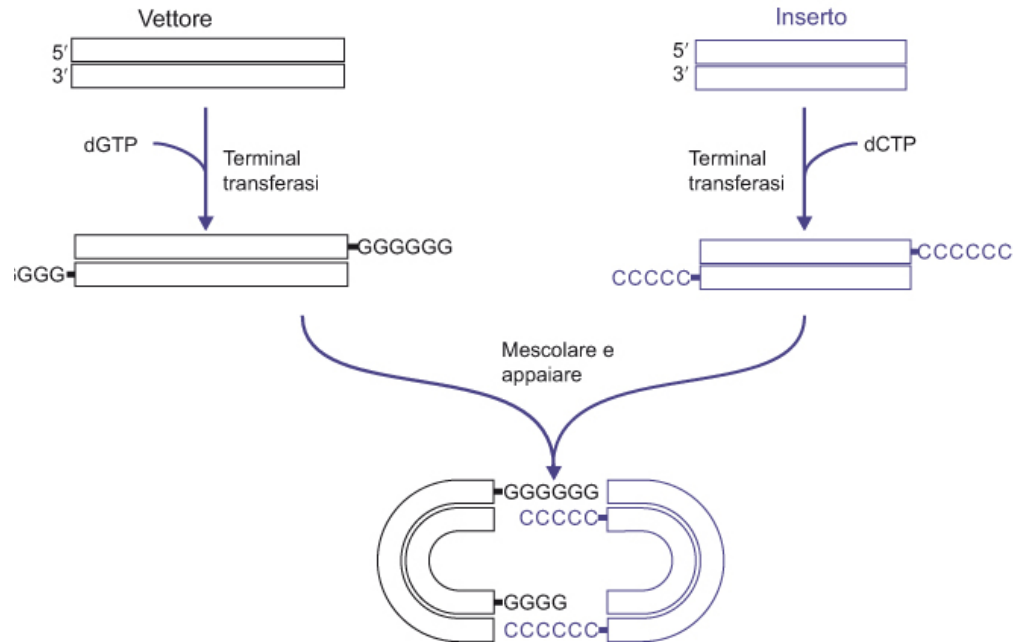


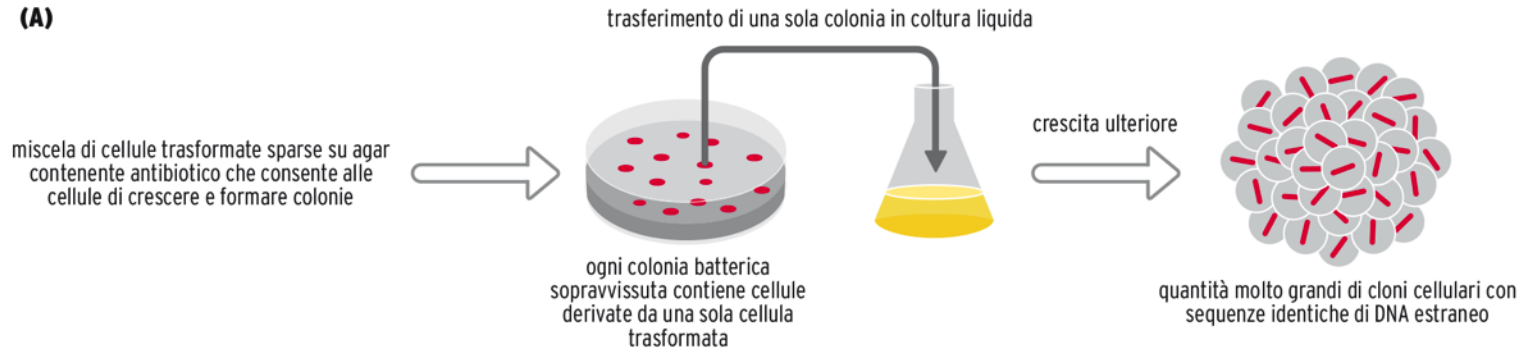
Figura 2.19 Allungamento con la terminal transferasi.



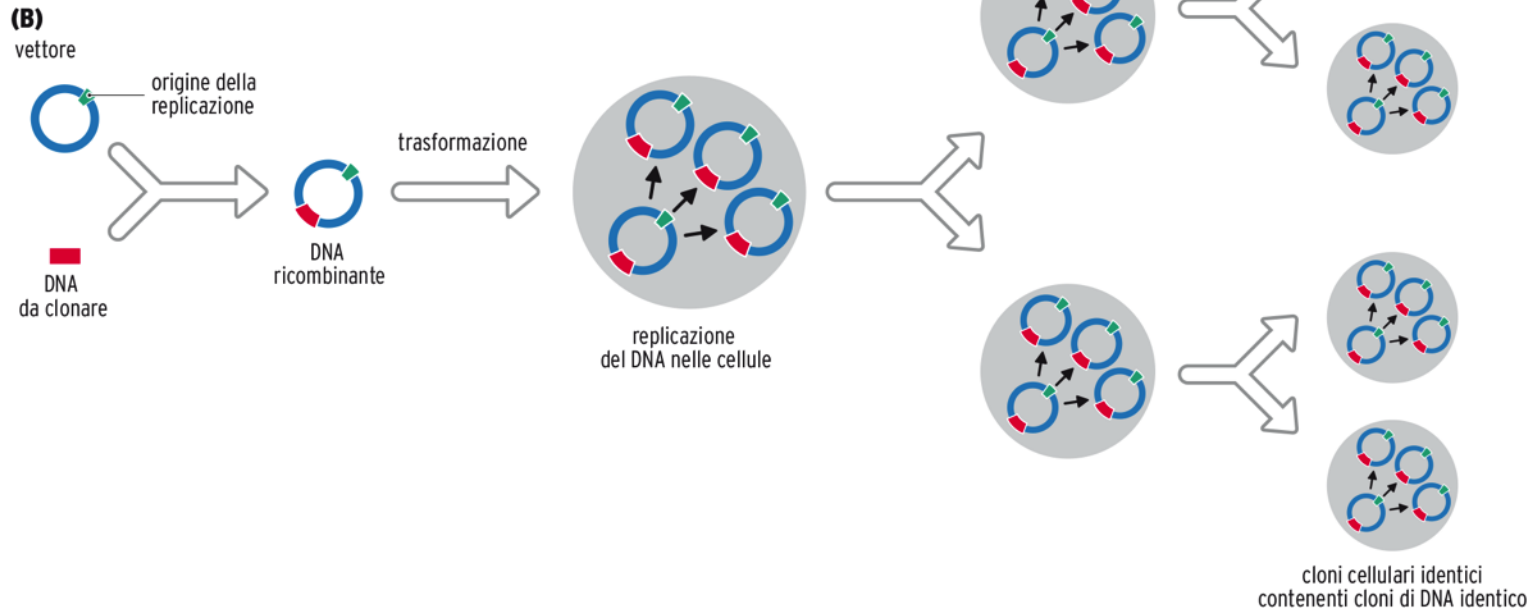
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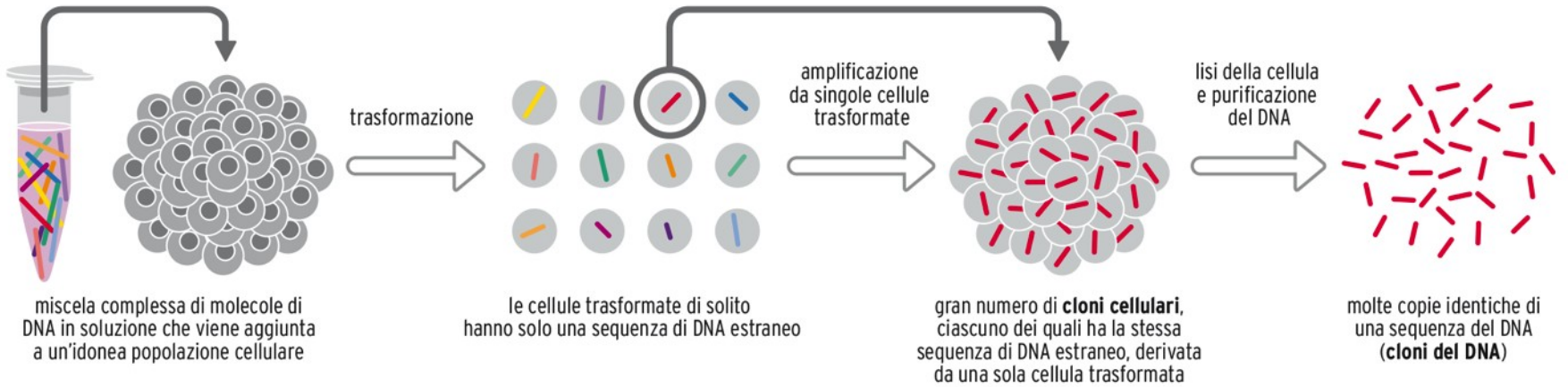


**(A)**

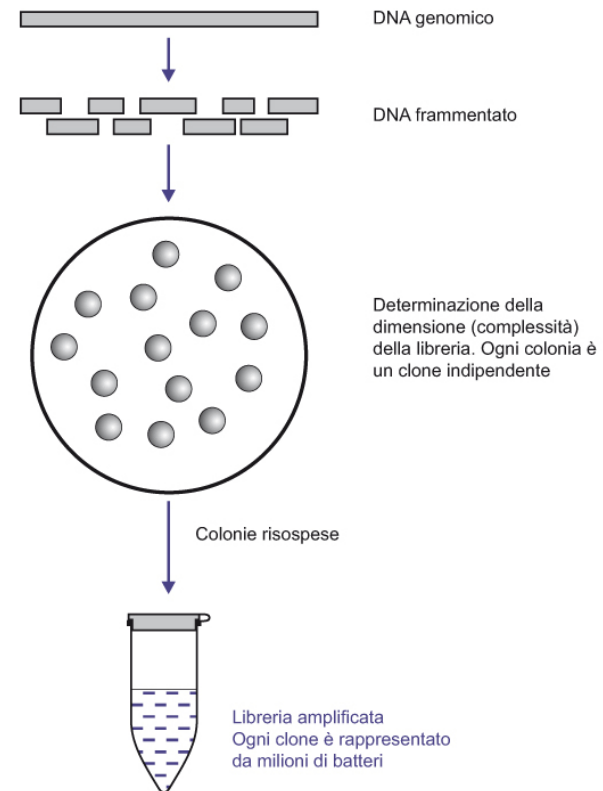
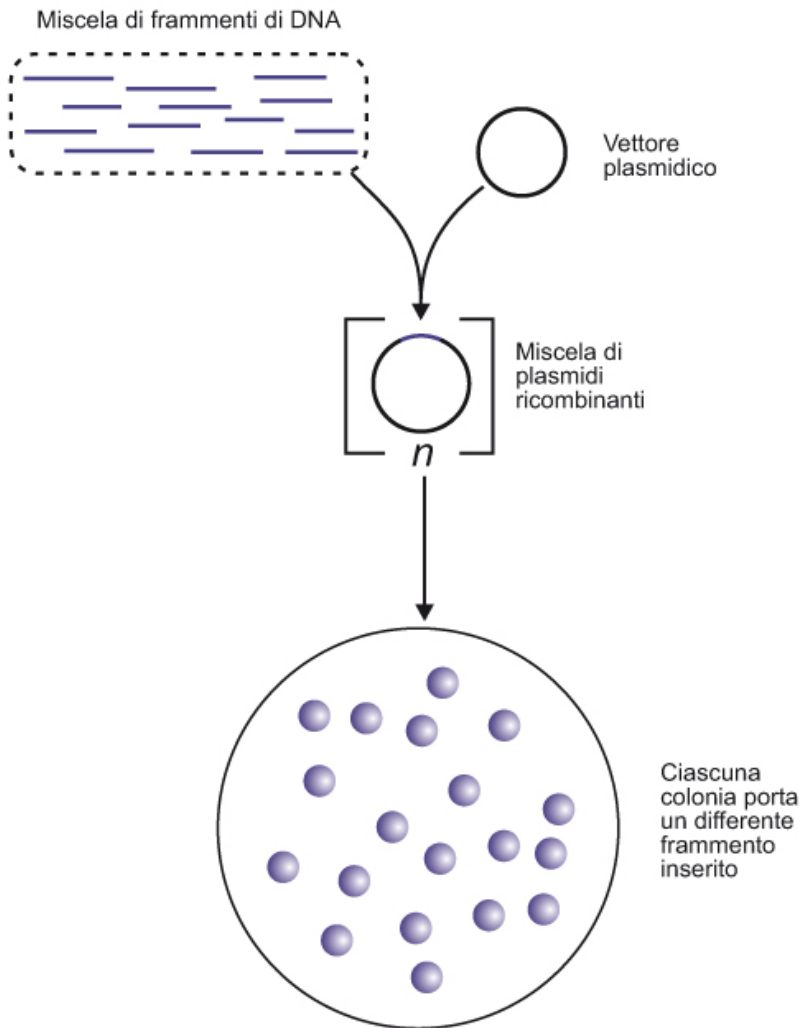


**(B)**

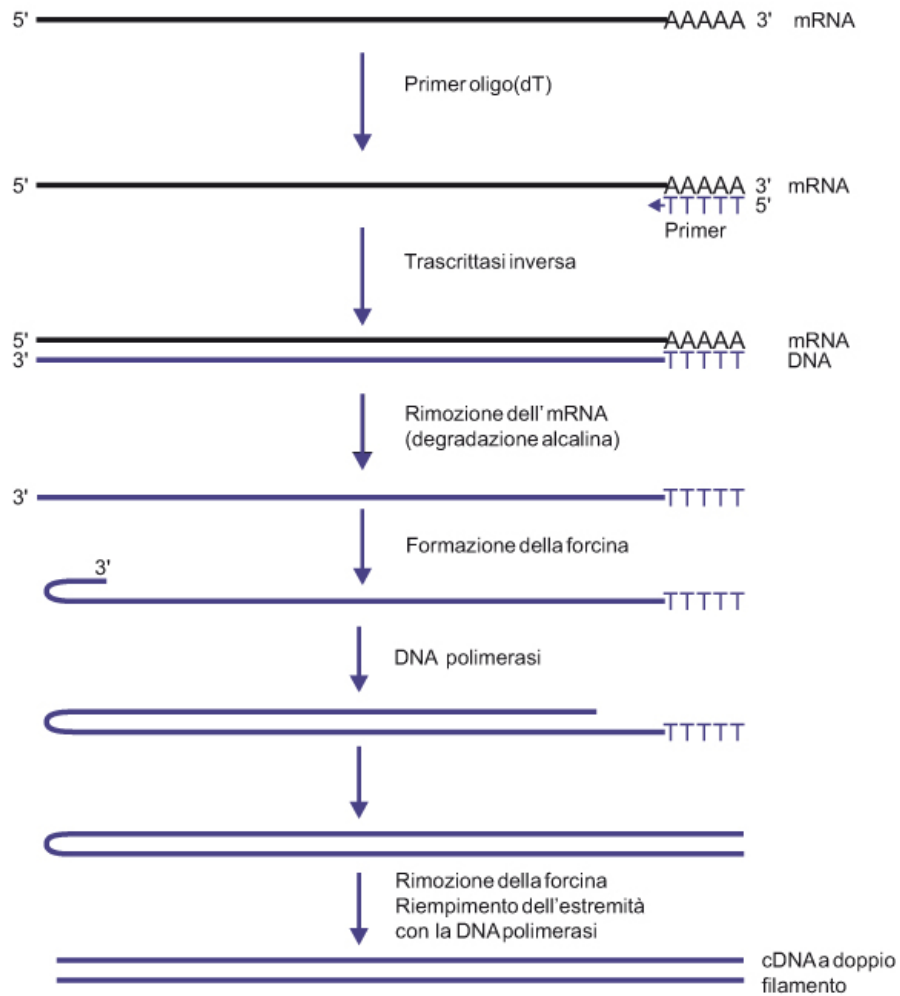




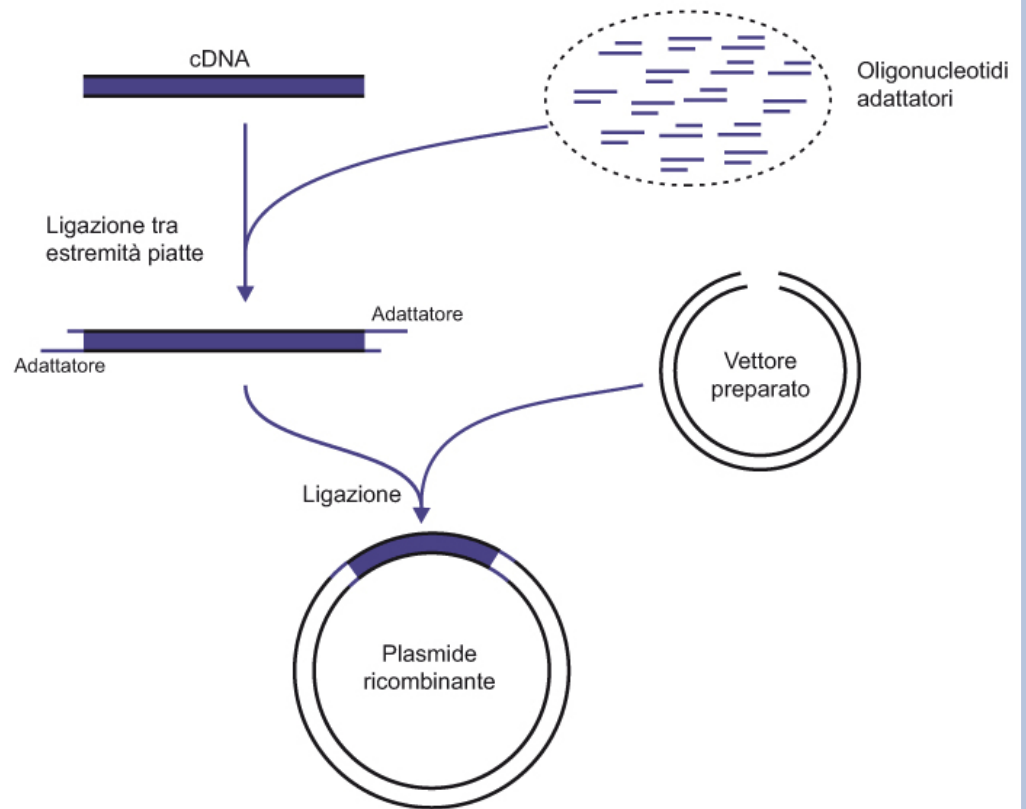
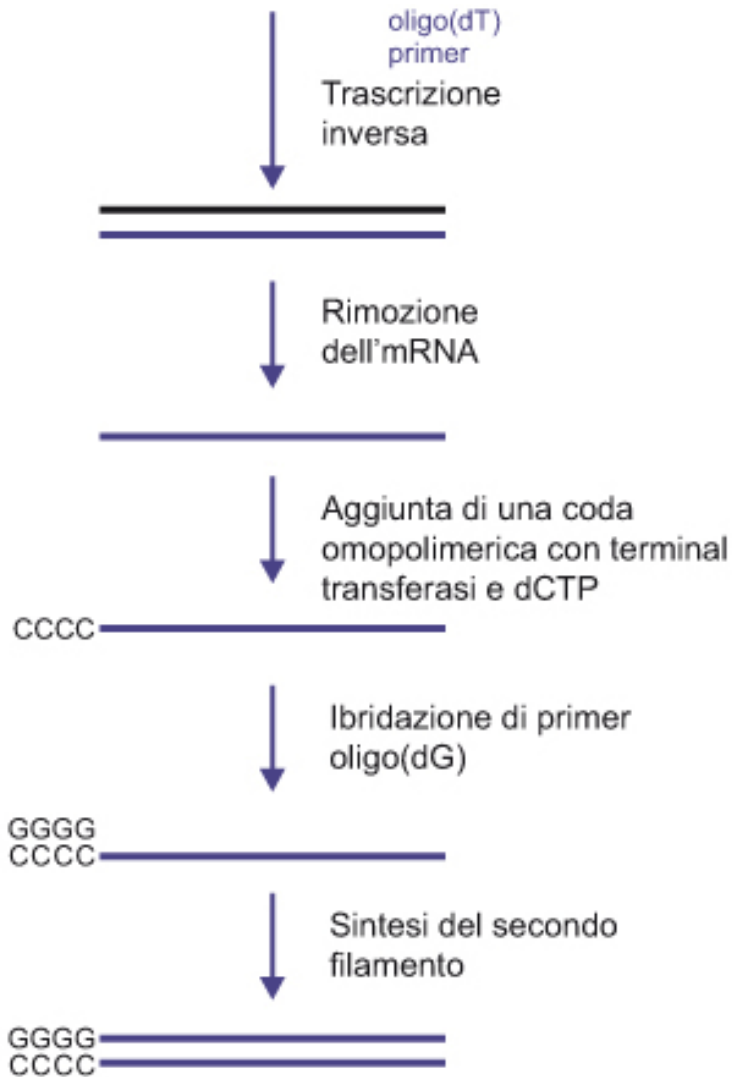
# CLONING GENOMIC DNA



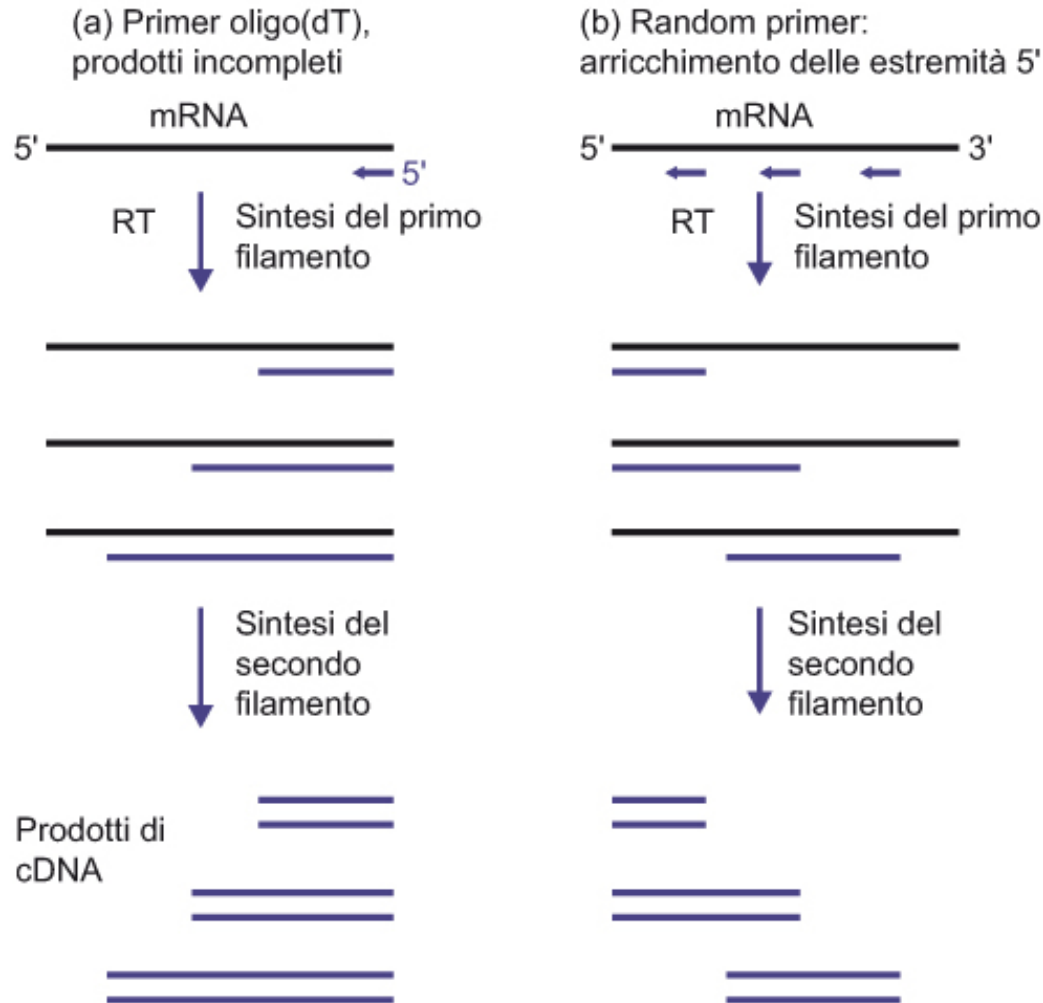
# MANIPULATING AND CLONING RNA: CDNA



# MANIPULATING AND CLONING RNA: CDNA



# MANIPULATING AND CLONING RNA: CDNA





# 11.1 Restriction and Modification Enzymes

- *Gel electrophoresis*: separates DNA molecules based on size (Figure 11.2a)
  - Electrophoresis uses an electrical field to separate charged molecules
  - Gels are usually made of agarose, a polysaccharide
  - Nucleic acids migrate through gel toward the positive electrode due to their negatively charged phosphate groups
  - Gels can be stained with *ethidium bromide*
  - and DNA can be visualized under UV light
- (Figure 11.2b)

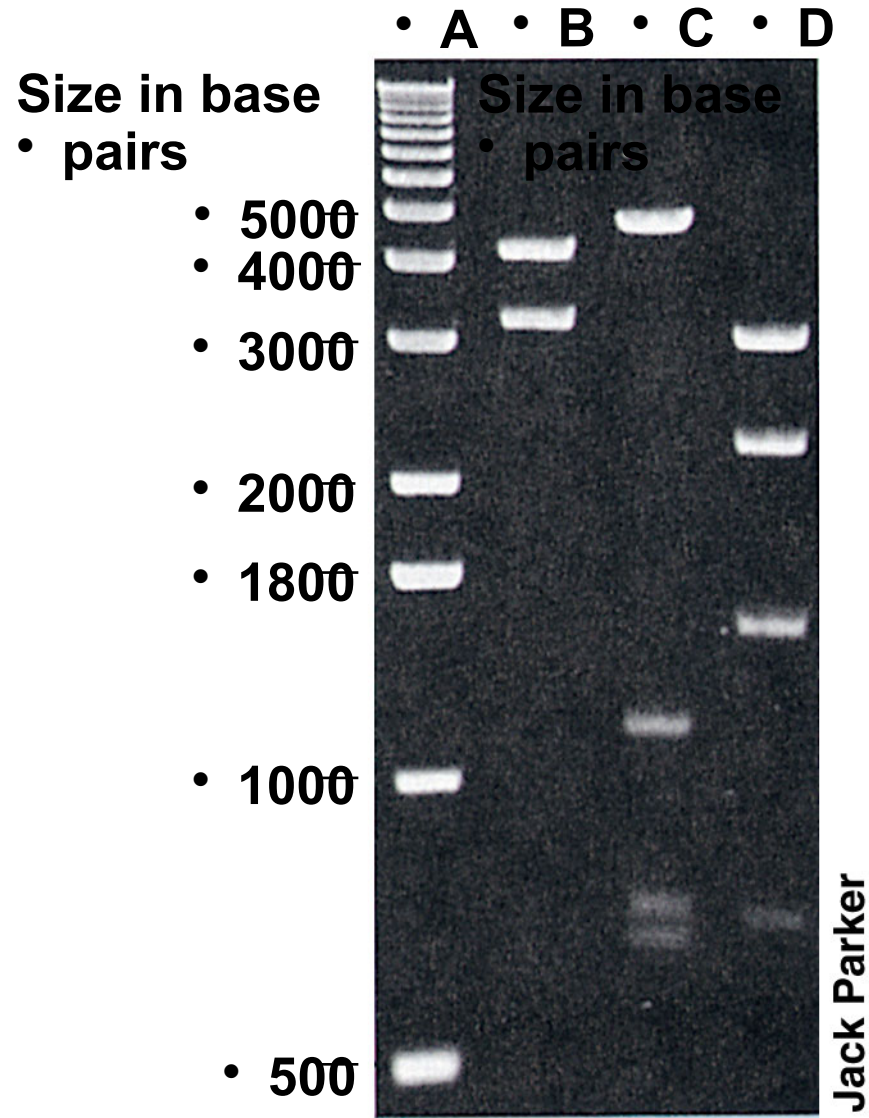
Figure 11.2a



Elizabeth Parker

(a)

Figure 11.2b



(b)

# THE HYBRIDIZATION

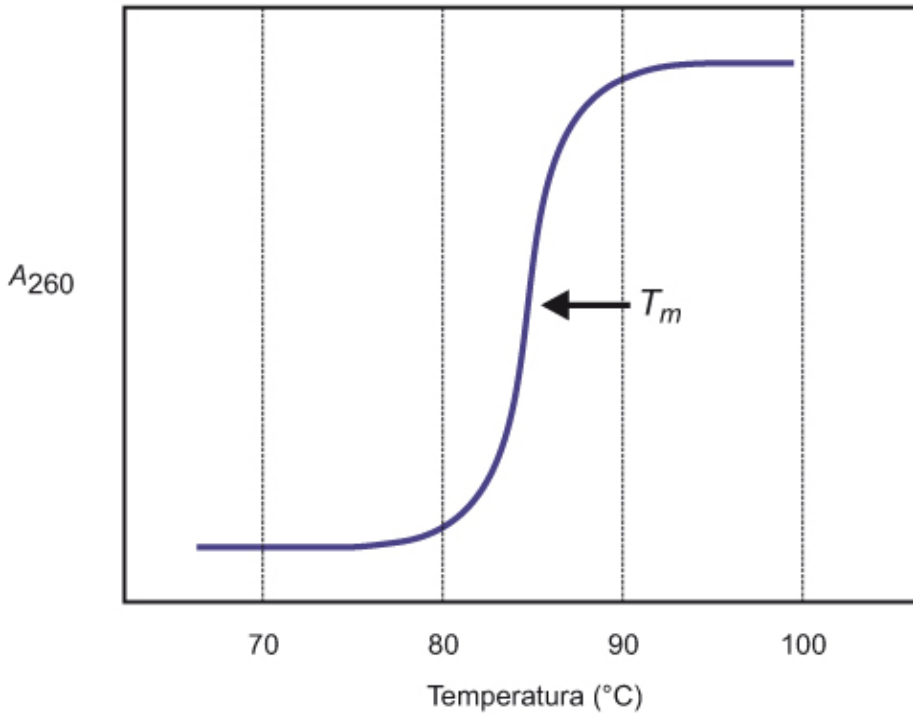
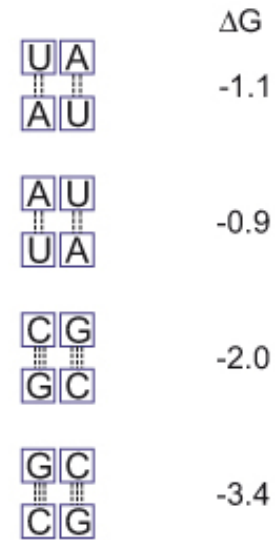


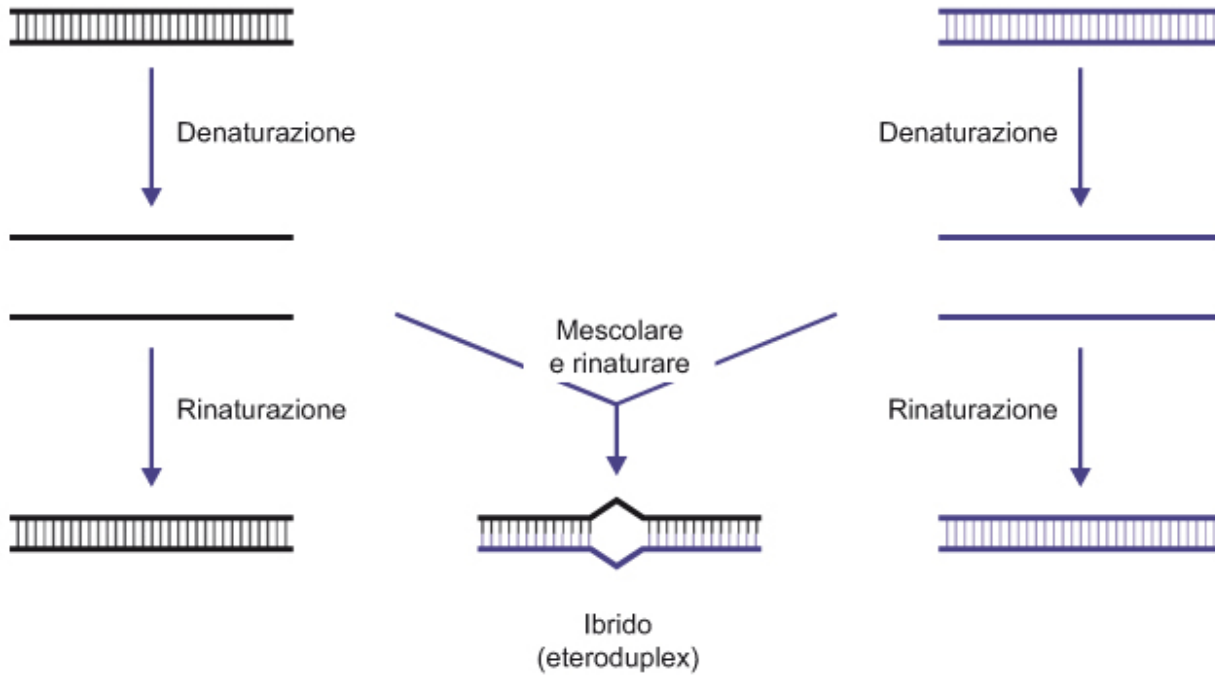
Figura 3.12 Melting (denaturazione) del DNA.



Energia rilasciata degli appaiamenti fra basi.



# THE HYBRIDIZATION



**Figura 3.14** Formazione di un DNA ibrido tra molecole di DNA simili, ma non uguali.



# THE HYBRIDIZATION

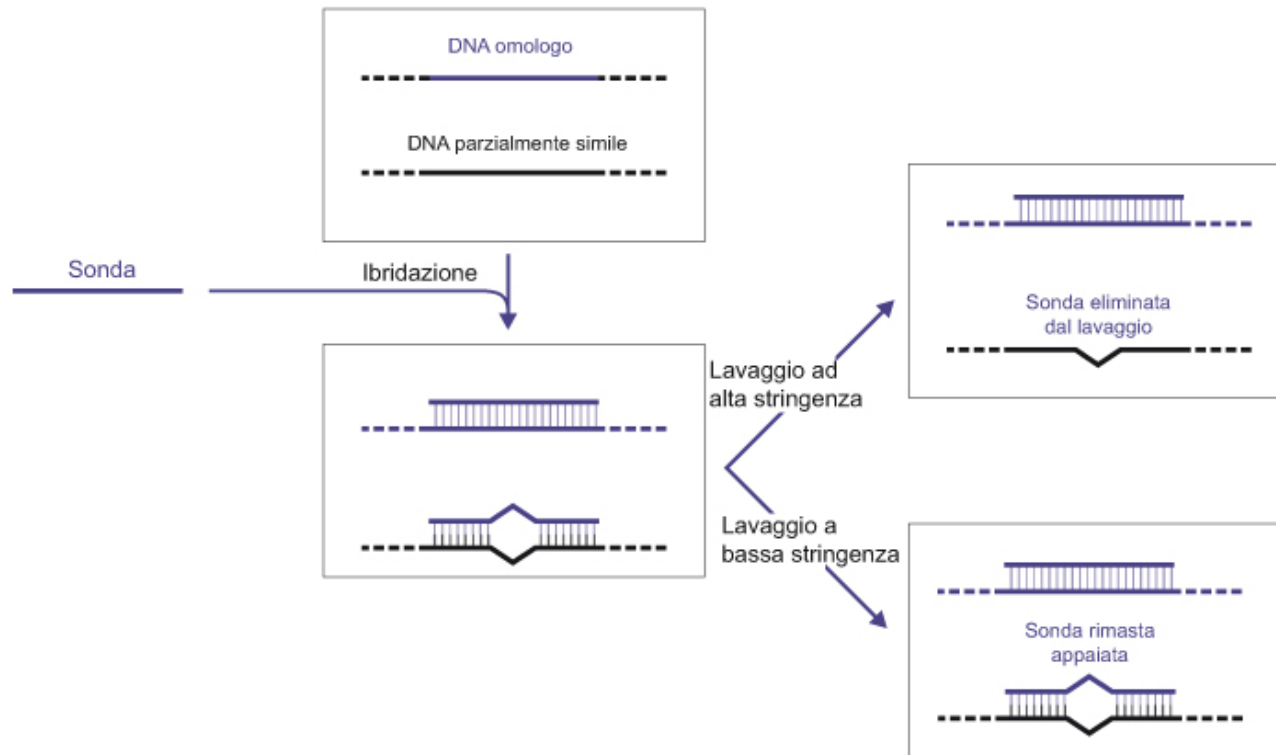


Figura 3.15 Lavaggi ad alta e bassa stringenza.



# THE HYBRIDIZATION

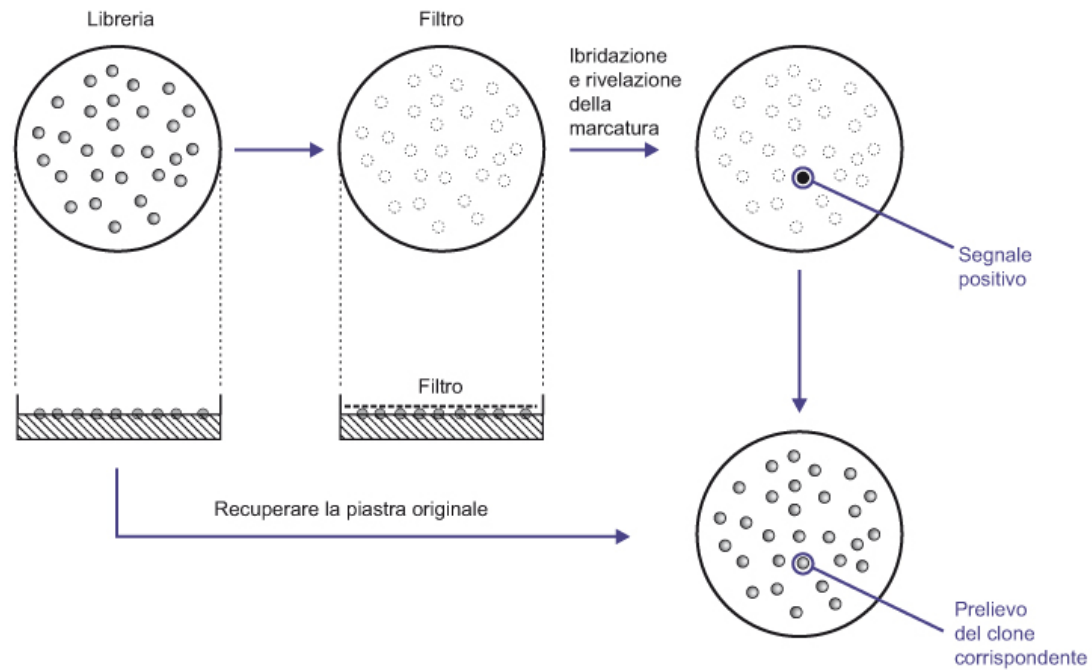


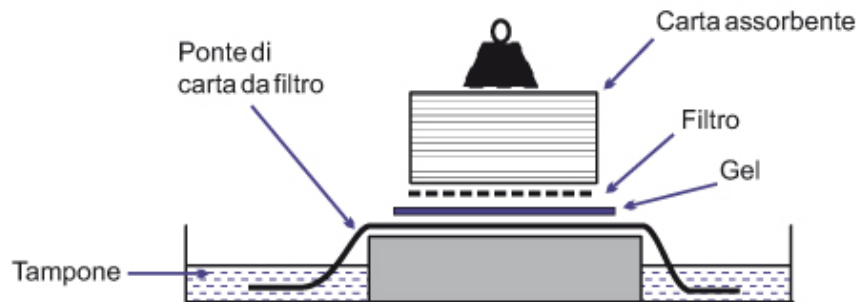
Figura 3.16 Screening di una libreria genica mediante ibridazione con una sonda genica.



# THE HYBRIDIZATION



Gel



Trasferimento  
su filtro



Ibridazione del filtro  
con una sonda  
marcata

Banda positiva

