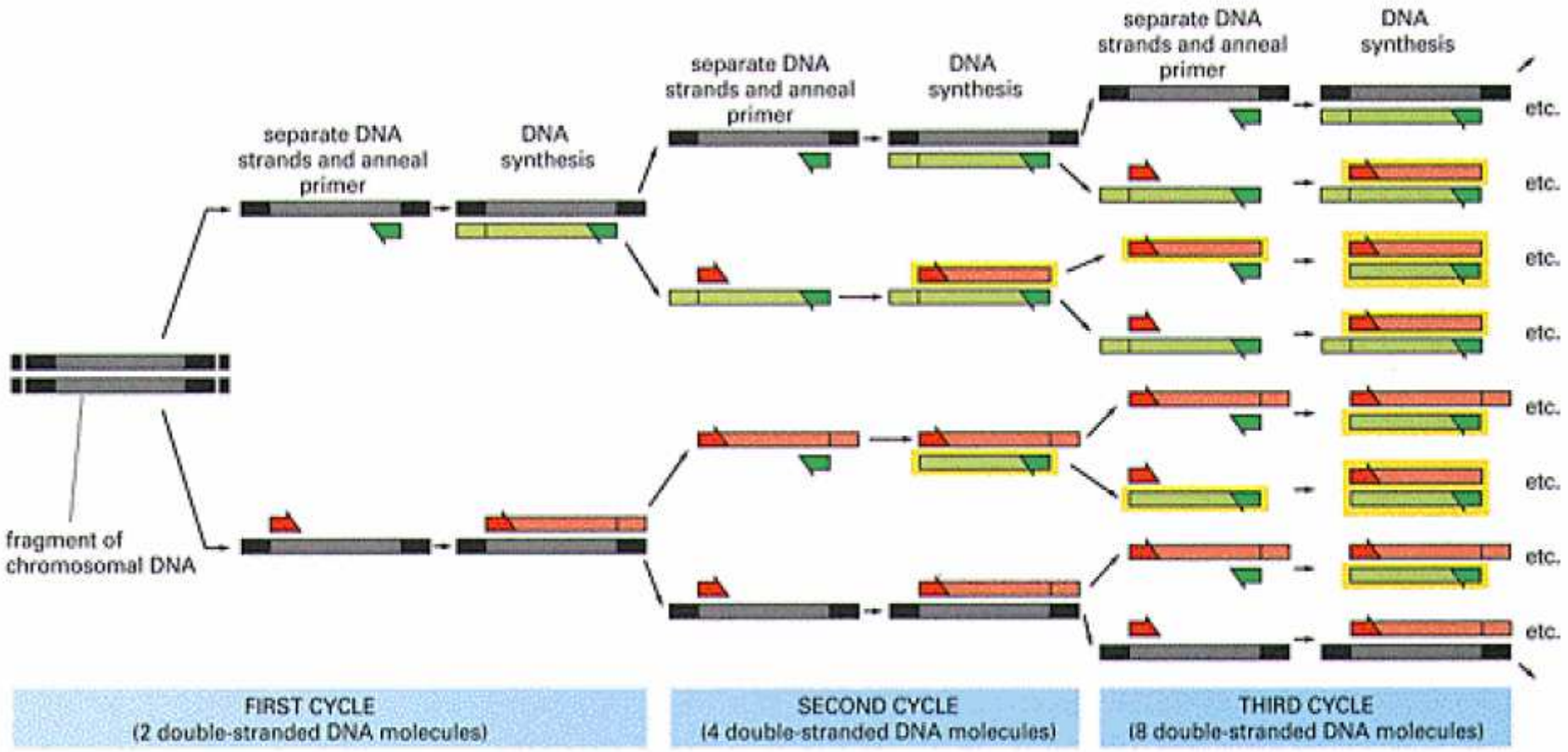
PCR

Il funzionamento della PCR

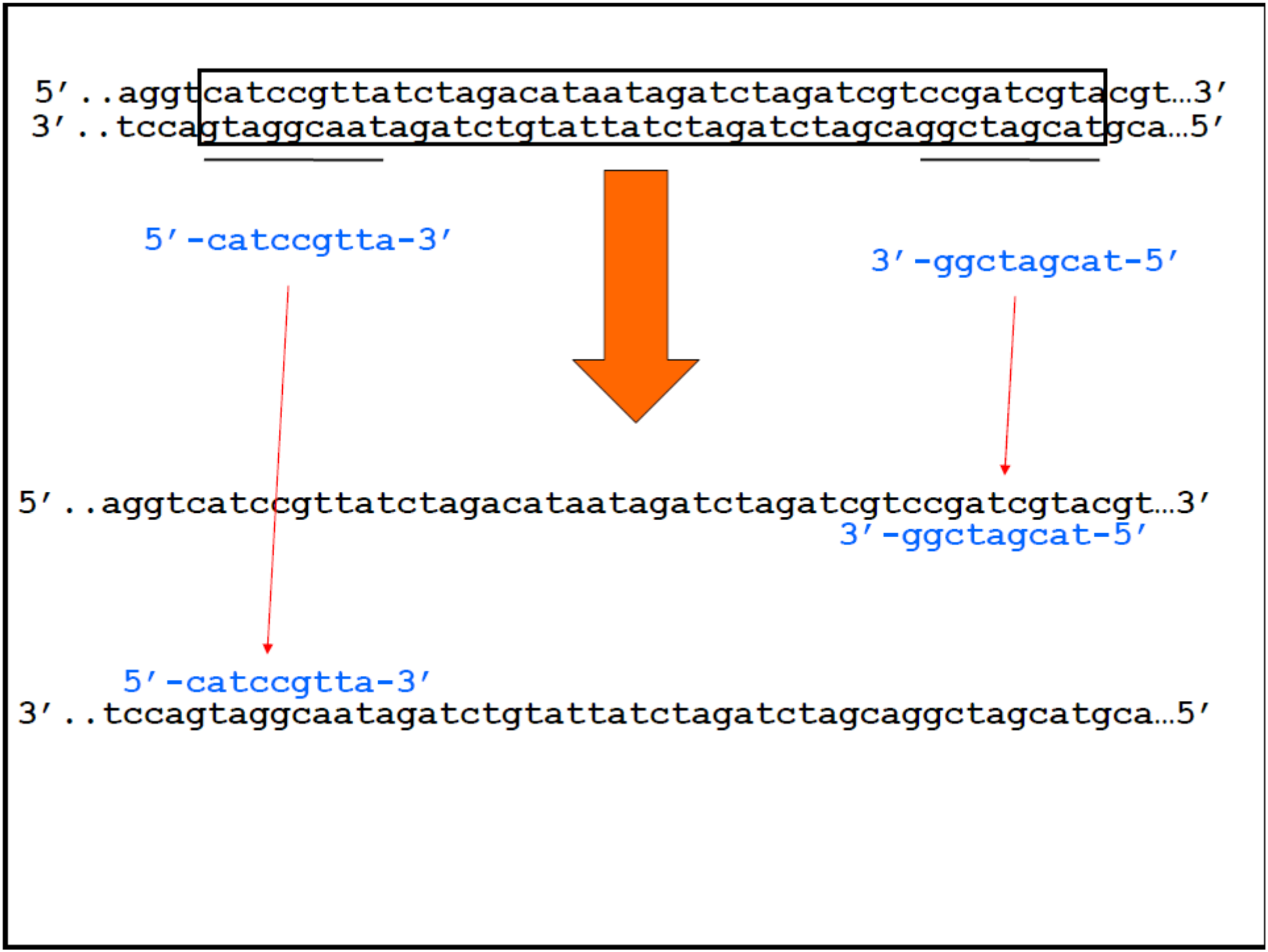


La reazione a catena della Polimerasi:
PCR: polymerase chain reaction

Si basa sulla esecuzione ciclica ripetuta più volte delle fasi di:
-denaturazione (denaturation)
-ibridazione (annealing)
-sintesi (extension) del DNA complementare allo stampo

utilizzando 2 primers complementari alle sequenze esterne (a sinistra ed a destra) del pezzo di DNA che si desidera amplificare.

Poiché la denaturazione richiede alte temperature, si utilizza un enzima termoresistente, la Taq DNA polimerasi (dal micro-organismo termofilo Termophylus aquaticus).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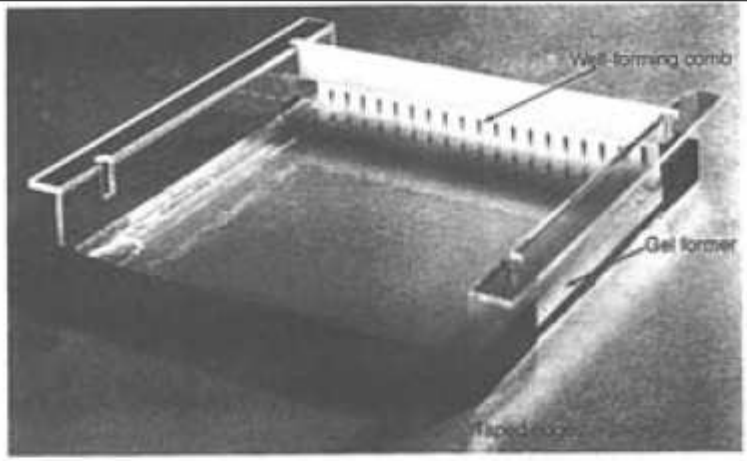
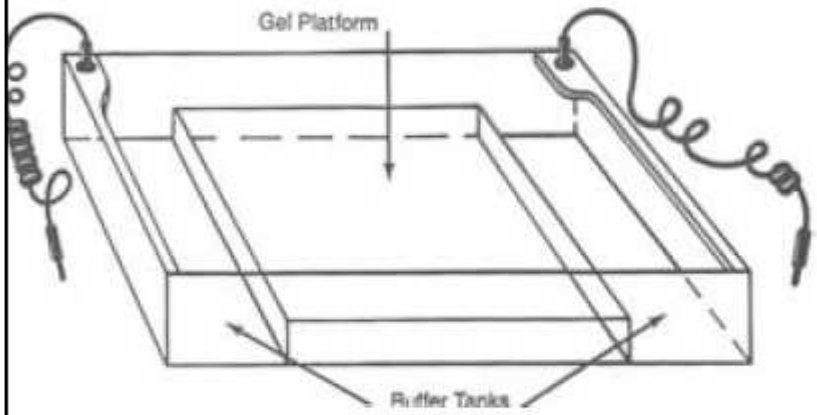
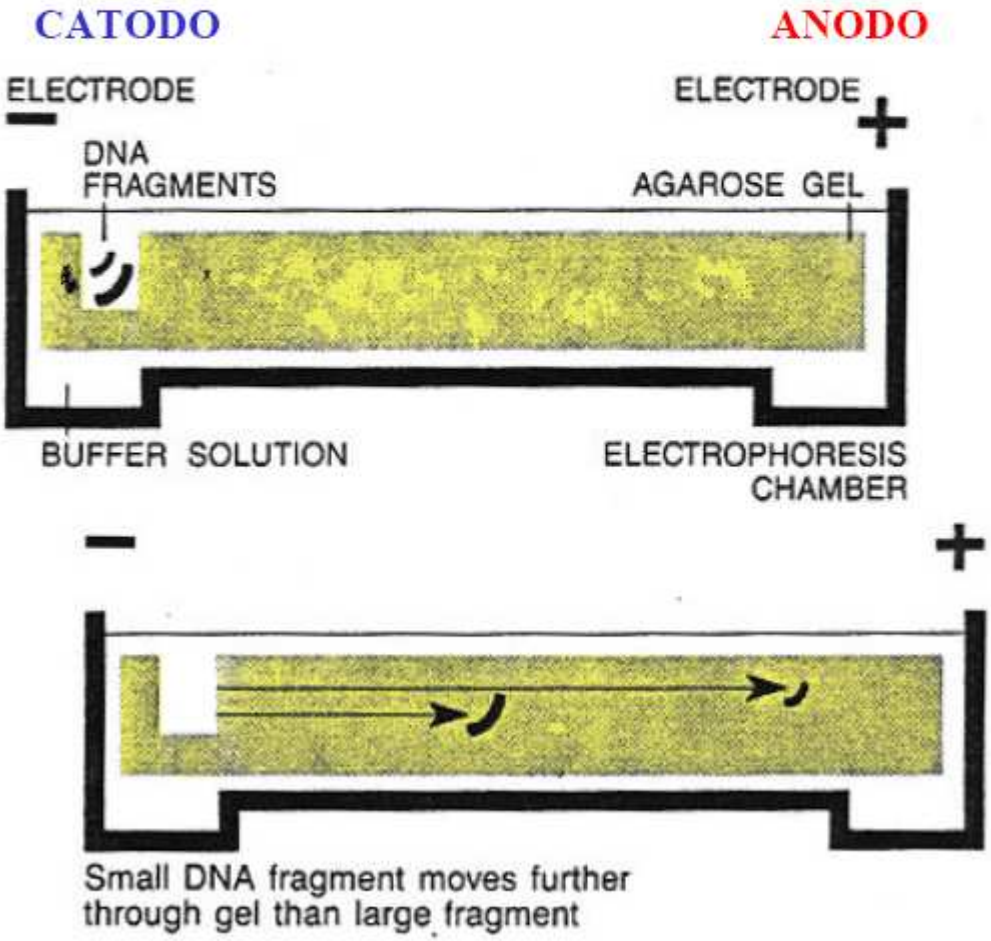
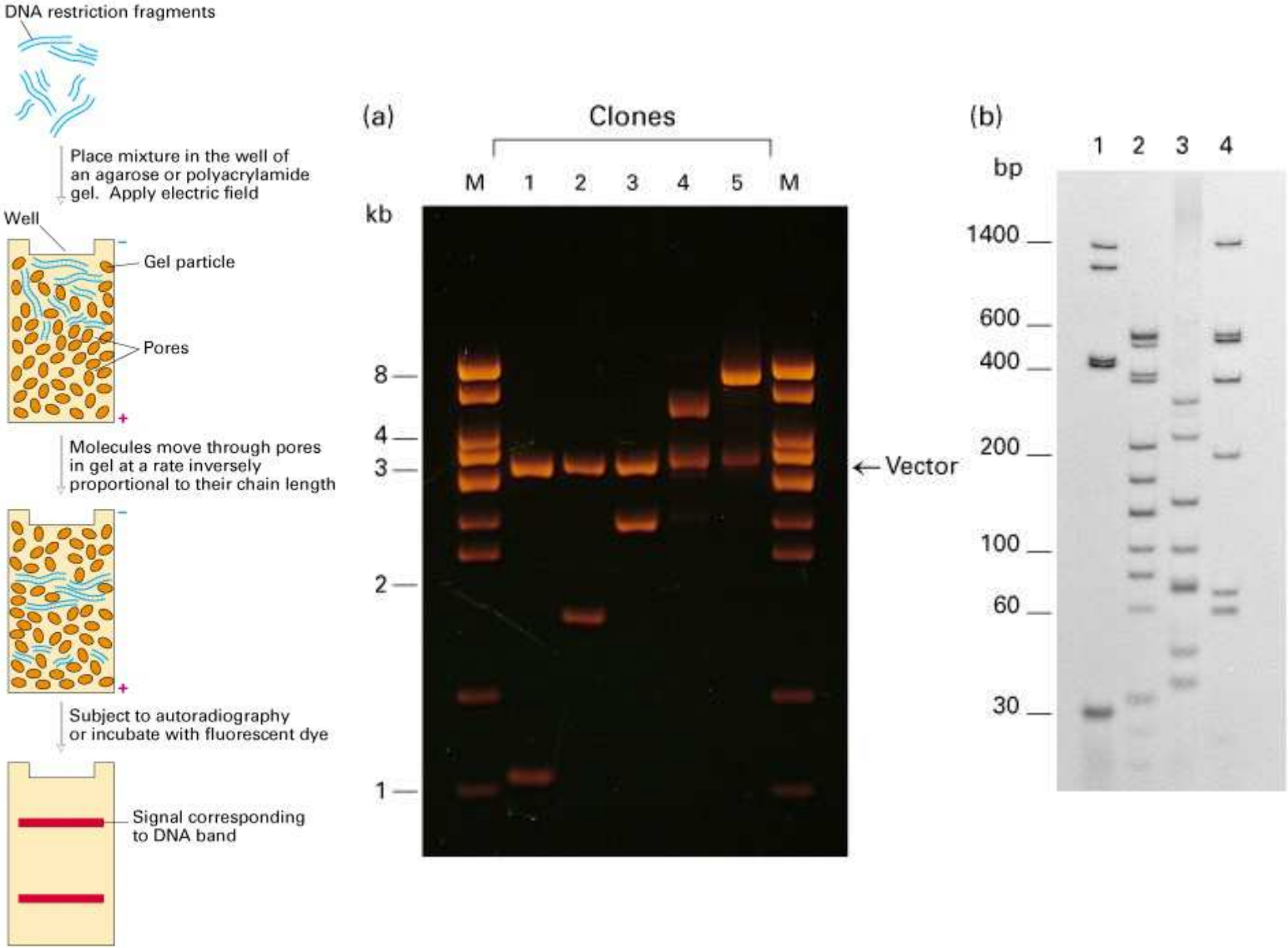
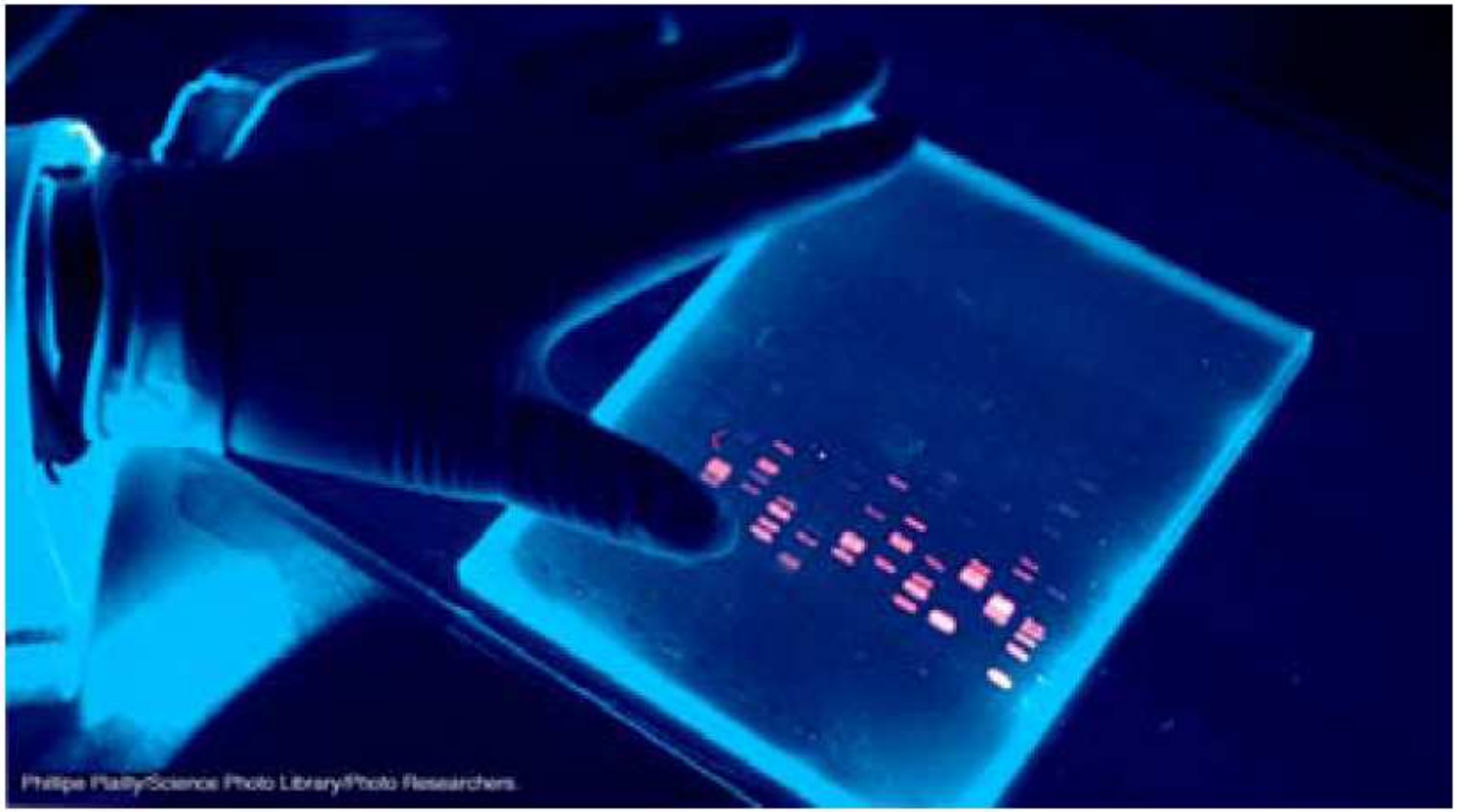

Well-forming comb
Gel former

A

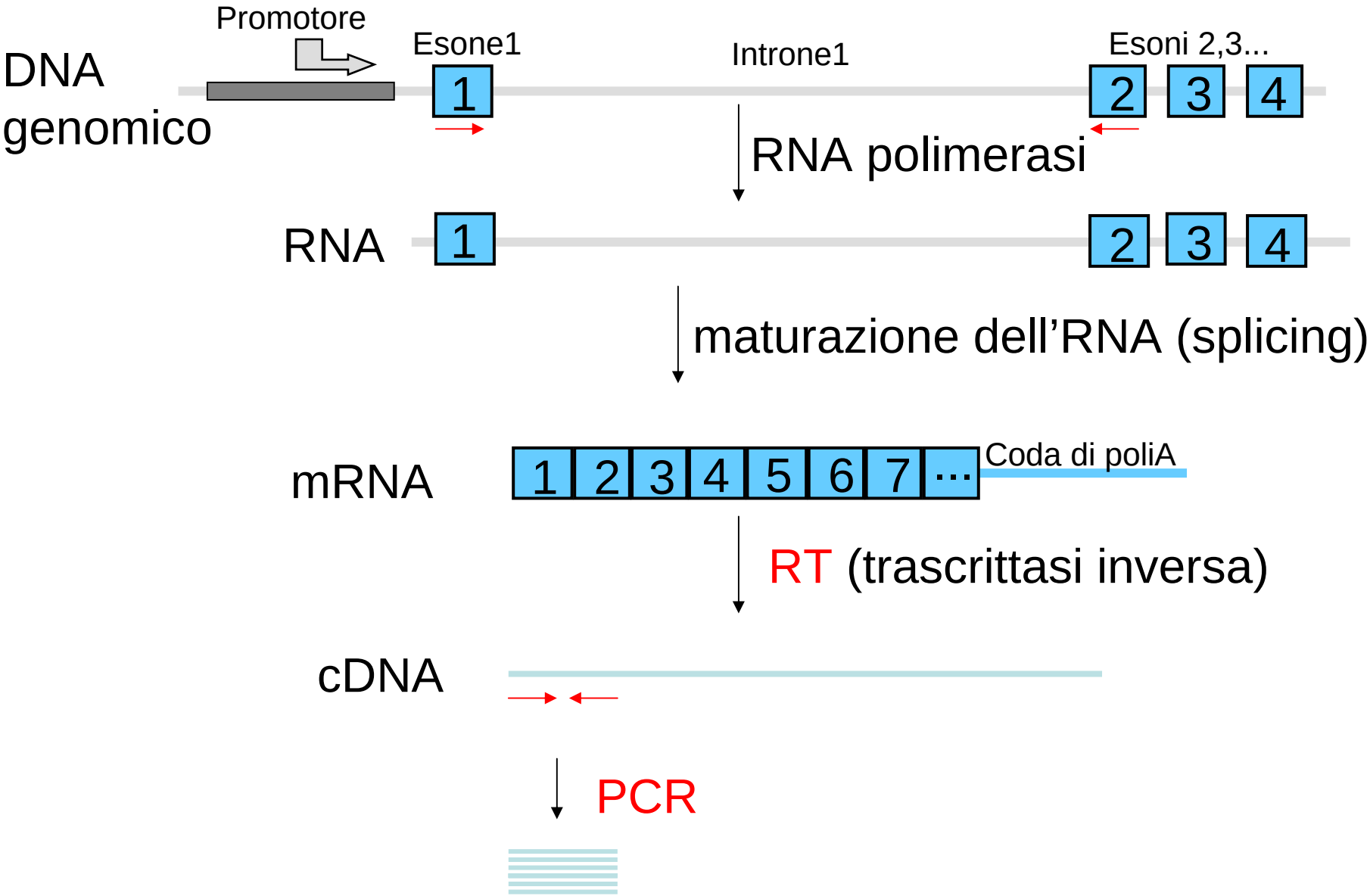

Gel Platform
Buffer Tanks


CATODO
ANODO
ELECTRODE
ELECTRODE
DNA FRAGMENTS
AGAROSE GEL
BUFFER SOLUTION
ELECTROPHORESIS CHAMBER
Small DNA fragment moves further through gel than large fragment


DNA restriction fragments
Place mixture in the well of an agarose or polyacrylamide gel. Apply electric field
Well
Gel particle
Pores
Molecules move through pores in gel at a rate inversely proportional to their chain length
Subject to autoradiography or incubate with fluorescent dye
Signal corresponding to DNA band
(a)
Clones
M 1 2 3 4 5 M
kb
8
4
3
2
1
← Vector
(b)
1 2 3 4
bp
1400
600
400
200
100
60
30

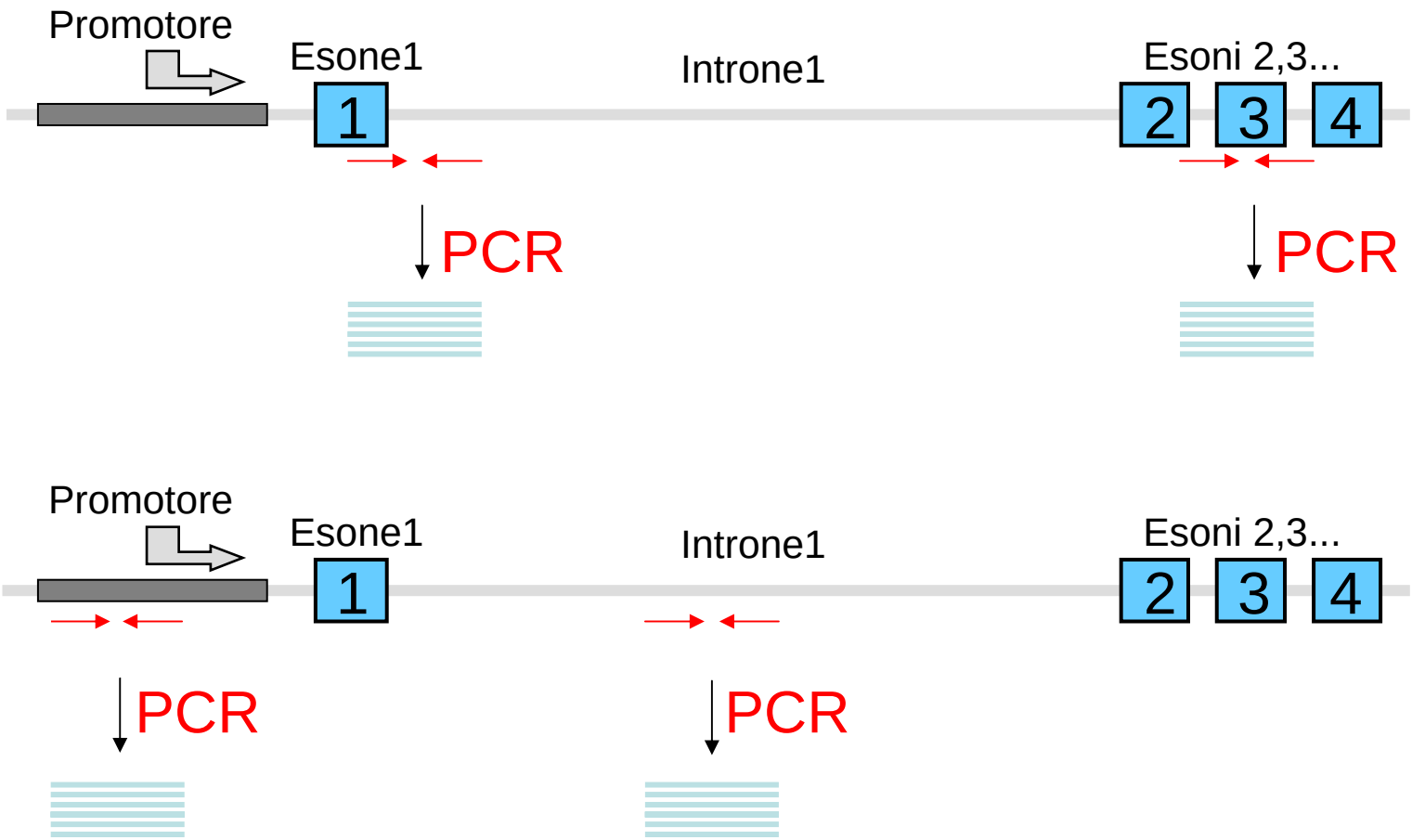

Philippe Plailly/Science Photo Library/Photo Researchers

RT-PCR


Promotore
Esone1
Introne1
Esoni 2,3...
DNA genomico
1
2
3
4
RNA polimerasi
RNA
maturazione dell'RNA (splicing)
mRNA
1 2 3 4 5 6 7 ...
Coda di poliA
RT (trascrittasi inversa)
cDNA
PCR

PCR

DNA genomico



• la PCR può essere effettuata su DNA genomico,
- per identificare un determinato individuo (medicina legale)
- per identificare la presenza di un determinato microorganismo (ad es. un microorganismo patogeno)
- per individuare eventuali mutazioni
- per clonare regioni genomiche

• la RT-PCR consente di amplificare solo i geni che vengono trascritti: la PCR utilizza come substrato il cDNA che è stato ottenuto mediante trascrizione inversa (RT) di un mRNA

-> la RT-PCR ci dà informazioni sui geni che vengono trascritti da un determinato tipo cellulare, in un determinato momento

- può essere utilizzata per clonare regioni codificanti