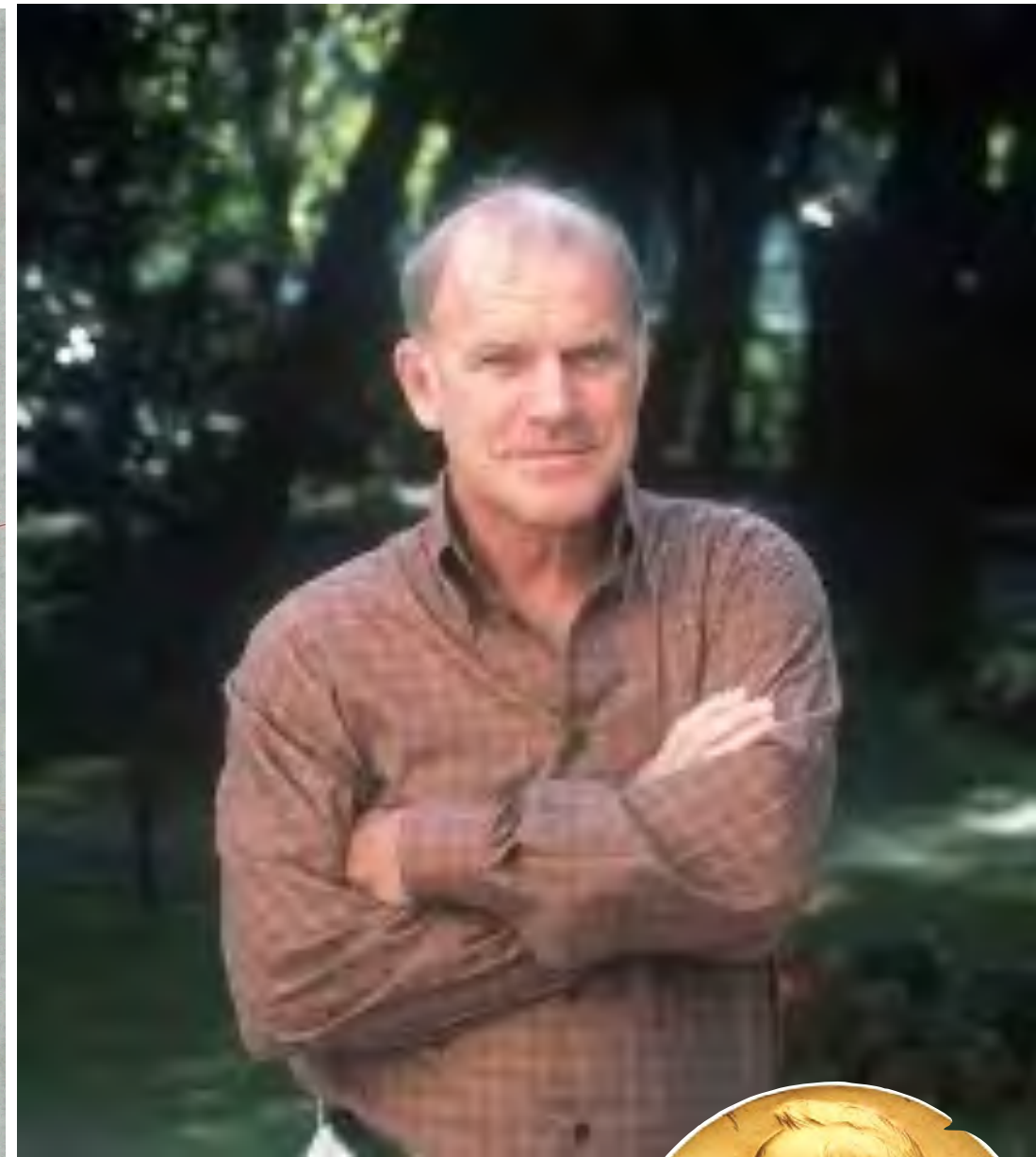
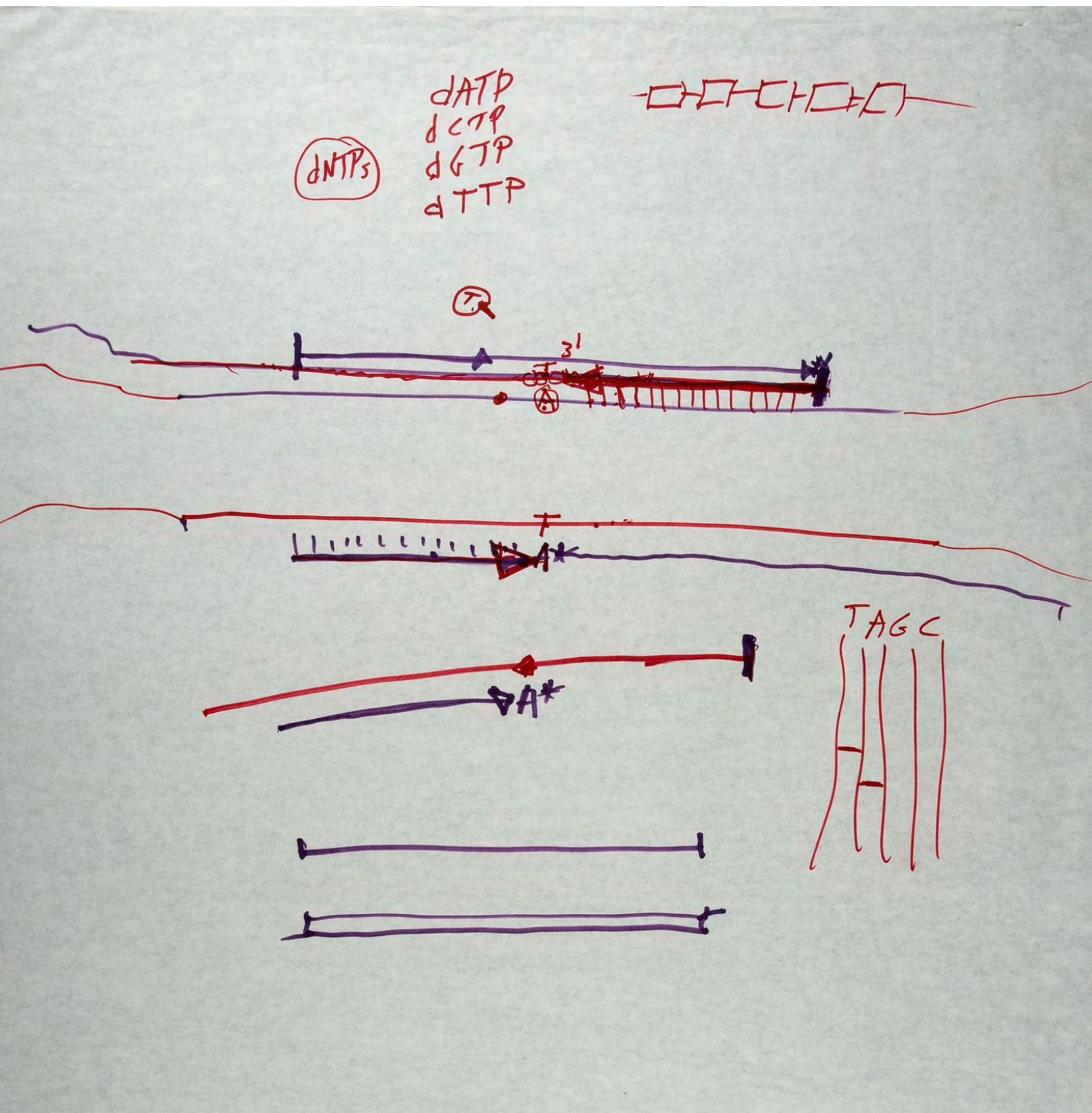


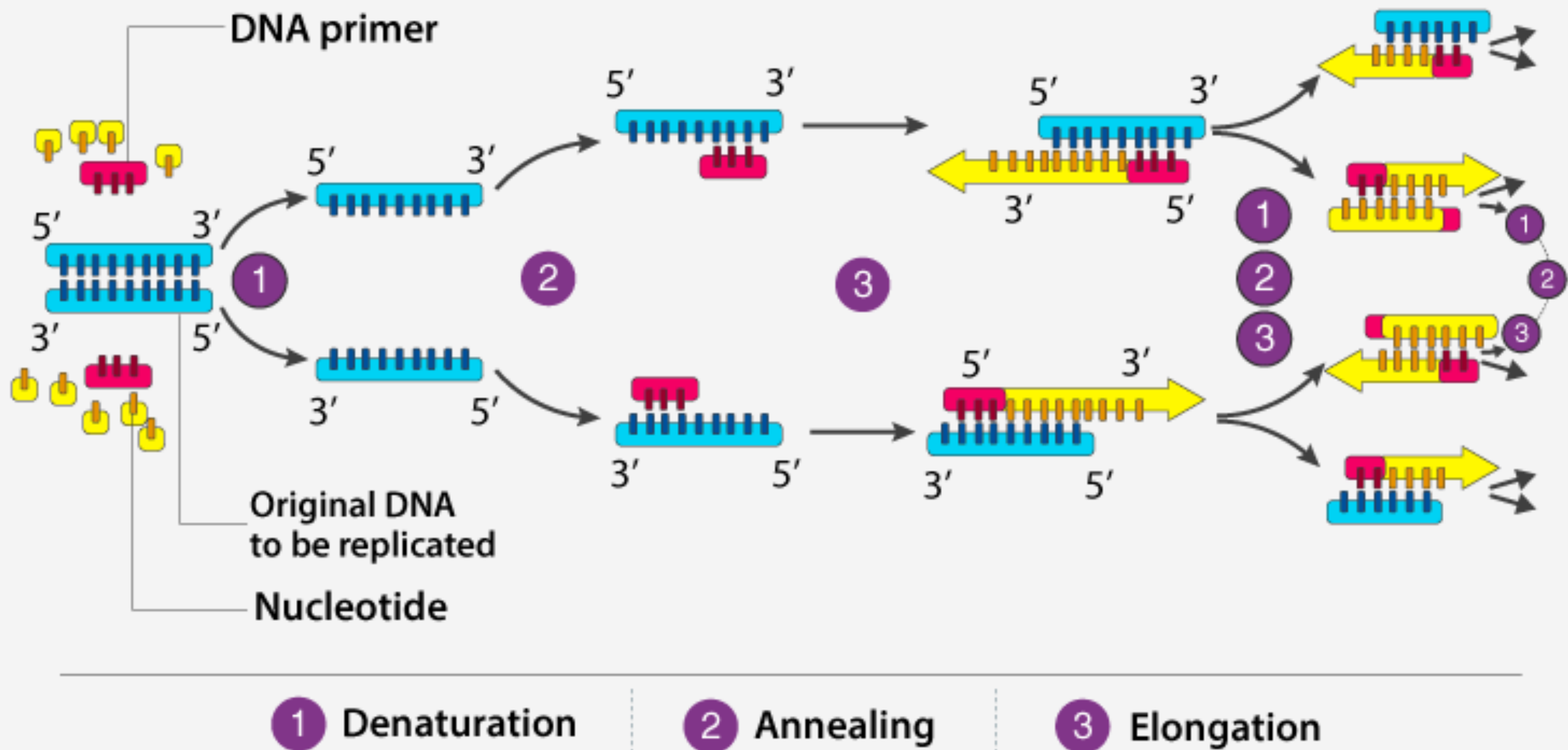
Введение в молекулярную биологию

Лекция 4. Методы анализа ДНК, секвенирование

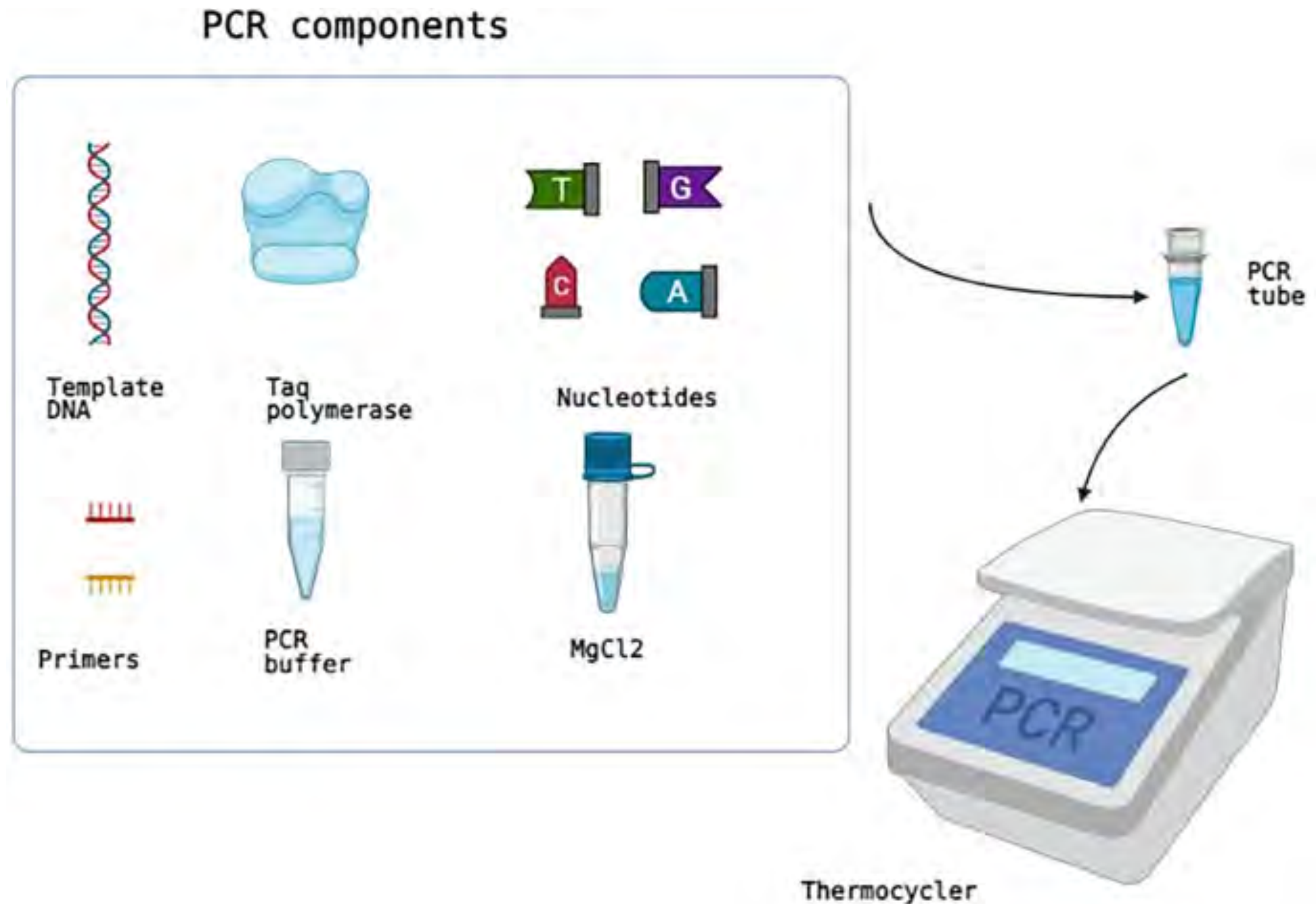
ПЦР: Введение



Классическая ПЦР: Принцип метода

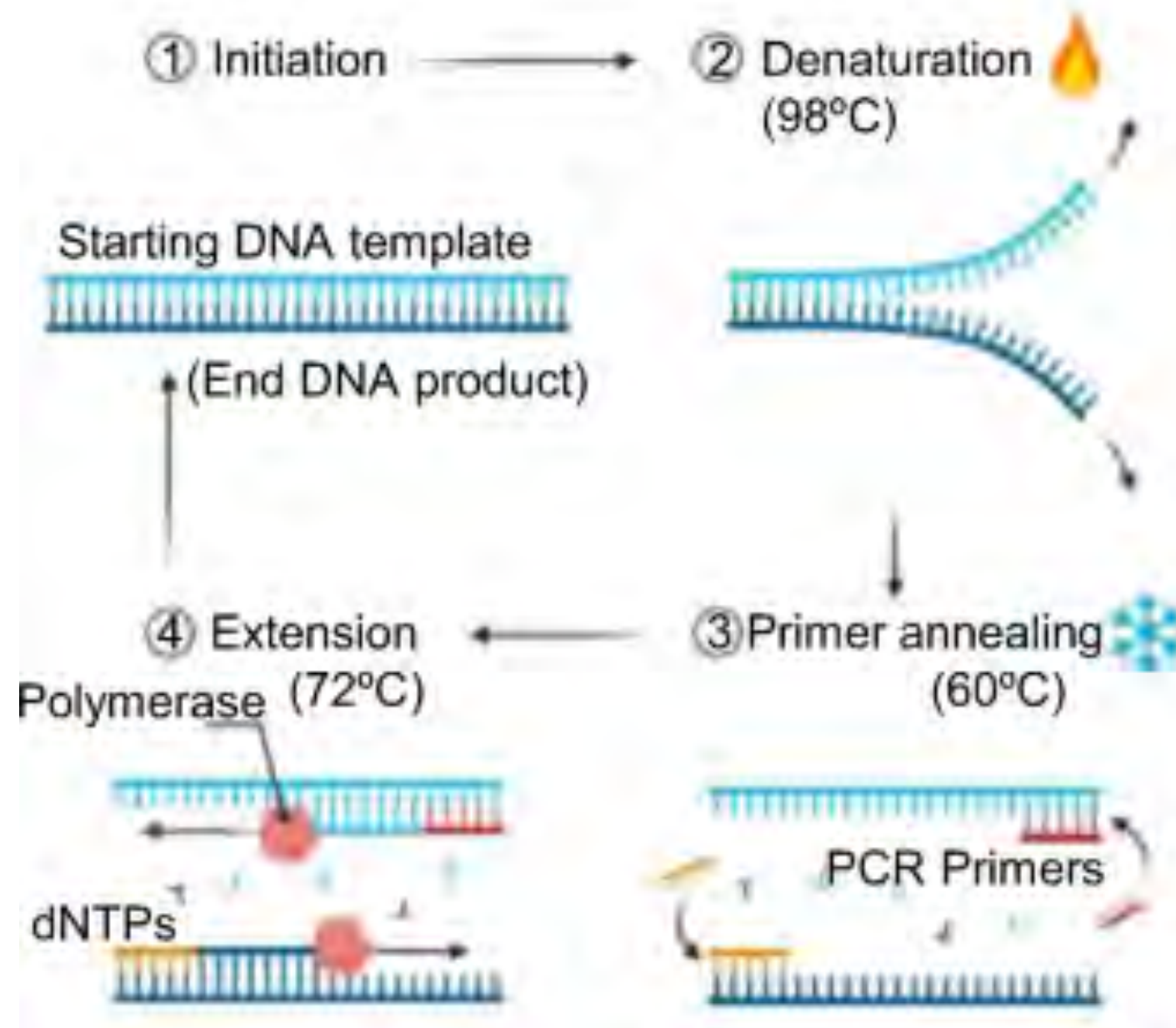


Классическая ПЦР: Компоненты реакции



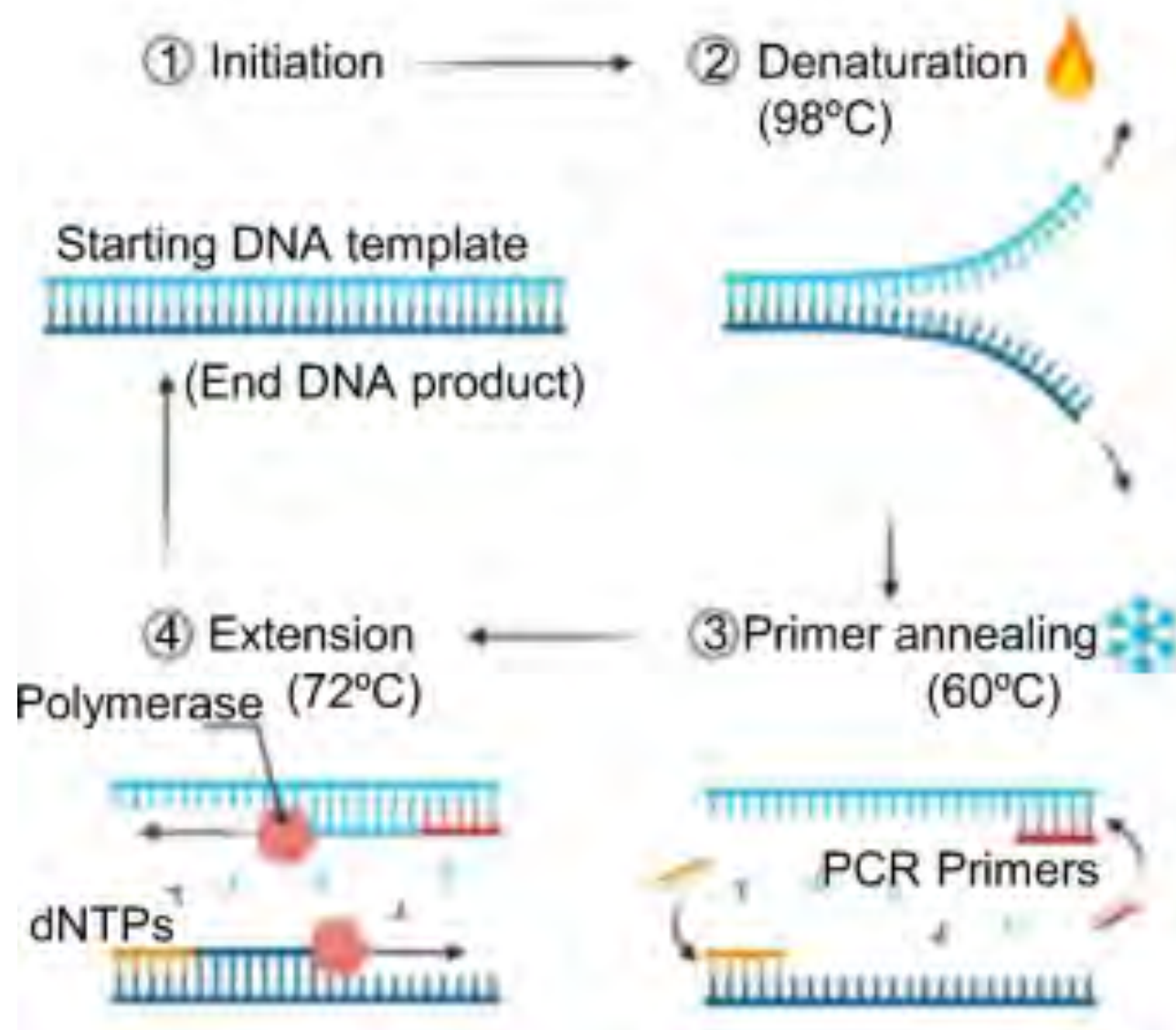
Классическая ПЦР: Циклы амплификации

a Thermocycler-based PCR (Polymerase Chain Reaction)

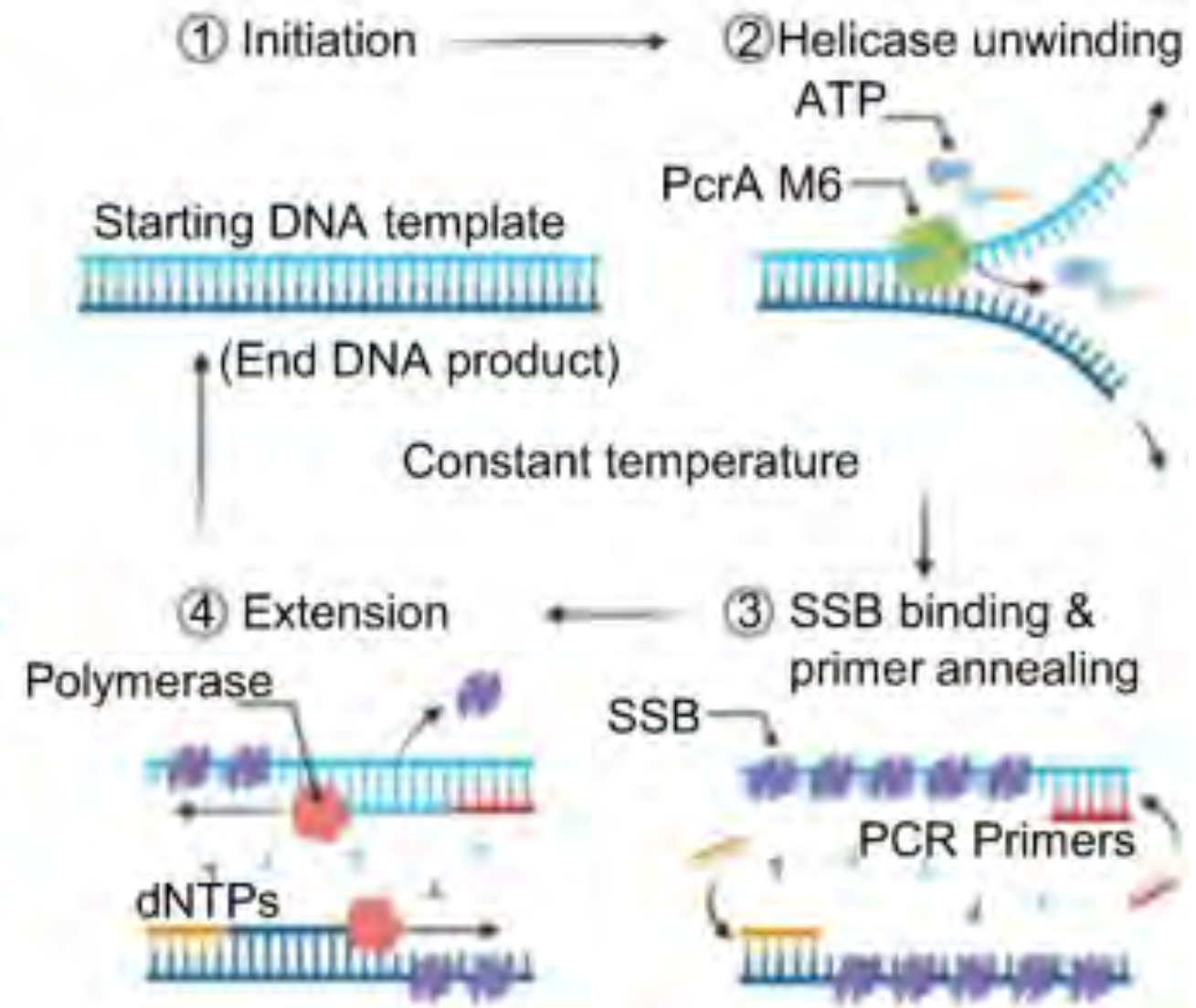


Классическая ПЦР: Циклы амплификации

a Thermocycler-based PCR (Polymerase Chain Reaction)



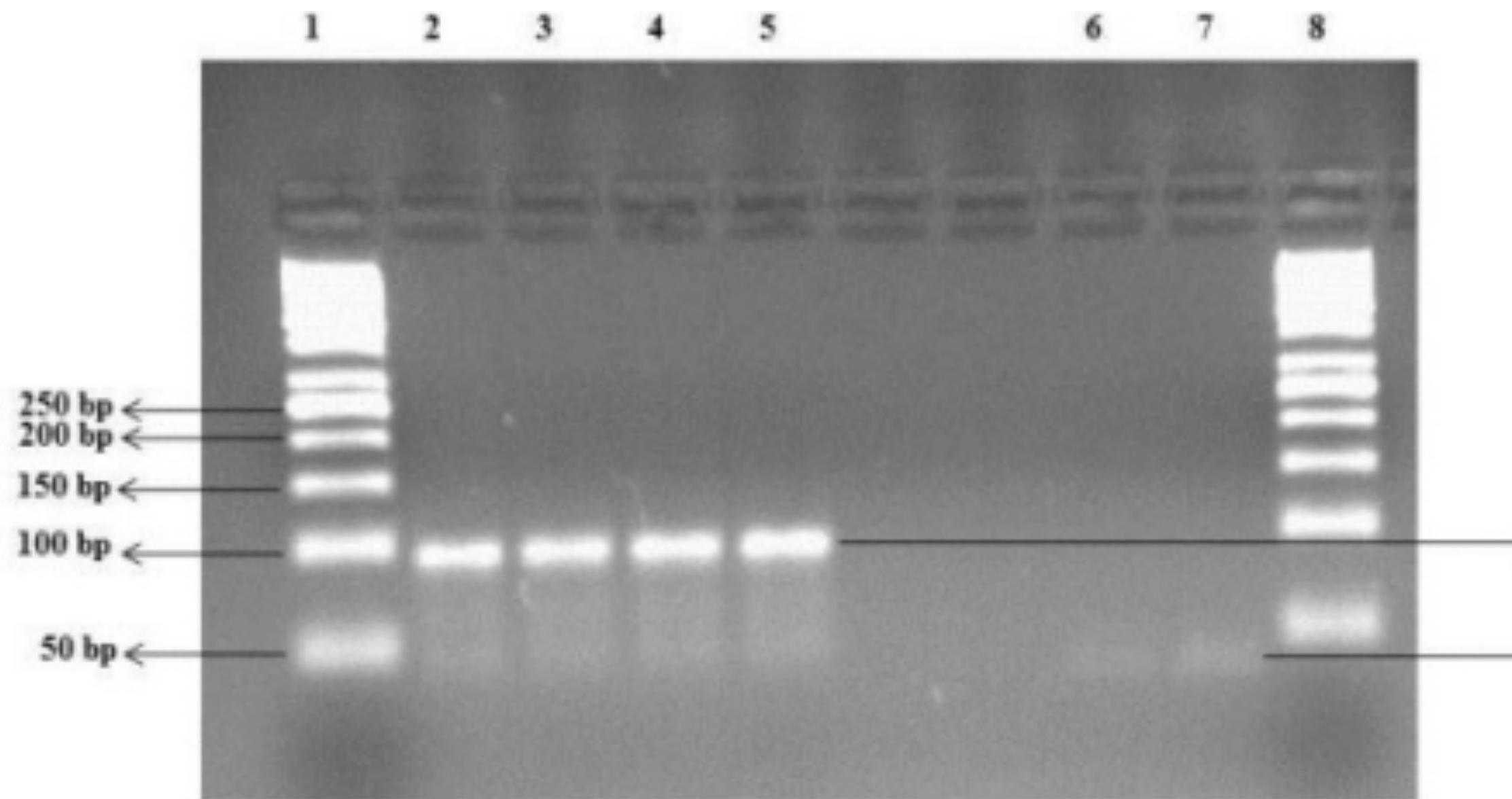
b Isothermal SHARP (SSB-Helicase Assisted Rapid PCR)



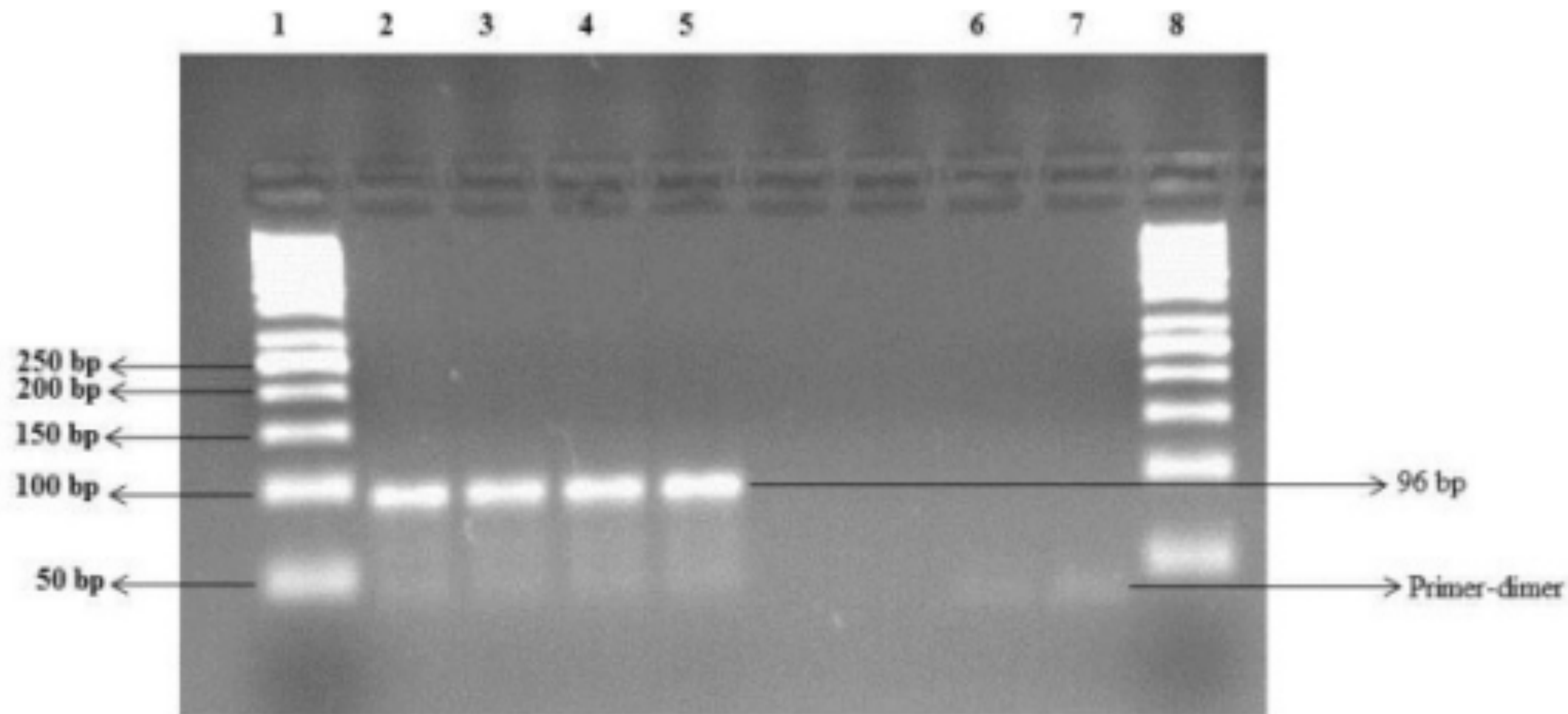
Классическая ПЦР: Применение



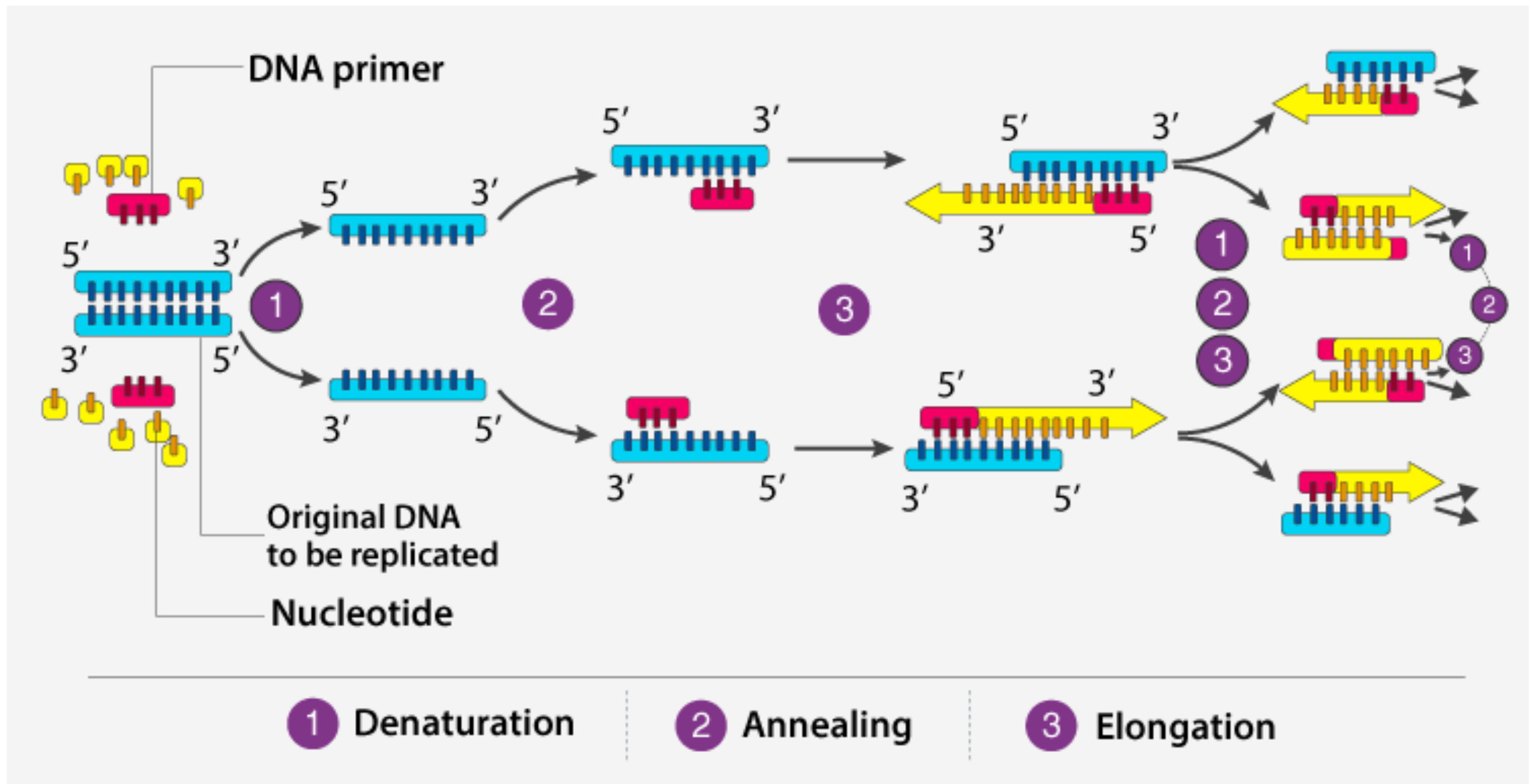
Классическая ПЦР: Выходные данные



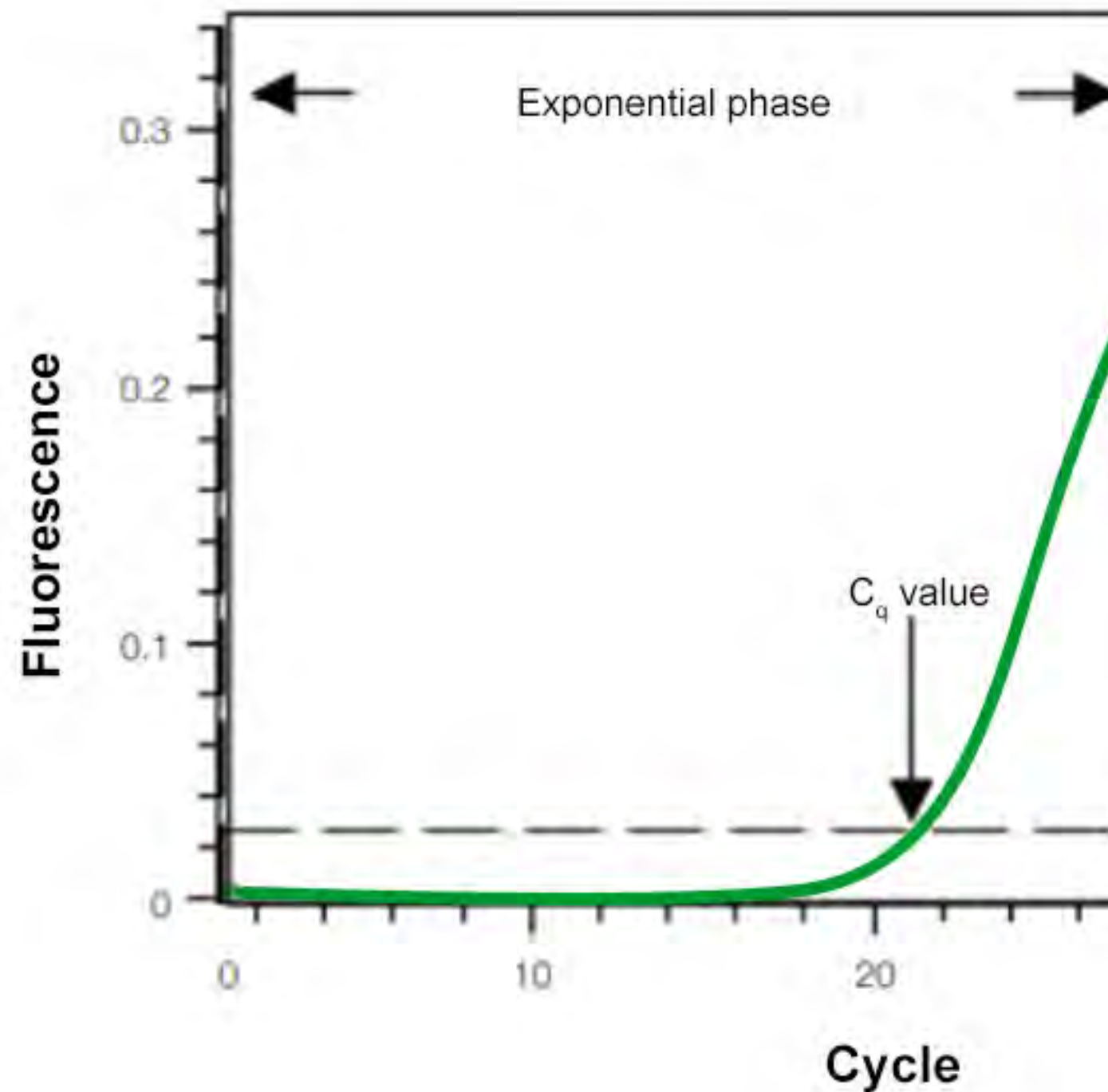
Классическая ПЦР: Выходные данные



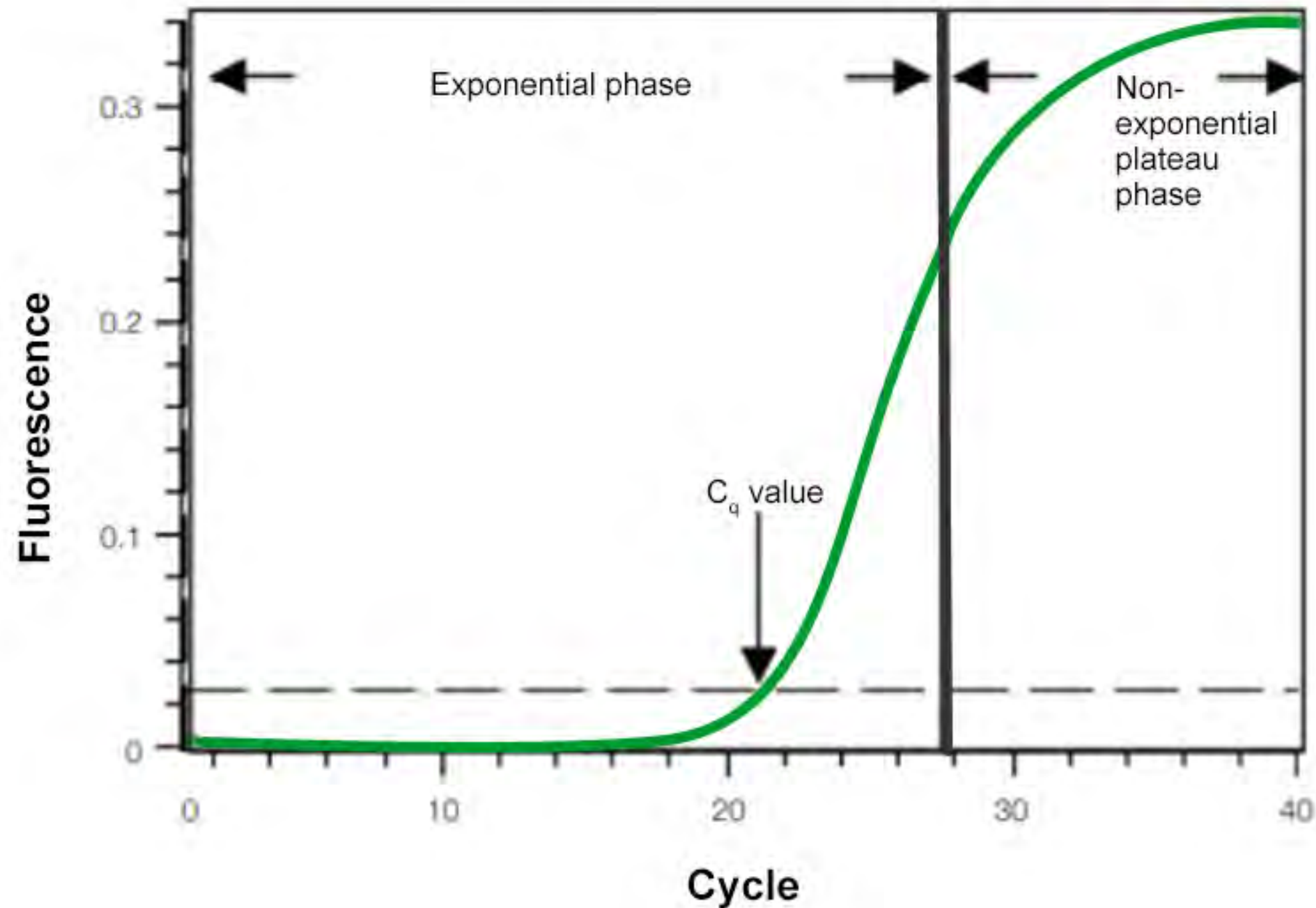
ПЦР в реальном времени (qPCR): Принцип



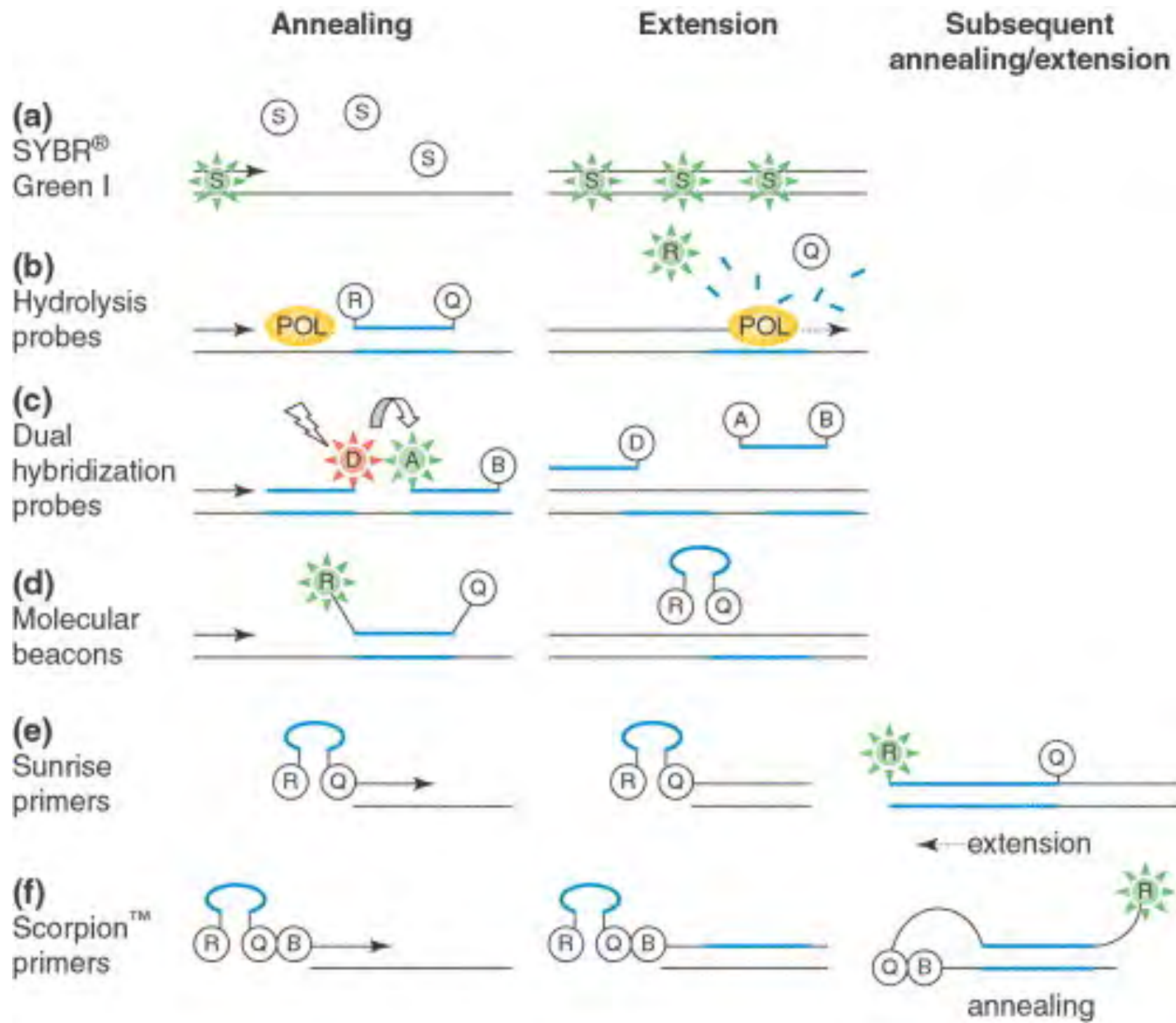
ПЦР в реальном времени (qPCR): Принцип



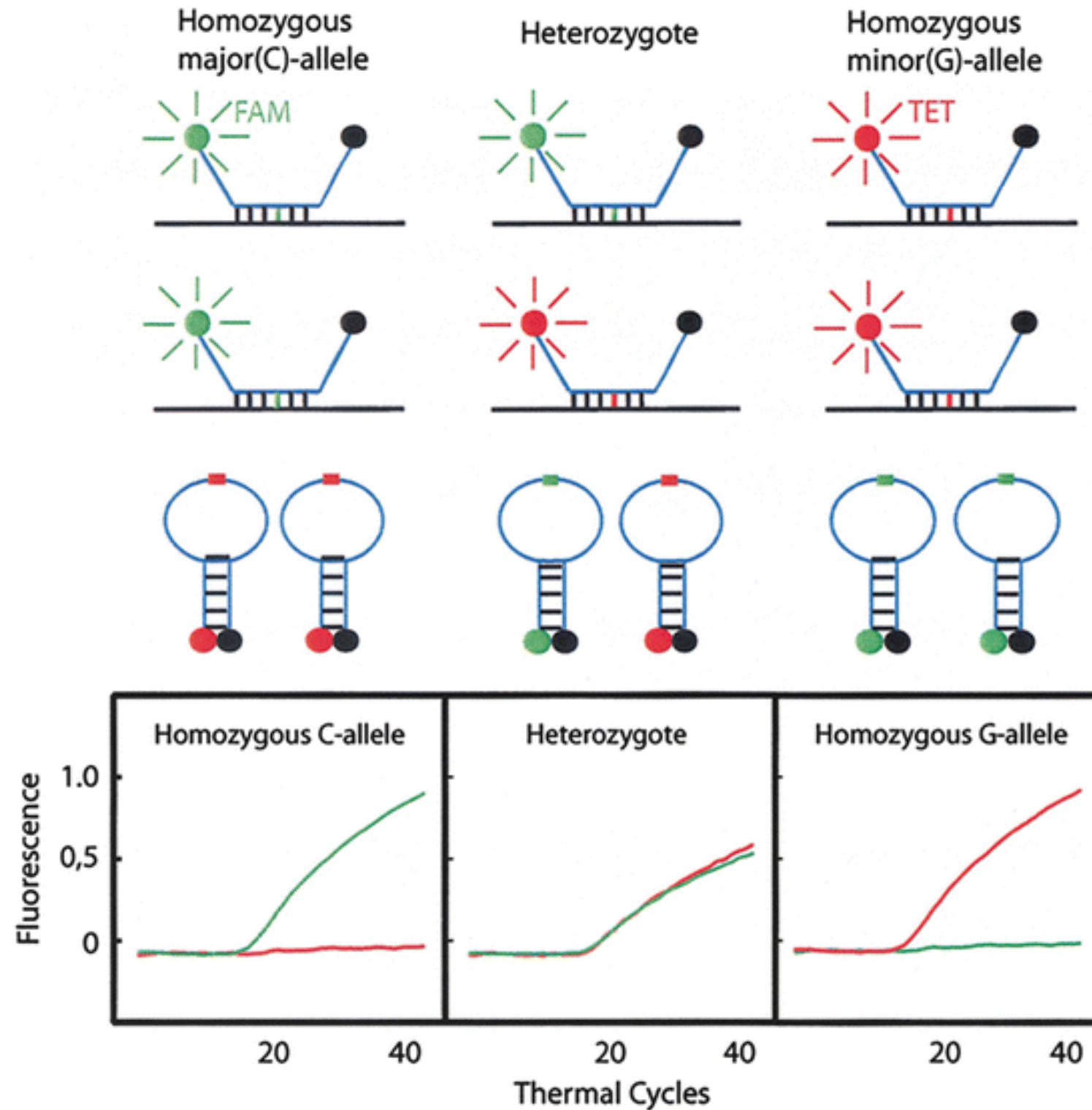
ПЦР в реальном времени (qPCR): Принцип



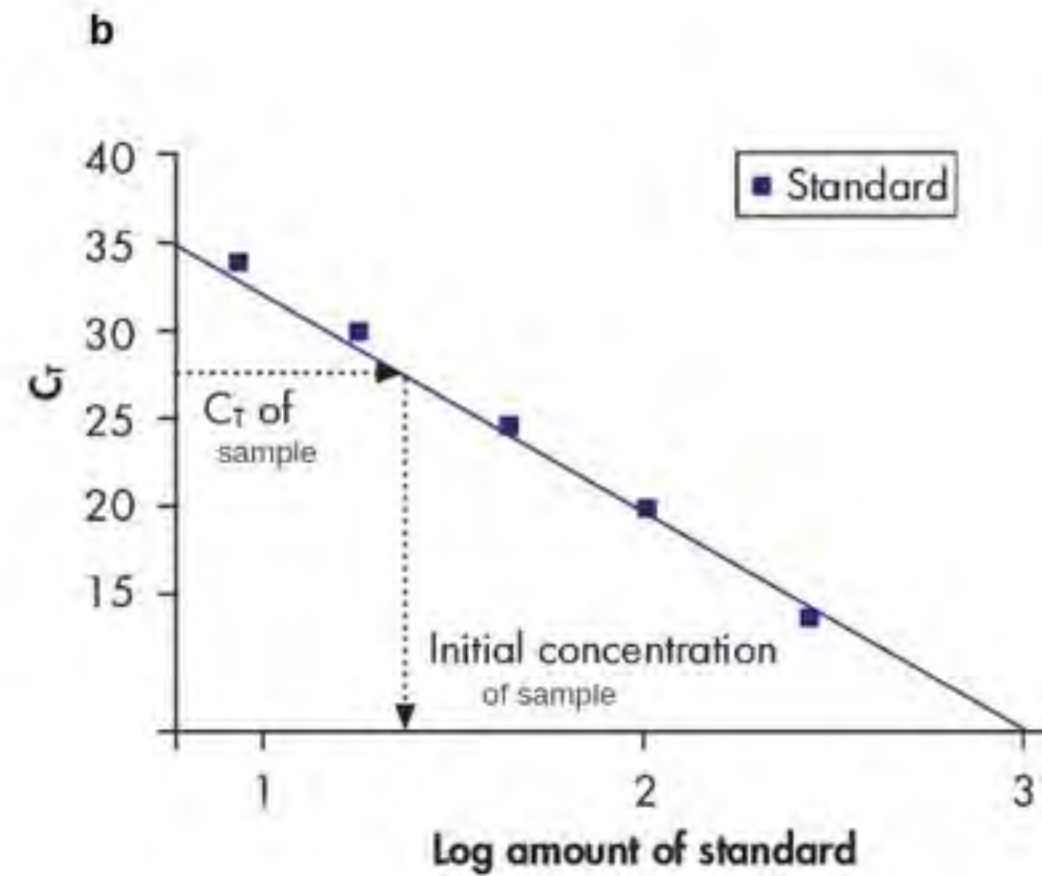
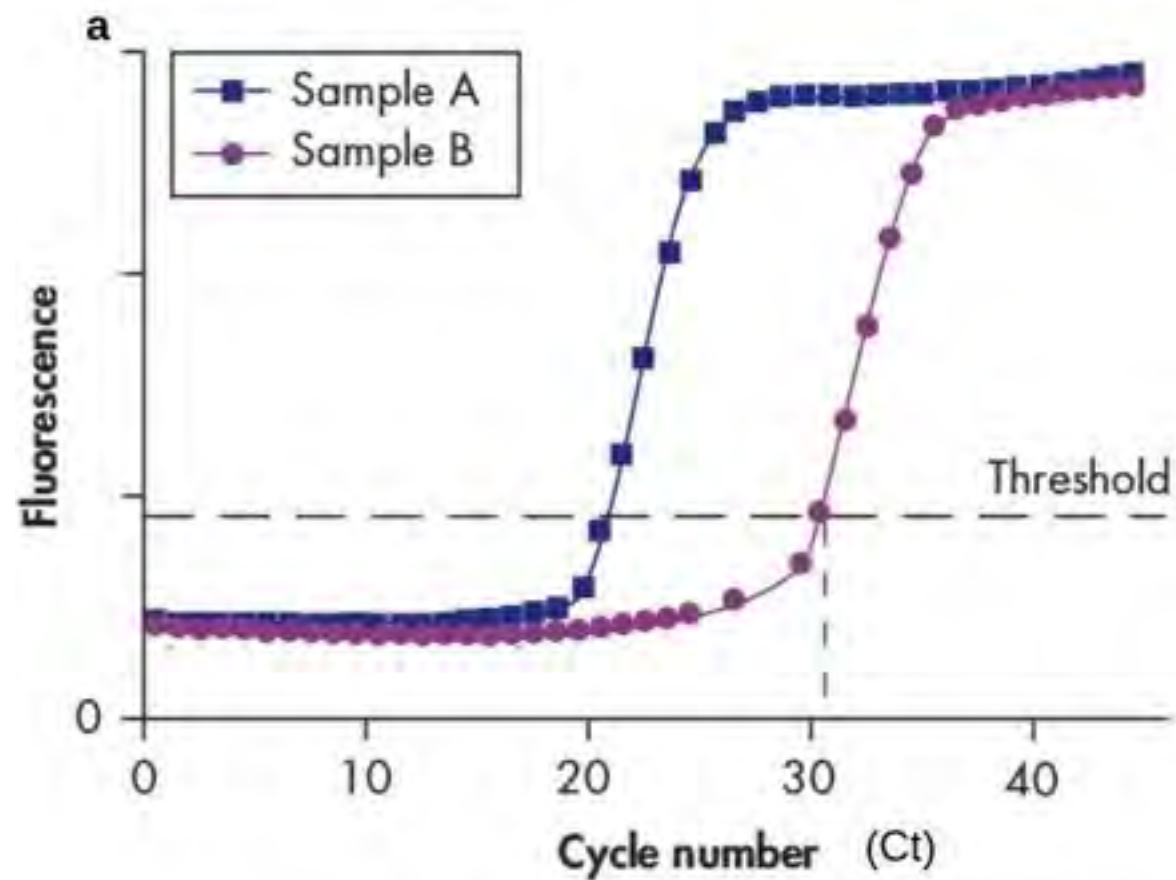
qPCR: Флуоресцентные методы



qPCR: применение

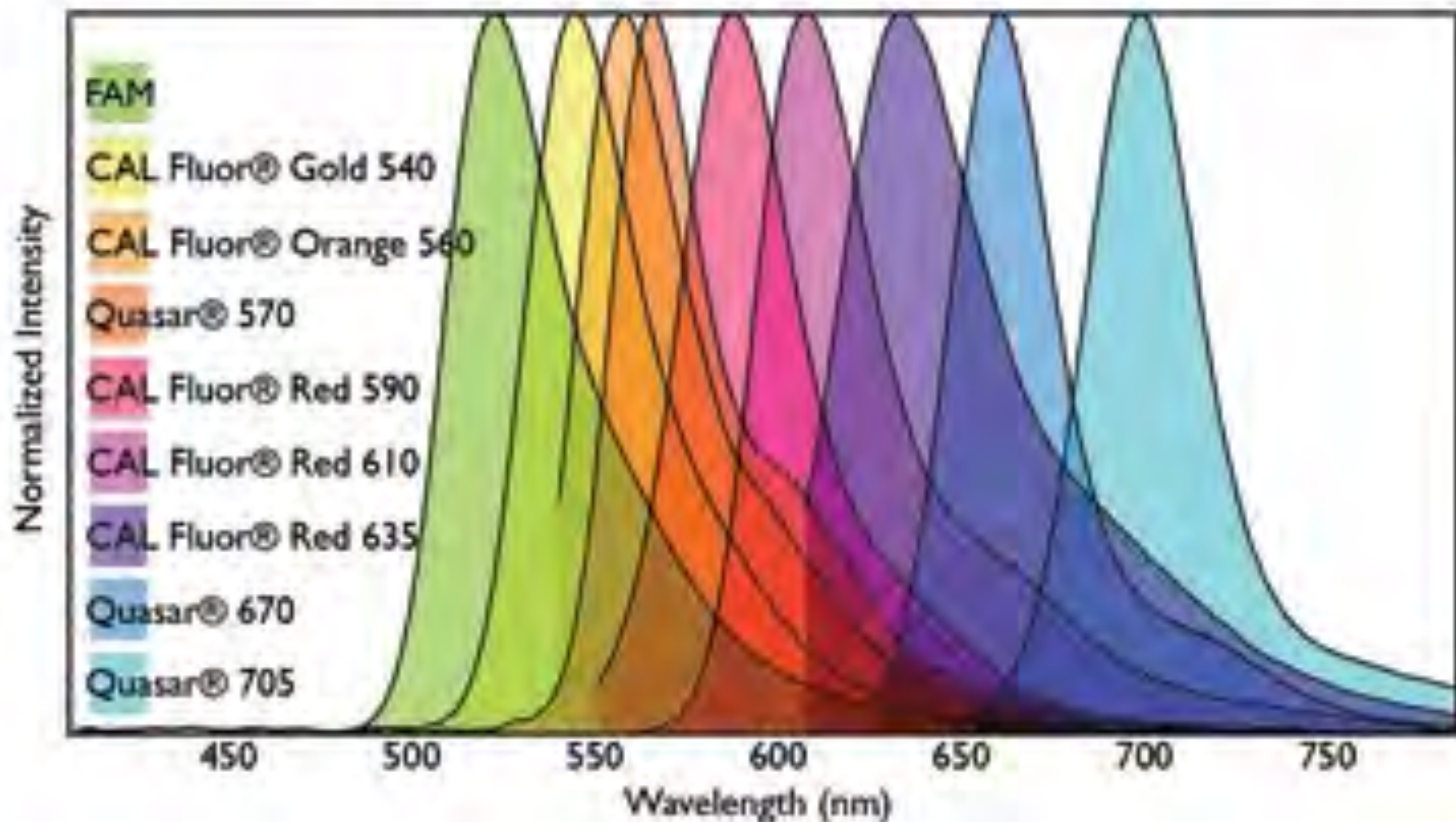


Обработка данных qPCR



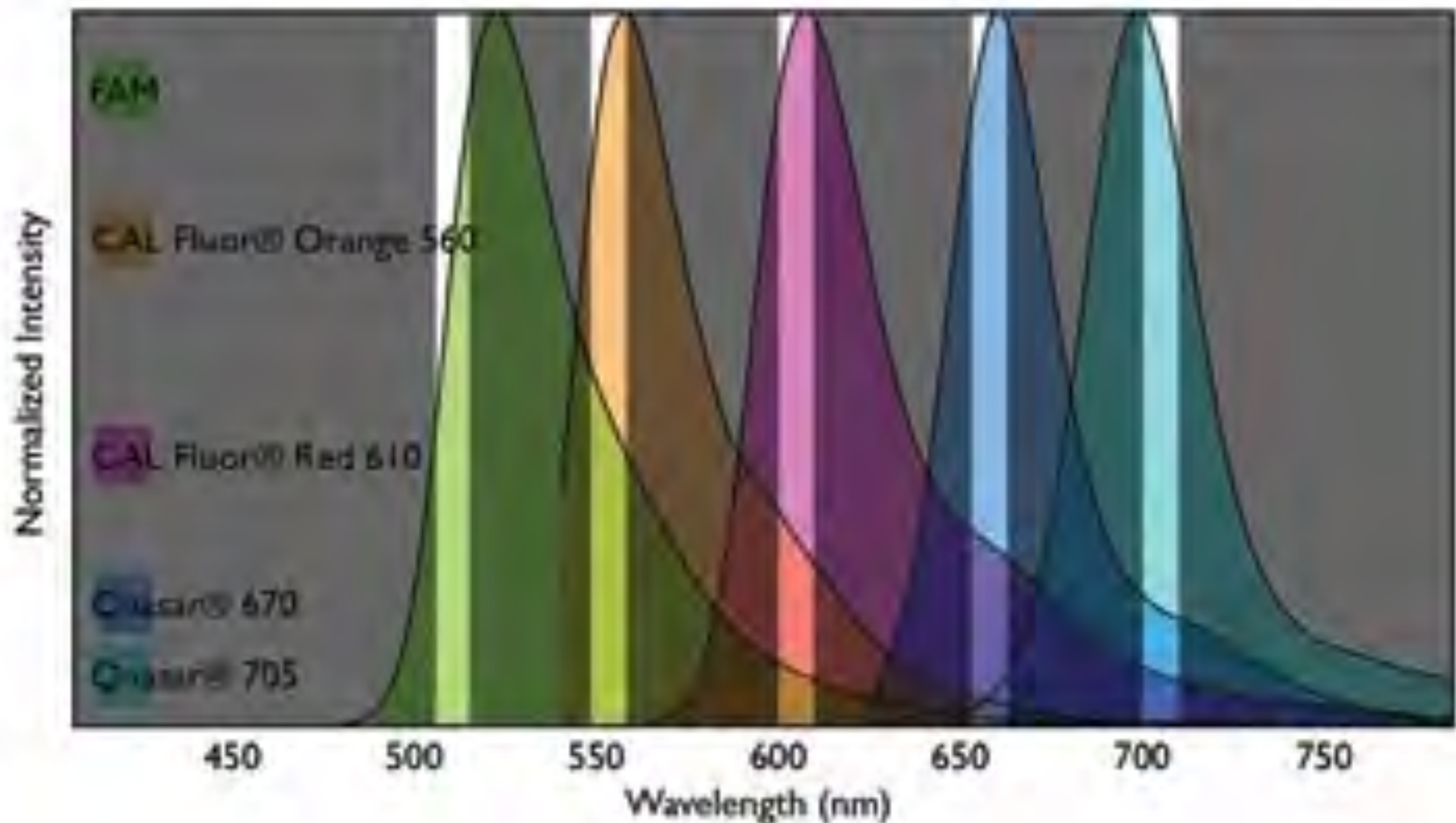
Другие варианты ПЦР: Мультиплексная ПЦР

Emission Spectra of Fluorophores for Multiplexing

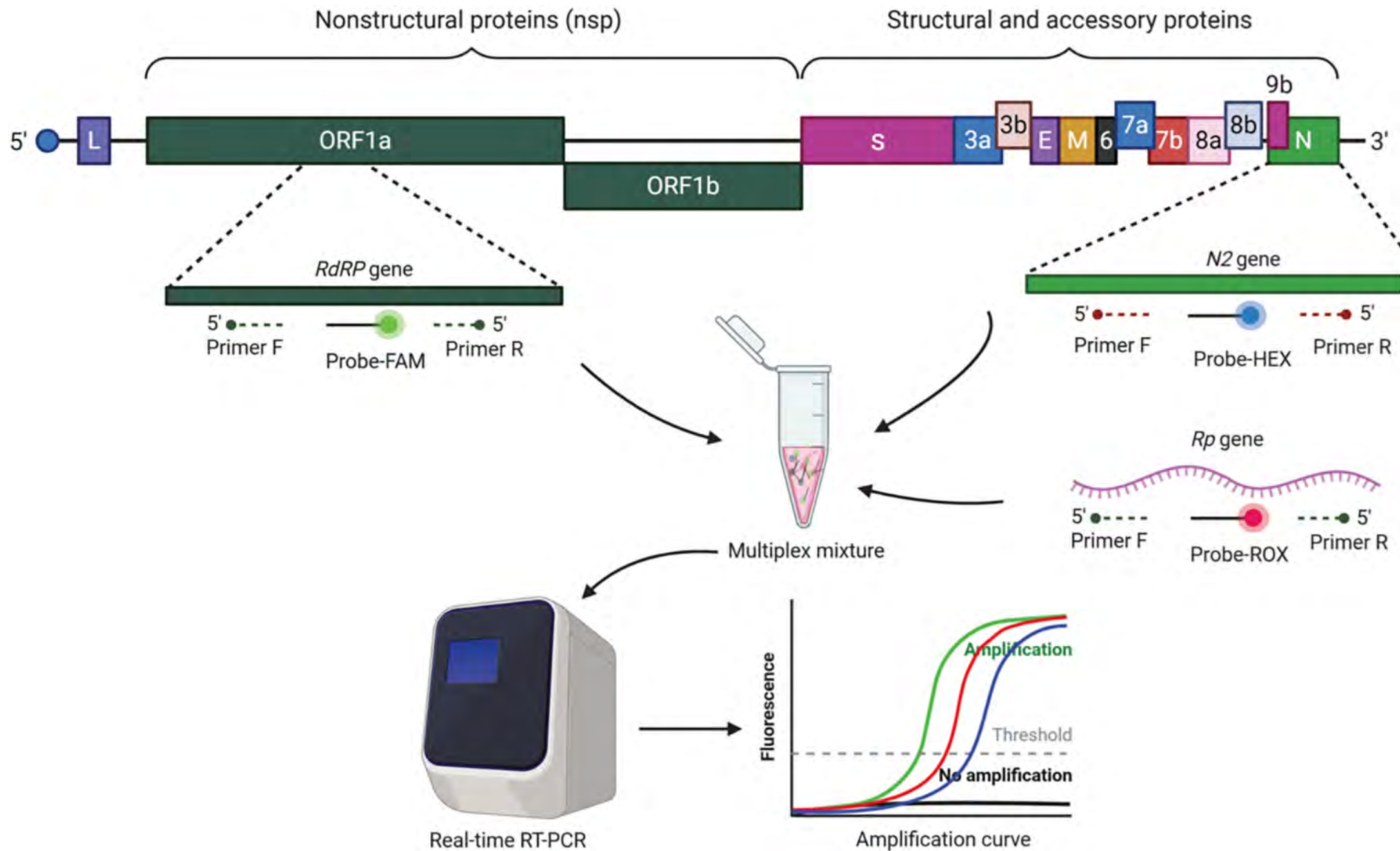


Другие варианты ПЦР: Мультиплексная ПЦР

Emission Filters Overlaid onto Reporter Spectra



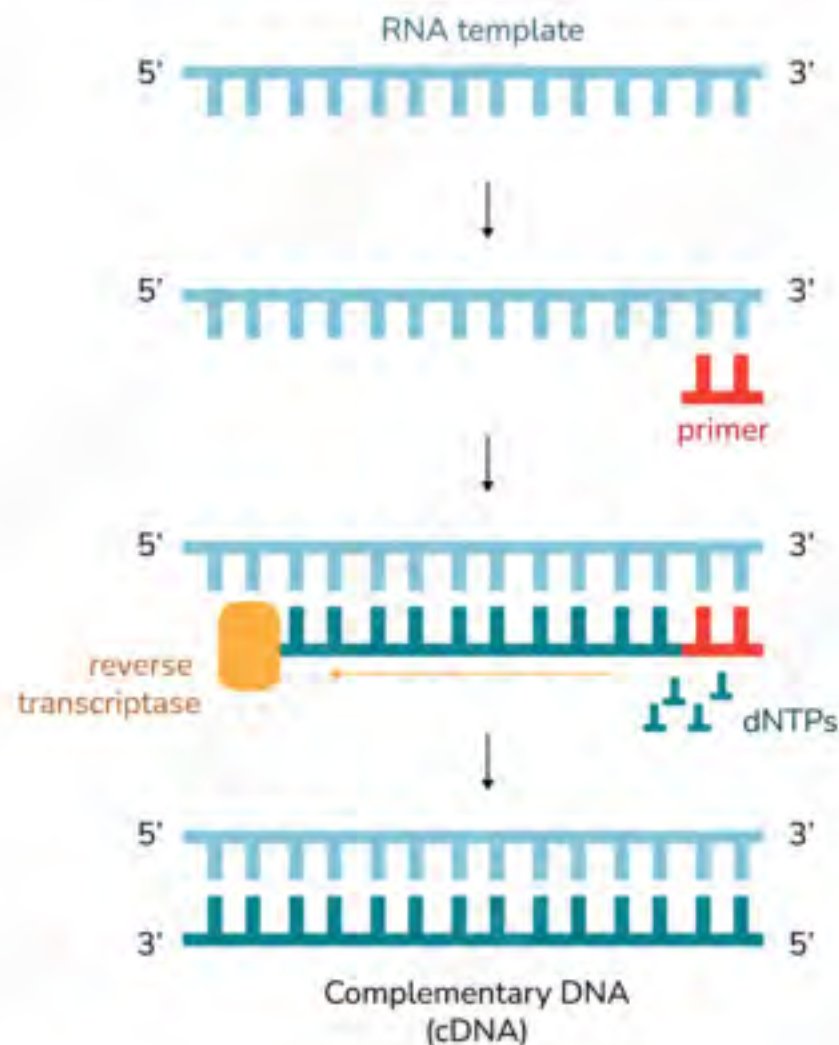
Другие варианты ПЦР: Мультиплексная ПЦР



Обратная транскрипционная ПЦР (RT-PCR)

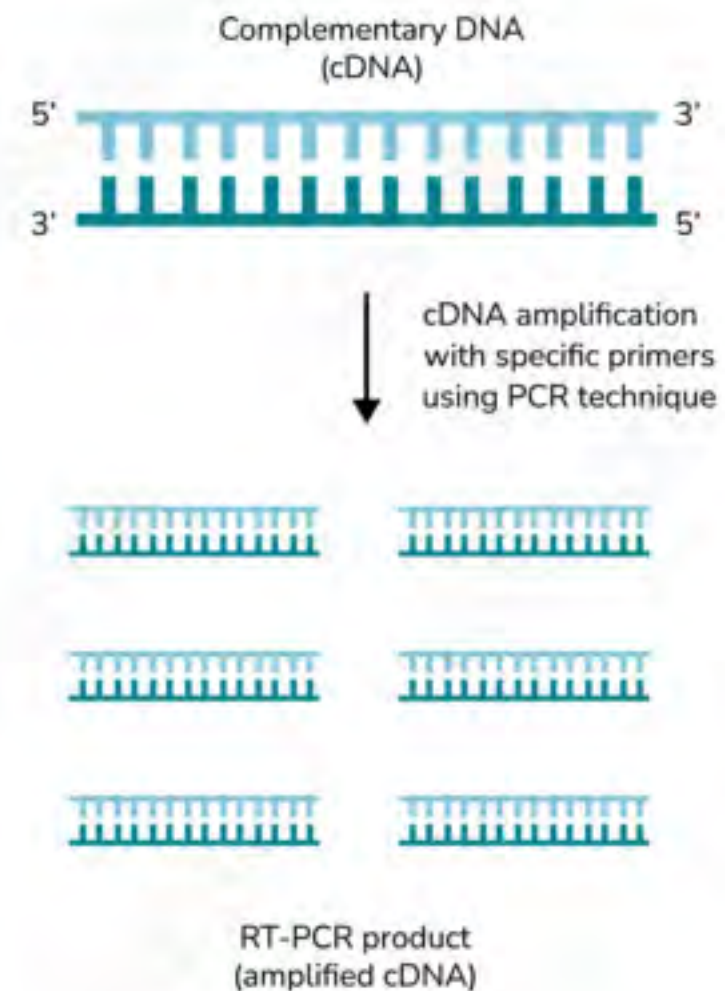
1 reverse transcription

(reverse transcribe of RNA to cDNA)

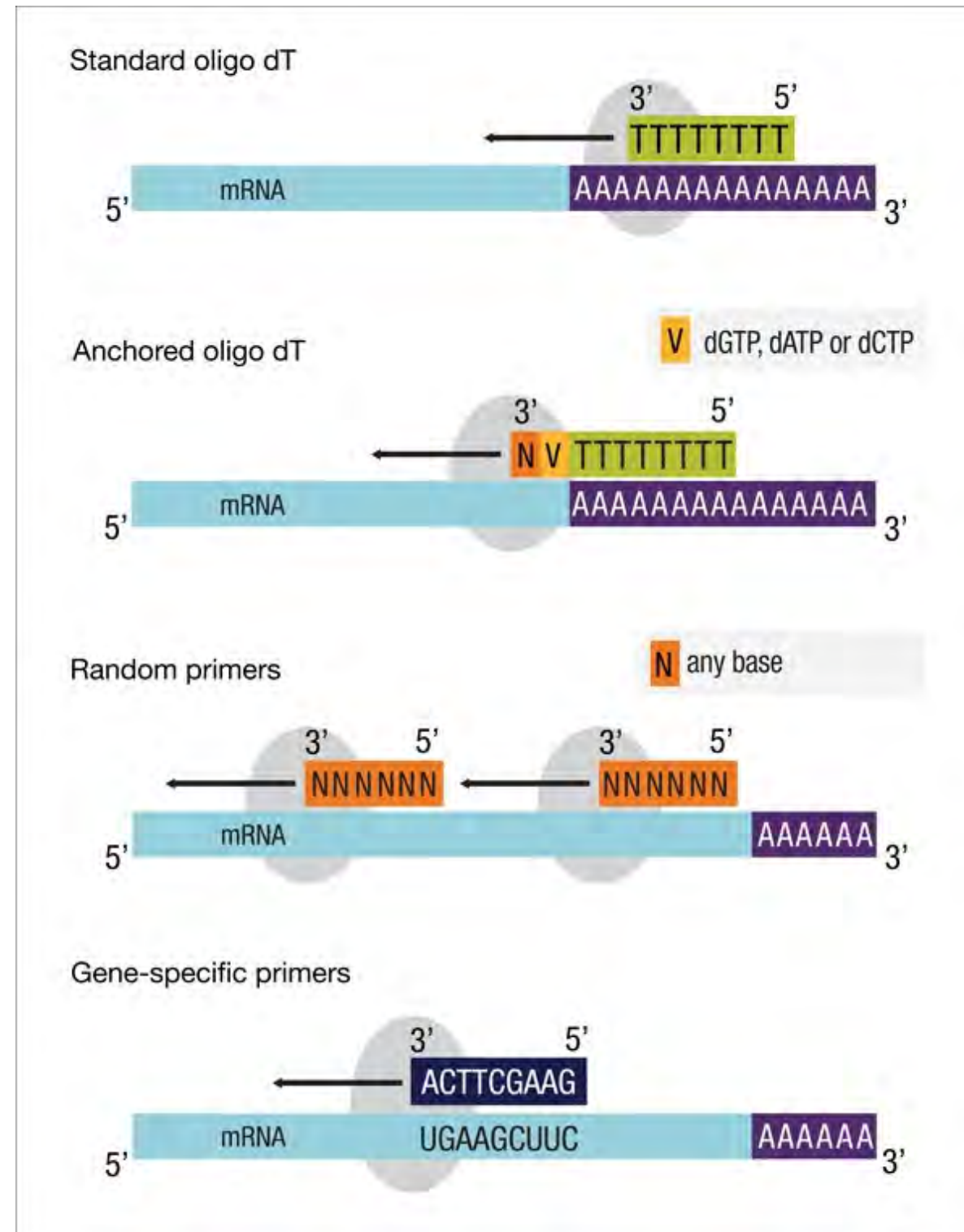


2 amplification

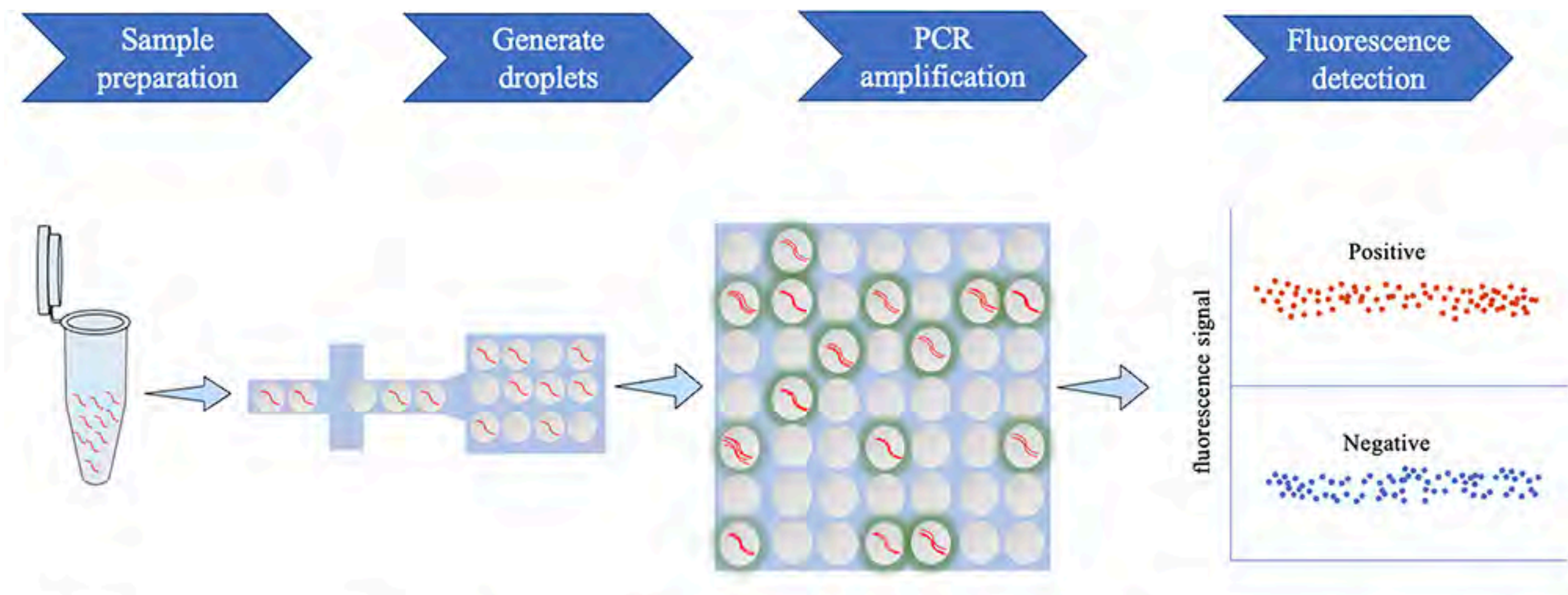
(amplify of cDNA by PCR)



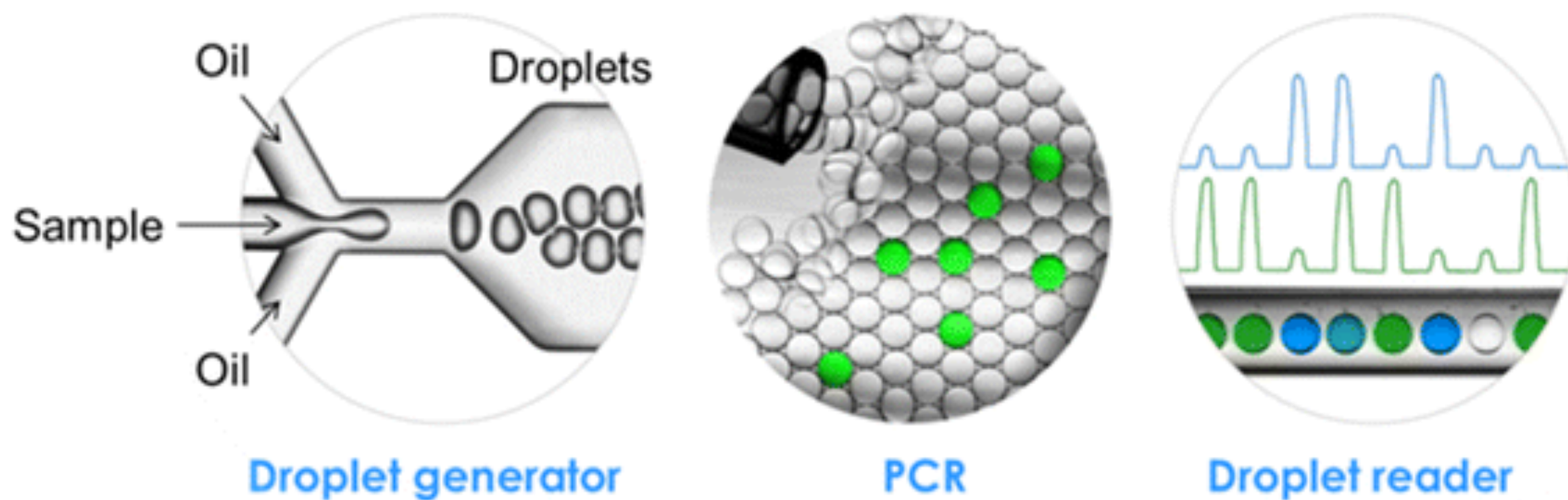
Обратная транскрипционная ПЦР (RT-PCR)



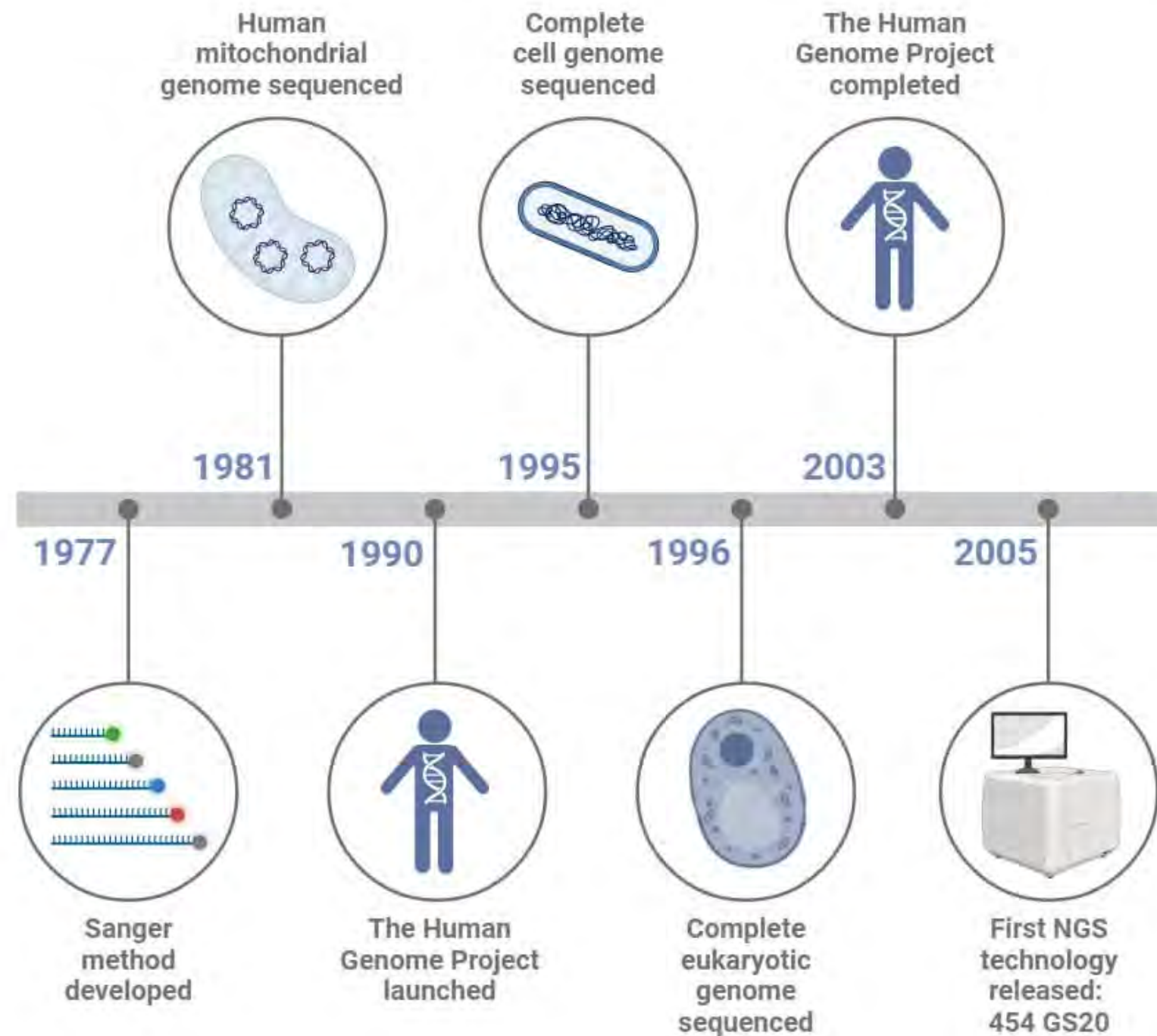
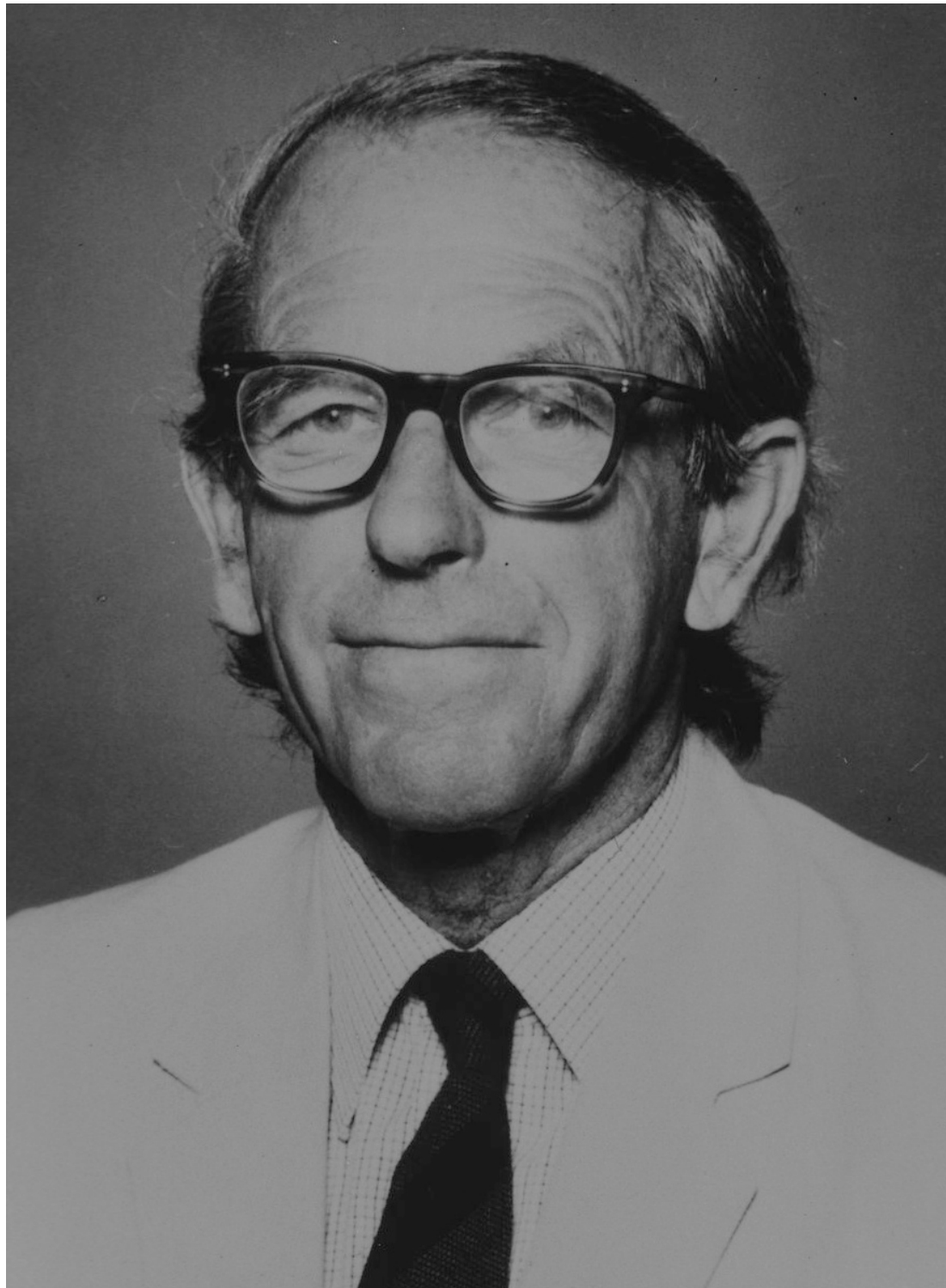
Цифровая ПЦР (dPCR): Принцип



Цифровая ПЦР (dPCR): Принцип

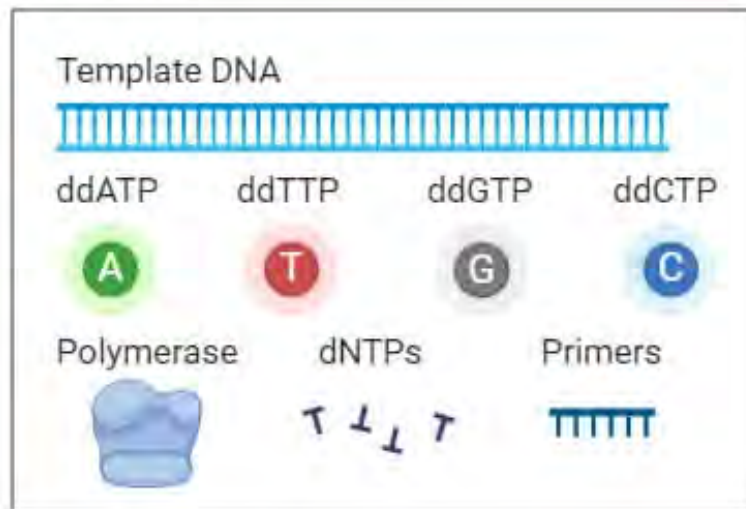


Секвенирование по Сэнгеру: История и значение

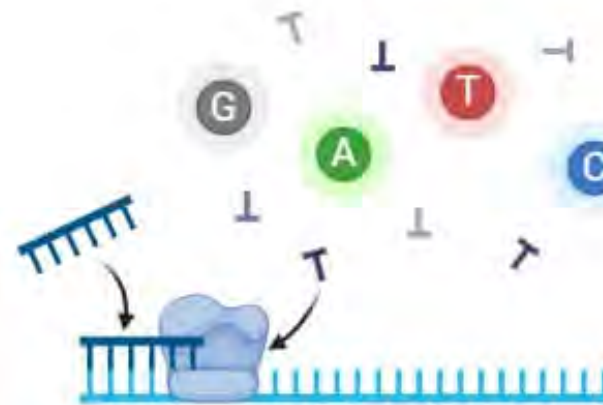


Секвенирование по Сэнгеру: Методика

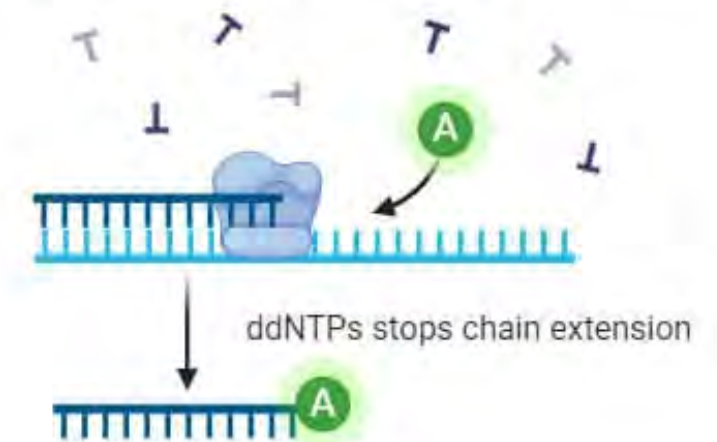
Reagents



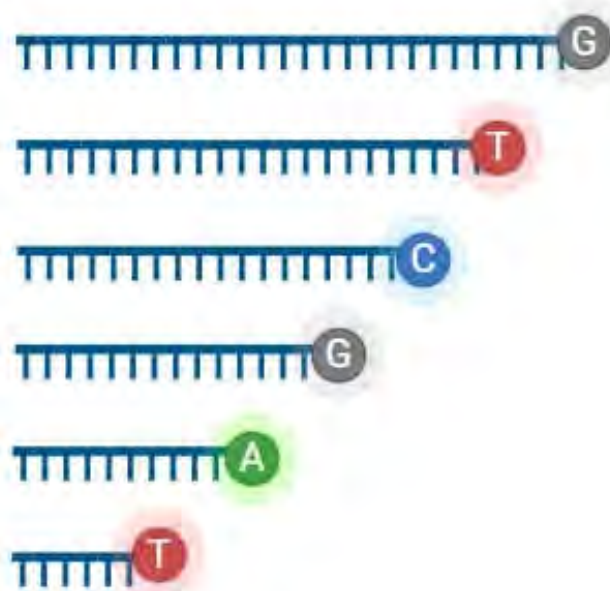
① Primer annealing and chain extension



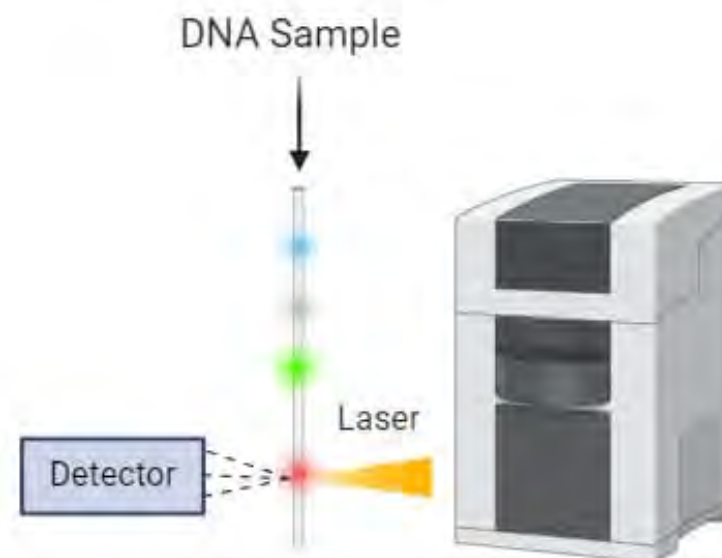
② ddNTP binding and chain termination



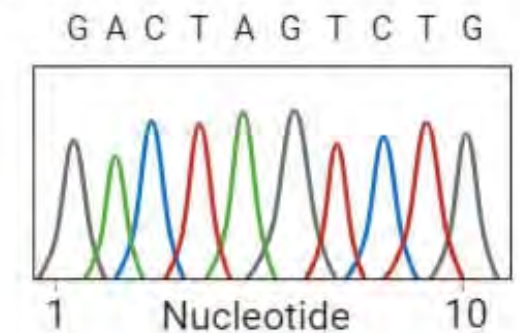
③ Fluorescently labelled DNA sample



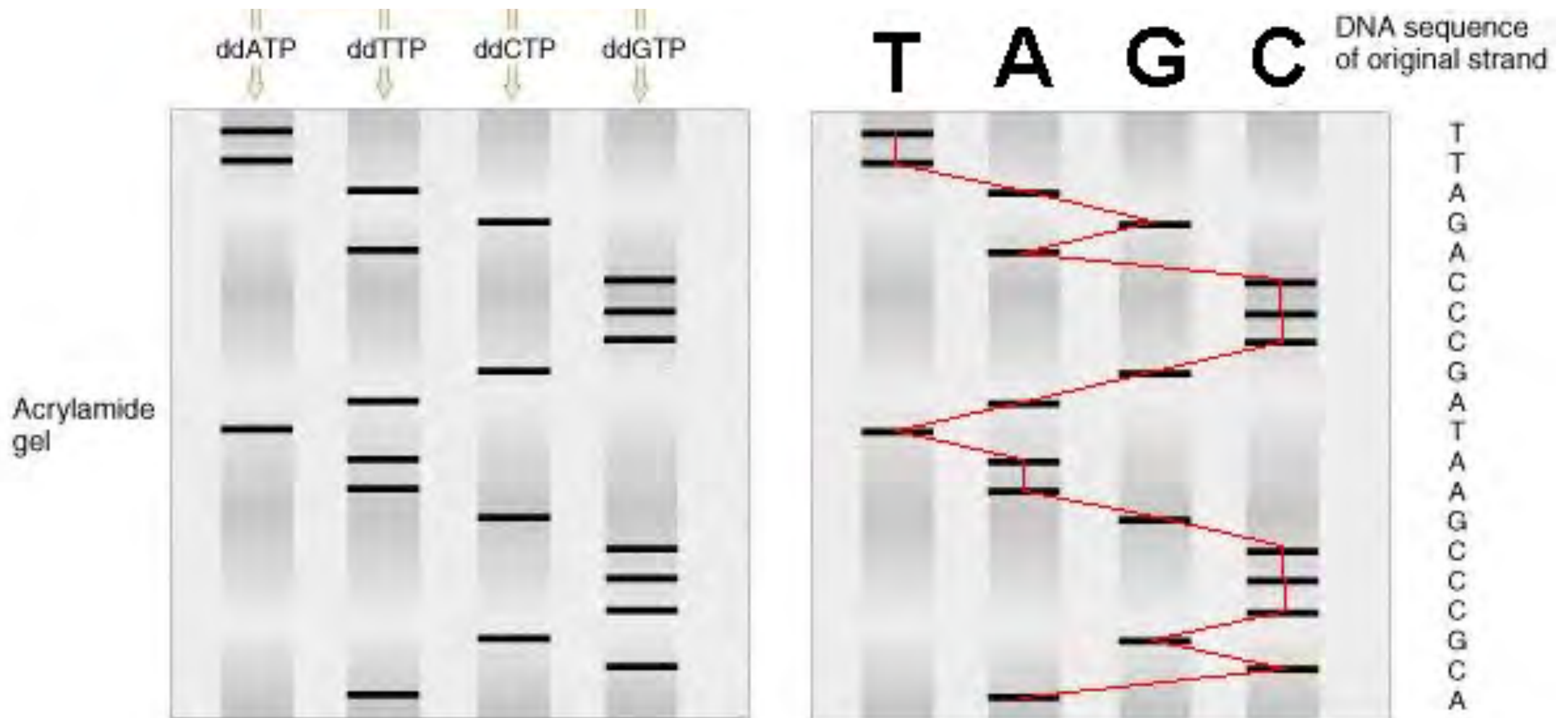
④ Capillary gel electrophoresis and fluorescence detection



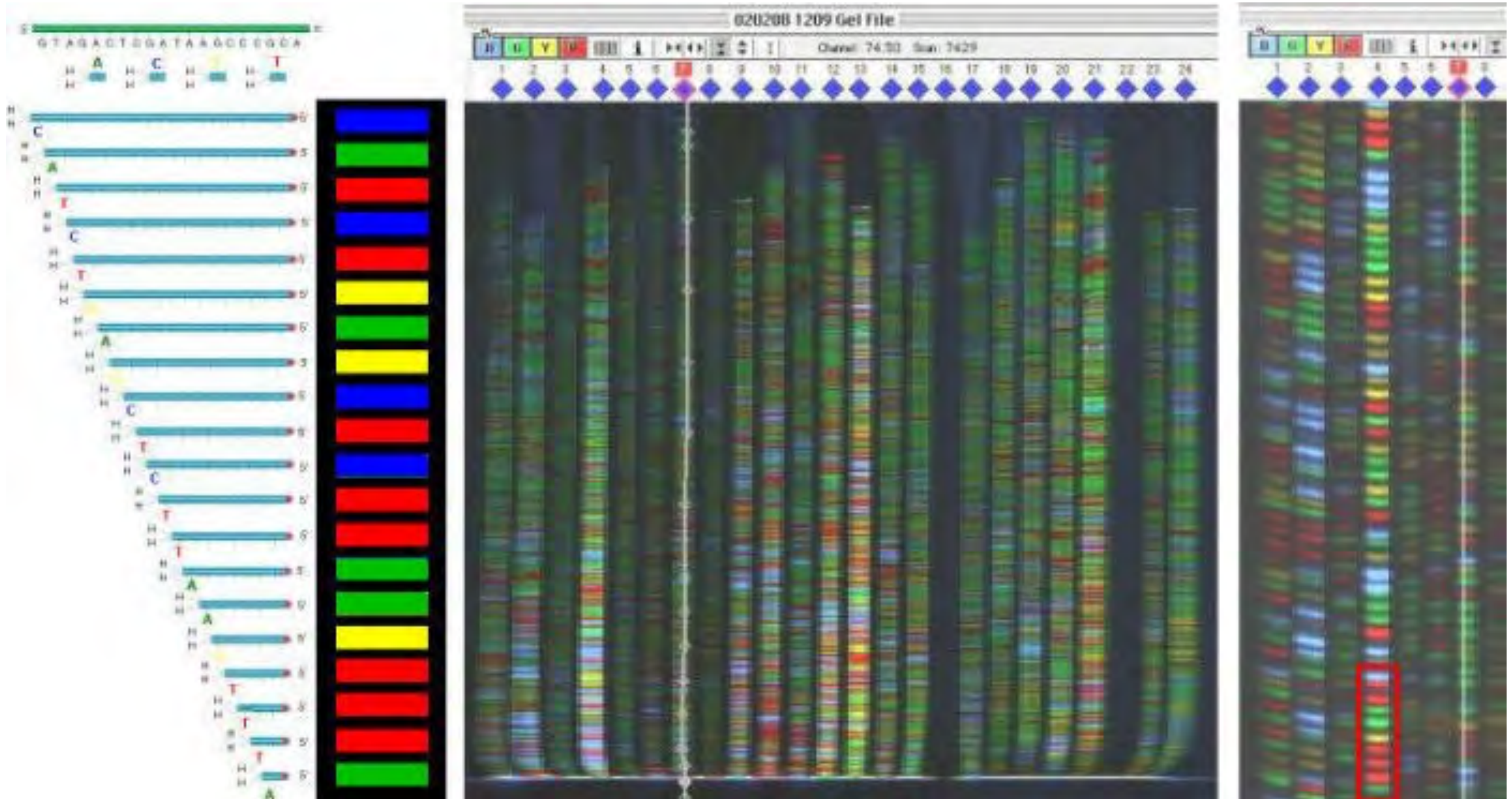
⑤ Sequence analysis and reconstruction



Секвенирование по Сэнгеру: Методика

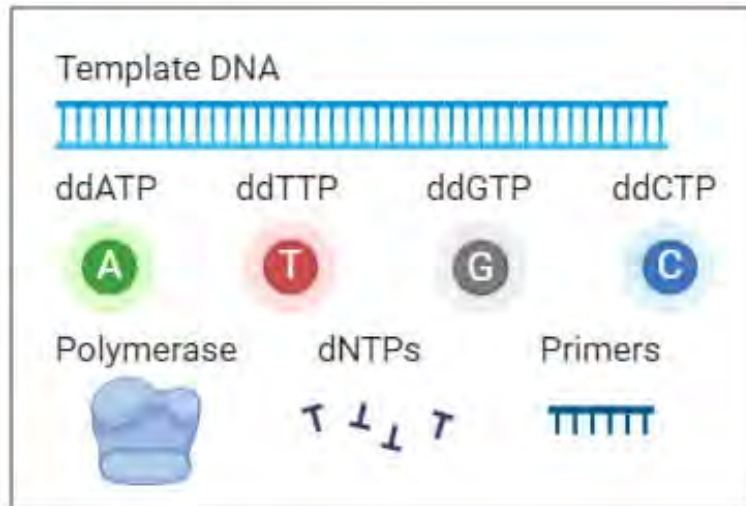


Автоматизация Сэнгер-секвенирования

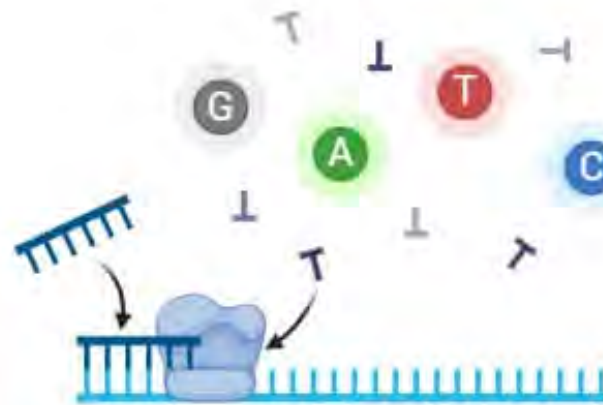


Автоматизация Сэнгер-секвенирования

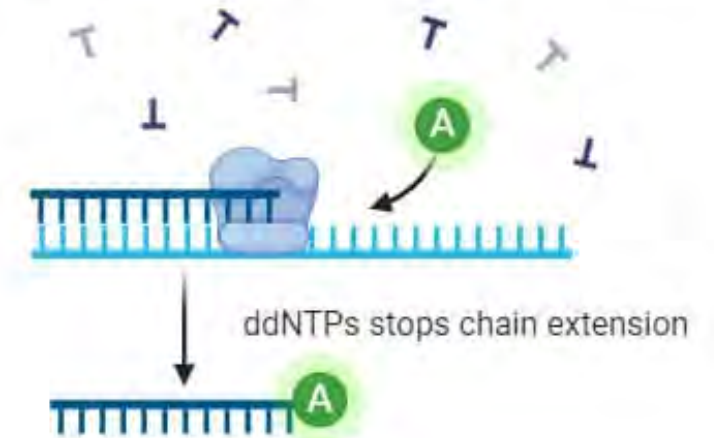
Reagents



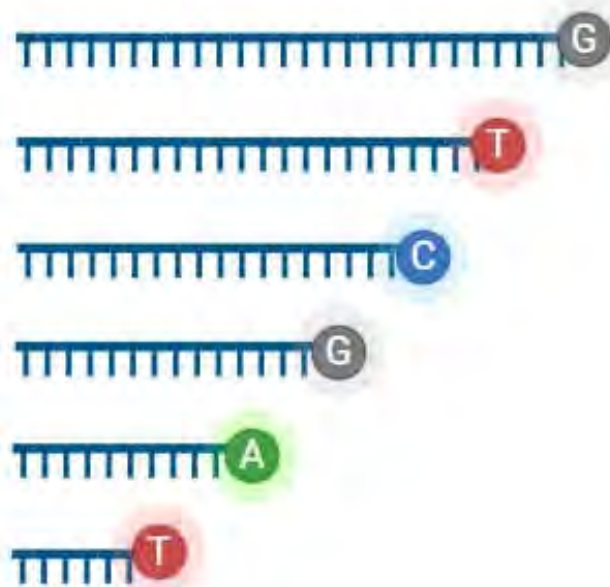
① Primer annealing and chain extension



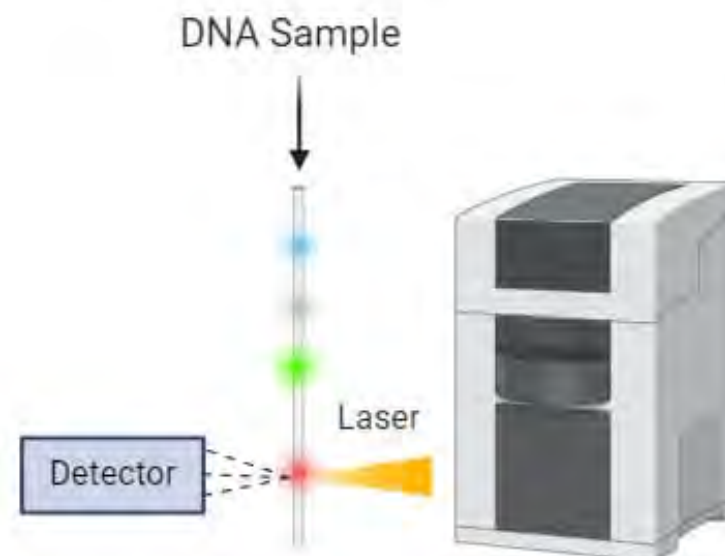
② ddNTP binding and chain termination



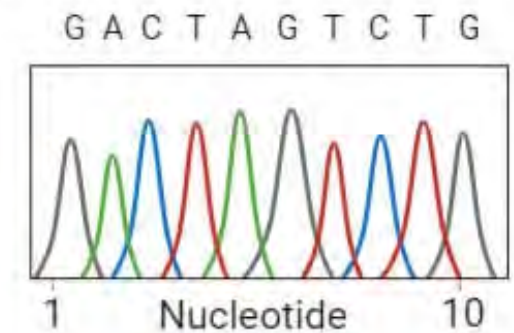
③ Fluorescently labelled DNA sample



④ Capillary gel electrophoresis and fluorescence detection



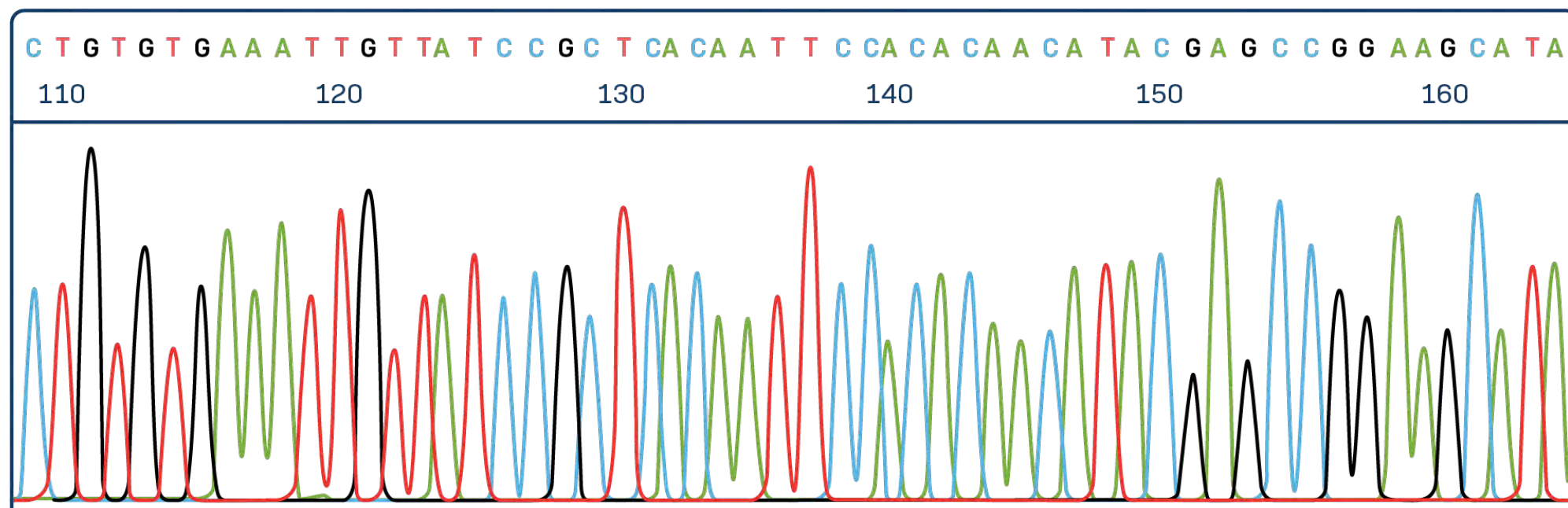
⑤ Sequence analysis and reconstruction



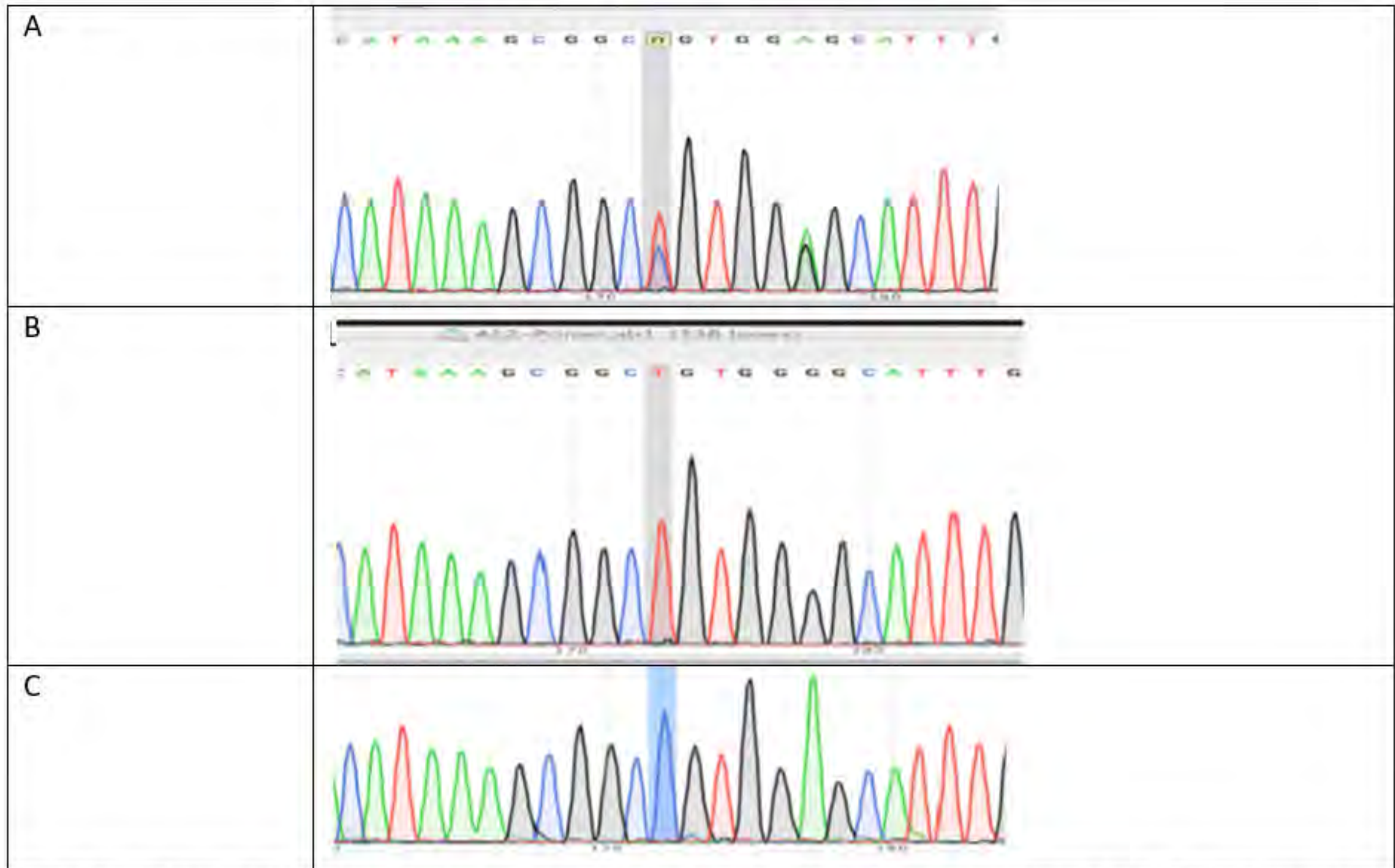
Сэнгер: Выходные данные



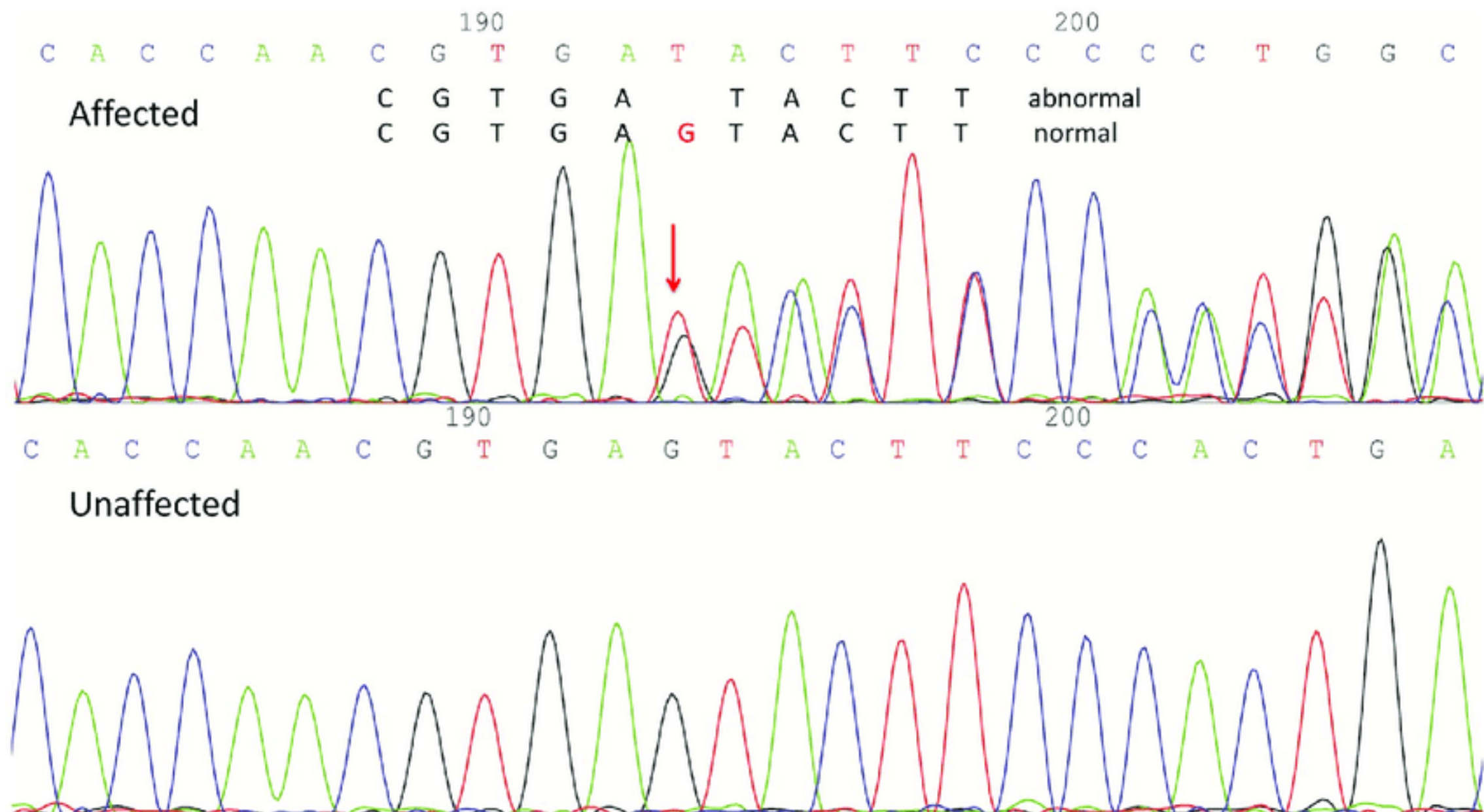
Сэнгер: Выходные данные



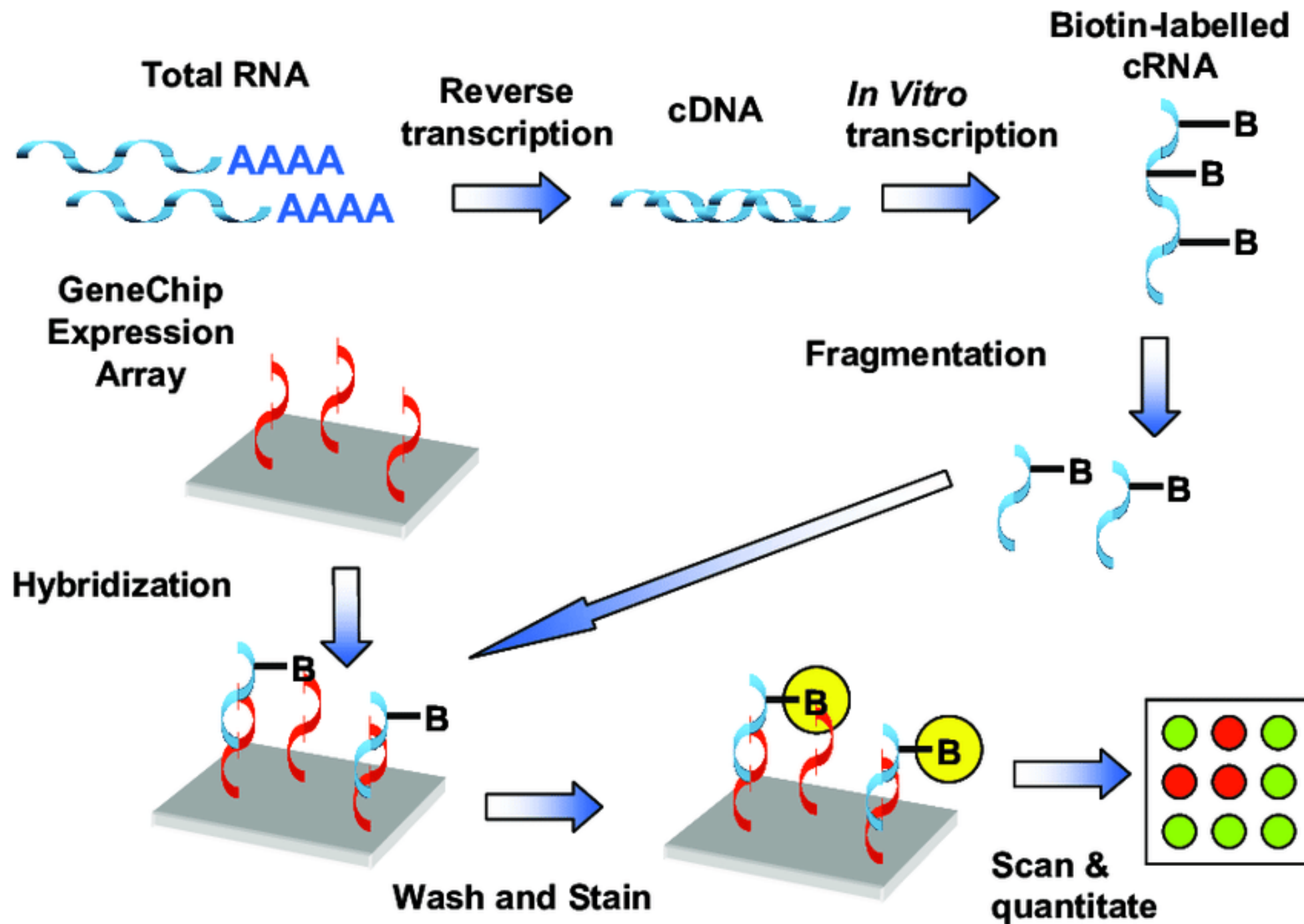
Сэнгер: Выходные данные



Сэнгер: Выходные данные



Микрочипы: Введение



Микрочипы Affymetrix: Особенности технологии

Figure 1A

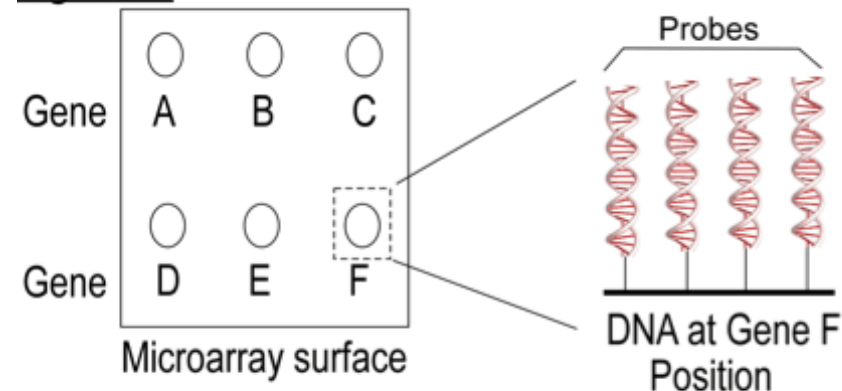


Figure 1B

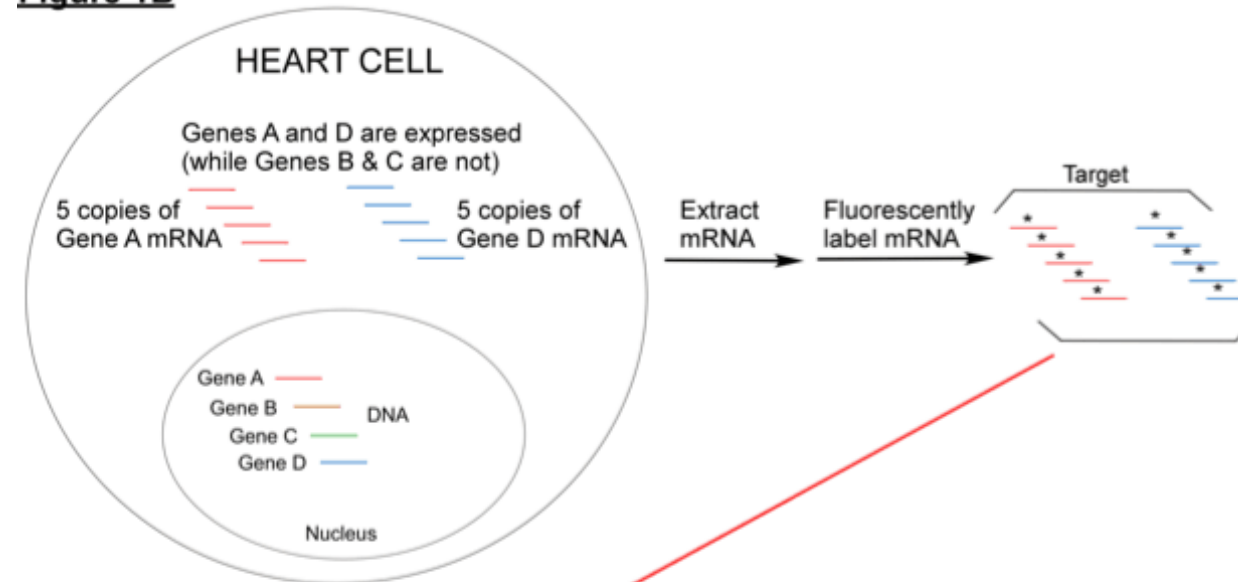
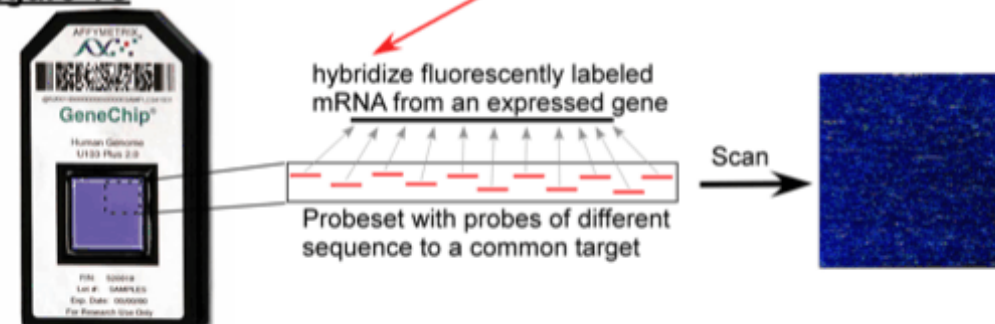
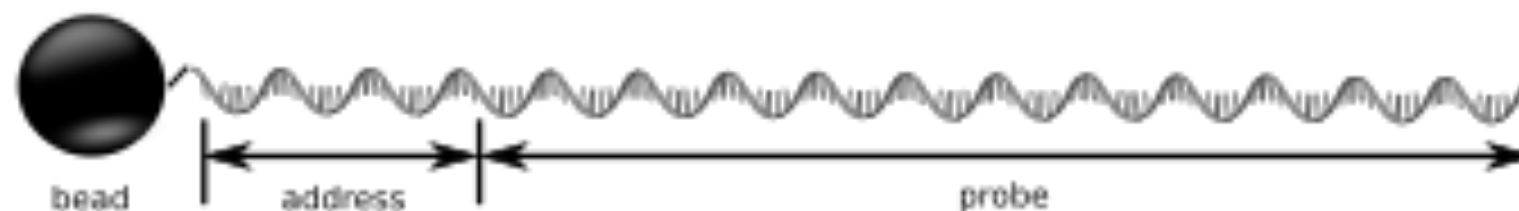
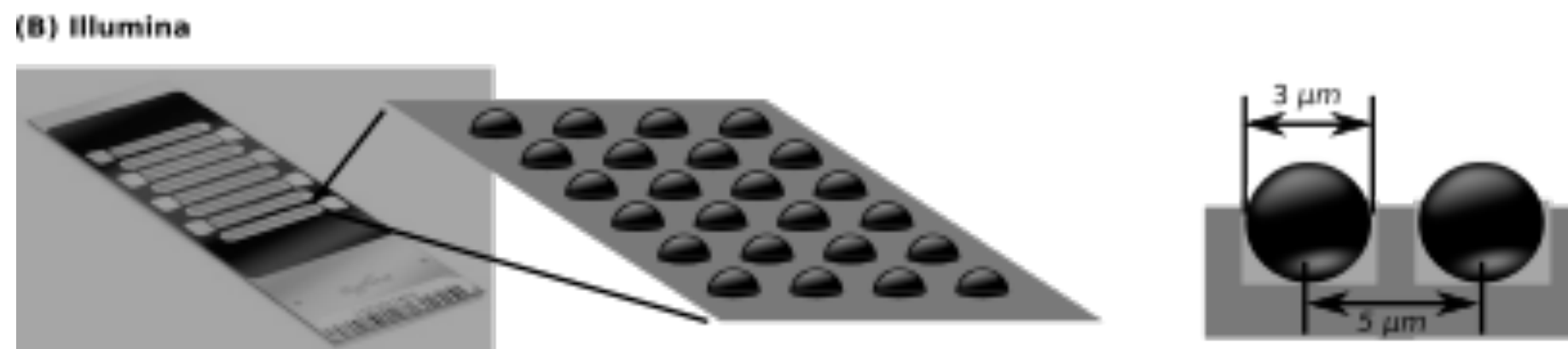
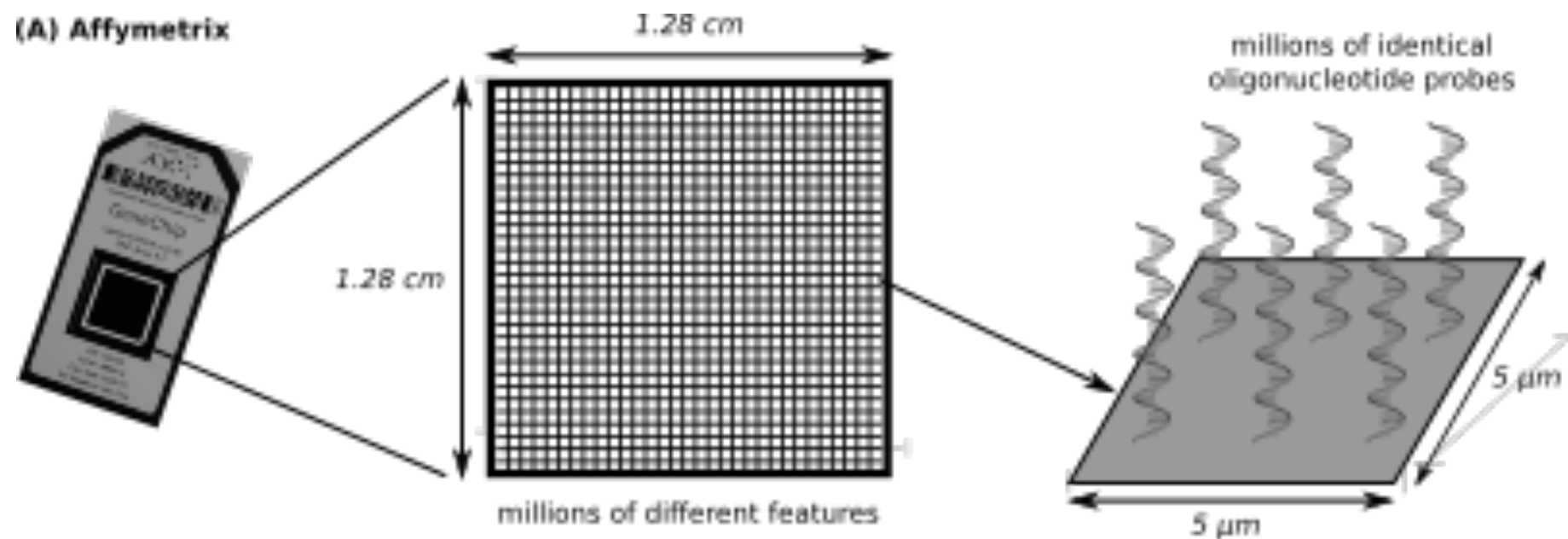


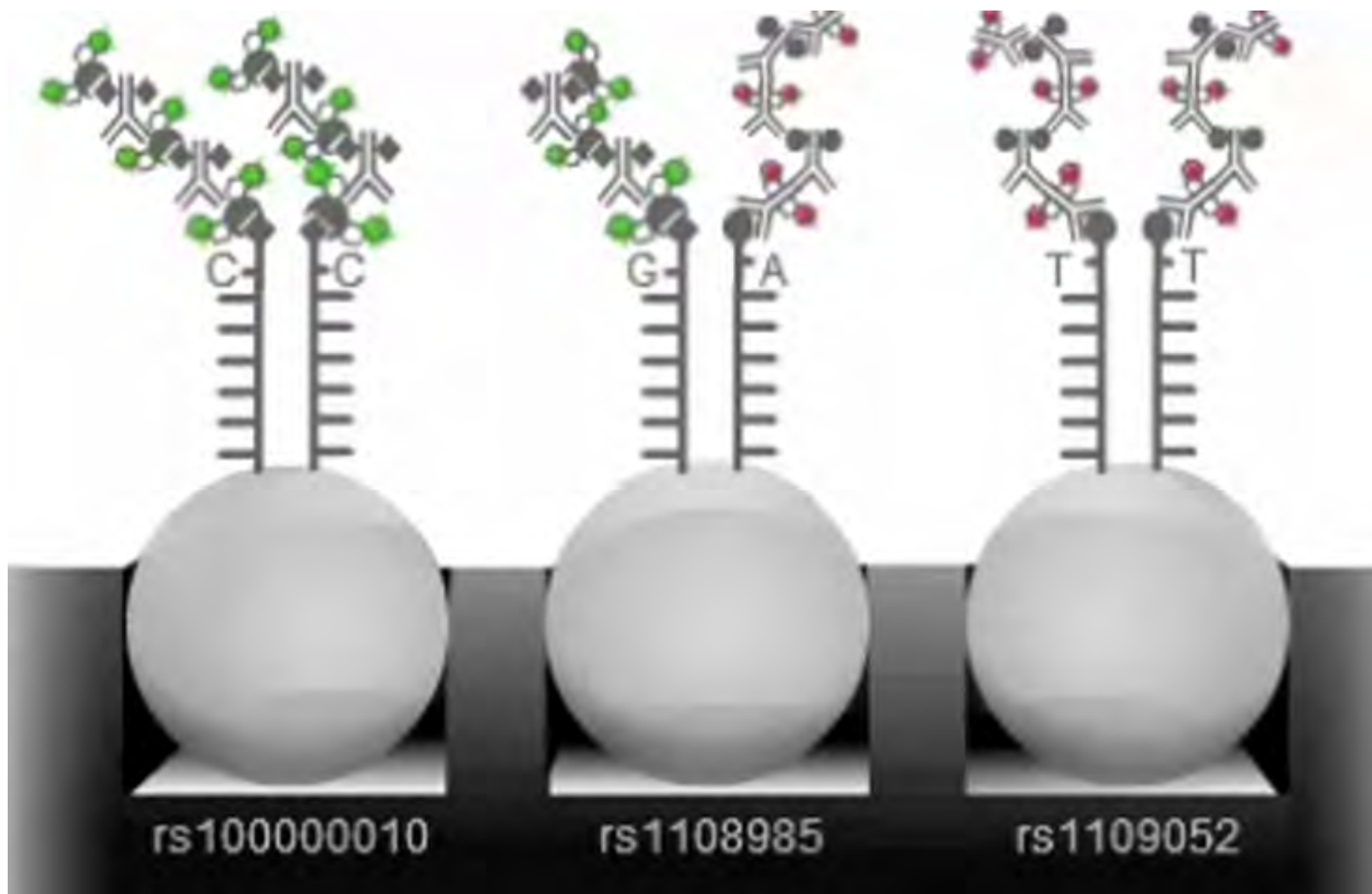
Figure 1C



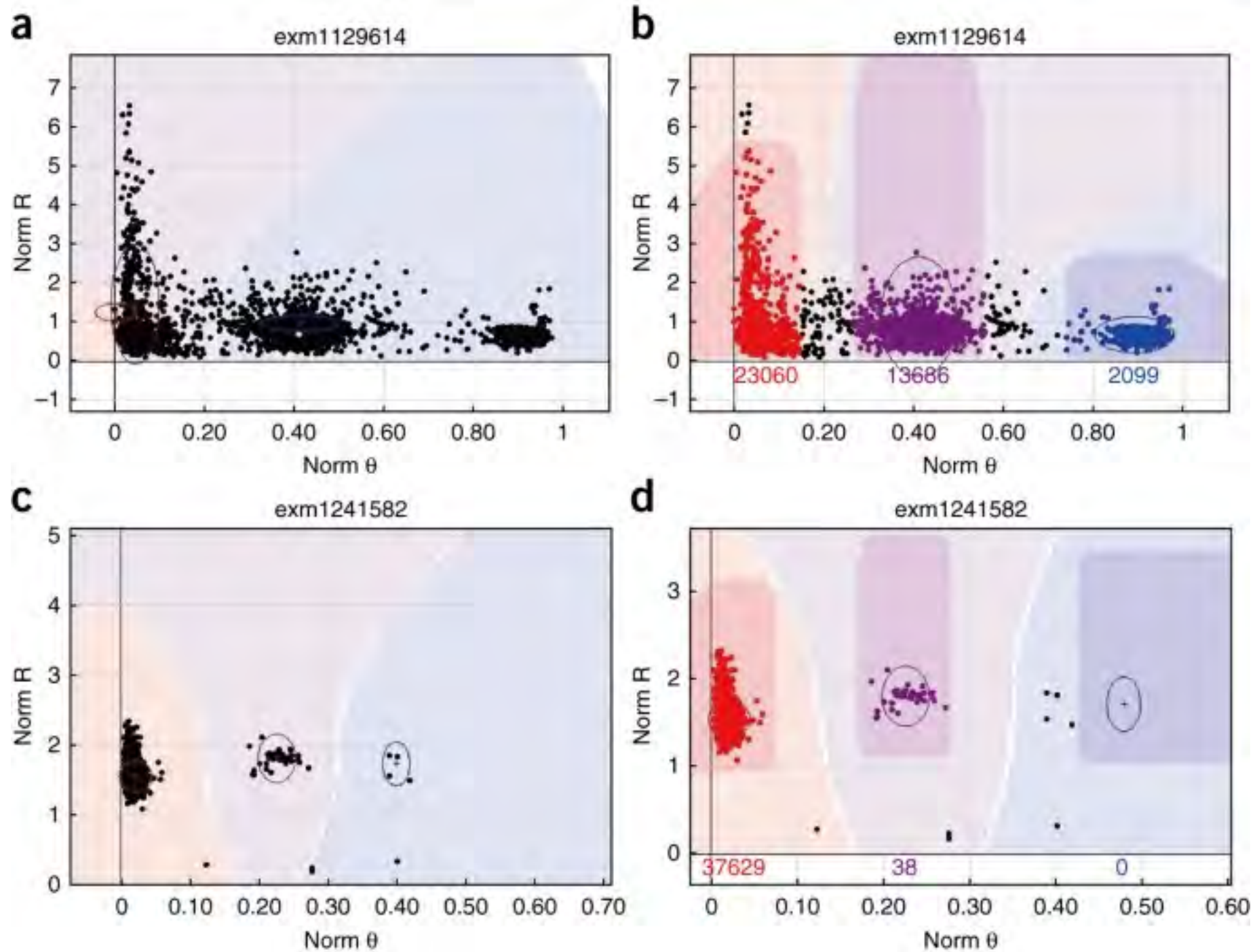
Микрочипы Illumina: Технология



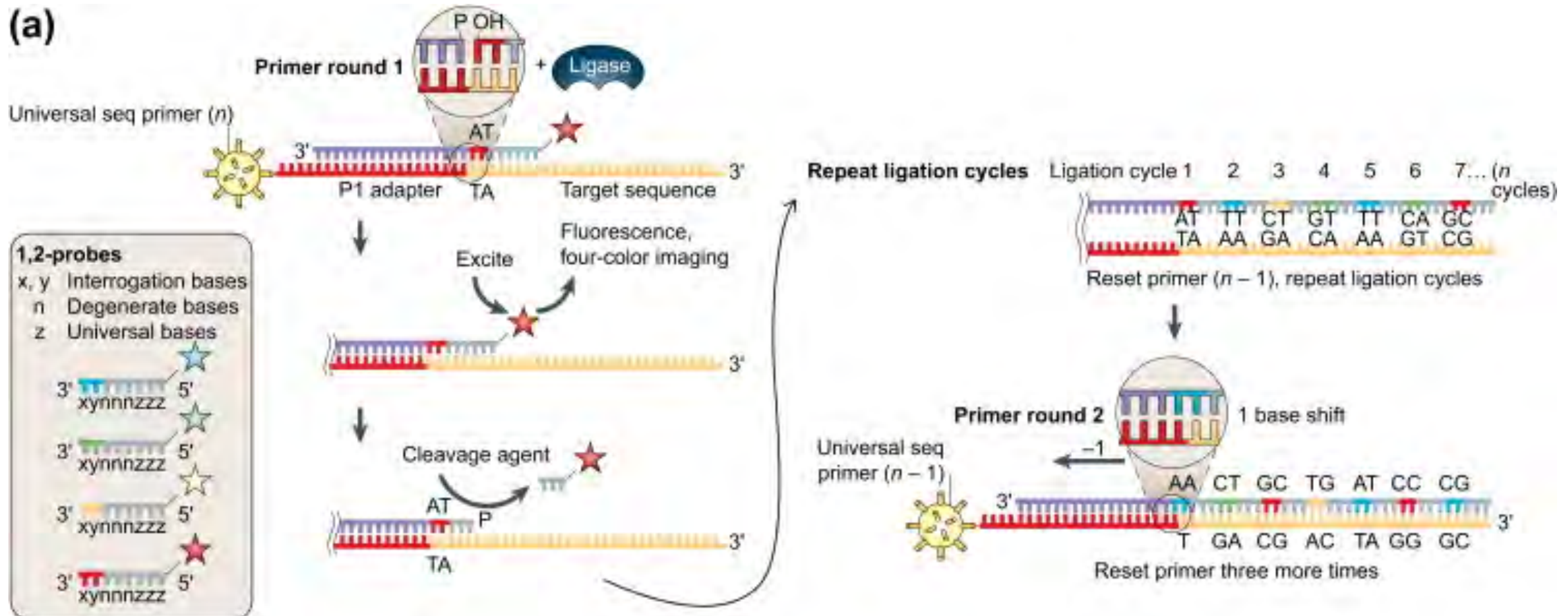
Микрочипы Illumina: Технология



Микрочипы Illumina: обработка данных



Исторические NGS методы: SOLiD



(b)

(b)

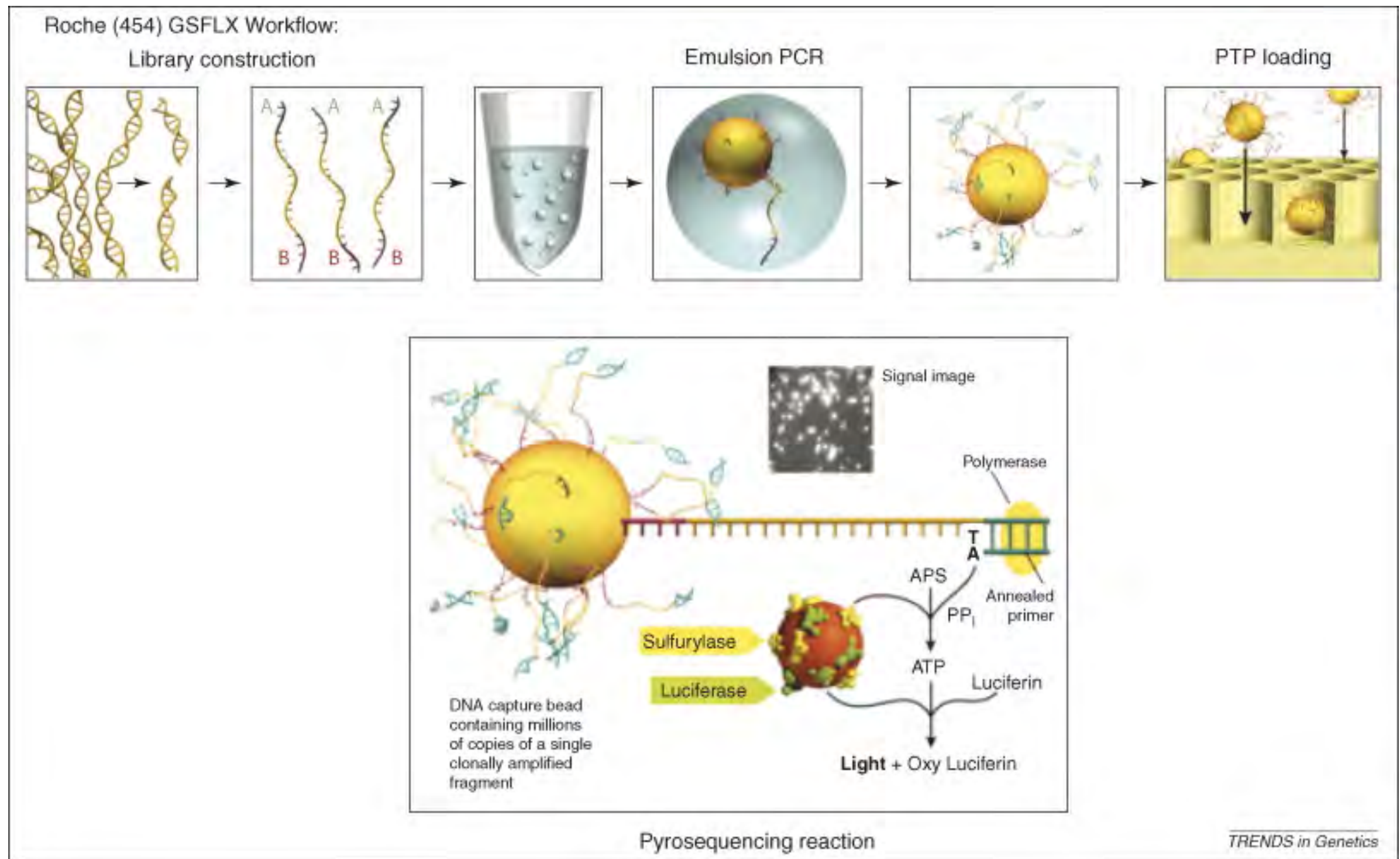
		Read Position																																					
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35		
Primer Round	1	Universal seq primer (n) 3' TTTTTTTTTT																																					
	2	Universal seq primer (n-1) 3' TTTTTTTTTT																																					
	3	Universal seq primer (n-2) 3' TTTTTTTTTT																																					
	4	Universal seq primer (n-3) 3' TTTTTTTTTT																																					
	5	Universal seq primer (n-4) 3' TTTTTTTTTT																																					

● Indicates positions of interrogation Ligation Cycle 1 2 3 4 5 6 7

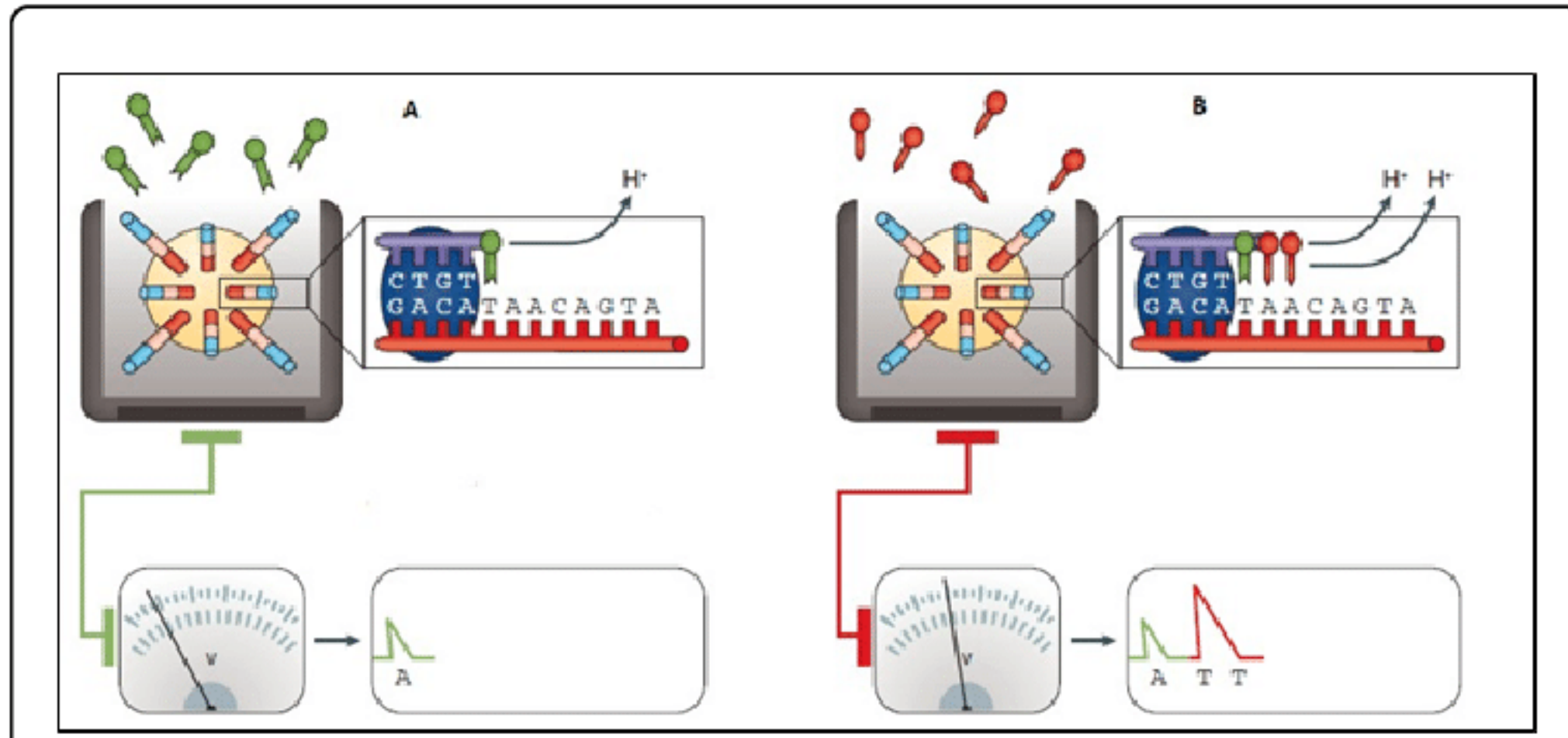
• Indicates positions of interrogation Ligation Cycle 1 2 3 4 5 6 7

DUAL INTERROGATION OF EACH BASE

Исторические NGS методы: 454 Pyrosequencing

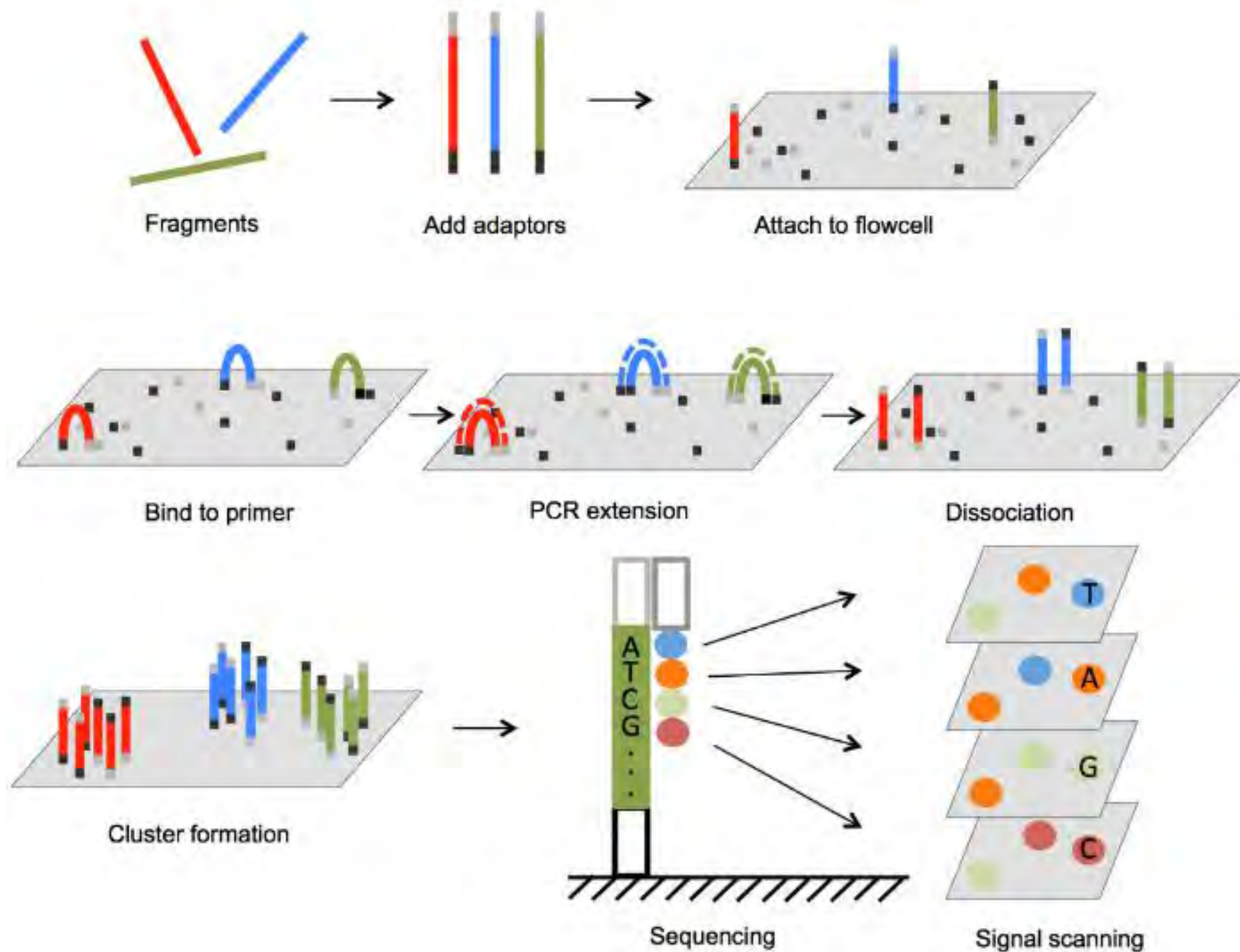


Ion Torrent: Принцип действия



Ion Torrent: Выходные данные

Illumina: Технология секвенирования



Illumina: Выходные данные

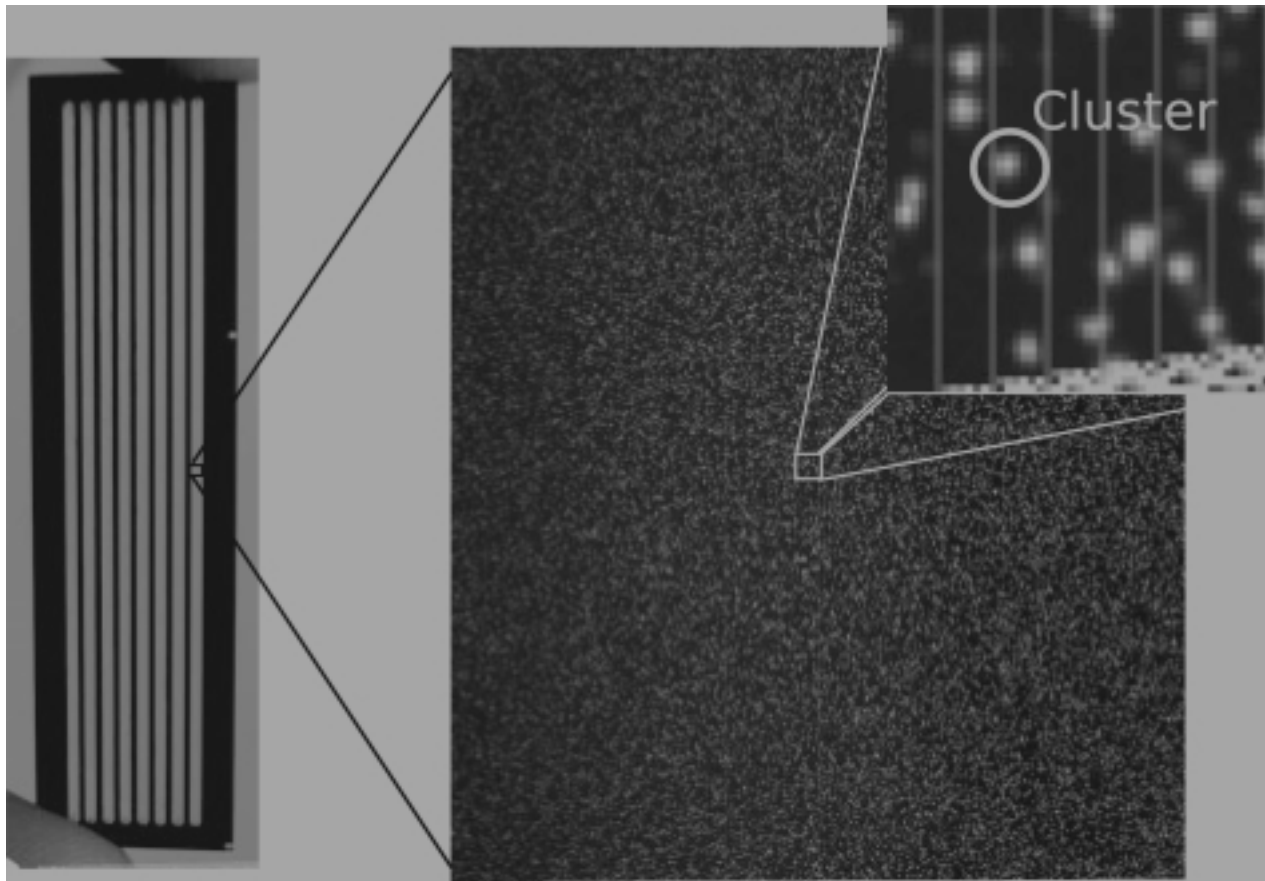


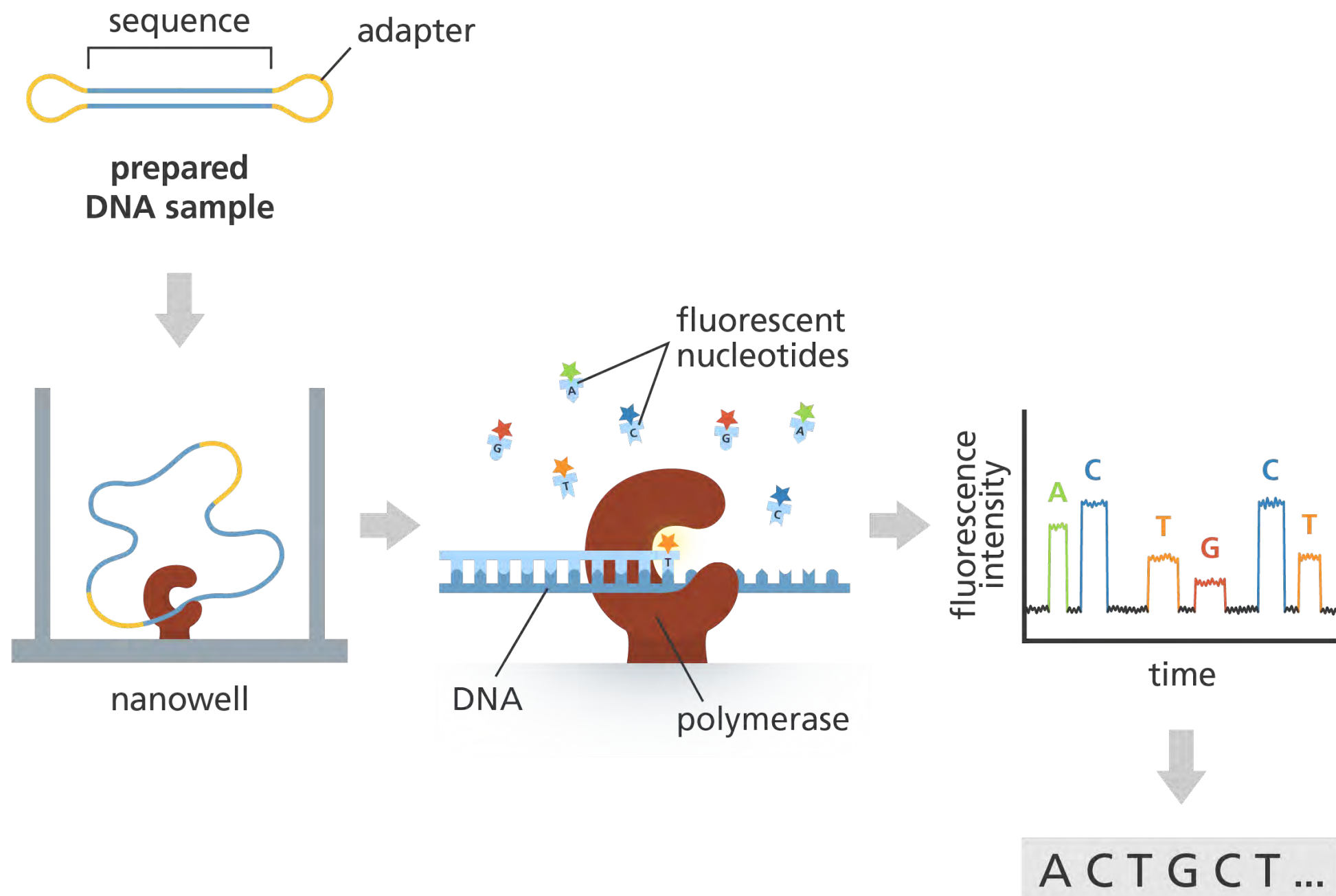
Diagram illustrating the structure of an Illumina sequencing output line, with labels pointing to specific components:

```
@FORJUSP02AJWD1  
CCGTCAATTCATTAAAGTTTAACTTGCGGCCGTACTCCCAGGCGGT  
+  
AAAAAAAAAAAA::99@:::??@@::FFAAAAACCAA:::BB@@?A?
```

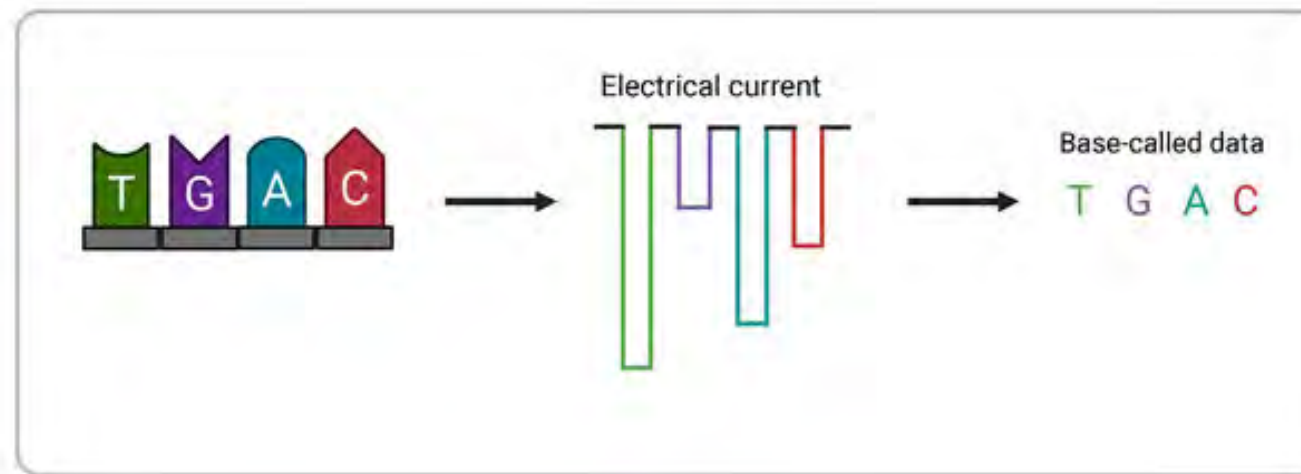
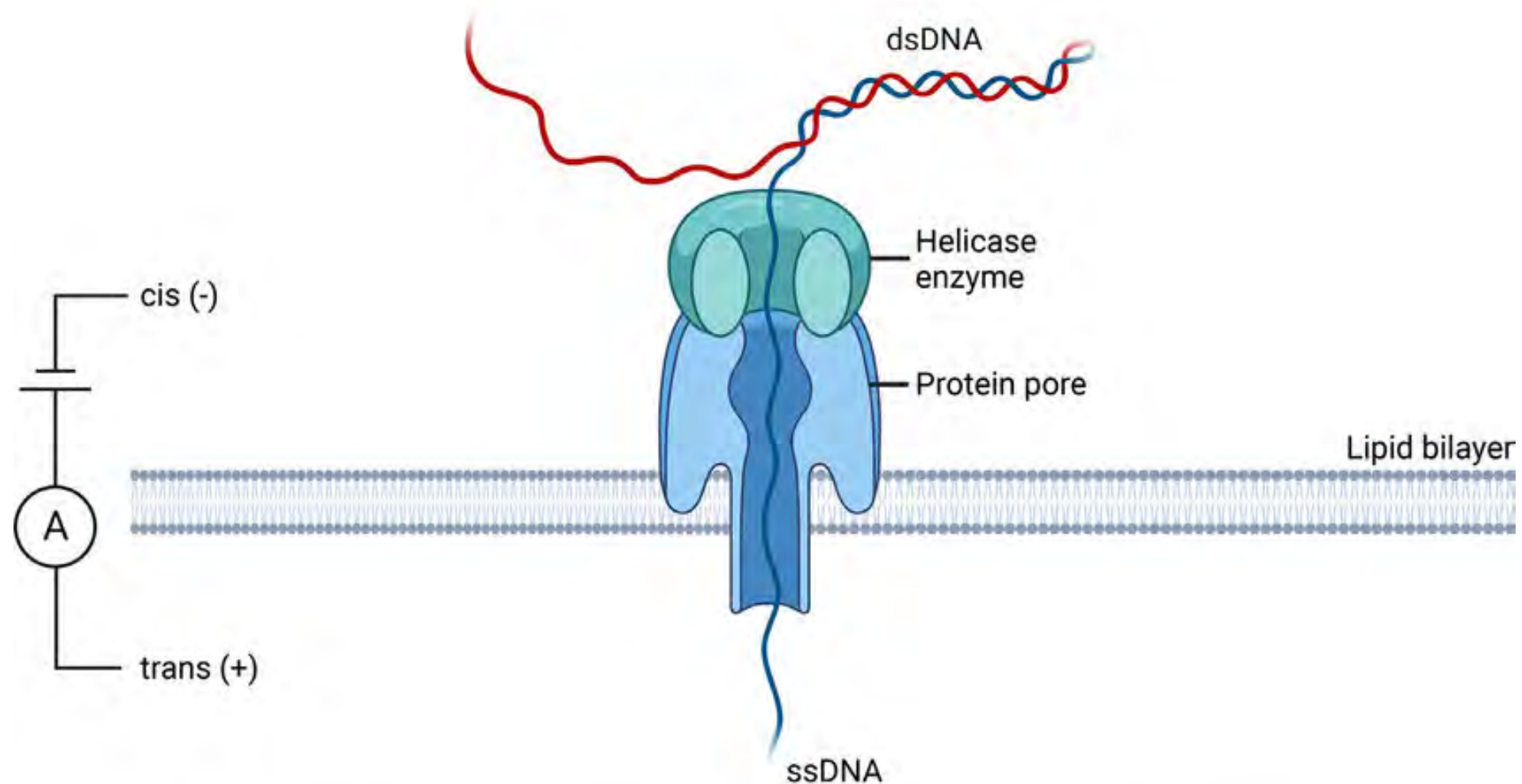
Labels and their corresponding parts:

- Label**: Points to the header line `@FORJUSP02AJWD1`.
- Sequence**: Points to the sequence line `CCGTCAATTCATTAAAGTTTAACTTGCGGCCGTACTCCCAGGCGGT`.
- Q scores (as ASCII chars)**: Points to the quality score line `AAAAAAAAAAAA::99@:::??@@::FFAAAAACCAA:::BB@@?A?`.
- Base=T, Q='!' = 25**: Points to the 10th character in the quality score line, which is `!`.

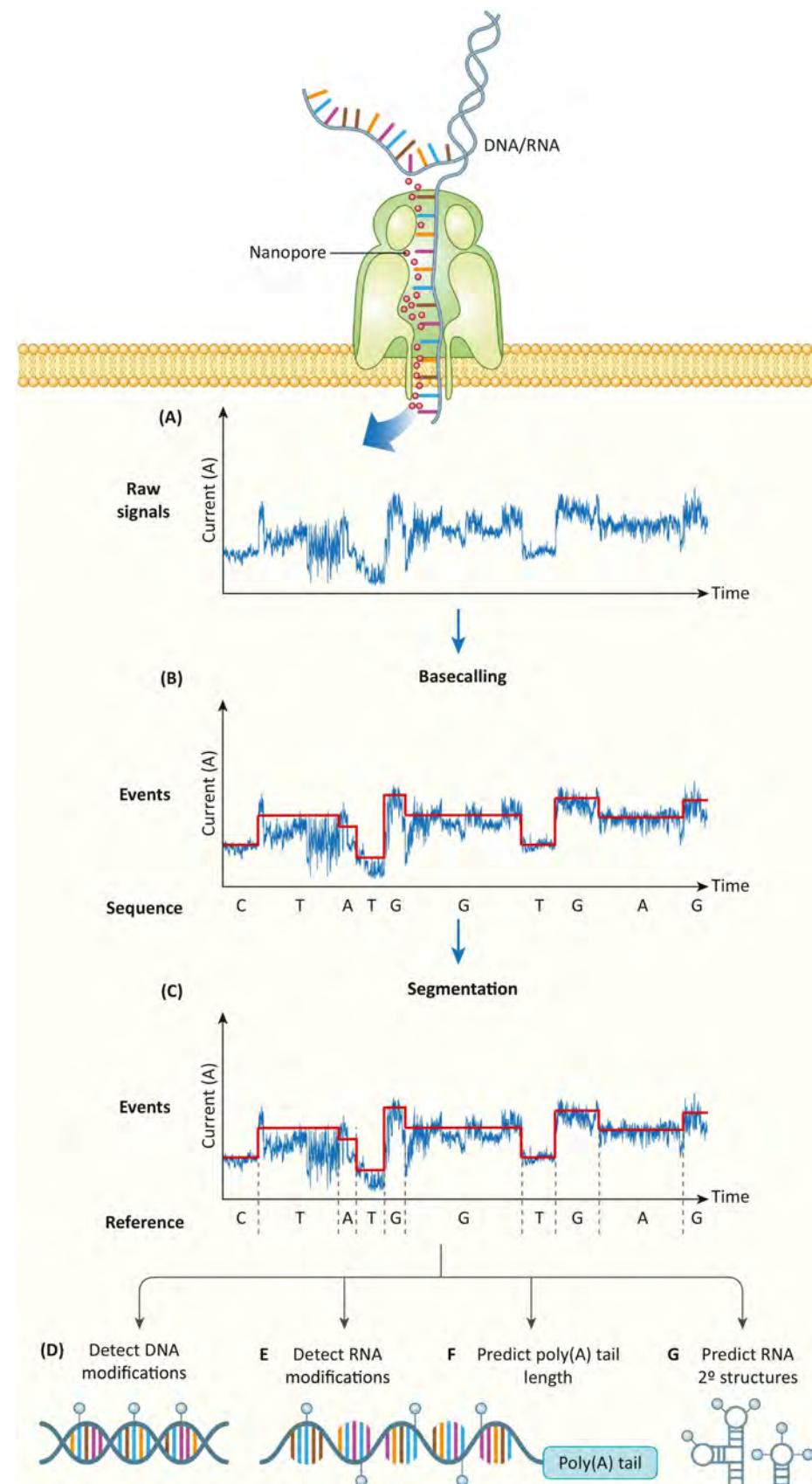
Pacific Biosciences (PacBio): SMRT секвенирование



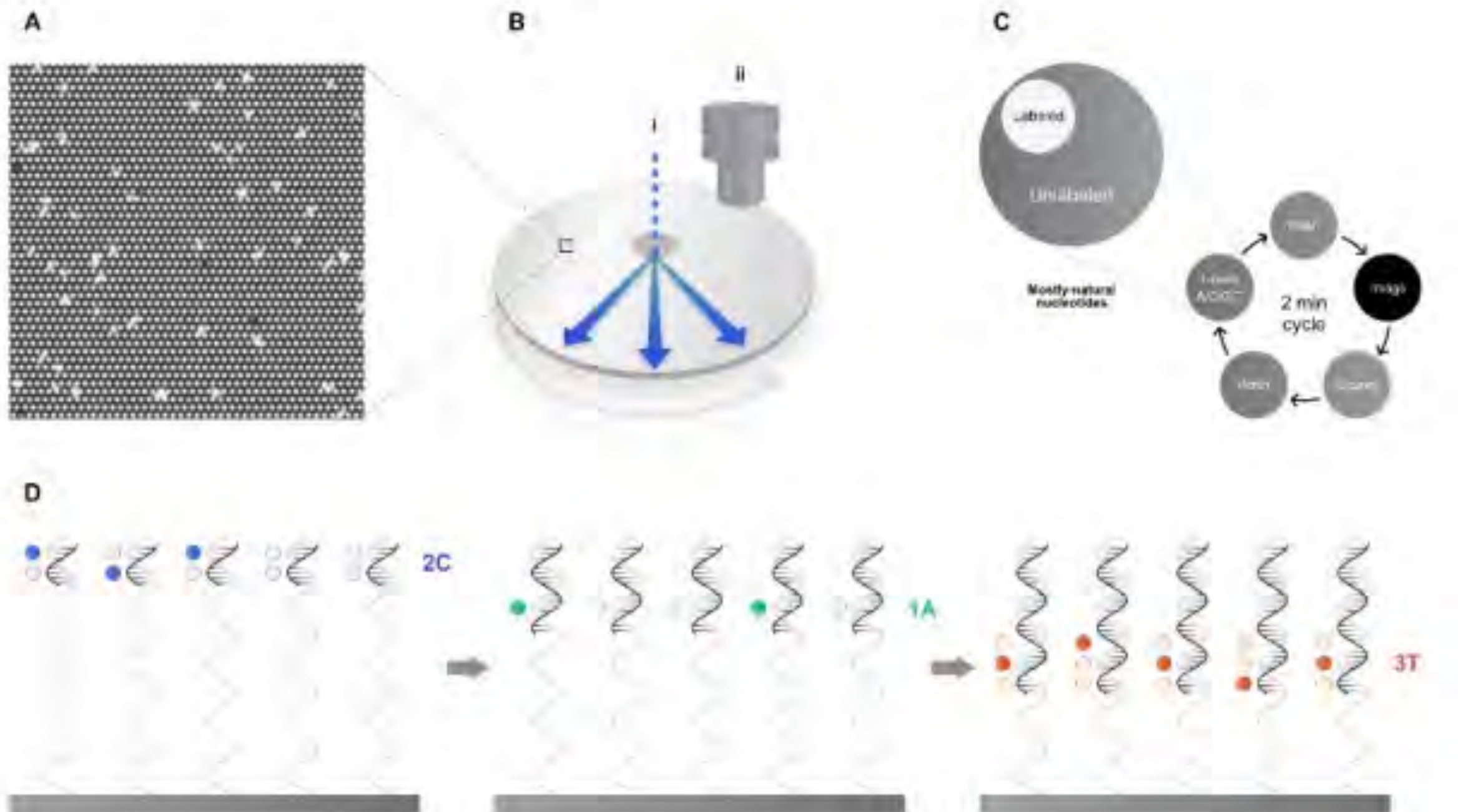
Oxford Nanopore Technologies: Нанопоровое секвенирование



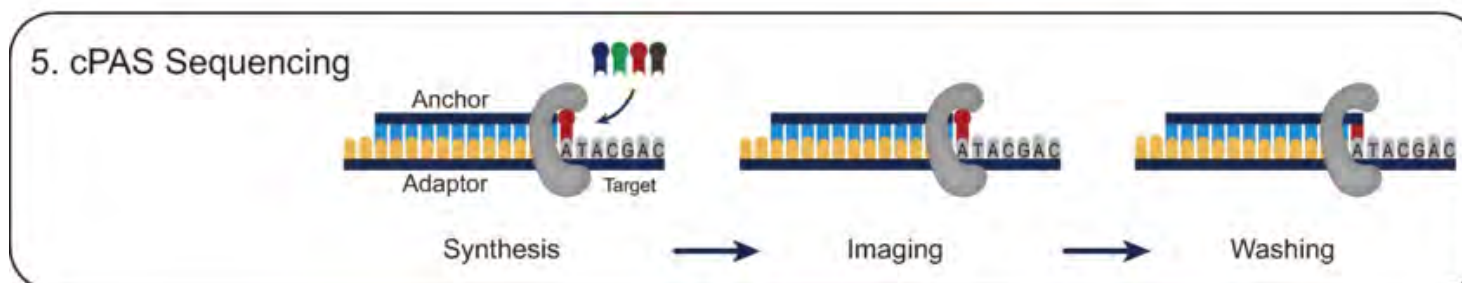
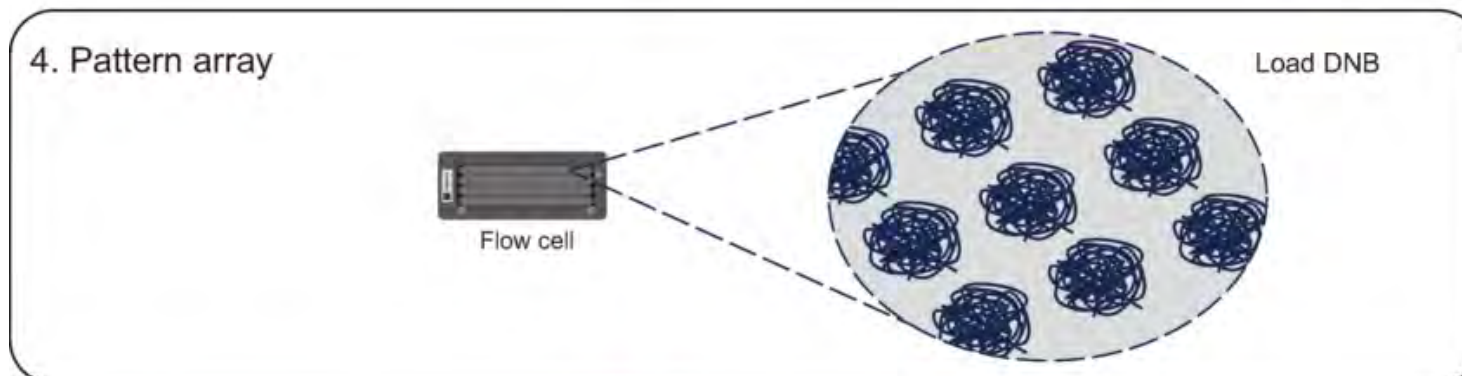
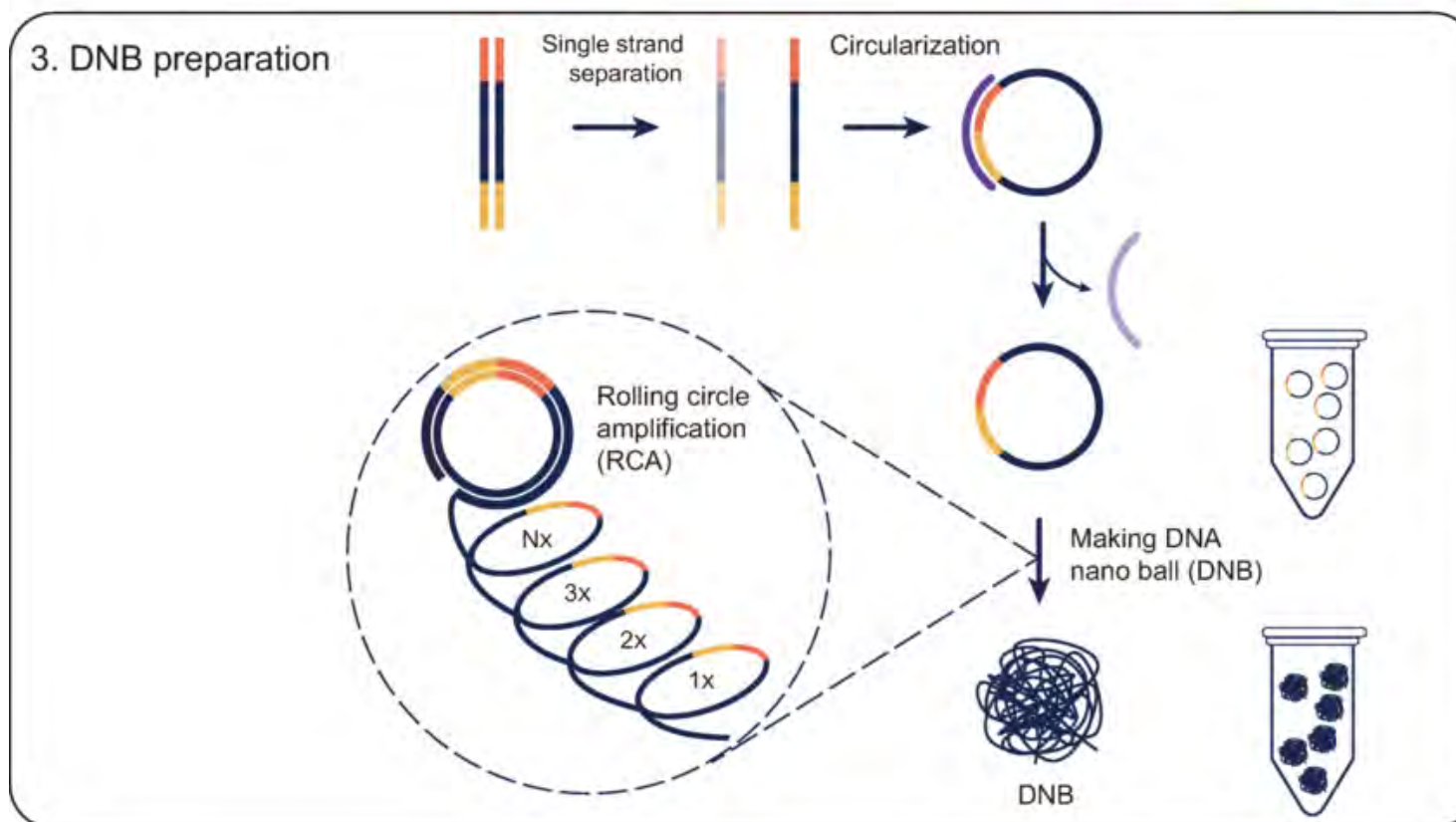
Анализ модификаций с Nanopore



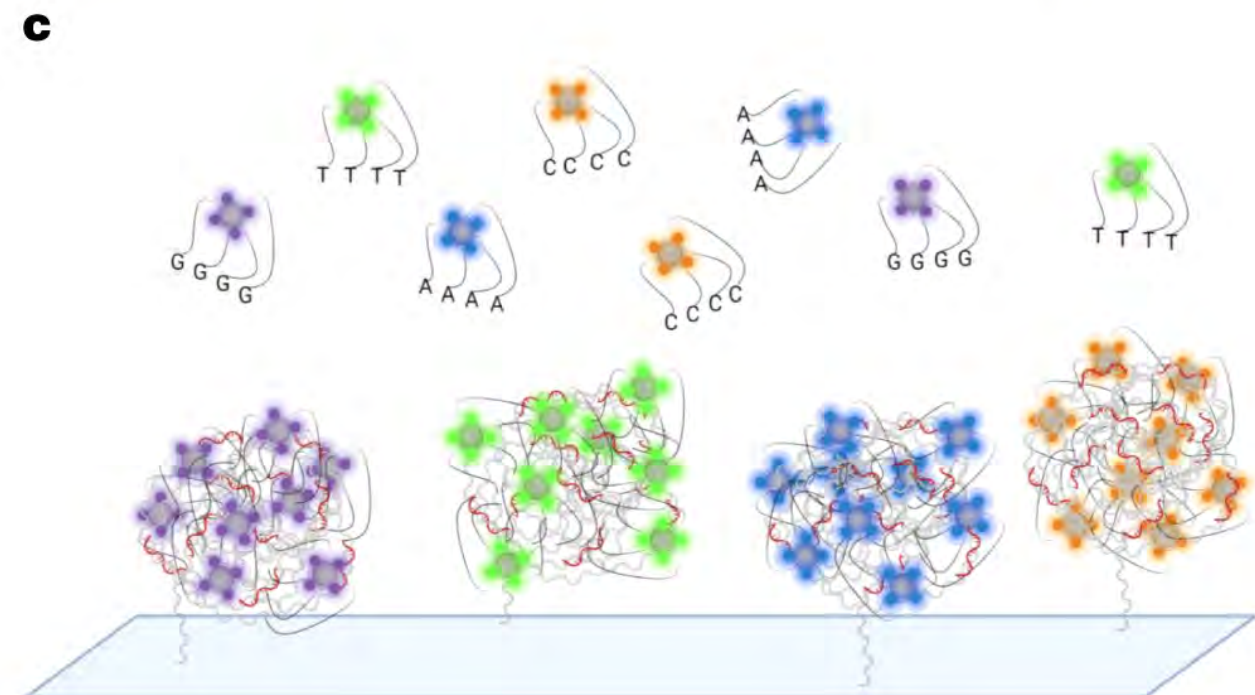
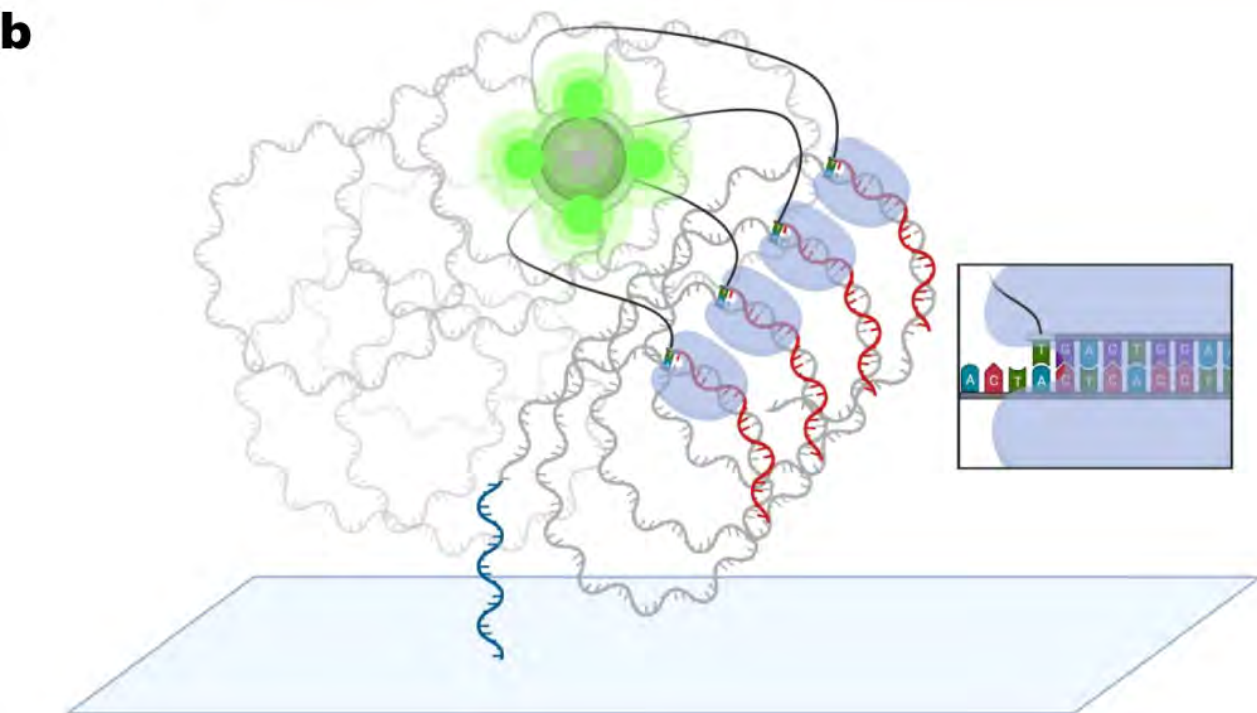
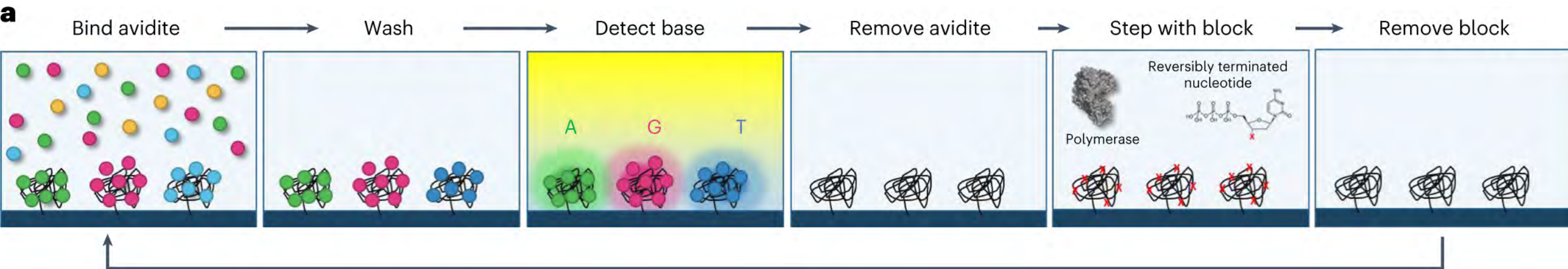
Новые подходы: Ultima Genomics



Новые подходы: MGI Tech

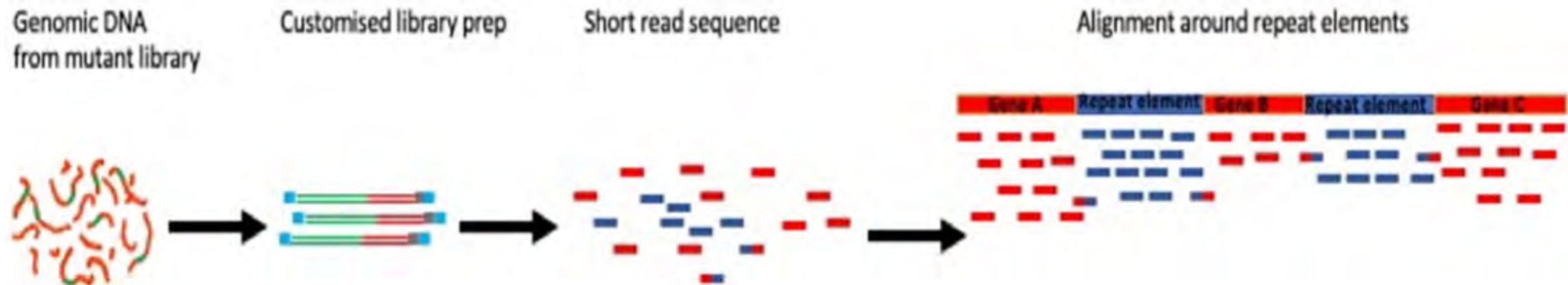


Новые подходы: Aviti

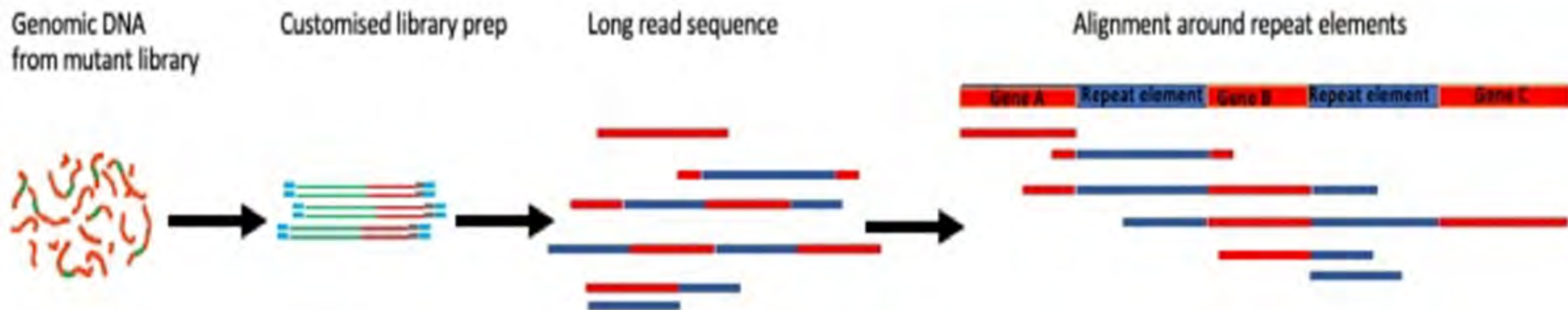


Выравнивание последовательностей

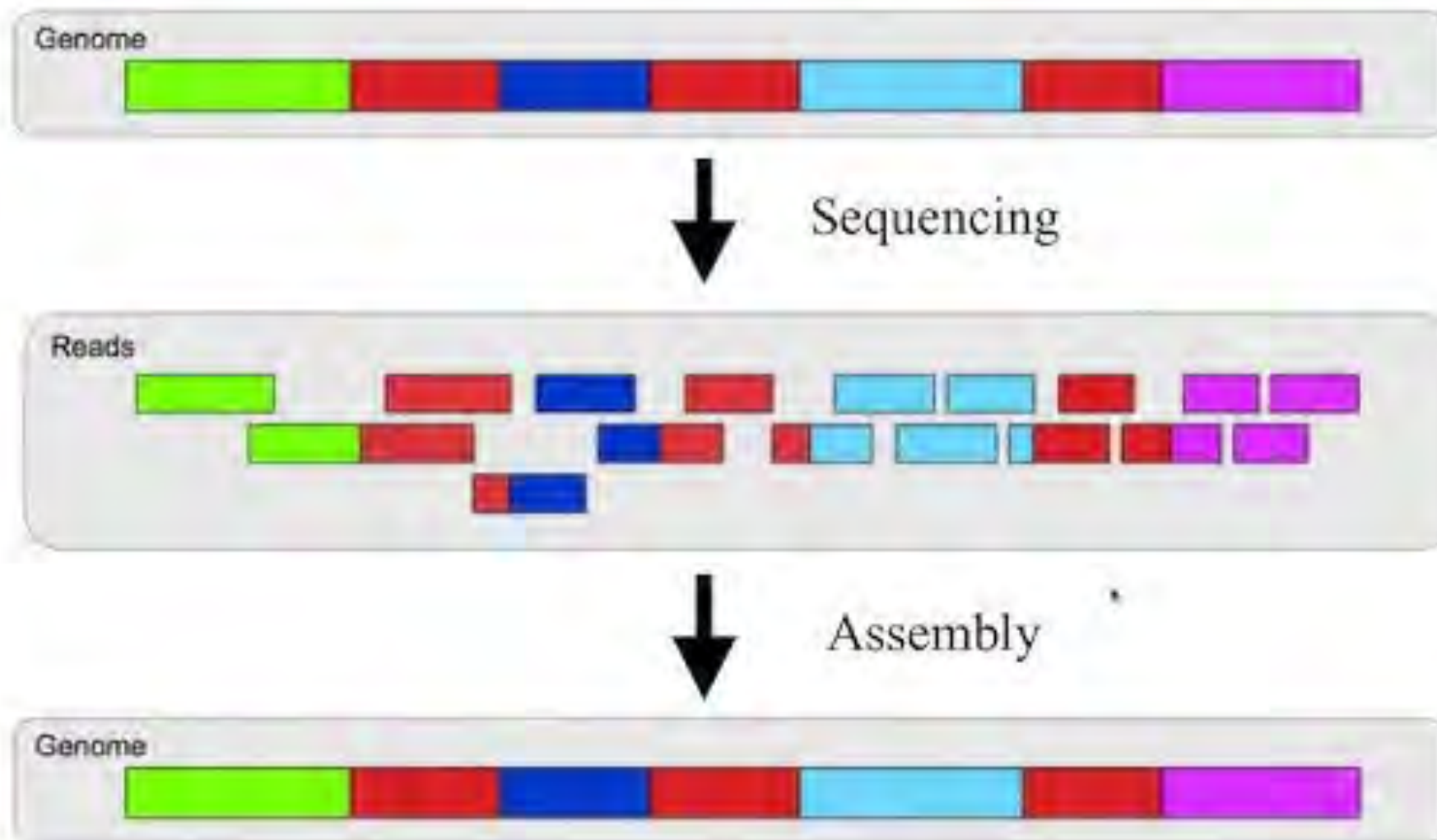
A



B



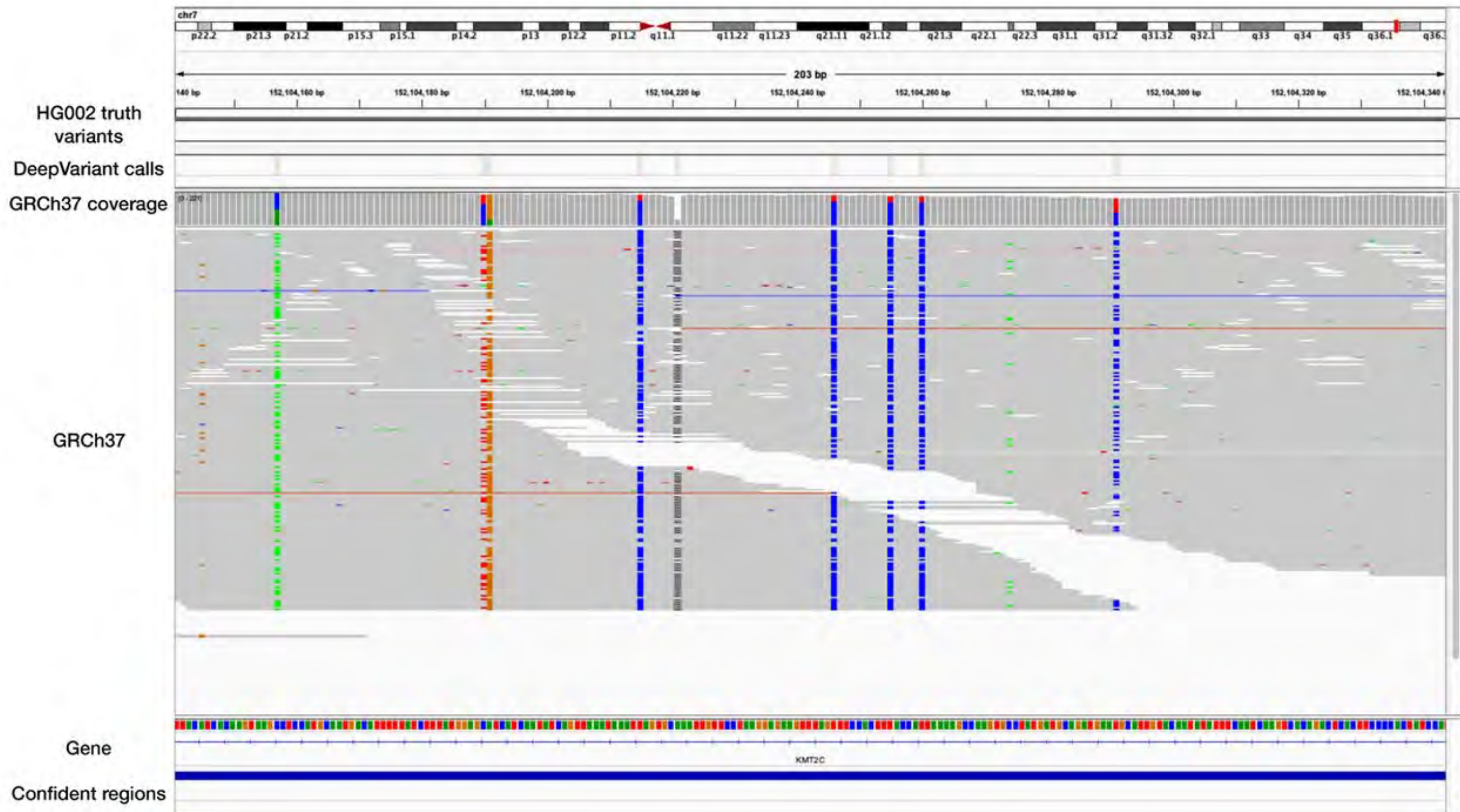
Сборка геномов de novo



Обнаружение генетических вариаций

Reference	CCGTTAGAGTTACAATTCGA
Read 2	TTAGAGTAAACAA
Read 3	CCGTTAGAGTTA
Read 4	TTACAATTCGA
Read 5	GAGTAAACAA
Read 6	TTAGAGTAAACAAT

Обнаружение генетических вариаций



DNA-seq: Подготовка библиотек

Fragmented input DNA

End repair

Input DNA blunting

Ligation 1

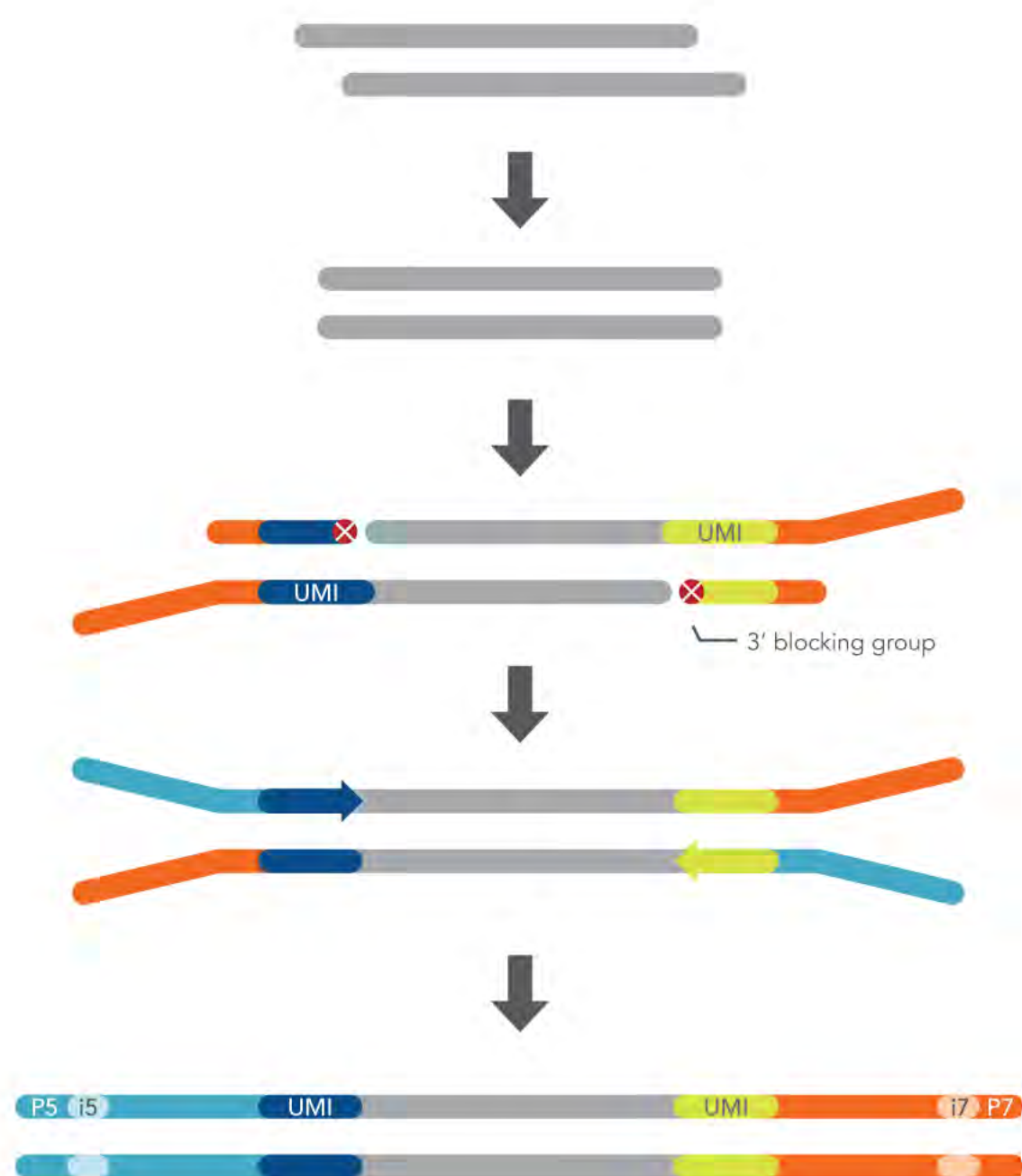
Single-stranded ligation of
Ligation 1 Adapter to 3' ends of insert

Ligation 2

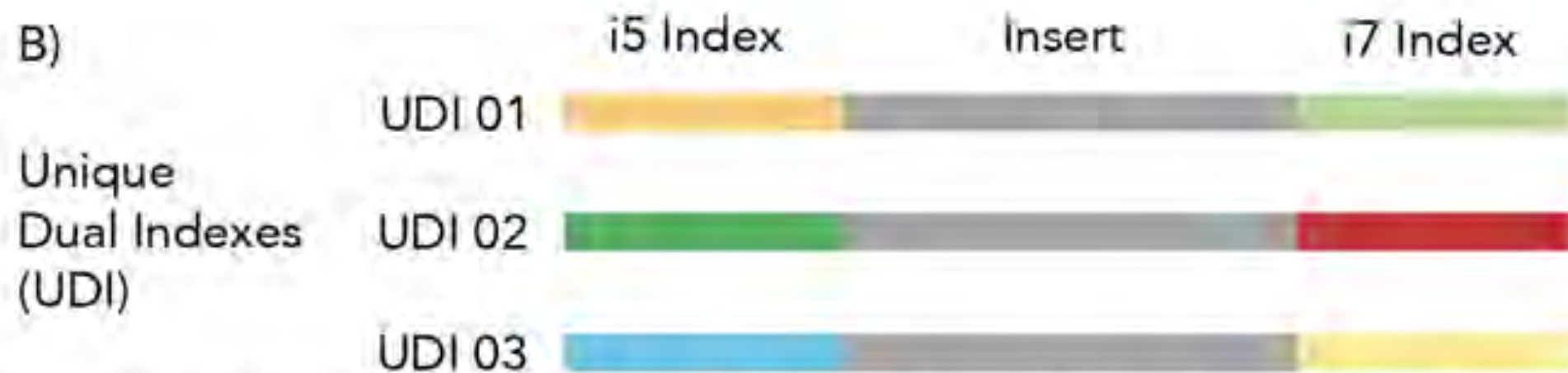
Ligation 2 Adapter primes gap filling across
the UMI followed by 5' ligation

PCR

Amplification with xGen™ Unique Dual Index (UDI)
Primer Pairs

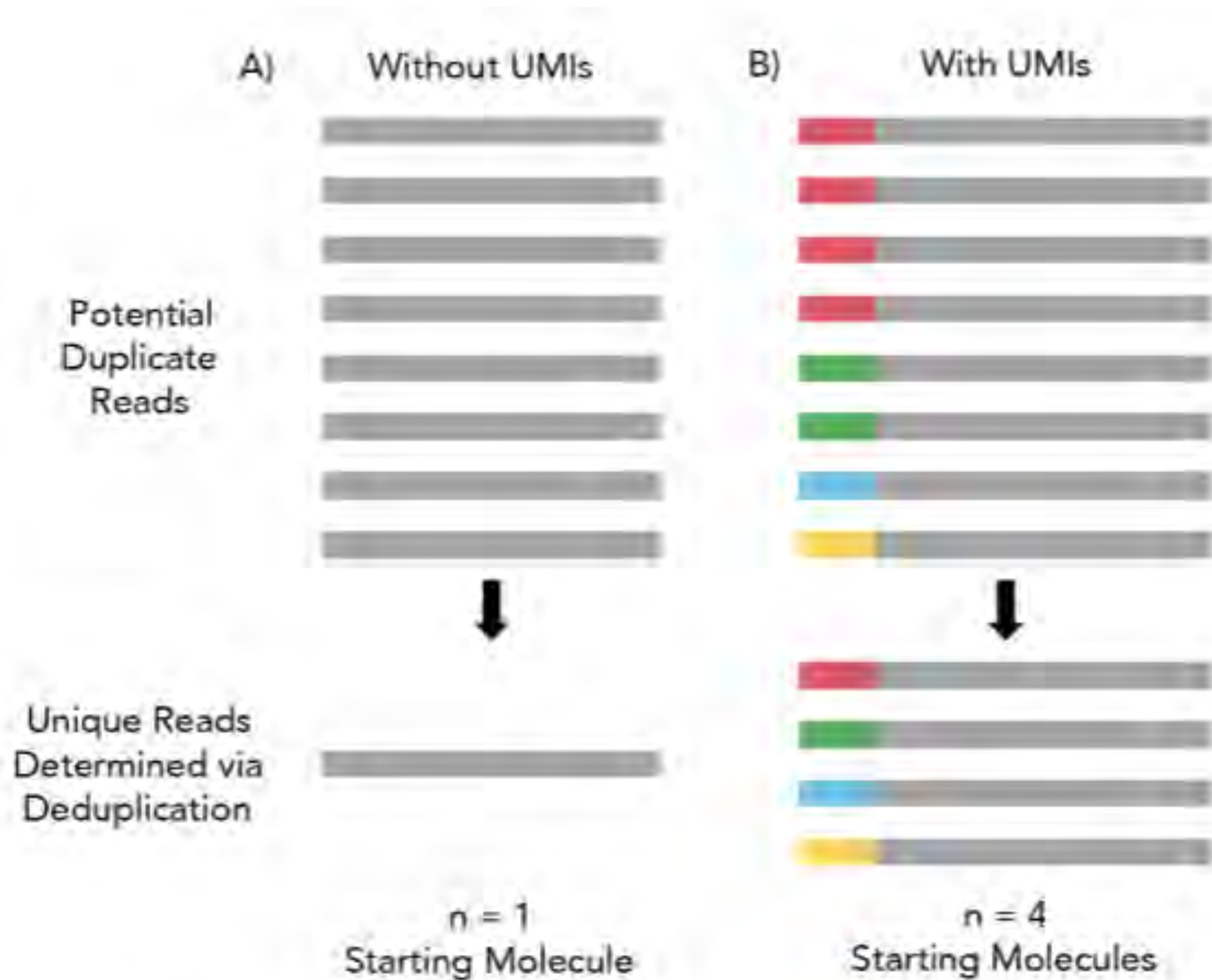


DNA-seq: Подготовка библиотек

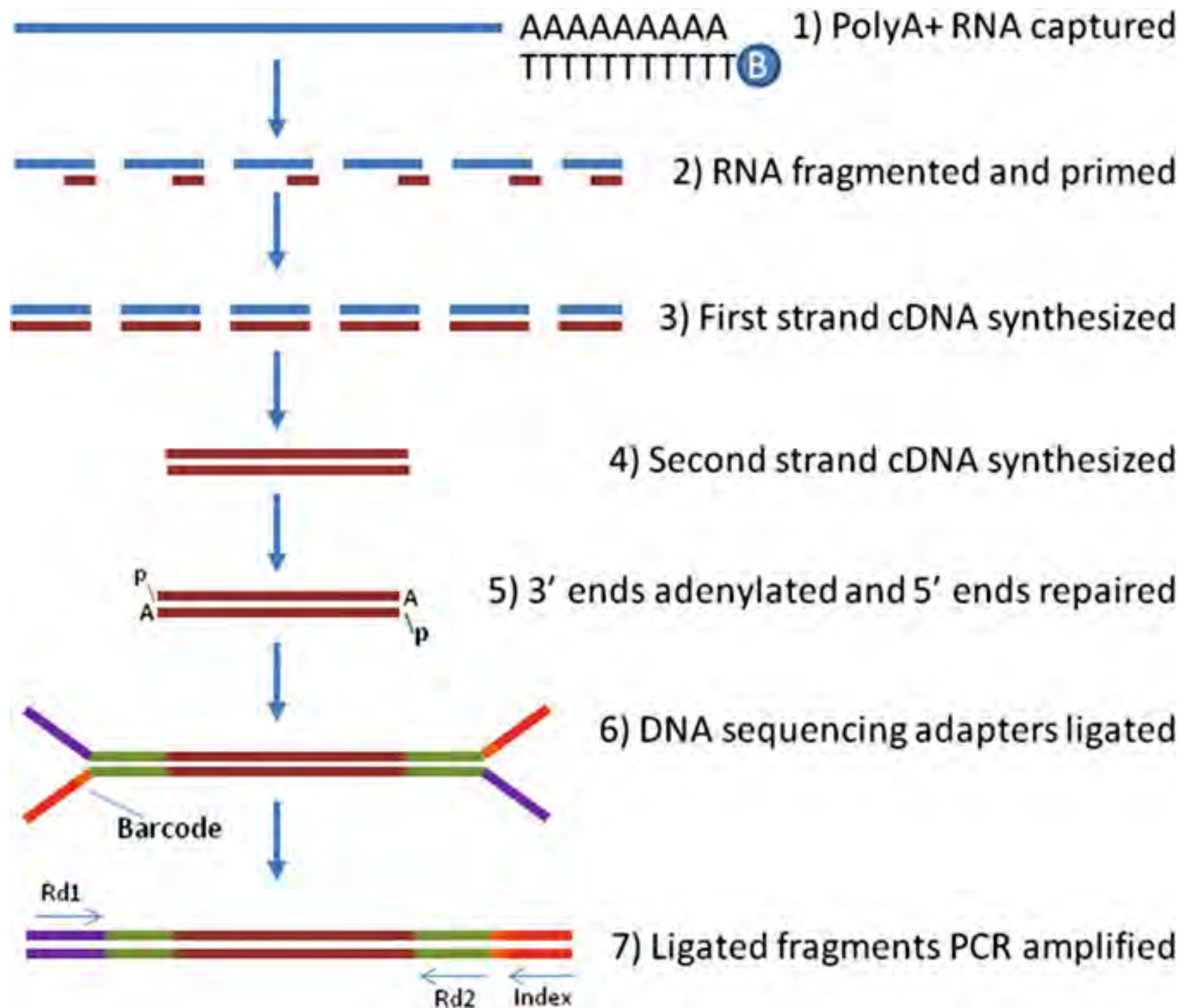


A) Combinatorial dual indexing has repeated sequences across the rows and columns of a primer plate in contrast to B) unique dual indexing where every sequence is unique.

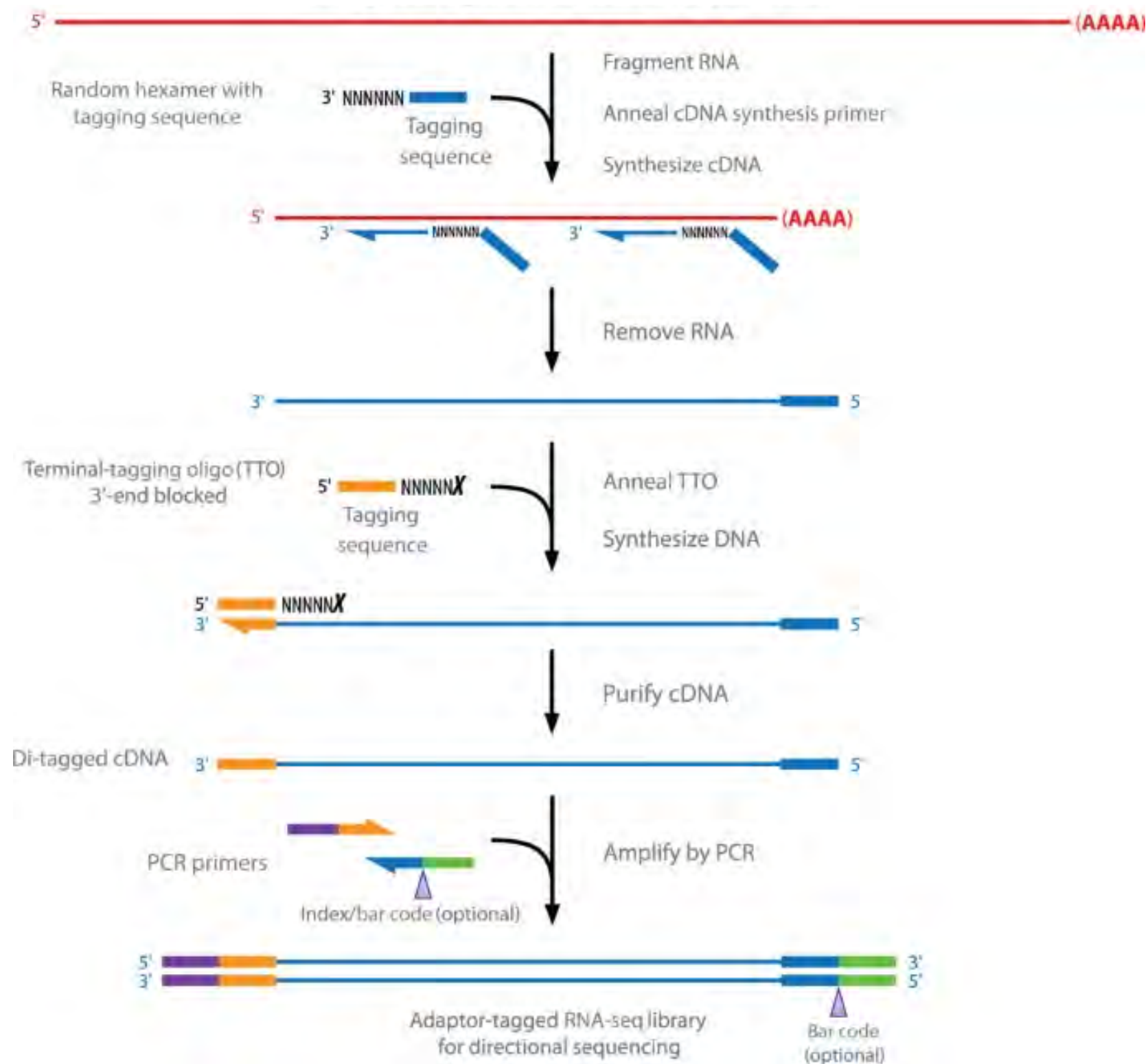
DNA-seq: Подготовка библиотек



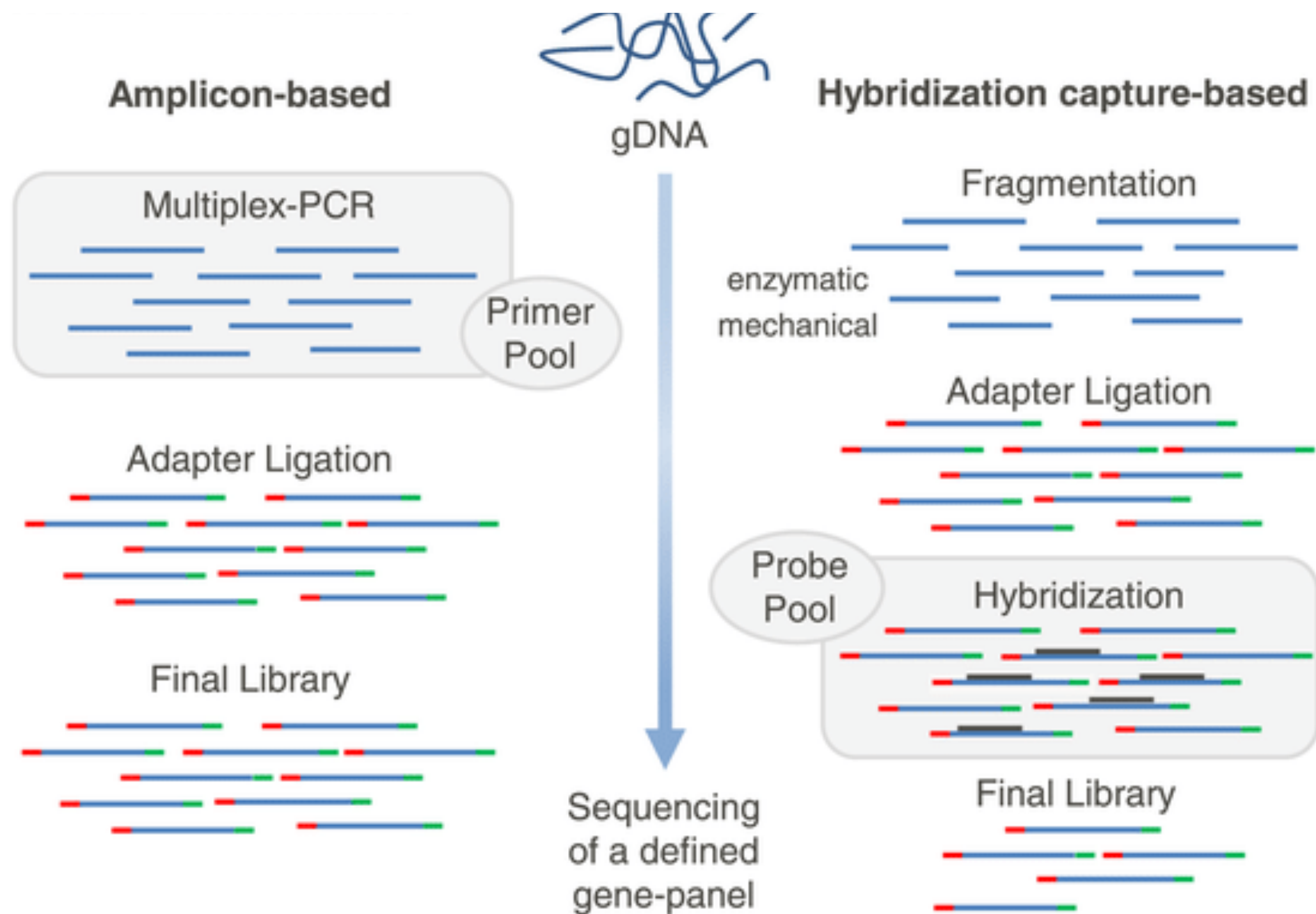
RNA-seq: Подготовка библиотек



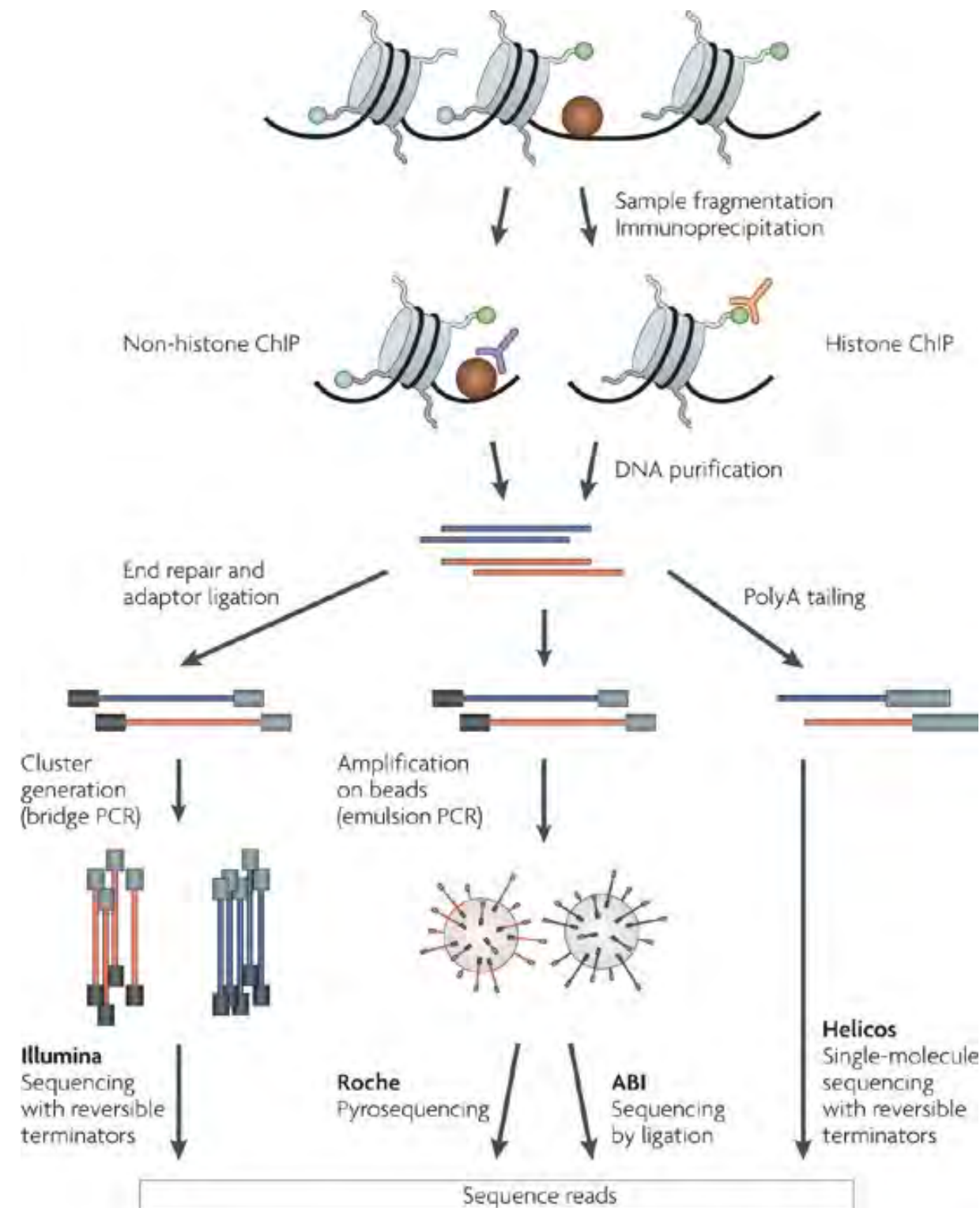
RNA-seq: Stranded vs. Non-stranded



Обогащение и экзомное секвенирование



ChIP-seq: Подготовка образцов

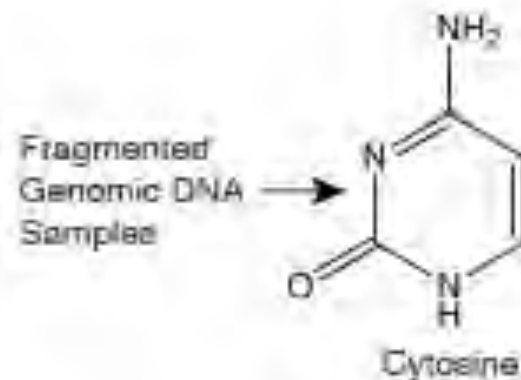


Methyl-seq: Анализ метилирования

STEP 1

Denaturation

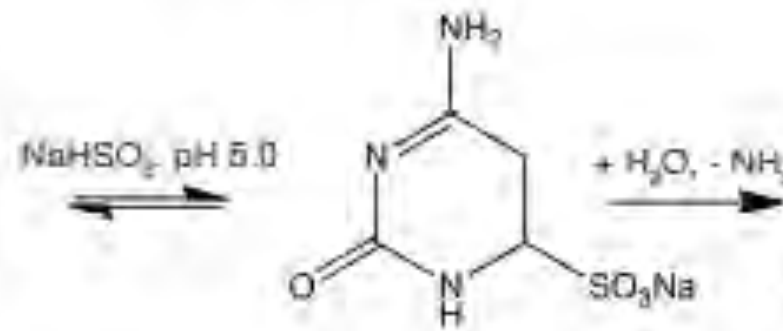
Incubation at 98°C
fragments genomic DNA



STEP 2

Conversion

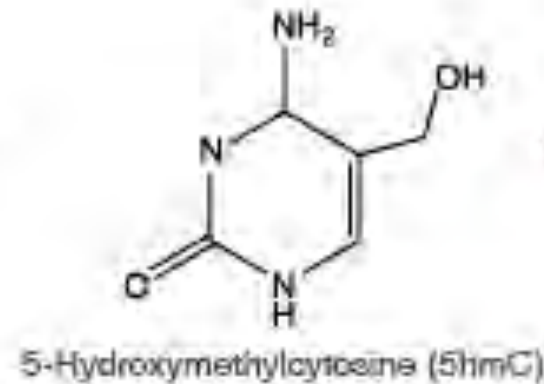
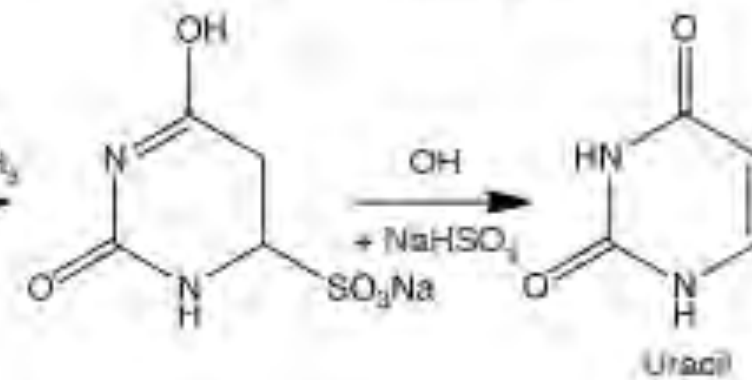
Incubation with sodium bisulfite
at 64°C and low pH (5-6)
deaminates cytosine residues
in fragmented DNA.



STEP 3

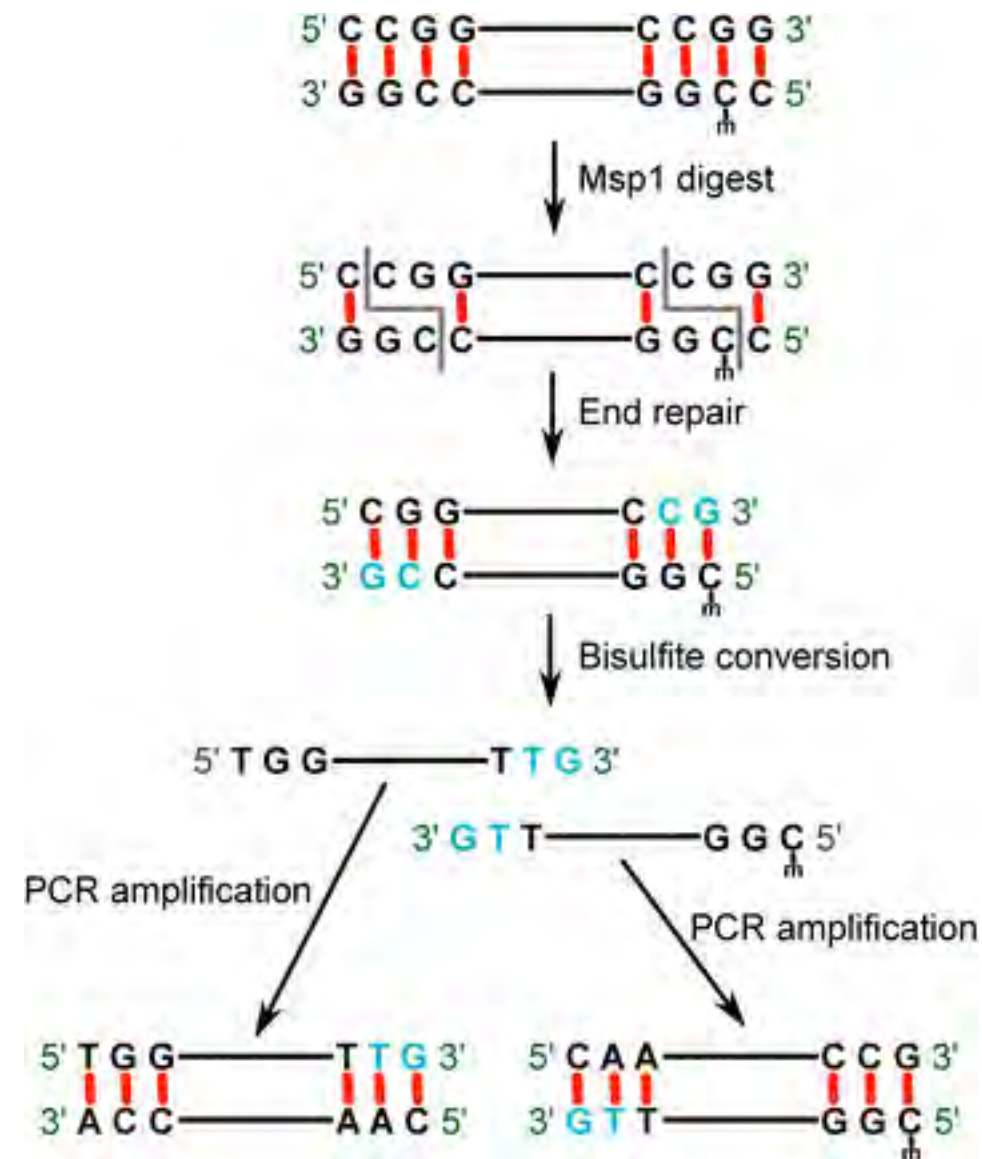
Desulphonation

Incubation at high pH
at room temperature for
15 min removes the
sulfite moiety,
generating uracil



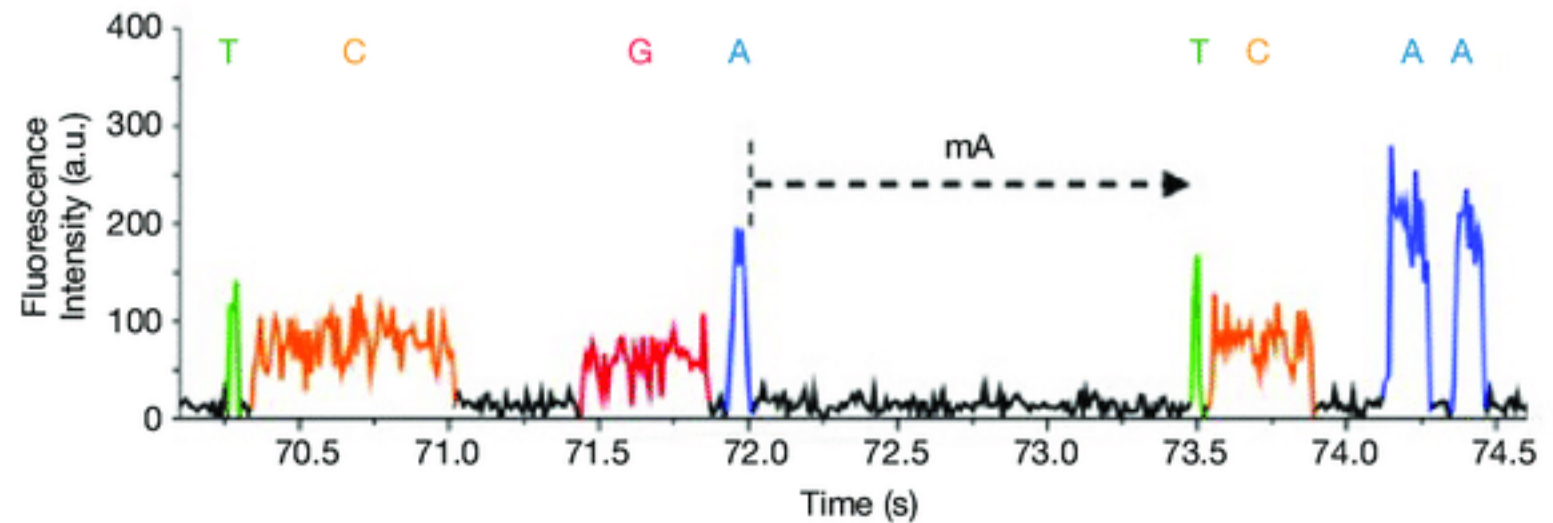
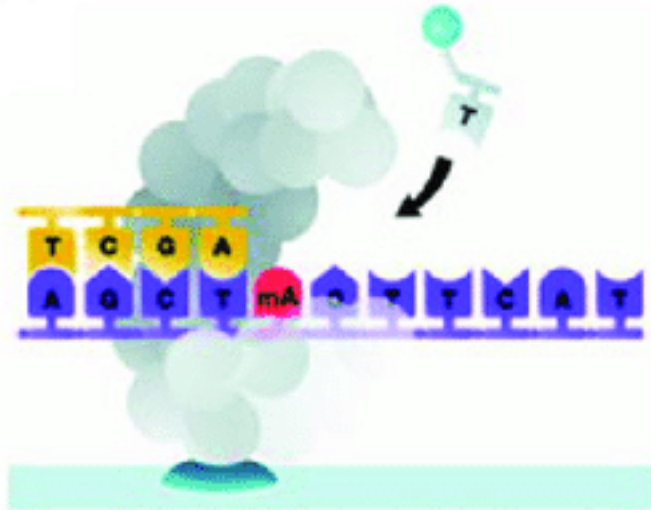
5mC and 5hmC are not
susceptible to bisulfite
conversion and remain intact

RRBS: Анализ метилирования

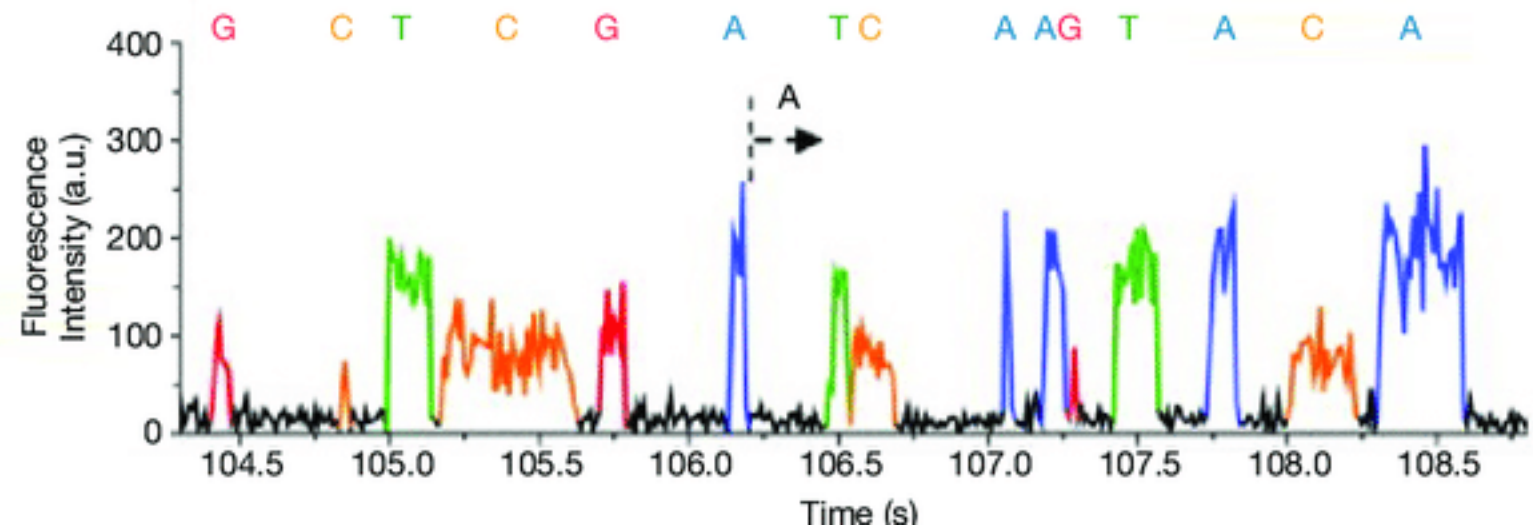
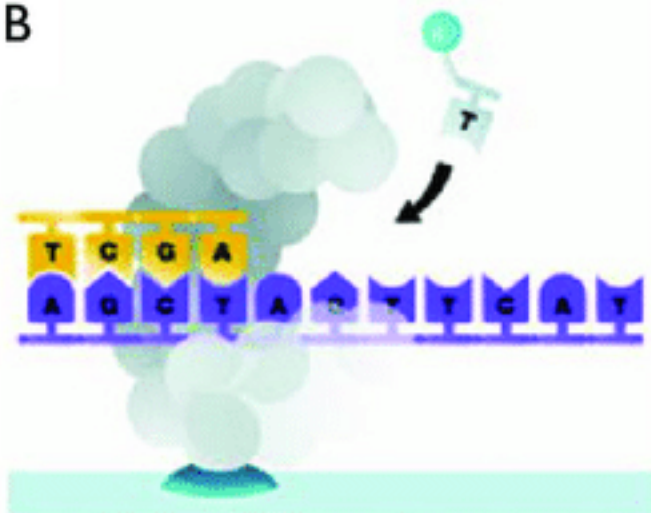


Анализ модификаций с PacBio

A



B



Вопросы и обсуждение