PM401
Basic Microbiology

Applied Microbial Genetics

(2015)

Applied Genetics

2. Basic DNA techniques

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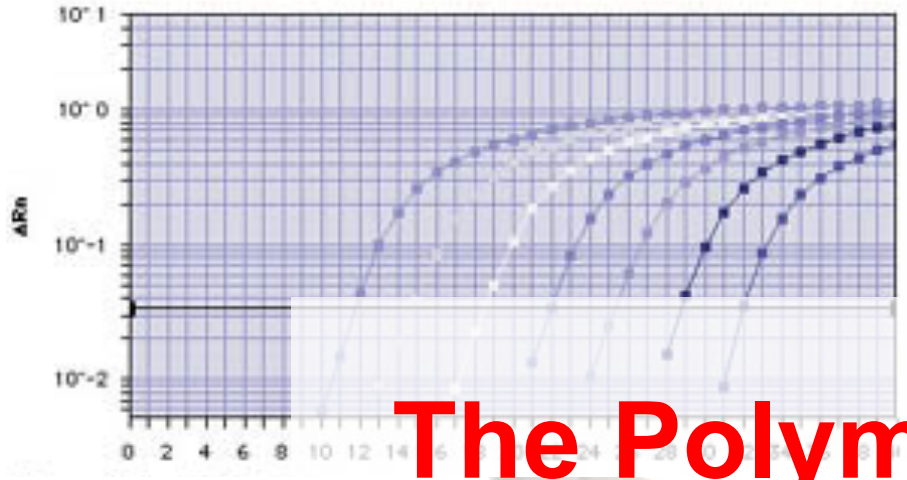
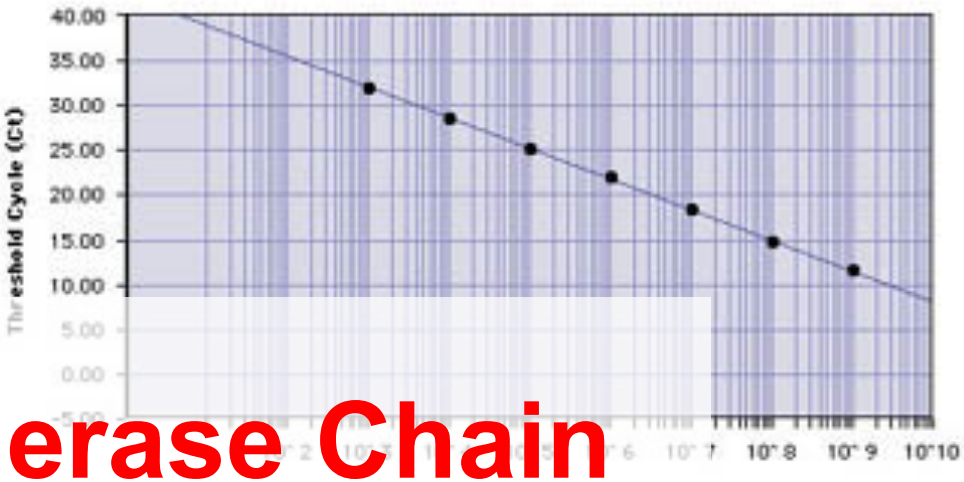
28 April 2015

Before we start

- **Nothing better than pictures and videos**

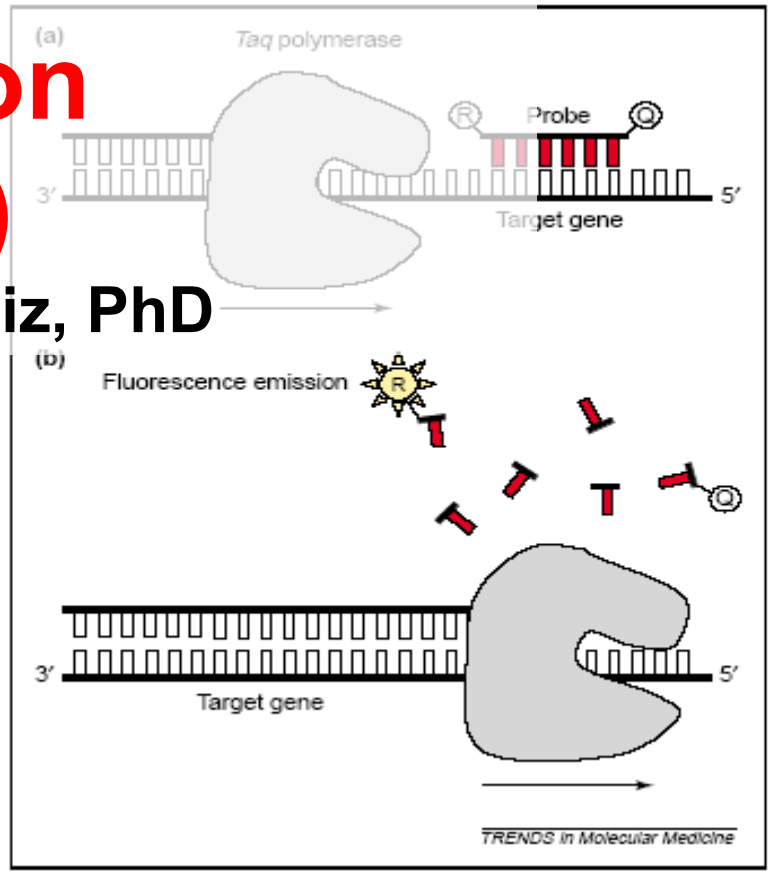
- **<u>ASSIGNMENT</u>:**
  1. Go to YouTube (http://youtube.com) or DNATube (http://www.dnatube.com/) or any other video server you like (e.g., Vimeo)
  2. Pick a good video on any concept you learned in this part of the course:
     - DNA processes
     - Gene regulation
     - PCR (today's lecture)
  3. Email it to me (to: ramy.aziz@pharma.cu.edu.eg) with Subject: PM401-April28 Assignment (No title → No see!)





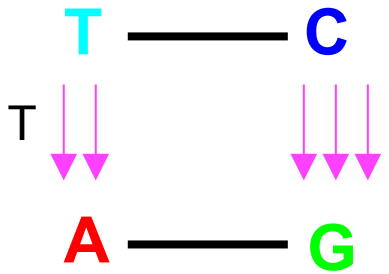
The Polymerase Chain Reaction (PCR)

Ramy Karam Aziz, PhD

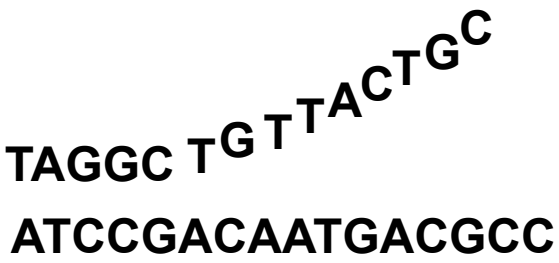


Principle of Hybridization Methods

1. **Hybridization** is binding two genetic sequences. The binding occurs because of the hydrogen bonds [pink] between base pairs.



2. When using hybridization, DNA must first be denatured, usually by using use heat or chemical.

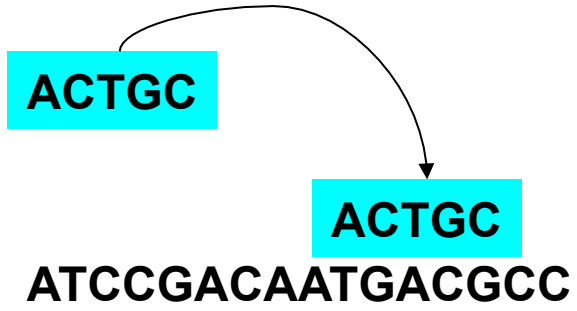


www.bioalgorithms.info

http://www.biology.washington.edu/fingerprint/radi.html

Principle of Hybridization Methods

3. Once DNA has been denatured, a single-stranded radioactive probe [light blue] can be used to see if the denatured DNA contains a sequence complementary to probe.



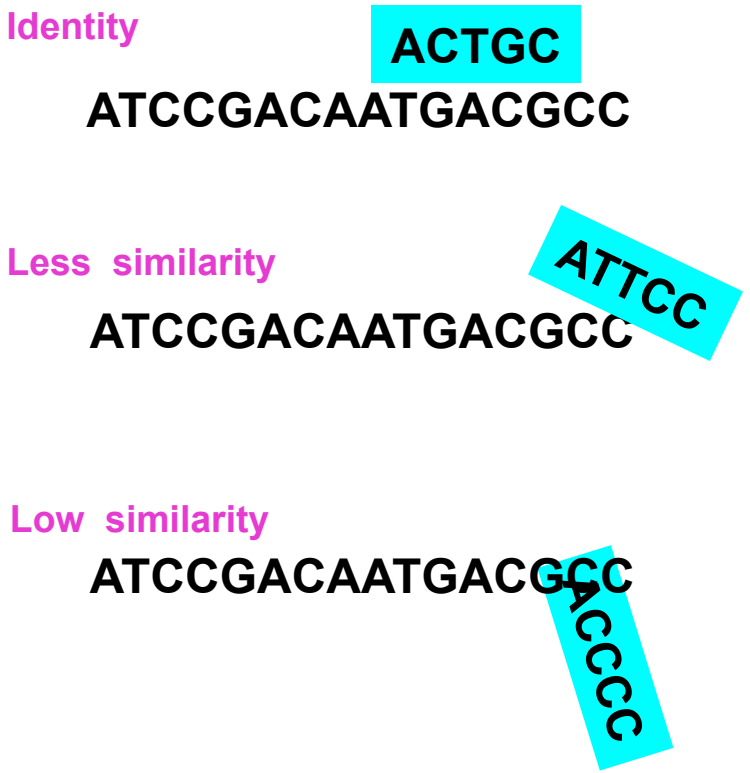
4. Sequences of varying <u>similarity</u> stick to the DNA even if the fit is poor.

http://www.biology.washington.edu/fingerprint/radi.html

Principle of Hybridization Methods

5. After washing, non-specific probes are washed away, and specific probes stay.

6. Probes can be labeled by a fluorescent or radioactive phosphate group.



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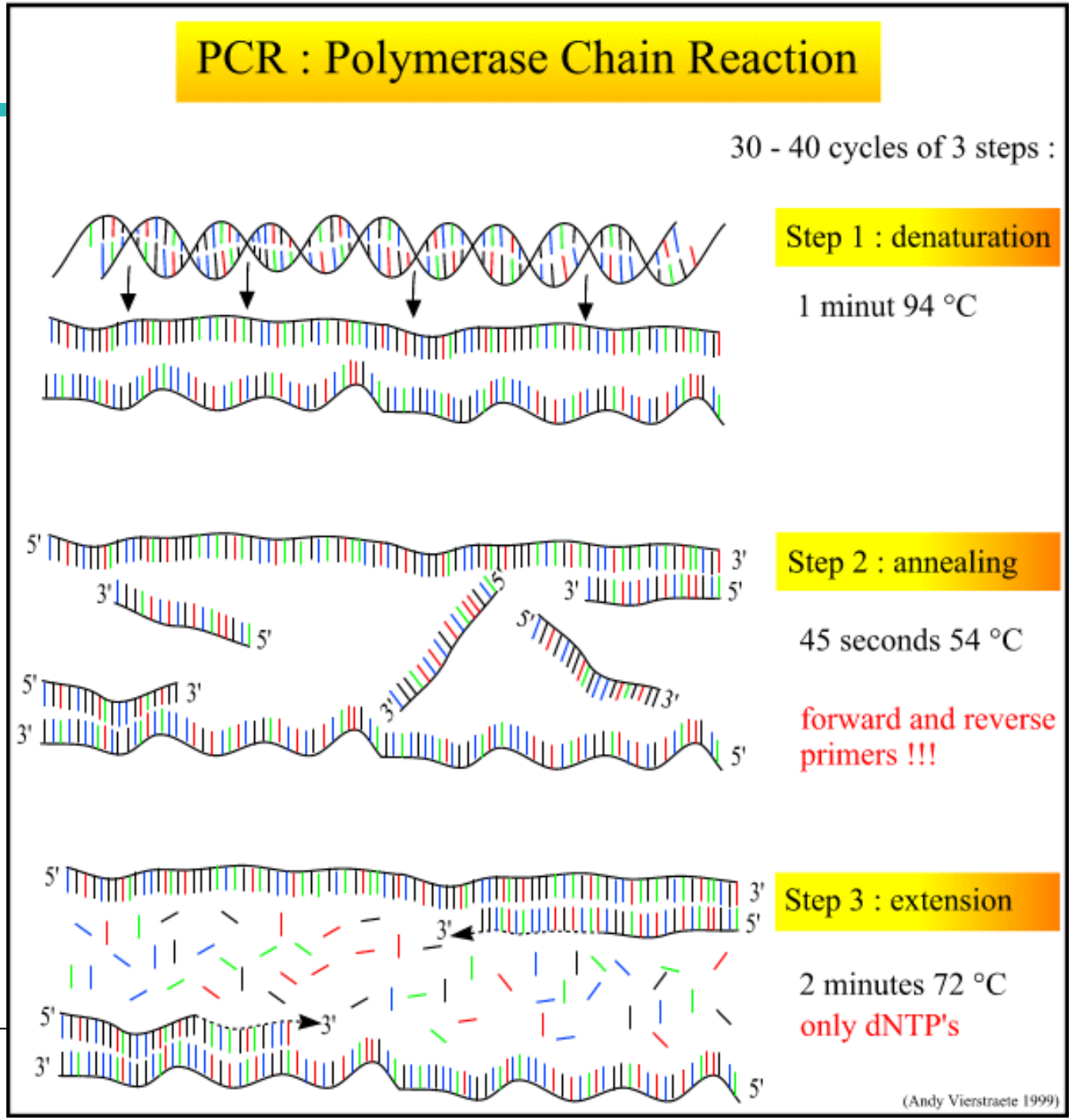
PCR principle

Template:

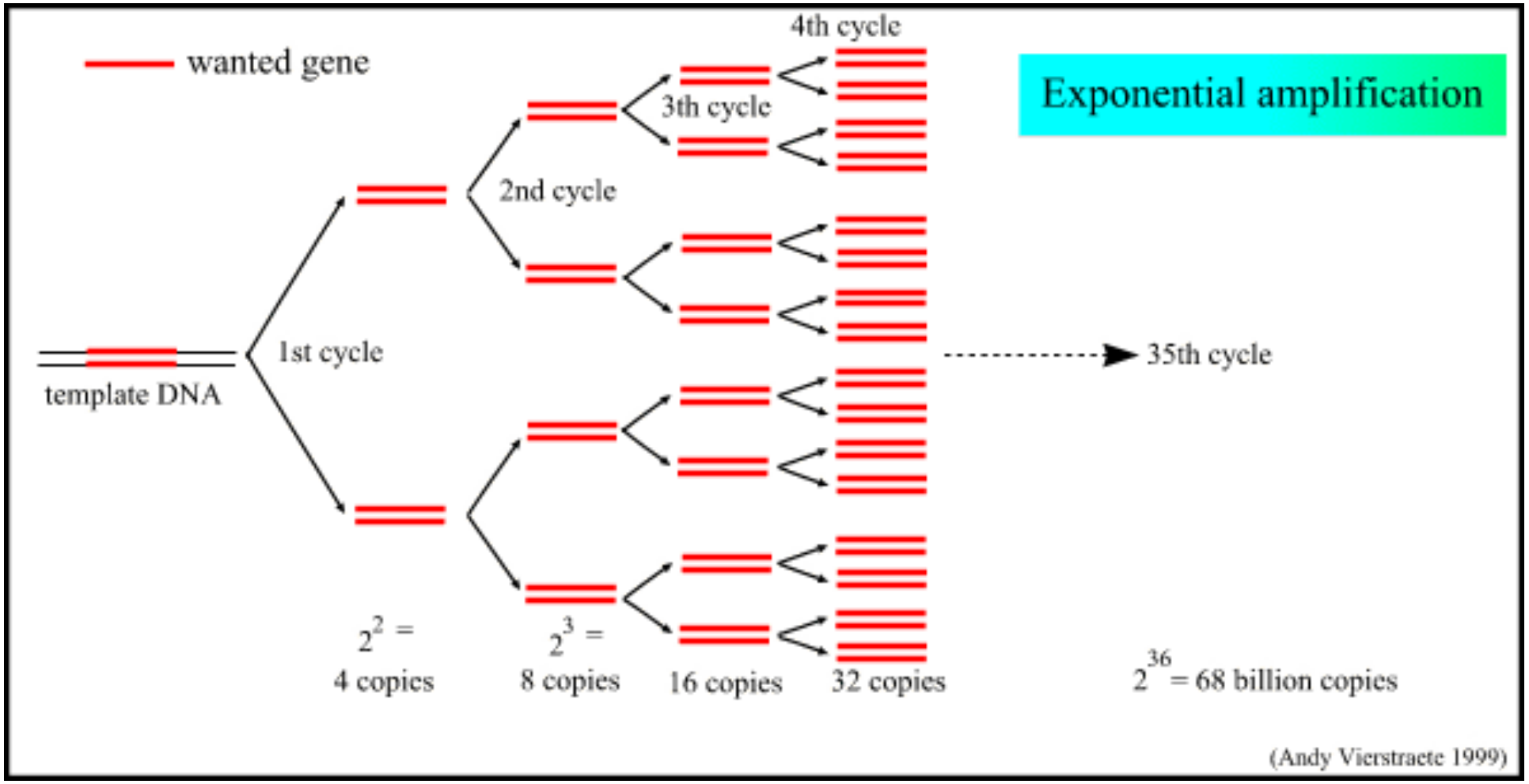
1) DNA

Or

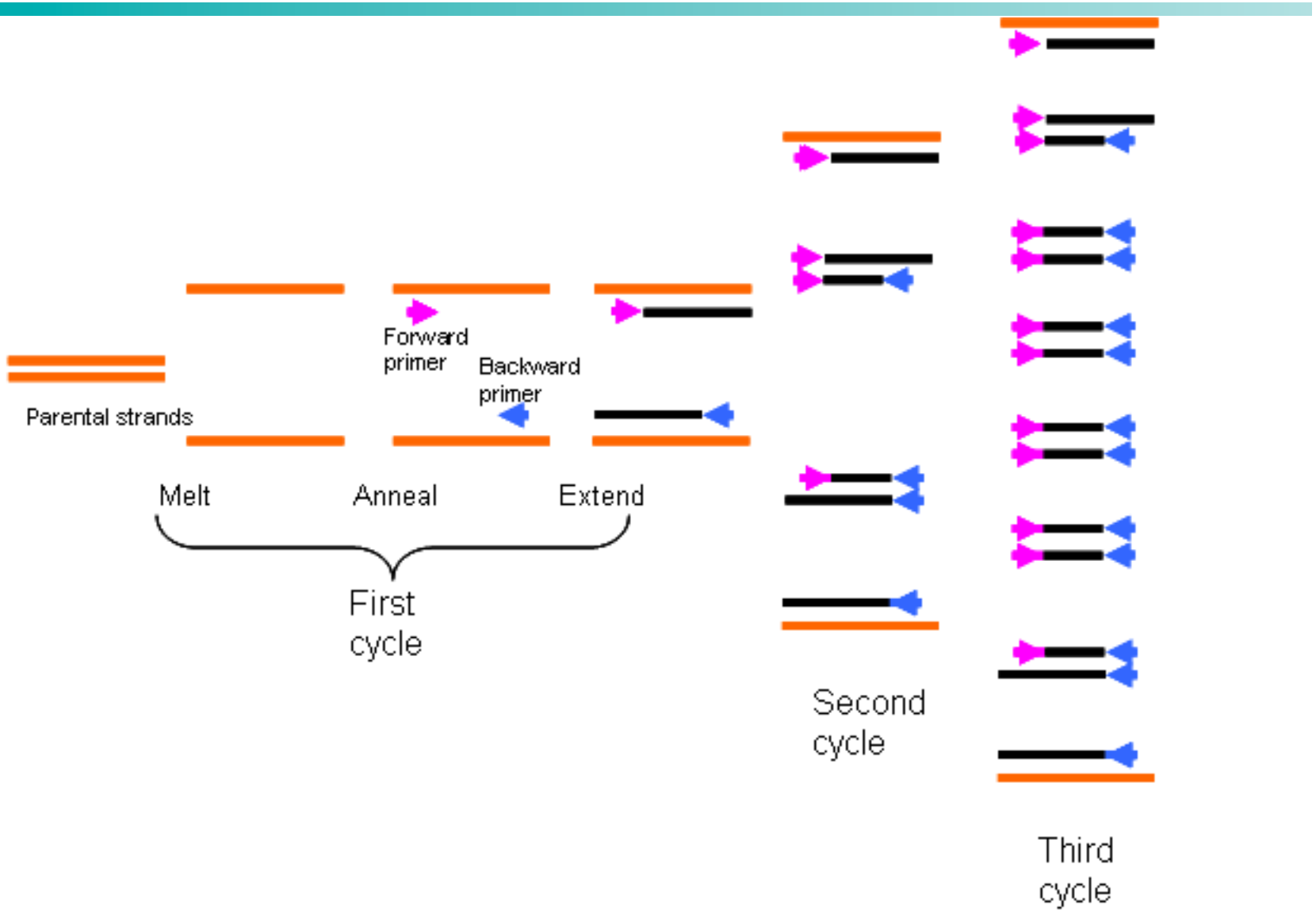
2) Transform RNA into cDNA



PCR principle



PCR principle



CYCLE NUMBER	AMOUNT OF DNA
0	1
1	2
2	4
3	8
4	16
5	32
6	64
7	128
8	256
9	512
10	1,024
11	2,048
12	4,096
13	8,192
14	16,384
15	32,768
16	65,536
17	131,072
18	262,144
19	524,288
20	1,048,576
21	2,097,152
22	4,194,304
23	8,388,608
24	16,777,216
25	33,554,432
26	67,108,864
27	134,217,728
28	268,435,456
29	536,870,912
30	1,073,741,824
31	1,400,000,000
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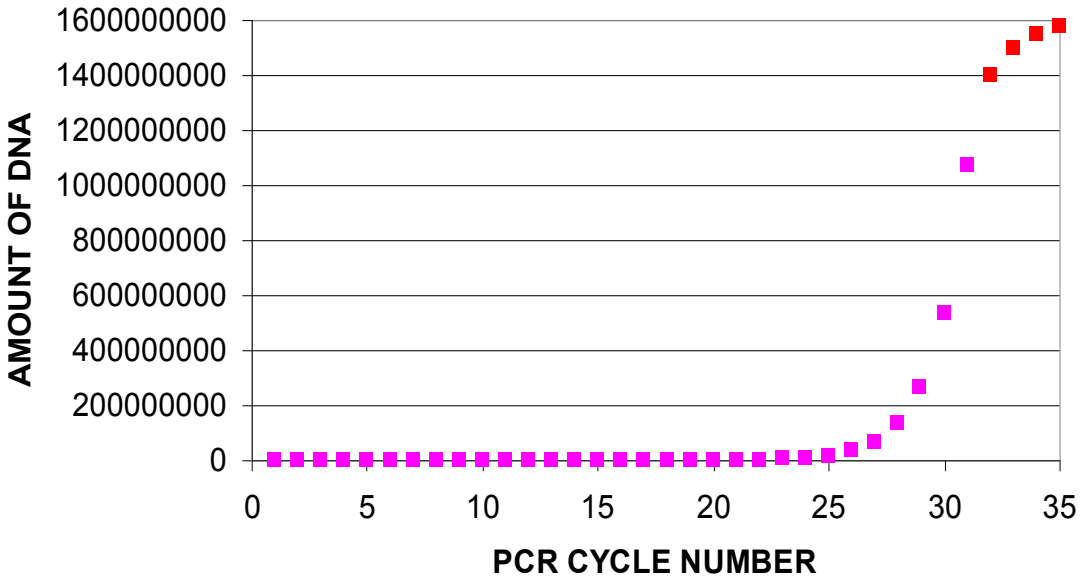
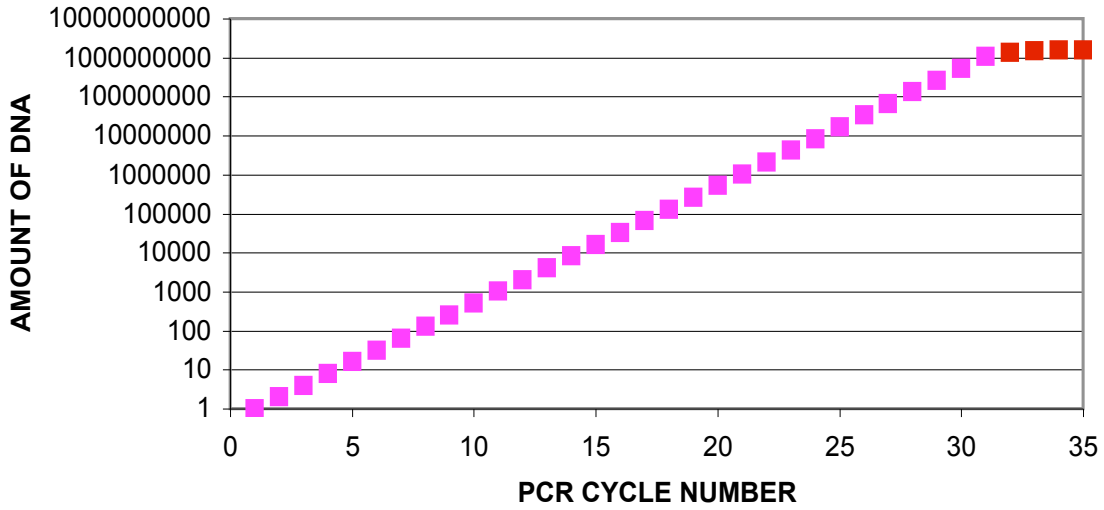
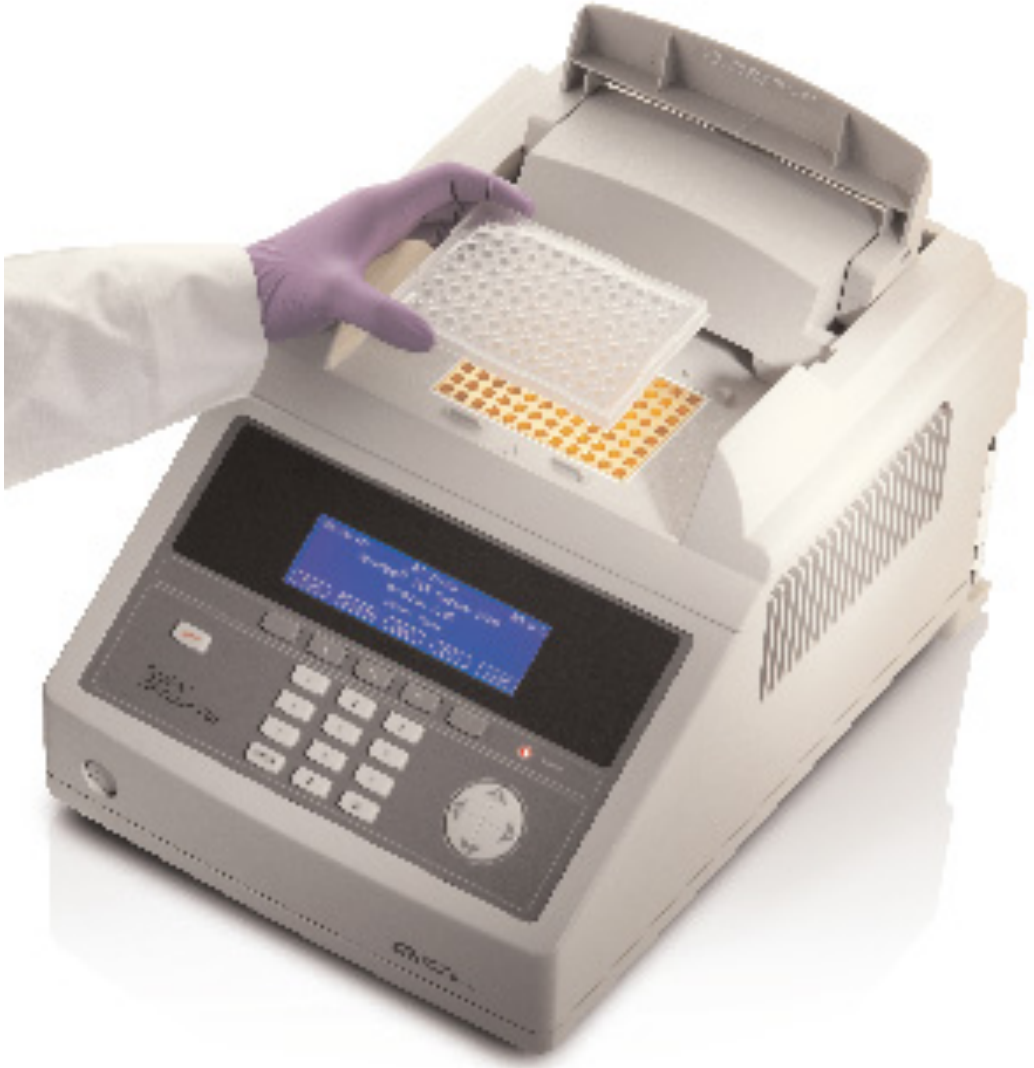




PCR principle: summary

Specific **Amplification** Coupled with **Detection**

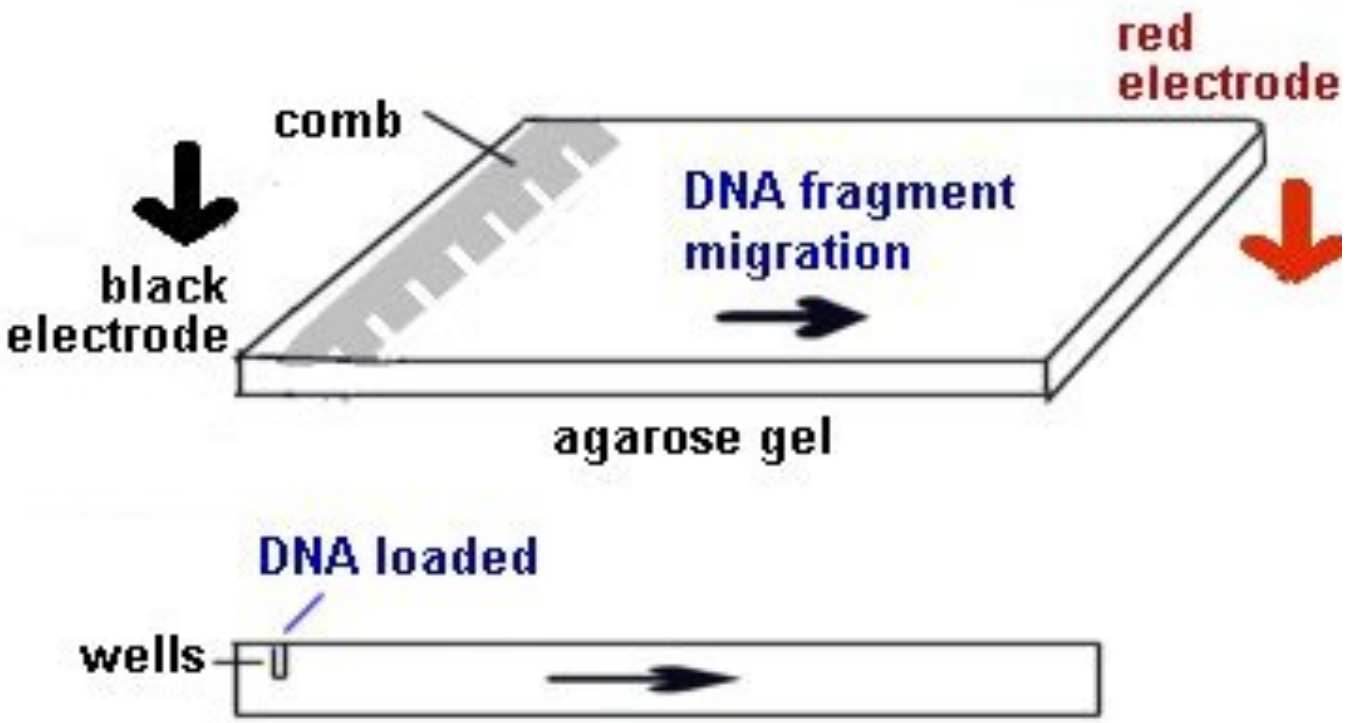
How to Run PCR?



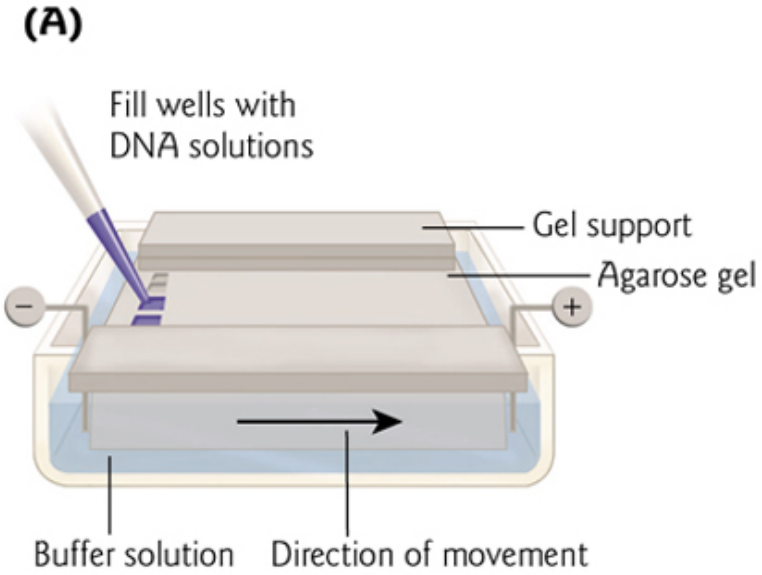
Detecting the end product: Gel electrophoresis

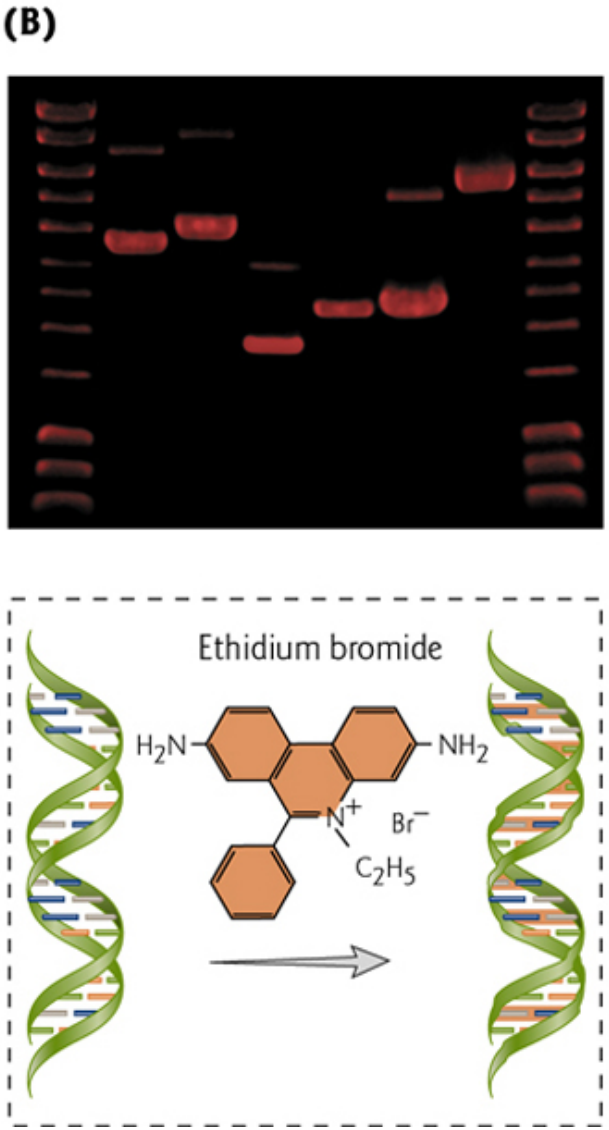
- After reaction is complete, the DNA is loaded into an agarose gel.
- The DNA is mixed with Glycerol and specific dyes to be visible when loading into the gel
- An electric current is applied so the DNA moves towards the + ve electrode according to it s size
- Small fragments move faster and reach the end of the gel, larger fragments move slower and are at the beginning the gel.
- Gel is stained to allow visualization of the DNA bands.

Detecting the end product: Gel electrophoresis



Gel Electrophoresis





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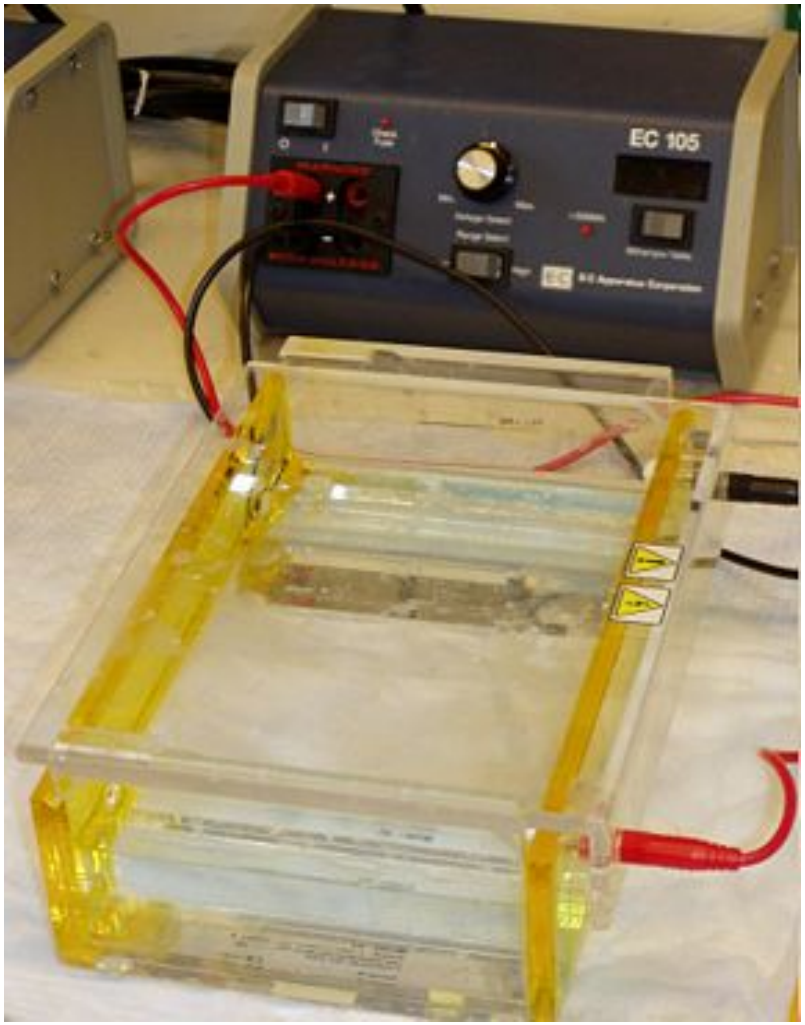
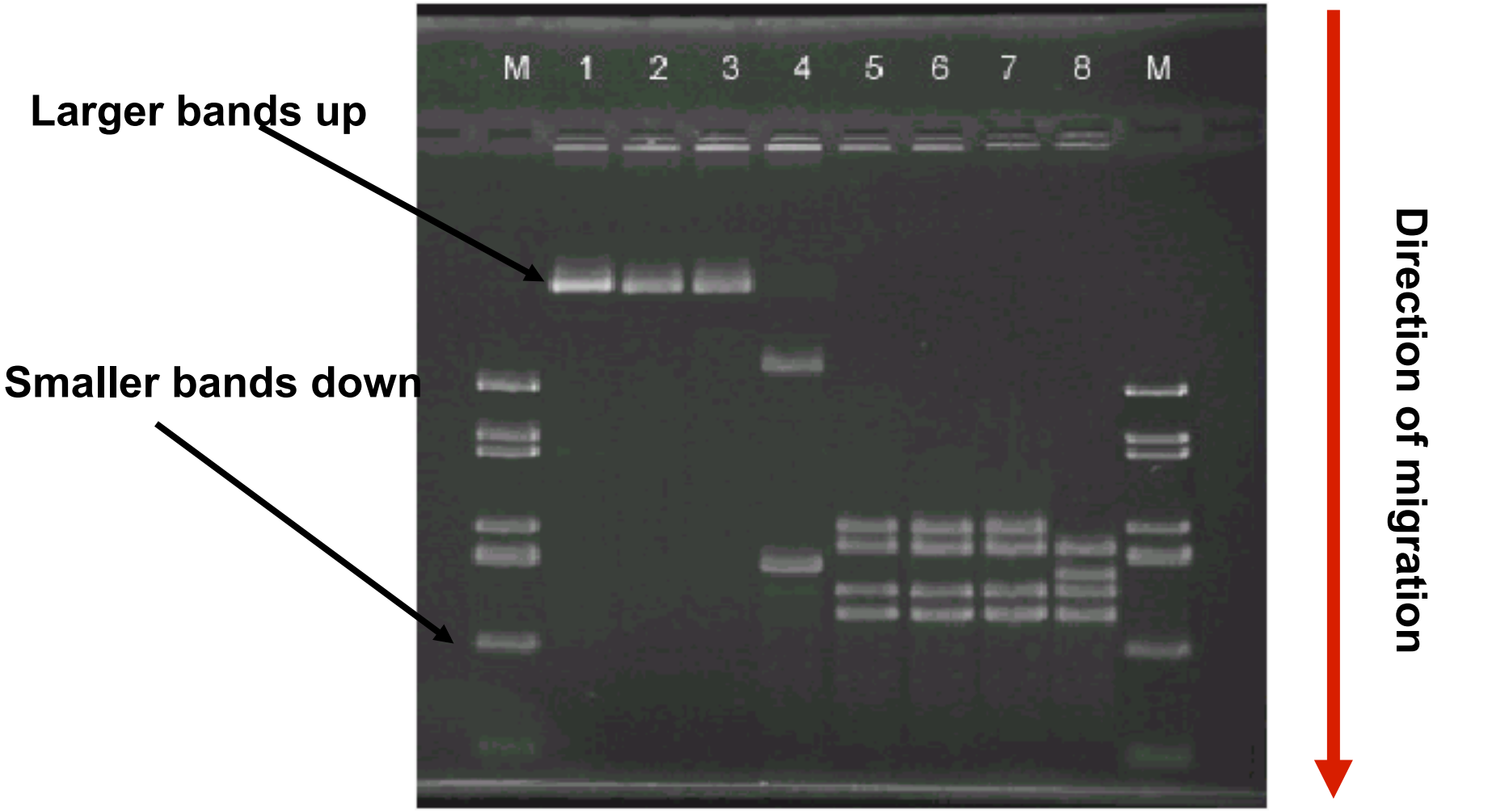


Gel Electrophoresis apparatus

Loading the gel

Gel Electrophoresis



Agarose gel stained with Ethidium bromide and visualized under UV

Applied PCR example: Avian flu

- The following is a part of the avian flu (H5N1) hemagglutinin gene (type H5):

CGATCTAGATGGAGTGAAGCCTCTAATTCTGAGAGATTGTAGTGT
AGCTGGATGGCTCCTCGGAAACCCAATGTGTGACGAATTCATCAA
TGTGCCGGAATGGTCTTACATAGTGGAGAAGGCCAGTCCAGCCAA
TGACCTCTGTTACCCAGGGGATTTCAACGACTATGAAGAACTGAA
ACACCTATTGAGCAGAATAAACCATTTTGAGAAAATTCAGATCAT
CCCCAAAAGTTCTTGGTCCAATCATGAAGCCTCATCAGGGGTGAG
CTCAGCATGTCCATACCTGGGGAAGTCCTCCTTTTTCAGAAATGT
GGTATGGCTCATCAAAAAGAACAATGCATACCCAACAATAAAGAG
GAGCTACAATAATACCAACCAAGAAGATCTTTTGGTACTGTGGGG
GATTCATCATCCTAA

- Suppose you want to use the underlined part of the gene (241 bases) as your diagnostic target DNA

Applied PCR example: Avian flu

- First, you design a pair of primers: the forward primer can be GAGAGATTGTAGTGTAGCTG while the reverse primer has to be the reverse complement of the last part of your target DNA, i.e., the reverse complement of AGCCTCATCAGGGGTGAGC, which is GCTCACCCCTGATGAGGCT.
- The next step is to synthesize these primers in a special laboratory.
- Once you have the primers and the rest of the reagents, you use them to test many suspected nasal secretion specimens. You have to have a positive control (i.e., a sample known to be positive for the hemagglutinin (type H5) and you also have to have a negative control (nasal secretions known to be negative).

Applied example: Avian flu