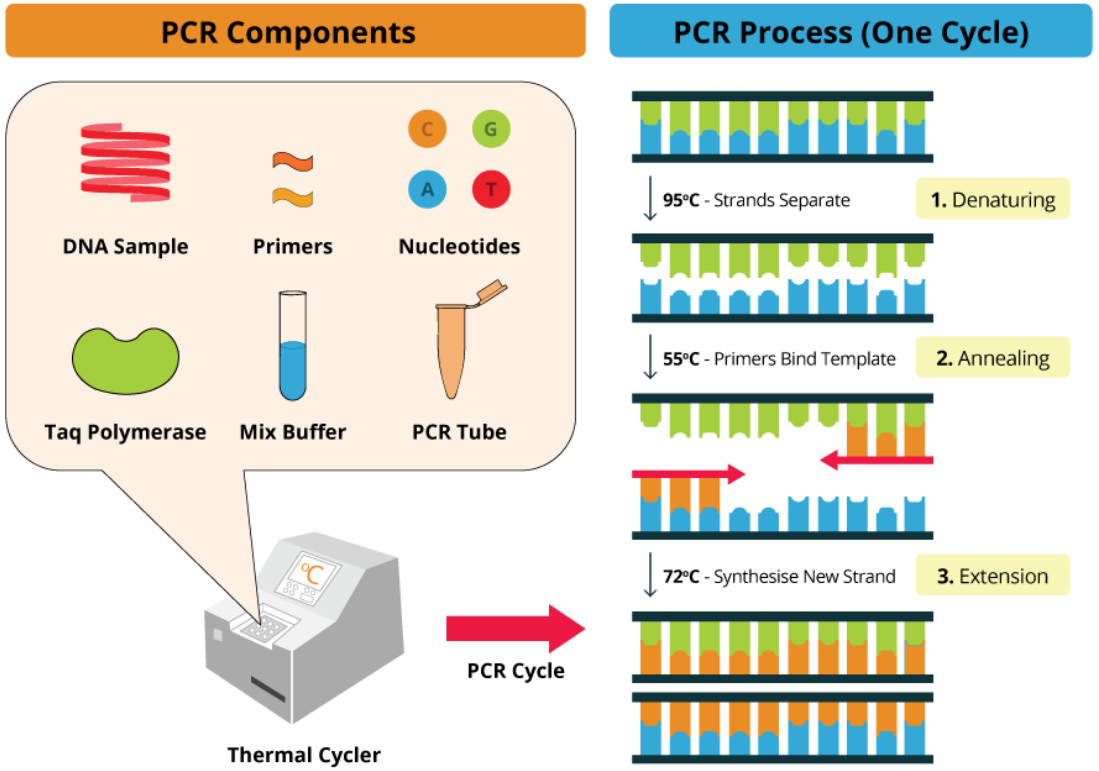
Lesson 19
Polymerase Chain Reaction (PCR)



PCR

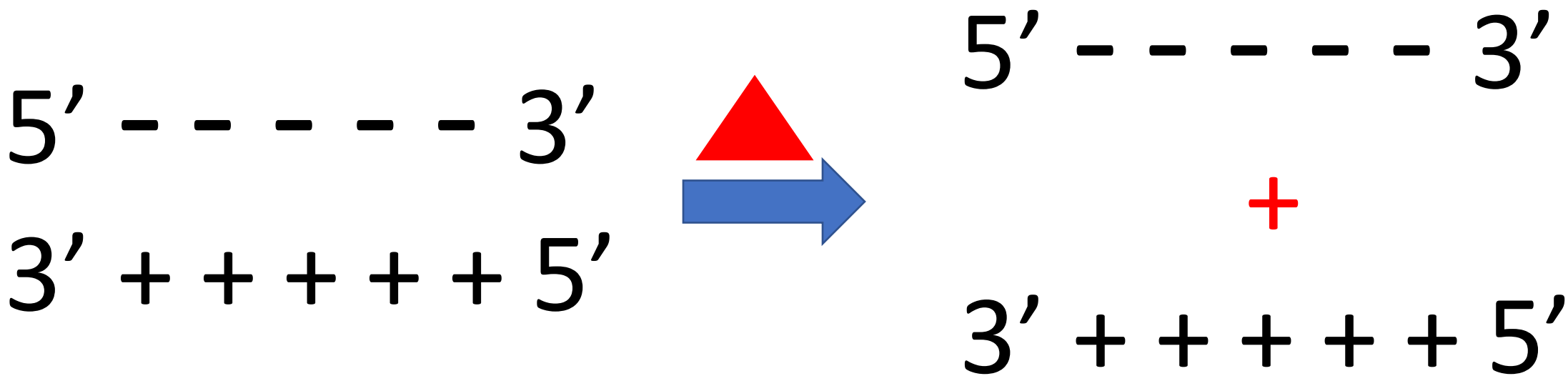
- **Polymerase Chain Reaction (PCR)** is a technique to exponentially amplify (= synthesize in enormous quantities) DNA in a lab test tube
  - Very small DNA quantity → very large quantities of the same DNA
- **3 major differences** between DNA cloning and PCR:
  - Molecular cloning replicates DNA within living cells (bacteria), while PCR replicates DNA in an *in vitro* solution (cell free technique)
  - Molecular cloning involves cutting and pasting DNA sequences, while PCR amplifies DNA by copying an existing sequence
  - DNA cloned by molecular cloning is usually faithfully copied and fully functional, whereas PCR introduces errors in sequence, resulting in mutation
- PCR is intensively used in (*e.g.*,):
  - Gene sequencing
  - Diagnostics
  - Forensic
    - Crime
    - Parental testing

Will see some example later

Main steps in PCR (very similar to cellular DNA replication)

1. Denaturation (95°C)

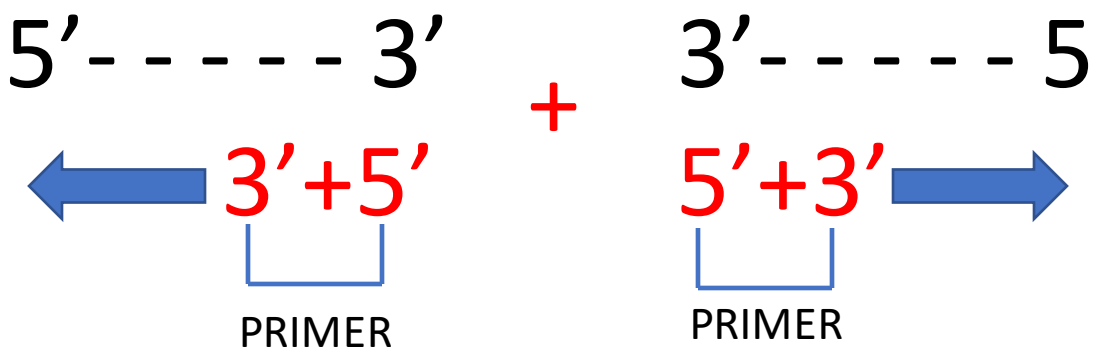
- Your template ds-DNA is **denatured** = separated into 2 single-stranded chains, via heating at **high temperature**



Main steps in PCR (very similar to cellular DNA replication)

2. Annealing (55°C)

- You add two small and specific nucleotide **ORI** sequences = **DNA PRIMERS** (see Lesson 9/10), each complementary to the 5' and 3' ends of the two template DNA single strands, respectively
- These will hybridize with your 2 template DNA single strands (**annealing**) and give you the required 3'-OH starting point for DNA synthesis
  - Remember, DNA synthesis goes 3'→5' (Lesson 9/10)



Main steps in PCR (very similar to cellular DNA replication)

3. Extension (75-80°C)

- You add a specific DNA polymerase (Lesson 11)
  - This enzyme will extend the 3’ end of each primer along the corresponding template DNA strand
  - The result is two copies of the template DNA

5’- - - - - 3’
3’+ + + + + 5’

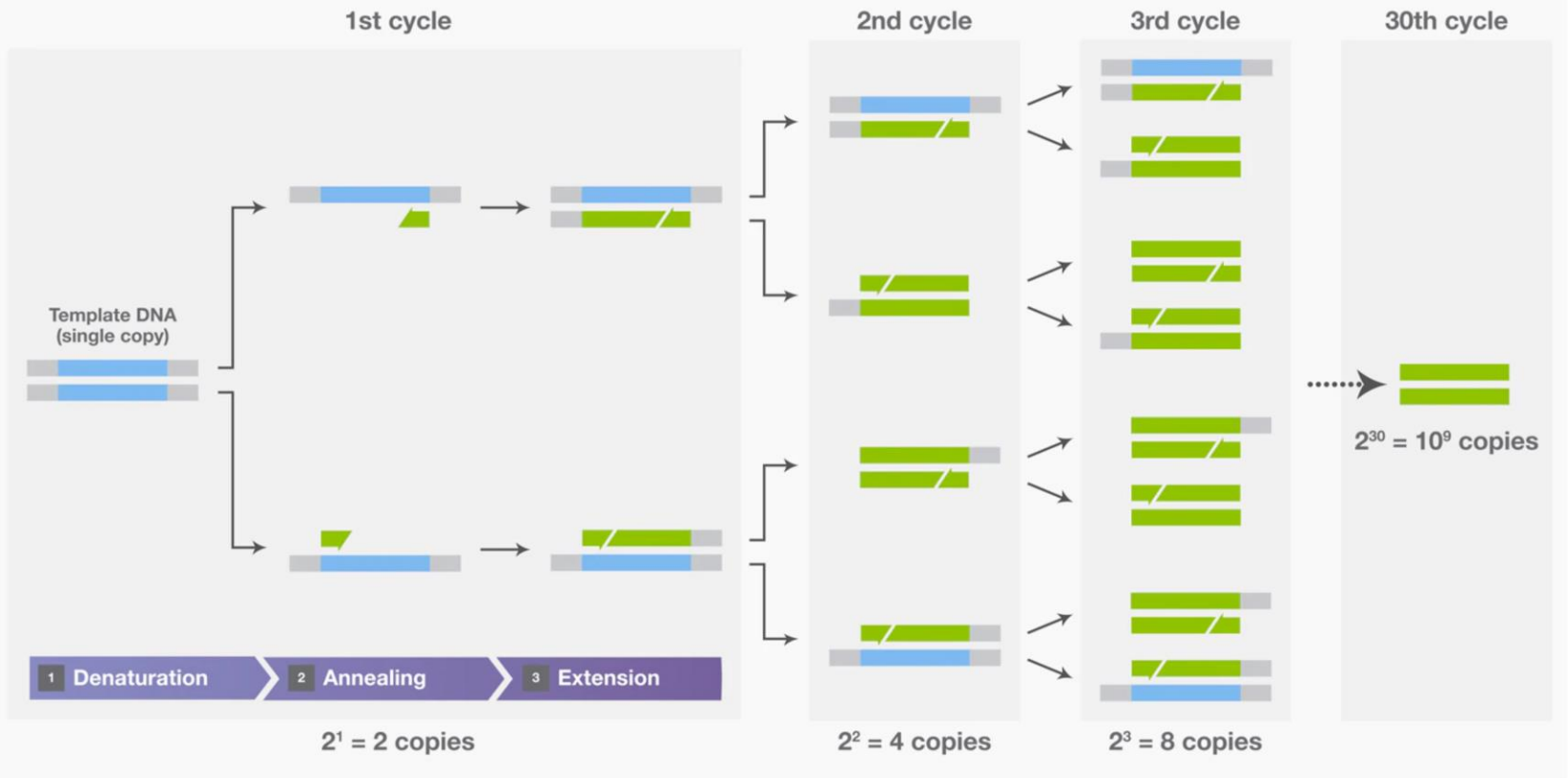
+

3’- - - - - 5’
5’+ + + + + 3’

Main steps in PCR (very similar to cellular DNA replication)

4. Cycling

- You repeat (**cycle**) steps 1-3 for 25-35 times in the same test tube



PCR – some details

- All PCR steps can be carried out automatically (PCR instruments)
- You can cycle the process as you use a specific DNA polymerase
  - **Taq DNA polymerase**
    - Taq is a thermostable DNA polymerase named after the thermophilic eubacterial microorganism *Thermus aquaticus* (hot spring bacteria)
    - Taq's optimum temperature for activity is 75–80°C, with a half-life of greater than 2 hours at 92.5°C, 40 minutes at 95 °C and 9 minutes at 97.5°C
    - At 75-80°C, Taq reaches its optimal polymerization rate of about 150 nucleotides per second per enzyme molecule, and any deviations from the optimal temperature range inhibit the extension rate of the enzyme
    - At temperatures above 90 °C, Taq demonstrates very little or no activity at all, but the enzyme itself does not denature and remains intact for a much longer time required

Designing primers

Double-stranded DNA

5’TGCATTGCCGAAGCCTTAGCAGGTACC3’ Top strand
3’ACGTAACGGCTTCGGAATCGTCCATGG5’ Bottom strand

Primer for TOP strand

5’TGCATTGCCGAAGCCTTAGCAGGTACC3’
3’CCATGG5’
OH

Primer for BOTTOM strand

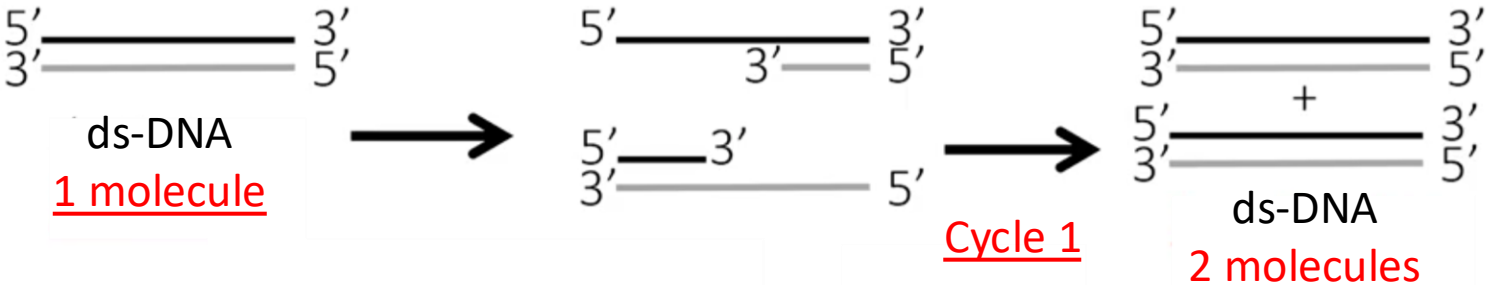
3’ACGTAACGGCTTCGGAATCGTCCATGG5’
5’TGCATT3’
OH

Allow DNA synthesis starting from the 3’OH of primers

5’TGCATTGCCGAAGCCTTAGCAGGTACC3’
3’ACGTAACGGCTTCGGAATCGTCCATGG5’

3’ACGTAACGGCTTCGGAATCGTCCATGG5’
5’TGCATTGCCGAAGCCTTAGCAGGTACC3’

PCR - recap



1. Denature (95°C)
2. Anneal primers (55°C)
3. Extend (Taq, 75-80 °C)

Repeat steps 1-3

Cycle 2

ds-DNA

4 molecules

Cycle 3

ds-DNA

8 molecules

Cycle 30

ds-DNA

10^9 molecules

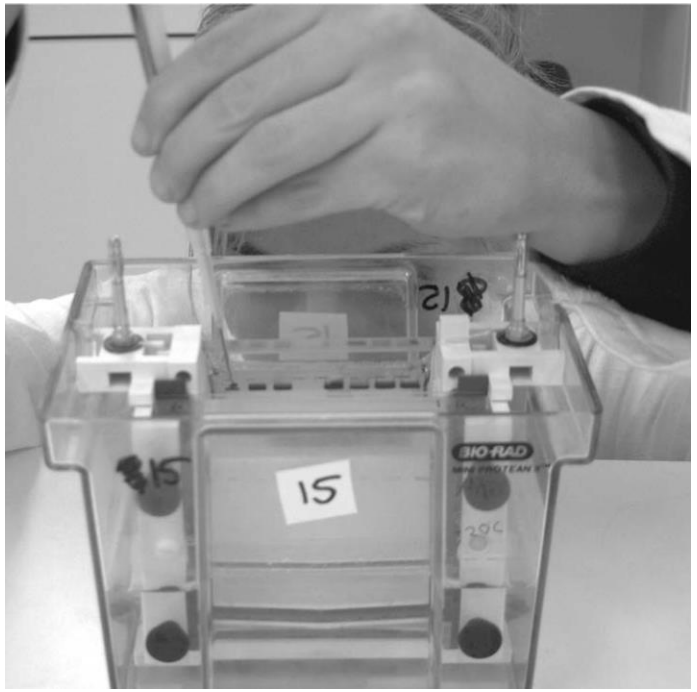
PCR → Exponential DNA amplification in a test tube using heat stable Taq DNA polymerase

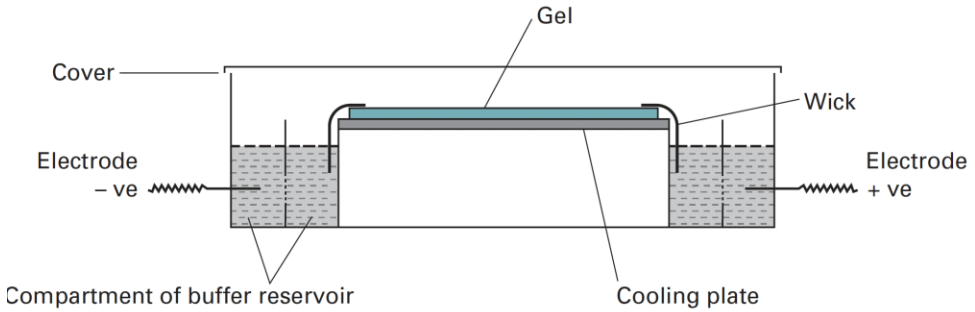
Visualizing PCR results

- The results of a PCR reaction are usually visualized using **gel electrophoresis**
- Gel electrophoresis concept:
  - fragments of DNA are pulled through a gel matrix by an electric current
  - DNA fragments are separated according to size

Dissecting a Protein for Study

- Protein separation by charge
- Electrophoresis
  - Relies on the migration of a charged particle under the influence of an electric field E
  - Under the influence E, these charged particles will migrate either to the cathode or to the anode, depending on the nature of their net charge





Visualizing PCR results

- The results of a PCR reaction are usually visualized using **gel electrophoresis**
- Gel electrophoresis concept:
  - fragments of DNA are pulled through a gel matrix by an electric current
  - DNA fragments are separated according to size
- A standard (**DNA ladder**) is typically included so that the size of the fragments in the PCR sample can be determined
- DNA fragments of the same length form a ***band*** on the gel
  - Bands can be seen by eye if the gel is stained with a DNA-binding dye

Visualizing PCR results

- The results of a PCR reaction are usually visualized using **gel electrophoresis** (will see later in the lab)
- Gel electrophoresis is a technique in which fragments of DNA are pulled through a gel matrix by an electric current and it separates DNA fragments according to size
- A standard (**DNA ladder**) is typically included so that the size of the fragments in the PCR sample can be determined
- DNA fragments of the same length form a ***band*** on the gel
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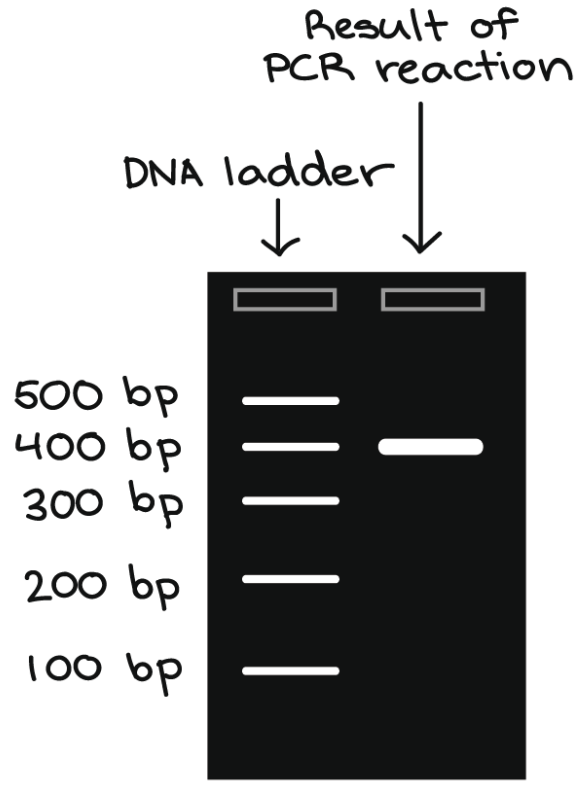
- Example gel electrophoresis
  - PCR reaction producing a **400** base pair (bp) fragment
- A DNA band contains 10^9 target DNA region (not just one or a few copies)
  - Importance of PCR tool: it produces enough copies of a DNA sequence that we can see or manipulate that region of DNA



DNA profiling

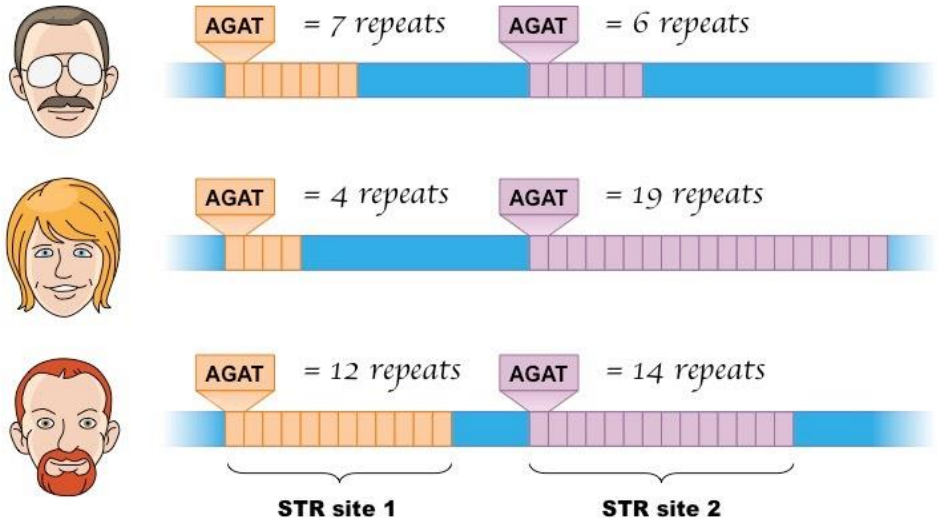
- DNA profiling is the process where a specific DNA pattern, called a profile, is obtained from a person or sample of body tissue
- Even though we are all unique most of our DNA is actually identical to other people's DNA
- However, specific regions vary highly between people
  - These regions are called **polymorphic**
  - Differences in these variable regions between people are known as **polymorphisms**
- **Each of us inherits a unique combination of polymorphisms from our parents**
- DNA polymorphisms can be analyzed to give a DNA profile

Forensic & Law

- DNA profiling is used by CSI to:
  - identify the probable origin of a body evidence sample associated with a crime or crime scene
  - identify disaster victims
    - Tsunami/earthquakes
    - Airplane crash
    - Fire/explosions…
- DNA profiling can be requested by parents/court to:
  - reveal family relationships

Short tandem repeat (STRs) (aka microsatellites)

- One of the current techniques for DNA profiling uses polymorphisms called **short tandem repeats (STRs) (or DNA microsatellites)**
- STRs are regions of **non-coding DNA** that contain **repeats of the same nucleotide sequence**
- For example
  - GATAGATAGATAGATAGATAGATA is an STR where the nucleotide sequence GATA is repeated six times
- STRs are found at different places or **genetic loci** in a person's DNA

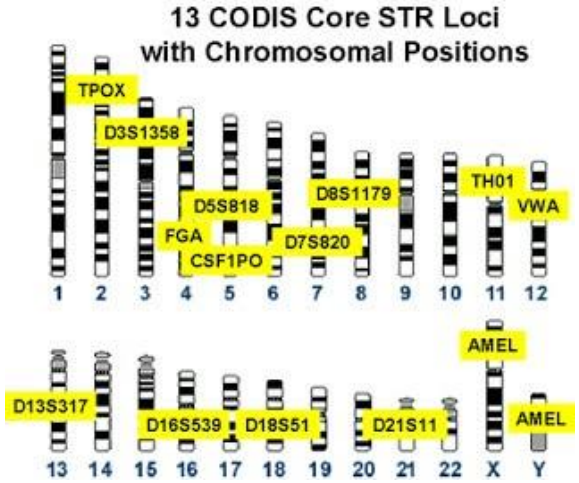


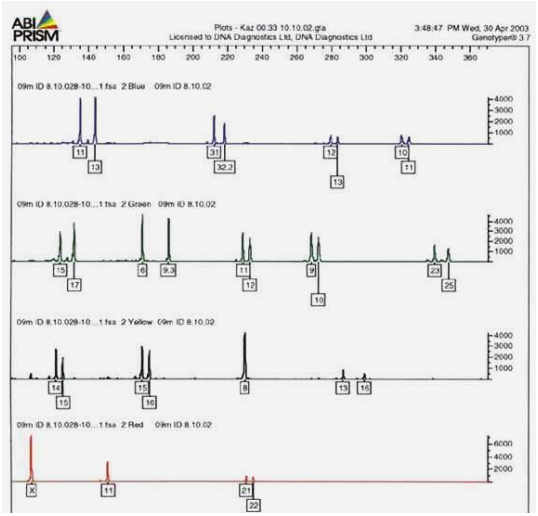
DNA profiling in brief

- One way to produce a DNA profile is for scientists to examine STRs at 10 or more **genetic loci**
  - A locus is the **specific physical location** of a gene or other DNA sequence on a chromosome, like a genetic street address
  - These genetic loci are usually on different chromosomes
- A DNA profile can tell the scientist *e.g.*,
  - the DNA is from a man or woman
  - if the sample being tested belongs to a particular person
  - reveal familiar relationships
- The DNA profiling procedure consists in the following steps:
  - A DNA sample is collected (*e.g.*, from blood, semen, saliva, etc.) and then amplified using PCR
  - **DNA microsatellites (= STR sequences)** are identified and cut with specific restriction enzymes to generate the corresponding fragments
  - Fragment length will differ between individuals due to the variable length of their STRs
  - The fragments are separated using gel electrophoresis and the resulting profiles are compared

DNA profiling in forensic - example

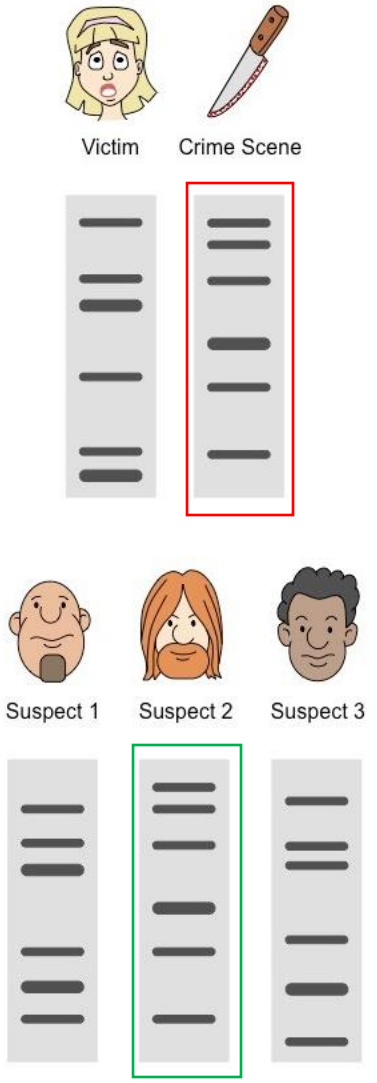
- **Forensic Investigations**
  - Suspects DNA should be a **complete match** with the DNA sample taken from the crime scene if a conviction is to occur
  - The number of loci used to generate a unique profile depends on the size of the population being compared
    - *e.g.,* America (population: ~ 320 million) uses **13** loci for comparison; Australia (population: ~ 25 million) uses only **9** loci
- There are several worldwide DNA databases collected from crime/tragedy scenes
  - *e.g.*, CODIS is the acronym for the Combined DNA Index System and is the generic term used to describe the FBI's program of support for criminal justice DNA databases as well as the software used to run these databases
  - In case of crime, the first thing is to check whether the STRs in collected DNA matches one of the database entry
    - If CODIS match is found → suspected identified





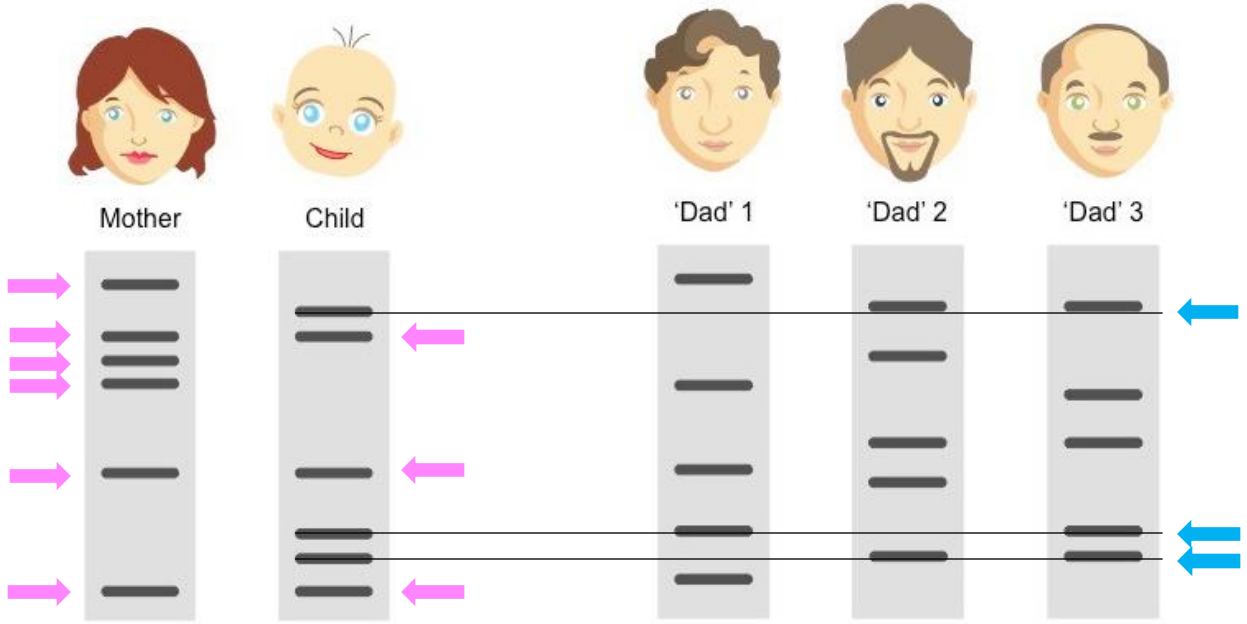
DNA profiling in forensic - example

- In case of CODIS negative result police proceed with investigation and possible suspect identification
- Court authorizes taking DNA samples from possible identified suspects
- The STRs from DNA collected from the crime scene is compared to those of possible suspects to determine who was present on the crime scene



DNA profiling in law - example

- Same STR identification procedure as for crime (except no CODIS mining)
- Compare the profiles of three men with that of a mother and child to determine the biological father



DNA mutations and cancer

- **Cancer is a genetic disease**
  - Genetic abnormalities cause the disease by affecting the way our cells function
  - These genetic mutations can interfere with cell reproduction processes
    - effectively switching off/on normal/abnormal growth controls and causing cells to grow indefinitely (tumor)

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- We can acquire **cancer-causing genetic mutations** anytime during our lifetime
  - by chance (*e.g.*, errors during transcription/translation) (**endogenous**)
  - through exposure to carcinogenic materials (*e.g.*, IRs or chemicals) that can damage DNA (**exogenous**)

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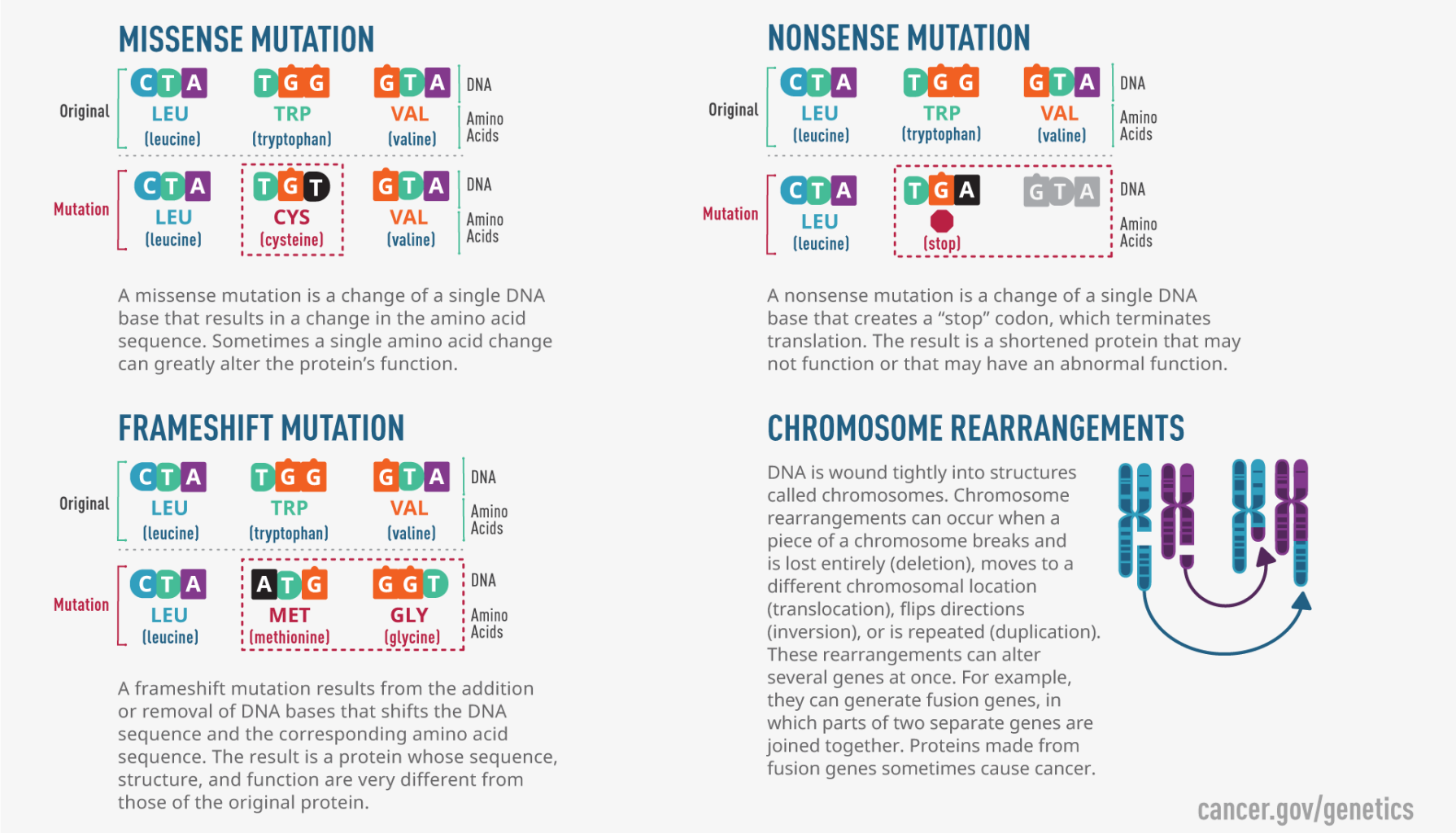
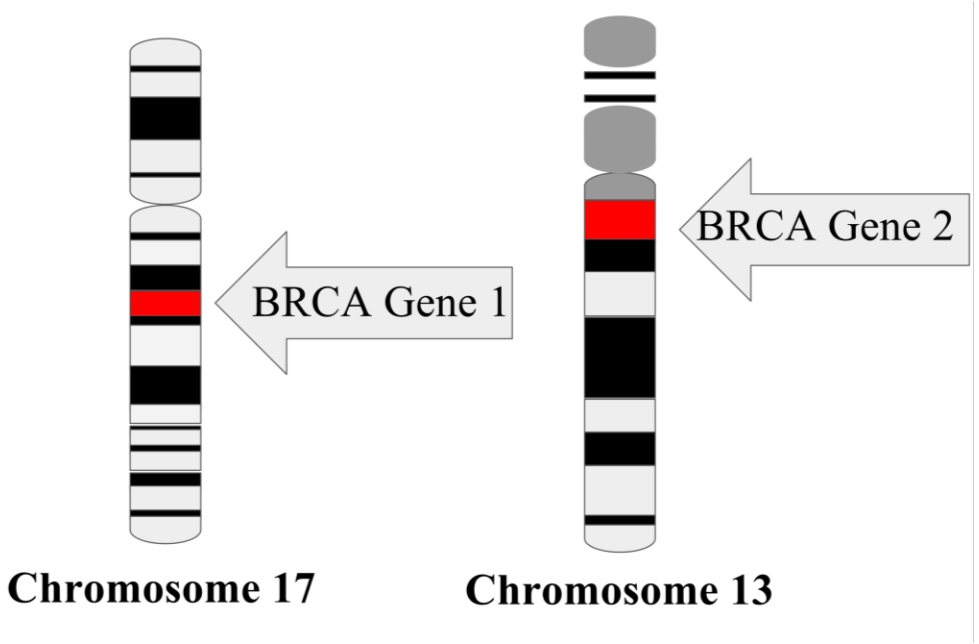
- These genetic mutations are found in virtually every cell of the body ("except" the heart)

Cancer diagnostics



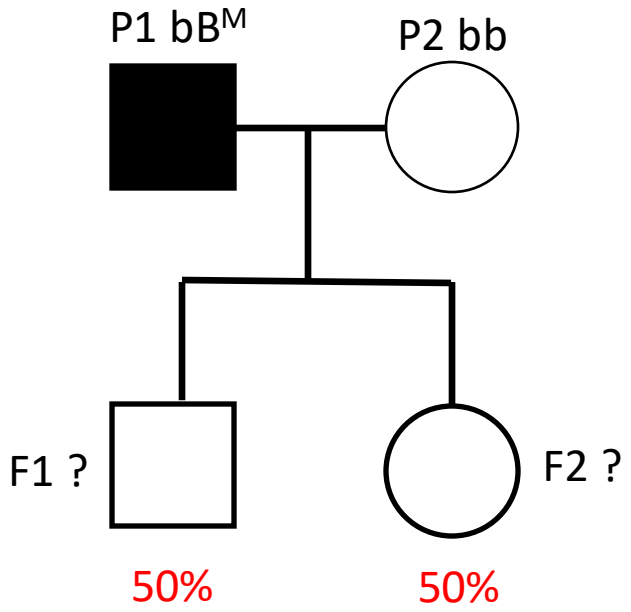
The BRCA genes

- The **BRCA** genes (***BRCA1* and *BRCA2***) are **tumor suppressor genes**
- Both genes normally produce two proteins (BRCA1 and BRCA2) that help **repair damaged DNA**
  - keeping the genetic material of the cell stable
- A **mutated BRCA gene** can lead to increased risk of cancer
  - particularly **breast or ovarian** cancer in women
- Mutated BRCA1 and BRCA2 become **oncogenes**



The BRCA genes

- BRCA mutations can be inherited from **either parent** and may be passed on to both sons and daughter
- Both BRCA1 and BRCA2 genes are inherited in an **autosomal dominant pattern**
  - **one copy of the altered gene in each cell (heterozygosity) is sufficient to increase a person's chance of developing cancer**
- Each child of a genetic carrier has a **50% chance** of inheriting the mutated gene from the parent who carries the mutation
- *BRCA* mutations can increase the risk of other cancers
  - *e.g.*, colon, pancreatic and prostate cancer



P1 / P2	b	B^M
b	bb	bB^M
b	bb	bB^M

Searching for BRCA mutations

- In the presence of BRCA-linked familiar cancer history
- *de novo* BRCA-related cancers
  1. DNA is extracted from a small sample of, *e.g.*, blood or saliva
  2. The BRCA1 and BRCA2 genes are isolated, amplified via PCR and checked via gel electrophoresis (each should have 1 single strong band)
  3. Each PCR product (from BRCA1 and BRCA2) can be automatically sequenced and compared to the wild type

Searching for BRCA mutations

Wild type BRCA1 gene

Mutated BRCA1 gene