BE/EE189 Design and Construction of Biodevices
PCR Lecture

Overview

- Why PCR?
- PCR History
- PCR Basics
- Post PCR Analysis
- Limitations of PCR (benefits of qPCR)
- qPCR Basics
- Melting curve analysis

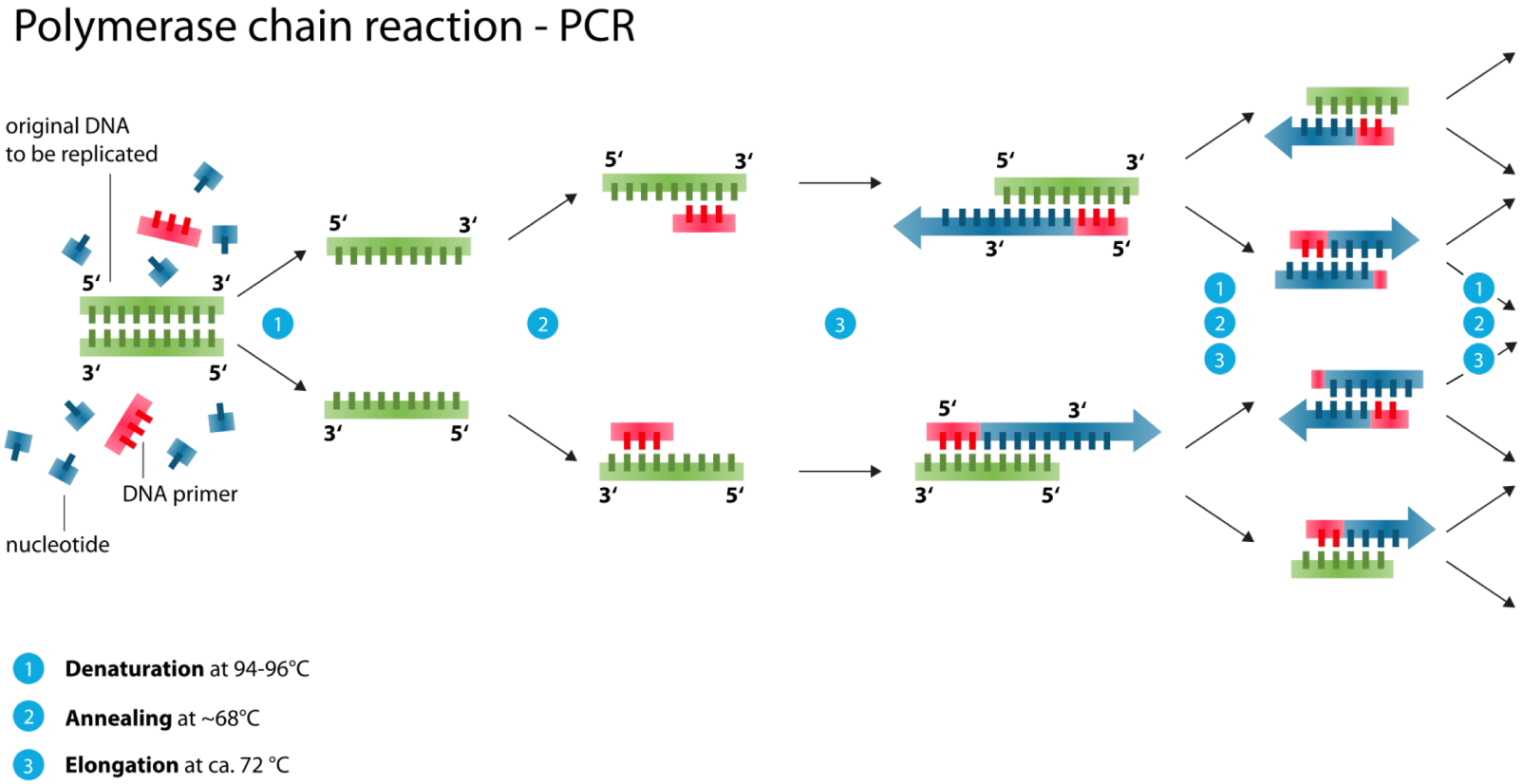
Why PCR?

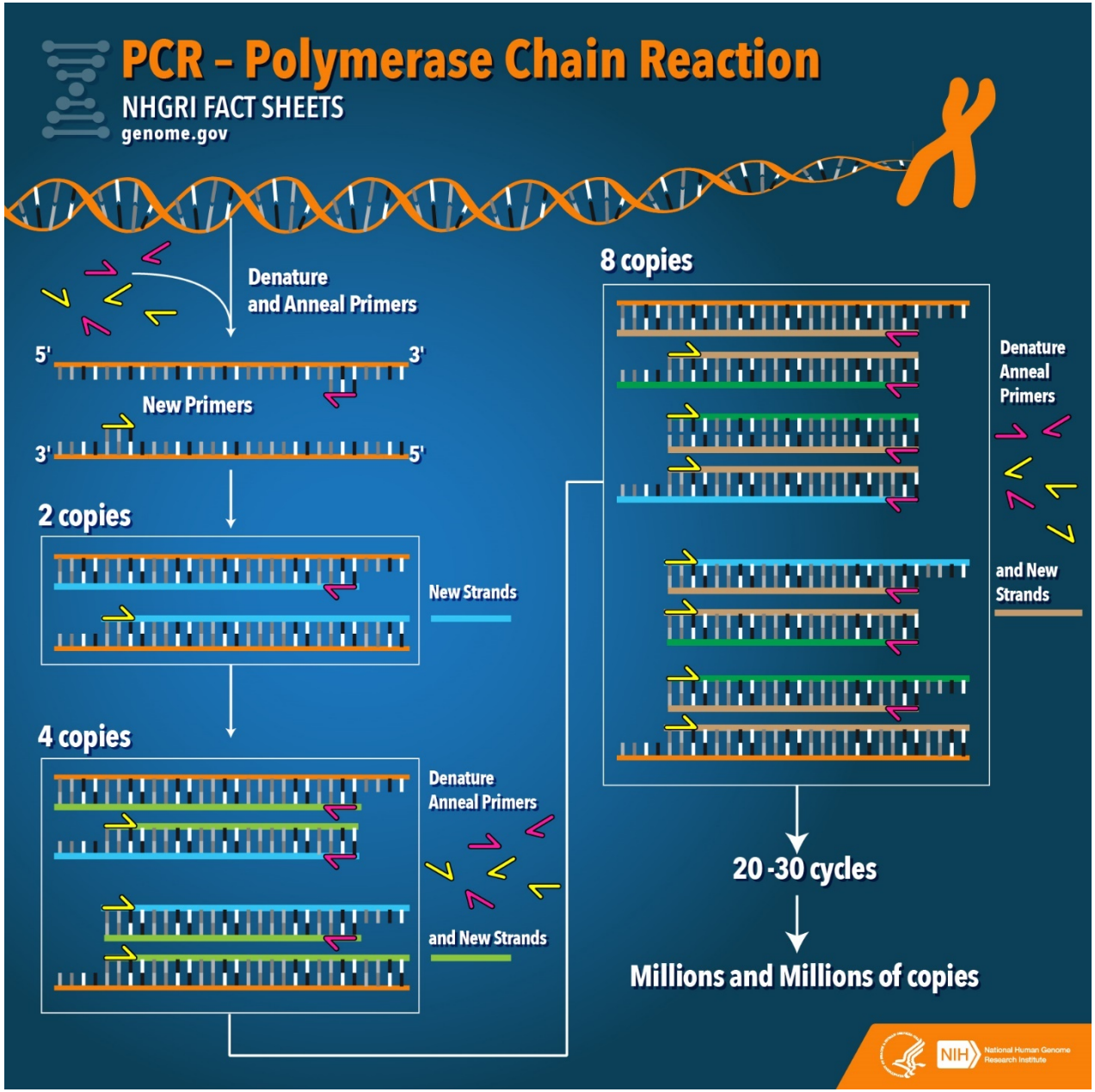
- Scientists need a method of amplifying enough DNA for quantitative/qualitative inspection
- Examples:
  - Comparing the relative amounts of a gene sequence in two different samples
  - Amplifying and preserving rare or trace samples
  - Amplifying several defining sequences for DNA identification

PCR history

- 1957 – First DNA polymerase is identified by Arthur Kornberg as an enzyme that replicates DNA
- 1971 – First paper suggesting a method similar to PCR for DNA amplification, using a 2 primer system instead of 1
- 1983 – Kary Mullis conceives idea for PCR.
  - Unlike existing methods, his uses 2 primers which allows for exponential amplification
  - Unfortunately, PCR needed temperature cycling to work, and most polymerases would denature when too hot
- 1988 – Kary Mullis commercializes *Taq polymerase* from a bacteria that lives in hot springs in Yellowstone

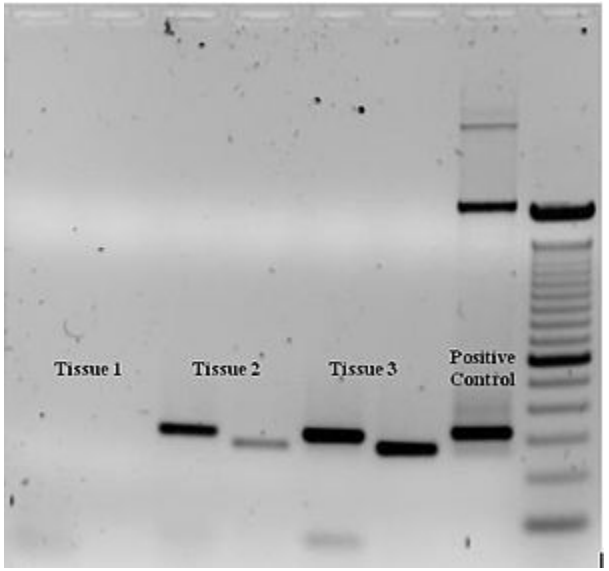
PCR Basics


Polymerase chain reaction - PCR
original DNA
to be replicated
5′
3′
3′
5′
DNA primer
nucleotide
1
2
3
1 Denaturation at 94-96°C
2 Annealing at ~68°C
3 Elongation at ca. 72 °C


PCR – Polymerase Chain Reaction
NHGRI FACT SHEETS
genome.gov
Denature
and Anneal Primers
5'
3'
New Primers
3'
5'
2 copies
New Strands
4 copies
Denature
Anneal Primers
and New Strands
8 copies
Denature
Anneal
Primers
and New
Strands
20 -30 cycles
Millions and Millions of copies
NIH
National Human Genome
Research Institute

Analysis of PCR Results

- DNA segments are separated via electrophoresis
- DNA is stained such that the intensity roughly provides a measurement of concentration
- This is an example of end point detection



Limitations with PCR

- PCR eventually plateaus as the reactants are used up
  - This plateau can occur at different rates for different PCR reactions of the SAME sample
  - In other words, tube A might have slightly more reactants than tube B and so PCR lasts longer in tube A than B.

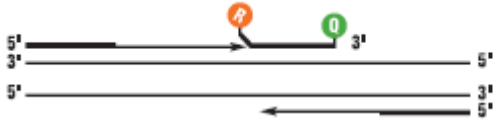
- Benefits of real time quantitative PCR (qPCR)
  - Detection is done as the reaction progresses (not just end point detection)
  - Quantitative results (not qualitative comparisons like with gel)
  - No need for electrophoresis at the end
  - Can perform melt curve analysis immediately afterwards

qPCR Basics

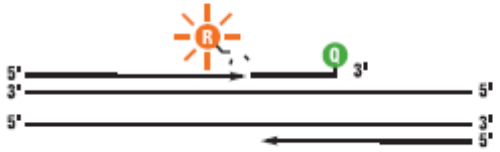
TAQMAN® PROBE-BASED ASSAY CHEMISTRY

1. **Polymerization:** A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' ends of a TaqMan® probe, respectively.

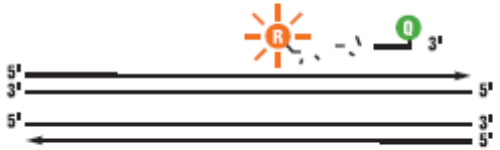
2. **Strand displacement:** When the probe is intact, the reporter dye emission is quenched.



3. **Cleavage:** During each extension cycle, the DNA polymerase cleaves the reporter dye from the probe.



4. **Polymerization completed:** Once separated from the quencher, the reporter dye emits its characteristic fluorescence.

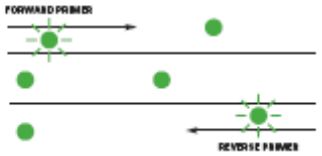


SYBR® GREEN I DYE ASSAY CHEMISTRY

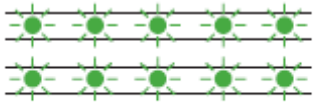
1. **Reaction setup:** The SYBR® Green I Dye fluoresces when bound to double-stranded DNA.

2. **Denaturation:** When the DNA is denatured, the SYBR® Green I Dye is released and the fluorescence is drastically reduced.

3. **Polymerization:** During extension, primers anneal and PCR product is generated.



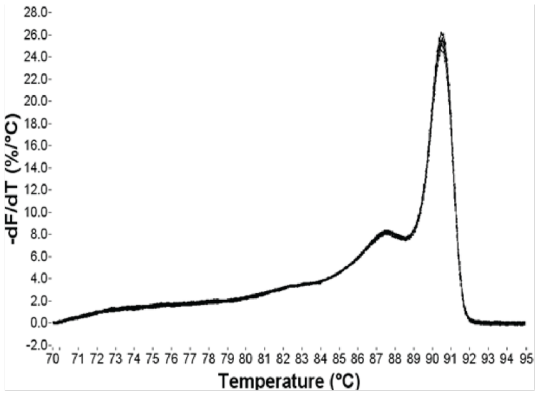
4. **Polymerization completed:** When polymerization is complete, SYBR® Green I Dye binds to the double-stranded product, resulting in a net increase in fluorescence detected by the 7900HT system.



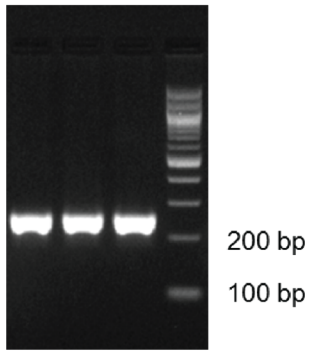
Melt Curve Analysis

- How do we assess whether we've produced specific sequences? (hint: read title of slide)
- The temperature is slowly increased after the DNA has been amplified
- As DNA "melts", it denatures and that particular DNA no longer fluoresces (via SYBR Green)
- The rate at which it melts peaks at a particular temperature
- This peak is unique to the sequence. Several peaks suggest several sequences

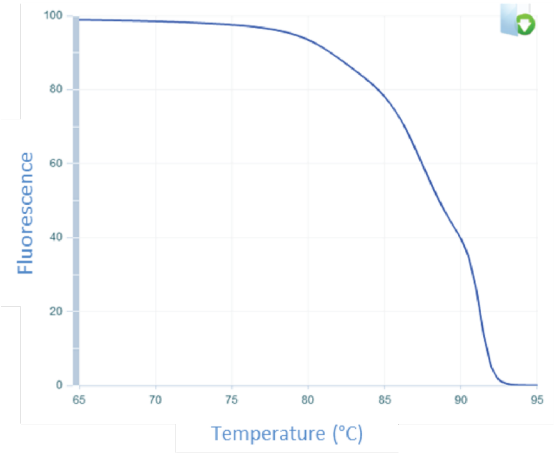
Example of a Melt Curve



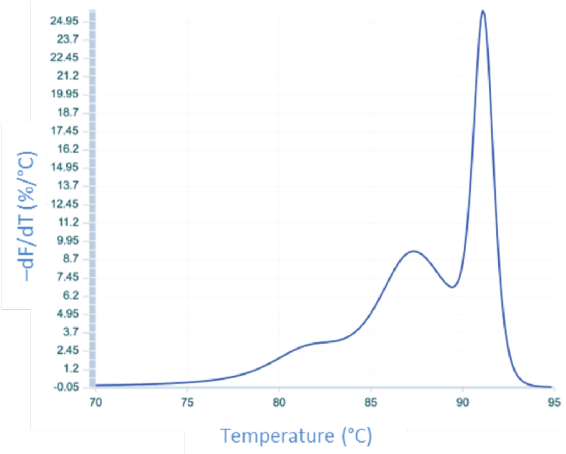
A. CFTR Exon 13 Melt Curve.



B. CFTR Exon 13 Agarose Gel.



C. uMelt Predicted Dissociation Curve for CFTR Exon 13.



D. uMelt Derivation Melt Curve for CFTR Exon 13.

https://www.idtdna.com/pages/decoded/decoded-articles/core-concepts/decoded/2014/01/20/interpreting-melt-curves-an-indicator-not-a-diagnosis