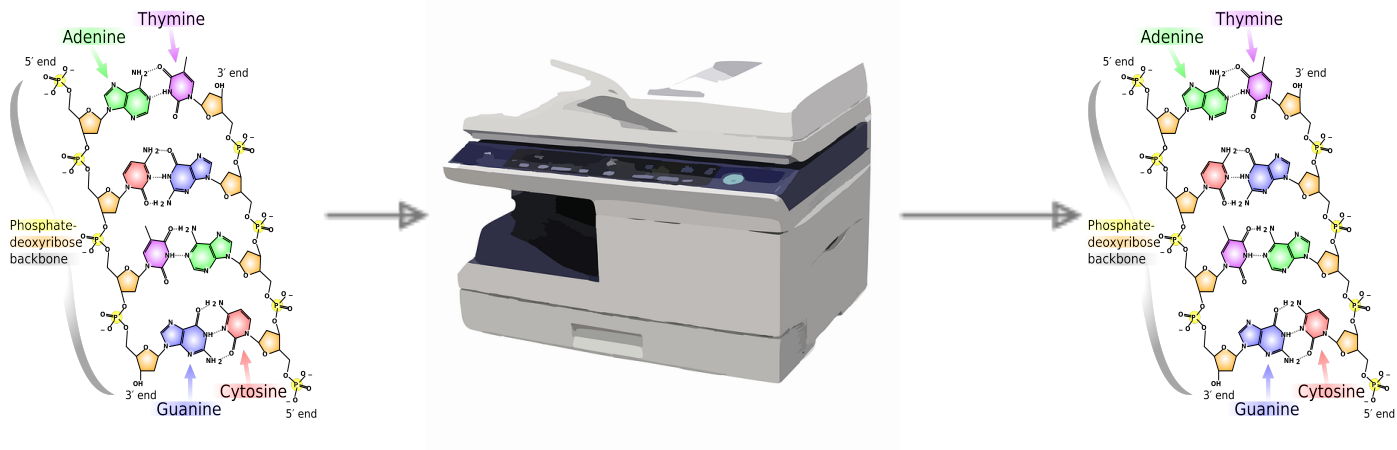


Polymerase Chain Reaction (PCR)

Steven Salzberg

How do we make copies of DNA?



Recall that DNA sticks to itself (“hybridizes”)

5' - ACACCGGTTCTAGAGCAT - 3'
3' - TGTGGCCAAGCATCTCGTA - 5'

Primers

5' -CTATTCATTCAATCATACACCCAA

3' -GATAAGTAAGTTAGTATGTGGGTTCTATCTATCTATCTATCTATCTATCTATCTATCTAAGAAACACCACCCAGAC-5'

5' -CTATTCATTCAATCATACACCCAA**GATAGATAGATAGATAGATAGATAGATAGATAGATAGATTCTTTGTGGTGGGTCTG**-3'
CTAAGAAACACCACCCAGAC-5'

Here we have 2 primers, one in green, one in blue

Melt (heat up gently)

5' -CTATTCATTCAATCATACACCCAA

3' -GATAAGTAAGTTAGTATGTGGGTTCTATCTATCTATCTATCTATCTATCTATCTATCTAAGAAACACCACCCAGAC-5'

5' -CTATTCATTCAATCATACACCCAA**GATAGATAGATAGATAGATAGATAGATAGATA**GATTCTTTGTGGTGGGTCTG-3'

CTAAGAAACACCACCCAGAC-5'

Anneal (cool down gently)

5' -CTATTCATTCAATCATACACCCAA

3' -GATAAGTAAGTTAGTATGTGGGTTCTATCTATCTATCTATCTATCTATCTATCTATCTAAGAAACACCACCCAGAC-5'

5' -CTATTCATTCAATCATACACCCAA**GATAGATAGATAGATAGATAGATAGATAGATA****GATTCTTTGTGGTGGGTCTG-3'**

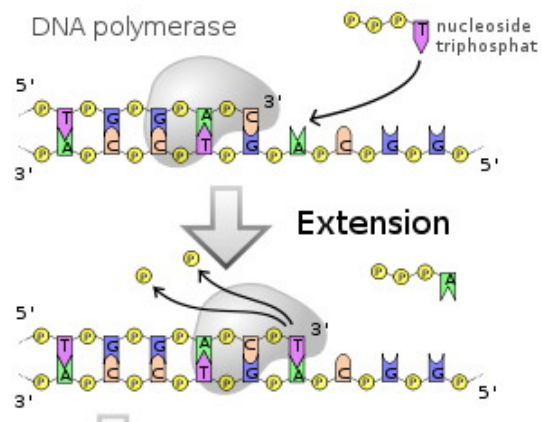
CTAAGAAACACCACCCAGAC-5'

Add copier molecule: DNA polymerase

5' -CTATTCATTCAATCATACACCCAA

3' -GATAAGTAAGTTAGTATGTGGGTTCTATCTATCTATCTATCTATCTATCTATCTATCTAAGAAACACCACCCAGAC-5'

5' -CTATTCATTCAATCATACACCCAA**GATAGATAGATAGATAGATAGATAGATA**GATTCTTTGTGGTGGGTCTG-3'
CTAAGAAACACCACCCAGAC-5'



Result after round one:

5' -CTATTCATTCAATCATACACCCAAGATAGATAGATAGATAGATAGATAGATAGATAGATTCTTTGTGGTGGGTCTG-3'
3' -GATAAGTAAGTTAGTATGTGGGTTCTATCTATCTATCTATCTATCTATCTATCTATCTATCTAAGAAACACCACCCAGAC-5'

5' -CTATTCATTCAATCATACACCCAAGATAGATAGATAGATAGATAGATAGATAGATAGATTCTTTGTGGTGGGTCTG-3'
3' -GATAAGTAAGTTAGTATGTGGGTTCTATCTATCTATCTATCTATCTATCTATCTATCTATCTAAGAAACACCACCCAGAC-5'

Result after round two:

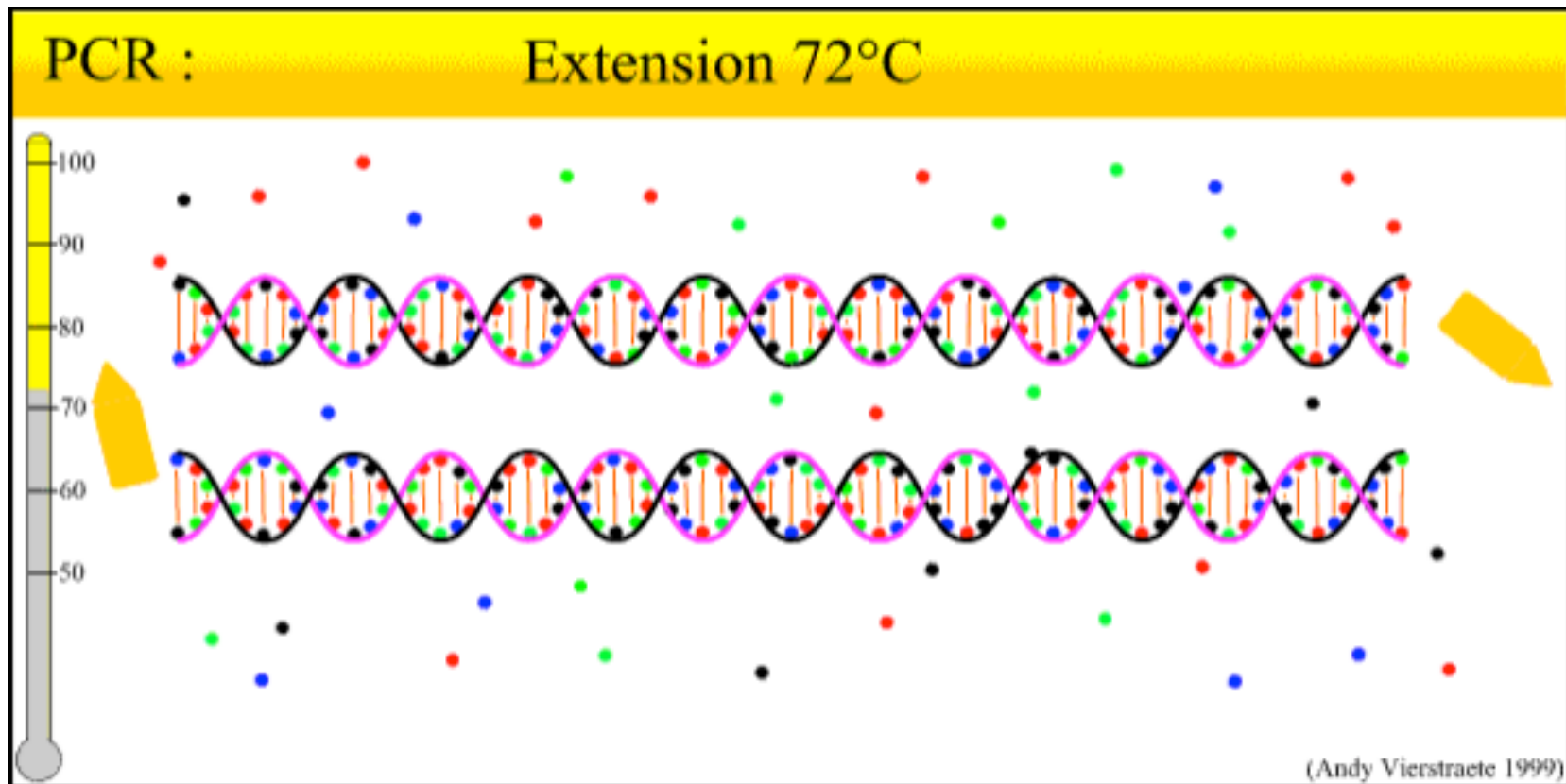
5' -CTATTCATTCAATCATAACCCAA**GATAGATAGATAGATAGATAGATAGATAGATAGAT**AGATTCTTTGTGGTGGGTCTG-3'
3' -GATAAGTAAGTTAGTATGTGGGTT**CTATCTATCTATCTATCTATCTATCTATCTATCTAT**CTAAGAAACACCACCCAGAC-5'

5' -CTATTCATTCAATCATAACCCAA**GATAGATAGATAGATAGATAGATAGATAGATAGAT**AGATTCTTTGTGGTGGGTCTG-3'
3' -GATAAGTAAGTTAGTATGTGGGTT**CTATCTATCTATCTATCTATCTATCTATCTATCTAT****CTAAGAAACACCACCCAGAC-5'**

5' -CTATTCATTCAATCATAACCCAA**GATAGATAGATAGATAGATAGATAGATAGATAGAT**AGATTCTTTGTGGTGGGTCTG-3'
3' -GATAAGTAAGTTAGTATGTGGGTT**CTATCTATCTATCTATCTATCTATCTATCTATCTAT**CTAAGAAACACCACCCAGAC-5'

5' -CTATTCATTCAATCATAACCCAA**GATAGATAGATAGATAGATAGATAGATAGATAGAT**AGATTCTTTGTGGTGGGTCTG-3'
3' -GATAAGTAAGTTAGTATGTGGGTT**CTATCTATCTATCTATCTATCTATCTATCTATCTAT****CTAAGAAACACCACCCAGAC-5'**

Repeat for 30 cycles or more...



Summary: the PCR recipe

Ingredients

- DNA
- primers
- DNA polymerase
- A's, C's, G's, T's

Summary: the PCR recipe

1. Melt at 94o C
2. Cool to 54o C
3. Warm to 72o C
4. Go to step 1



The Nobel Prize in Chemistry 1993

Kary B. Mullis, Michael Smith

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Nobel Lecture

Nobel Lecture, December 8, 1993

The Polymerase Chain Reaction

In 1944 [Erwin Schroedinger](#), stimulated intellectually by [Max Delbrück](#), published a little book called *What is Life?* It was an inspiration to the first of the molecular biologists, and has been, along with Delbrück himself, credited for directing the research during the next decade that solved the mystery of how "like beget like."

Max was awarded this Prize in 1969, and rejoicing in it, he also lamented that the work for which he was honored before all the peoples of the world was not something which he felt he could share with more than a handful.

[Samuel Beckett](#)'s contributions in literature, being honored at the same time, seemed to Max somehow universally accessible to anyone. But not his. In his