PCR: introduction

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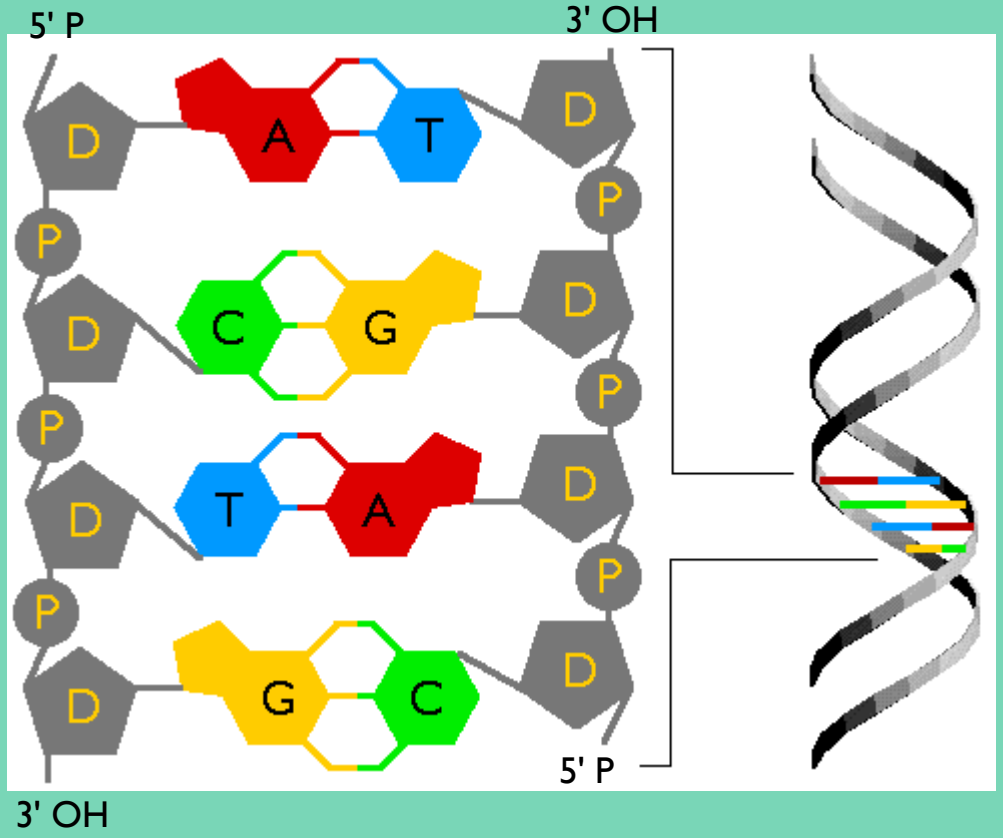
Polymerase chain reaction is a laboratory technique used to make multiple copies of a specific segment of DNA

Basics

principle of complementarity

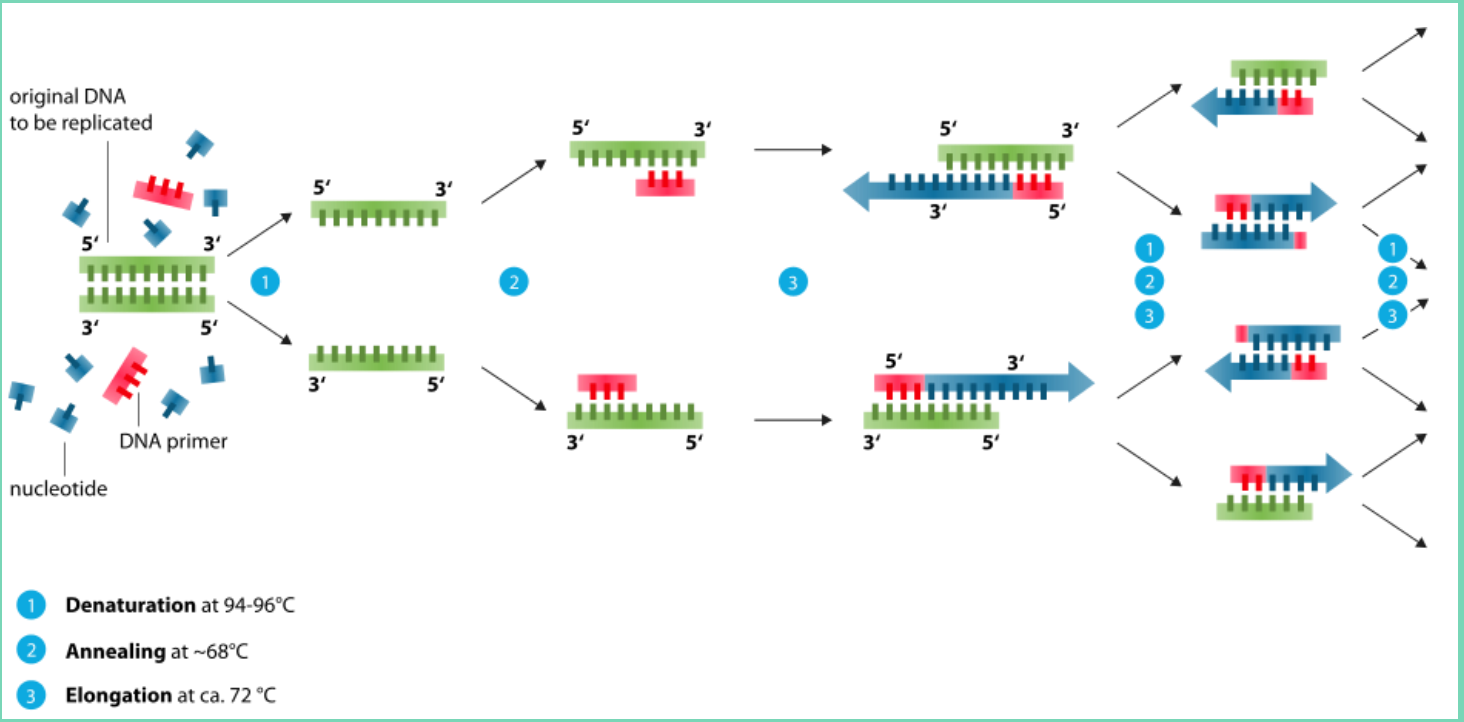


Jerome A. Montvilo http://www.ric.edu/

Glossary

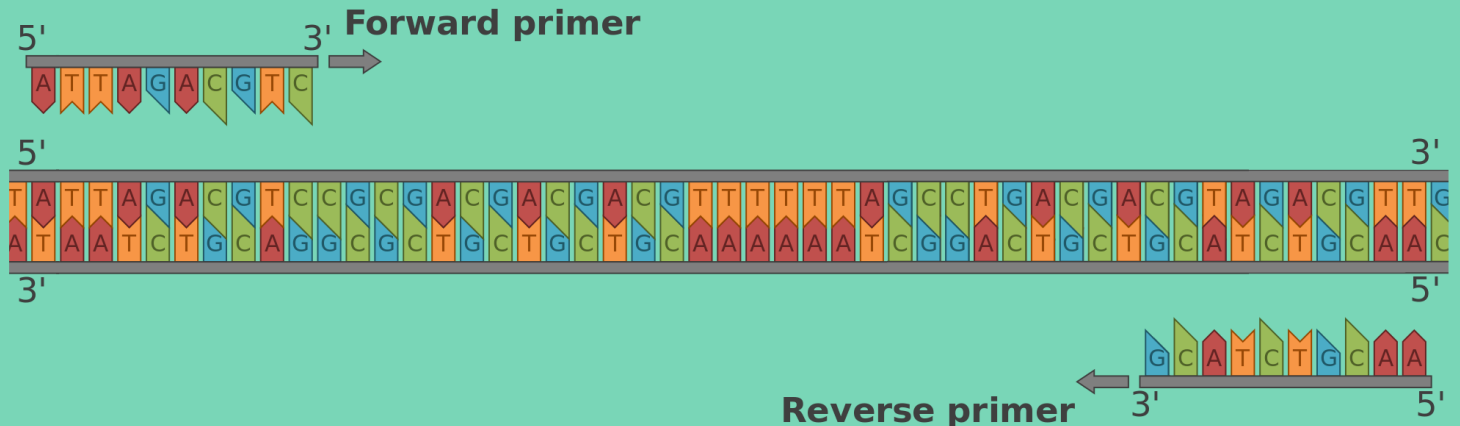
- DNA template – a sample of DNA that contains the target sequence
- DNA Polymerase – enzyme that synthesizes new strands of DNA
- Primers – short molecules of single-stranded DNA, used to identify and set borders to the reaction (~20nt)

Principle



Primers

- Forward and reverse primers: synthesis goes from 5' to 3', thus they are complementary to different DNA strands
- Define the specificity of the target



Zephyris [CC BY-SA 3.0]

Polymerase

- Has to withstand high temperatures:
  temperature of denaturation: ~93 °C
- Thus DNA-dependent DNA-polymerases are taken from thermophile bacteria

daveynin [CC BY 2.0]

Pol-s for different purposes

For different purposes, different DNA polymerases:

- slow but accurate for cloning
- fast but with 0,3% error rate (3 wrong nucleotides per 1000) for gene expression analysis etc.

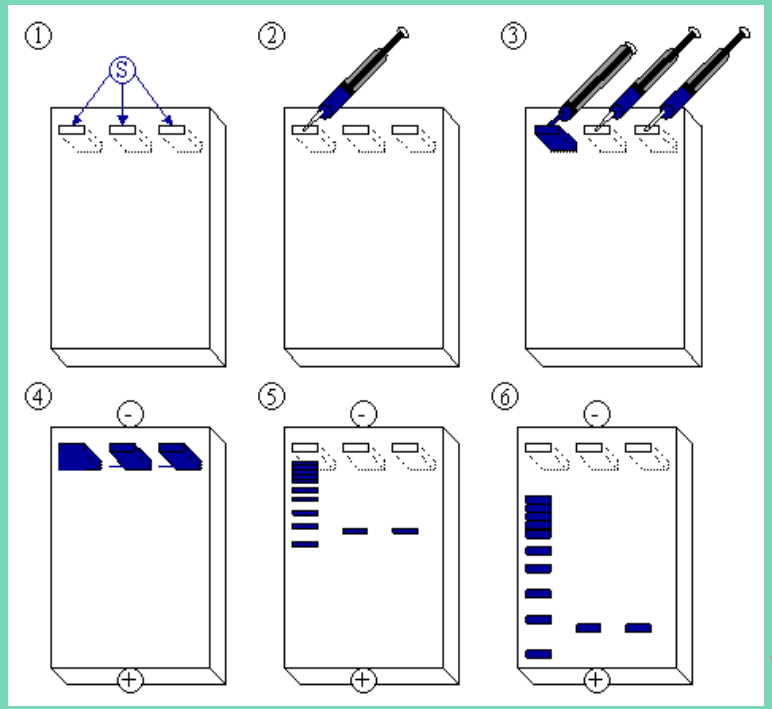
- Basic one is *Taq*Pol from *Thermus aquaticus*

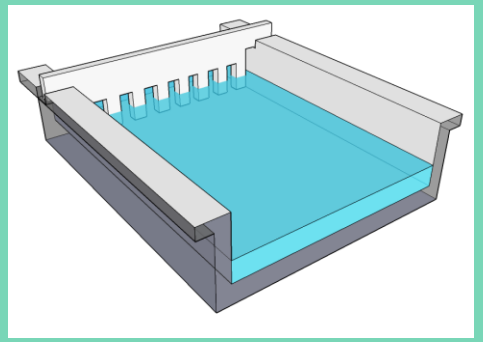
	KOD (%)	Phusion HF (%)	Pt *Taq* (%)	Expand HF (%)	FastStart HF (%)	Sequal Prep Long (%)	*Pfu* Ultra HF (%)
Overall error rate[a]	0.21	0.11	0.34	0.25	0.23	0.29	0.23
Insertions	0.10	0.07	0.14	0.11	0.11	0.11	0.12
Deletions	0.06	0.02	0.08	0.07	0.05	0.06	0.05
Substitutions	0.01	0.01	0.07	0.04	0.03	0.07	0.01

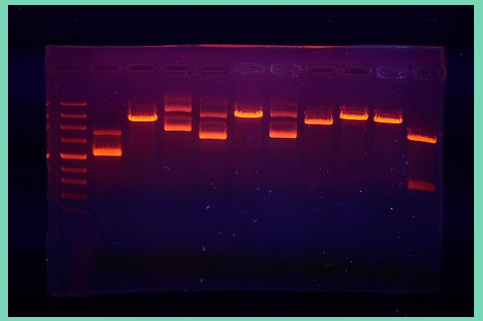
thermofisher.com

PCR results

- The most basic one:
  agarose gel electrophoresis







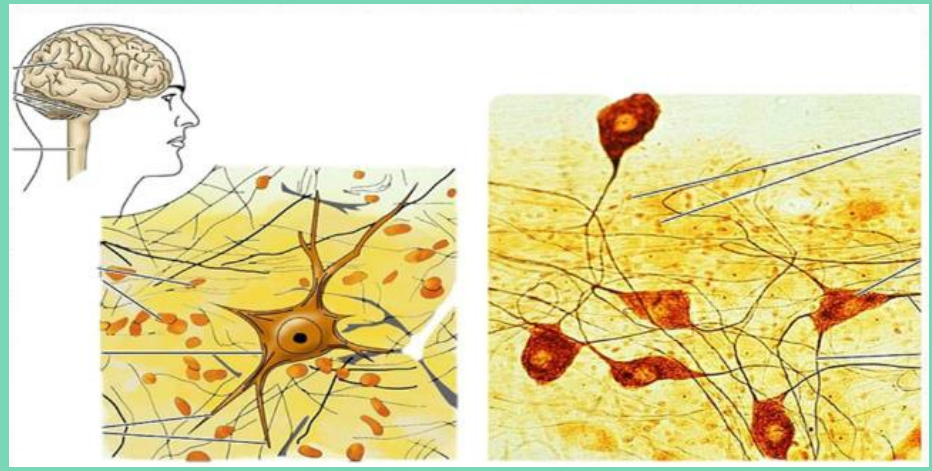
Applications of PCR

myriad of ways...

Gene expression

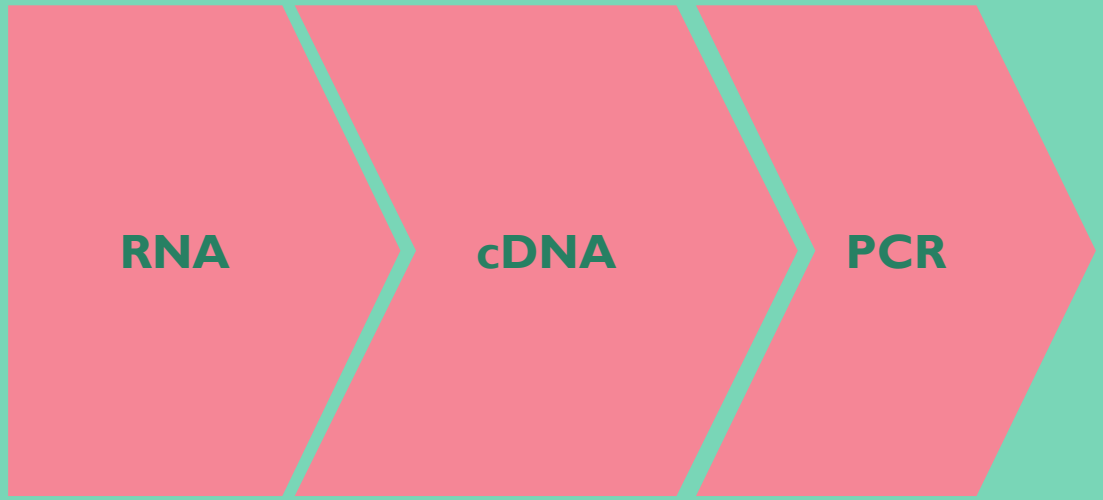
- DNA is ~the same in all cells
- Cells are different: different genes are expressed at different levels

- Thus mRNAs and ncRNAs must be studied



RT-PCR
Reverse transcription PCR

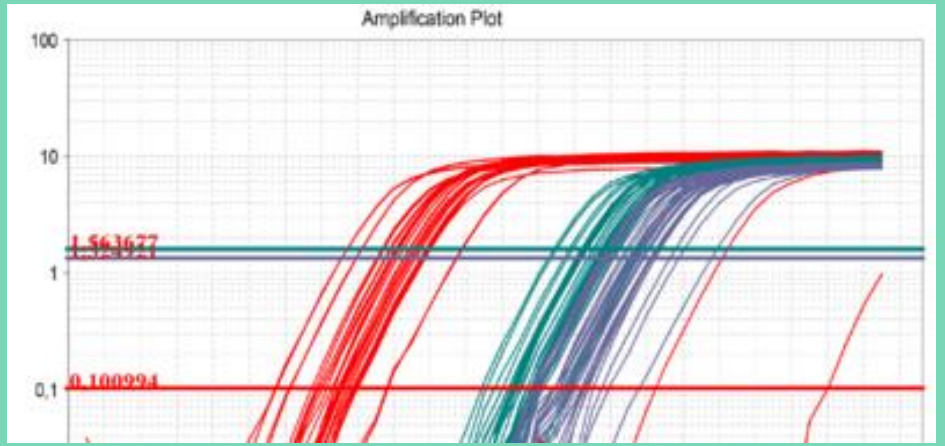
- RNA is revers transcribed to cDNA (RNA-dependent DNA-polymerases)
- M-MLV Reverse Transcriptase (Moloney Murine Leukemia Virus)



Real time PCR (qPCR)

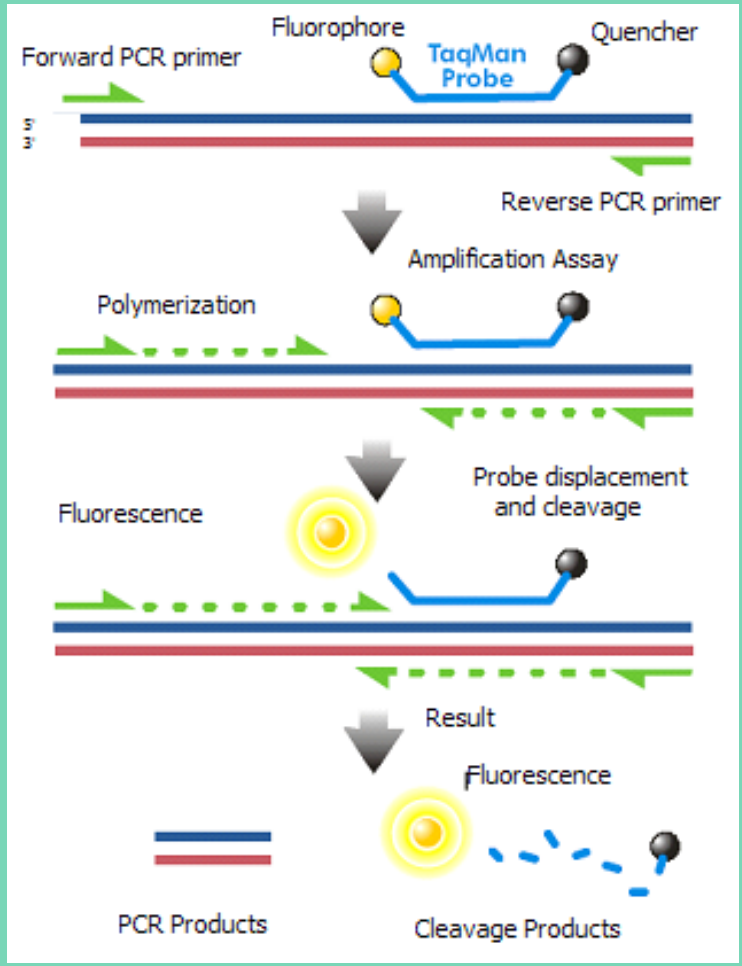
- In theory every step of PCR is 2^n
- In practice it reaches a plateau

- qPCR monitors the reaction due to fluorescent dyes



TaqMan qPCR

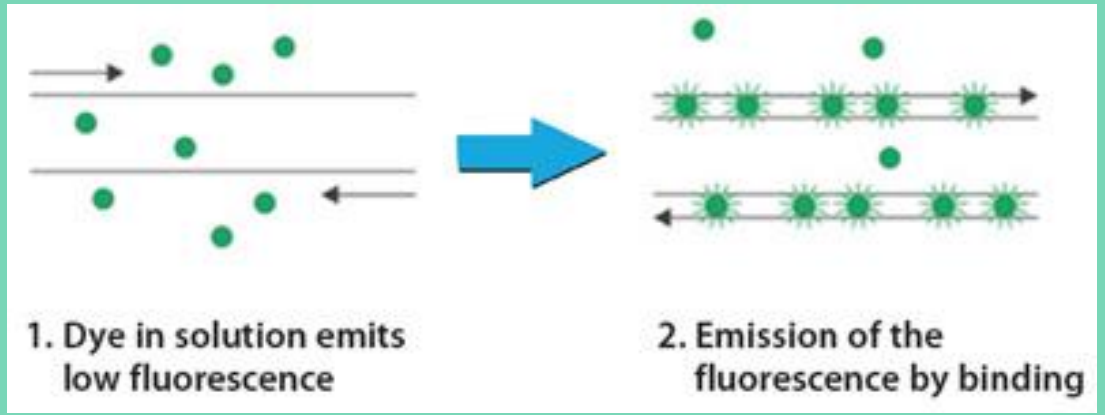
- Very sensitive technique
- 3 primers
- Has quencher and reporter
- Fluorescence only with the use of the target primer



Braindamaged [Public domain]

SYBR GREEN

- SYBR green inserts into dsDNA and emits light
- As PCR progresses more emission is detected



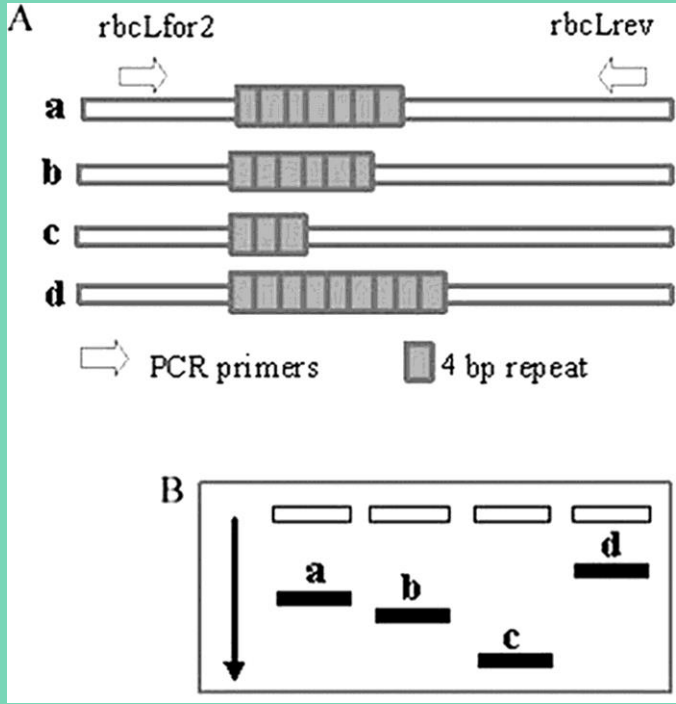
Sigma-Aldrich

DNA profiling (fingerprint)

- DNA sequence is different in different people
- It should not take up to whole genome DNA sequencing to identify a person

Variable number tandem repeats

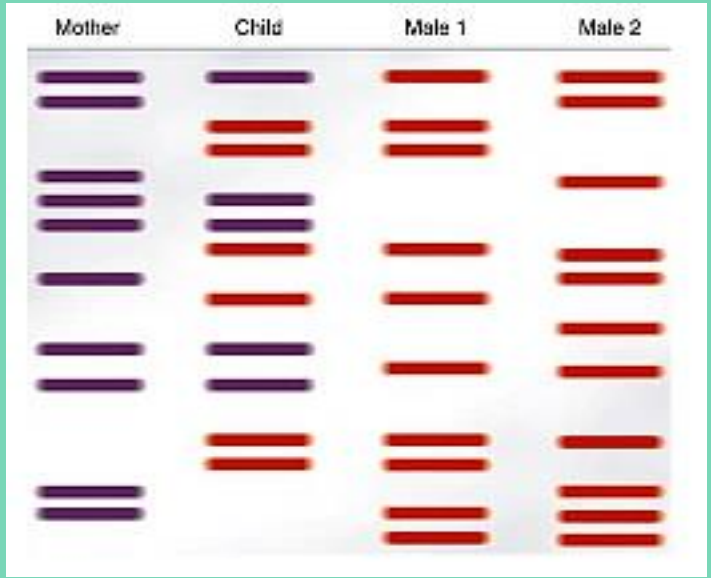
- Each person has different number of VNTR
- microsatellites: 3-5 nucleotide repeats (mostly 4 bp)
- repeated different number of times n=[3;70]
- ~ 20 loci for an ID



Monica GuhaMajumdar et al. Eukaryotic Cell 2008;7:639-646

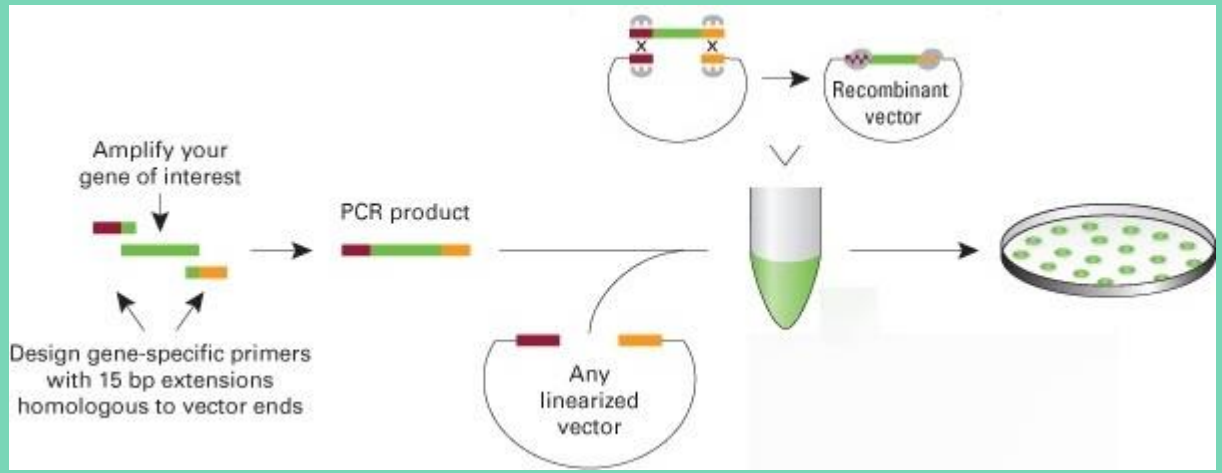
Paternity test

- A child DNA has a combination of their maternal and paternal VNTR patterns



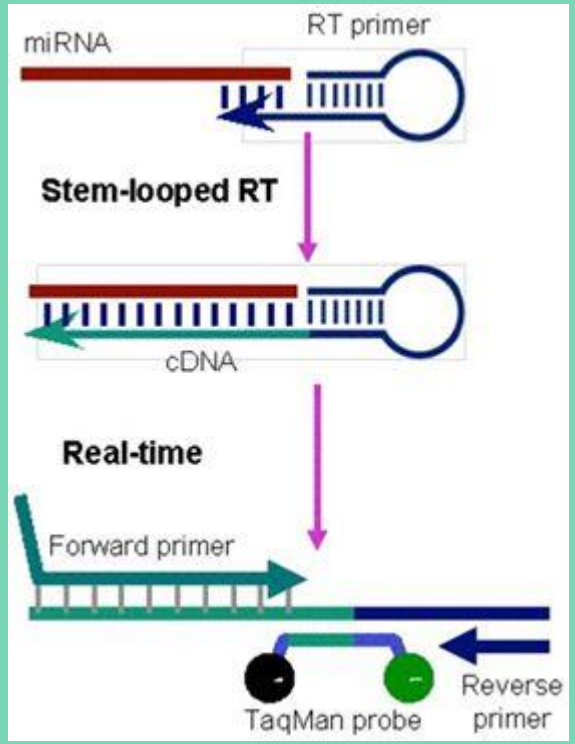
Cloning: deadly sin

- Cloning: making an identical fragment of DNA
- Gene from genome: PCR, insert into plasmid, cloned in bacteria
- Further use: study the gene/protein



Interesting: catch that miR

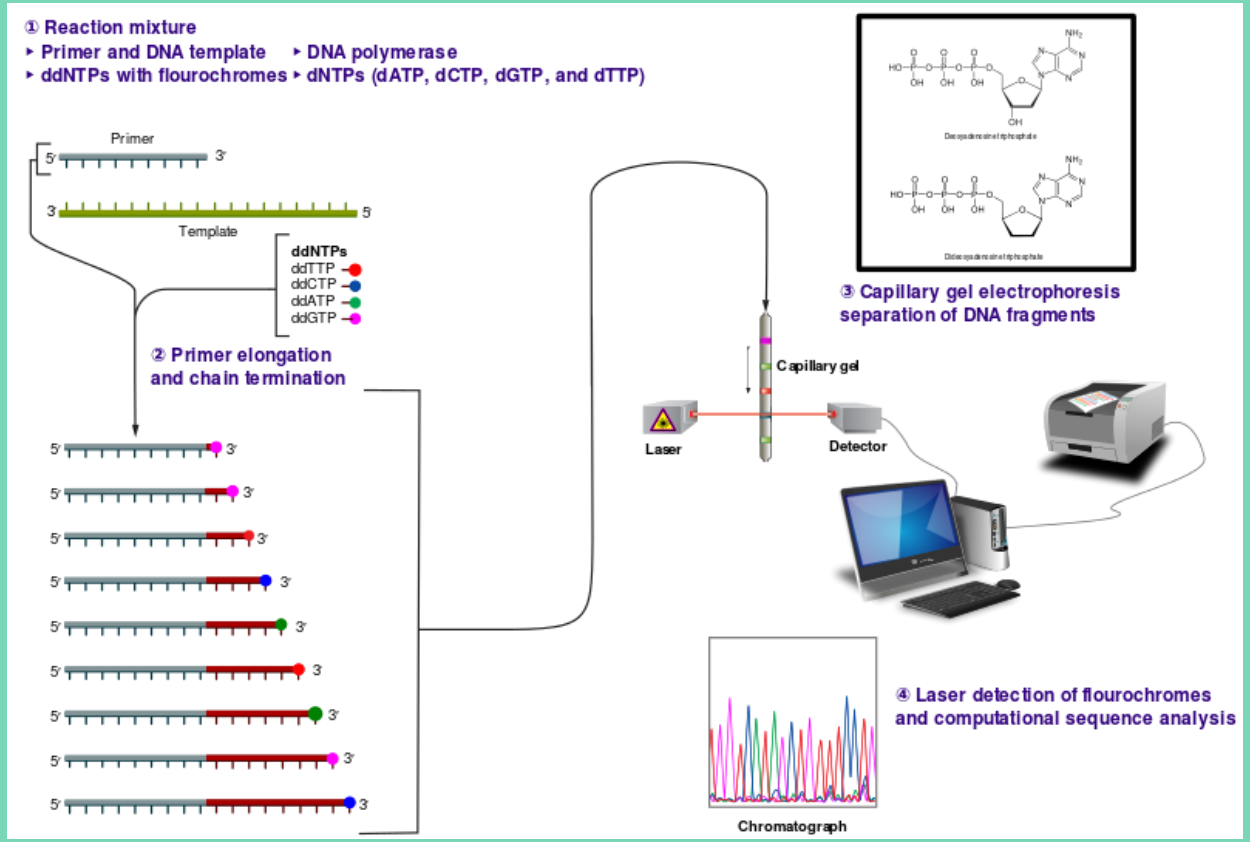
- microRNAs are equal/shorter than primers
- PCR is still possible :)
- RT primers are designed to have a stem-loop structure
- Followed by TaqMan qPCR



PCR Troubleshooting and Optimization: The Essential Guide | Book 2011
ISBN: 978-1-904455-72-1

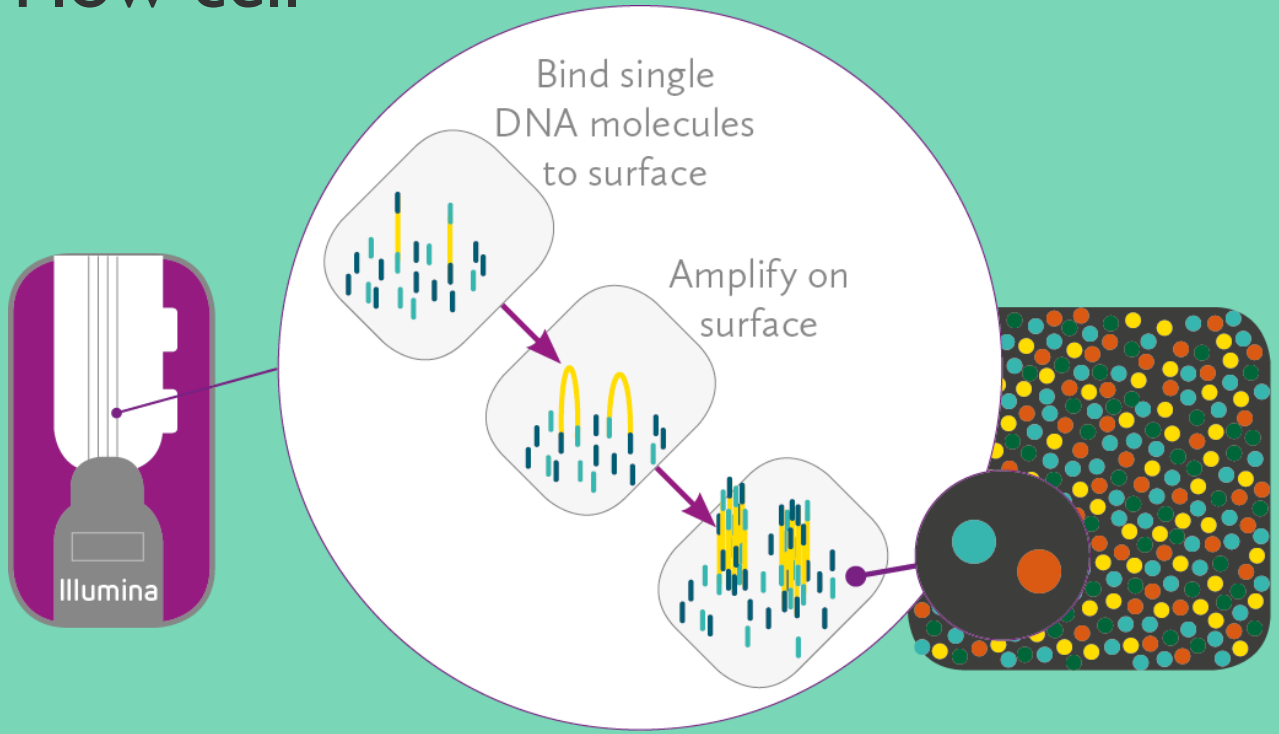
Sequencing vs PCR

Chain-termination method (Sanger)



Bridge PCR
next-gen seq

- Colonies of identical DNA fragments
- Each PCR cycle: +1 bp, fluorescent
- Flow cell



MDA

Multiple displacement amplification

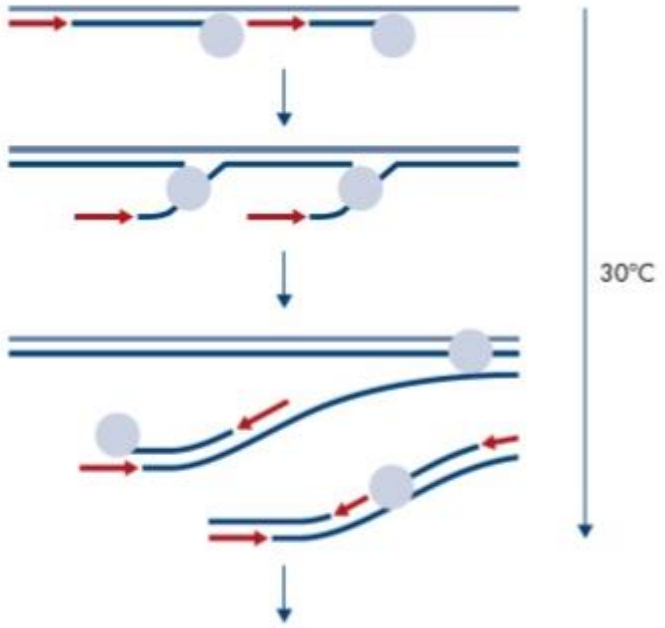
Amplification
but not PCR

MDA

- Non specific primers
- Constant amplification and displacement

QIAGEN's REPLI-g technology

- Primers (arrows) anneal to the template
- Primers are extended at 30℃ as the polymerase moves along the gDNA or cDNA strand displacing the complementary strand while becoming a template itself for replication
- In contrast to PCR amplification, MDA:
  - Does not require different temperatures
  - Ends in very long fragments with low mutation rates



MDA good for whole genome

- 30˚C constant, no high temperatures
  less DNA damage
- Phi29 polymerase — high fidelity
  low error rate
- Long fragments, up to 70 kbp

video! (?)

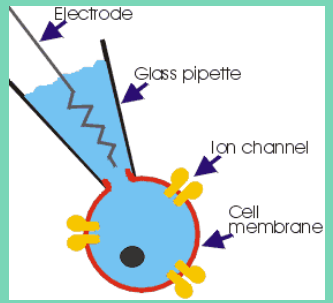
Thank you

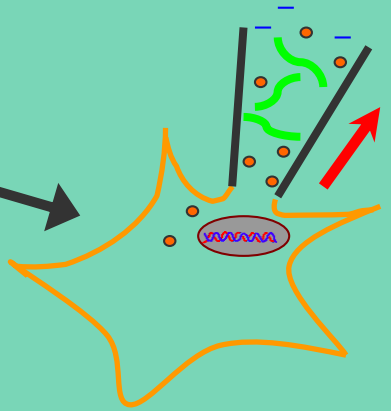
Further reading

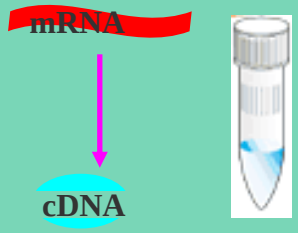
- Molecular Cloning:
  A Laboratory Manual
  aka "Maniatis"
- protocol-online.org + bio-forum
- ResearchGate
- molbiol.ru

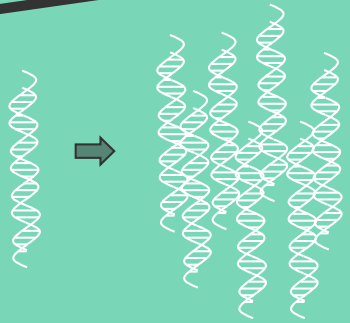
Single cell PCR One cell — a lot of results

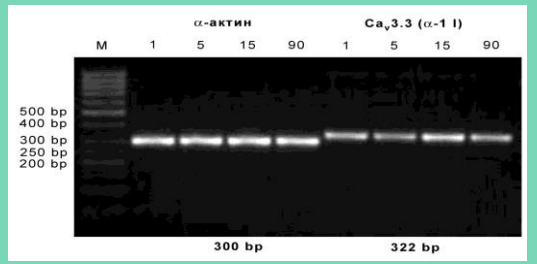
- PCR is capable of detecting even one DNA molecule!





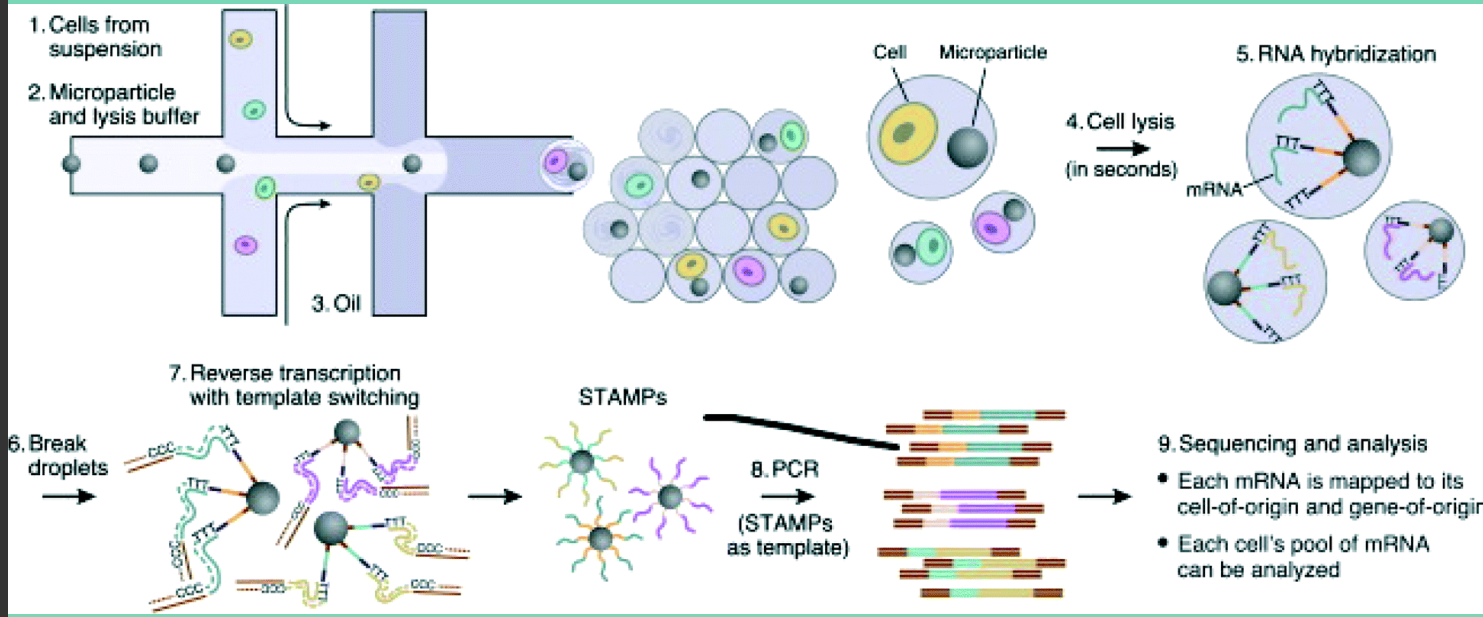






DropSeq

- Barcoded droplet-based mRNA sequencing
- The microparticles carry a cell-specific barcode, a unique molecular identifier, and an oligo-dT for capturing polyadenylated mRNAs.



DOI: 10.1039/C5LC90101D *Lab Chip*, 2015, **Manjima Dhar**
Research highlights: microfluidic-enabled single-cell epig

Слава Україні