qPCR course

Alyona Minina
Department of Plant Biology
SLU

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A scientific representation of challenges to be met during this course

- Waste of time
- Not learning a thing
  - Boredom
- Lack of understanding
- Basic stuff was usually studied not in English
  - Language barrier
    - Fear of looking stupid
      - Not asking questions
• work together (you will learn stuff better while teaching each other)

• climb over your language barrier, there are no stupid questions

• tell me what do you want to know?
an example of a language barrier

\[ x = a \times b^c \]

\[ \log_k l = m \]

Please interrupt and ask!!
What do you want to know?

Data Analysis and interpretation

Statistical analysis of qPCR

Application of qPCR

How qPCR works

Basics

Troubleshooting

Normalization

Everything

More
• The ends of DNA
• Polymerase
• Concept of PCR
DNA
Deoxyribonucleic Acid

5'-Carbon sugar

Nucleobase

H^+ acid <-> H^+ + acid^-
H^+ + base^- <-> H^+base
H^+ PO_4^{2-} <-> H^+ + PO_4^{3-}

purines

pyrimidines

5-Carbon sugar

H = prime to distinguish numeration of atoms in nucleobases and sugar backbone
DNA is a polymer molecule.
This image represents a diagram of a nucleic acid strand, specifically DNA or RNA, showing the 5' and 3' ends. The diagram highlights the structure of three nucleotides connected by phosphodiester bonds. The 5' end is marked with a blue arrow, and the 3' end is marked with a red arrow. The nucleotides are labeled as Base #1, Base #2, and Base #3, with specific positions marked for each base.
small and charged  \( \Rightarrow \) hydrophilic

mostly hydrophobic

cell environment is mostly hydrophilic
14 pages long article in PNAS (February 1953) describing a model with phosphates looking inwards
Double helix model

Rosalind Franklin
Maurice Wilkins
Ray Gosling
Double helix model

Rosalind

Ray Gosling
What will hold two strands together?

1:1 1:1
But Francis, do you think we were lucky to have solved it?
WE wish to suggest a structure for the salt of deoxyribonucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

As a residue on each chain every 3-4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues. In both chains, the distance of a phosphorus atom from the fibre axis is 18 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together, by the purines and pyrimidine base pairs. The bases of the chains are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a base from the other chain.
which one is which?
hydrophobicity effect

5'  

Hydrogen bond  

3'  

rigid  

?  

5'
It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.
Arthur Kornberg

“Enzymic synthesis of deoxyribonucleic acid”

Kornberg et al, 1956
Biochimica et Biophysica Acta
DNA polymerase I  E. coli
$PPI = \text{pyrophosphate}$
DNA polymerase I exonuclease activity
DNA polymerase

- Can synthesise only in one direction

- Needs (among other things):
  1. ssDNA (single strand) as a template
  2. 3’-OH group to add new nucleotides to
Draw replication of one circular DNA molecule and how you think it works in an E. coli cell.
Concept of PCR

Yellowstone National Park

Thermus aquaticus
50-80°C

Taq DNA polymerase

Thomas D. Brock
Concept of PCR

why are the strands so easy to separate without breaking them?

melting = denaturation

95°C

dsDNA

ssDNA

+ primer annealing

60°C

Taq pol

does this process require energy?

synthesis = extension

72°C

Look up this: Paul Rothemund: The astonishing promise of DNA folding
Inventor of PCR Kary Mullis

4 Nobel Prize Winners Who Were Clearly Insane

#3. Kary Mullis

Prize: Chemistry

 Talks Out Of His Ass About: Everything Else

On the other hand, Kary Mullis, 1993 chemistry Nobel Prize winner, is by all accounts a dick.
Concept of PCR

1 cycle
1. why Mg²⁺ is important?
   what will happen if you add too much/too little?

2. why concentration of dNTP is important?
   what will happen if you add too much/too little?

3. why concentration of primers is important?
   what will happen if you add too much/too little?

4. why GC content of the template is important?
   what to do if it is too high/too low?

5. What is two step and what is three step PCR?