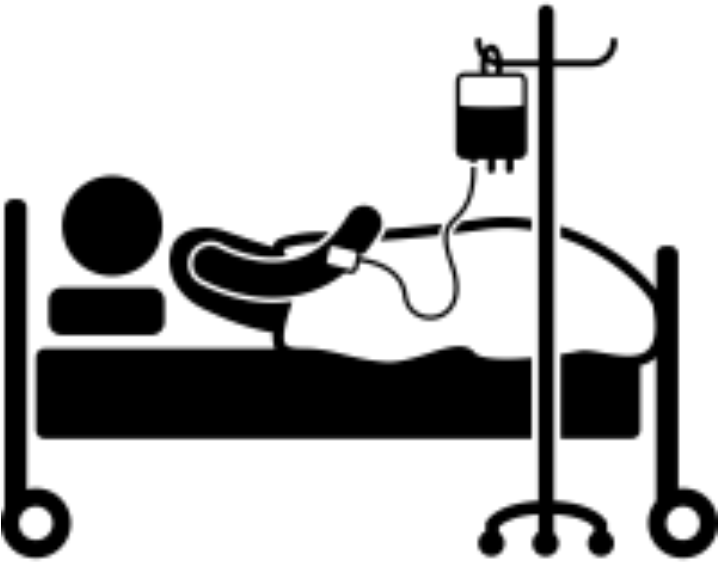

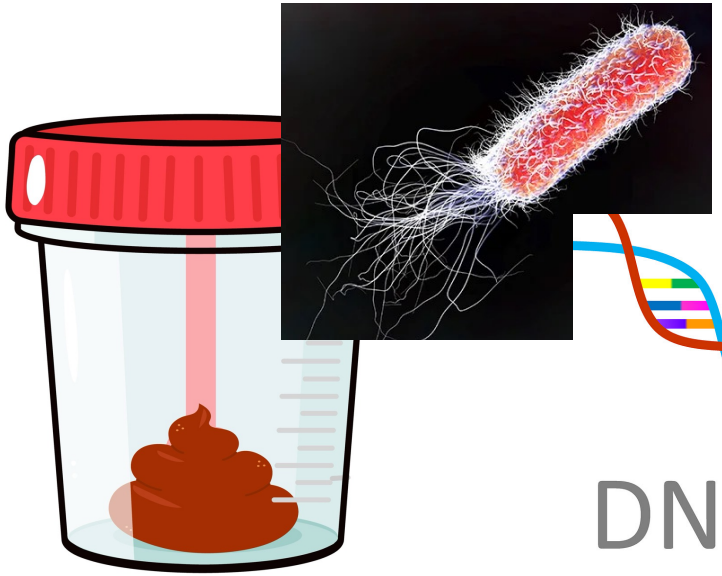


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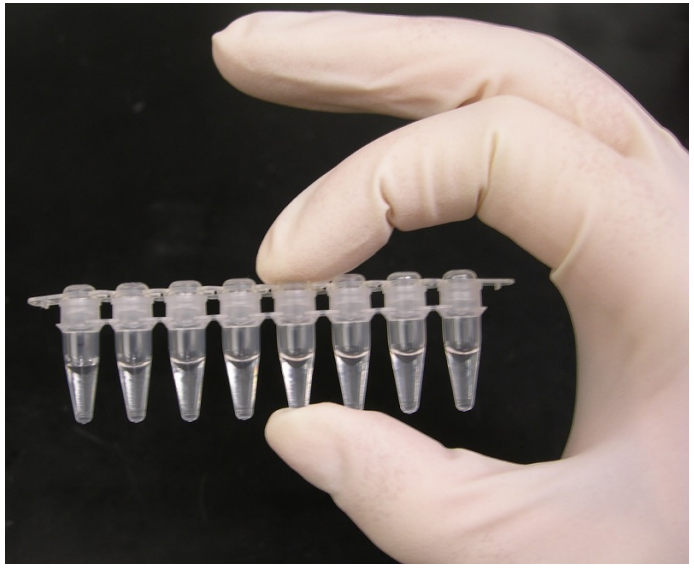
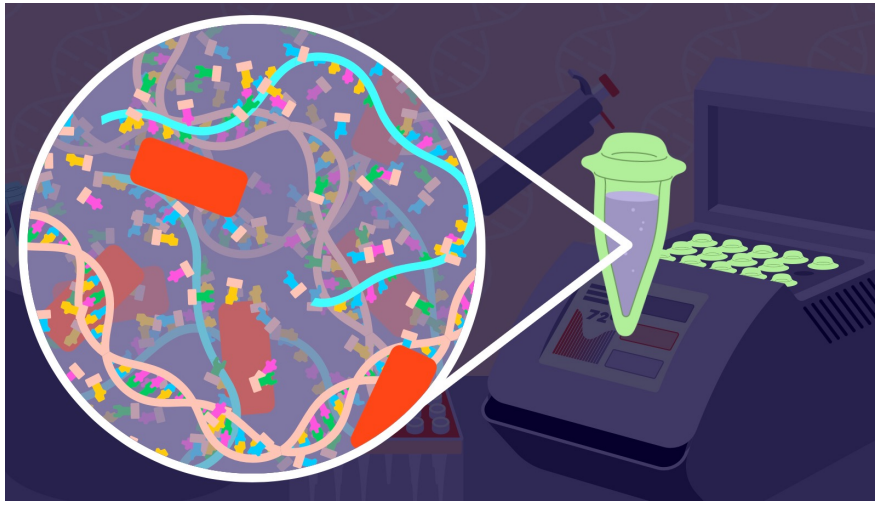
TTCTTTCTGCTGTATCAGCAAGCTTTATTCTGGGAAACAGTGACGCACATACAGGAACAAAAGCTGCAGC
```

PCR reaction- practical

- Mix:
  - DNA extracted from the environmental samples - template
  - Primers – to target the amplicon sequence
  - Nucleotides – to build new DNA molecules
  - Taq- Polymerase
  - Salts – Ca^{+2} for the enzyme to function

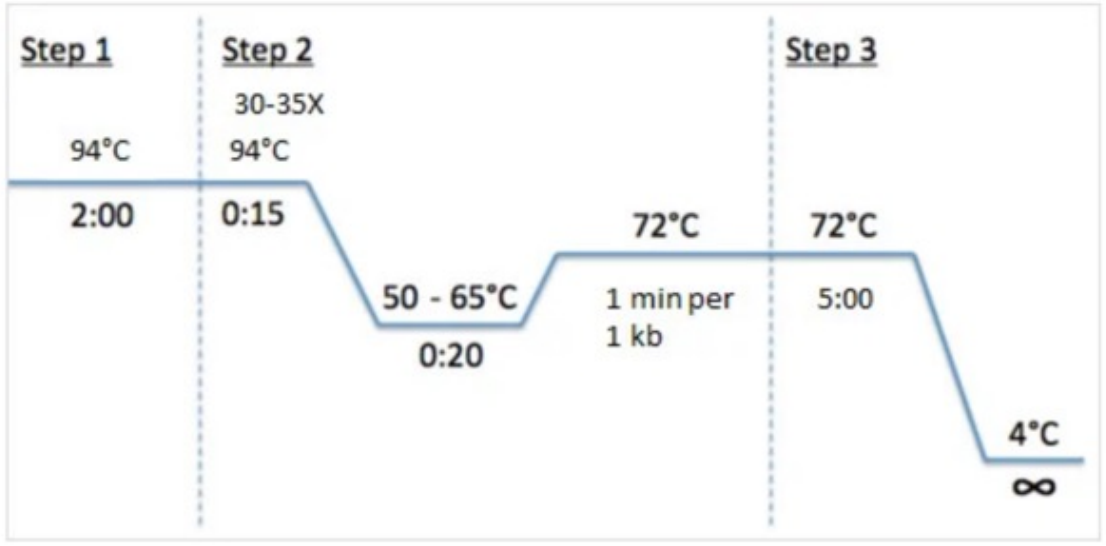




PCR reaction- practical

PCR Steps

- Program your thermocycler for your PCR reaction using the following guidelines:



Step	Temp	Time	# of cycles
Initial Denaturation	94°C	5 min	1 cycle
Denaturation	94°C	30 sec	25-35 cycles
Primer Annealing	T_m-5°C	45 sec	
Extension	72°C	1 min per kb	
Final Extension	72°C	5 min	1 cycle

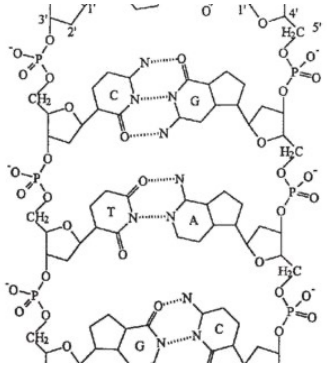
Taq Polymerase

- Why denature at high heat?
- In cells during DNA replication ->
  - Multitude of enzymes/cofactors needed to unwind DNA and replicate it.
- With PCR we are able to do with with just the Taq polymerase
- But high heat denatures enzymes…

Why do we need PCR?

Visualization of DNA: Gel electrophoresis

- DNA has a –ve charge



Visualization of DNA: Gel electrophoresis

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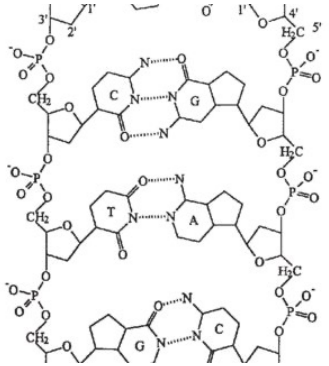
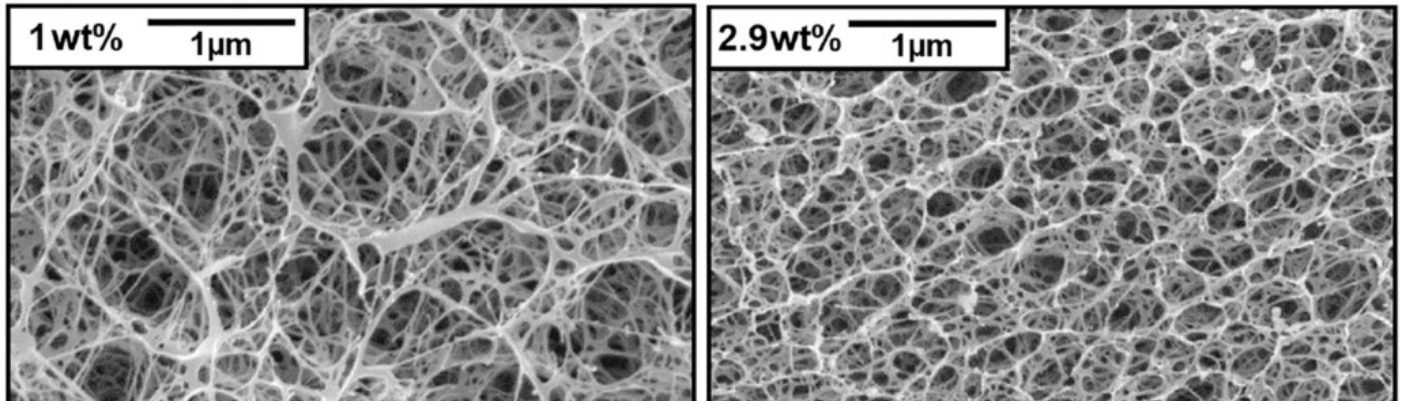
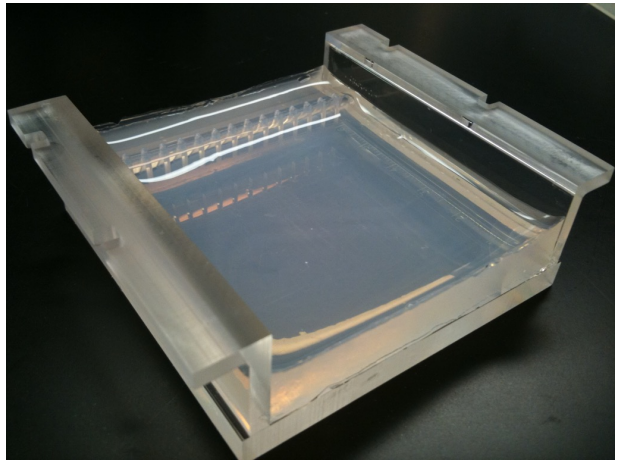


- Agarose makes a polymer gel with pores





Visualization of DNA: Gel electrophoresis

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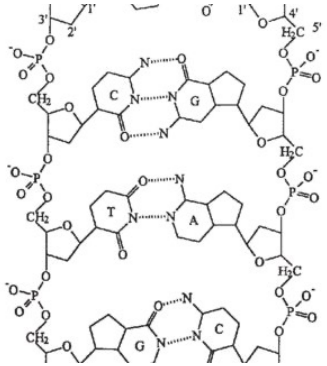
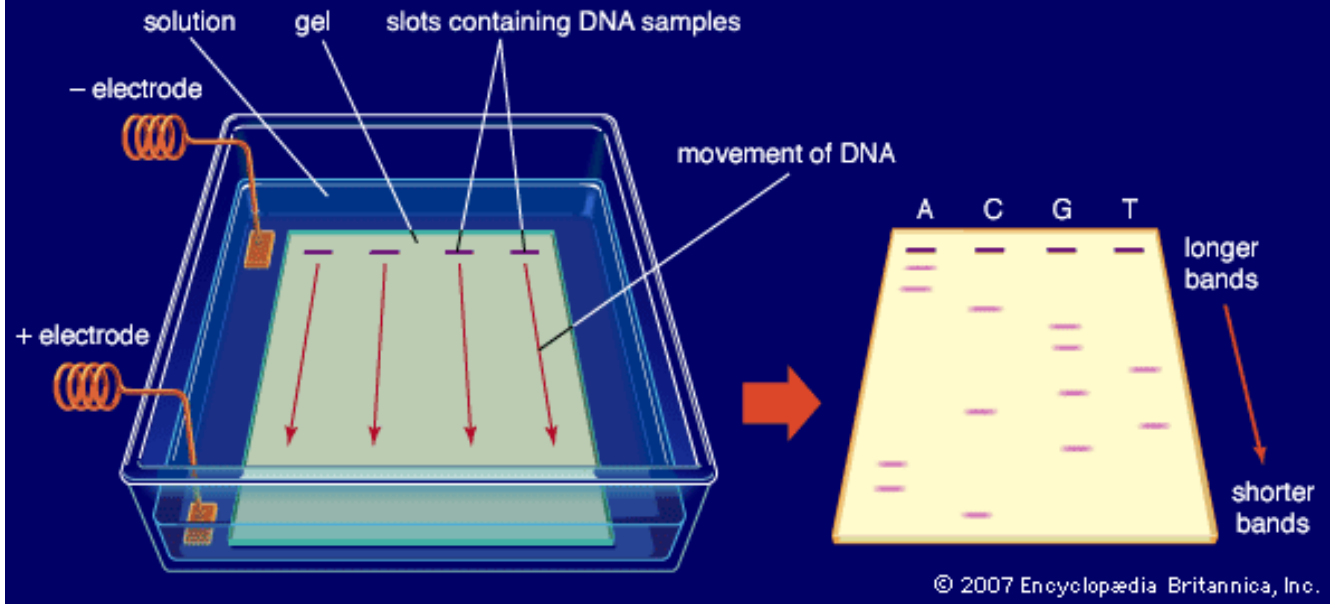
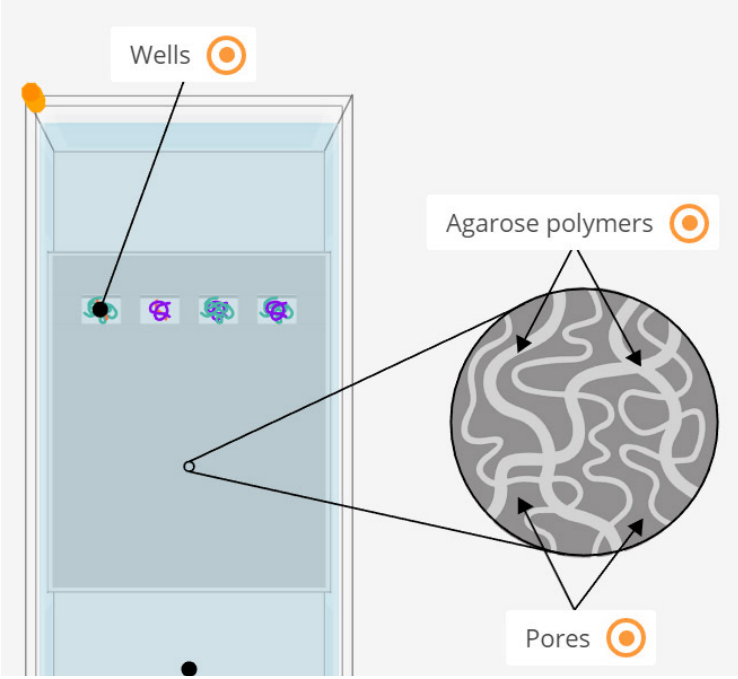
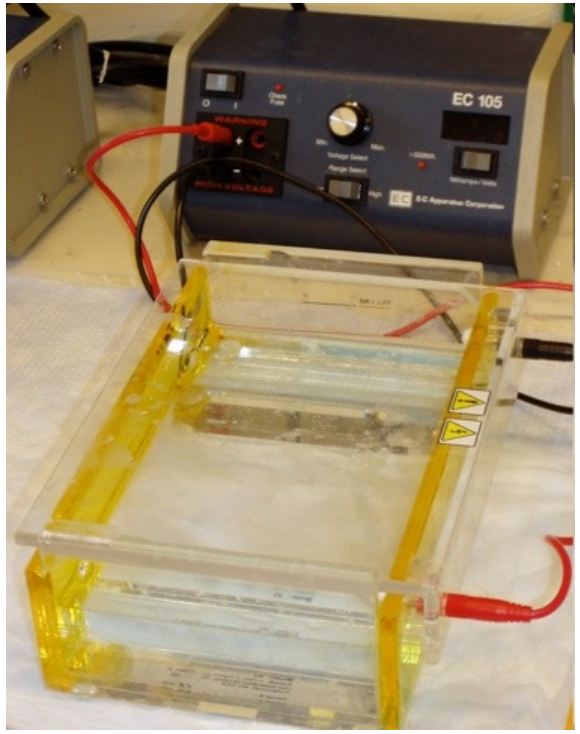


- Agarose makes a polymer gel with pores
- When an an electric current is applied, DNA moves towards the positive electrode
- Larger DNA pieces move through the gel slower







Visualization of DNA: Gel electrophoresis

- Fluorescent organic dyes can bind to DNA
- When exposed to UV, the DNA lights up
- Higher concentration of DNA leads to brighter bands