INTRO TO BIOHACKING

Or “How I learned to stop worrying and love the zombie apocalypse”

Structure

- What is Biohacking?
- Crash course in Molecular Biology
- Key Concepts and Tools for a bio lab

- What are other biohackers up to?
- What problems are the community facing, how can we solve them?

- Discussion – our project ideas and goals

But First:

A disclaimer...

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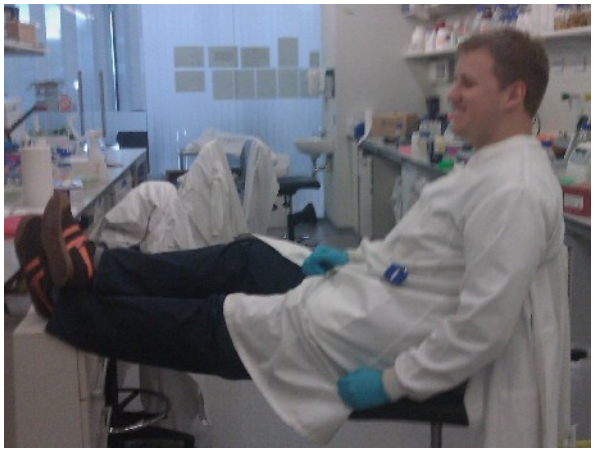


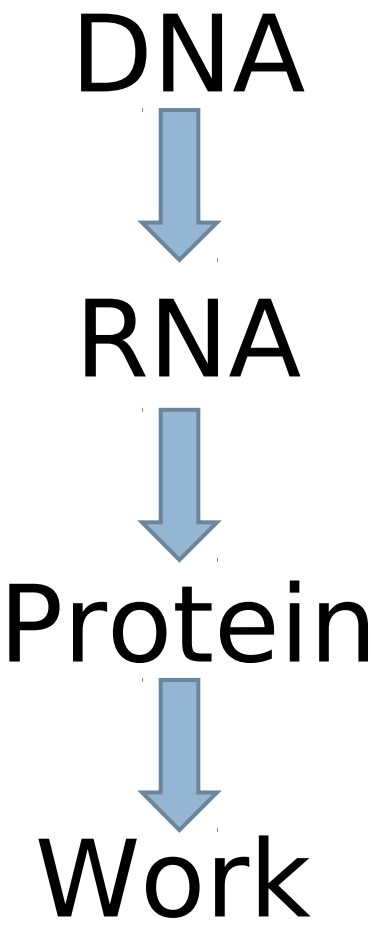
Dr Robert Neville

Me

So... Biohacking, WTF?

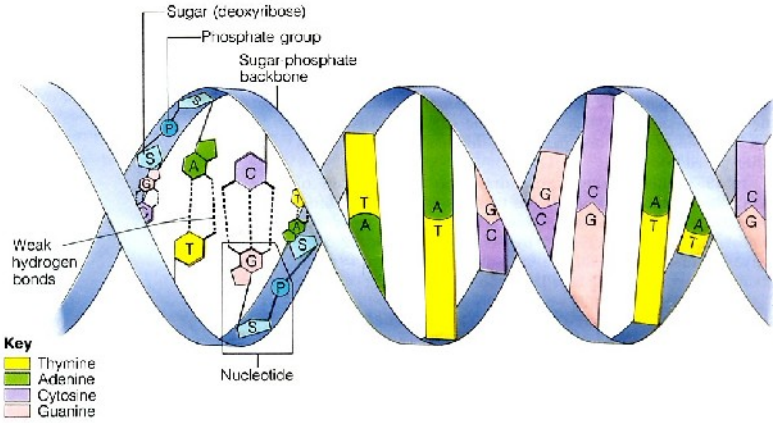
- Two main streams of development / research:
  - Biological research / engineering
    - Cloning
    - (random) mutagenesis
  - Physical / chemical research and engineering
    - Building open-source, cost-effective lab equipment

“Central Dogma” of mol biol

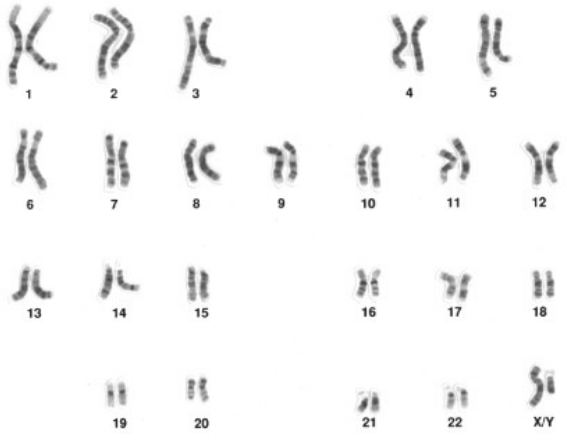


“Central Dogma” of mol biol

Information is encoded in our DNA



DNA
RNA
Protein
Work



Our DNA contains four bases: A C T G

These sequences are “recipes” for manufacturing proteins, which do all the work in the cell

Humans have ~3 billion base pairs encoding approx 20-25,000 protein-coding genes

“Central Dogma” of mol biol

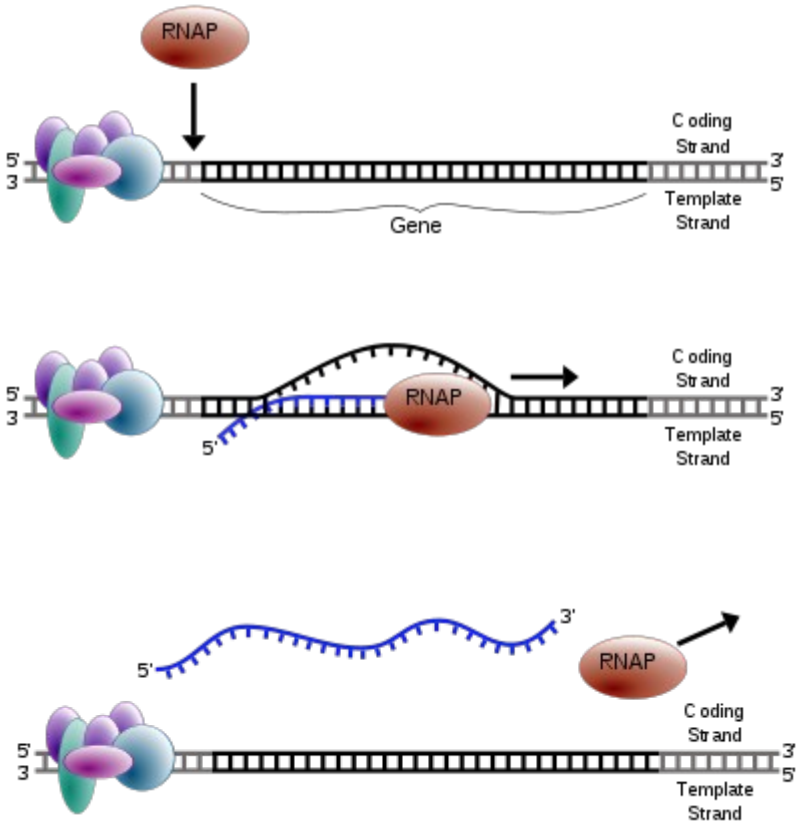
“Transcription”:
A complex of proteins recognise a “promoter sequence” at the start of a gene

This protein complex recruits RNA polymerase

RNA polymerase uses DNA as a template to make a complementary strand of RNA

RNA floats away for processing...

DNA is left unchanged



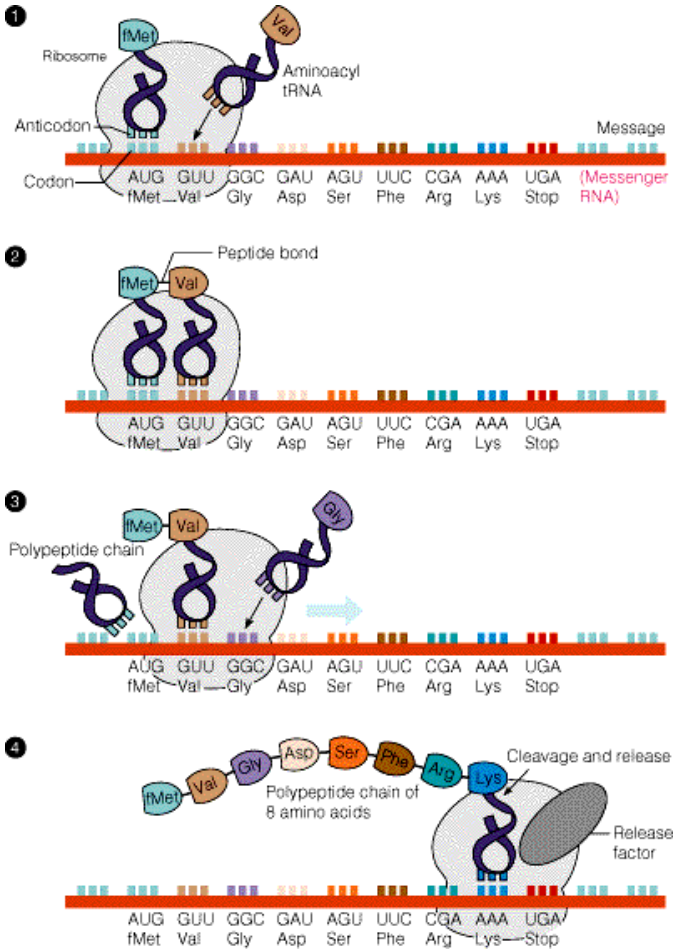
DNA
RNA
Protein
Work

“Central Dogma” of mol biol

mRNA is a template for protein manufacture

mRNA feeds through a **ribosome**, which recruits protein subunits

Subunits get assembled into peptide chain, until end of the mRNA is reached



DNA

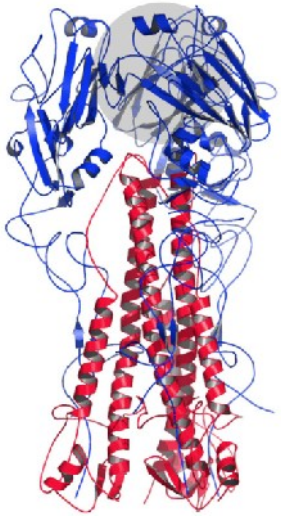
RNA

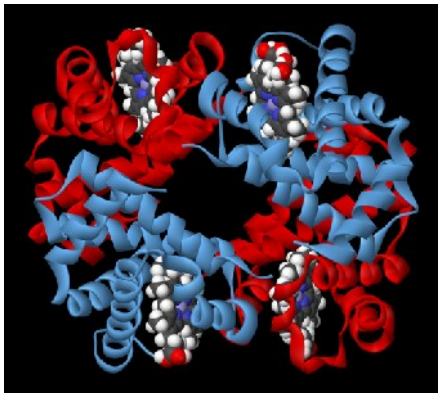
Protein

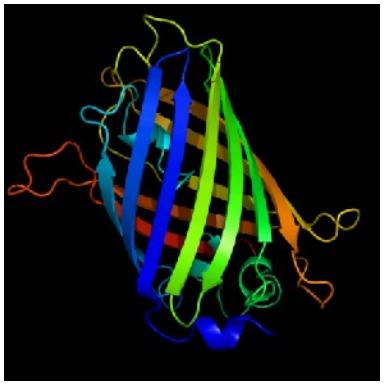
Work

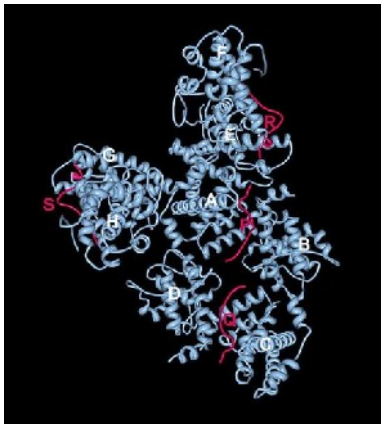
“Central Dogma” of mol biol

Protein folds into complex shape, ready for function









DNA

RNA

Protein

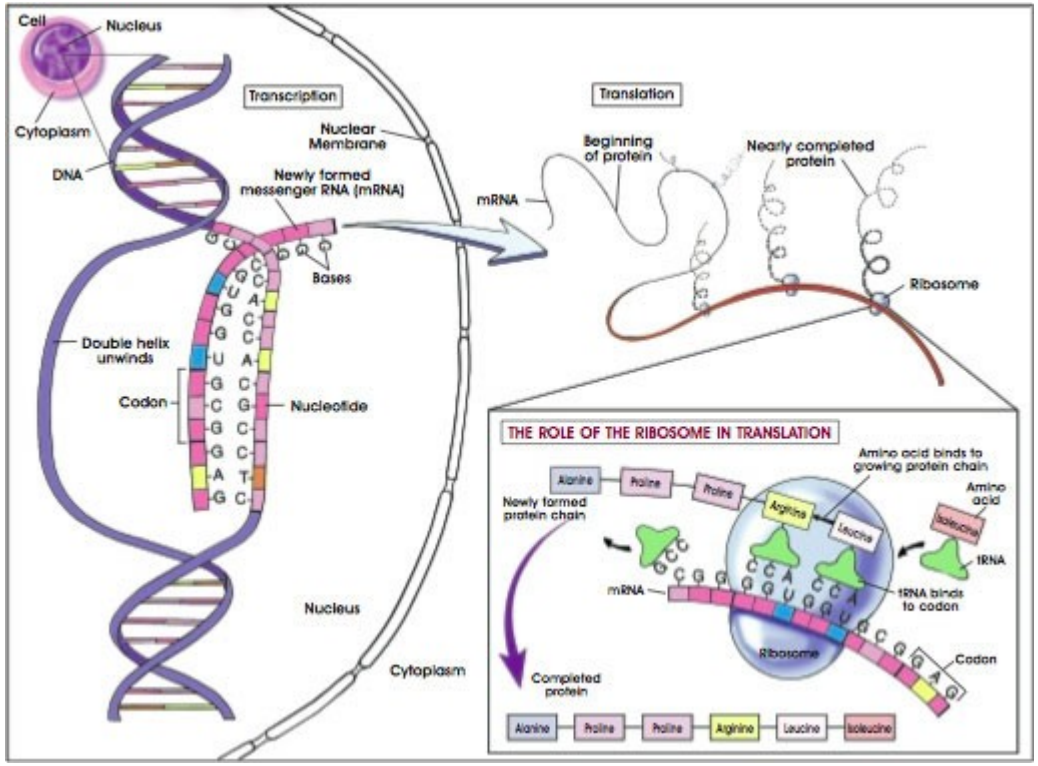
Work

Huge range of potential functions!

“Central Dogma” - recap

DNA – central information store
...transcribed to...
RNA – working copy, acts as a template
...translated to...
Protein – the completed machine

DNA
⇩
RNA
⇩
Protein
⇩
Work



“Central Dogma” – Er... sort of...

- All the above is correct... but very incomplete!
  - DNA sequences can be structural, regulatory or coding (or any combination of these!)
  - RNA sequences can be structural, regulatory, enzymatically active, coding (or combinations of these!)
  - Meta-information:
    - Splice variants
    - Localisation signals
    - Modification signals
    - ...etc, etc...

- However, this is good enough for most cloning work!

Introduction to cloning

Cloning is (usually) NOT about making copies of whole organisms!

I know, I was disappointed too…

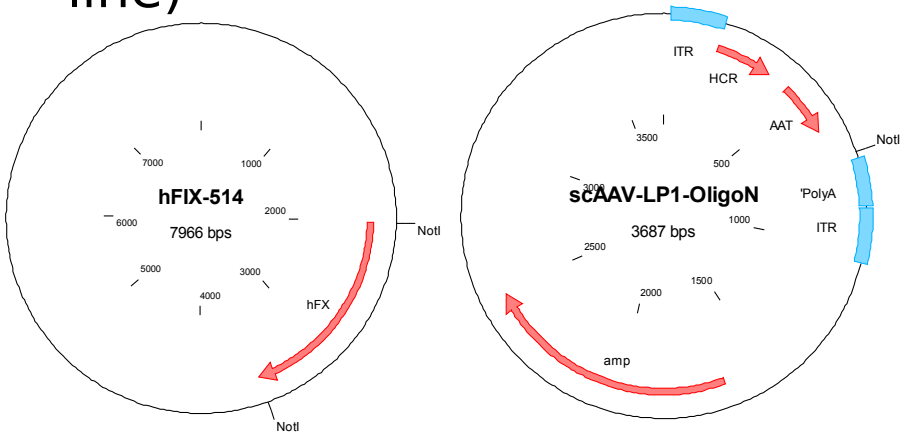
Introduction to cloning

- The aim of cloning is to end up with a plasmid that we can put into our target organism, where it will efficiently express our desired gene.
  - Uses include:
    - protein production (e.g. Insulin manufacture)
    - improved survival (e.g. Antibiotic resistance)
    - Reduced survival (new vulnerabilities to drugs)
    - modified behaviour (new reactions to stimuli)
    - Creating fusion proteins
    - ...etc

Introduction to cloning

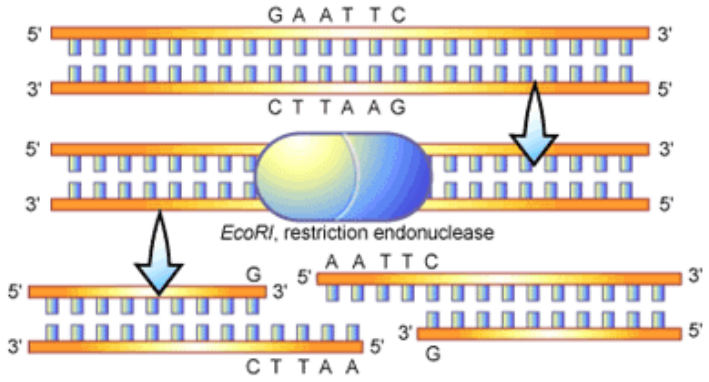
Plasmids are circles of DNA that can be put into many cell types (bacteria, yeast, animal, plant, etc...)

We can use them to carry our gene of interest into a target organism (typically bacteria, yeast or a cell line)



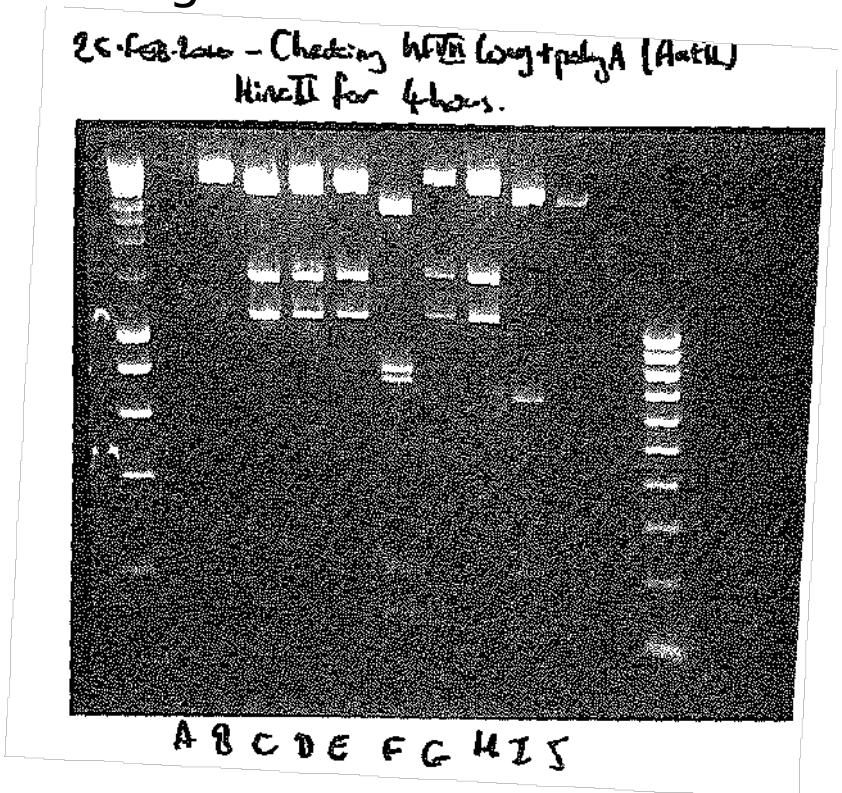
Restriction Enzymes recognise specific short DNA sequences, cut the DNA at that point

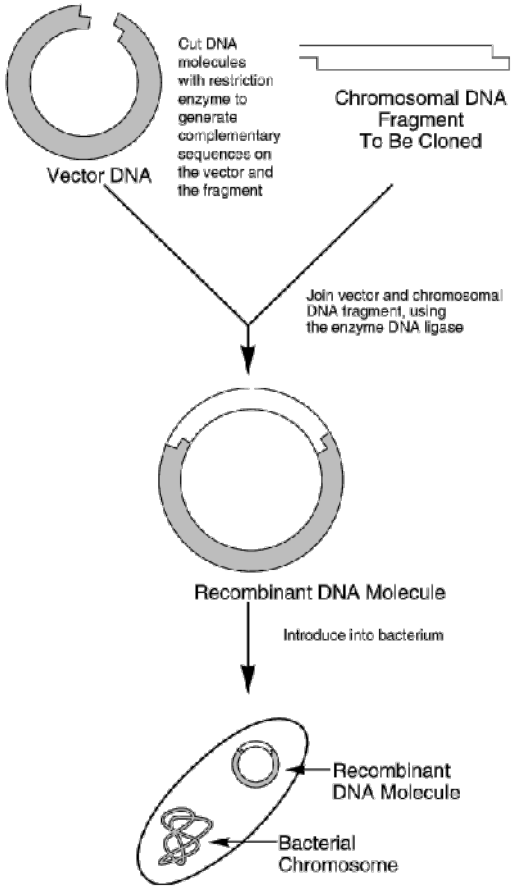
Sticky ends / overhangs allow DNA fragments to be ligated together



Introduction to cloning

Electrophoresis allows us to separate, visualise and extract desired fragments, ready to put the right ones together...



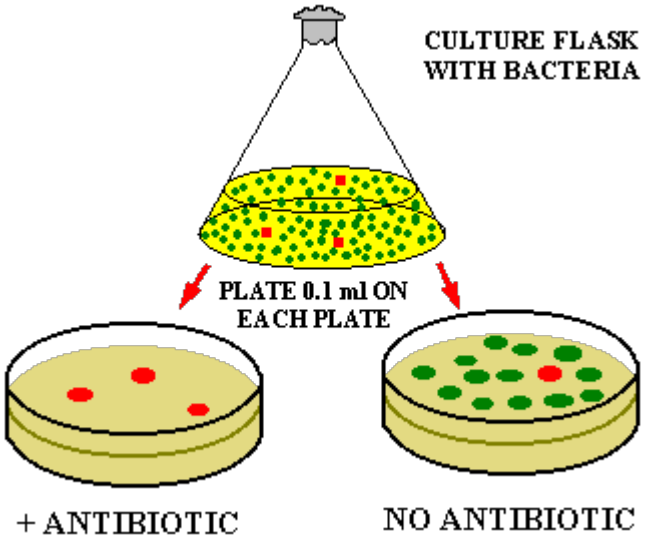


Introduction to cloning

Transfect / Transform / Transduce plasmid into your cells

Then need to select for cells that have successfully taken up the gene

Most common approach is antibiotic selection



End result: a pure culture of beasties expressing the novel combination of gene(s) you've chosen, in the conditions you've designed

Core techniques

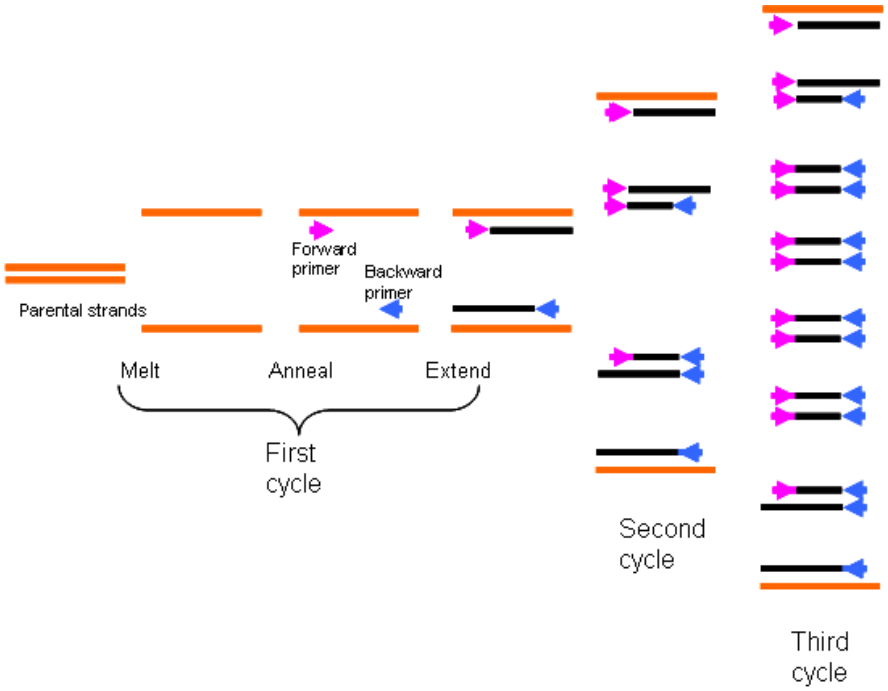
Polymerase Chain Reaction

A method to rapidly create many copies of a DNA sequence; essential for modern molecular biology

Two “primer” sequences are mixed with the template DNA, a DNA polymerase, and dNTPs.

The temperature is cycled to progress the reaction; each complete cycle doubles the number of copies present

Resulting DNA can be used for detection, quantification, cloning, mutagenesis, etc...



Core techniques

Variants on the PCR:

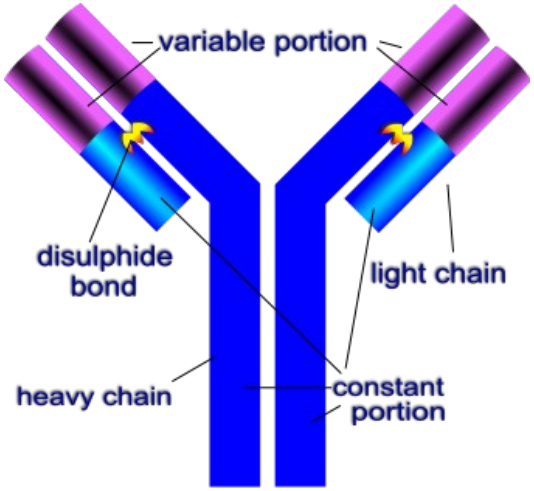
RT-PCR to detect RNA strands

qPCR (or RT-qPCR) to count number of strands

Mutagenic PCR – to create “daughter” strands including a new mutation

Core techniques

Western blotting and ELISA



Antibodies bind to and recognise specific proteins

Can be used to immobilise your proteins or just to detect them

Make your own (hard, time consuming, unethical and very illegal!) or commercially available

Core techniques

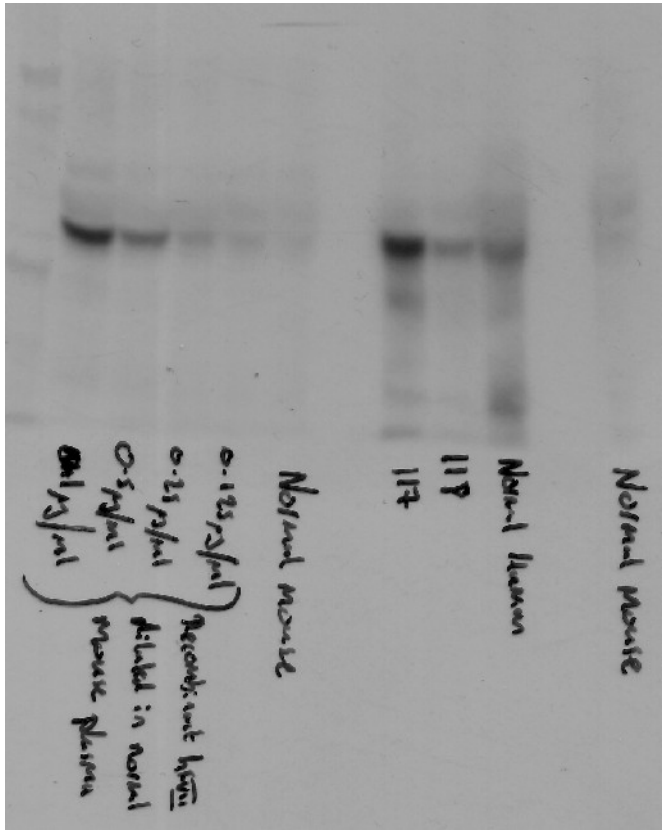
Western blotting and ELISA

These techniques used to confirm or measure expression of your protein

Electrophoresis is used to separate proteins by size

Then protein “smear” is probed with antibody to detect your specific target; should all be at the same height on the gel

ELISA is similar, but no electrophoresis...



Core techniques

Obtaining and checking DNA

Many thousands of annotated gene sequences available for free, online (start at http://www.ncbi.nlm.nih.gov/)

DNA made to order is constantly getting cheaper... ~15p / base

Limitations on length of commercial DNA synthesis, but workarounds exist

Efficient sequencing is hard... but cheap to outsource. Many dedicated services to do it by post / drop in.

Key tools & resources for a bio lab

- Environment: cleanliness, containment, disposal
- Reagent handling: need to measure tiny volumes / masses
- Centrifuge
- Thermal cycler
- Electrophoresis tank (& power supply)
- Incubator & water bath

Key tools & resources for a bio lab

Pipettes

Handling a wide range of volumes, some in sterile conditions

Key tools & resources for a bio lab

Centrifuge

Need to spin samples for separating layers of chemicals, pelleting DNA for purification, using various lab kits... An essential tool.

Sensible minimum is to safely spin a 2 gram sample at 11,000*g*

- Achievable with a “dremelfuge”, if you get someone else to hold it ;)

Larger masses are desirable (e.g. 50ml or 500ml tubes up to 11,000*g*) but very challenging to do safely. Enormous energies involved!

Key tools & resources for a bio lab

Electrophoresis tank

Separating DNA, RNA or protein according to size/charge ratio. Buffers are used to give everything in the sample the same charge, then samples are dropped into a matrix.

Voltage is applied, forcing the (charged) fragments to migrate.

Speed (and therefore distance) of migration is determined by fragment size.

Key tools & resources for a bio lab

Incubator / Water bath

Various organisms strongly prefer particular temperatures and should be grown in a fairly narrow temperature range.

As a consequence, many enzymes (esp restriction enzymes and polymerases) are very inefficient outside narrow temp ranges.

Therefore, need somewhere warm-but-not-hot to grow bugs and run reactions!

(Most bugs and enzymes in regular use are best at 37.5º)

Key tools & resources for a bio lab

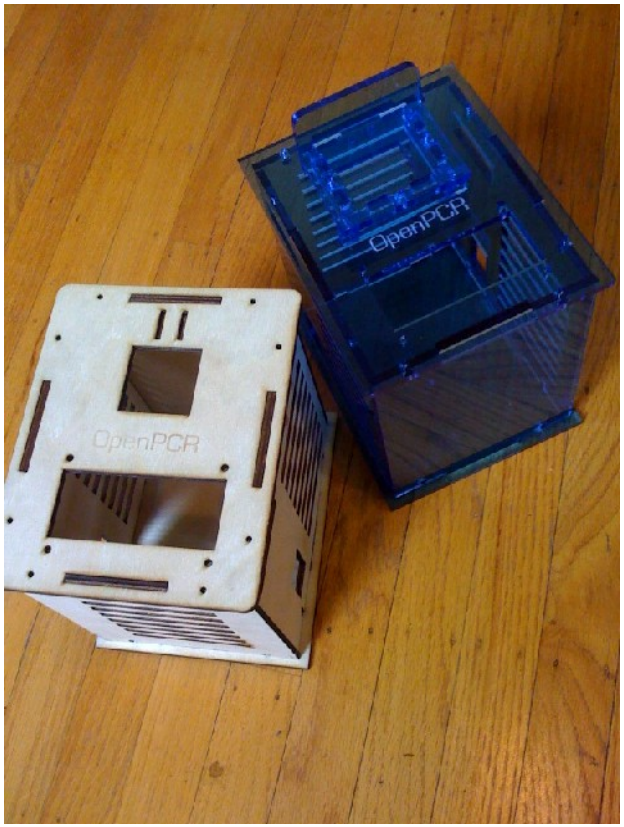
Thermal cycler

Essential for PCR

Basically just a programmable hotplate; a typical programme is:

START:
  95º for 10 minutes
  Do 40 times {
                95º   for 1 min
                54.5º for 1 min
                60º   for 2 min
                                }
  60º for 10 min
END

±0.5º is a sensible target...



openpcr.org

Key tools & resources for a bio lab

Spectrophotometer

Measuring colour change to track reactions, measure protein production, etc.

Key tools & resources for a bio lab

Reagents

A lot of molecular biology can be done with kits, which streamline processes and avoid nasty chemicals... Expensive

A lot of work going into avoiding nastier and more expensive chemicals within the community

Careful planning (e.g. Biobricks) can minimise the necessary reagent library

Restriction enzymes are a big challenge

The community needs to settle on model organisms to work with... Versatility and safety are concerns!

Key tools & resources for a bio lab

Safety Equipment

Most of modern molecular biology is pretty safe if you're careful.

Main hazards:

- Power sources (up to 200V, 400mW)
- Centrifugues – tubes or entire rotors flying off if imbalanced!
- A few nasty chemicals (acids, alkilis, mutagens) – generally OK with gloves, lab coat, goggles and working in a well-ventilated area
- Burns working with bunsen flames
- ...and the organisms you're working with!