KING'S College LONDON

INSTITUTE OF PSYCHIATRY,
PSYCHOLOGY & NEUROSCIENCE

Module:
Techniques in Neuroscience

Week 5:
Molecular biology: Going inside the cell

Dr Elizabeth Glennon

Topic 1:
An introduction to molecular biology methods
Part 1 of 3

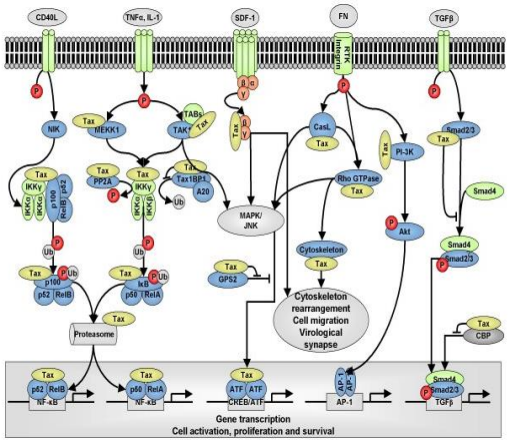
Topic list

This week, we will be looking at the following topics:

- ***Topic 1: An introduction to molecular biology methods***
- Topic 2: Video of procedures
- Topic 3: Focused journal club

Click **Next** to continue

Topic outline: introduction to methods in molecular biology



Cell signalling proteins targeted by the Tax1 oncoprotein

This topic is divided in 3 parts, which will explore:

1. Protein production using recombinant DNA technologies
2. Protein separation using SDS-PAGE
3. Protein identification using western blotting

Boxus et al. (2008)

Part 1

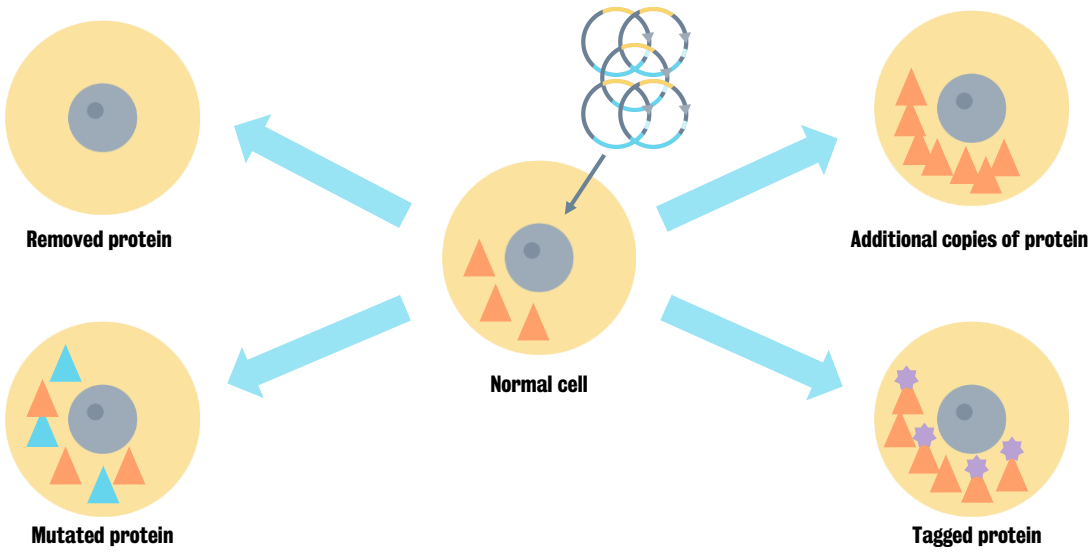
Part 1 outline

In this section, we will explore the use of recombinant DNA technologies as we focus on:

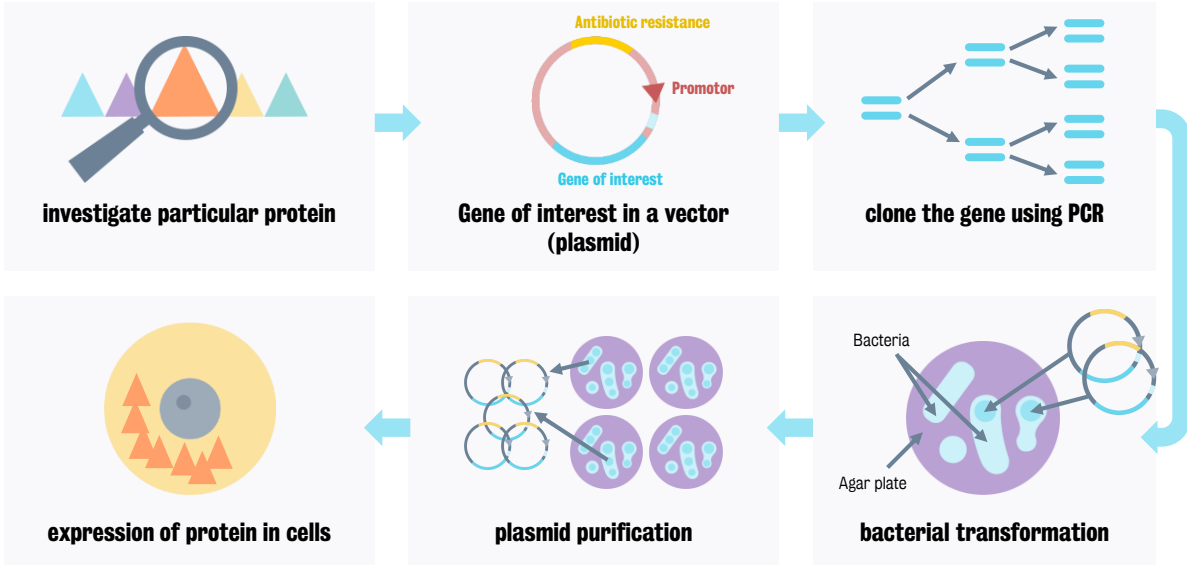
1. Overview of recombinant DNA technologies
2. The plasmid vector
3. Introduction to polymerase chain reaction (PCR)
4. Cloning

Click **Next** to continue

Recombinant DNA technologies

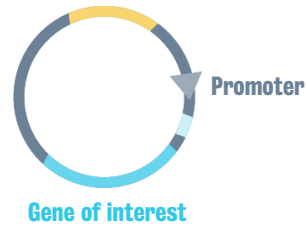


Recombinant DNA technologies



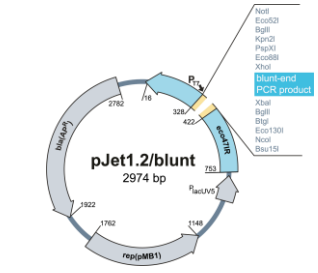
Recombinant DNA technologies

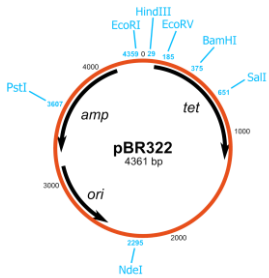
Plasmid vector



- circular piece of DNA
- the gene in the plasmid is cDNA
- may have an eukaryotic resistance marker
- exact sequence of the gene
- no introns or up/downstream sequences
- can only express one isoform

Examples of plasmid vectors





Definitions:

Promoter: where transcription of a gene is initiated.
Transcription: when a section of DNA is copied to make RNA. RNA may then be used as a template for making a protein.
cDNA (complementary DNA): DNA that is synthesised by using RNA as a template. cDNA is often used to clone eukaryotic genes in prokaryotes.
Prokaryote: a unicellular organism that lacks a membrane bound nucleus, like a bacterium.
Eukaryotic cells: have a membrane bound nucleus that houses the genetic material. The cells that make up our body are eukaryotic cells.
Introns: sections of DNA or RNA that do not code for proteins.
Up/downstream sequences: a gene has two ends, a 5′ end and a 3′ end. Upstream refers to sequences that are towards the 5′ end, and downstream refers to sequence that are towards the 3′ end. There sequences are involve in the control of transcription.
Gene isoforms: different versions of RNA transcripts make from the same gene.

Introduction to polymerase chain reaction (PCR)

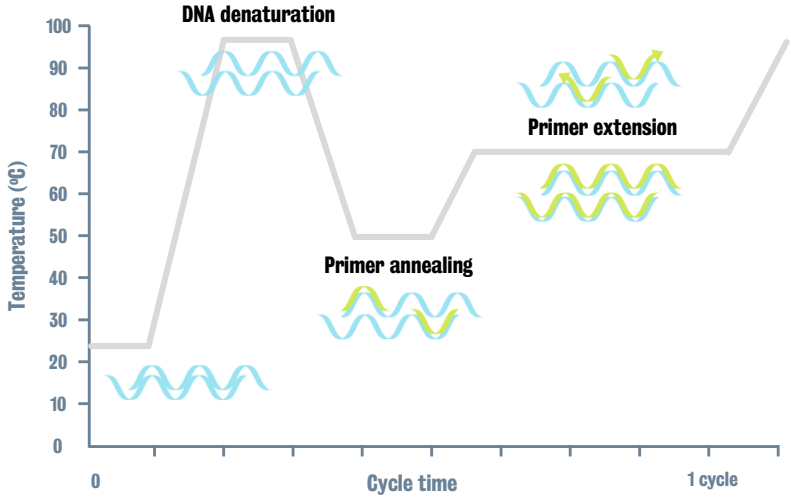
Template DNA

DNA polymerase

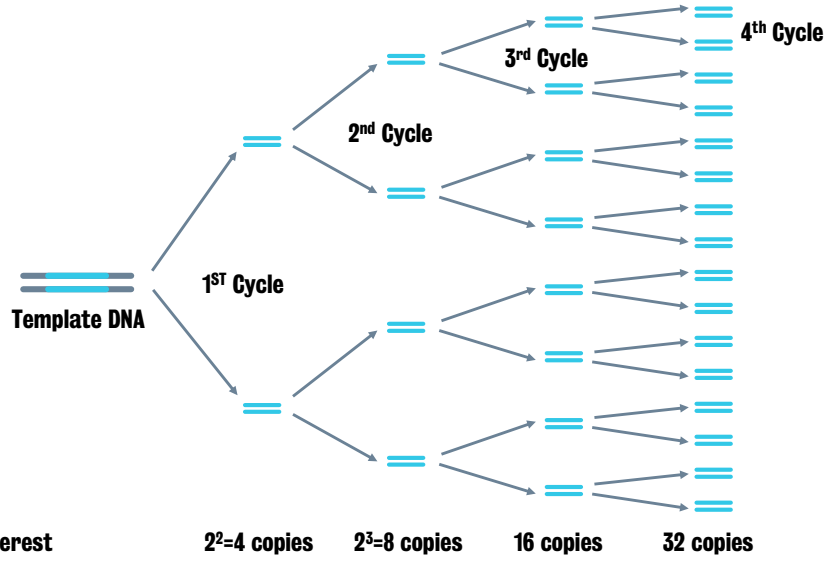
Primers

Nucleotides

PCR components



Introduction to polymerase chain reaction (PCR)



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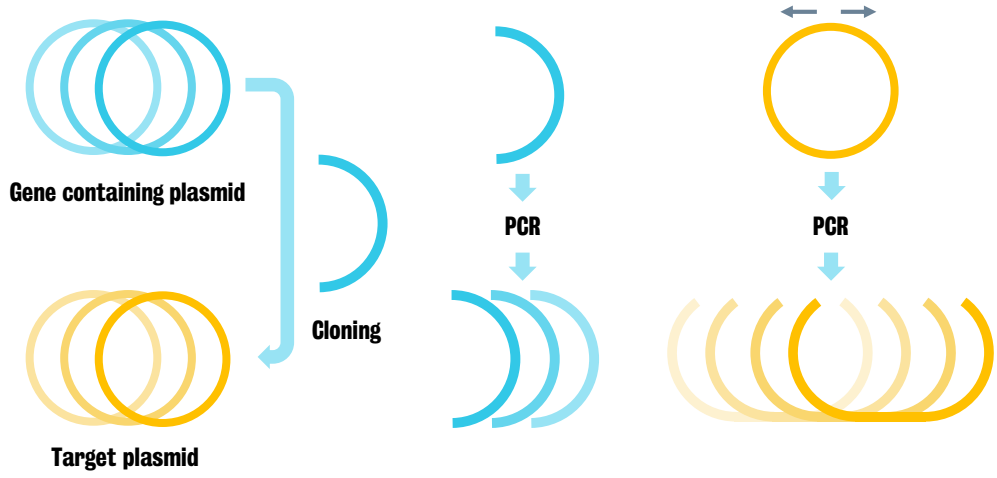

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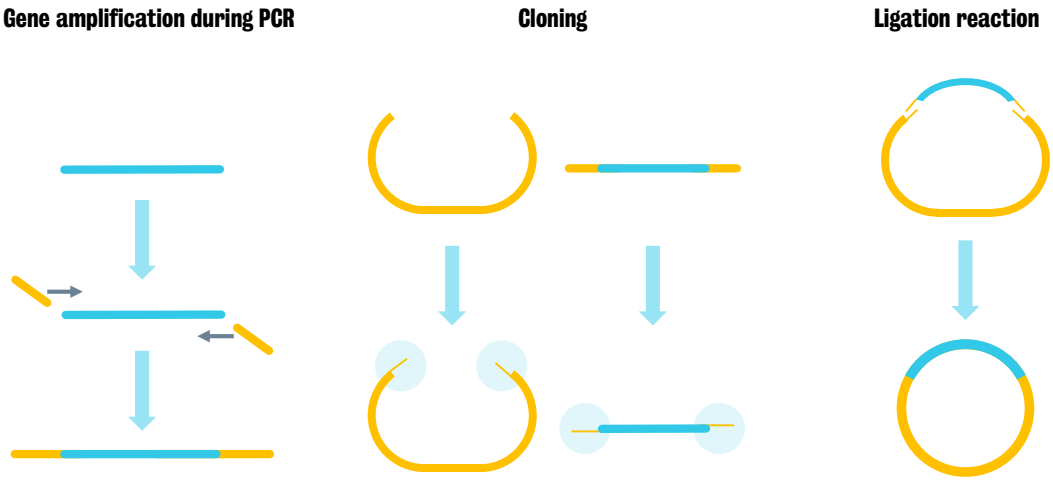
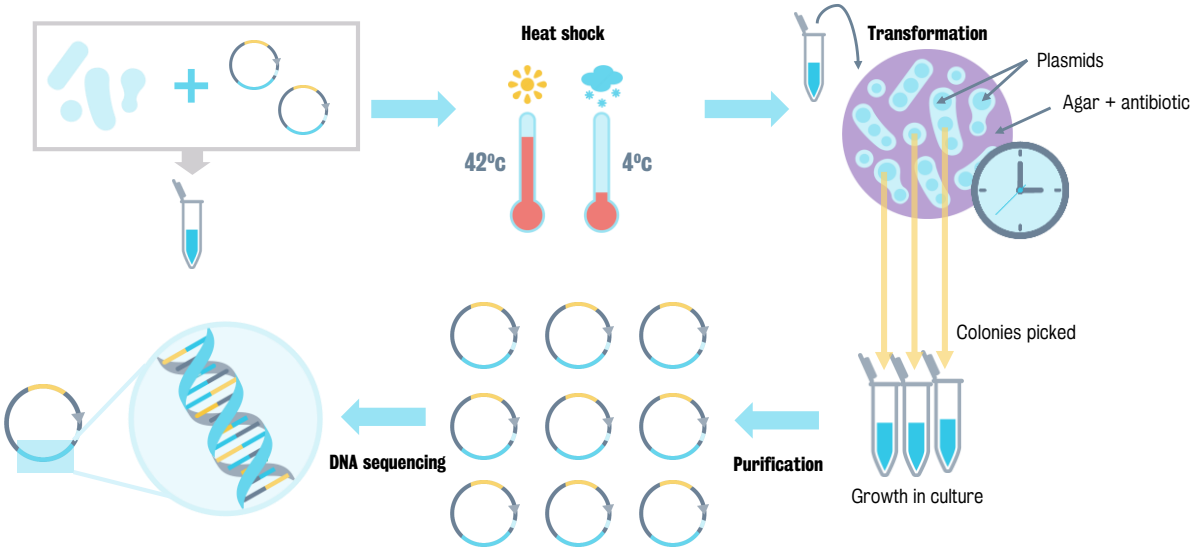


Introduction to polymerase chain reaction (PCR)



End of part 1