

# Crash Course in Research Methods

**Proteins**

In solution:

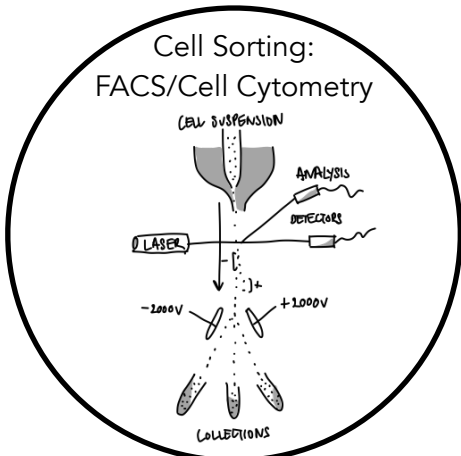
- A. Gel Electrophoresis
- B. Western Blotting

In situ:

- C. Immunofluorescence
- D. GFP-tagged

**B**

See blotting comparison table and SNOW DROP mnemonic



**Genetic Editing/Engineering**

DNA repairs mechanisms:

- HDR/Homologous recombination
- NHEJ (requires template)

Gene Knockout:

- older methods
- TALENs
- CRISPR

Gene Silencing (~10% function)

- RNAi

CRISPR - uses Cas9, requires PAM sequence near the target to bind, uses sgRNA

**Conditional Knock Out**

- Cre-LoxP recombinase system
- Can also use drugs
  - Tamoxifen
  - Doxycycline
  - Tetracycline

**Gene Knock-In**

- Addition, insertion or swapping of genetic material
- eg. fluorescent reporter

**FACS/Cell Cytometry:**

FSC = 'cell size'

SSC = 'granularity'/complexity

For sorting see gating

**Cell Behaviours**

**Proliferation:**

1. BrdU
  - Replaces thymine
  - Measures new DNA synthesis

**Apoptosis:**


3. DAPI - membrane viability!
4. Annexin-V
5. TuNEL

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**DNA Detection**

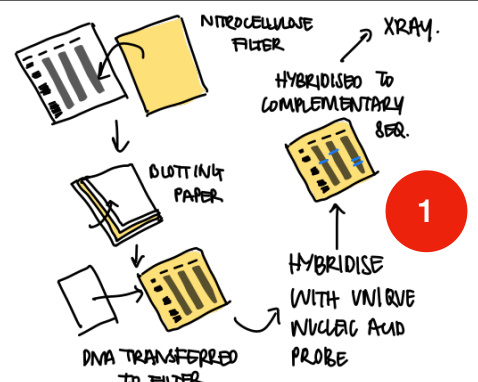
1. Southern Blotting
2. PCR
3. qPCR (/real time PCR)
4. Genomics

Next-Gen Seq.

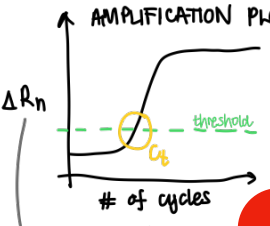


Sanger Seq.





**AMPLIFICATION PLOT**

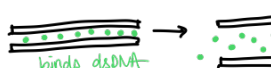


$C_t = \frac{1}{\text{nucleic acid amount in sample}}$

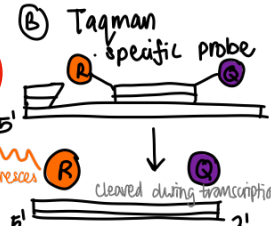
Δ in fluorescence

**(A) SYBR green** - intercalating dye

binds dsDNA



**(B) Taqman specific probe**



fluoresces

cleaved during transcription.

**PCR Cycle Diagram**

TEMPLATE DNA (SINGLE COPY)

FIRST CYCLE → n CYCLE

2<sup>1</sup> = 2 COPIES

2<sup>2</sup> = 4 COPIES

(DENATURATION → ANNEALING → EXTENSION)

**PROBE (SPECIFIC)**

**TARGET GENETIC MATERIAL** w/ tissue

- (A) DENATURE
- (B) HYBRIDISE
- (C) PROBE DETECTION
- (D) ANALYSIS (MICROSC.)

IN TISSUE

**ONE / TWO STEP**

from dNTPs

RT

mRNA 5' → 3'

cDNA

cDNA AMPLIFICATION via Taq polymerase

**SNOW DROP - blotting mnemonic**

S - southern for DNA

N - northern for RNA

O - western for protein

**RNA Detection**

1. Northern Blotting
2. RT-PCR
3. qRT-PCR
4. Transcriptomics

RNA/DNA transcripts in tissue: In-Situ Hybridisation (5)

Micro Array

RNA-Seq.

Southern Blotting	Northern Blotting	Western Blotting
DNA	RNA	Protein
Agarose Gel	Agarose Gel	SDS-PAGE
NA probe	DNA/RNA/ODN probe	1°/2° antibodies w/ fluoro/enzyme

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