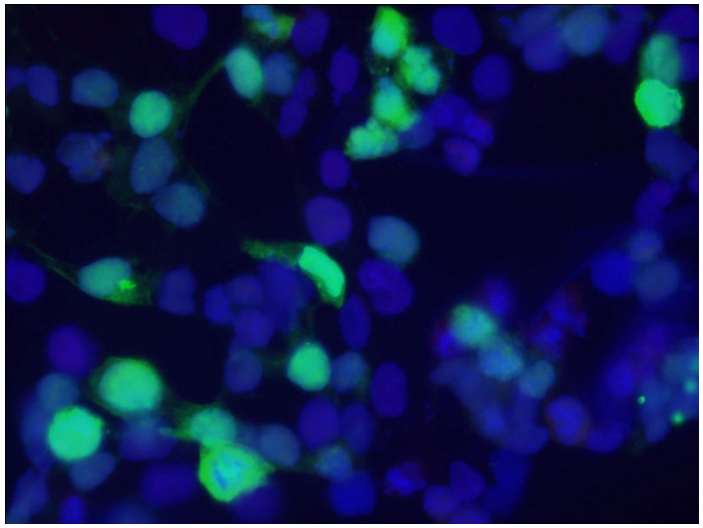
Project: Protein Localization in Mammalian Cells

- Idea: Compare localization of proteins (ZFP568 & GALT) in two types of mammalian cells
- Significance: Protein location is essential for proper function, mislocalization is associated with disease
- Biological Techniques:
  - DNA extraction & purification
  - Transfection of DNA into HEK293T (human embryonic kidney) & NIH3T3 (mouse embryonic fibroblast) cells
  - Cell staining & fluorescence microscopy to visualize protein location with GFP



Diseases associated with Protein Mislocalization

- Cystic fibrosis (CF)
- Familial hypercholesterolaemia (FH)
- Congenital sucrase-isomaltase deficiency (CSID)

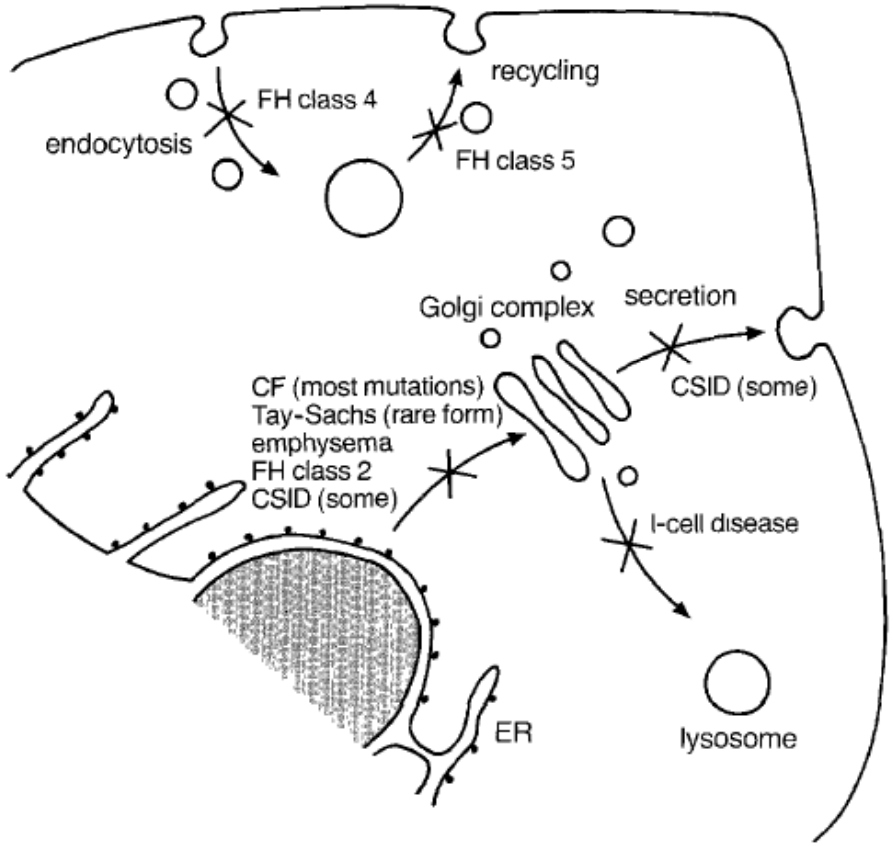


FIGURE 1

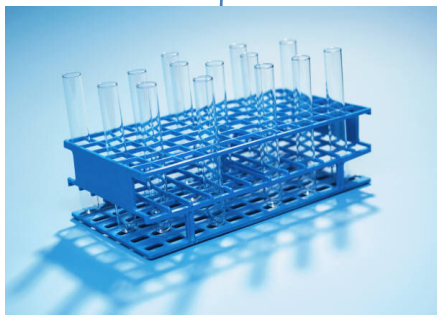
Protein trafficking events disrupted in some genetic diseases. CF, cystic fibrosis; FH, familial hypercholesterolaemia; CSID, congenital sucrase–isomaltase deficiency.

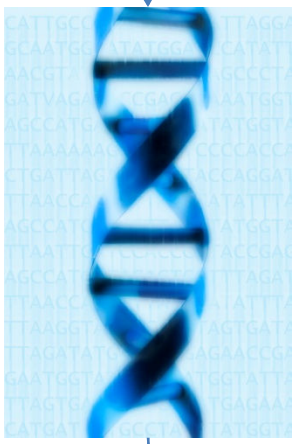
Project Goal

- Compare the localization of two proteins (ZFP568 & GALT) in two types of mammalian cells (human embryonic kidney cells & mouse embryonic fibroblasts)
- We'll do this by
  - Making DNA that codes for our proteins (DNA extraction & purification)
  - Putting that DNA in mammalian cells (transfection)
  - Determining where our proteins are within the cells (cell staining & fluorescent microscopy)

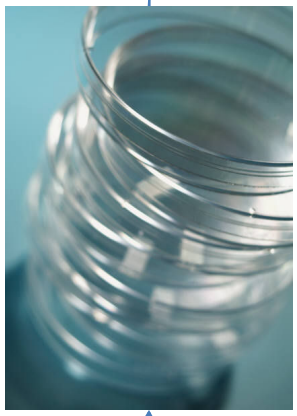
Our Project Plan

Miniprep to extract DNA from bacteria (Tues)

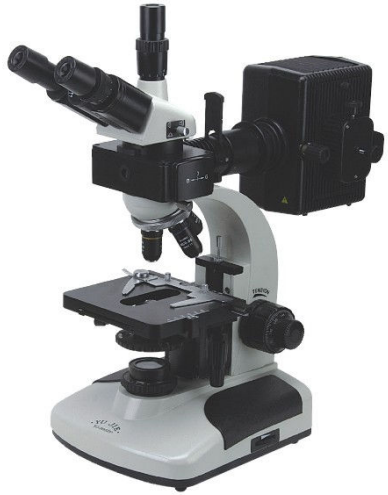




Transfect to put our DNA in mammalian cells (Wed)



Stain & look at our cells to see where the protein is (Thurs/Fri)



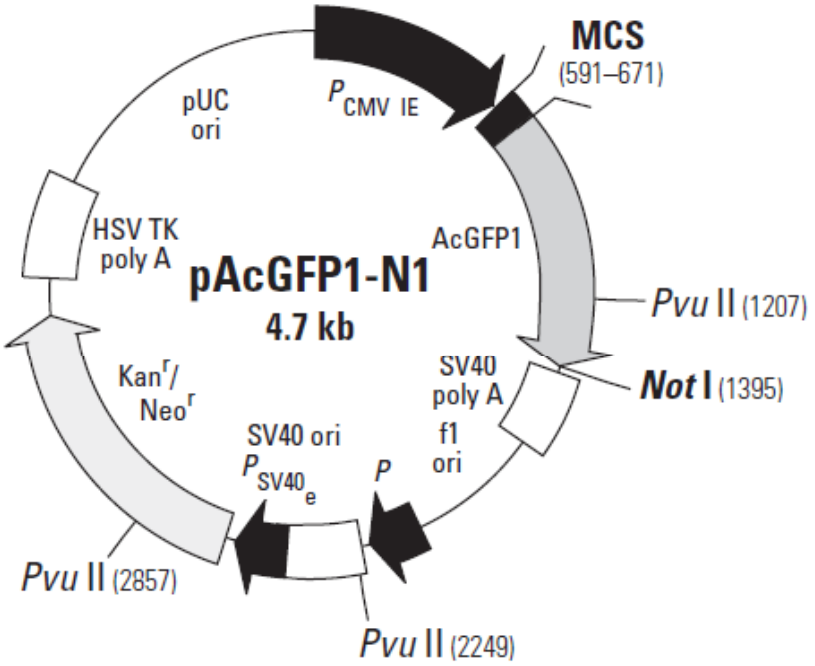
The Two Cell Types We'll Use

- HEK293T cells (human embryonic kidney)
  - Good for transfections
  - Sturdy cells, but small (not much cytoplasm)
- NIH 3T3 cells (mouse embryonic fibroblast)
  - Not as good for transfections
  - But bigger (more cytoplasm)

The Two Proteins We'll Look At

- GFP-ZFP568 (Zinc finger protein 568)
  - Transcription factor important in mouse development
- GFP-GALT (Galactose-1-phosphate uridylyltransferase)
  - Enzyme important for converting galactose to glucose

GFP vector – a “plasmid”



591 601 611 621 631 641 651 661 671 AcGFP1

G CTA GCG CTA CCG GAC TCA GAT CTC GAG CTC AAG CTT CGA ATT CTG CAG TCG ACG GTA CCG CGG GCC CGG GAT CCA CCG GTC **ATG GTG**

Nhe I *Eco47* III *Bgl* II *Xho* I *Sac* I *Hind* III *Eco*R I *Pst* I *Sal* I *Acc* I *Kpn* I *Asp*718 I *Sac* II *Apa* I *Bsp*120 I *Bam*H I *Xma* I *Sma* I *Age* I

Restriction Map and Multiple Cloning Site (MCS) of pAcGFP1-N1 Vector. Unique restriction sites are shown in bold. NOTE: The *Xba* I and *Bcl* I sites are methylated in the DNA provided by Clontech Laboratories, Inc. If you wish to digest the vector with these enzymes, you will need to transform the vector into a dam– host and make fresh DNA.

Restriction Enzymes

- Digestions and Ligations
  - Allowed us to put the DNA coding for ZFP568 or GALT in the GFP vector