Final DSAP Question

If you were continuing to work on this gene, what would you do next?

7   As a scientist investigating this protein in duckweed, what experiments would you do next?

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Follow-up questions about the protein

When is the gene/protein expressed?

Where is the protein located in the cell or organism?

What other proteins inteact with the protein?

What would happen if we removed the protein?

Can we determine its enzymatic activity?

What is the structure of the protein?

When/Where is your gene expressed?

Is it on all the time, in all cells?
    If so, what does that tell you?

Or, Is it only expressed at certain times?
    At specific points during the cell cycle? (G1, S, G2, M)
    At specific points during the life cycle? (seeds vs plants)

Or, Is it only expressed under specific conditions?
    Light vs dark?
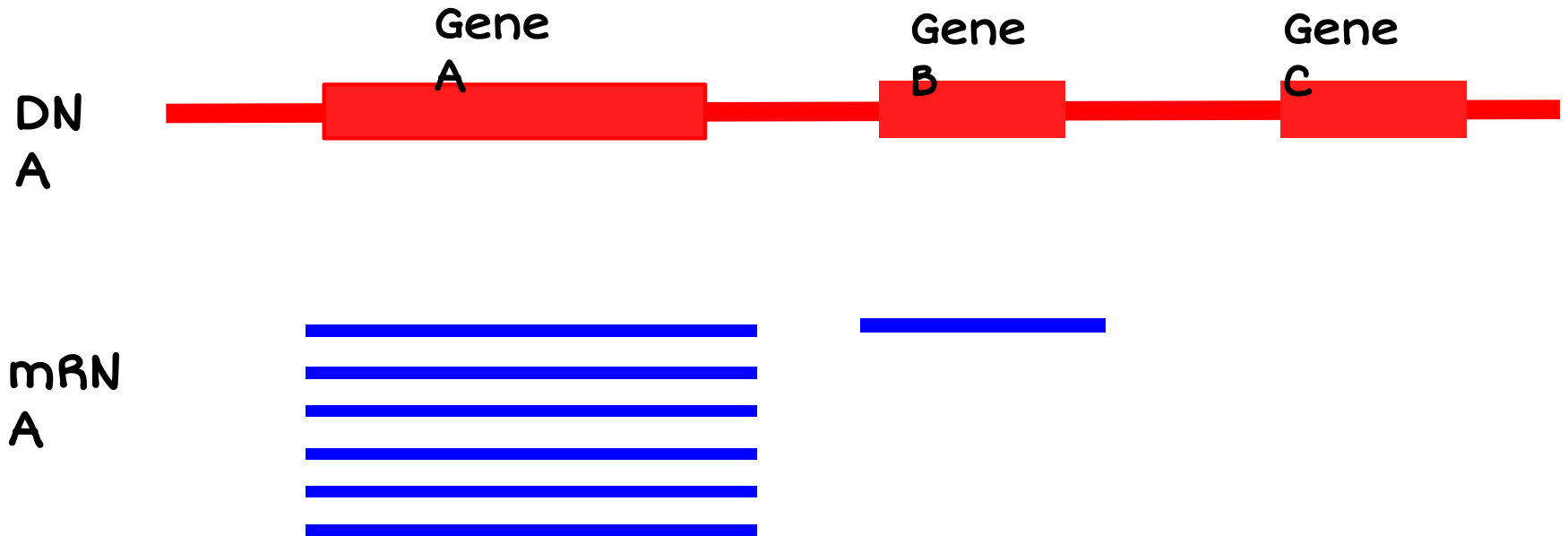   Under stress (low pH, heavy metals, high T°)

Or, Is it only expressed in certain places?
    Nucleus, golgi, membrane surface, cell wall?
    Roots, leaves, flowers?

Assaying Gene Expression

Which genes are transcribed and at what level?

DNA
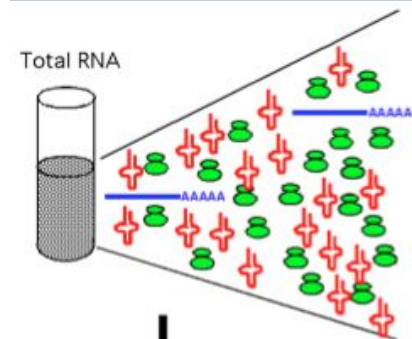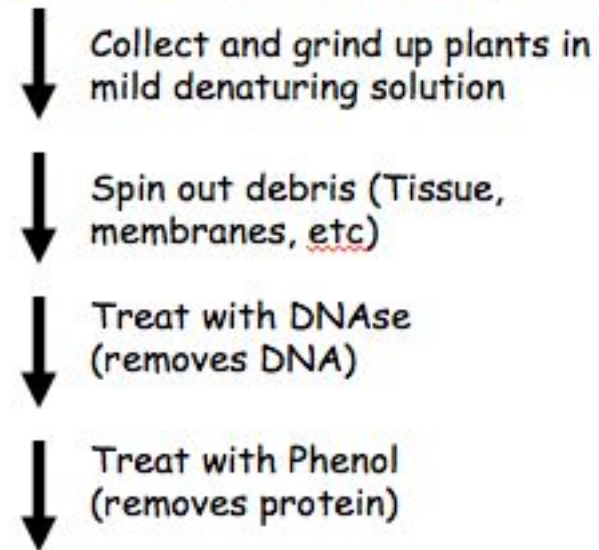
Gene A   Gene B   Gene C

mRNA

P.

How can we assay the expression of a specific mRNA?
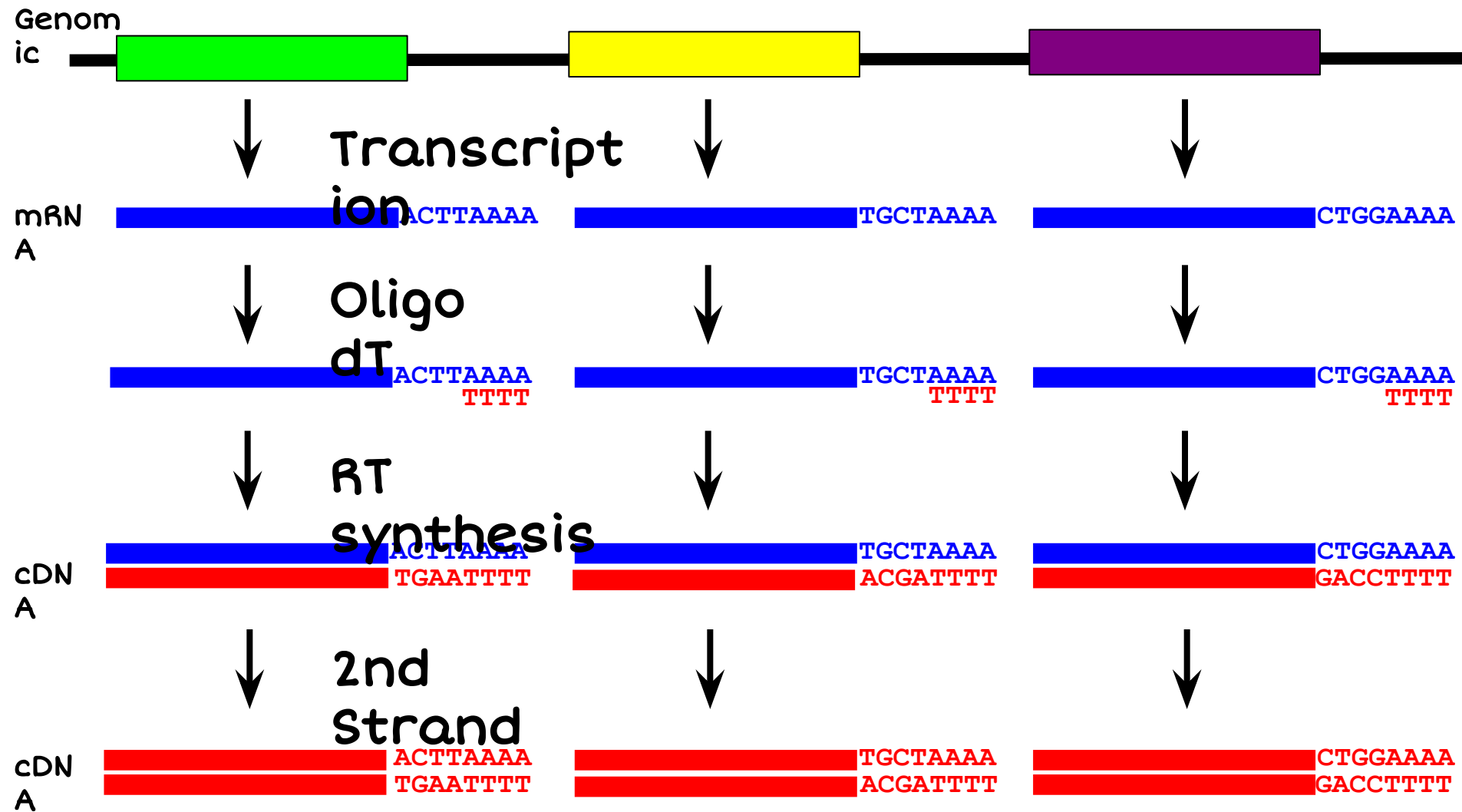
RNA purification
cDNA synthesis
PCR
agarose gels

cDNA Synthesis
vs
Reverse Transcriptase-PCR
(RT-PCR)

Purify mRNAs

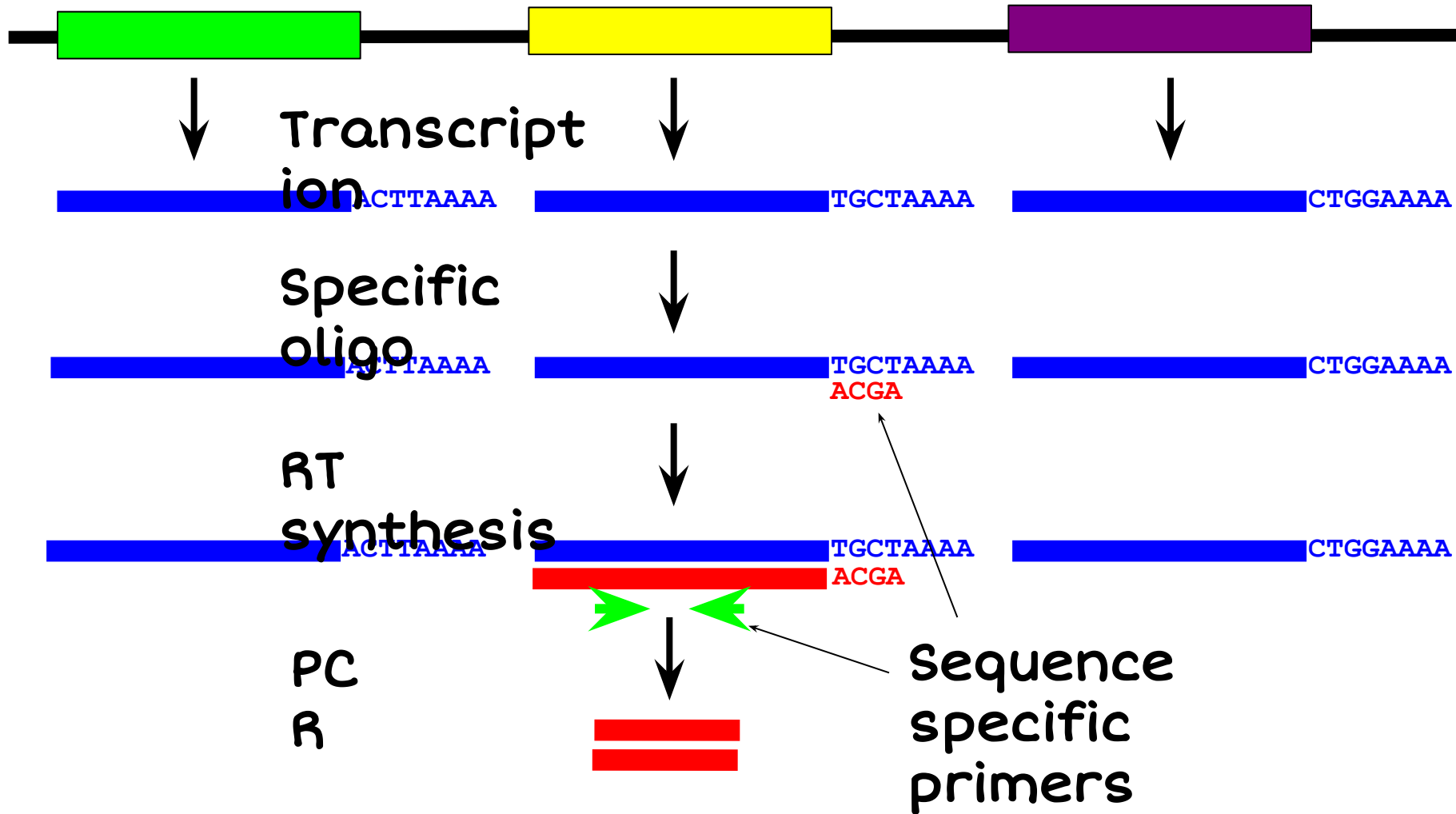

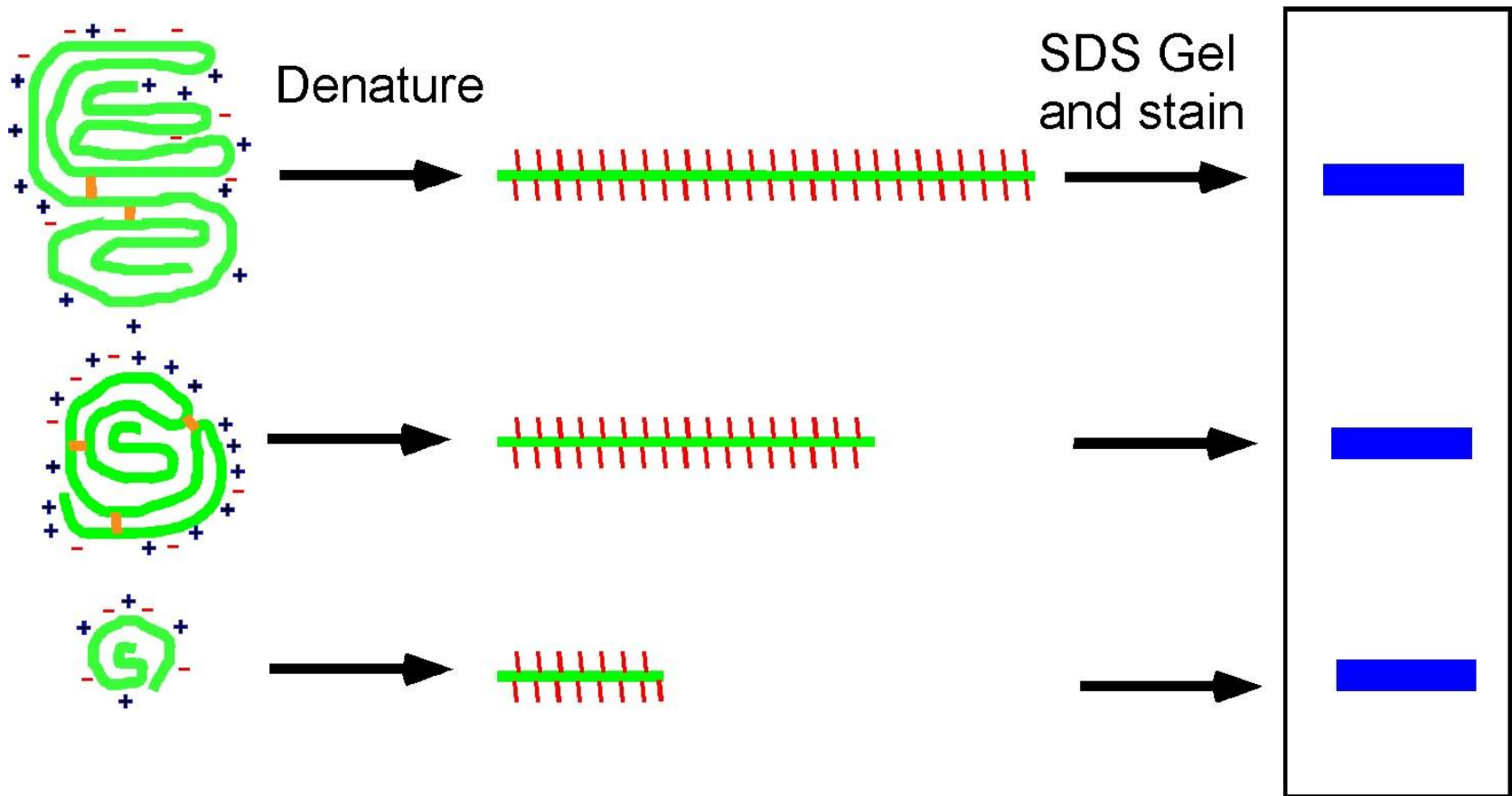Collect and grind up plants in mild denaturing solution

Spin out debris (Tissue, membranes, etc)

Treat with DNAse (removes DNA)

Treat with Phenol (removes protein)

Total RNA

cDNA Synthesis with oligo-dT

Amplifies every mRNA in the cell

RT-PCR with specific cDNA oligos

Transcription

ACTTAAAA   TGCTAAAA   CTGGAAAA

Specific oligo

ACTTAAAA   TGCTAAAA   CTGGAAAA
            ACGA

RT synthesis

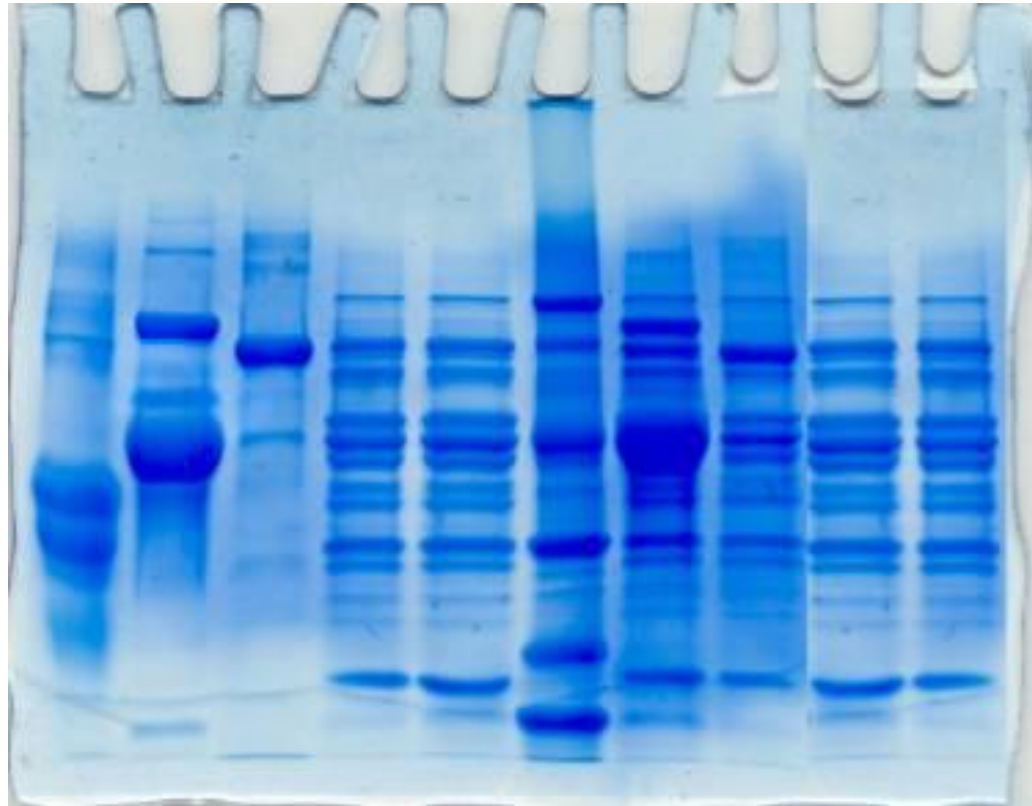ACTTAAAA   TGCTAAAA   CTGGAAAA
            ACGA

Sequence specific primers

PCR

Amplifies only a specific mRNA in the cell

Proteins of different shape and charge run according to size on a denaturing gel

Denature

SDS Gel and stain

Coomassie stained protein gel

Western blot reaction

Proteins on blot separated by size

Specific Protein

Specific Protein Band

Substrate

Light or Color

Primary Antibody

Secondary Antibody linked to enzyme
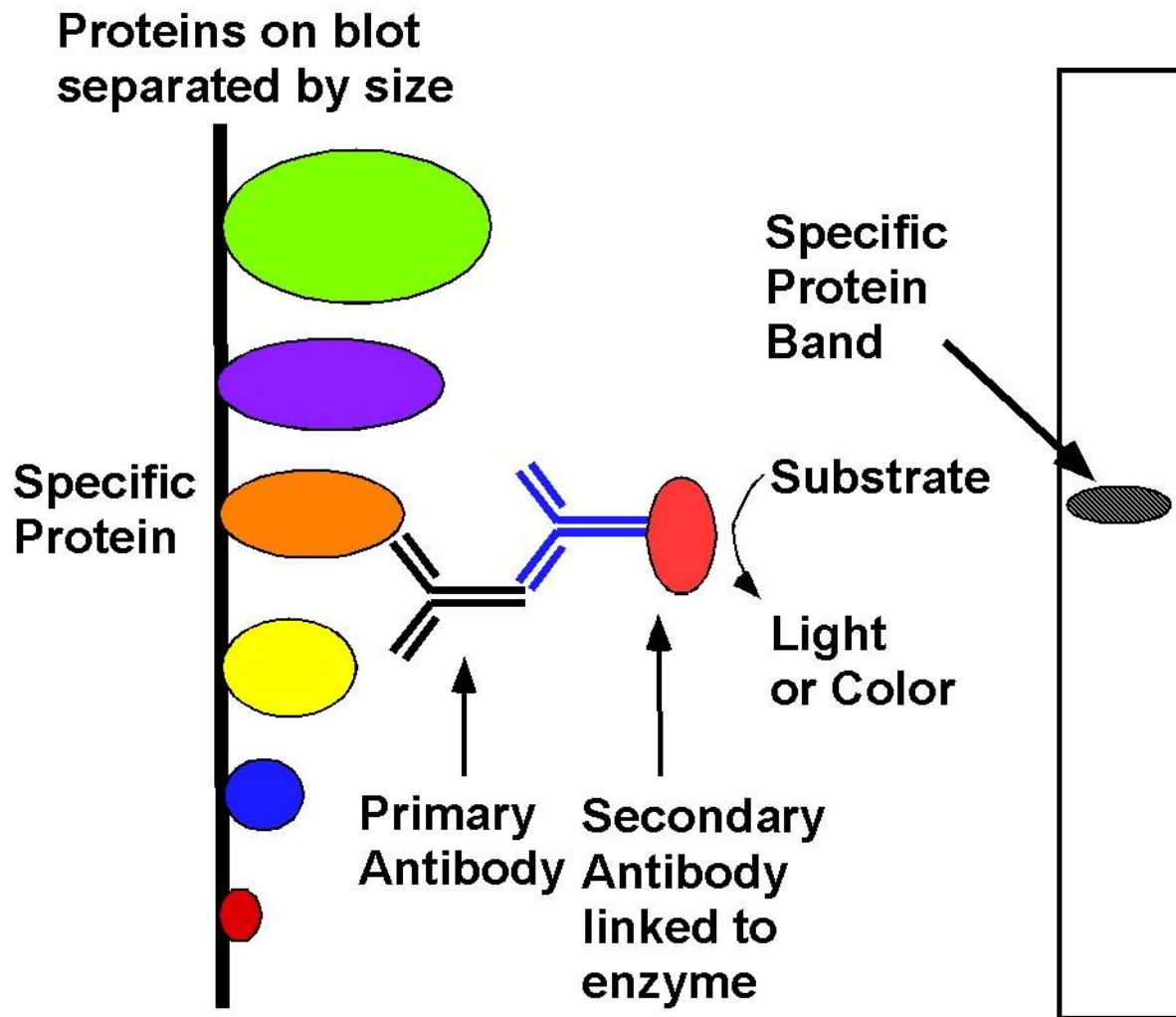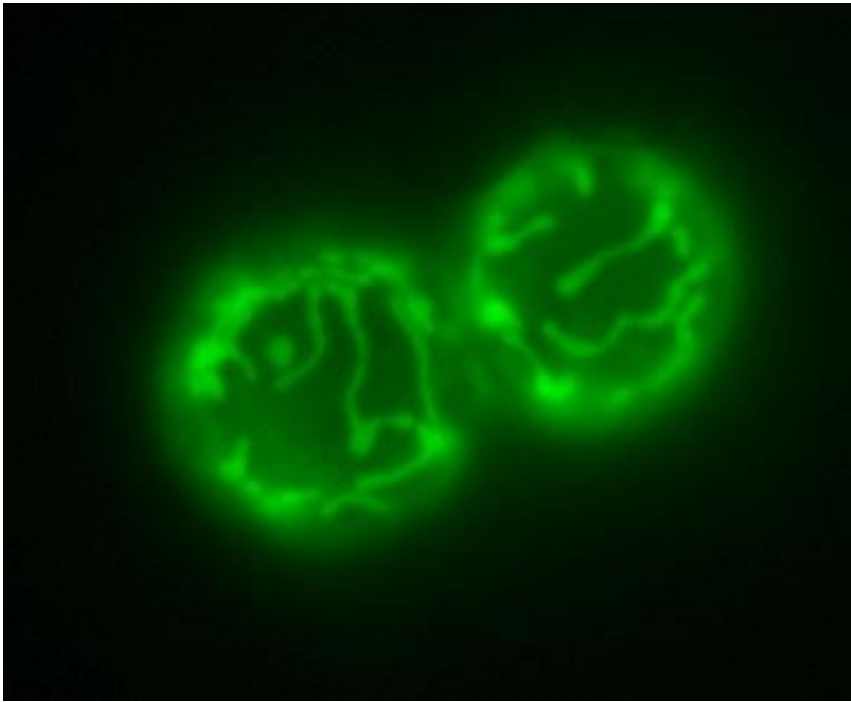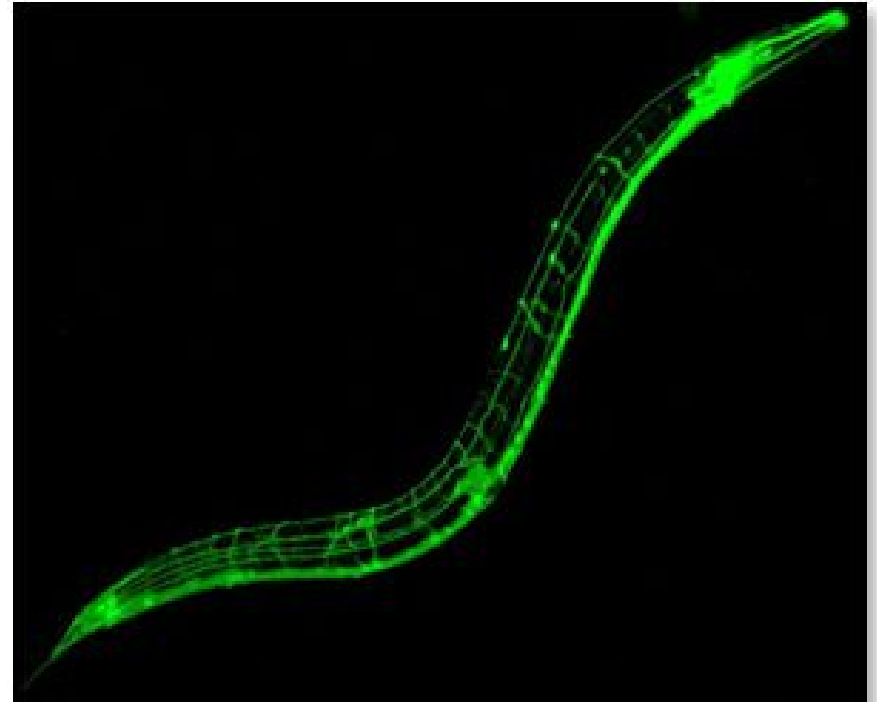
Where is the protein localized in the cell or organism?

Green Fluorescent Protein (GFP) tagged proteins
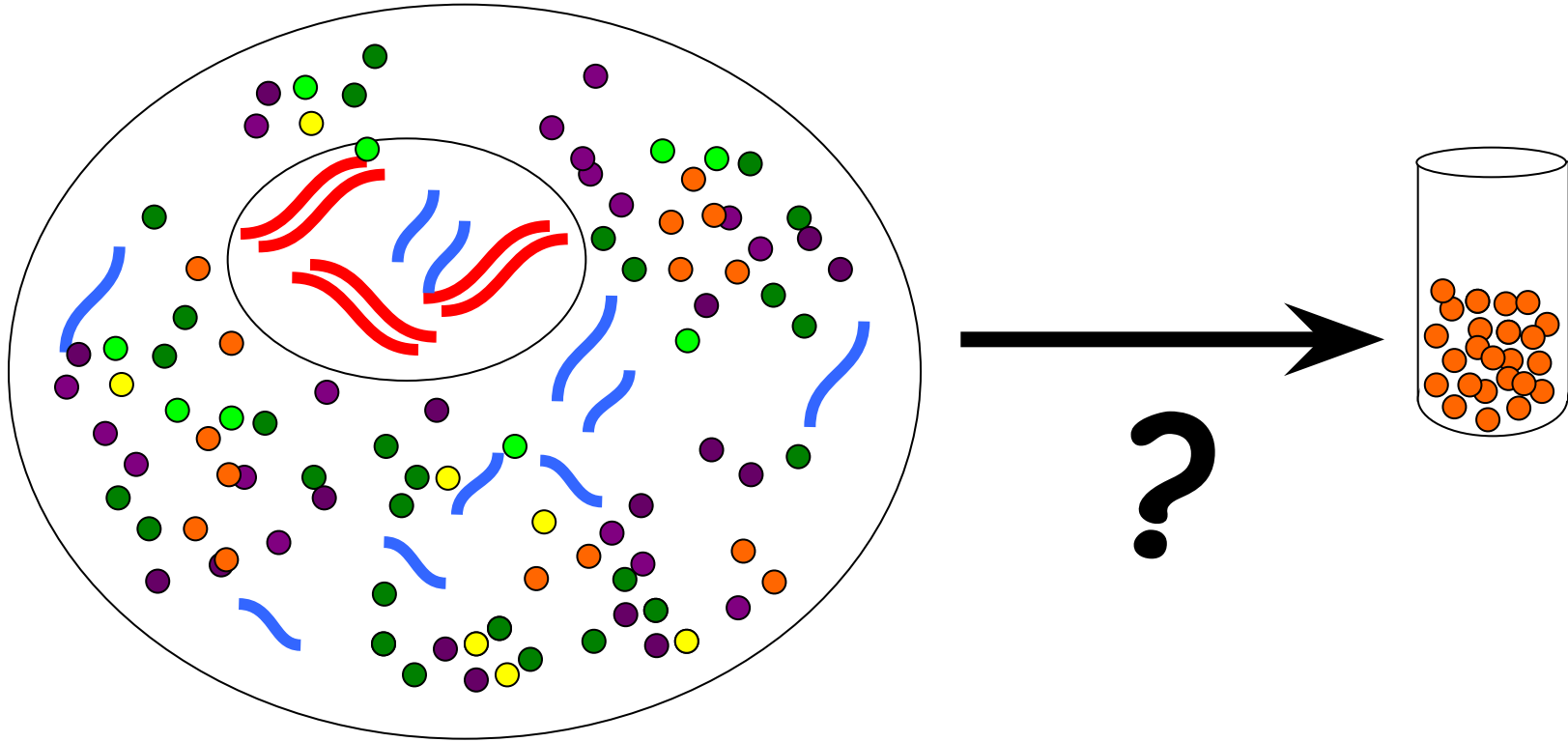- Used to localize proteins in the cell

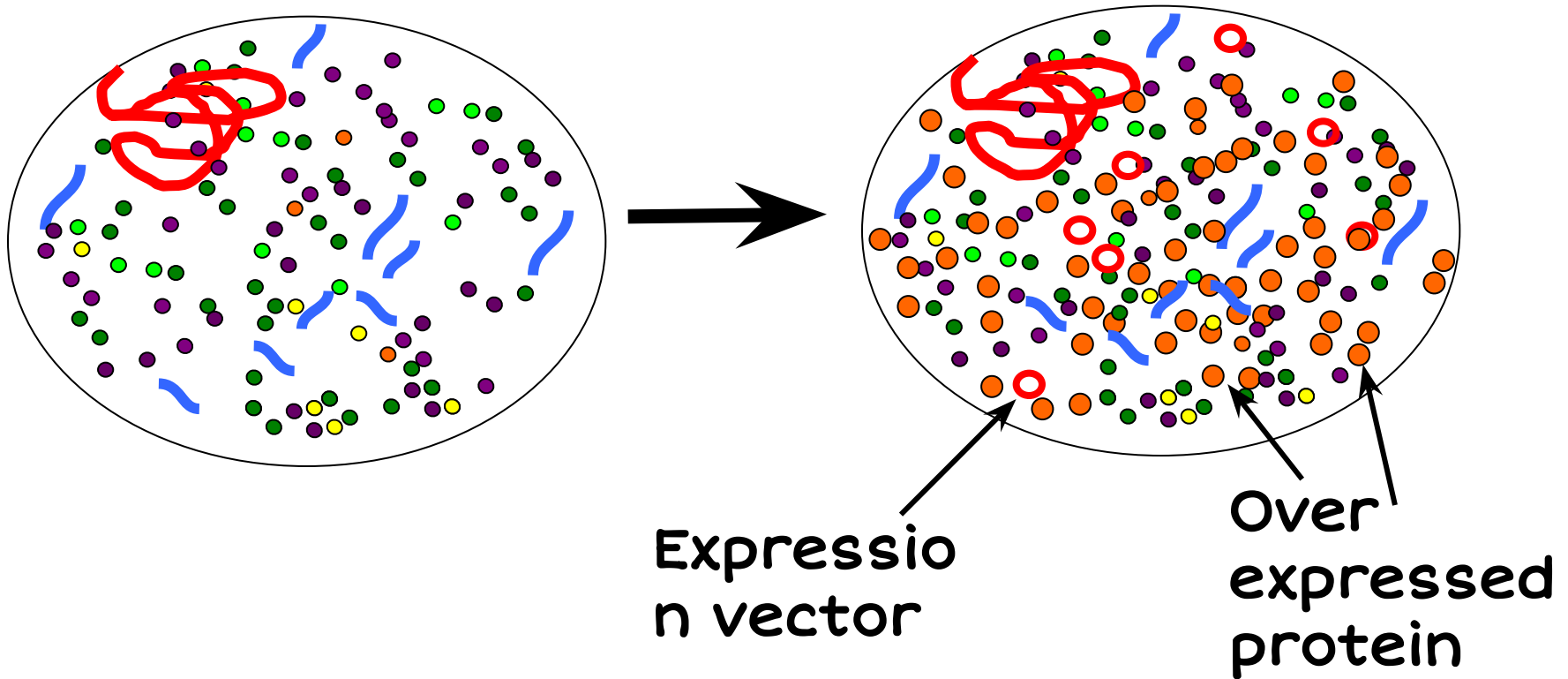|	Gene X	GFP	

Yeast Mitochondria

Worm nervous system

How can we obtain enough of a protein to make antibodies, crystals or enzymatic assays?

Overexpression of a protein in a bacteria

Expressio n vector

Over expressed protein

Determination of the a protein structure by crystallography

Cell expressing proteins

Purify specific proteins

Grow crystals

Expose crystals to ~~X-rays~~

Protein Crystal
X-ray Beam
Diffraction Pattern
Diffraction Process

Collect X-ray diffraction pattern

Diffraction Pattern from NSLS

Develop electron map

Electron Density Map

Develop structure from map