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Institute for
Critical Technology and Applied Science

Protein expression, detection and purification

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Some researchers will stop
before this point
(TA cloning)
Store you gene in *E. coli*

TA cloning

Not suitable for gene expression

You need expression vector

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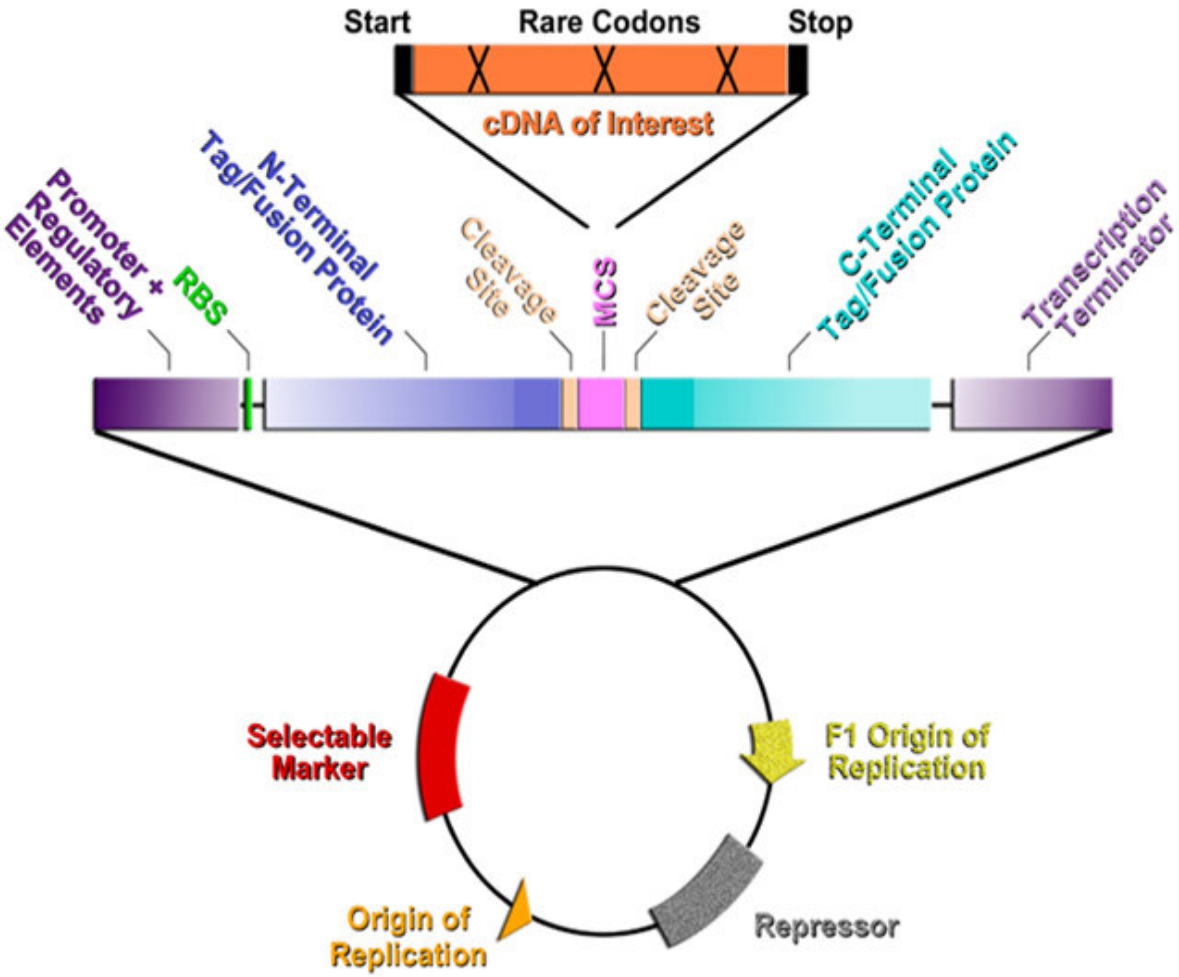
Expression vector

- Promoter
- Terminator
- Repressor
- Fusion tag



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Points to consider for protein expression

- Post-translational modification
- Folding
- Host (more choices of bacteria)
- Location (secreted, periplasmic or cytoplasmic)
- Inclusion bodies

Gene amplification vs protein expression

PCR vs expression system

- Protein vs DNA (**Proteins are not so simple**)
- Proteins are tough to predict
- Proteins can be high maintenance (**invitation for frustration**)
- **Protein work is more fun(?)**: interesting to note that when pressed for opinion, most will say protein work tends to be more rewarding, interesting, and challenging.

Detection of your Protein

Gel Electrophoresis (SDS-PAGE)

This is what your **sample buffer and gel** is all about.

* has **dye** (so that you can see stuff)
* has **glycerol** (makes sample heavy so that sample will flow into wells)
* has **SDS (VERY IMPORTANT)** coats proteins with negative charge (now all proteins have same charge), and denatures proteins to uniform shape (rod-like shape). Now all proteins have equivalent shape as well.
•has **beta-mercaptoethanol** or DTT (dithiothreitol) these are reducing agents. will reduce and break disulfide bonds.
•Run gel electrophoresis **polyacrylamide gel electrophoresis (PAGE)**

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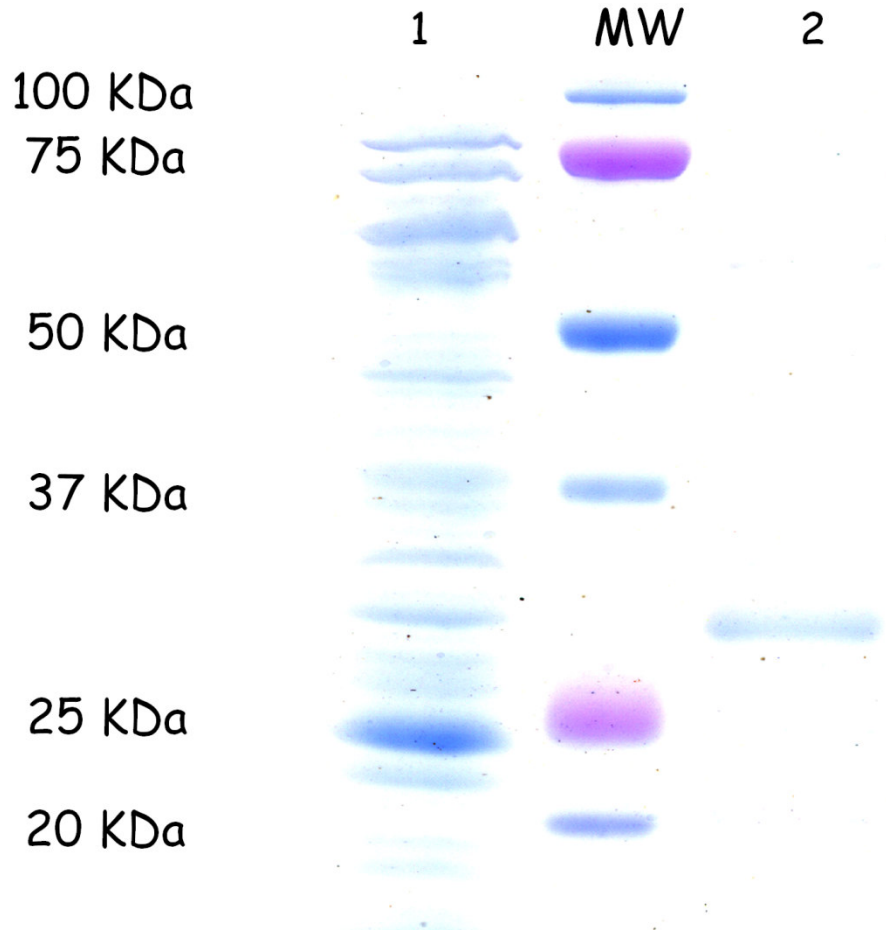
Gel Electrophoresis (SDS-PAGE)

STAINING:

coomassie blue (0.3ug to 1ug per band) can see a band as faint as 100ng

silver staining 2 to 5ng (MUCH MORE SENSITIVE)



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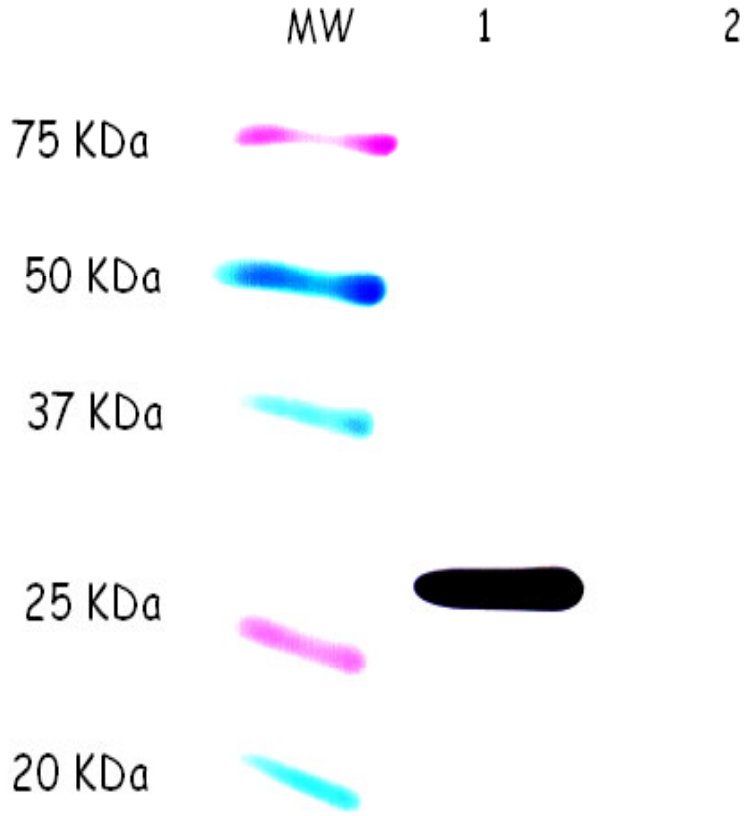
Protein Detection
Western blot

essentially a procedure that allows you to probe for a specific protein using an ANTIBODY. main idea. USE A MEMBRANE. (this is why it's called a blot)



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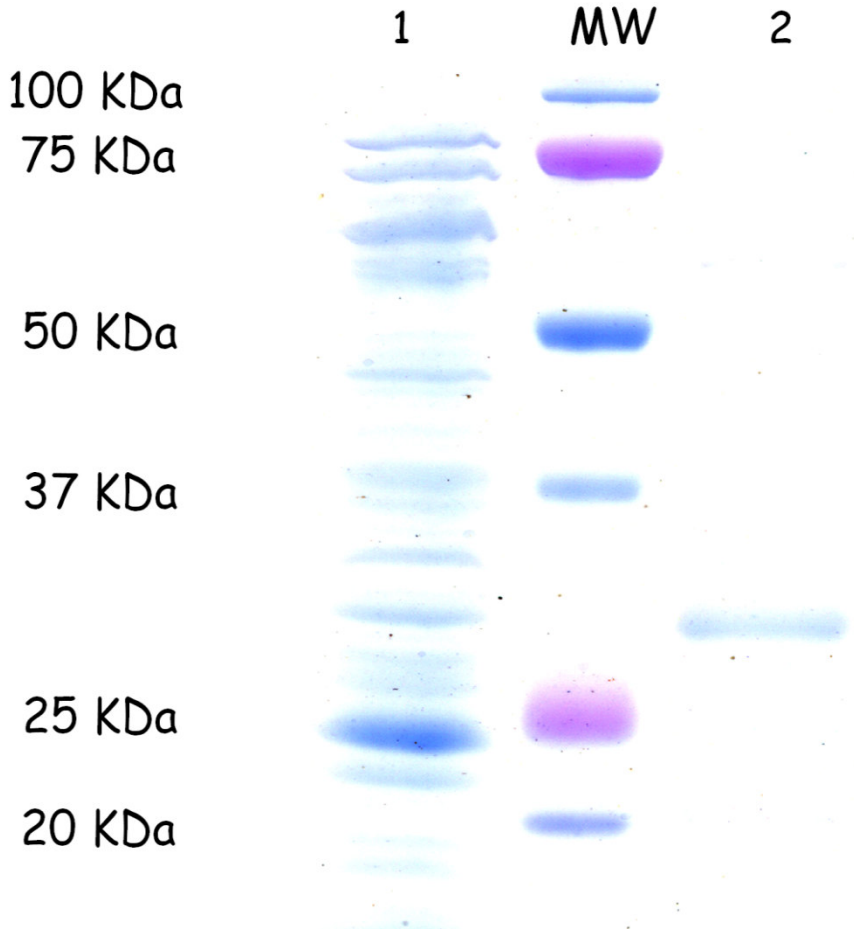
Protein Purification

How to purify your protein out of the total proteins



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Protein Purification

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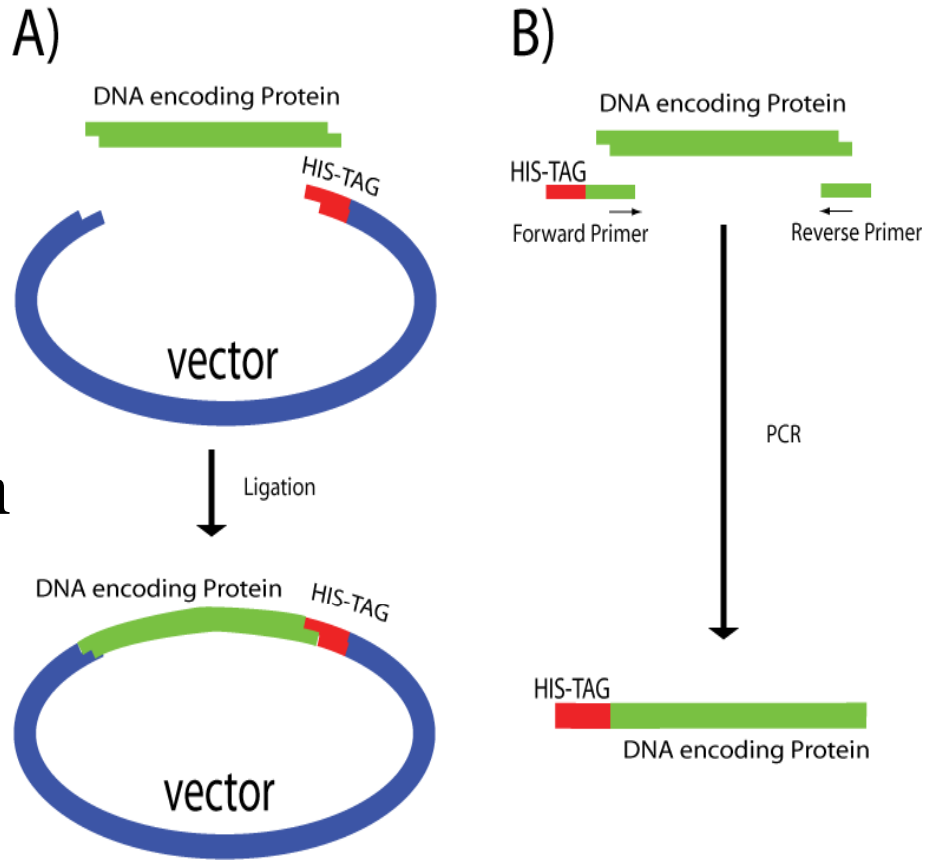

His-Tag

His-His-His-His-His-His

CATCATCATCATCATCAT

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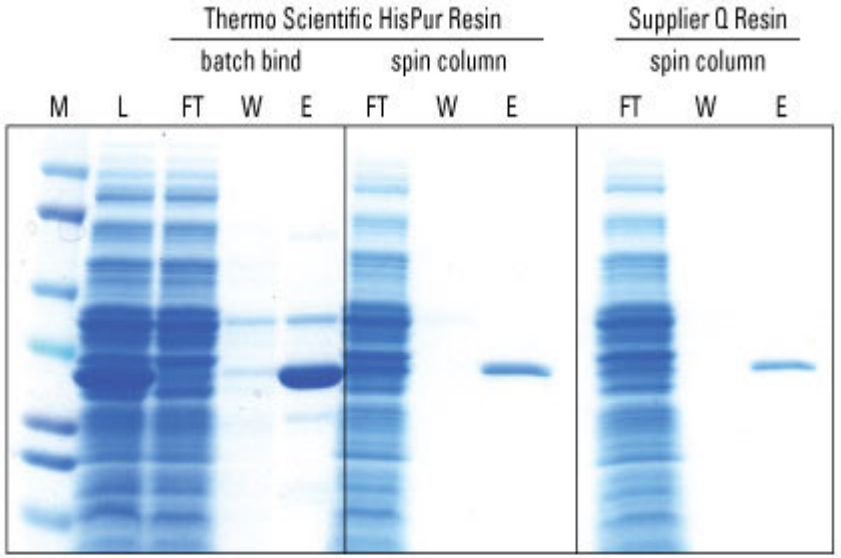
Protein Purification

Nickel (**Ni**) or Cobalt

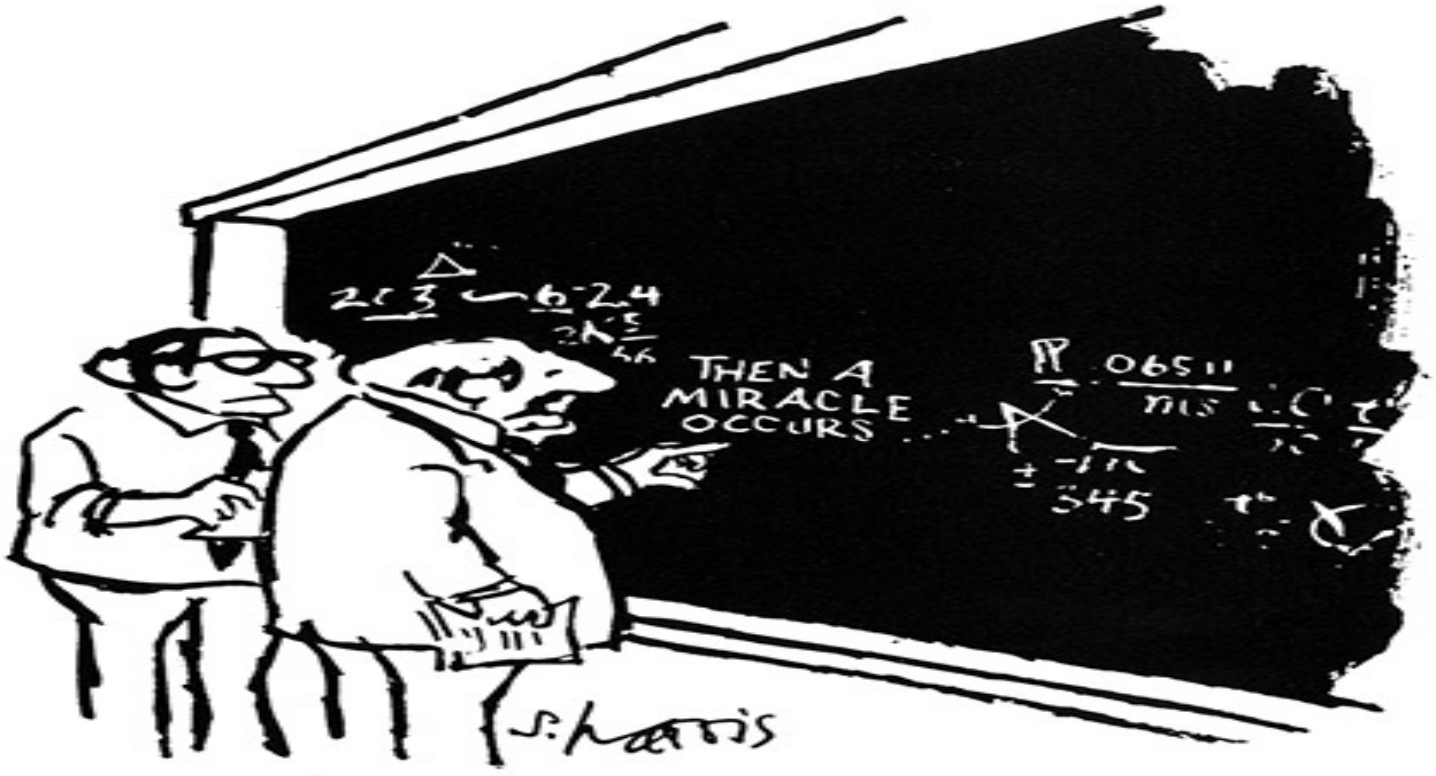


proteins are usually eluted with 150-300 mM imidazole

Or cleaved His-tag with proteases

Send for Sequencing

Questions



"I think you should be more explicit here in step two."

If we knew what it was we were doing, it would not be called research, would it?" - Albert Einstein