

# Protein Engineering

20.109 Module 2 Day 4  
Thursday Oct 16th, 2008

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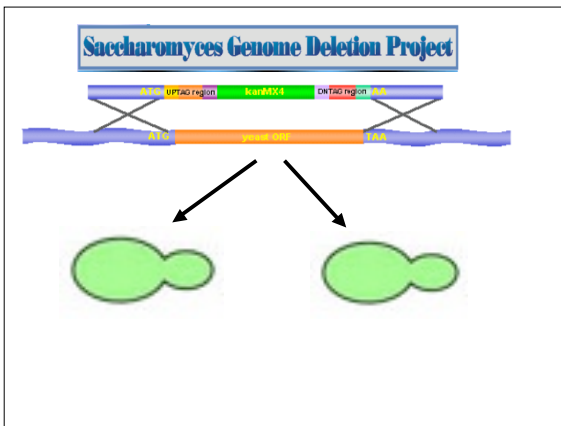
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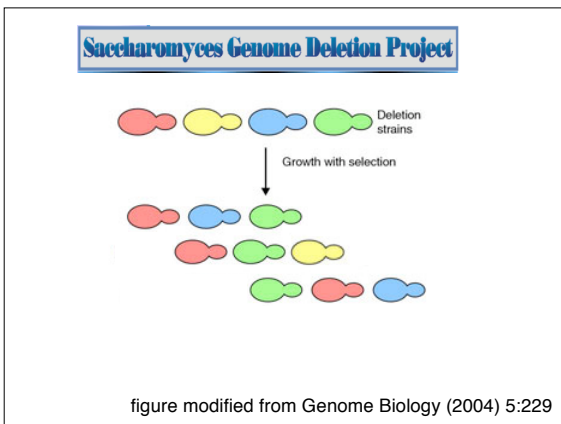
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### Techniques for phenotyping yeast

REPLICA PLATING AND INDIRECT SELECTION OF BACTERIAL MUTANTS  
 JOSHUA LEDERBERG AND ESTHER M. LEIDERBERG

J Bacteriol. (1952) 63(3): 399-406  
 EMBO J. (1992) 11: 4145

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### Techniques for phenotyping yeast

**Spot Test analysis.** Equal numbers of wild type or mutant yeast cells were spotted on media with or without 400 ug/ml of cobalt. The relative numbers of yeast cells spotted on the plates are indicated. In relation to wild type, the mutant strain did not produce as many colonies in the presence of cobalt. This suggests sensitivity to cobalt for the mutant cells. W/T is the wild type (normal) strain; Mut is the gene deletion mutant strain for the *rox1* gene.

Bioinformatics 2007 8:117

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### Protein Purification: why bother...

- Essential for structural studies
- Required to study enzyme function *in vitro*
- Needed for raising antibodies to use as molecular probes
- Useful to study protein:DNA and protein:protein interactions
- Can be used for "reverse genetics" (isolate gene from protein)

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### Protein Purification: where to begin?



cell suspension or tissue



- Best starting sources are cheap
- readily available
- enriched for protein of interest
- To follow purification: nice to have an assay that is
- cheap
- readily available
- specific for protein of interest

SDS-PAGE can be used to determine how pure but not how functional

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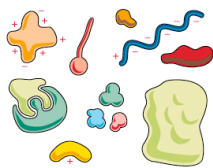
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### Physical properties enable fractionation



- Mixture contains proteins of various size,
- shape,
- charge,
- hydrophobicity,
- affinity for different molecules

**These properties can be exploited to separate individual protein from mixture**

Alberts "Essential Cell Biology"

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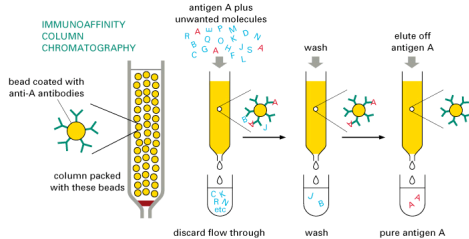
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### Affinity Chromatography



Alberts "Essential Cell Biology"

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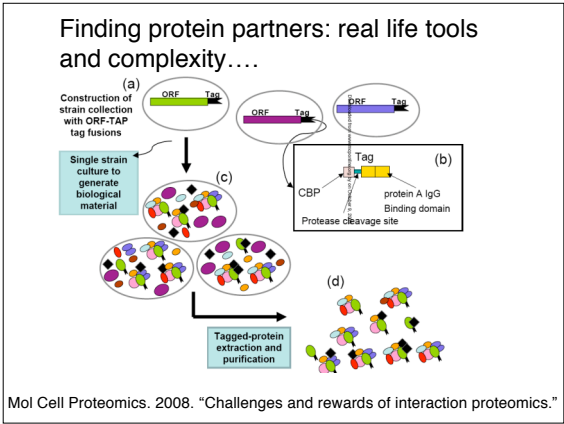
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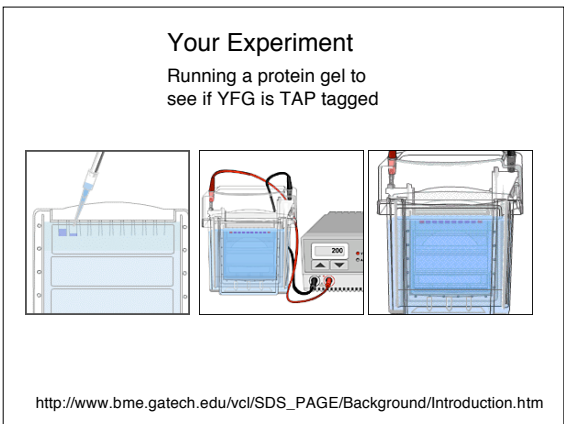
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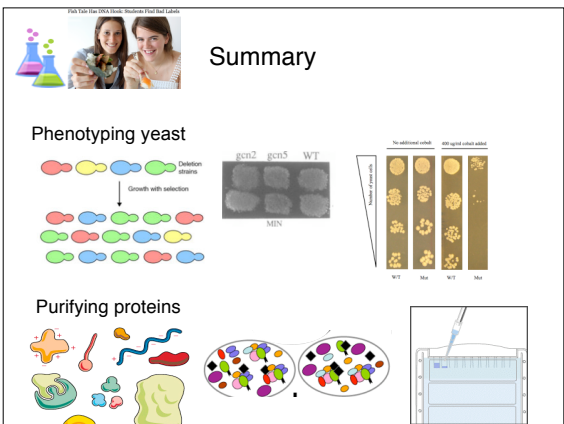
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