Identification of Host Factors Involved in 2 µm Plasmid Maintenance in Yeast

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1) Introduction:

The yeast 2 µm plasmid, a selfish genetic element, is a simple genome that is nonetheless able to maintain a stable copy number. By purifying complexes containing the 2 um- encoded proteins, we hope to learn which host proteins have been hijacked to facilitate plasmid partitioning. Our approach is to purify Tandem Affinity Purification (TAP)-tagged proteins and to identify the 2 µm- plasmid associated proteins by mass spectrometry . We can then test whether purified proteins are important for segregation using genetic and biochemical methods. Using this unbiased approach, we can develop a testable model describing host proteins involved in segregation.

2) 2 µm Plasmid:

The 2 µm plasmid of *Saccharomyces cerevisiae* is a simple genome that reflects much of the same characteristics of a chromosome.

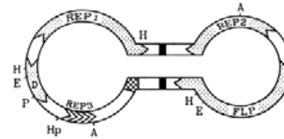
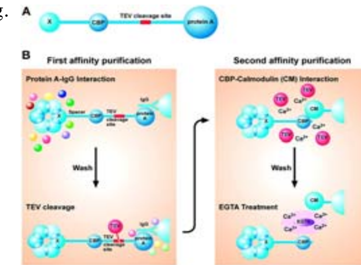


Diagram 1: 2 µm Plasmid

Proteins	Function
FLP	Recombination Enzyme
REP1	Vital Component to Segregation
REP2	Vital Component to Segregation
RAF1	Unknown

3) TAP:

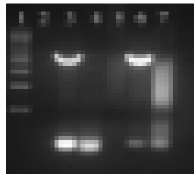
Tandem affinity purification (TAP) efficiently separates epitope-tagged protein complexes from crude extracts as seen in diagram B. Diagram A reveals the structure of a TAP-tag.



4) PCR:

Polymerase chain reaction (PCR) amplifies small chains of DNA. In the diagram below, DNA was extracted from yeast cells and was transformed with TAP- Rep1 or TAP- Rep2 cassettes. They were then subjected to PCR using primers specific for each integration.

Figure 1: Confirmation of TAP Cassette in Rep1 and Rep2 Genes



Lanes	Strains
1	500 base pair ladder
2	(-) DNA
3	Rep 1#1
4	Rep 1#2
5	Rep 2#1
6	Rep 2#2
7	Parent Strain

5) Western Blot:

To detect protein in a given sample, the technique Western blot (Immunoblot) can be utilized. The proteins are transferred to a membrane and are then probed using antibodies specific to protein.

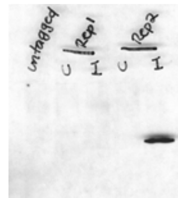


Figure 2: Western Blot of Rep1and Rep2 (uninduced and induced)

Name	Description
Untagged	Cir+ strains grown in sRaff (u)
U	Rep1 or Rep2 TAP tagged strain grown in sRaff
I	Tagged strains grown in YPRG overnight

6) Silver Staining:

Silver staining was applied to the proteins in order to confirm that similar amounts of total protein were loaded.

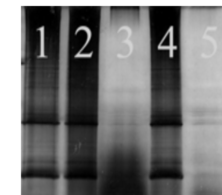
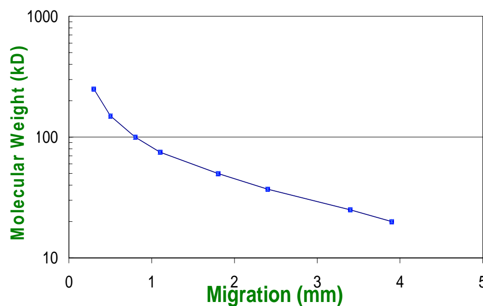


Figure 3: Silver Staining of Rep1 and Rep2 Genes

Lanes	Strains
1	Wild Type Untagged
2	Rep 1 Uninducing
3	Rep 1 Inducing
4	Rep 2 Uninducing
5	Rep 2 Inducing

7) Protein Molecular Weight Curve:



8) Results:

- TAP tag amplified by PCR and transformed into yeast
- Integration verified by PCR
- Expression confirmed by the Western blot
- Correct size
- Gal-inducible

9) Conclusion and Future Work:

From what the results indicate, it is suitable to proceed with purification. After the proteins are purified, they can be tested in future experiments whether they are vital for segregation using the methods like partitioning in deletion strains, co-immunoprecipitation, and GST- pull-down assays.

10) References:

1) Bauer, Andreas; Kuster, Bernhard. 2003. Affinity purification- masspectrometry: Powerful tools for the characterization of protein complexes. *Eur J Biochem.* 270: 570-578.

2) Burke, Dan; Dawson, Dean; Stearns, Tim. *Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual.* Cold Spring Harbor Laboratory Press. 2000 ed. 2000.

3) Schimanski, Bernd; Nguyen, Tu N.; Gunzl, Arthur. 2005. Highly Efficient Tandem Affinity Purification of Trypanosome Protein Complexes Based on a Novel Epitope Combination. *Eukaryotic Cell.* 4: 1942-1950.

4) Velmurugan, Soundarapandian; Ahn, Yong-Tae; Yang, Xian-Mei; Wu, Xu- Li; Jayaram, Makkuni. 1998. The 2µm Plasmid Stability System: Analyses of the Interactions among Plasmid- and Host-Encoded Components. *Mol. Cell. Biol.* 18: 7466-7477.

5) Volkert, Frederic; Wilson, Duncan; Broach, James. 1989. Deoxyribonucleic Acid Plasmids in Yeasts. *Microbiological Reviews,* 53: 299-316.

11) Acknowledgements:

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