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Creation of a gene selection method using a Ste13-Cps1 construct in S. cerevisiae

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The trans-Golgi network (TGN) reporter protein A-ALP, which is composed of the luminal (LD) and transmembrane domain (TMD) of TGN resident protein ALP fused to the cytosolic domain (CD) of Ste13, allows us to detect its arrival to the vacuole and therefore its rate of processing in S. cerevisiae. A-ALP is useful in gene screenings, being cleaved at a C-terminal site by a pep4 dependent carboxypeptidase in the vacuole, enabling detection for an assortment of mutations in the CD region. Alternatively, the Cps1 gene codes for the vacuolar enzyme, carboxypeptidase yscS; this cleaves the substrate CBZ-Gly-Leu. This mechanism allows for the leucine auxitrophic Cps1 strain to grow without the presence of any other leucine source, making it a good strategy for a gene selection, allowing identification for specific mutations. A Ste13(CD +TMD)-Cps1(LD) reporter construct was made to see if it would behave in the same manner and remains inactive until reaching the vacuole. For comparison, four derivatives of this strain were made with mutations in the Ste13 region with known processing rates when using A-ALP, verified by DNA sequencing. Western blotting was used to assess the expression and steady state processing of each protein. To ensure the proteins could not be processed prior to reaching the vacuole, the strains were grown up on plates with the CBZ-Gly-Leu substrate. An imunoprecipitation experiment will now be done to show processing by using radioisotope markers and to determine a half-time to determine the functional ability of each Ste13-Cps1 construct.