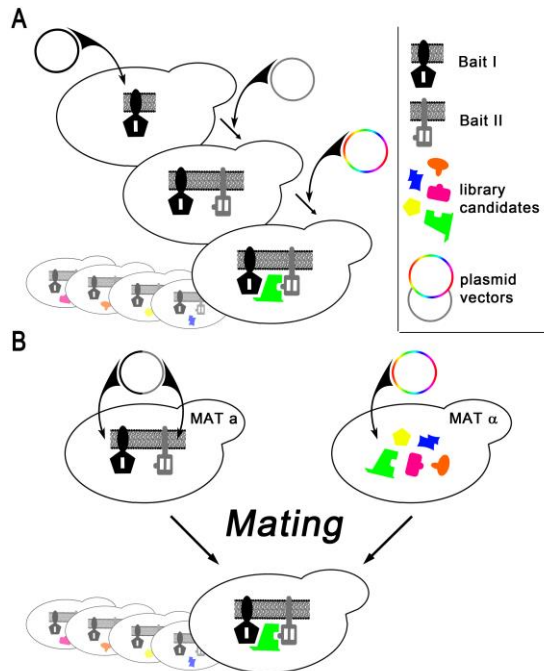




A Novel Screen Identifying Ternary Binding Partners Of Known Interacting Proteins

Technology Description



Many biological processes depend on oligomeric protein-protein interactions (PPI). However, state-of-the-art *in vivo* PPI techniques focus on analysing binary interactions (i.e. the Split-Ubiquitin System (SUS)¹). The SUS can also be used to analyse binding of three proteins in the so-called SUS Bridge Assay (SUB)² – see Figure A. Nevertheless, this assay makes an unbiased screening approach cumbersome and its low efficiency restricts the identification of meaningful candidates.

Here, we present the first screening system in yeast that permits high-throughput screening of cDNA libraries for ternary binding partners of a known interaction couple. This is achieved by a unique combination of SUB and yeast mating. Technical prerequisites are special “2in1”-vectors³, which allow simultaneous transformation of “Bait I” and “Bait II” on a single plasmid in yeast of one mating type and the cDNA library in the other – see Figure B.

Innovation

Up to now: cDNA-libraries can be screened for binding partners in “dimeric” systems – unknown interacting partners for one known protein

Now: cDNA-libraries can be screened for interacting partners in “trimeric” systems – unknown interacting partners for two known binding proteins

Applications

- ❑ cDNA libraries from virtually all species can be tested
- ❑ System can be expanded to identify quarternary interactions

Advantages

- ❑ Efficient – mating-based approach guarantees 10-100 times higher coverage of primary interactions compared to standard approaches
- ❑ Fast – can be done in less than a month
- ❑ Reliable – the new screen identifies real trimeric interaction partners as verified by our data (manuscript in preparation)
- ❑ Low-cost – libraries can be re-used and stored in yeast, ready-to-use

Proof of Concept

Please refer to the next page

Requested Cooperation/IP Status

Industrial Licensing Partner/
Patent Pending, Priority Date 2015-05-15

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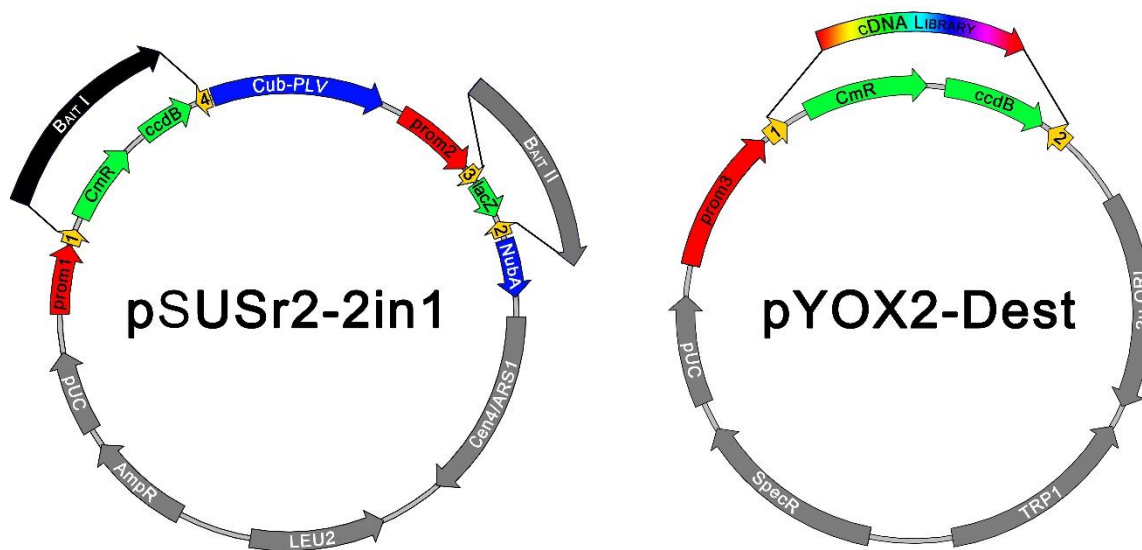
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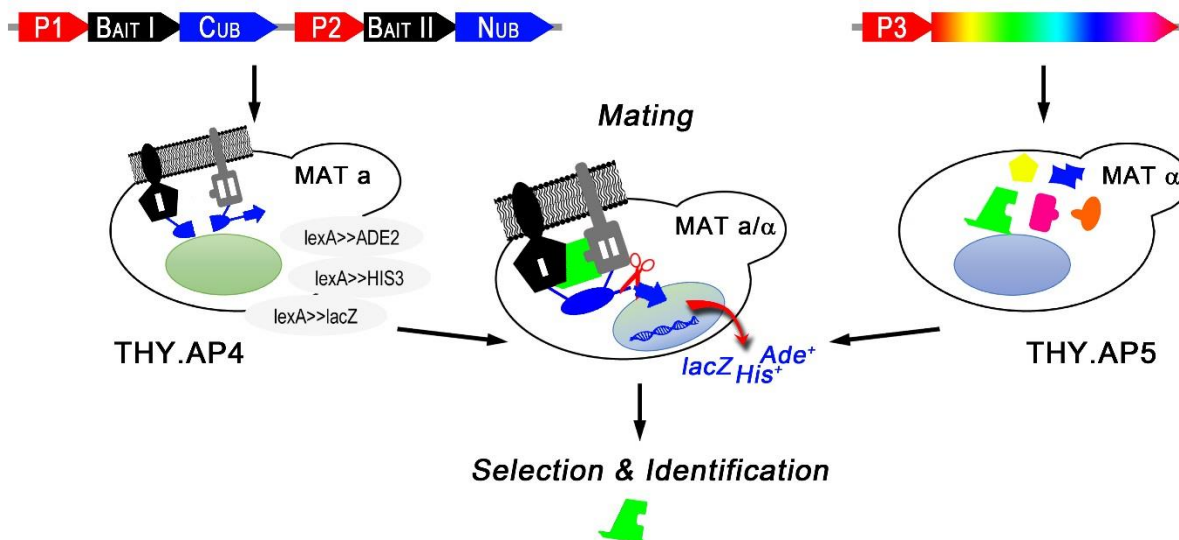
OVERALL DESIGN OF MATING-BASED SUB-SCREENING

The new Cloning Vectors



Different promoters regulate expression of either Bait I or Bait II. Both baits are maintained on a low copy vector backbone. The library is maintained in a 2 μ high copy plasmid and expressed through a strong promoter. For counterselection in *E. coli* a different resistance marker (Spectinomycin) is used in pYOX2-Dest as opposed to pSUSr2-2in1 (Ampicilin).

Overall Design of the new Screening Tool



A mating-based approach is facilitated through simultaneous cloning of both bait proteins on one plasmid backbone and the cDNA library on a second; each of which is either transformed in mating type *a* or α haploid yeast cells, respectively. As proof of concept we have identified novel interaction partners of the Brassinosteroid receptor complex of *Arabidopsis thaliana* (manuscript in preparation).

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