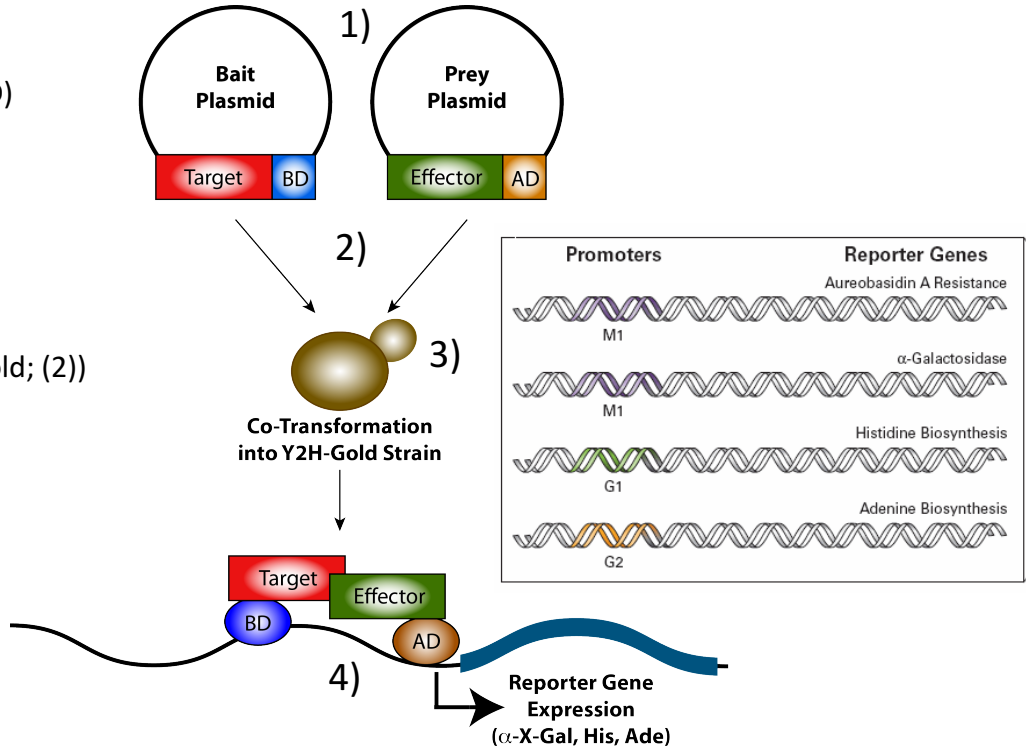
Yeast two hybrid: Basics

- Method to investigate protein-protein interaction
- Based on the transcription factor Gal4
- Gal4 consists of a DNA-binding domain (BD) and an activation domain (AD)
- Both domains are functional even when seperated
- Both domains need to be in close proximity to activate transcription

- Generate fusion proteins in autonomously replicating plasmids (1)
- Fusion proteins: 1st POI-Gal4-BD (Bait) / 2nd POI-Gal4-AD (Prey)
- Both plasmids are co-transformed into Gal4-deficient Yeast strain (Y2H Gold; (2))
- Plasmids complement Leucine and Tryptophan auxotrophy (3)
  (Selection on SD –leu/-trp medium)

- Bait fusion protein can bind to upstream activating sequences (UAS)
  of Gal4-responsive promoter regions
- Interacting proteins bring BD and AD in close proximity
- POI1-BD/POI-AD complex activate transcription of reporter genes (4)
  (Histidine / Adinine / α-Galactosidase / Aureobasidine)
  (slection on SD –leu/-trp/-his/-ade +α-X-Gal)

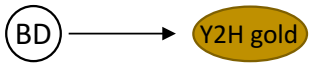


Additional information: Matchmaker III user manual (Clontech)

Yeast two hybrid: The two main methods (Library Screen)

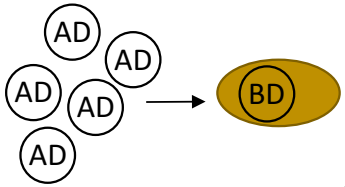
Library screen

Pre-transform bait plasmid

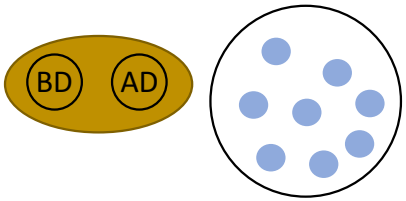


Grow primary transformant

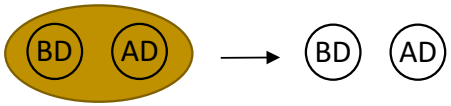
Transform prey library



Select positive interactors
(SD –leu/-trp/-ade/-his +X-α-Gal)

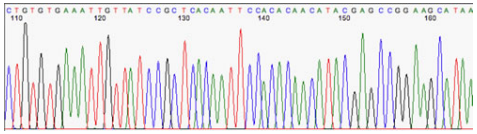


Extract plasmids



Sequence AD-Plasmid
(to identify interaction partner)

Your Gene of interest



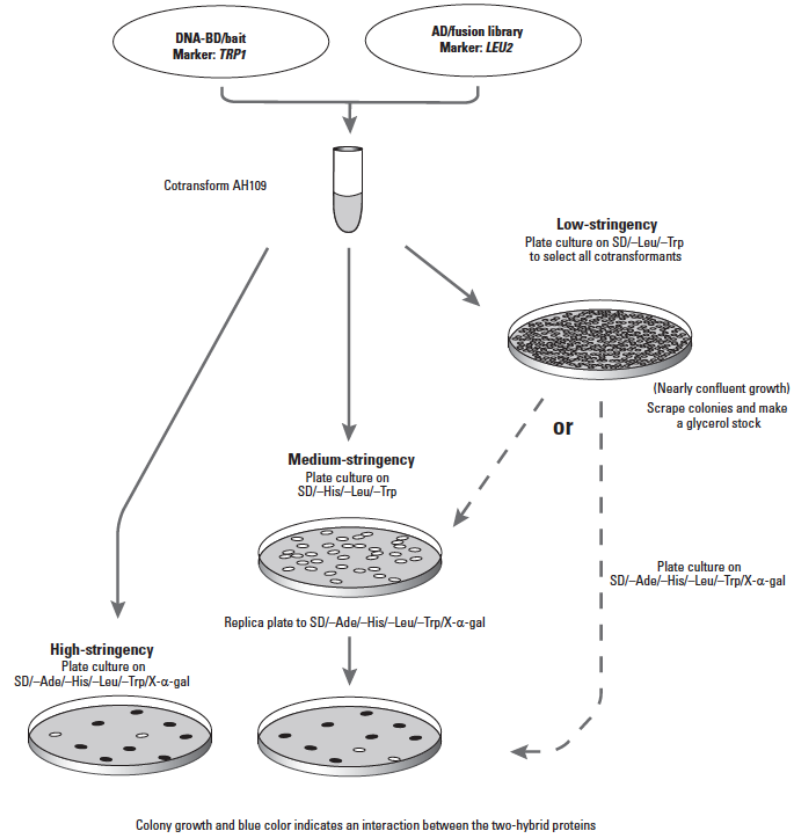
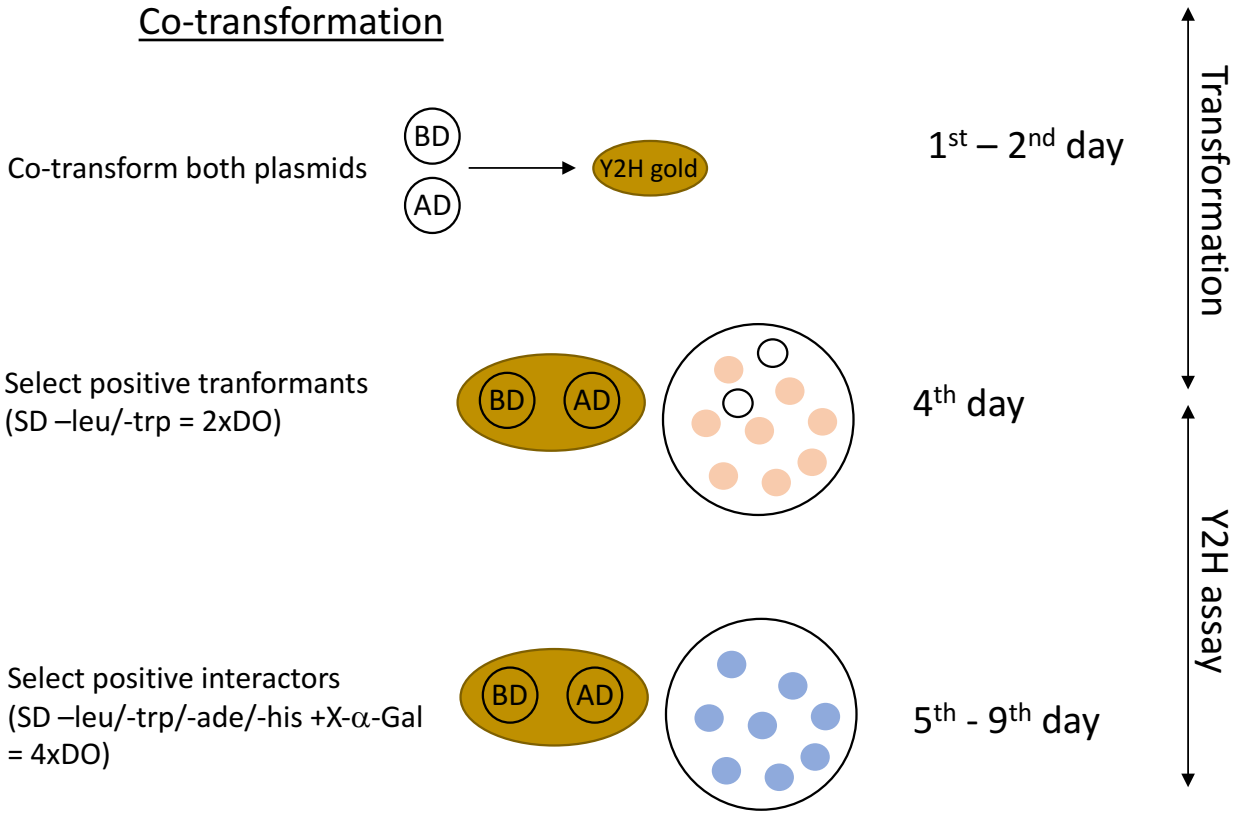


Figure 5. Screening an AD fusion library using strain AH109. Use the stringency of your choice to screen for interacting proteins. Note: high stringency selections result in fewer colonies, and reduce the number of false positives. However, weak interactions may be missed.

From: Matchmaker™ GAL4 Two-Hybrid System 3 & Libraries User Manual

Yeast two hybrid: The two main methods (Co-transformation)

Co-transformation



Material provided:

- Y2H Gold o/n culture (in YPDA medium)
- Solutions to prepare competent cells (H2O, Te/LiAc)
- Solutions for Yeast transformation (PEG/LiAc/TE, DMSO)
- Plasmids for Yeast Transformation (3x bait plasmid containing target genes 2x prey plasmid containing effectors)

- Test strains o/n culture (in SD 2XDO medium)
- Control strains o/n culture (in SD 2XDO medium)
- Y2H Media (2x DO, 4x DO Medium)

Yeast two hybrid: Pros and Cons

Pros:

- Quick and easy screening method
- Yeast is easy to handle in the lab (growth, transformation, selection)
- Semi *in-vivo* conditions
- Allows for post-translational modifications (e.g. Glycosylation, Prenylation etc.)
- Yeast chaperones support proper protein folding

Cons:

- Depends on nuclear localization
- Post-translational modifications in yeast can be different to the natural system
- Co-factors might not be present in yeast
- False negatives:
  - Wrong fold of proteins can alter interaction
  - TM-domains or Prenylation can interfere with nuclear localization
- False positives:
  - Wrong fold can cause unnatural interaction
  - Proteins which are naturally separated (spatial or temporal) can interact in yeast
  - Intrinsic activation domain can cause autoactivation in BD-fusion proteins

  → Needs downstream verification by additional experiments

Yeast two hybrid: Verification of Interactions

Methods to verify interaction partners:	Pros:
- FRET (Förster resonance energy transfer)	- *in planta* conditions, transient expression, does not require protein extraction
- Co-Immunoprecipitation (CoIP)	- *in planta* conditions, transient expression, quick
- *in-vitro* interaction studies (e.g. analytical gel filtration)	- "clean" environment (no protein modifications/additional proteins), detection of binding affinity, allows to study the mode of interaction (e.g. by protein structure resolution)

Additional Yeast based assays:

- Yeast-one-Hybrid	- screen for Protein-DNA interaction
- Yeast-three-hybrid	- screen for Protein-RNA interaction
- Split-ubiquitin system	- screen for Protein-Protein interaction of membrane proteins