YEAST TWO-HYBRID AND RELATED ASSAYS

Advanced Genetics

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2/19/24
FUNCTIONAL PROTEOMICS

- Critical aspects required for understanding protein function:
  - Protein sequence and structure
  - Expression profile
  - Post-translational modifications
  - Intracellular localization
  - Interactions with other proteins or nucleic acids.
BIOLOGICAL EFFECTS OF PROTEIN-PROTEIN INTERACTIONS

• Alter kinetic properties of enzymes
• Facilitate substrate channeling in biochemical pathway
• Inactivate or regulate degradation of another protein
• Change specificity of a given protein for its substrate
• Serve a regulatory role in an up or downstream biological event
YEAST 2-HYBRID—BASIC PREMISE

- Bares greater resemblance to higher eukaryotic systems than a bacterial system
- Quantity of biological reagent (i.e. protein/s of interest) is less limiting on assay readout
- Detects weak and/or transient interactions due to signal amplification via reporter gene
• **GAL4/UAS system** commonly used
  • DNA binding domain (BD)
  • Transcription activating domain (AD)
HOW DOES IT WORK?

- GAL4 domains are separated and fused to proteins of interest: BD-Bait and AD-Prey
• Interaction status of bait and prey protein fusions will dictate reporter expression
What are the five critical components of the Y2H system?
5 KEY COMPONENTS

Yeast Two Hybrid System
www.technologyinscience.blogspot.com
• Auxotrophic reporters (eg. HIS3)
  • Interaction enables growth in the presence of HIS3 inhibitor 3AT
• Colorimetric reporters (eg. lacZ+ x-gal)
  • Interaction turns colonies a different color
REPLICA PLATING

1. Press sterile velvet onto plate to pick up cells from bacterial colonies.

2. Transfer cells to new plates.

3. Incubate.

4. Compare growth on plates to identify auxotrophic mutants that grow on medium containing histidine but do not grow on medium lacking histidine.
CAVEATS—DO YOUR HOMEWORK AHEAD OF TIME!

- Is the bait protein known to activate/repress transcription?
- Is the bait known to be in a certain protein family?
- When/Where are proteins expressed in the cell?
  - Important for construction of prey library
- How will you confirm interactions picked up in screen?
  - Co-IP of endogenous proteins
HOW TO: STEP 1

- Construct BD-Bait fusion
  - Clone bait DNA into DB containing vector
  - Transform into bacteria
  - Plate on selection media (usually antibiotic)
  - Sequence colonies to confirm correct reading frame
HOW TO: STEP 2

- Construct AD-Prey fusion
  - Clone prey DNA into AD containing vector
  - Transform into bacteria
  - Plate on selection media (usually antibiotic)
  - Sequence
  - cDNA library prepped for high through-put
HOW TO: STEP 3

- Grow bacteria and purify plasmids prior to yeast transformation
HOW TO: STEP 4

- **Test for self activation!**
  - Assay strains singly transfected for bait or prey plasmids
  - Measure HIS3 basal expression due to self-activation. Basal level expression of amino acid reporter can skew readout

- **Optimize sensitivity**
  - Vary the number of operator sequences upstream reporter
  - Control cell’s dependency on the reporter
    - 3AT inhibits HIS3 in dose-dependent manner. Vary concentration of amino acid in growth media
    - Competitive inhibition: use 3-AT to titrate the minimum level of histidine expression required for survival
HOW TO: STEP 5

• Perform your screen!
ADVANTAGES

• Low-tech—no sophisticated equipment required
• Offer important first hints at interactions
• Can detect transient interactions that may be missed by co-IP
• Immediate identification of gene products
  • Colony selection and sequencing
• Study known interactions
  • Identify necessary residues/domains for binding
POTENTIAL WEAKNESSES

• High rate of **False Positives**…
  • Unnatural concentrations
  • Interactors may not be expressed together naturally
  • Non-specific interactions

• And **False Negatives**
  • Hybrid protein conformation interferes with physiologic binding
  • Proteins may not localize to the nucleus
  • Interactions may be host dependent (post translational modifications, chaperones, etc.)
MINIMIZING FALSE RESULTS

FALSE POSITIVES:
- Run replicate experiments
- Vary expression level of bait and prey proteins
- Always validate with independent method
  - Co-IP

FALSE NEGATIVES:
- Run system with positive control—two known interactors
- Test other models—circumvent problems due to heterologous expression
- Design and test N-term and C-terminal fusions
- Screen with multiple different fusion vectors—alter expression levels
VARIATIONS

• Reverse Yeast-2Hybrid
  • Assess when an interaction is disrupted

• One-hybrid
  • Detect protein-DNA interactions
VARIATIONS

- Three-hybrid
  - Detect protein-RNA interactions
- One-two-hybrid
  - Assays simultaneous protein-DNA and protein-protein interactions
- Split ubiquitin system
  - Detect transmembrane protein interactions
SCREENING FOR CO-FACTORS

ULTimate Y2H+1 Principle

- "+1" PROTEIN
- BAIT
- PREY
- No growth on medium lacking Histidine

Promoter  HIS3

INTERACTION
- Transcription machinery
- Growth on medium lacking Histidine

Promoter  HIS3

BAIT = your protein of interest
PREY = protein partner of the bait

LexA or Gal4
DNA Binding Domain

Gal4
Activation Domain

The bait requires a cofactor or a post-translational modification for a folding allowing interactions

Cofactor, enzyme ...
BAIT⁺ = Modified Bait
SPLIT UBIQUITIN SYSTEM

SPLIT-UBIQUITIN

- Cub
- Nub
  - Spontaneous reassociation
  - "Split-ubiquitin"

- Cub
- NubG
  - No spontaneous reassociation

Point mutation in Nub Ile3 → Gly

MBMATE Y2H PRINCIPLE

INTERACTION

- Forced reassociation of ubiquitin
  - LEXA-VP16 release after proteolytic cleavage

- Transcription machinery

ON
- Growth on medium lacking Histidine

OFF
- No growth on medium lacking Histidine

Promoter
- HIS3
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Could you use Y2H in your proposal? How?
OTHER ADAPTABLE MODELS

- **S. cerevisiae** = original Y2-H model
- *Candida albicans* (alternative yeast species)
- **E. coli** = B2H
  - Screen larger libraries, lower false positive rate
- Mammalian two-hybrid = M2H
  - Uses transfected cell lines
- *Arabidopsis thaliana* = P2H
  - Plant model
- **Bombyx mori**
  - Insect model system
• https://www.hybrigenics-services.com/contents/our-services/discover/ultimate-y2h-2
• https://blog.addgene.org/tips-for-screening-with-yeast-two-hybrid-systems
• https://www.researchgate.net/publication/6215295_Chick_Hairy1_protein_interacts_with_Sap18_a_component_of_the_Sin3HDAC_transcriptional_repressor_complex
• https://www.researchgate.net/figure/A-Workflow-of-a-yeast-two-hybrid-screen-Prey-library-was-transformed-into-the-NMY63_fig6_242334601
• https://en.wikipedia.org/wiki/Two-hybrid_screening
• https://www.youtube.com/watch?v=InA8wOzFg1U
• https://www.slideshare.net/25071987/yeast-two-hybrid-9235978
COURSE ANNOUNCEMENTS

• Proposal: Topic due Wednesday, February 28th
  • Email to advancedgenetics@genetics.wustl.edu

• Homework deadlines (email to Doris and Ena):
  • Dr. Dutcher’s HW due 2/20.
  • Dr. Schedl’s HW due 2/27.