TECHNICAL MANUAL

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Yeast cross and capture collection

Cat #YSC5049 (Bait MATa Collection), YSC5092 (Prey MATα Collection)

Product Description:

Developed by Dr. Stagljar of University of Toronto, the Yeast Cross-and-Capture Collection assay is a novel method that permits rapid analysis of protein-protein interactions (PPIs). This system uses differentially tagged ORF arrays in the two haploid yeast (*Saccharomyces cerevisiae*) mating types MATa and MATα. In MATa cells, "bait" ORFs are tagged at the 3' end with a sequence encoding six histidines (6xHIS), while "prey" ORFs in MATα cells are tagged with a sequence encoding a triple VSV tag (3xVSV). Both tags also contain a V5 epitope to allow identification of both bait and prey proteins. To examine a particular PPI, a bait strain is crossed with a prey strain to generate diploid yeast expressing the desired bait- and prey-tagged proteins. Following diploid growth and cell lysis, extracts are incubated with nickel beads, allowing isolation of the 6xHIS-tagged bait and its associated proteins. Bound proteins are examined by immunoblot analysis for the presence of the bait and prey proteins using anti-V5 and anti-VSV antibodies. If the prey protein binds to the nickel beads in a bait-dependent manner, a PPI is inferred (Figure 1A). Conversely, the absence of the prey protein in a pulldown reaction suggests that the two proteins fail to interact (Figure 1B).

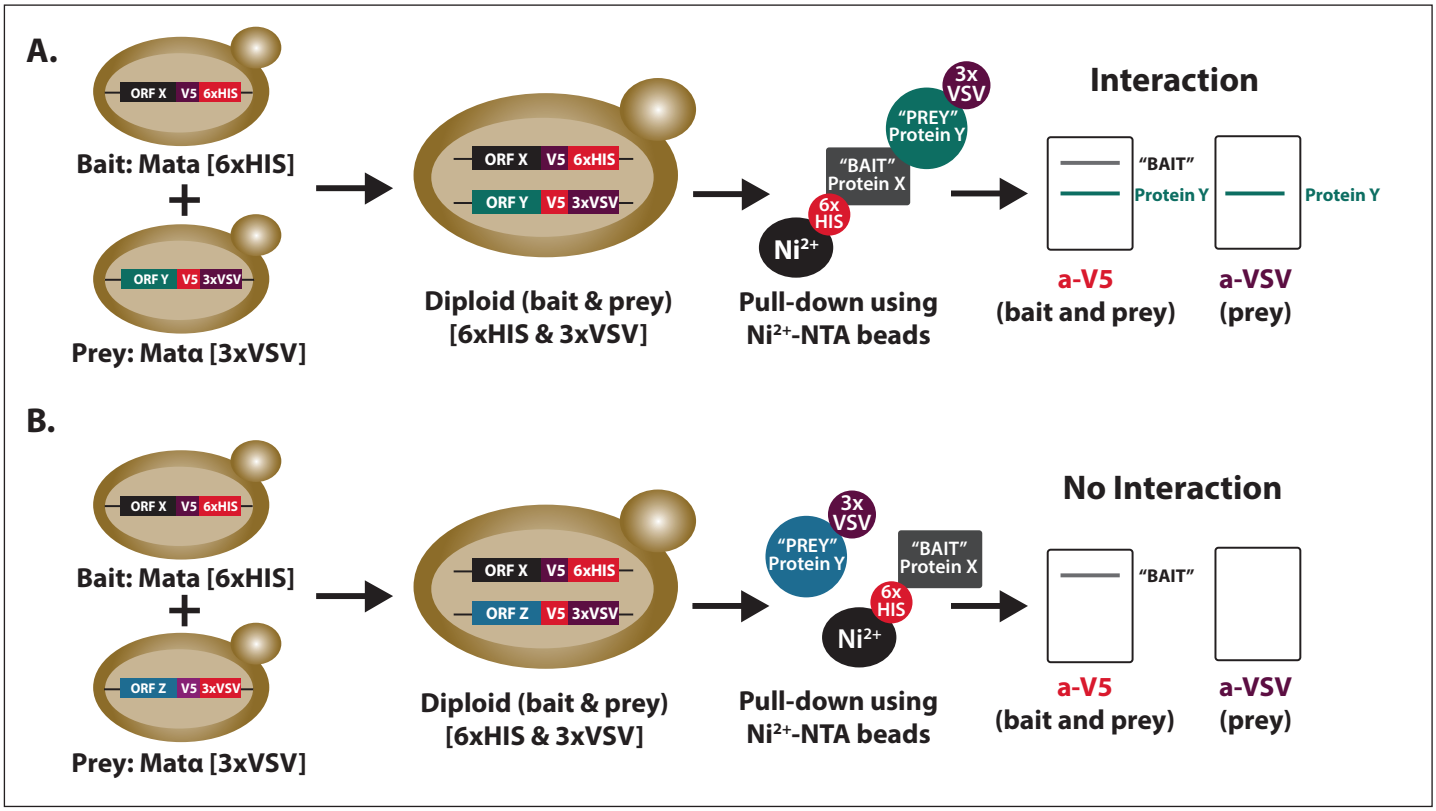

Figure 1. Cross-and-Capture Assay. A strain containing the bait ORFX tagged with a V5 epitope and six histidines (6xHIS) is crossed with strains that contain prey ORFY or ORFZ tagged with a V5 epitope followed by a triple VSV tag (3xVSV). Diploids, which express both tagged bait and prey, are grown on selective medium. Protein extracts from the diploids are then incubated with nickel beads (Ni2+-NTA), allowing isolation of bait (Protein X–6xHIS) and bait-associated prey protein (Protein Y–3xVSV) **A.**, whereas a non-interacting protein (Protein Z–3xVSV) will not bind **B.** Proteins are then separated by SDS-PAGE, and blots are probed for bait and prey (anti-V5 antibody) and specifically for the prey (anti-VSV antibody) by immunoblot (Suter *et al,* 2007).

In total 506 yeast ORF-baits and a same number of ORF-preys constitute the Yeast Cross and Capture Collection. The ORF composition consists in 258 ORFs encoding proteins involved in DNA repair, replication and recombination (*Saccharomyces* Genome Database, yeastgenome.org), as well as 248 ORFs encoding proteins of unknown function that were assigned to the nucleus based on their localization patterns (Huh *et al*. 2003).

To generate bait and prey strains, PCR products containing the desired tags and the Kan^r cassette were produced from bait- and prey-specific plasmids and transformed into MATa and MATα strains, respectively (Figure 2). Confirmation of the successful tagging of all 506 ORFs was achieved by colony PCR via sequencing of the ORF/tag junction and by immunoblotting.

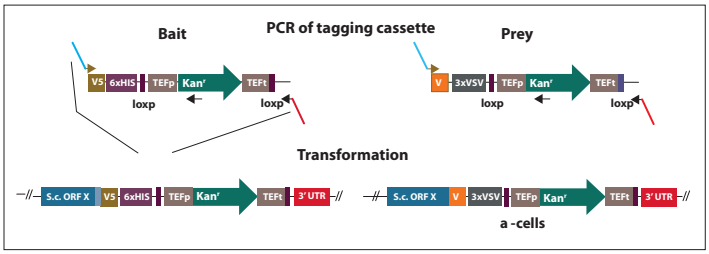

Figure 2. Generation and verification of tagged protein arrays. To tag ORFX as bait (V5–6xHIS) and prey (V5–3xVSV), a set of primers is used that anneal to identical binding sites within the template plasmids and have flanking sequence homologous to ORFX. PCR products generated from the bait and prey templates are transformed into a- and α-cells, respectively. Homologous recombination occurs between the variable portion of the 5′ primer (light blue) and the 3′ terminus of the ORF, and between the variable portion of the 3' primer (red) and the 3' UTR of ORFX. Transformants are selected on G418 plates, and colony PCR is performed to verify integration of the Kan^r downstream of the desired ORF. Abbreviations: TEF, translational elongation factor; TEFp, TEF promoter; TEFt, TEF terminator: Kan^r, kanamycin resistance; loxp, site for CRE specific homologous recombination (Suter et al, 2007.

Shipping and storage

Plates are shipped on dry ice and should be stored at -80 ˚C.

Strain information

Baits are in the BY4741 (MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0) background strain. Preys are in BY4742 (MATα ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0) background strain.

Table 1. Antibiotic resistance in bait and prey libraries.

Antibiotic	Concentration	Utility
Kanamycin resistance (G418)	200 µg/mL	selection marker

Plate replication

To allow any CO_2 that may have dissolved into the medium from the dry ice in shipping to dissipate, please store plates at -80 ˚C for at least 48 hours before thawing.

Table 2. Materials for replication.

Item	Vendor	Cat #
Yeast Extract, 500 g, granulated	VWR	EM1.03753.0500
Peptone, granulated, 2 kg - Difco	VWR	90000-368
Glucose (D(+)-Glucose Monohydrate)	EMD	1.08342.2500
Glycerol	VWR	EM-4760
G418	Calbiochem	345810
96-well microplates	Nunc	260860
Aluminum seals	Nunc	276014
Disposable replicators	Genetix	X5054

*** When preparing medium for yeast clones, do not add the glycerol to the medium until after the clones have grown. Glycerol inhibits the growth of yeast.**

YPD medium 1 liter

YPD mix:

Yeast extract	10 g
Peptone	20 g
dH_2O	900 mL

Autoclave mixture for 20 minute at 121 °C

Glucose/dH_2O mix:

Dextrose	20 g
dH_2O	100 mL

Shake until in solution and autoclave for 20 minutes at 121 °C. Under hood, add YPD mix to 100 mL of sterile Glucose/dH_2O mix.

Replication

Prepare target plates

1. Prepare 96-well target plates by dispensing 150 µL of YPD + G418 (200 µg/mL) into each well.

Prepare source plates

1. Remove the lids and the aluminum seal from the source plates. Removing the seals while the source plates are frozen will minimize cross-contamination.
2. Allow the source plates to thaw completely with the lids on. Wipe any condensation that may appear under the lids with ethanol and an absorbent wipe.

Replicate

1. Gently place a sterile, disposable replicating tool into the source plate and lightly mix the yeast cells. Make sure to scrape the bottom of each well thoroughly ensuring maximum transfer of cells.
2. Gently remove the replicating tool from the source plate and gently insert the tool into the target plate. Mix the replicating tool around in the target plate.
3. Dispose of the plastic replicating tool.
4. Replace the lid of the target plate and the source plate.
5. Repeat steps 1-6 until all plates have been replicated.
6. Return the source plates to an ultralow freezer. Do not seal the plates until they are completely frozen. This will help ensure that the plates do not become cross-contaminated.
7. Place the target plates in a 30 °C incubator for at least 48 hours.
8. When sufficient growth has been noted in the target plates, add 65 µL of a sterile 50/50 mixture of glycerol and YPD to each well and mix. This will bring the total glycerol percentage in each well to 15%.
9. Place the target plates in an ultralow freezer. Do not seal the plates until they are completely frozen. This will help ensure that the plates do not become cross-contaminated.

Bait and prey strains in MATa and MATα backgrounds are both marked with G418 resistance. Growing them in 200 µg/mL G418 in YPD medium is recommended since it prevents some frequent contaminations. However, growth on YPD is also fine because the resistance is in the genome.

Obtaining clone information

Individual clone information and plate locations can be found on the USB that accompanies the purchased collection. Alternatively, the datasheet can be downloaded from the Yeast Cross and Capture product page at dharmacon.horizondiscovery.com.

Additional information

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Reference

1. Examining protein–protein interactions using endogenously tagged yeast arrays: The Cross-and-Capture system. Bernhard Suter, Michael J. Fetchko, Ralph Imhof, Christopher I. Graham, Ingrid Stoffel-Studer, Caroline Zbinden, Maanasa Raghavan, Lianet Lopez, Lucija Beneti, Jacqueline Hort, Jeffrey Fillingham, Jack F. Greenblatt, Guri Giaever, Corey Nislow, and Igor Stagljar. *Genome Res.* 17:1774-1782, 2007.

FAQS/troubleshooting

For answers to questions that are not addressed here, please email technical support ts.dharmacon@horizondiscovery.com with your question, your sales order or purchase order number and the catalog number or clone ID of the construct or collection with which you are having trouble.

If you have any questions, contact

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