



**Molecular Medicine
and Genetics**

Wayne State University Yeast Two Hybrid Service

2 February 2006

Dear [REDACTED]:

The following is an explanation of the results from the two-hybrid screen we performed for you.

We performed the screen as described on our web site (http://proteome.wayne.edu/2hybrid_fc.html).

Each screen (combination of a particular bait and library) was assigned a Project Number (e.g., MPCproj72). Some of the details for each project are shown in the Excel sheet, [REDACTED]Projects.xls .

The activation domain libraries that we screened, "Normal Human Prostate" and "Human Prostate Tumor", are described on our website above.

After mating your bait strain with the library we picked colonies and determined the galactose-dependence of the leu and lacZ phenotypes. For all gal-dependent clones, we isolated the ORF by PCR and re-cloned it into the AD vector by gap repair in yeast. We then mated against your bait strain to demonstrate that the leu and lacZ phenotypes were dependent on the ORF. If the interaction was detected, we considered this a "repeated" phenotype. Those that repeated are listed in the Excel sheet named "**All_interactors**".

We then digested the ORFs and assigned them to restriction fragment classes. We then took a representative from each restriction class and performed a specificity test by mating it to 7 bait strains expressing unrelated baits (in this case, the baits were Campylobacter proteins known to interact in two-hybrid assays). The column labeled "**nonspecific baits tested**" shows the number of nonspecific baits tested (7) and the number that interacted with each AD clone is listed under the column labeled "**nonspecific baits interacting**".

We determined the 5' end sequence of all AD clones with a specific interaction. The sequences may be found in the file "MPCY62sequencestrimmed.txt". These sequences have had vector sequence trimmed to the *EcoRI* ORF insertion site.

We used the sequences to perform a BLAST search against Genbank. This identified very high probability matches with human genes, designated by the Genbank ID.

The “**Times isolated**” column refers to the number of times a particular transcript was isolated with a particular bait.

At the same time as the specificity test was performed, we re-tested the original bait used to isolate each clone. From this test we derived reporter activation scores. Our scoring system uses a 0-3 scale for growth –leu plates (0= no growth; 3= maximal growth) and a 0-5 scale for lacZ activity on X-Gal plates (0=white; 5=dark blue). Note that absence of lacZ activity indicates a weak, but reproducible interaction.

The specificity test is shown in the image file MPCY62.jpg. Each column (1-12) is a different AD clone. The top row (A) was mated with the original bait strain used to isolate each clone. The next 7 rows (B-H) were mated with 7 unrelated baits. The plate on the left is an image of the “A” row of interactors and the partial plate on the right is from the “B” row. For example, the sixth column corresponds to the phenotype of clone A6.

Please note that the screen of the Normal Human Prostate library identified only non-specific interactions. The Prostate Tumor screen revealed three interactions, however one of these interactions was with a fusion that was out of frame relative to the Genbank entry.

If you have any questions please feel free to contact us.

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