

Where to Start when testing out the omega-based B1H selection system:

The best place to start is to streak the selection strain out on tetracycline/Zeoicin (50 ug/ml) containing media and make competent cells and a glycerol stock. You will also want to make NM media for doing some positive control experiments. The recipe for the NM media along with plasmid maps, etc. is present on-line.

3-AT can be dissolved to a stock concentration of 1M in ddw for the positive selections. To try the system in your hands I would recommend transforming the 1352 omega-Zif268 plasmid (amp) and either the pH3U3-mcs (kan) or the pH3U3-zif268 (kan) plasmids into the strain. Once you have cells containing both of these sets of plasmids, you will want to grow the cells from a single colony to an OD (600) ~ 0.2 in rich media with Kan and Amp (5 ml culture) and then pellet the cells by centrifugation. Remove the excess media and resuspend your cells in NM media containing 0.1% histidine, 0.2 mM uracil with Kan and Amp. Grow at 37°C for 2hrs and then pellet 1 ml of the cells in a microfuge. Remove the excess media. Resuspend the cells in 1 ml ddw and pellet the cells again. Repeat this wash two more times. Then resuspend the cells in NM media lacking histidine and make 10 fold dilutions of the cells in the same NM media. Spot 5 ul of each dilution on an NM plate with Kan and Amp and 10 uM IPTG also containing either

0.1% histidine & 0.2 mM uracil,
no histidine & 0.2 mM uracil,
no histidine & 0.2 mM uracil + 1 mM 3-AT,
no histidine & 0.2 mM uracil + 3 mM 3-AT,
no histidine & 0.2 mM uracil + 5 mM 3-AT,
no histidine & 0.2 mM uracil + 10 mM 3-AT
also one plate with rich media (Kan/Amp) as a control for the number of cells plated.

Only the cells containing the pH3U3-zif268 reporter should survive the selection conditions (3-AT). Both sets of cells should grow on His+ plates, and there should be intermediate growth of the pH3U3-MCS strain in the absence of histidine.

An alternate option is that you transform the electrocompetent cells of the strain with 1352 omega-Zif268 plasmid and pH3U3-Zif268 or pH3U3-MCS plasmid simultaneously and recover the cells in SOC for 1 hour. Then pellet the cells and resuspend the cells in NM medium containing Amp(100 ug/ml) and Kan (25 ug/ml), 0.1% histidine and 0.2 mM uracil, grow at 37°C for 2 hour. Following the washing and titering steps as above.

If this control looks good, then you are ready to attempt a selection. For each selection you cotransform 100 ng of your 1352 expression plasmid and 1 ug of the pH3U3 target site into electrocompetent US0 cells (selection strain). We

sometimes run a control using the 1352-omega-odd^{ori} with the pH3U3 target site so that there is information about the background level of colonies at each selection stringency. When we do a selection histidine is omitted from the selective media, if desired uracil can also be omitted to increase the stringency.

More info on the omega system can be found in (Noyes, M.B., Meng, X., Wakabayashi, A., Sinha, S., Brodsky, M.H.; Wolfe, S.A. "A systematic characterization of factors that regulate *Drosophila* segmentation via a bacterial one-hybrid system" *Nucleic Acids Research*, 2008, **36**, 2547-2560) as well as the Meng et al Nat. Biotech paper. Please let us know if you encounter any problems with the reagents.

Please don't hesitate to ask if you have any questions about these components.