There should be 5 plasmids and one stab of the *hisBpyrF* selection strain (which contains the F’ episome (TetR) from XL1-blue cells) that comprise the system available from addgene. The five plasmids are:

pB1H1 : chloramphenicol resistance plasmid containing the RNAP alpha subunit and a MCS occupied by the Dorsal gene. This plasmid is the primary expression vector that was used in our studies

pB1H1-Zif268 : pB1H1 containing Zif268 as an alpha fusion. This expression plasmid can be used as a positive control to test out the system in your hands.

pB1H2 : ampicillin resistance plasmid containing the RNAP alpha subunit and a MCS occupied by the Dorsal gene. This plasmid is the secondary expression vector that was used in selections of the Runt/Bgb heterodimer.

pH3U3-MCS kanamycin resistance plasmid containing the HIS3/URA3 co-cistronic reporter with a MCS upstream of the weak promoter controlling the reporter genes.

pH3U3-Zif268 kanamycin resistance plasmid containing the HIS3/URA3 co-cistronic reporter with a Zif268 binding site upstream of the weak promoter controlling the reporter genes. This expression plasmid can be used as a positive control to test out the system in your hands.

The best place to start is to streak the strain out on tetracycline 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. will be sent by e-mail.

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 pB1H1-Zif268 plasmid (chlor) 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 Chlor (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 with Kan and Chlor. 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 the wash one more time. Then resuspend the cells 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 Chlor and 10 uM IPTG also containing either

0.1% histidine,

no histidine,

no histidine + 1 mM 3-AT,

no histidine + 3 mM 3-AT,

no histidine + 5 mM 3-AT,

no histidine + 10 mM 3-AT

also one plate with rich media (Kan/Chlor) 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 stain in the absence of histidine.

Another alternate option is that you transform the electrocompetent cells of the strain with pB1H1 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 Chlor(30 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 tittering steps as above.

If this control looks good, then you are ready to attempt a selection.

A detailed protocol for the system can be found at Nature Protocols 1(1), p30, 2006.

Please let me know if you have any concerns,

 Scot A. Wolfe