List of recommended plasmids for the ZFN selection using the B1H system. These are available from addgene (www.addgene.org) under "Scott Wolfe Lab". We also have an on-line tool for finding target sites and designing libraries at (http://pgfe.umassmed.edu/ZFPsearch.html). You will also need to get the US0*ΔhisBΔpyrFΔrpoZ:Zeo* selection strain (**Plasmid 18049;** which contains the F' episome (Tet^R) from XL1-blue cells).

Plasmid Nomemclature:

1352 UV2 omega-Zif268 (Plasmid 18045): 1352 expression plasmid containing Zif268 as an omega fusion. This expression plasmid can be used as a positive control to test out the system in your hands.

1352 UV2 omega-odd^{ori} (Plasmid 18044): 1352 expression plasmid containing only omega. This expression plasmid can be used as a negative control for experiments in the absence of a TF.

pH3U3-MCS (Plasmid 12609) kanamycin resistance plasmid containing the HIS3/URA3 co-cistronic reporter with a MCS upstream of the weak promoter controlling the reporter genes. Binding sites should be placed 10 bp upstream of the -35 box to select ZFPs. See pH3U3-Zif268 map for example.

pH3U3-Zif268 (Plasmid 18046) kanamycin resistance plasmid containing the HIS3/URA3 co-cistronic reporter with a Zif268 binding site (**GCGTGGGCG**) 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.

1352wUV2 F1bbs kan GCGTGGNNN (Plasmid 18753) 1352-based vector for building finger 1 libraries between the bbs1 sites. There is a kanomycin cassette between the bbs1 sites to facilitate cloning. The anchor fingers recognize GCGTGG, where your subsite is represented by NNN. Details of the library oligos are in the supplementary data for our paper.

1352wUV2 F2bbs kan GCANNNAGGk (Plasmid 18752) 1352-based vector for building finger 2 libraries between the bbs1 sites. There is a kanomycin cassette between the bbs1 sites to facilitate cloning. The anchor fingers recognize GCANNNAGGk, where your subsite is

represented by NNN. Details of the library oligos are in the supplementary data for our paper.

1352wUV2 F3bbs kan NNNTGGGCGk (Plasmid 18756) 1352-based vector for building finger 3 libraries between the bbs1 sites. There is a kanomycin cassette between the bbs1 sites to facilitate cloning. The anchor fingers recognize TGGGCGk, where your subsite is represented by NNN. Details of the library oligos are in the supplementary data for our paper.

pCS2-HA-GAAZFP-FokIRR (Plasmid 18754) pCS2-based vector containing Exon 2 *krdl* fingers with the RR version of the foci endonuclease domain. The Zinc fingers are cloned in frame between the KpnI and BamHI sites. This should serve as a control for functional ZFNs as the lesion frequency generated by these fingers is 10 to 20% at the optimal dose.

pCS2-FLAG-TTGZFP-FokIDD (Plasmid 18755) pCS2-based vector containing Exon 2 *krdl* fingers with the DD version of the foci endonuclease domain. The Zinc fingers are cloned in frame between the KpnI and BamHI sites. This should serve as a control for functional ZFNs as the lesion frequency generated by these fingers is 10 to 20% at the optimal dose.

UV2 = low expression promoter for omega-ZFP selections

The kan gene in the F1/2/3 bbs plasmids generates a 1.2 kb dropout when the plasmids are digested with *BbsI*. This simplified the purification of double-cut backbone DNA for the ligation of the finger libraries into each plasmid.

There are also 3 control plasmids:

1352 UV2 omega-Zif268 and **pH3U3-Zif268** (reporter vector) that provide a complementary pair of constructs for testing the activity of the system. We have also included a negative control **1352-omega-oddori** that does not express a DNA-binding domain fused to omega.

We have also included the pH3U3-MCS, this is a reporter vector with a multiple cloning site that can be used for introducing sites of interest to test, or for building your own binding site libraries if you are interested.

Where to Start:

The best place to start is to streak the strain out on tetracycline/Zeocin (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. 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 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 the wash two more times. 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 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 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 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 tittering 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 el at 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.