List of recommended plasmids for the ZFN selection using the B1H system. These are available from addgene (www.addgene.org) under “Scot Wolfe Lab”. We also have on-line tools for finding target sites and designing libraries at (http://pgfe.umassmed.edu/ZFPSearch.html). You will also need to get the US0ΔhisBΔpyrFΔrpoZ:Zeo selection strain (Plasmid 18049; which contains the F’ episome (Tet^R) from XL1-blue cells).

Plasmid Nomenclature:

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 GCANNAGGk (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 GCANNAGGk, 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 krndl 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 krndl 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 for selecting zinc finger proteins with novel specificity, or for building your own binding site libraries if you are interested. The on-line tools will aid in the design of the zinc finger libraries as well as the appropriate positioning of the binding sites in the reporter vector for the selections.