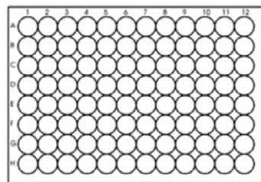


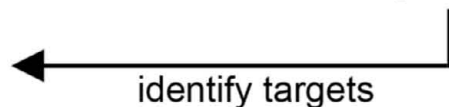
Assemble ZFP archive

- collection of ZFPs recognizing 27 triplet sequences in each of 3 finger positions



Construct target database

- database of ZFN targets in the zebrafish genome (Zv7)
- assembled using our corresponding archive



construct 3-finger ZFP cassettes by overlapping PCR

subclone into p1352

Determine binding specificity

- bacterial 1-hybrid analysis of ZFPs

subclone into pCS2-EL/KK

Determine in vivo activity

- inject ZFN mRNAs into 1-cell zebrafish embryos
- assess somatic lesion frequency

Assess Germline Transmission

- identify founder fish bearing mutagenic alleles for selected ZFN targets