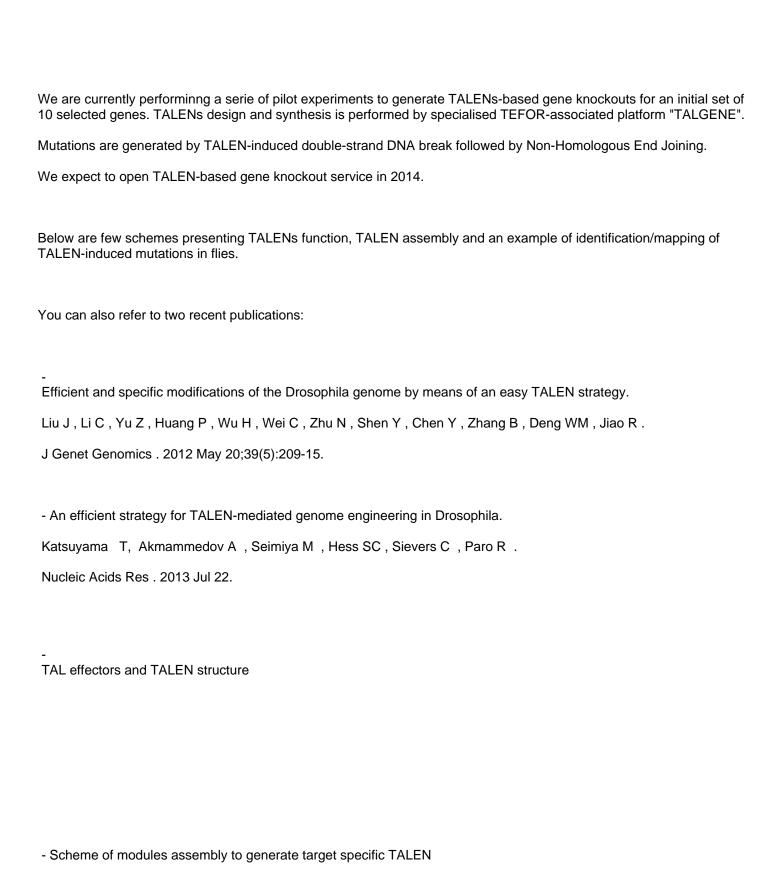
## TALEN-based Drosophila gene knock-outs



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- Synthesize 8 gene sets ea	ch with 4 core RVD-coding	modules and bearing 5'	and 3' termini as	dictated by the	table to
the right					

- Digest with BsmB1 to generate unique 5' and 3' overhangs with single nucleotide polymorphisms (as dictated by table to the right)
- Select a 16 (or 24) bp DNA recognition site

(e.g., AGGTACTCGAATCCTG)

- For 1st 8-mer repeat, pick an NI from set 1, an NN from set 2, an NN from set 3, etc.. to create a pool of 8 single-repeat genes
- Anneal and ligate the 8 single-repeat genes into a predetermined order to produced the desired 8-mer

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- Pooled ligation of 8-mer arrays
- Cloning into the pAvrAa7-FN scaffold plasmid
- Generation of dTALEN recognizing 16 (or 24) bp target site
- Scheme of TALEN based gene knockout procedure in Drosophila
- Injection of TALEN mRNAs into wild type Drosophila embryos
- Enzyme digestion to screen for mosaic F0
- Enzyme digestion and sequencing to identify expected mutants

- Establishing stocks

- Genotyping - testing TALENS in vivo by identification of somatic mosaics
Yellow mosaics observed in F0
TALENs against yellow gene
Positioning of TALENs target sequences around an unique restriction site.
Positioning of TALENs target sequences around an unique restriction site.  Genotyping FO mosaics by identification of incomplete digestion of PCR products encompassing mutation site

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