TALEN-based Drosophila gene knock-outs

We are currently performinng a serie of pilot experiments to generate TALENs-based gene knockouts for an initial set of 10 selected genes. TALENs design and synthesis is performed by specialised TEFOR-associated platform "TALGENE".

Mutations are generated by TALEN-induced double-strand DNA break followed by Non-Homologous End Joining.

We expect to open TALEN-based gene knockout service in 2014.

Below are few schemes presenting TALENs function, TALEN assembly and an example of identification/mapping of TALEN-induced mutations in flies.

You can also refer to two recent publications:

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Efficient and specific modifications of the Drosophila genome by means of an easy TALEN strategy.

Liu J , Li C , Yu Z , Huang P , Wu H , Wei C , Zhu N , Shen Y , Chen Y , Zhang B , Deng WM , Jiao R .

J Genet Genomics . 2012 May 20;39(5):209-15.

- An efficient strategy for TALEN-mediated genome engineering in Drosophila.

Katsuyama T, Akmammedov A , Seimiya M , Hess SC , Sievers C , Paro R .

Nucleic Acids Res . 2013 Jul 22.

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TAL effectors and TALEN structure

- Scheme of modules assembly to generate target specific TALEN

- Synthesize 8 gene sets each 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

- 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.

Genotyping FO mosaics by identification of incomplete digestion of PCR products encompassing mutation site