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Introducing a targeted mutation in the *Cyclin G* gene of *Drosophila melanogaster* by developing a specific zinc finger nuclease



Colloque Zinc Finger Nuclease
Saint-Raphaël
6-8 octobre 2010

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I - INTRODUCTION

Understanding the role of *Drosophila melanogaster* Cyclin G

Different results show the involvement of Cyclin G in:

- Transcriptional regulation (Salvaing et al., 2008)
- Growth control and cell proliferation (F. Faradji et al., in preparation).

• We have got different strains allowing *Cyclin G* (*CycG*) overexpression (S. Boyer, unpublished), or inactivation by RNAi (Salvaing et al., 2008) but no mutant has been obtained till now.

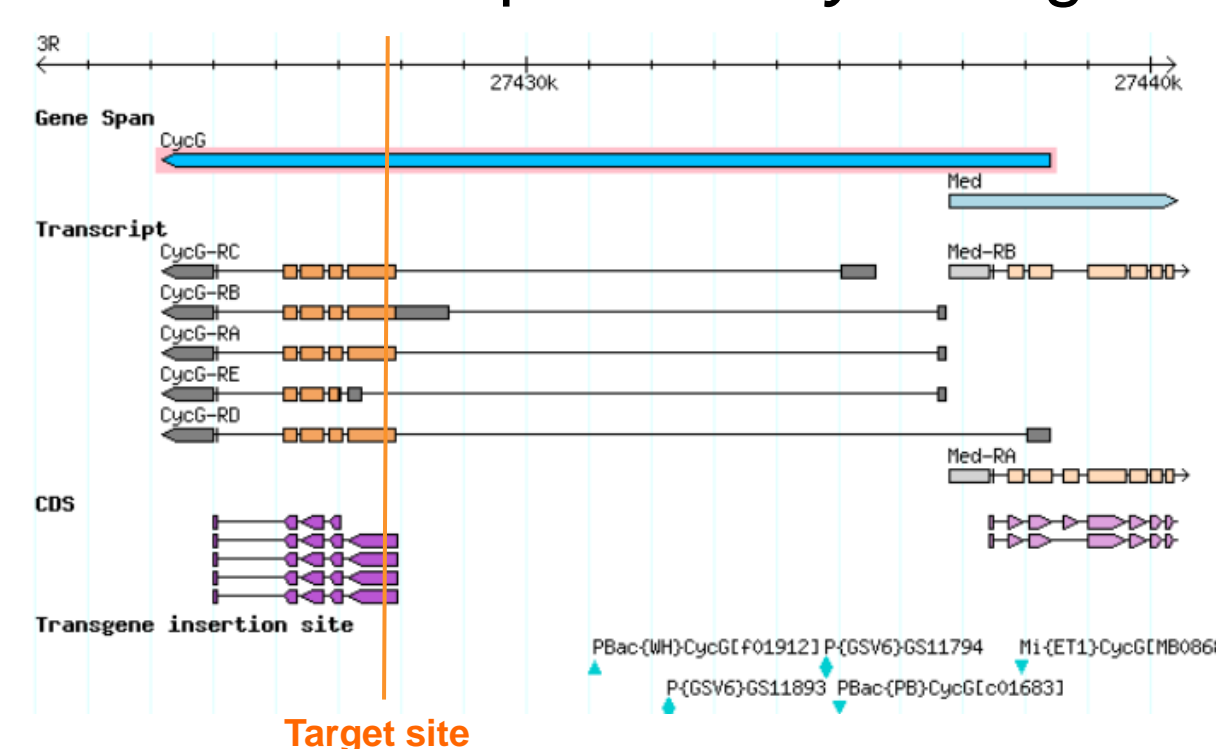
II - Materials and Methods

Make custom zinc finger nucleases (ZFN)

1) Search for sequences that could be ZFN target sites in proper position within the *CycG* gene:

- Carlos Barbas'lab = <http://zincfingertools.org>
- ZiFDB = <http://bindr.gdcb.iastate.edu/ZiFDB>

Genomic map of the *CycG* region



Results for Cyclin G (Zinc finger Tools)

Spans residues (relative to top strand)	Score	Half sites and core sequence
59	58.89	5'-CGC TAC TCC TCTGCT GCC GCC GAA-3'
36	42.00	3'-CGC ATG AGG AGACGA CCG CGG CTT-5'

Results for Cyclin G (ZiFDB)

35 aCGC TAC TCC TCTGCT GCC GCC GAA-3' 60 Right GNN Score: 0.59
35 tCGC ATG AGG AGACGA CCG CGG CTT-5' 60 Left GNN Score: 0.59

A score of 0.59, suggests that the protein has a 59% likelihood of recognizing its target (Ramirez et al. Nature Methods 2008)

Check by BLAST that this sequence does not exist elsewhere in the *Drosophila* genome.

2) Amino-acid sequences of the ZFNs

ZFN1 : GAA GCC GCC

proteic sequence:

LEPGEKPYKCEPGKSFSSQSSNLVLRHQRTHHTGEKPYKCEPGKSFSSDCRDLARHQRTHHTGEKPYKCEPGKSFSSDCRDLARHQRTHHTGKKT

ZFN2: GCG GTA GGA

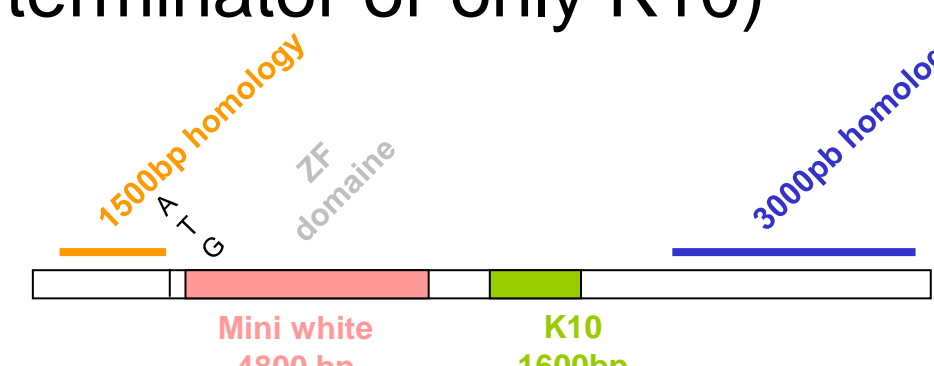
proteic sequence:

LEPGEKPYKCEPGKSFSSRSDDLVRHQRTHHTGEKPYKCEPGKSFSSQSSSLVLRHQRTHHTGEKPYKCEPGKSFSSQRAHLERHQRTHHTGKKT

3) Plasmid constructions

• Insertion of the ZFN cDNA sequences (synthesized by Genecust) into pENTR-NLS-ZFNs then transfer into pCS2-DEST (a kind gift from Pr D. Carroll)

• Donor constructions (complete *mini-white* + K10 transcription terminator or only K10)



4) Preparation of RNA using RiboMax Large Scale RNA, AmpliCap kit (Promega)

5) Injections of RNA plus a donor construction or RNA into *w¹¹¹⁸ lig4¹⁶⁹* or *w¹¹¹⁸ Drosophila* embryos.

III - Results

A- Homologous Recombination (HR)

- RNA (ZFN1+ZFN2) and DNA donor (*miniwhite* + *K10*) were injected into *w¹¹¹⁸ lig4¹⁶⁹ Drosophila* embryos.

• First injections:

nb	Total	% survival
Embryos injected	423	-
Larvae obtained	100	24
Pupae obtained	83	19.3

Injection mix :

- Data from literature : RNA = 250 to 1000 mg/ml
- DNA donor = 0.125 mg/ml to 6 mg/ml
- Chosen conditions of injection: RNA = 0.25mg/ml
- DNA donor = 0.6mg/ml

• Second injections:

	Concentration mg/ml RNAs	Concentration mg/ml Donor DNA	Embryos
A	0.5	1	87
B	0.38	1.5	194
C	0.28	2	200
D	0.25	1.5	250
(future)	1	1.5	200
	1	1	200
	0.65	1.5	200
	0.65	1	200

Screen of offspring for red eyes: no insertion of the donor DNA was obtained on 1154 injected embryos.

B - Non-Homologous End-Joining (NHEJ)

- RNA (ZFN1+ZFN2) were injected into *w¹¹¹⁸ Drosophila* embryos

• Injection conditions:

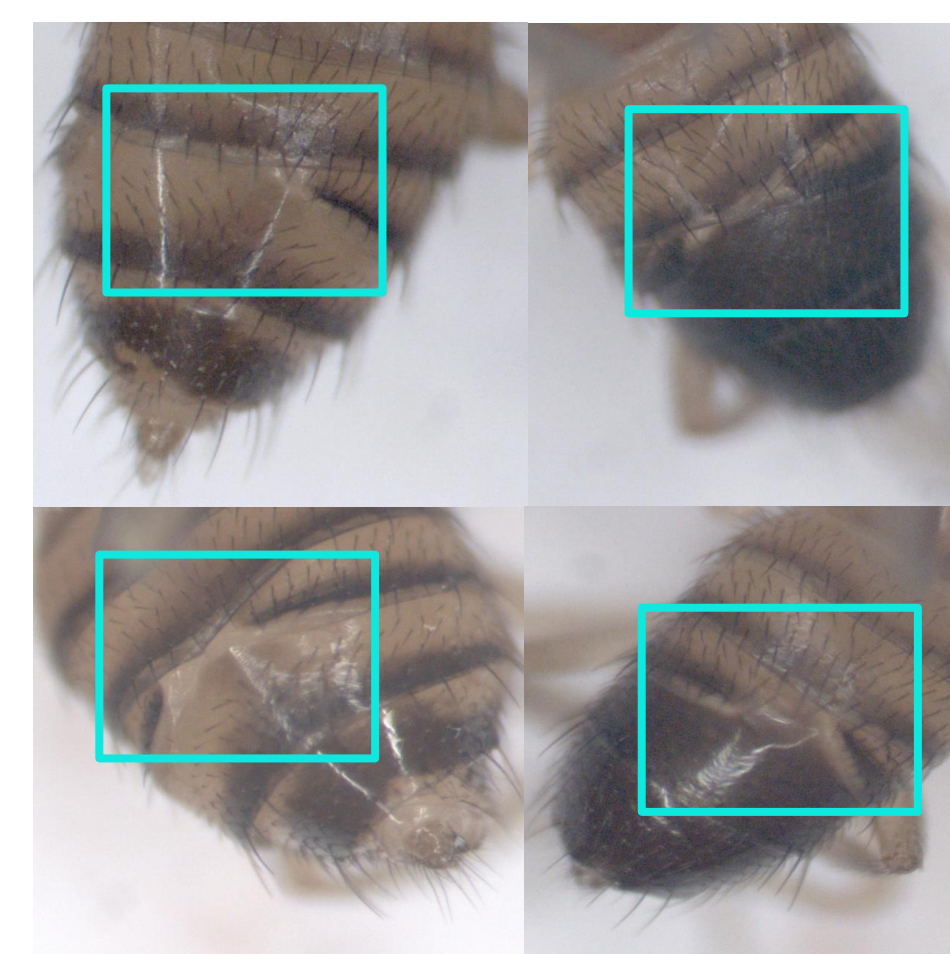
Concentration mg/ml RNAs	Embryos
0.25	152
0.5	147
0.6	123

• Genetic screen of offspring:

- Adults were crossed with a double-balanced strain (*TM2, Ubx/TM6c, Sb*).

- Individuals were crossed with *Df(3R)ED13109/TM6, Sb*, a deficiency that uncovers the *CycG* gene (see part #C).

- Phenotype analysis of the offspring:



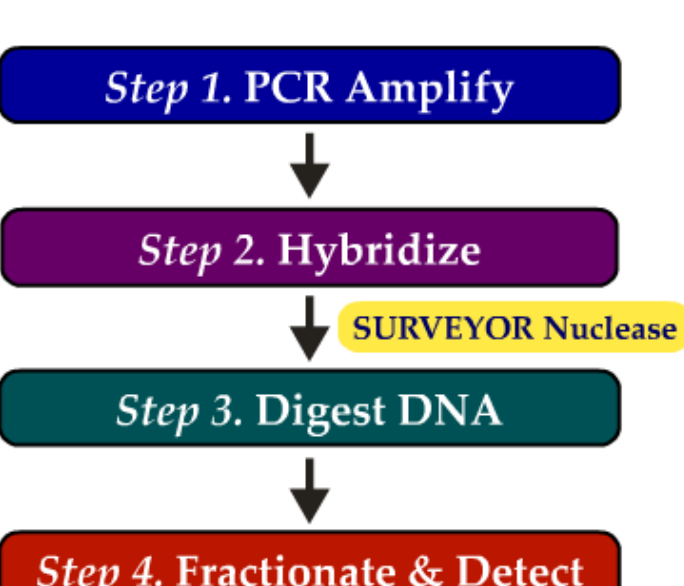
Similar cuticle defects are observed in flies where *CycG* has been inactivated by RNAi.

- Based on phenotype analyses, 11 potential mutants were obtained.

• Molecular analysis of potential mutants:

- Using Surveyor nuclease (Transgenomic)

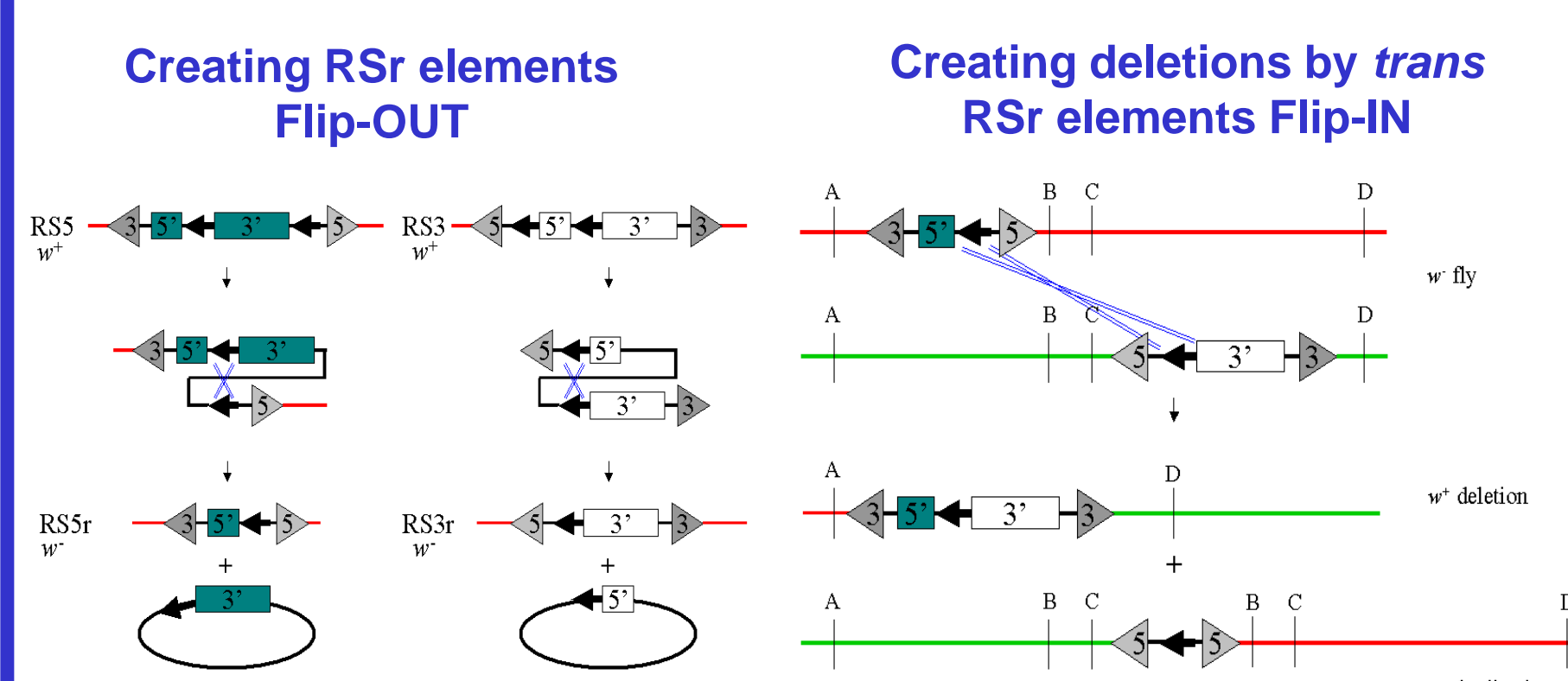
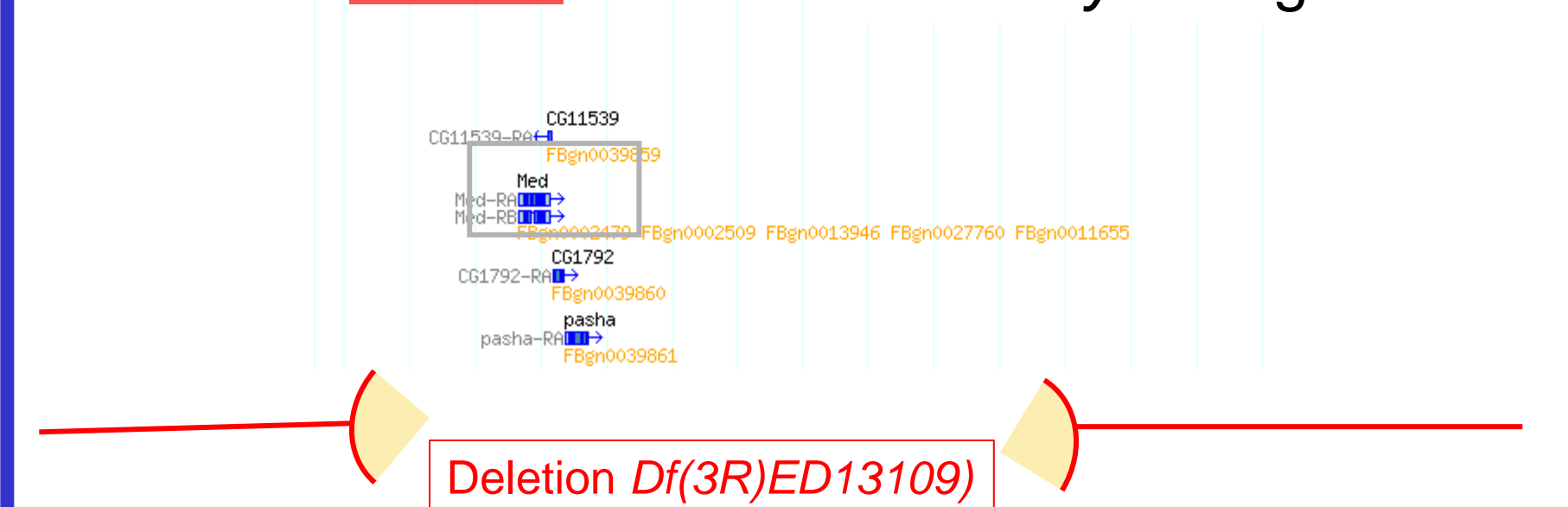
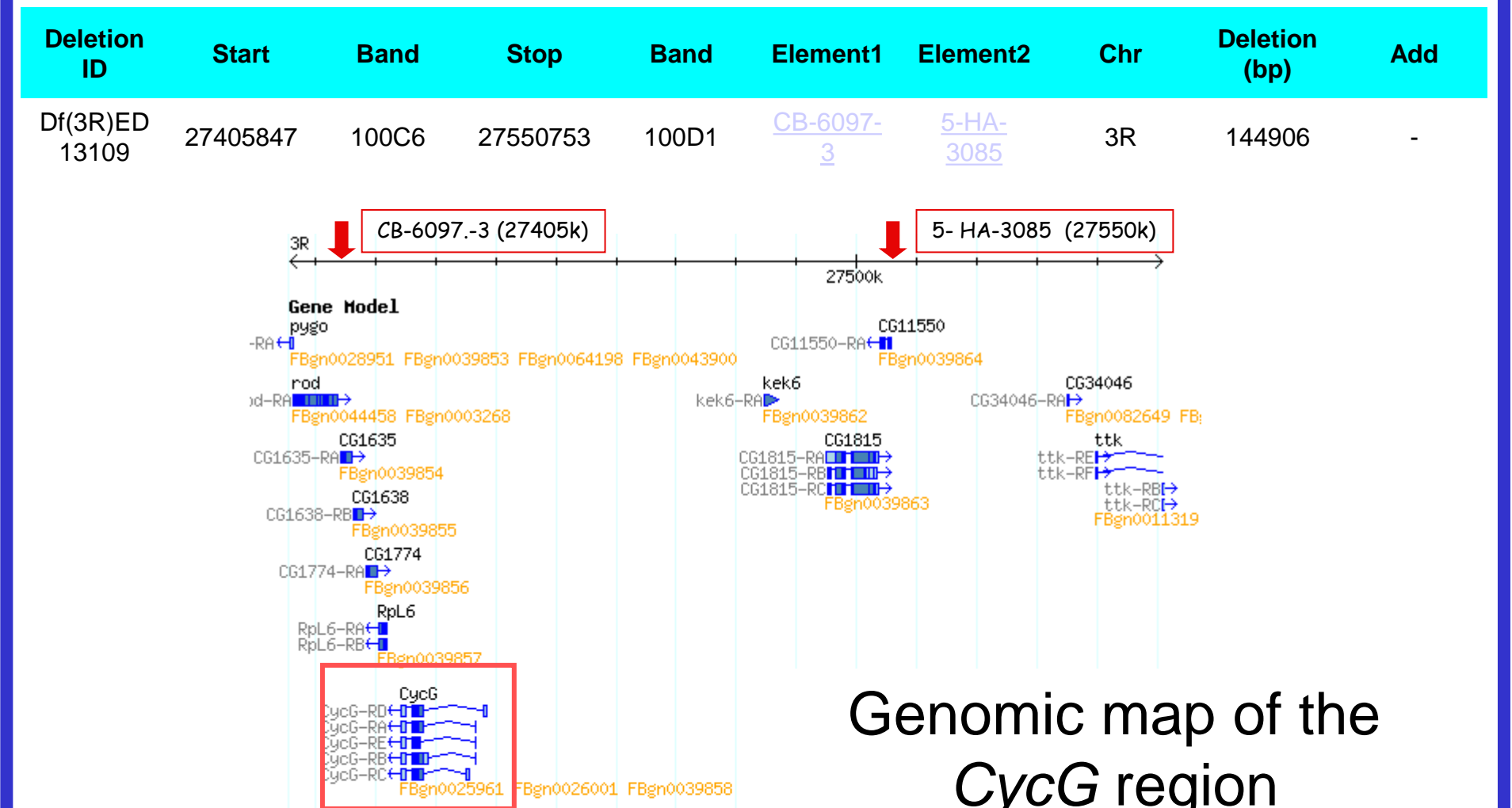
Mutation Detection in Four Straightforward Steps



C - Deletion construction: (DrosDel)

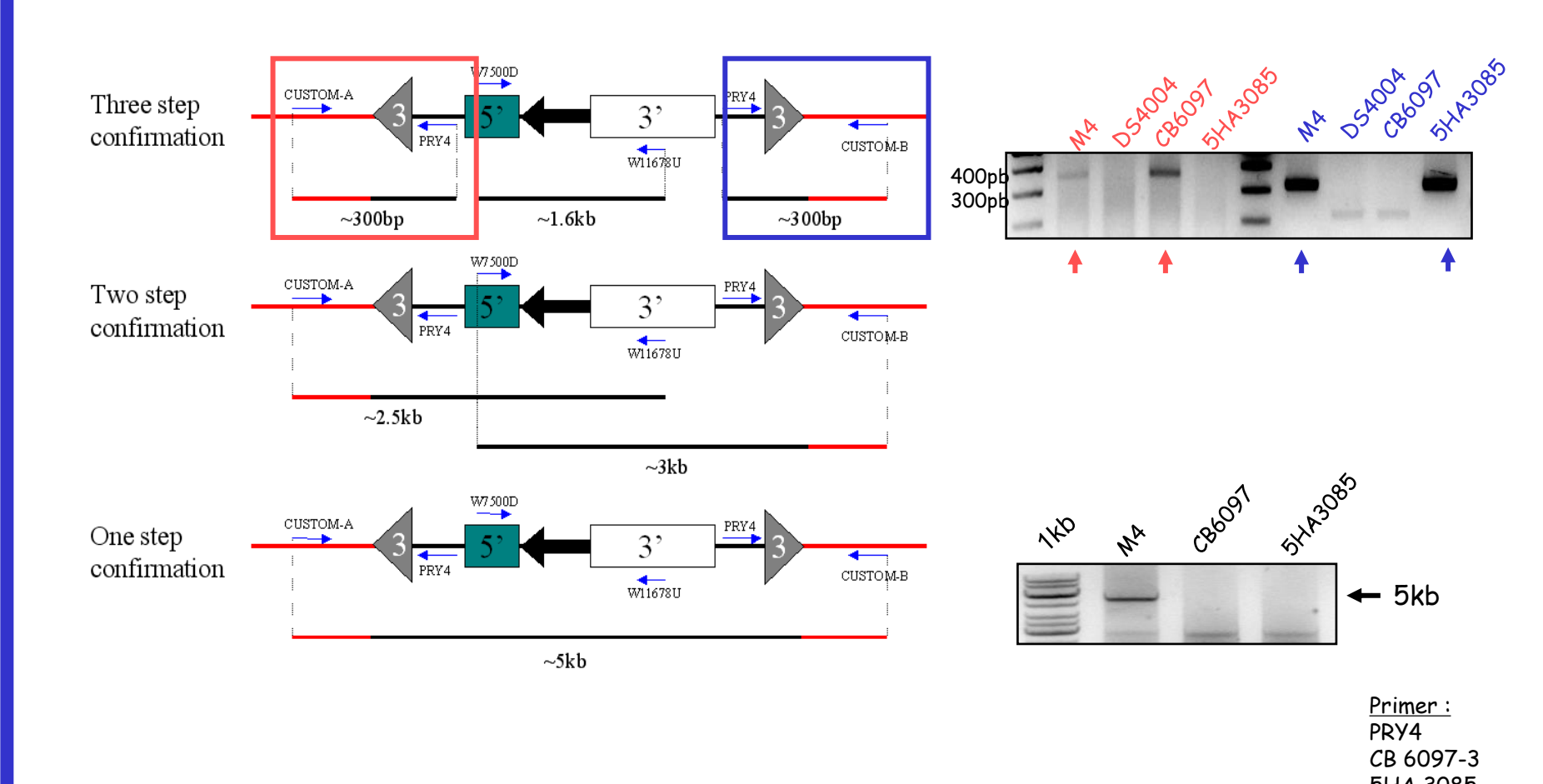
(<http://www.drosdel.org.uk>)

The deletion was constructed using RS P-element (Golic and Golic, 1996)



- Individual crosses with a double-balanced strain *TM2, Ubx/TM6 Sb* were performed and one potential mutant *Df(3R)ED13109* was obtained.

• Testing *Df(3R)ED13109* by genomic PCR:



• Testing *Df(3R)ED13109* by genetics:

- Cross *Df(3R)ED13109* individuals with a mutant allele of *Medea*, an essential gene which is supposed to be contained in the deficiency.
- Check for absence of *Medea* / *Df(3R)ED13109* flies among the offspring.

IV - Future

• Complete the analysis of potential NHEJ mutants with Surveyor nuclease and sequencing.

• Obtain a *CycG* mutant by replacement using the K10 Donor construction.

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