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Effect of co-expression of alpha-synuclein and leucine-rich repeat kinase 2 on the hallmark features of Parkinson's disease in *Drosophila melanogaster*

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Supplementary Data

1. Details of fly stocks procured:

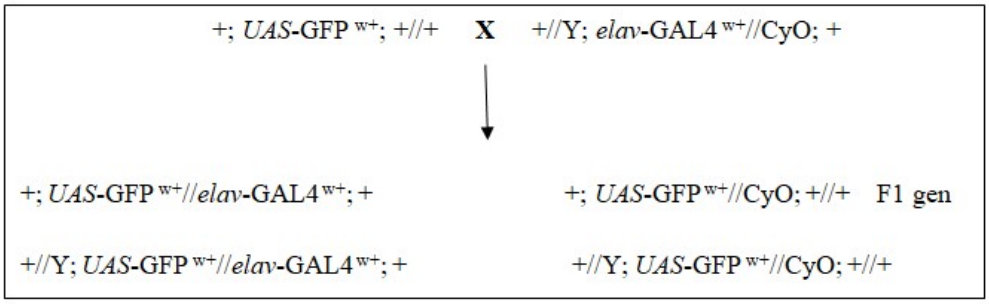
Sr. No.	Fly stocks	Genotype	Description	Source
1	*Elav*-GAL4	*elav-GAL4*;+;+	The fly stock is homozygous for *elav-GAL4* and the gene is located on 1st chromosome. It is specific for the expression of any *UAS* associated target genes in all neurons of the fly brain.	NCBS, Bangalore
2	*Elav*-GAL4	+; *elav-GAL4*//CyO;+	The fly stock is heterozygous and the gene is located on one of the 2nd chromosomes. The curly of oster (*CyO*) gene (more information provided under point 10) is located on the other 2nd chromosome.	Department of Biotechnology, University of Hyderabad
3	*Repo*-GAL4	+; +;*repo-GAL4//TM6,Tb*	The fly stock is heterozygous and the gene is located on one of the 3nd chromosomes. The TM6, Tb gene (more information provided under point 10) is located on the other 3rd chromosome. It is specific for the expression of any *UAS* associated target genes in all glial cells of the fly brain.	IISER, Pune
4	*TH*-GAL4	+; +, *TH-GAL4// TH-GAL4*	The fly stock is homozygous for *TH-GAL4* and the gene is located on the 3rd chromosome. It is specific for the expression of any *UAS* associated target genes in dopaminergic neurons of the fly brain.	NCBS, Bangalore
5	*UAS-wild type SNCA*	*yw*; *UAS-SNCA// UAS-SNCA;+*	The fly stock is homozygous for *UAS-SNCA* and the gene is located on the 2nd chromosome. It is one of the human PD-associated target genes expressed in *Drosophila.* The *y* gene stands for the yellow gene causing the yellow body colour of the flies. The *w* gene stands for the white gene	Department of Biotechnology, University of Hyderabad

			responsible for red eye colour.	
6	*UAS-wild type LRRK2*	+; *UAS-LRRK2// CyOGFP;+*	The fly stock is heterozygous for *UAS-LRRK2* and the gene is located on the 2nd chromosome. It is another of the human PD-associated target genes expressed in *Drosophila.* The *CyO* gene is also associated with the green fluorescent (GFP) gene, which can cause larvae to emit green fluorescence and help in identification (The larvae exhibiting fluorescence will not contain the *LRRK2* gene.)	VIB Centre for Biology and Disease, Leuven, Belgium
7	*UAS-LRRK2-G2019S*	+; *UAS-LRRK2 G2019S//CyOGFP;+*	The fly stock is heterozygous for *UAS-LRRK2 G2019S* and the gene is located on the 2nd chromosome. It is a mutated form of *LRRK2* with elevated kinase activity.	VIB Centre for Biology and Disease
8	*UAS-GFP-S65T*	+; *UAS- GFP-S65T// UAS-GFP-S65T*	The fly stock is homozygous for *UAS-GFP* and the gene is located on the 2nd chromosome. It emits green fluorescence on expression.	IISER, Pune
9	First chromosomal balancer	*FM3//FM7*	*FM* stands for first multiply inverted and the numbers are to identify their place in a series of balancers that have been created. This chromosome carries a marker mutation of *Bar* eyes which reduces the eyes of flies into slits.	Department of Biotechnology, University of Hyderabad
10	Second chromosomal balancer	*Pin//CyO*	*Pin* is a marker mutation on the second balancer chromosome responsible for needle-like bristles *CyO* is another marker that causes the curling of wings	Department of Biotechnology, University of Hyderabad
11	Third chromosomal balancer	*TM3//Sb*	*TM* stands for third multiply inverted and the numbers are to identify their place in a series of balancers that have been created. This chromosome carries a marker mutation of *Sb* (stubble) responsible for short blunt bristles.	IISER, Pune
12	Double chromosomal balancer for first and second chromosomes	*FM7i//Y; Tfd//CyOGFP*; +	It's a combination of marker mutations on the first and second chromosomes. *Tfd* represents tufted thoracic bristles	Department of Biotechnology, University of Hyderabad
13	Double chromosomal balancer for second and third chromosomes	+//Y; *If // CyO*; *MKRS // TM6, Tb*	It's a combination of marker mutations on the second and third chromosomes. *If the* gene causes small slit-shaped eyes with fused ommatidia *Tfd* gene causes tufted thoracic bristles *MKRS* gene causes stubble-like bristles	Department of Biotechnology, University of Hyderabad

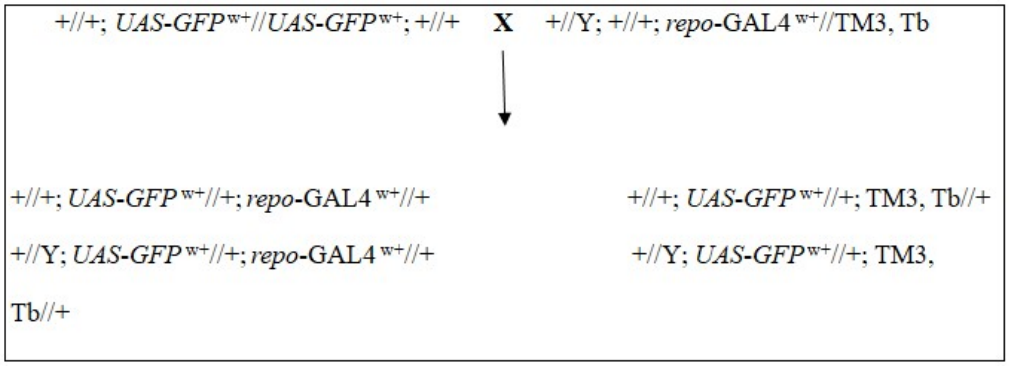
NCBS: National Centre for Biological Sciences
IISER: Indian Institute for Scientific Education and Research
VIB Centre for Biology and Disease, Centre for Human Genetics, Laboratory of Neuronal Communication, University of Leuven, Belgium

2. Crosses set up for the generation of experimental flies:
2.1 Validation of GAL4 lines:

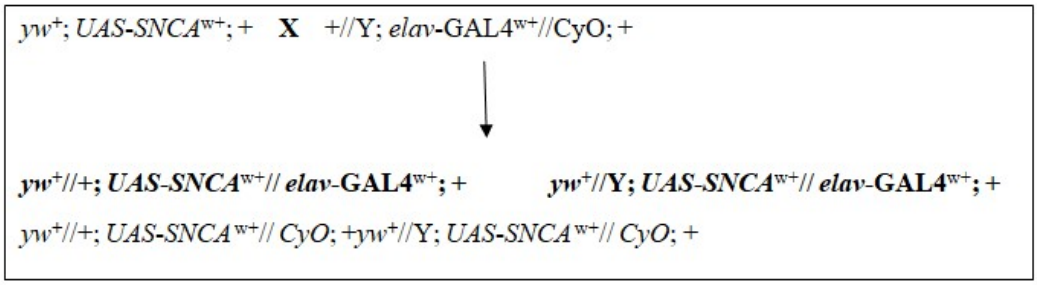
Cross 1: *UAS-GFP* females crossed with *elav-GAL4* males for validation of *elav-Gal4* stock:



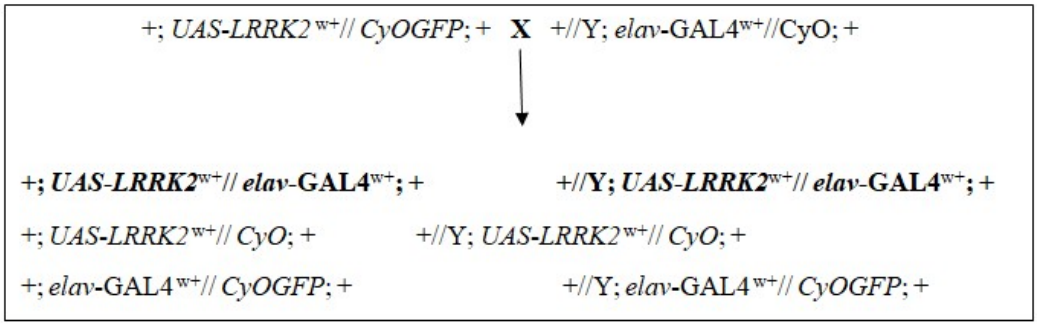
Cross 2: *UAS-GFP* females crossed with *repo*-GAL4males for validation of *repo-Gal4* stock:



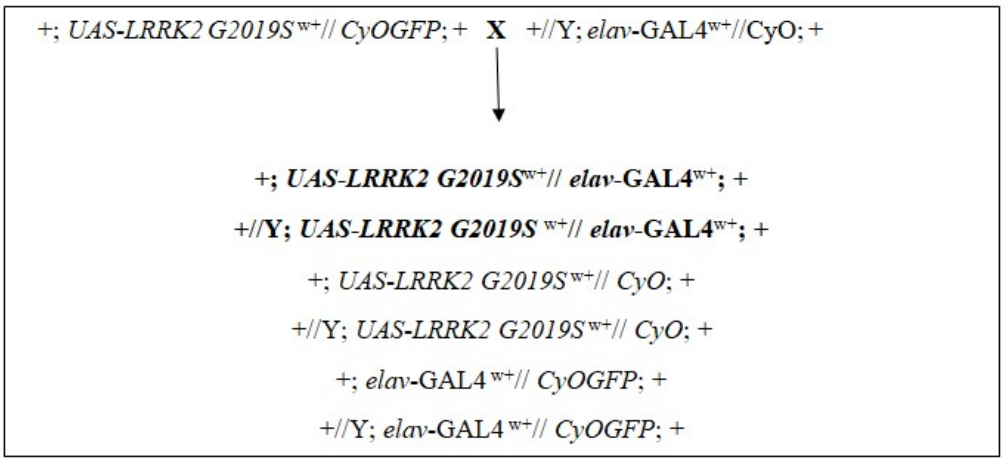
2.2 Generation of fly lines individually expressing *SNCA, LRRK2,*and *LRRK2-G2019*in cell subtypes:
Cross 3: *UAS-SNCA* females crossed with *elav*-GAL4males for generation of *UAS-SNCA// elav*-GAL4flies:



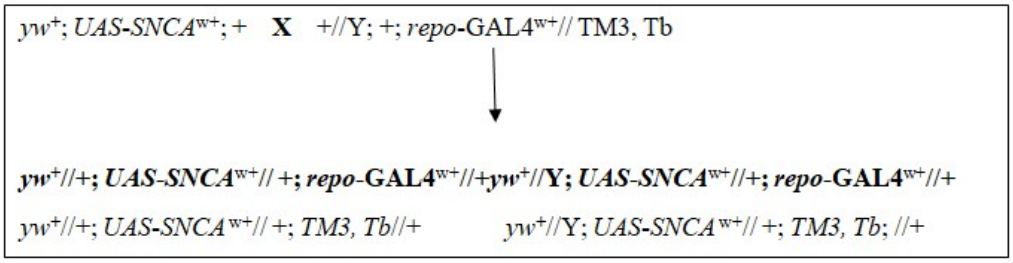
Cross 4: *UAS-LRRK2* females crossed with *elav*-GAL4malesfor generation of *UAS-LRRK2*// *elav*-GAL4flies:



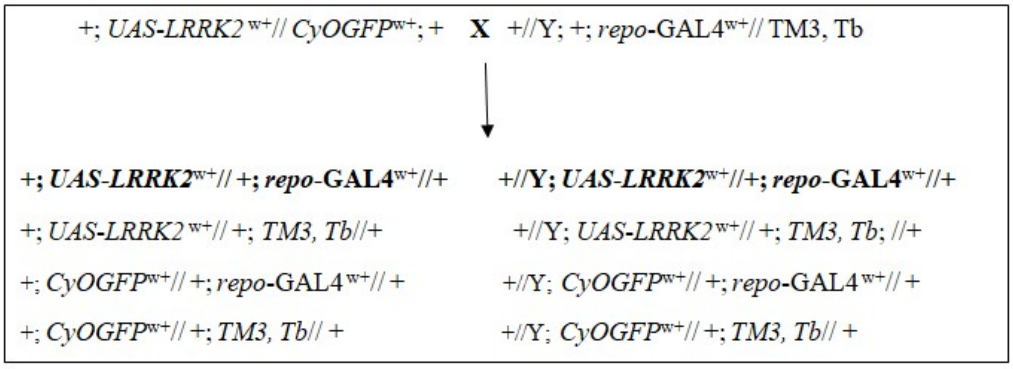
Cross 5: *UAS-LRRK2 G2019S* females crossed with *elav*-GAL4males for generation of *UAS-LRRK2-G2019S*// *elav*-GAL4flies:



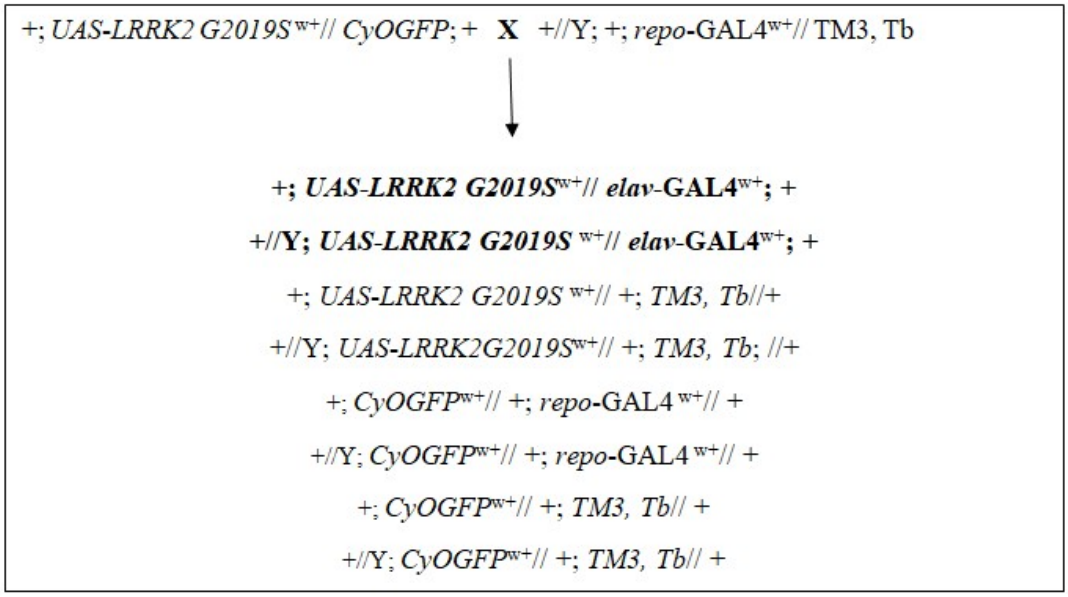
Cross 6: *UAS-SNCA* females crossed with *repo*-GAL4males for generation of *UAS-SNCA*// *repo*-GAL4flies:



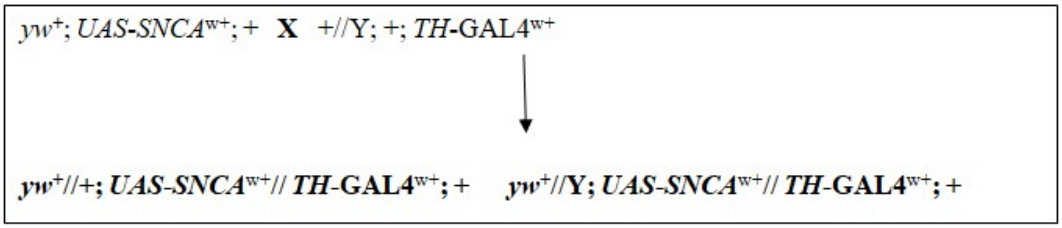
Cross 7: *UAS-LRRK2* females crossed with *repo*-GAL4males for generation of *UAS-LRRK2*// *repo*-GAL4flies:



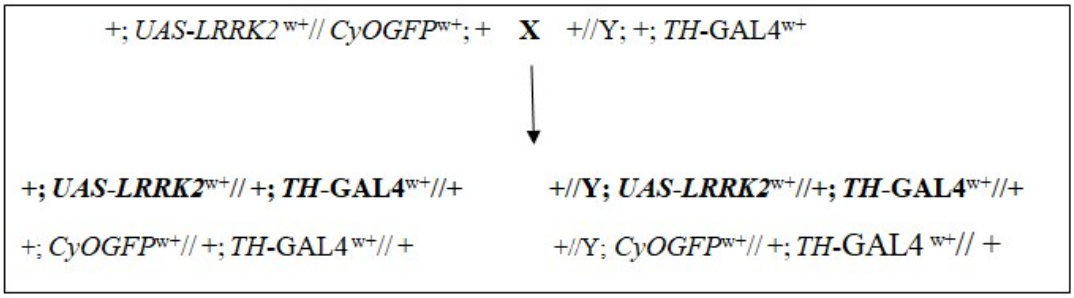
Cross 8: *UAS-LRRK2 G2019S* females crossed with *repo*-GAL4males for generation of *UAS-LRRK2-G2019S*// *repo*-GAL4flies:



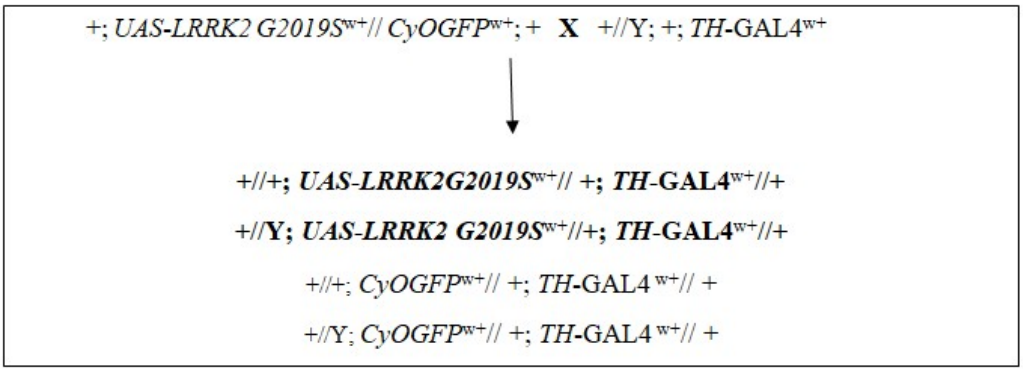
Cross 9: *UAS-SNCA* females crossed with *TH*-GAL4males for generation of *UAS-SNCA*// *TH*-GAL4flies:



Cross 10: *UAS-LRRK2* females crossed with *TH*-GAL4males for generation of *UAS-LRRK2*// *TH*-GAL4flies:

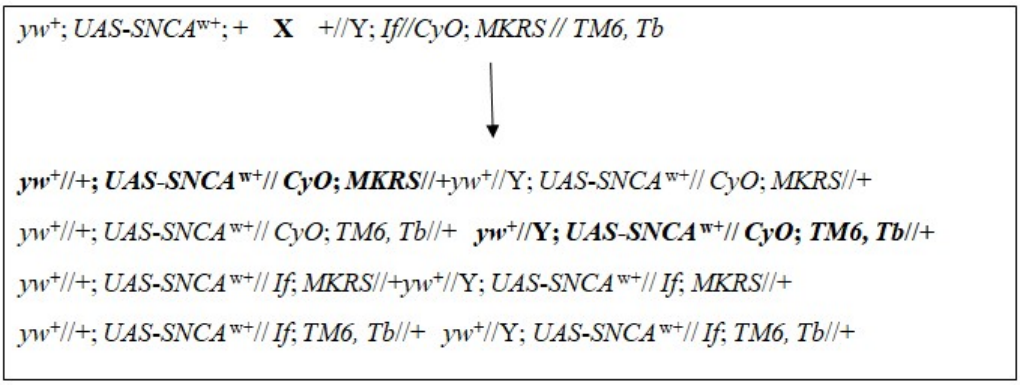


Cross 11: *UAS-LRRK2 G2019S* females crossed with *TH*-GAL4males for generation of *UAS-LRRK2-G2019S*// *TH*-GAL4flies:



2.3 Generation of fly lines co-expressing *SNCA* with *LRRK2* and *G2019S*in cell subtypes:
2.3.1Flies co-expressing SNCA and LRRK2 in the presence of *repo*-GAL4 driver:

Step 1:
Cross 12: Cross set between *UAS-SNCA* female and double balancer (2nd and 3rd chromosomes) male flies:



Step 2:
Cross 13: Cross set between *UAS-SNCA* male and female flies (genotypes highlighted in step 1):

yw^{+}//+; *UAS-SNCA*$^{w+}$// *CyO*; *MKRS*//+**X***yw*$^{+}$//Y; *UAS-SNCA*$^{w+}$// *CyO*; *TM6, Tb*//+

↓

yw^{+}//+; *UAS-SNCA*$^{w+}$; *MKRS*//*TM6, Tb* *yw*$^{+}$; *UAS-SNCA*$^{w+}$; *MKRS*//*TM6, Tb*

yw^{+}//+; *UAS-SNCA*$^{w+}$// *CyO*; *MKRS*//*TM6, Tb* *yw*$^{+}$; *UAS-SNCA*$^{w+}$; *MKRS*//+

yw^{+}//+; *UAS-SNCA*$^{w+}$// *CyO*; *MKRS*//*TM6, Tb* *yw*$^{+}$; *UAS-SNCA*$^{w+}$; *TM6, Tb*//+

yw^{+}//+; *UAS-SNCA*$^{w+}$// *CyO*; *MKRS*//+ *yw*$^{+}$; *UAS-SNCA*$^{w+}$// *CyO*; *MKRS*//*TM6, Tb*

yw^{+}//+; *UAS-SNCA*$^{w+}$// *CyO*; *TM6, Tb*//+ *yw*$^{+}$; *UAS-SNCA*$^{w+}$// *CyO*; *MKRS*//+

yw^{+}//+; *UAS-SNCA*$^{w+}$; *TM6, Tb*//+*yw*$^{+}$; *UAS-SNCA*$^{w+}$// *CyO*; *TM6, Tb*//+

yw^{+}//Y; *UAS-SNCA*$^{w+}$; *MKRS*//*TM6, Tb* *yw*$^{+}$//Y; *UAS-SNCA*$^{w+}$; *MKRS*//+

yw^{+}//Y; *UAS-SNCA*$^{w+}$; *TM6, Tb*//+ ***yw***$^{+}$***//Y; UAS-SNCA***$^{w+}$***// CyO; MKRS//TM6, Tb***

yw^{+}//Y; *UAS-SNCA*$^{w+}$// *CyO*; *MKRS*//+ *yw*$^{+}$//Y; *UAS-SNCA*$^{w+}$// *CyO*; *TM6, Tb*//+

Step 3:
Cross 14: Cross set between *repo*-GAL4 female and double balancer (2nd and 3rd chromosomes) male flies:

+; +; *repo*-GAL4^{w+}// *TM6, Tb***X** +//Y; *If*//*CyO*; *MKRS*//*TM6, Tb*

↓

+; *If*//+; *repo-GAL4*$^{w+}$***//MKRS***+//Y; *If*//+; *repo-GAL4*$^{w+}$*//MKRS*

+; *If*//+; *repo-GAL4*$^{w+}$// *TM6, Tb* +//Y; *If*//+; *repo-GAL4*$^{w+}$// *TM6, Tb*

+; *If*//+; *MKRS*//*TM6, Tb* +//Y; *If*//+; *MKRS*//*TM6, Tb*

+; *CyO* //+; *repo-GAL4*$^{w+}$*//MKRS*+//**Y; *CyO***//**+; *repo-GAL4***$^{w+}$***//MKRS***

+; *CyO* //+; *repo-GAL4*$^{w+}$// *TM6, Tb* +//Y; *CyO*//+; *repo-GAL4*$^{w+}$// *TM6, Tb*

+; *CyO* //+; *MKRS* // *TM6, Tb* +//Y; *CyO*//+; *MKRS*//*TM6, Tb*

Step 4:
Cross 15: Cross set between *repo-GAL4* male and female flies (genotypes highlighted in step 3):

+; *If*//+; *repo-GAL4*$^{w+}$//*MKRS***X** +//Y; *CyO*//+; *repo-GAL4*$^{w+}$//*MKRS*

↓

+; *If* //*CyO; repo*-GAL4^{w+}//*repo*-GAL4^{w+}+//Y; *If*//*CyO; repo*-GAL4^{w+}//*repo*-GAL4^{w+}

+; *If*//*CyO; repo*-GAL4^{w+}//*MKRS*+//Y; *If*//*CyO; repo*-GAL4^{w+}//*MKRS*

+; *If* //+; *repo*-GAL4^{w+}//*repo*-GAL4^{w+}+//Y; *If*//+; *repo*-GAL4^{w+}//*repo*-GAL4^{w+}

+; *If*//+; *repo*-GAL4^{w+}// *MKRS*+//Y; *If*//+; *repo*-GAL4^{w+}//*MKRS*

+; *CyO*//+; *repo*-GAL4^{w+}//*repo*-GAL4^{w+}+//Y; *CyO*//+; *repo*-GAL4^{w+}//*repo*-GAL4^{w+}

+; *CyO*//+; *repo*-GAL4^{w+}// *MKRS*+//Y; *CyO*//+; *repo*-GAL4^{w+}//*MKRS*

Step 5:
Cross 16: Cross set between selected progeny (genotypes highlighted in bold in steps 2 and 4):

+; *If*//*CyO; repo*-GAL4^{w+}// *MKRS***X***yw*$^{+}$//Y; *UAS-SNCA*$^{w+}$// *CyO*; *MKRS*//*TM6, Tb*

↓

yw^{+}//+; *UAS-SNCA*$^{w+}$// *If; repo*-GAL4^{w+}// *MKRS*

yw^{+}//+; *UAS-SNCA*$^{w+}$// *If; repo*-GAL4^{w+}// *TM6, Tb*

yw^{+}//+; *UAS-SNCA*$^{w+}$// *CyO; repo*-GAL4^{w+}// *MKRS*

yw^{+}//+; *UAS-SNCA*$^{w+}$// *CyO; repo*-GAL4^{w+}// *TM6, Tb*

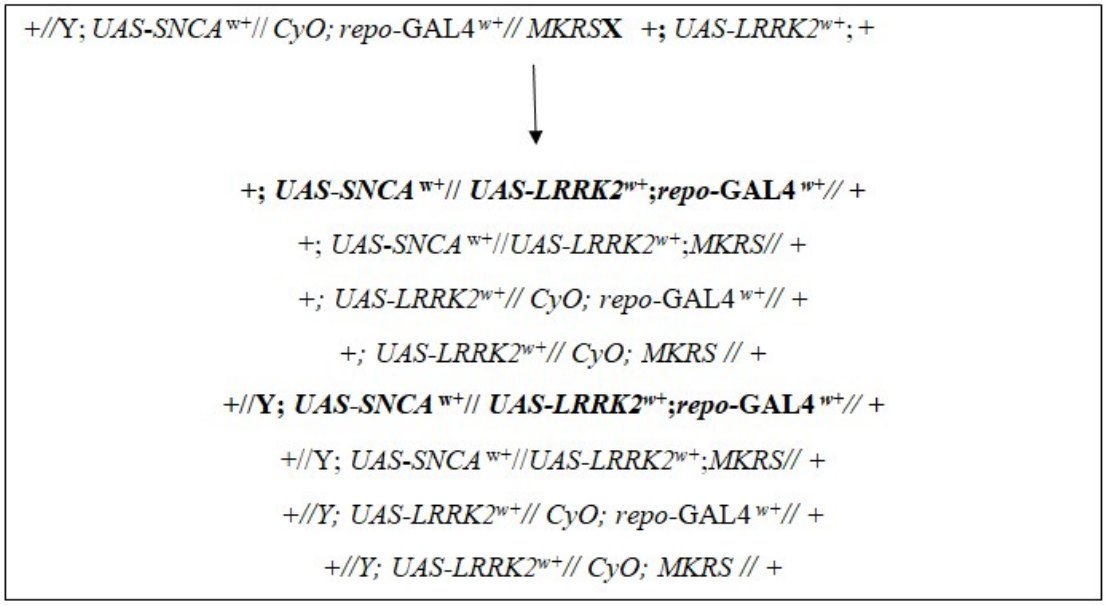
+//Y; *UAS-SNCA*$^{w+}$// *If; repo*-GAL4^{w+}// *MKRS*

+//Y; *UAS-SNCA*$^{w+}$// *If; repo*-GAL4^{w+}// *TM6, Tb*

+//Y; *UAS-SNCA*$^{w+}$// *CyO; repo*-GAL4^{w+}// *MKRS*

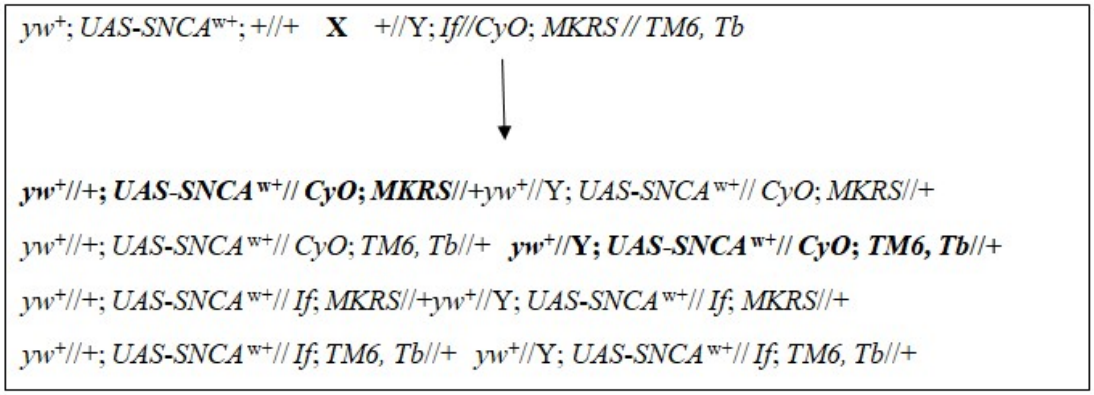
+//Y; *UAS-SNCA*$^{w+}$// *CyO; repo*-GAL4^{w+}// *TM6, Tb*

Step 6:
Cross 17: Cross between selected progeny (highlighted in bold in step 5) generated in step 5 and *UAS-LRRK2*



2.3.2Flies co-expressing SNCA and LRRK2 in the presence of *TH*-GAL4 driver:

Step 1:
Cross 18: Cross set between *UAS-SNCA* female and double balancer (2nd and 3rdchromosomes) male flies:



Step 2:
Cross 19: Cross set between *UAS-SNCA* male and female flies (genotypes highlighted in step 1):

yw^{+}//+; *UAS-SNCA*$^{w+}$// *CyO*; *MKRS*//+**X***yw*$^{+}$//Y; *UAS-SNCA*$^{w+}$// *CyO*; *TM6, Tb*//+

↓

yw^{+}//+; *UAS-SNCA*$^{w+}$; *MKRS*//*TM6, Tb* *yw*$^{+}$; *UAS-SNCA*$^{w+}$; *MKRS*//*TM6, Tb*

yw^{+}//+; *UAS-SNCA*$^{w+}$// *CyO*; *MKRS*//*TM6, Tb* *yw*$^{+}$; *UAS-SNCA*$^{w+}$; *MKRS*//+

yw^{+}//+; *UAS-SNCA*$^{w+}$// *CyO*; *MKRS*//*TM6, Tb* *yw*$^{+}$; *UAS-SNCA*$^{w+}$; *TM6, Tb*//+

yw^{+}//+; *UAS-SNCA*$^{w+}$// *CyO*; *MKRS*//+ *yw*$^{+}$; *UAS-SNCA*$^{w+}$// *CyO*; *MKRS*//*TM6, Tb*

yw^{+}//+; *UAS-SNCA*$^{w+}$// *CyO*; *TM6, Tb*//+ *yw*$^{+}$; *UAS-SNCA*$^{w+}$// *CyO*; *MKRS*//+

yw^{+}//+; *UAS-SNCA*$^{w+}$; *TM6, Tb*//+*yw*$^{+}$; *UAS-SNCA*$^{w+}$// *CyO*; *TM6, Tb*//+

yw^{+}//Y; *UAS-SNCA*$^{w+}$; *MKRS*//*TM6, Tb* *yw*$^{+}$//Y; *UAS-SNCA*$^{w+}$; *MKRS*//+

yw^{+}//Y; *UAS-SNCA*$^{w+}$; *TM6, Tb*//+ ***yw*$^{+}$//Y; *UAS-SNCA*$^{w+}$// *CyO*; *MKRS*//*TM6, Tb***

yw^{+}//Y; *UAS-SNCA*$^{w+}$// *CyO*; *MKRS*//+ *yw*$^{+}$//Y; *UAS-SNCA*$^{w+}$// *CyO*; *TM6, Tb*//+

Step 3:
Cross 20: Cross set between *TH*-GAL4 female and double balancer (2nd and 3rd chromosomes) male flies:

+; +; *TH*-GAL4^{w+}**X**+//Y; *If*//*CyO*; *MKRS*//*TM6, Tb*

↓

+//+; ***If***//+; ***TH-GAL4^{w+}//MKRS***+//Y; *If*//+; *TH-GAL4^{w+}//MKRS*

+//+; *If*//+; *TH-GAL4^{w+}//TM6, Tb* +//Y; *If*//+; *TH-GAL4^{w+}//TM6, Tb*

+//+; *CyO*//+; *TH-GAL4^{w+}//MKRS*+//**Y**; ***CyO***//+; ***TH-GAL4^{w+}//MKRS***

+//+; *CyO*//+; *TH-GAL4^{w+}//TM6, Tb* +//Y; *CyO*//+; *TH-GAL4^{w+}//TM6, Tb*

Step 4:
Cross 21: Cross set between *TH-GAL4* male and female flies (genotypes highlighted in step 3):

+; *If*//+; *TH-GAL4*$^{w+}$//*MKRS***X** +//Y; *CyO*//+; *TH-GAL4*$^{w+}$//*MKRS*

↓

+; *If* //*CyO; TH*-GAL4^{w+}//*TH*-GAL4^{w+}+//Y; *If* //*CyO; TH*-GAL4^{w+}//*TH*-GAL4^{w+}

+; *If* //*CyO; TH*-GAL4^{w+}//*MKRS* +//Y; *If* //*CyO; TH*-GAL4^{w+}//*MKRS*

+; *If* //+; *TH*-GAL4^{w+}//*TH*-GAL4^{w+}+//Y; *If* //+; *TH*-GAL4^{w+}//*TH*-GAL4^{w+}

+; *If* //+; *TH*-GAL4 *w*+// *MKRS*+//Y; *If* //+; *TH*-GAL4^{w+}//*MKRS*

+; *CyO*//+; *TH*-GAL4^{w+}//*TH*-GAL4^{w+}+//Y; *CyO*//+; *TH*-GAL4^{w+}//*TH*-GAL4^{w+}

+; *CyO*//+; *TH*-GAL4^{w+}// *MKRS*+//Y; *CyO*//+; *TH*-GAL4^{w+}//*MKRS*

Step 5:
Cross 22: Cross set between selected progeny (genotypes highlighted in bold in steps 2 and 4):

+; *If* //*CyO; TH*-GAL4^{w+}// *MKRS***X***yw*$^{+}$//Y; *UAS-SNCA*$^{w+}$// *CyO*; *MKRS*//*TM6, Tb*

↓

yw^{+}//+; *UAS-SNCA*$^{w+}$// *If; TH*-GAL4^{w+}// *MKRS*

yw^{+}//+; *UAS-SNCA*$^{w+}$// *If; TH*-GAL4^{w+}// *TM6, Tb*

yw^{+}//+; *UAS-SNCA*$^{w+}$// *CyO; TH*-GAL4^{w+}// *MKRS*

yw^{+}//+; *UAS-SNCA*$^{w+}$// *CyO; TH*-GAL4^{w+}// *TM6, Tb*

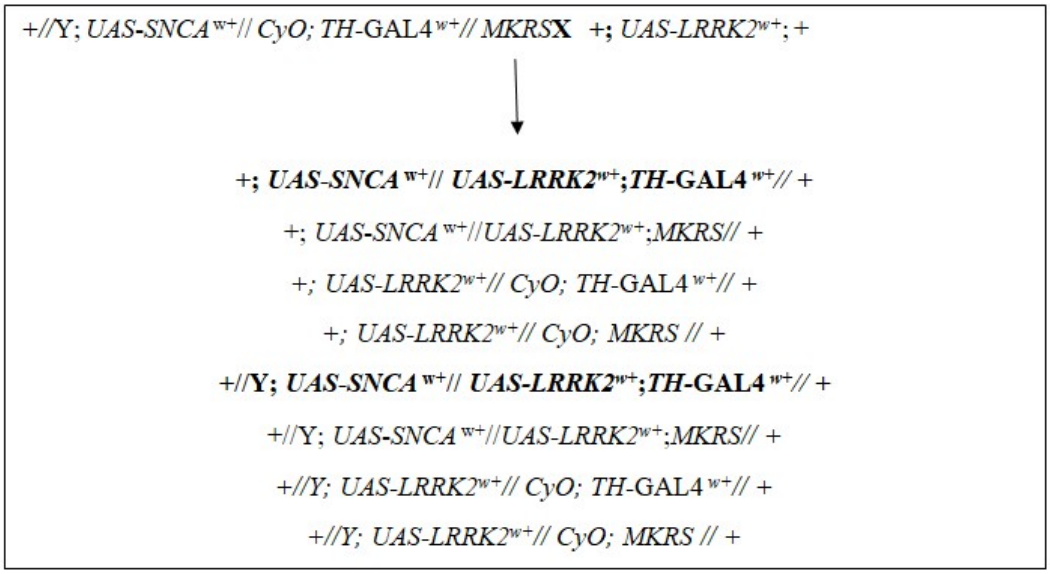
+//Y; *UAS-SNCA*$^{w+}$// *If; TH*-GAL4^{w+}// *MKRS*

+//Y; *UAS-SNCA*$^{w+}$// *If; TH*-GAL4^{w+}// *TM6, Tb*

+//Y; *UAS-SNCA*$^{w+}$// *CyO; TH*-GAL4^{w+}// *MKRS*

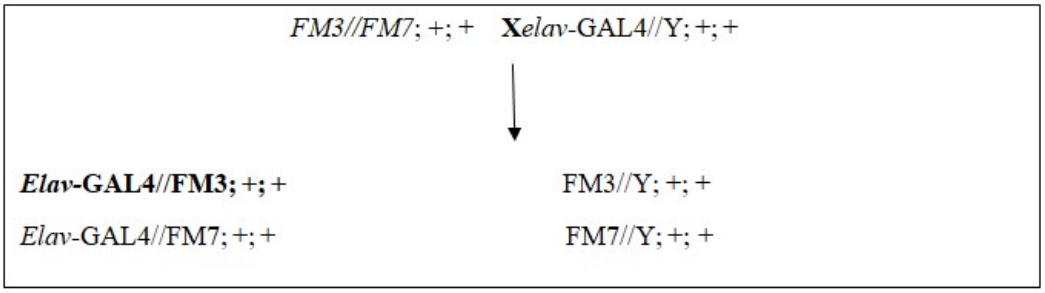
+//Y; *UAS-SNCA*$^{w+}$// *CyO; TH*-GAL4^{w+}// *TM6, Tb*

Step 6:
Cross 23: Cross between selected progeny (genotypes highlighted in bold in step 5) obtained from step 5 and *UAS LRRK2*:

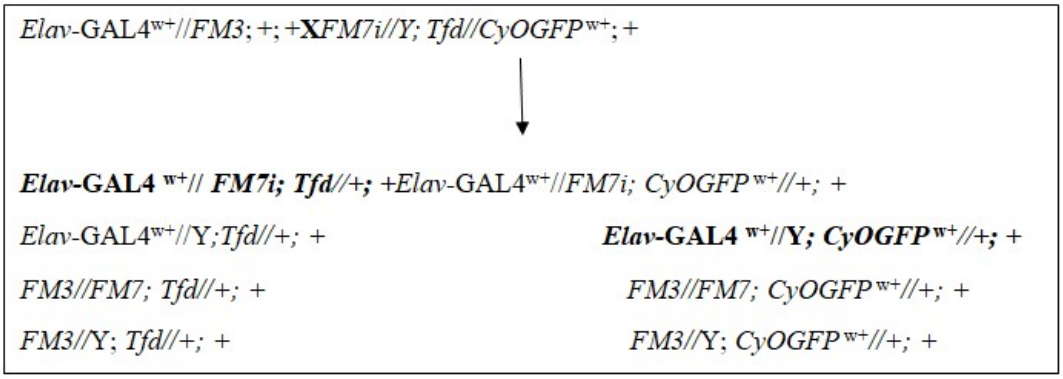


2.3.3Flies co-expressing SNCA and LRRK2 in the presence of *elav*-GAL4 driver:

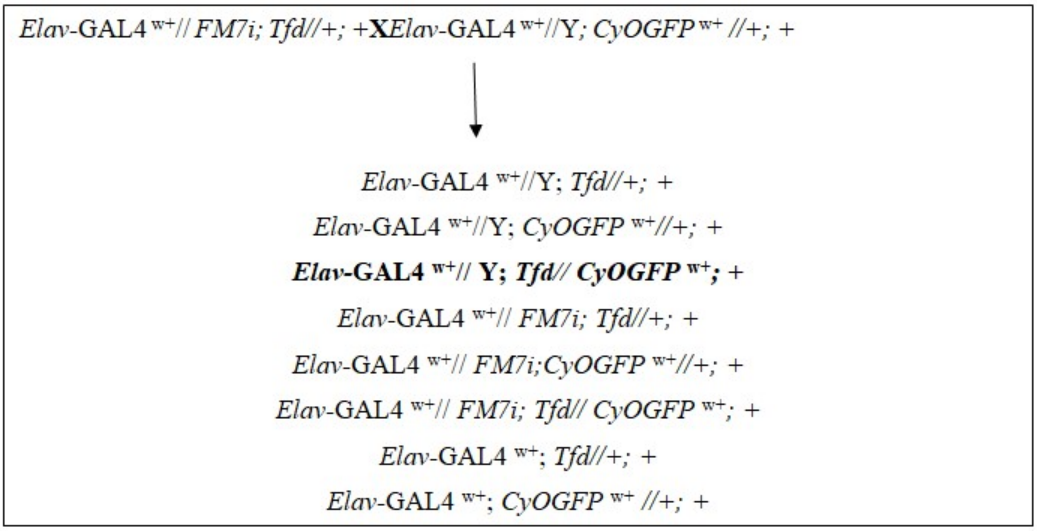
Step 1:
Cross 24: Cross set between *FM3//FM7* female and *elav*-GAL4 male flies:



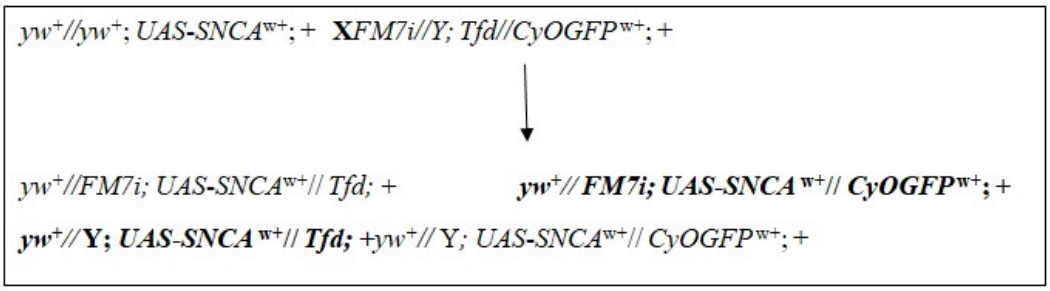
Step 2:
Cross 25: Cross set between progeny (genotype highlighted in bold in step 1) and double balancer (1st and 2nd chromosomes) flies:



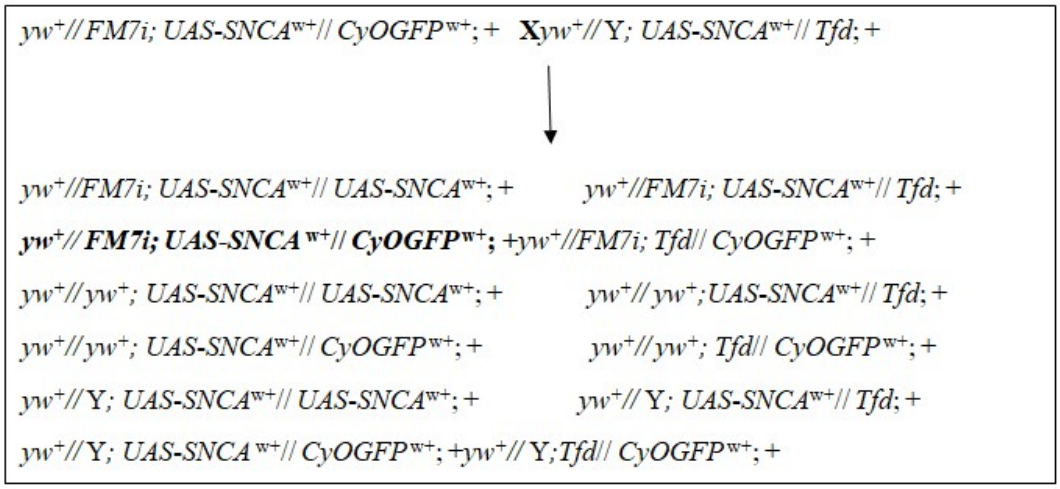
Step 3:
Cross 26: Cross set between *elav-GAL4* male and female flies (genotypes highlighted in step 3):



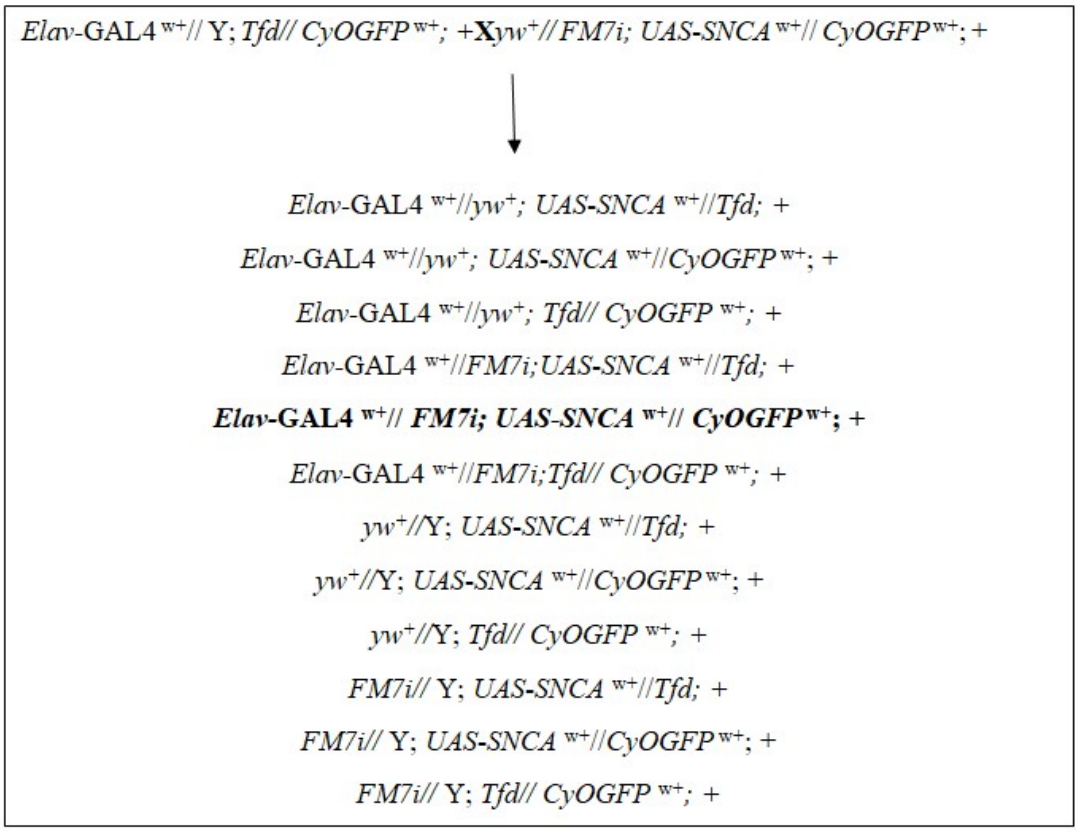
Step 4:
Cross 27: Cross set between *UAS-SNCA* male and double balancer (1st and 2ndchromosomes) female flies:



Step 5:
Cross 28: Cross set between *UAS-SNCA* male and female flies (genotypes highlighted in step 4):



Step 6:
Cross 29: Cross set between selected progeny (genotypes highlighted in bold in steps 3 and 5):



Step 7:
Cross 30: Cross between selected progeny (in bold in step 6) obtained from step 6 and *UAS-LRRK2*: