THE INTERNATIONAL JOURNAL OF
DEVELOPMENTAL
BIOLOGY
www.intjdevbiol.com

doi: 10.1387/ijdb.140190LS

SUPPLEMENTARY MATERIAL

corresponding to:

Gliolectin positively regulates Notch signalling during wing-vein specification in *Drosophila*

NAVEEN PRASAD and LINGADAHALLI S. SHASHIDHARA

***Address correspondence to:** Naveen Prasad. Indian Institute of Science Education and Research (IISER) Pune, Pashan Road, Pune 411008, India.
E-mail: novino11@gmail.com

Full text for this paper is available at: http://dx.doi.org/10.1387/ijdb.140190LS

Experimental procedure

Generation of* glec *cDNA clone

Full length *glec* gene was amplified from cDNA of *Drosophila* embryos. Embryos were collected and matured so that majority of them were at stage 13 (10 hours AEL) at the time of RNA isolation. RNA was isolated using Trizol reagent (Invitrogen) according to manufacturer's guidelines. Reverse Transcription was carried out using MMLV Reverse Transcriptase (Promega) and the resulting cDNA was used to amplify *glec*. The primers that were used to amplify the CDS of *glec* are:

Forward: GCGGAAAGAACAAAGC

Reverse: TTGGACCGTCAAGTCTTGGT

The 977 bp amplicon was cloned into pGEM-T easy vector (Promega).

***In situ* hybridisation**

Preparation of glec *probe*

In order to generate template for *in vitro* transcription, M13 Forward and reverse primers were used to amplify DNA fragment containing the full length *glec* from the pGEM- T easy vector containing *glec*. Anti-sense *glec* RNA probe was prepared using SP6 polymerase (Roche).

Hybridisation of the probe was carried out as described earlier (Sturtuvent *et al*., 1993), with the exception of the last step where detection was carried out using Fast Red tablets. In order to perform fluorescent *in situ* hybridisation, one Fast Red tablet (Roche) was dissolved in 2 mL of 0.1 M Tris Cl, pH 8.2. Following incubation with anti-DIG antibodies, the larval heads containing wing discs were incubated for 30 minutes in 0.1 M Tris Cl, pH 8.2. Fast red solution was added and the colour reaction was monitored under microscope. The reaction was stopped using PBS with 0.1 % triton 100 X. The wing discs were mounted and image was captured using fluorescent microscope.

Generation of UAS-* glec *lines

In order to insert *glec* in pUAST vector, primers containing ECoR1 and Xho1 site were designed and used to amplify 995 bp amplicon (containing the full length *glec*) from the full length *glec* clone in pGEM T easy that we generated (described above).The following primer pair was used for amplification:

Forward: CCGGAATTCGCGGAAAGAAGAAACAAAGC

Reverse: CCGCTCGAGTTGGACCGTCAAGTCTTGGT

The resulting amplicon was purified, restricted using ECoR1 and Xho1 and subsequently cloned in pUAST vector. The resulting clone was sequence-verified before using to generate transgenic UAS-*glec* lines.

Generation of UAS-*glec^{RNAi} *transgenic lines

Two RNAi constructs were prepared in SympUAST vector (Giordano *et al*., 2002) to target two non overlapping regions of *glec*.

The 5' *glec* ^{RNAi} construct spanned 7- 214 bp of the CDS of *glec*.

The 3' *glec* ^{RNAi} construct spanned 289- 682 bp of the CDS of *glec*. BLAST search against annotated genes of *Drosophila melanogaster* did not reveal any other gene or fragment with matching sequences of 19 bp or longer in the regions that we used to generate the UAS-*glec^{RNAi}* transgenic lines. As RNA interference is mediated by 21 nucleotide short RNA fragments, this suggests that the UAS-*glec^{RNAi}* transgenic lines by us using these fragments do not have any potential off targets.

In order to generate 5' *glec* ^{RNAi} construct, primers were designed containing XhO1 and Not1 sites. The primers are:

Forward: CCGCTCGAGTGTTGTGTCCGCCAATGGC

Reverse: ATAAGAATGCGGCCGCGATGTCATATC CGTAGCGTTC

In order to generate 3' *glec* ^{RNAi} construct, primers were designed containing ECoR1 and Xho1 sites. The primers are:

Forward: CCGGAATTCGACCTCAAGGACGATATCCAGCAC

Reverse: CCGCTCGAGGCTTCTGATCTGCCAATTCGCTAG

Using the primers, the two different regions of *glec* gene were amplified and amplicons were cloned in sympUAST vector. The resulting clones were sequenced and used to generate UAS- *glec* ^{RNAi} transgenic lines.

All the three clones (UAS-*glec* and UAS-*glec* ^{RNAi}) were used to generate transgenic flies by injecting them in poleplasm of 0-1 hour old embryos of w^{1118} flies along with a helper plasmid that served as a source of transposase. Transgenic flies were then screened on the basis of eye colour using standard techniques.