

## Microarray comparison of anterior and posterior *Drosophila* wing imaginal disc cells identifies novel wing genes

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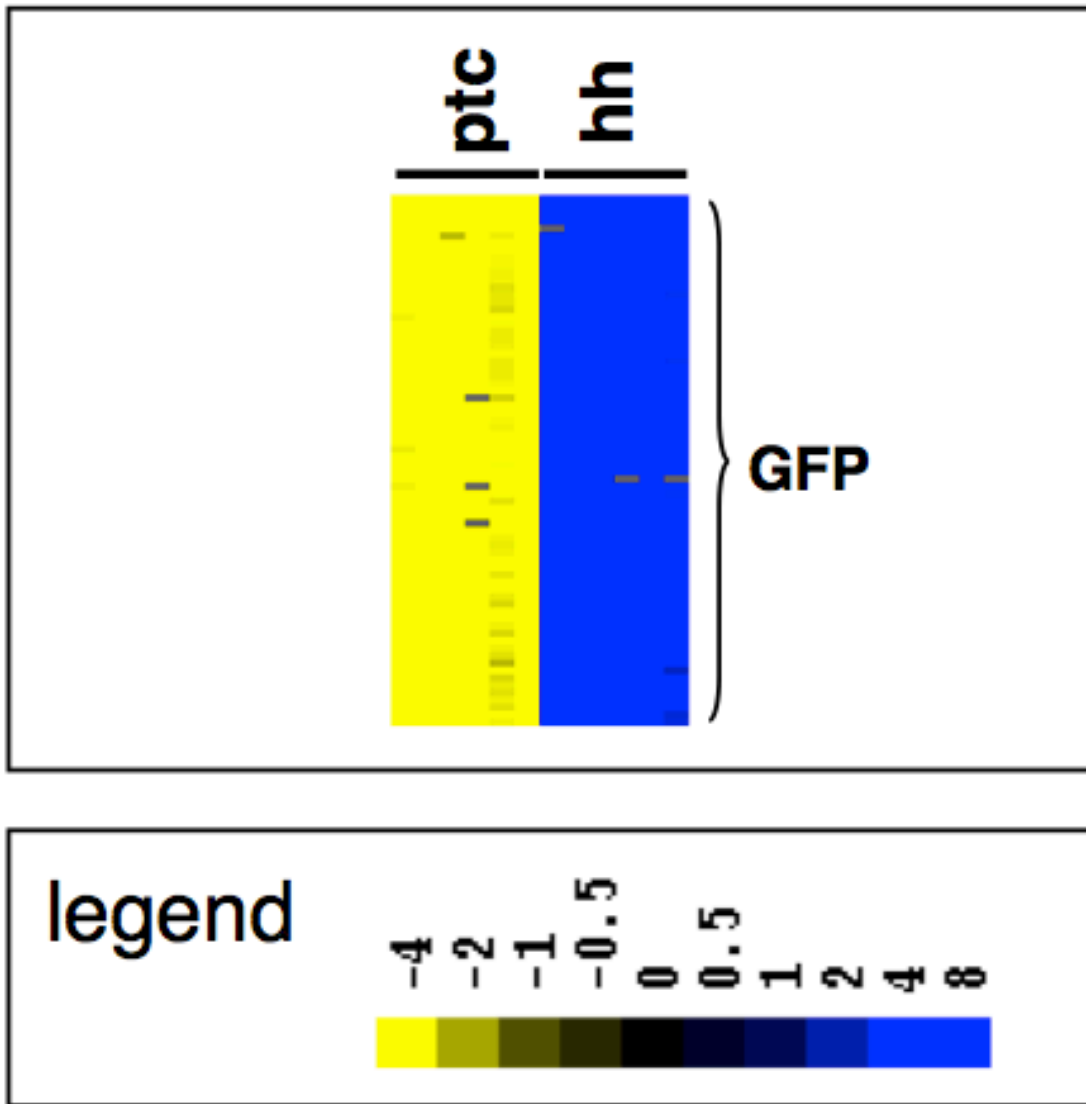
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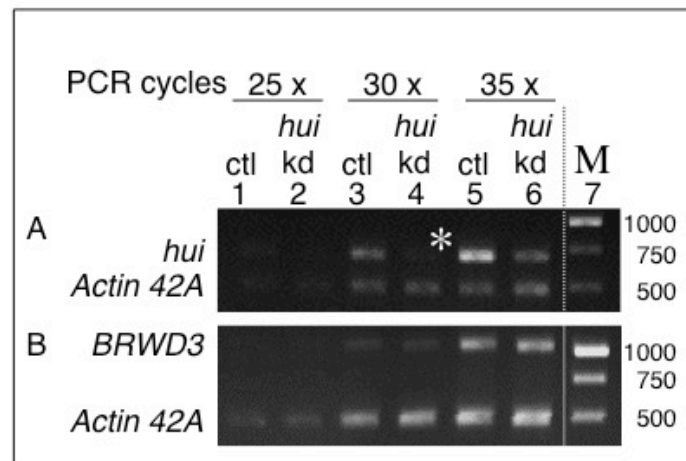
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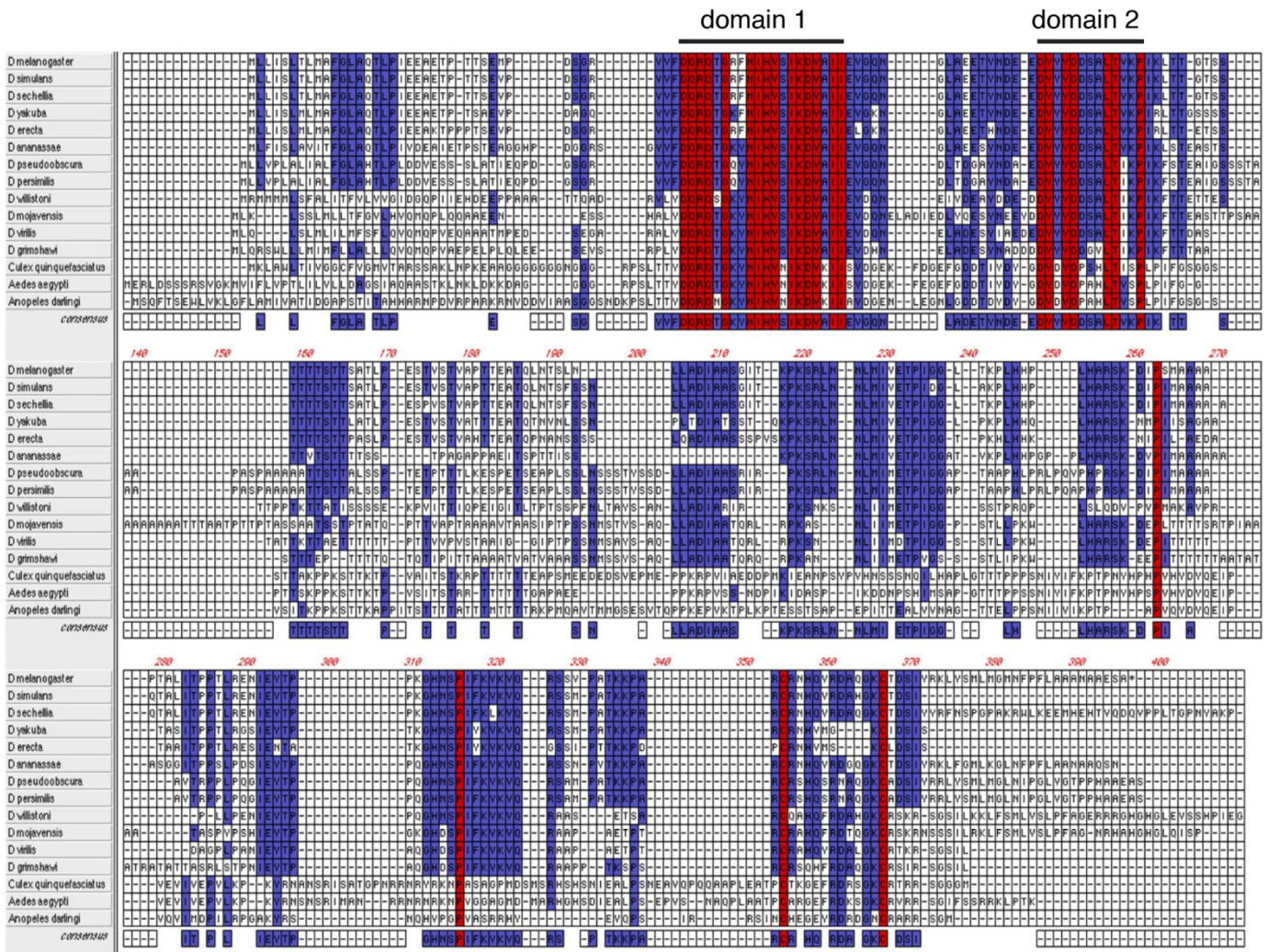
**DOI: 10.1534/g3.113.006569**



**Figure S1** Quality control of A/P microarray comparison. Heat-map of GFP expression in 72 replicate GFP spots. *ptc-Gal4* activated GFP expression resulted in anterior hybridization to GFP (yellow); and *hh-Gal4* activated GFP expression resulted in posterior hybridization to GFP (blue) (compare to Figure 1A). The twelve individual array hybridizations are indicated in the columns and the 72 replica GFP spots in the rows. Legend of the color-coded expression ratios in log<sub>2</sub>-transformed values.



**Figure S2** RT-PCR quantification of *hui* expression levels after RNAi knockdown. (A, B) RNA was isolated with RNA purification Mini kit (Zymo Inc.) from wing discs of control larvae (ctl, *white*<sup>1118</sup>) and larvae that expressed the transgenic *hui* RNAi construct under control of the *C765-Gal4* (*hui* kd). Duplex RT-PCR for *hui* and *Actin 42A* (as reference) or *BRWD3/CG31132* and *Actin 42A* was performed with the OneStep RT-PCR kit (Qiagen). Primers were: GTCGTTTTGGAACCTTTTCACGCGTTC (*hui*-forward), GTTCTATTTGGATGAGTAAGTGGATTCGG (*hui*-reverse), CTGGGTCTGCCGATCACCATGTG (*BRWD3*-forward), CATTACATTAACCGACTGCCGATTGGC (*BRWD3*-reverse) GATGGCAACATACATGGCCG (*Actin 42A*-forward), GTGTGCAGCGGATAACTAG (*Actin 42A*-reverse). The annealing temperature was 50° C and 10 µl aliquots were removed after 25, 30, and 35 PCR cycles. (upper) Note that *hui* transcript levels (\*) are reduced in the RNAi discs relative to *Actin 42A* levels. (lower) Quantification of the predicted off-target transcript *BRWD3* in control discs, showing no reduction after *hui* knock-down. M - marker. Numbers indicate size (base pairs).



**Figure S3** Hui protein alignment. The putative Hui protein of *D. melanogaster* was aligned with homologs from eleven other *Drosophila* and three other insect species. Domains of highest conservations indicated; blue indicates strong conservation; red, complete identity. The positions of the arbitrarily determined Domains 1 and 2 are indicated.

**Table S1 List of genes with differential A and P expression.** Datasheet that includes A. the list of A and P genes that were identified with SAM, B. the list of 102 A and P genes that were identified with a combination of cluster analysis and SAM, C. the list of the notum sub-cluster, and D. the list of 29 genes that were analyzed by *in situ* detection. For a description of data analysis see Material and Methods. The original microarray data is available at: <http://www.ncbi.nlm.nih.gov/projects/geo/index.cgi>; accession number GSE46601.

Table S1 is available for download at <http://www.g3journal.org/lookup/suppl/doi:10.1534/g3.113.006569/-/DC1>.

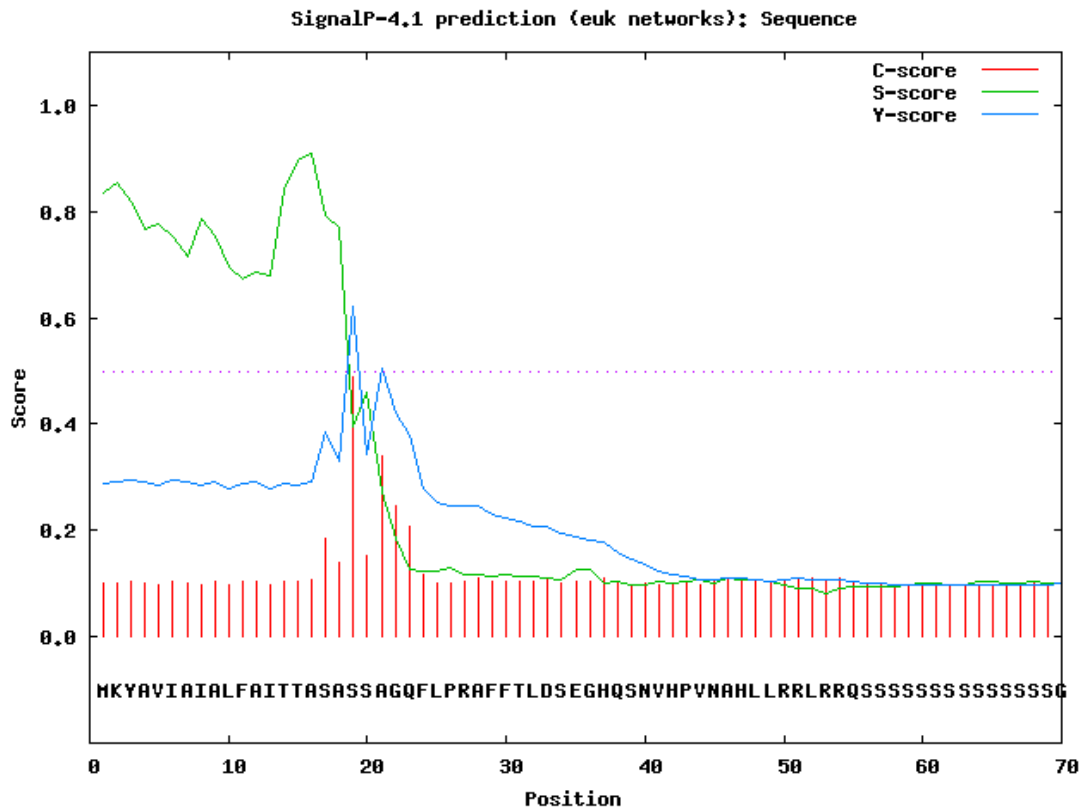
**Table S2 Protein sequences and prediction of signal peptides.** The predictions for signal peptides were generated with the SignalP 4.1 software (<http://www.cbs.dtu.dk/services/SignalP/>) from the Center for Biological Sequence Analysis of the Technical University of Denmark Lyngby.

SignalP 4.0: discriminating signal peptides from transmembrane regions  
 Petersen TN., Brunak S., von Heijne G. & Nielsen H.  
 Nature Methods, 8:785-786, 2011

**CG10200 (*hui*) predicted Protein 1**

MKYAVIAIALFAITTAASSAGQFLPRAFFFTLDSEGHQSNVHPVNAHLLR  
 RLRRQSSSSSSSSSSSSSSSGGNVFTYASHSVQNADGSGHSGSSYTQQAAA  
 PVLGGVSFDERFGESSLTGGDNYYPTYNGYTSAAISSNSGVGSTGYSYQH  
 ISGVGPIPATQIQQTAVVVDASGNQKVTNIQGATDSNGVLNIAIKKTESTYS  
 SK

# SignalP-4.1 euk predictions  
 >Sequence



# Measure	Position	Value	Cutoff	signal peptide?
max. C	19	0.490		
max. Y	19	0.621		

max. S	16	0.910		
mean S	1-18	0.778		
D	1-18	0.706	0.340	YES

Name=Sequence SP='YES' Cleavage site between pos. 18 and 19: ASA-SS D=0.706  
D-cutoff=0.340 Networks=SignalP-noTM

# [data](#)

# [gnuplot script](#)

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Signal peptides: 1

# [processed fasta entries](#)

# [gff file of processed entries](#)

## **CG10200 (*hui*) predicted Protein 2**

MKYAVIAIALFAITTASASSAGQFLPRAFFFTLDSEGHQSNVHPVNAHLLR  
RLRRQSSSSSSSSSSSSSSGGNVFTYASHSVQNADGSGHSGSSYTQQAAA  
PVLGGVSFDERFGESLTTGGDNYYPTYNGYTSAAISSNSGVGSGTGSYQH  
ISGVGPIPATQIQQTVAVVDASGNQKVTNIQGATDSNGVLNIKKTESTYS  
SK

# SignalP-4.1 euk predictions

**>Sequence**



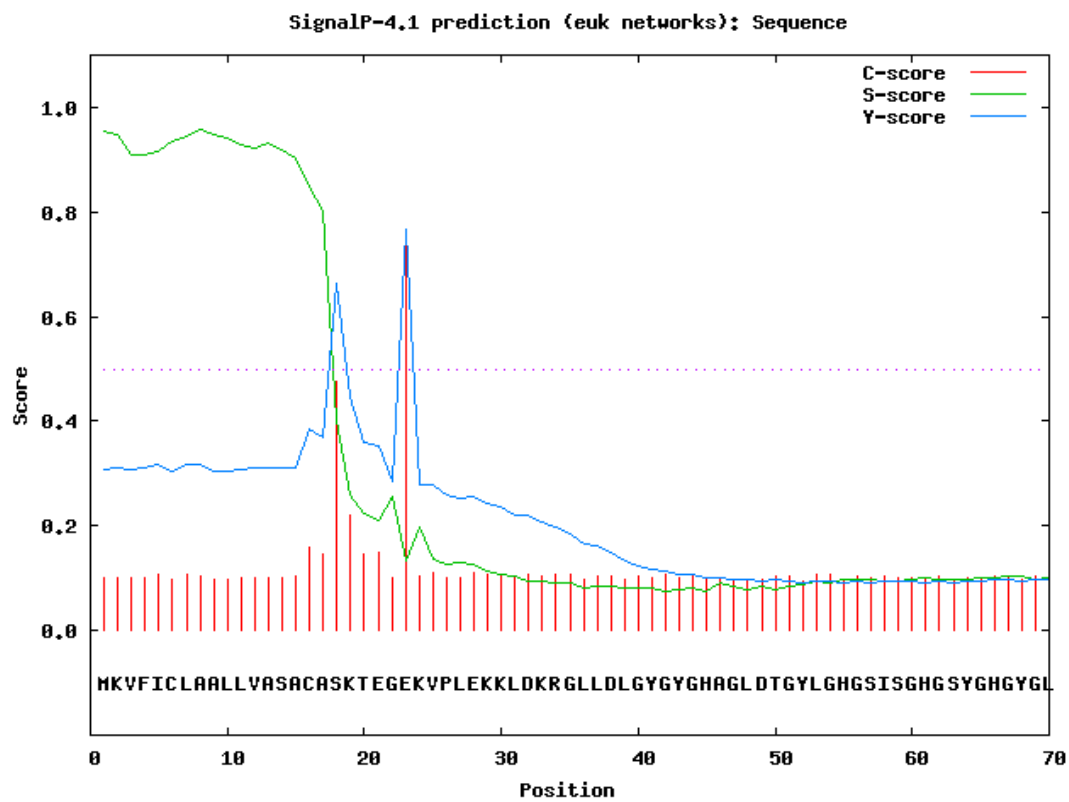


## CG16884 predicted Protein

MKVFICLAALLVASACASKTEGEKVPLEKKLDRGLLDLGYGYGHAGLDT  
 GYLGHGSI SGHGSYGHGYGLTGYSAPA AVAVGHSGPAI AVGHTAPAVAVH  
 HAPAPYVISKQADVHK TITITKGI PVPVHVDRPY PVPVHEKRPVPEVKVPV  
 PQPYEVIRKVPVTVKEYVKVPVPVPPQPYEVIRHEKVPVHVVPVDRPVPVEV  
 PRPYVPVAKPYPVYVEKAVNVQVPVHVDRPYPVYVKVPVSVSHSVVKHAP  
 TVAVSSYPVSAIGHDATVYSDHHGYHK

# SignalP-4.1 euk predictions

>Sequence



# Measure	Position	Value	Cutoff	signal peptide?
max. C	23	0.762		
max. Y	23	0.767		
max. S	8	0.958		
mean S	1-22	0.772		

D 1-22 0.770 0.340 YES

Name=Sequence SP='YES' Cleavage site between pos. 22 and 23: TEG-EK D=0.770  
D-cutoff=0.340 Networks=SignalP-noTM

# [data](#)

# [gnuplot script](#)

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Signal peptides: 1

# [processed fasta entries](#)

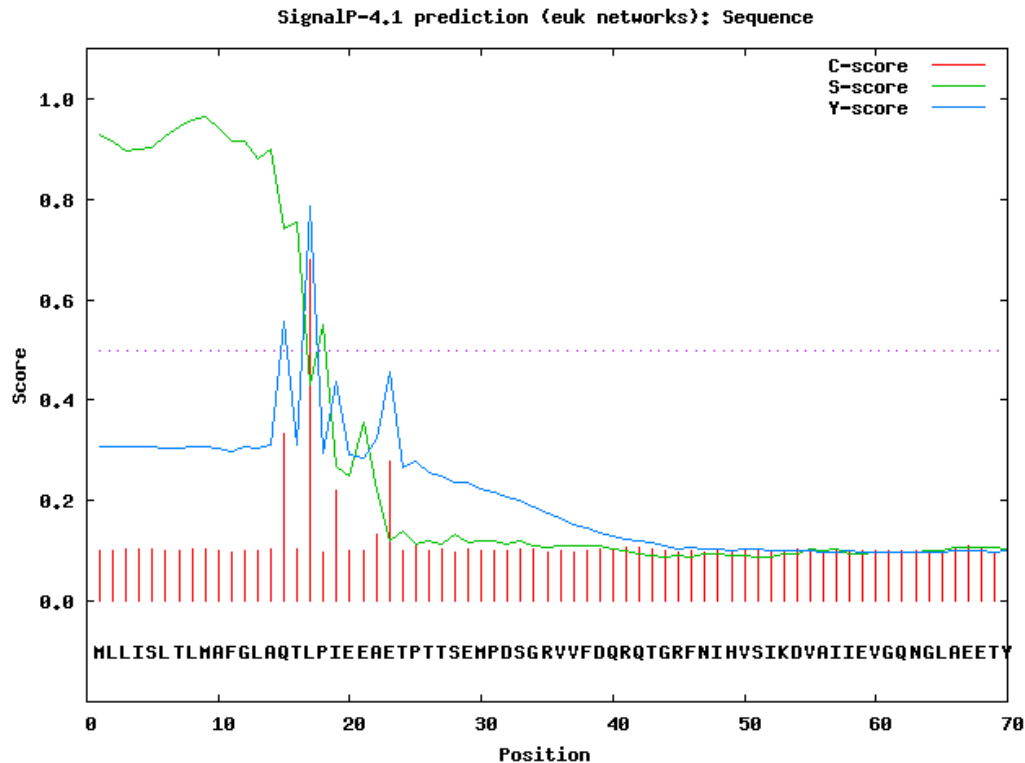
# [gff file of processed entries](#)

## CG15905 predicted Protein

```
MLLISLTLMAFGLAQTLPiEEAETPTTSEMPDSEGRVVFDRQTGRFNIHV  
SIKDVAIIEVGQNGLAEEETYNDDEEDYYYDDSAITVKPIKLTGTSTTTT  
STTSATLPESTVSTVAPTTEATQLNTSLNLLADIAASGITKPKSRLNNLM  
IVETPIGGLTKPLHHPLHARSKDIPSMAAAAPTALITPPTLRENIEYTPP  
KGHNSPIFKVKVQRSSVPATKKPARCRNHQVRDAQGKCTDSIYRKLYSML  
MGMNFPFLAANAESA
```

# SignalP-4.1 euk predictions

>Sequence



# Measure	Position	Value	Cutoff	signal peptide?
max. C	17	0.680		
max. Y	17	0.785		
max. S	9	0.963		
mean S	1-16	0.899		
D	1-16	0.846	0.340	YES

Name=Sequence SP='YES' Cleavage site between pos. 16 and 17: AQT-LP D=0.846  
D-cutoff=0.340 Networks=SignalP-noTM

# [data](#)

# [gnuplot script](#)

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Signal peptides: 1

# [processed fasta entries](#)

# [gff file of processed entries](#)