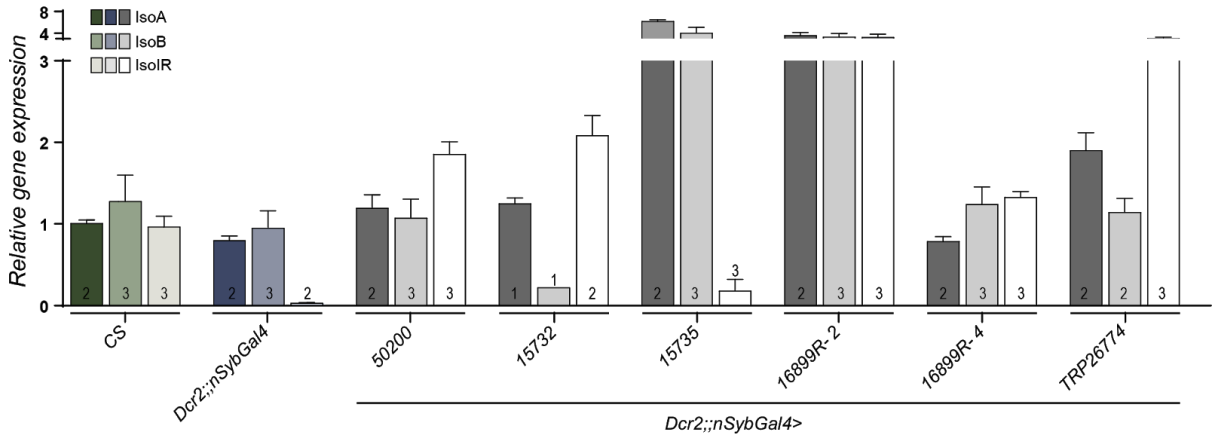
qPCR on available RNAi lines



Materials and Methods and Results

We analyzed the efficiency of the available *FoxP* RNAi lines using RT-qPCR. We extracted RNA from about 20 flies for each genotype (Table 1), following the TriFast™ protocol from peqlab (a VWR company). The RNA was subsequently transcribed into cDNA using the OneStep RT-PCR Kit from QIAGEN (thermocycler program used: 42°C for 2 minutes, 4°C pause until manual restart at 42°C for 30 minutes, 95°C for 3 minutes and finally 10°C ∞). Subsequently, the qPCR was performed. The primers were identical to those used in (Mendoza et al. 2014). For the qPCR reaction we used a Biorad CFX Connect Real-Time PCR Detection System thermocycler and the Biorad CFX manager software to store and analyze the data. Every sample was run in triplicate in a 96-well plate in a total volume of 10 µl. The mixture contained 5 µl sybrGreen master mix (QuantiTect SYBR Green from QIAGEN), 0.5 µl from each primer, 1 µl of 1:10 diluted cDNA and 3 µl sterile H_2O (thermocycler program in Table 2). As reference, we used the housekeeping gene rp49 (ribosomal protein 49), while as a negative control we used the same reaction mix without cDNA. The experiments were repeated 1 to 3 times.

Fly line	**Source**
CS	own laboratory
UAS-Dcr2;; nSyb-GAL4	Stephan Sigrist

50200	VDRC
15732	VDRC
15735	VDRC
16899R-2	NIG-Fly
16899R-4	NIG-Fly
TRP26774	?

Table 1: Fly lines used for the experiment

Step	Temperature	Time	Nr. of Cycles
Denaturation	95 °C	3 min	
Denaturation	95 °C	10 s	
Annealing	55 °C	10 s	
Elongation, measurement	72 °C	30 s	GoTo step 2 x39
Denaturation	95 °C	10 s	
Melting curve, measurement	65 °C – 95 °C	+ 0.5 °C / 5s	

Table 2: qPCR thermocycler program

We determined the relative abundances of the mRNAs for all the isoforms of *FoxP* in each RNAi line by calculating the 2^(-ΔΔCq) of each line against CS.

References

Mendoza, Ezequiel, Julien Colomb, Jürgen Rybak, Hans-Joachim Pflüger, Troy Zars, Constance Scharff, and Björn Brembs. 2014. “Drosophila FoxP Mutants Are Deficient in Operant Self-Learning.” *PloS One* 9 (6): e100648.