

CLONING OF SOXB ORTHOLOGS IN A SERPULID ANNELID

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Sox family members of transcription factors are found throughout the animal kingdom and are characterized by the presence of a DNA-binding HMG (High Mobility Group) domain, and are involved in a diverse range of developmental processes (Bowles, 2000). Degenerate primers were designed based on conserved regions of amino acids to isolate and characterized SoxB genes in a serpulid annelid. No positive clone has been isolated so far and further efforts will be required.

The Sox family of transcription factors is characterized by the presence of a DNA-binding HMG domain and the members are involved in a variety of developmental processes throughout the animal kingdom (Bowles, 2000). For example, Sox family members are implicated in the regulation of germ layer formation (ectoderm), cell type specification and organ development (Wegner, 1999). In particular, early neural expression of Sox homologues has been found in the chordate *amphioxus* (Holland et al. 2000), in the hemichordate *Ptychordera flava* (Taguchi et al. 2002), and in *Drosophila* (Cremaze et al. 2001) suggesting that the function of Sox B genes in early neurogenesis is ancestral to bilaterians.

In this experiment, we intend to clone and analyze the expression pattern during embryogenesis of a SoxB gene in a Serpulid annelid. Annelids belong to one of the three great branches of bilaterians, the lophotrochozoa (Adoutte et al. 2000). The particular usage of Sox B genes in this animal will contribute to the understanding of the evolution of bilaterian embryogenesis. In particular to the evolution of ectodermal and neural specification functions.

Methods and Materials

Sequences of amino acids of Sox family members were analyzed and identified based on their conserved regions (Figure 1). One of those conserved region is the High Mobility Group DNA-binding domain. Then, using degenerate primers we have isolated, amplified, and cloned a fragment of the protein-encoding gene in a *H. Elegans*. The gene in question was isolated from an *H. Elagans* cDNA library by degenerated Polymerase Chain Reaction (PCR) using the Advantage 2 PCR Enzyme System. By using agarose gel electrophoresis the PCR product size was identified as being of about 1000 bp (Figure 2), within the range of the expected fragment. Subsequently, the fragments were ligated in the DK101 vector (a modified pGEM5Zf (+) vector). The colonies with the insert were isolated and their plasmids sequenced. Then, these sequences were analyzed and characterized using the NCBI PubMed database software to identify any SoxB orthologs.

Consensus	DHKKREMNAT MVKSRGERRK IAQQNPEOMN SEISKRLGKR MKLLSESEKR EFIEEAERLQ AQHMKDYPCY KYREPRRKK (C/G/P)	79 Source	Acc. No.
ca-SoxB1*	-v-----q-k-m-s-k-----l-a--a-q-----d-k--i-l-eh-----l-	G	D38377
dr-SoxB1*	-v-----q--m-s-d-k-----a-q--d-----d-k--v-eh-----t-	C	AC018183
se-SoxB1	-v-----q--l-s-e-k-----a-e-----d-k--v-eh-----t-	C	AF157389
ch-SoxB1	-v-----q--m-e-k-----a-e--v-a-a-----d-k--l-eh-----t-	C	AB011802
n/h-SoxB1	-v-----q--m-e-k-----a-e--v-a-a-----d-k--l-eh-----t-	C	X94125/Y11436
xe-SoxB2	-v-----q--m-e-k-----a-e-----a-----d-k--l-eh-----t-	C	A:005476
ch-SoxB2	-v-----q--m-c-k-----a-c-----a-----d-k--l-eh-----t-	C	D12532
n/s/h-SoxB2	-v-----q--m-e-k-----a-e-----t-----d-k--l-eh-----t-	C	X94127/X96997/Z31560
xc-SoxB3	-v-----q--m-c-k-----a-d-----d-d-----d-k--v-eh-----t-	C	Y07542
ch-SoxB3	-v-----q--m-s-k-----a-d-----d-----d-k--v-eh-----t-	C	AB0011803
n/d/h-SoxB3	-v-----q--m-l-c-k-----a-d-----d-a-----d-k--v-eh-----t-	C	X94125/A53143/ X71135
zf-SoxB19	-kv-----q--m-e-k-----a-e-----t-d-a-----d-k--l-eh-----k-pp	C	X79821
ce-SoxB2*	-v-----q--m-s-k-----a-e--l-q-q-----d-k--l-eh-----p-	G	Z69792
dr-SoxB2.1f	g-----l-q-q--k-d-k-----a-e--a-----d-k--l-eh-----p-	C	X96419
dr-SoxB2.2*	e-----l-q-q--d-k-----a-e--l-e-----d-k--l-eh-----p-	G	AC015146
dr-SoxB2.3*	-v-----q--m-d-k-----a-e--t-g-q-----d-k--l-eh-----p-	G	AC015146
se-SoxB2	-v-----q--l-s-e-k-----a-e-----d-d-----d-k--l-eh-----p-	C	AF157388
ch-SoxB14	-v-----q--m-e-k-----a-e-----a-----y-d-k-----eh-----p-	C	AB026622
n/h-SoxB14	-v-----q--m-e-k-----a-e-----a-----y-d-k-----eh-----p-	C	AF193437/AJ006230
zf-SoxB21	-v-----a-q--m-l-d-k-----a-e-----d-----d-k--v-eh-----p-	C	U85092
ch-SoxB21	-v-----q--m-e-k-----a-e-----a-----d-k--v-eh-----p-	C	BAR77266
hu-SoxB21	-v-----a-q--m-e-k-----a-e-----t-----d-k--v-eh-----p-	C	AF107044

Figure1. Conserved regions below red lines were used to design the primers.

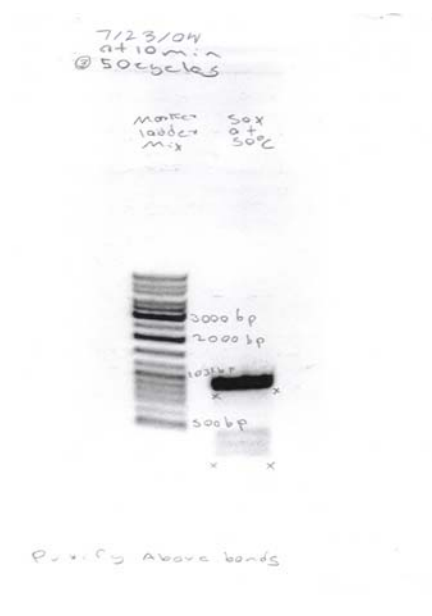


Figure2. PCR product of 1000bp.

Results

Using computational analysis (NCBI PubMed) the expected amino acid sequences of the serpulid annelid SoxB shows negative results. Therefore, more Sox B-Orthologs should be analyzed and eventually their expression pattern will be characterized during embryogenesis in *Hydroides elegans*.

Conclusion

In summary, without a full sequence of the gene, the role of SoxB orthologs in a serpulid annelid cannot be identified. The negative result is due in part to a first attempt at cloning SoxB subclass genes. A more general set of primers and different embryonic stages will be used during the successive attempts.

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