CONSTRUCTION AND SCREENING OF DOGFISH (SQUALUS ACANTHIAS), HAGFISH (MYXINE GLUTINOSA), AND TOADFISH (OPSANUS TAU), HEART AND BRAIN cDNA LIBRARIES.

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Understanding in almost all fields of biological research is currently being advanced rapidly by the application of recombinant DNA technology to the processes under investigation. One of the key methods in this advance is that of cDNA cloning and sequencing. This permits the identification and characterization of genes (and therefore proteins) without the necessity of purifying the protein. For example the recent identification of the Squalus cardiac CNP by the authors required only a single animal. The aim of this project was to extend this work by the construction of cDNA libraries from the hearts and brains of the hagfish Myxine glutinosa, and the toadfish Opsanus tau as well as the rectal gland from Squalus acanthias. These were then to be screened in order to isolate cDNAs encoding natriuretic factors, the homologues of oxytocin and vassopressin and novel 7-transmembrane domain receptors.

Hagfish and toadfish hearts (8 and 4 respectively) and brains (10 and 6) were dissected from anaesthetised animals and immediately frozen in liquid nitrogen. A single rectal gland was similarly frozen after removal from a dog fish. Messenger RNA was prepared using a Fast Track kit from InVitrogen. Double stranded cDNA was synthesized and after adapter ligation, was size fractionated by preparative agarose gel eletrophoresis. This entire process yielded approximately 100ng of purified cDNA. This was then ligated to the λgt10 vector (which had previously digested with EcoRI). After in vitro packaging the resulting bacteriophage were plated on the selective E.coli strain, C600 Hfl. The background in each case was less than 3% and the total number of recombinants shown in the table below:

Hagfish brain	1.3×10^5
Hagfish heart	2.6×10^5
Toadfish brain	5.0×10^5
Toadfish heart	1.3×10^5
Dogfish rectal gland	5.5×10^5

These libraries were then screened with several probes: The previously isolated squalus CNP, and two oligonucleotides encoding the highly conserved amino acid motif KLDRIG. The latter resulted in 2 positively hybridizing regions from the toadfish heart library which are currently being characterised in Cambridge. The rectal gland library (and also another similar library provided by Dr Benz) was screened with the two orphan receptor probes (see the Bulletin article by JPS). These screens produced no positively hybridizing clones indicating that the mRNA corresponding to these receptors is rare in rectal gland tissue.

DSCJ and JPS received fellowships from the Lucille P. Markey Charitable Trust and JPS also from the Blum-Halsey Trust. JNF is in part supported by the American Heart Association (Maine Affiliate). We also acknowledge the kind gifts of reagents from United States Biochemicals, Cleveland OH and Invitrogen Corporation San Diego CA.