Expressed sequence tags in a normalized cDNA library prepared from multiple tissues of the American lobster *Homarus americanus*

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Changes in temperature and salinity are known stressors in American lobsters that induce expression of chaperone proteins, including hsp70 and hsp90, as well as polyubiquitin, a component of the protein degradation pathway[2,3]. Although induction of these genes is likely to be protective during environmental fluctuations normally encountered, they may be insufficient to resist extremes of temperature or salinity, particularly on a background of environmental contamination. To develop a more complete view of gene transcriptional changes during stress in adult lobsters, we are developing a library of expressed sequence tags (ESTs) that will be employed in future microarray analyses.

Total RNA was prepared from three tissues in the branchial chamber (gill, epipodite, and branchiostegite) as well as examples of major organ groups (brain, heart, antennal gland, hepatopancreas, and abdominal muscle) and reproductive tissues (ovary and testis). Analysis of total RNA with an Agilent Bioanalyzer revealed two closely-spaced peaks of rRNA, the crustacean 28S rRNA fragmenting to two smaller products, one or both of which may coincide with the 18S rRNA peak. cDNA was reverse transcribed from pooled total RNA using oligo-dT as primer and was directionally ligated into the pCMV Sport 6.1 vector. The resulting cDNA library was normalized by subtraction at two different Cot values (Invitrogen). Normalization reduced the abundance of a highly-expressed transcript (arginine kinase) from 2.47% in the un-normalized library to 0.0465% in the final product, a 53-fold reduction. The average insert size was 2.41 kb.

Plasmids were purified from overnight cultures of randomly-picked clones using a Biomek 2000 liquid handling robot and 5,568 of these were 5'-end sequenced. Nucleotide sequences were processed by automated editing of trace files and local blast analysis for direct submission to dbEST, using *trace2dbest* on a dedicated Linux computer (Parkinson, Anthony and Blaxter, unpublished software, University of Edinburgh). Resulting were 4,604 dbEST-submissable sequences with an average length of 572 nucleotides, including an average high-quality length of 453 nucleotides. Clustering by *partigene* software[1] revealed 1,412 ESTs in 579 individual clusters, plus 3,192 singletons. Sequencing 5,568 clones thus produced a total yield of 3,771 unique sequences, representing a return of two unique sequences for every three clones picked and sequenced. Following blastx analysis against the non-redundant database from NCBI, 41.6% of the unique sequences were tentatively identified via high-scoring hits. ESTs of *Homarus americanus* and their blastx identifications are presently available at www.ncbi.nlm.nih.gov.

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3. **Spees, J.L., S.A. Chang, M.J. Snyder, and E.S. Chang.** Thermal acclimation and stress in the American lobster, *Homarus americanus*: equivalent temperature shifts elicit unique gene expression patterns for molecular chaperones and polyubiquitin. *Cell Stress & Chaperones* 7: 97-106, 2002.