

well as their overall quantity as a subfraction of DOC, is only summarily known^{2,3,8,28}. Likewise, we lack precise knowledge about the real straining (and aggregation or agglutination) capacity of oikopleurid food-concentrating filters¹². But even a very conservative estimate of 10% of DOC as grazable by oikopleurids, means that this source of food is as important for the oikopleurids as is total POC. This agrees well with previous findings that POC accounts for a maximum of 30% of the energy needs of *O. dioica*^{19,20}.

Oikopleurid tunicates often occur in high densities in discrete strata at various depths^{21,22}, and may under such conditions clear 30–60% of the water mass in 24 h^{1,4}. Obviously, they may remove and repack colloidal DOC (>0.2 µm particle size) rapidly under such conditions. On the basis of filter parameters, this ability to graze on colloidal DOC is probably shared by caddisfly larvae²³, pedal worms²⁴, ascidians²⁵, salps²⁶ and amphioxus⁹. □

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Sequence identification of 2,375 human brain genes

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WE recently described a new approach for the rapid characterization of expressed genes by partial DNA sequencing to generate 'expressed sequence tags'¹. From a set of 600 human brain complementary DNA clones, 348 were informative nuclear-encoded messenger RNAs. We have now partially sequenced 2,672 new, independent cDNA clones isolated from four human brain cDNA libraries to generate 2,375 expressed sequence tags to nuclear-encoded genes. These sequences, together with 348 brain expressed sequence tags from our previous study, comprise more than 2,500 new human genes and 870,769 base pairs of DNA sequence. These data represent an approximate doubling of the number of human genes identified by DNA sequencing and may represent as many as 5% of the genes in the human genome.

Most (83%) of the 2,375 partial cDNA sequences reported here (Table 2) are not related to any previously described sequences. Based on database matches to known genes from humans and from such evolutionarily distant organisms as *Escherichia coli*, yeast, *Caenorhabditis elegans*, *Drosophila*, barley, *Arabidopsis*, rice and green algae, we have putatively identified 217 of the expressed sequence tags (ESTs; Table 1). These include a novel gene similar to *Notch/TAN-1* (refs 1, 2), a new neurotransmitter transporter gene, and a new member of the multidrug resistance gene family. Several genes involved in development or cell differentiation in *Drosophila* are represented by similar human ESTs, including *seven in absentia*³, *big-brain*⁴, the *discs-large* tumour suppressor⁵ and the homeotic gene *orthodenticle*⁶. New members of previously known gene families in humans include a Ca²⁺-transporting ATPase, an ADP ribosylation factor and a new neural-cell adhesion molecule gene.

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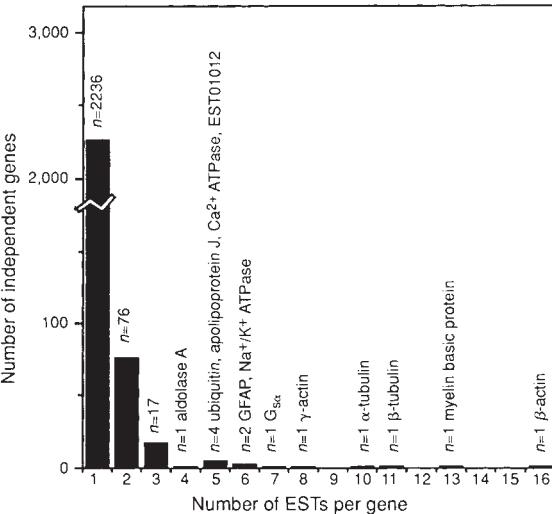


FIG. 1 Redundancy of sequencing of ESTs. The number of putatively identified EST clones plus the groups of ESTs that form contigs are plotted against the number of independent genes represented. The number of genes is given above each bar, with the names of the genes for redundancies of 4–16. We define redundancy as the number of times each gene is represented by an EST; for example β-actin has an EST redundancy of 16. One five-member EST contig was constructed that did not match any known sequences, indicating that at least one common transcript in brain, EST 01012, has not been reported previously. GFAP, glial fibrillary acidic protein.

The 1,971 ESTs without a putative identification were analysed using the coding-region prediction program CRM with the GRAIL server⁷. Some of the unknown ESTs (15%) scored a likely probability of containing protein-coding sequence. Half of the ESTs to known human genes contain protein-coding sequences, so at most half of the unknown ESTs are likely to contain coding sequences. We have found no evidence that genomic DNA or cDNA to unspliced precursor RNA is a major contaminant of either the hippocampus or fetal brain library.

The limited extent of redundancy of EST sequencing is shown in Fig. 1. Of the nuclear-encoded messenger RNAs, the most common ESTs were to the β-actin (0.6% of the EST clones)

TABLE 1 Gene composition of human brain cDNA libraries

EST	Putative Identification	Species	DB	Accession	Length	%ID	EST	Putative Identification	Species	DB	Accession	Length	%ID
EST02243	14,3,3 protein (PKI regulator)	H (G) HUM1433	272	98.9	EST01651	Lactate dehydrogenase A	H (G) HUMLDHA	378	99.5				
EST01784	2',3'-cyclic nucleotide phosphodiesterase	H (P) A28209	272	98.5	EST01764	Lactate dehydrogenase B	H (G) HUMLDHB	401	99.5				
EST01455	60K filarial antigen	A (P) A28209	88	50.6	EST01764	Lamin B receptor	C (P) A36427	76	71.4				
EST01982	80K-H protein	H (G) HUMG1991A	263	97.3	EST01724	Ion protease	E (P) J00901	103	41.3				
EST02077	ADP-ribosylation factor 1	H (P) B3283	84	41.2	EST02463	Long-chain-fatty-acid-CoA ligase	R (P) A36275	36	62.2				
EST01620	AMP deaminase, brain	H (G) HUMTICA	200	96.5	EST02418	MARCKS homolog	M (E) MHE52	237	92.4				
EST01504	Actin, beta, cytoskeletal	R (P) A37056	57	100.0	EST01962	MHC class I HIA-O2 heavy chain	H (G) HUMHCA2B	231	97.8				
EST01543	Actin, gamma, cytoskeletal	H (G) HUMACTGN	359	98.9	EST02503	MHC class III HSP70-1 gene (HEA)	H (G) HUMHBBP	181	100.0				
EST01891	Actinin, alpha	H (G) HUMACTAR	315	81.6	EST01918	Mycobacterium tuberculosis	R (G) RATMTRN3	137	93.5				
EST01801	Adaptin, beta	H (G) HUMADPTA	380	99.5	EST01863	Microlute-associated protein 1B	H (G) HUMMET	236	99.2				
EST01710	Adenosine deaminase	H (G) HUMADAG	316	100.0	EST01673	Microlute-associated protein 4	R (G) RATNEU	293	86.4				
EST01625	Argin	R (G) RATAGR	103	84.6	EST01673	Milk fat globule membrane protein	H (G) HUMMAP4	249	97.6				
EST02113	Ala	H (G) HUMALA	92	82.8	EST01704	Monooxygenase A (MOXA)	M (P) A36479	48	61.2				
EST00675	Alcohol dehydrogenase	I (H) RICGOS2_1	38	59.0	EST01629	Myelin basic protein	H (G) HUMMOA	255	98.8				
EST01660	Alkalase A	H (G) HUMALDA	346	98.0	EST01629	Myelin basic protein	H (G) HUMMBB	314	99.4				
EST01761	Alkalase C	H (G) HUMALDC	317	99.0	EST02585	Myeloid differentiation primary response	M (H) MUSMD118_1	76	88.3				
EST02688	Alpha-Endonuclease	H (G) HUMENGA	345	98.8	EST01614	Mycobacterium cell surface antigen 24.1D5	H (G) HUMA21D5	177	98.3				
EST01635	Alpha-2-Macroglobulin	H (G) HUMENGA	319	99.4	EST01672	Myoisin heavy chain, non-muscle	H (G) HUMMMY	291	99.3				
EST01664	Amyloid A4	H (P) A29030	52	54.7	EST01744	N-formylpeptide receptor	H (G) HUMMP	237	99.2				
EST01701	Anion exchanger homolog AE3	M (P) A33638	95	97.9	EST01338	NAD(P)+ transhydrogenase (B-specific)	B (P) DEBOMX	86	93.1				
EST01585	Apolipoprotein J	H (G) HUMPOJ	317	99.0	EST01805	Na+/K+ ATPase, alpha subunit	H (G) HUMPSNFB	98	98.0				
EST01825	Aspartate aminotransferase, cytosolic	H (G) HUMASAM	309	98.7	EST02610	Neural cell adhesion molecule 1	H (G) HUMATPIA2	277	99.6				
EST02671	Aspartate aminotransferase, mitochondrial	H (G) HUMASAT	231	99.1	EST01473	Neuraxin	M (P) S05479	82	43.4				
EST01634	Axonal glycoprotein TAG-1	R (P) A34695	69	87.1	EST01519	Neurofibromatosis type 1	R (P) S06017	120	84.3				
EST02530	B cell-specific Mo-MLV integration site	M (G) MUSM11A	111	87.5	EST01749	Neurofilament heavy chain	H (G) HUMF1MNA	369	98.6				
EST02306	Biotin protein	D (P) S06969	57	53.4	EST02455	Neurofilament subunit M (NF-M)	H (G) HUMFFH	356	99.7				
EST01443	CDP-SO-P	E (P) J00368	33	41.2	EST02603	Neutrophil oxidase factor	H (G) HUMFFM	164	99.4				
EST01800	Ca2+-transporting ATPase	H (G) HUMK1KA	380	99.2	EST00632	Nodulation protein I	K (P) A24400	63	39.1				
EST02146	Calbindin D28	R (G) RATALB028	81	87.8	EST01643	Nodulation protein I	K (P) A24400	71	50.0				
EST01823	Calcineurin A2	H (G) HUMCRNAB	207	99.7	EST01961	Notch/Xtch homologue	H (H) HUMDNL_1	85	57.0				
EST02055	Calcium channel	D (P) S05054	33	67.6	EST02423	Nuclear factor l-like protein (NFI)	H (G) HUMF1IA	111	92.0				
EST01849	Calmudulin	H (G) HUMC1A	327	98.5	EST01573	Nucleoside diphosphate kinase	H (P) A33386	71	52.8				
EST01446	Calmudulin-dependent PKI, BII	R (P) A26464	93	98.9	EST01657	Osteonectin/SPARC	H (G) HUMSPARC	348	99.7				
EST02378	CAMP-dependent PKI inhibitor	M (G) MUSPK1	234	91.5	EST01822	Osteopontin	H (G) HUMOP	170	96.5				
EST01644	CAMP-dependent PKI regulatory subunit RIIB	H (G) HUMRIB	198	97.5	EST01826	Otto homeotic protein	D (P) A35912	35	52.8				
EST01628	CAMP-dependent PKI regulatory subunit RIIB	H (G) HUMPKCMD	294	98.3	EST01486	Pancreatic tumor-related protein	H (G) HUMPCAN	422	99.3				
EST01041	CAMP-regulated phosphoprotein	B (P) B35308	21	86.4	EST01798	Peptidylprolyl isomerase (cyclophilin)	H (G) HUMCYC	382	99.5				
EST02447	CAMP-specific phosphodiesterase	H (G) HUMDEA	363	69.0	EST01751	Phe-4,5-BPI	R (P) A28807	40	90.2				
EST01536	Cannabinoid receptor	H (S) CANRHUMAN	97	93.9	EST01656	Phosphoglycerate kinase	H (G) HUMBGK11	374	98.7				
EST01795	Carboxypeptidase E	H (G) X1405	330	99.4	EST00992	Polymyxin B resistance	Y (P) A32714	20	76.2				
EST01606	Casein kinase II alpha subunit	H (G) HUMCKII	186	97.3	EST01805	Prohibitin	R (H) RATPROHIB	120	97.5				
EST01733	Catalase	H (G) HUMCATR	270	96.3	EST01775	Prohormone cleavage enzyme	M (H) MUSMPCA1_1	91	93.5				
EST01810	Cathepsin D	H (G) HUMCHD	246	98.4	EST01461	Protein kinase C, beta subunit	H (G) HUMPCB	155	94.9				
EST01799	Cell surface glycoprotein MUC18	H (G) HUMMUC1B8	413	97.9	EST02087	Protein kinase C, zeta	H (G) HUMPCCL	382	58.7				
EST01487	C/D PG40	H (G) HUMCSPG1A	261	100.0	EST01650	Protein phosphatase 2A beta subunit	H (G) HUMPCP2AB	286	76.6				
EST01913	Clathrin coat assembly protein homologue	Y (H) YSCYPA54_1	62	63.5	EST01584	PR65 (alpha)	H (G) HUMPCP2A	242	98.8				
EST01796	Coagulation factor VII	H (G) HUMCFVII	227	97.8	EST01786	Protein-tyrosine kinase (clone JTK14)	H (P) C38269	38	100.0				
EST01676	Cofilin	P (P) PICOFOL	132	89.5	EST01572	Protachlorophyllide reductase	W (P) S04783	34	57.1				
EST01774	Cysteine proteinase inhibitor	H (G) HUMCYSTR	163	97.6	EST01538	Pryvate dehydrogenase alpha subunit	H (G) HUMDHA	322	99.7				
EST01824	Cysteine-rich intestinal protein	R (P) GY71	66	67.7	EST01587	Pryvate kinase isozyme M2	H (G) HUMPK2L	395	98.5				
EST01502	Cytochrome P-4501E1	H (G) HUMCYP1E	175	97.2	EST02683	Rab1	H (H) HUMRASLP_1	78	97.5				
EST01951	Cytochrome c	H (G) HUMCYCAA	172	96.0	EST02515	Rab5b	H (P) F34323	91	82.6				
EST01808	DNA binding protein YAVR667	H (G) HUMYARBP	429	98.8	EST01072	Rac protein kinase	H (G) HUMAPCP	91	100.0				
EST01721	DNA repair helicase (ERCC3)	H (G) HUMERCC3A	334	98.5	EST01389	Radial spoke protein 3	F (P) S05962	58	52.5				
EST01622	DNFL152 (lung) mRNA	H (G) HUMDNFA	137	96.4	EST01787	Retinaldehyde-binding protein	H (G) HUMRA1BPC	129	100.0				
EST02577	Dicylglycerol kinase, lymphocyte	P (P) S09156	44	42.2	EST01579	Retrovirus-related gag polyprotein	H (P) F0082	95	77.1				
EST02477	Diamine acetyltransferase	H (S) ATADPHAM	74	45.3	EST02550	Retrovirus-related pol polyprotein	H (P) CNLQL	50	54.9				
EST01508	Dihydrolipoamide acetyltransferase	H (G) HUMPC02	254	98.4	EST01578	Ribophorin II	H (G) HUMRBIIR	289	99.3				
EST00642	Dilute (myosin heavy chain)	M (H) MUSDILUTE_1	27	100.0	EST01928	Ribosomal phosphoprotein P0	H (G) HUMPARP0	273	98.2				
EST01779	Discs-large tumor suppressor	D (H) DRDGLA	53	63.0	EST02158	Ribosomal phosphoprotein P1	H (G) HUMPARP1	126	91.3				
EST02627	Elongation Factor 1 alpha	H (G) HUMEF1A	361	99.2	EST01583	Ribosomal protein L18a	R (P) A5R118	68	95.7				
EST01165	Elongation Factor 1 beta	N (P) A24066	36	64.9	EST01627	Ribosomal protein L1a	X (P) A24579	75	63.1				
EST02596	Enolase, gamma-2, neuron specific	H (G) HUMENOG	345	98.5	EST01667	Ribosomal protein L3	O (P) J00771	74	80.0				
EST01743	Epoxide hydrolase	H (G) HUMENPM	362	99.7	EST01826	Ribosomal protein S10	M (P) R3M10	36	51.4				
EST01946	Ezrin	H (G) HUMEZRIN	153	99.3	EST01459	Ribosomal protein Y110	Y (P) S11581	40	68.3				
EST01971	Familial adenomatous polyposis coli	H (G) HUMFAPC	316	98.1	EST01608	Ribosomal protein S3	H (G) HUMHBM3	279	95.7				
EST01325	Fatty acid synthase	R (G) RATTFS	98	79.8	EST02442	Seven in absentia	D (P) A36195	46	80.8				
EST01476	Fibrillarin	H (G) HUMFIBA	201	99.5	EST01960	Spectrin, beta	H (G) HUMSPIT	268	67.7				
EST01790	Fibroblast growth factor receptor	H (G) HUMFGF1A	376	99.2	EST01699	Sperm membrane protein	R (P) A35981	52	58.5				
EST01967	Filoblastin	H (G) HUMFGM	120	98.3	EST01760	Spermidine/spermine N1-acetyltransferase	H (G) HUMSPDMAT	102	97.1				
EST02186	Fodrin, alpha	H (G) HUMFASXP	282	98.2	EST01575	Sphingomyelin phosphodiesterase	H (G) HUMSM2M	159	97.5				
EST02428	Fumarate hydratase, mitochondrial	H (G) HUMFMU	96	96.9	EST01984	Stathmin (p18)	H (G) HUMSP	180	100.0				
EST01665	G(i) alpha	H (G) HUMG1A	310	98.1	EST01697	Succinate dehydrogenase flavoprotein	B (H) BOVSUDHPL_1	44	100.0				
EST02389	G(i) alpha	H (G) HUMGSA1	275	99.3	EST00742	Synaptotagmin (p65)	H (S) SY65SHUMAN	27	53.6				
EST02362	Gα binding protein, beta subunit	M (H) MUSGAC_1	86	90.8	EST01586	T-cell surface glycoprotein E2	H (G) XI6996	277	99.6				
EST01745	GTP-binding protein beta chain homologue	H (G) HUMMB123	319	99.1	EST01809	TAPA-1, 26-kDa cell surface protein	H (G) HUMTAP1	271	98.5				
EST00825	Gamma-aminoacidic acid transporter	R (P) A35918	26	59.3	EST01939	Thy-1 glycoprotein	H (G) HUMTCB10	162	98.8				
EST01738	Gelatinase precursor, plasma	H (P) A37098	74	80.0	EST01575	TFEB transcription factor	H (G) HUMTEB	369	99.1				
EST01776	Gelsolin precursor, plasma	H (G) HUMGPGS	171	99.4	EST02402	Talin	M (H) MUSTALIN_1	79	81.2				
EST01649	Glial fibrillary acidic protein	H (G) HUMGFAP	399	99.2	EST01601	Thiosulfate sulfurtransferase (rhodanese)	B (P) ROBO	65	81.8				
EST01965	Globin, gamma	H (G) HUMGG1J	147	100.0	EST01435	Threonine 1-tRNA synthetase	H (G) HUMHSYNT	228	99.6				
EST02192	Glutamate decarboxylase	H (G) HUMGDA	213	100.0	EST02420	Thrombospondin precursor	H (G) HUMSPA	58	98.3				
EST0702	Glutamate dehydrogenase	H (G) HUMGDH	223	96.4	EST01783	Ty-1 glycoprotein	H (G) HUMTHY10	353	98.6				
EST02446	Glutamate-aspartate carrier protein	E (P) JV0092	57	37.9	EST01235	Tyrosin kinase	H (G) HUMTHY10	88	97.8				
EST02034	Glutaminase	P (P) JV0092	34	74.3	EST02451	Transcription factor ETR101	H (G) HUMTR101	287	99.3				
EST01576	Glyceraldehyde-3-phosphate dehydrogenase	H (G) HUMG3PD	428	99.8	EST01705	Trasducin beta-2 subunit	H (G) HUMTRNB	126	100.0				
EST01621	GNA-B-associated transcript 2 (BAT2)	H (G) HUMBAT2	301	98.7	EST02047	Triose-phosphate isomerase	H (G) HUMTR1	258	98.8				
EST01462	Heat shock cognate protein 70	H (G) HUMMSC70	290	97.2	EST01512	Tubulin, alpha	H (G) HUMTBAG	223	75.0				
EST01637	Heat shock protein 90	H (G) HUMMS90	265	99.6	EST01490	Tubulin, beta	H (G) HUMTBBS	298	93.6</				

TABLE 2 Gene composition of human brain cDNA libraries

	Total	Hippocampus unscreened	Hippocampus prescreened	Fetal brain*	Fetal brain†	Whole brain‡
No database match	1,942	474	394	1,025	32	17
Exact human match	255	76	88	87	0	4
Non-exact human match	51	8	10	33	0	0
Non-human match	99	34	26	35	2	2
Alu repeat	313	70	70	172	1	0
L1 repeat	58	13	5	39	0	1
THE-ltr sequence	17	1	1	14	1	0
Other repeat	9	3	4	2	0	0
Mitochondrial	181	115	33	27	0	6
rRNA	57	29	7	16	5	0
poly(A) insert only	339	171	161	5	0	2
Total	3,321	994	799	1,455	41	32

Four cDNA libraries were used as sources of clones for sequencing. Human hippocampus and fetal brain (*) libraries, plasmid template preparation, sequencing reactions, and automated sequencing were performed as described¹. A pooled probe consisting of inserts from 10 different EST clones with sequences that matched either mitochondrial genes or the 18S or 28S rRNAs was used to prescreen a gridded filter array of the hippocampus library; nonhybridizing clones are referred to as the 'prescreened library'. Another fetal brain library (†) was constructed by and was a gift from B. Soares (Columbia University). A directionally-cloned library (‡) was prepared¹⁴ using human adult brain mRNA from Clontech (Palo Alto). Of 482 clones analysed by restriction-enzyme digestion, 33% contained inserts at least 1,500 base pairs long. Stratagene hippocampus and fetal brain library totals include data from ref. 1. Sequences of nuclear-encoded cDNAs that did not include the interspersed repeats Alu, L1 or THE-ltr^{15–17} were searched against GenBank and, in 6-frame translation, against a comprehensive, non-redundant peptide database using the network BLAST¹⁸ server at the National Center for Biotechnology Information. For significant similarities, a putative gene name and protein identification resource (PIR) gene family identification¹⁹ for the EST were assigned. ESTs without significant matches using BLAST were searched in translation against PIR using the program FASTA. Ten additional marginal matches were found. A total of 2,300 new EST sequences comprising 765,505 nucleotides from the current data set have been submitted to GenBank and assigned accession numbers M77851–M79278 and M85308–M86179. All ESTs except those multiply representing actin, tubulin, and myelin basic protein clones were submitted. cDNA clones from which ESTs were derived are available from the American Type Culture Collection (Rockville, Maryland) with accession numbers 77501–78999 and 81000–81756. The Genome Data Base²⁰ expressed D-segment numbers for these clones are DOS1E–DOS2300E. We have developed a database which includes the clone and sequence data, sequence analysis results, physical mapping data, tissue localization and cross-references to the public databases and distribution of the clones, mapping and sequence data using the Sybase relational database management system (Sybase Inc., Emeryville, California). Comprehensive reports on the sequences described in this paper are available in electronic form. A README file describing how to access the EST database reports is available via anonymous file transfer protocol (FTP) to briggs.ninds.nih.gov. Questions on data access and database structure can be addressed via electronic mail to arkerlav@briggs.ninds.nih.gov.

and myelin basic protein genes (0.5% of the clones). Myelin basic protein, a highly expressed structural component of nerve tissue⁸, displays four alternate splicing forms, of which at least two are present among the ESTs reported here. Other common ESTs were G-protein subunit G_{αs}, γ-actin and both α- and β-tubulin.

All of the genes for which four or more ESTs were found have been sequenced in humans, except for one which was matched by five unknown ESTs. Assuming that most brain mRNAs are rare transcripts⁹, the chance of finding a new gene by EST sequencing is fairly high when ribosomal and mitochondrial transcripts are eliminated. Therefore, although normalization may be important as we near closure in sequencing every human gene, it is not necessary at this stage to reduce sequencing redundancy or to increase gene diversity. Furthermore, a certain amount of redundancy is desirable to the extent that it promotes assembly of EST contigs into full-length cDNA sequences.

By matching ESTs to known database sequences, a phenotypic characterization of the tissue begins to emerge. Protein superfamilies matched by ESTs were grouped into three broad functional categories to assess the biological spectrum represented

by these randomly selected cDNA clones. Structural and metabolic classes comprised about 30% of the ESTs each, 25% were involved in regulatory pathways and the remainder were not classifiable. Eleven of the eighteen enzymes of glycolysis and the citric acid cycle are represented by at least one subunit or isozyme. In addition, several genes not previously known to be expressed in the brain were matched, including spermine/spermidine acetyltransferase¹⁰ and osteopontin¹¹. Isolation of 171 ESTs from mouse testes was recently reported¹², including four with database matches in common with human ESTs.

The genomic mapping of these new human expressed genes is among our highest priorities. Physical mapping of the 2,375 EST clones reported here would provide human chromosome markers spaced an average of 1.2 megabases apart and would roughly double the number of expressed sequences that have been localized to chromosomes¹³. Mapped ESTs are a new resource for identifying candidate genes for the estimated 5,000 single-locus diseases¹³. All the sequences and clones described here are publicly available (Table 2). We shall update EST clone identification and map information through the NIH cDNA database. □

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