STACK: Sequence Tag Alignment and Consensus Knowledgebase

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ABSTRACT

STACK is a tool for detection and visualisation of expressed transcript variation in the context of developmental and pathological states. The data-system organises and reconstructs human transcripts from available public data in the context of expression state. The expression state of a transcript can include developmental state, pathological association, site of expression and isoform of expressed transcript. STACK consensus transcripts are reconstructed from clusters that capture and reflect the growing evidence of transcript diversity. The comprehensive capture of transcript variants is achieved by the use of a novel clustering approach that is tolerant of sub-sequence diversity and does not rely on pairwise alignment. This is in contrast with other gene indexing projects. STACK is generated at least four times a year and represents the exhaustive processing of all publicly available human EST data extracted from GenBank. This processed information can be explored through 15 tissue-specific categories, a disease-related category and a whole-body index and is accessible via WWW at http://www.sanbi.ac.za/Dbases.html. STACK represents a broadly applicable resource, as it is the only reconstructed transcript database for which the tools for its generation are also broadly available (http://www.sanbi.ac.za/CODES).

INTRODUCTION

Expressed sequence tags (ESTs) represent single pass reads from the 5′ and/or 3′ end of cDNA clones. These error-prone sequences gain significant biological value if they are reconstructed into consensus transcripts. The complete combined sequence of all expressed exons within a gene, a truly complete mRNA, would be an ideal scaffold for constructing a set of non-redundant transcripts. However, complete cDNA sequences such as these are not broadly represented in cDNA libraries and seldom extend to the 5′ cap of the transcript. The scarcity of full-length mRNA sequences and the abundance of ESTs has led a number of groups to attempt to add value to EST data by constructing gene indices (1–3). STACK differs from other gene indexing projects in that it is focused on the capture of context and form of expression and through this a reconstruction of a contextual transcript is attempted. The broad implementation of alignment based clustering tools can narrow the capture of potential variation within gene expression products. STACK does not rely on alignment based clustering tools to perform clustering, but instead incorporates a novel, loose clustering approach, d2_cluster, that performs comparisons via non-contextual assessment of the composition and multiplicity of words within each sequence (4,5). The non-alignment-based approach tends to capture transcript variants and contaminating sequences that could represent chimeric clones or aberrant transcripts associated with disease (4–6). The use of a loose clustering approach also allows for the incorporation of sequences that would otherwise be discarded as poor quality sequence. The inclusion of low quality sequence in STACK has led to the development of error checking tools to assess the integrity of each cluster, and in some cases, to elongate reconstructed transcripts.

STACK GENERATION

Tissue data sets

The STACK database represents the processing of all publicly available human EST data extracted from GenBank. ESTs are first partitioned arbitrarily into tissue/state context bins followed by removal of contaminating sequences such as vector, low complexity, mitochondrial and ribosomal sequences. Each tissue-grouped set is sent through a pipeline comprising of clustering, assembly, consensus generation and clone linking (7). ESTs are clustered using d2_cluster (5), which attempts to capture alternate expressed forms of a gene within the same cluster, in contrast to other protocols that may discard sequences containing ‘noise’. Alignment assemblies are generated using the PHRAP package (http://www.phrap.org) and the aligned clusters are used as input for assembly analysis using stack_Analyze and CRAW (6,7). Assembly artifacts and isoforms are partitioned within a cluster and the longest consensus sequence is assigned to a given cluster, while additional sub-consensus sequences are captured within each record if they exist. Sequences that originate from the same

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Table 1. STACK clustering and clonelinking information

Tissue	Input sequences	Singletons	Multi-sequence clusters	Linked sequences	Average length of linked sequences (bases)
Adipose	2376	1693	181	0	
Brain	229 005	46 336	22 588	11 018	1128.8
Cochlea	8494	3543	1220	265	746.8
Connective	47 081	14 433	5197	1626	973.2
Digestive	142 659	20 430	10 643	1971	1444.7
Disease	327 613	53 074	14 423	2582	948.6
Eye	28 554	14 308	3486	4207	789.5
Genomic	166 358	42 795	16 918	1740	749
Gland	172 314	34 629	16 972	4734	1245.2
Heart	73 941	21 902	8795	3728	922
Hemato-lymph	307 285	60 009	24 947	11 674	928.3
Lung	96 917	22 218	12 669	4213	978.9
Muscle	30 512	5454	2338	1165	881.1
Olfactory	2600	1478	248	458	664
Other	26 175	9637	4356	3762	1016.6
Reproductive	293 311	50 199	28 218	10 353	1342.6
Whole-body index[a]	937 782	159 743	66 081	7099	1948.3

[a] The whole-body index represents the processing of dbEST (GenBank110) and whereas the STACK tissue-level data represents processing of dbEST (GenBank115).

cDNA clone are traced and corresponding clusters are joined by a simple linker sequence producing extended STACK linked consensus entries (7; Table 1).

Whole-body index

Tissue-based clusters, their consensus sequences and mRNAs extracted from GenBank have been used to create the STACK whole body index. Tissue-level consensus sequences are decomposed to their constituent ESTs prior to a PHRAP assembly in order to maximise the alignment accuracy over EST reads that are of low quality. The whole-body index data is subjected to assembly analysis and consensus generation. Radiation hybrid mapping information is integrated into the consensus sequences using ePCR (8). The final consensus sequence is presented in FASTA format where the header line captures the unique STACK-ID, the GenBank accession numbers for each of the constituent ESTs, the original clone libraries and mapping information if it exists.

INCORPORATING RADIATION HYBRID (RH) MAPPING INFORMATION

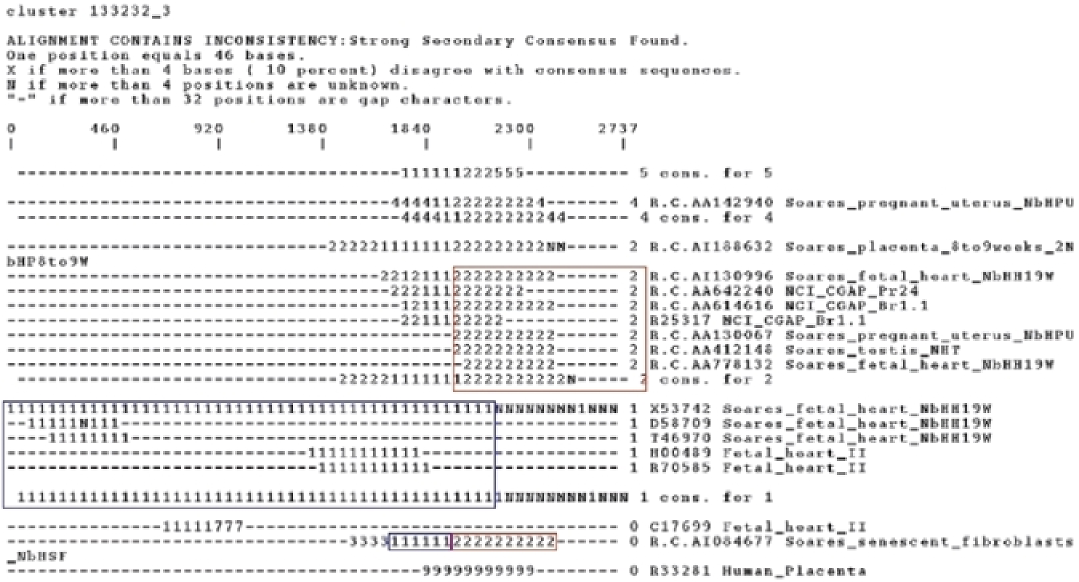
Mapping methodologies have centred around the use of sequence tag sites (STSs) as unique landmarks across the genome (9). EST-based landmarks entered the realm of feasibility when it was demonstrated that single-pass sequences provide suitable templates for the design of gene-based STSs (10). An international consortium was established to develop STSs from ESTs for mapping studies using primarily RH techniques (2). Approximately 1000 genetic markers from the Genethon map were included in the analysis to serve as a mapping framework and to allow gene positions to be related to genetic linkage information. Recently, about 41 000 markers were placed onto RH panels and formed the basis of Genemap'98 (11).

EST sequencing is intrinsically inadequate for identifying truly rare genes (12). Therefore, the STS pool that was generated from the EST data will tend not to capture rare transcripts and as a result, STSs would provide optimistic estimates of cluster accuracy. We have used RH markers to assess the fidelity of the STACK consensus sequences. A total of 52 882 RH markers were used to assign mapping information to STACK indices using ePCR (8). An analysis of our mapping information suggests a 1.08-fold redundancy in STACK clusters. The assignment of more than one cluster to a STS could represent the capture of paralogous genes in different clusters. Clusters containing inconsistent map locations account for 0.6% of the total clusters, which correlates with the error rates reported in the mapping laboratories. The availability of map locations within the STACK database provides a resource for positional candidate gene selection relevant to both physical location and source of gene expression.

GENE DISCOVERY RESOURCE

Capture of alternate gene expression forms

Databases such as TIGR and UniGene have focused on reconstructing the gene complement of the human genome and their technological developments have been directed towards achieving that goal. STACK, however, focuses on the detection



```
cluster 133232_3

ALIGNMENT CONTAINS INCONSISTENCY:Strong Secondary Consensus Found.
One position equals 46 bases.
X if more than 4 bases ( 10 percent) disagree with consensus sequences.
N if more than 4 positions are unknown.
"-" if more than 32 positions are gap characters.

0       460       920      1380      1840      2300      2737
|         |         |         |         |         |        |

 -------------------------------------11111222555---------- 5 cons. for 5

-------------------------------------444411222222224------ 4 R.C.AA142940 Soares_pregnant_uterus_NbHPU
 -------------------------------------4444112222222244----- 4 cons. for 4

-------------------------------2222211111112222222222NN--- 2 R.C.AI188632 Soares_placenta_8to9weeks_2N
bHP8to9W
------------------------------------22121112222222222----- 2 R.C.AI130996 Soares_fetal_heart_NbHH19W
-------------------------------------2221112222222-------- 2 R.C.AA642240 NCI_CGAP_Pr24
--------------------------------------121112222222222----- 2 R.C.AA614616 NCI_CGAP_Br1.1
--------------------------------------2211122222---------- 2 R25317 NCI_CGAP_Br1.1
-------------------------------------------2222222222----- 2 R.C.AA130067 Soares_pregnant_uterus_NbHPU
-------------------------------------------2222222222----- 2 R.C.AA412148 Soares_testis_NHT
--------------------------------------------222222222----- 2 R.C.AA778132 Soares_fetal_heart_NbHH19W
 -------------------------------2222211111112222222222N---- 2 cons. for 2

11111111111111111111111111111111111111111111111NNNNNNN1NNN 1 X53742 Soares_fetal_heart_NbHH19W
--11111N111----------------------------------------------- 1 D58709 Soares_fetal_heart_NbHH19W
----11111111---------------------------------------------- 1 T46970 Soares_fetal_heart_NbHH19W
-----------------------------11111111111------------------ 1 H00489 Fetal_heart_II
------------------------------11111111111----------------- 1 R70585 Fetal_heart_II

 11111111111111111111111111111111111111111111111NNNNNNN1NNN 1 cons. for 1

---------------11111777----------------------------------- 0 C17699 Fetal_heart_II
---------------------------------33331111112222222222----- 0 R.C.AI084677 Soares_senescent_fibroblasts
_NbHSF
----------------------------------------9999999999-------- 0 R33281 Human_Placenta
```

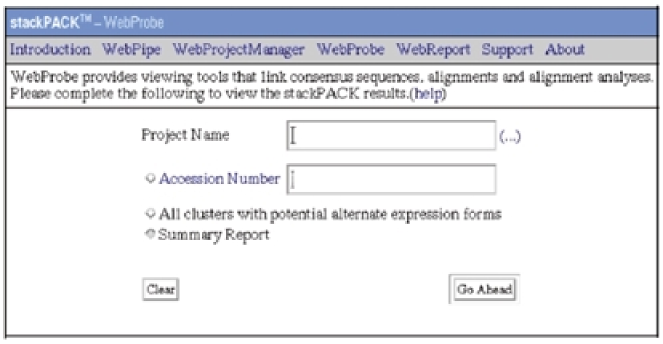
Figure 1. Craw output for a whole-body index cluster displaying alternate gene isoforms of the fibulin gene. The blue box indicates the region capturing the fibulin-1B isoform whereas sequences capturing fibulin-1C are surrounded by a red box.

and visualisation of transcript variation in the context of developmental and pathological states.

An apparent splice variant identified in a different cluster from its parent gene could be a close paralogue with a distinct genomic locus. However, alternatively spliced transcripts that capture important biological information within the same cluster assembly have to be handled appropriately. Two alternatively spliced transcript isoforms may contain regions of identity as well as disparate regions and so require specialised tools to capture the regions of dissimilarity. STACK applies CRAW as a post-assembly step to clustering and alignment in order to facilitate discrimination between distinct gene isoforms (6). Transcript variants are partitioned into sub-clusters that allow for simultaneous viewing of inconsistencies within a cluster. An example is fibulin (expressed in brain, parathyroid tumour, placenta, fibroblast, pancreas, heart, lung, testis, skin tumour) which exists as four or more isoforms (A–D) and each is clearly partitioned within the STACK whole-body index cluster 133232_3 (Fig. 1). Fibulin's B isoform (X53742) and its corresponding ESTs are displayed as a stretch of 1s in the ASCII representation of each sequence (Fig. 1, blue box). Sequences corresponding to isoform C have been partitioned into a sub-cluster displayed as a string of 2s (Fig. 1, red box).

Tissue specificity

STACK allows the user to rapidly explore 15 tissue categories, a disease-related category and a whole-body index. The tissue-based segmentation speeds disease analysis and functional annotation by providing the user with an immediate representation of areas of the body where a gene is expressed as well as detailed library information that pinpoints the expression location for primary and alternate expression transcripts. For example, STACK was used in the characterisation of the retinitis pigmentosa (*RP1*) gene. Matches with an eye-specific transcript in STACK were used successfully in the mapping of retina-specific ESTs to chromosomal regions that coincide with the *RP1* locus (13).



stackPACK™ – WebProbe

Introduction WebPipe WebProjectManager WebProbe WebReport Support About

WebProbe provides viewing tools that link consensus sequences, alignments and alignment analyses. Please complete the following to view the stackPACK results.(help)

Project Name [ ] (...)

○ Accession Number [ ]

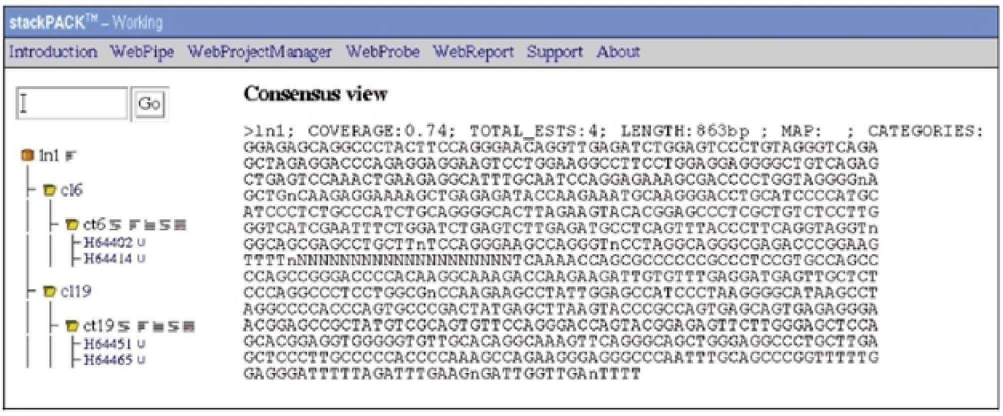
○ All clusters with potential alternate expression forms

● Summary Report

Figure 2. WebProbe, the STACK database extraction and viewing tool. The STACK tissue category is used as input to the 'project name' field that returns a list of all the clustered information.

DATA ACCESS VIA THE WEB

The STACK database can be queried via the Web at http://www.sanbi.ac.za/stacksearch.html using a sequence as input. The BLAST search algorithms implemented in the search engine allow for both DNA and protein queries. The results of a blast query are hyperlinked to the STACK viewer, which allows for the extraction of detailed information pertaining to the matching STACK sequence (Figs 2 and 3). STACK consensus sequences matched to *Drosophila* sequences are searchable on the Drosophila Related Expressed Sequences



```
>ln1; COVERAGE:0.74; TOTAL_ESTS:4; LENGTH:863bp ; MAP:  ; CATEGORIES:
GGAGAGCAGGCCCTACTTCCAGGGAACAGGTTGAGATCTGGAGTCCCTGTAGGGTCAGA
GCTAGAGGACCCAGAGGAGGAAGTCCTGGAAGGCCTTCCTGGAGGAGGGGCTGTCAGAG
CTGAGTCCAAACTGAAGAGGCATTTGCAATCCAGGAGAAAGCGACCCCTGGTAGGGGnA
GCTGnCAAGAGGAAAAGCTGAGAGATACCAAGAAATGCAAGGGACCTGCATCCCCATGC
ATCCCTCTGCCCATCTGCAGGGGCACTTAGAAGTACACGGAGCCCTCGCTGTCTCCTTG
GGTCATCGAATTTCTGGATCTGAGTCTTGAGATGCCTCAGTTTACCCTTCAGGTAGGTn
GGCAGCGAGCCTGCTTnTCCAGGGAAGCCAGGGTnCCTAGGCAGGGCGAGACCCGGAAG
TTTTnNNNNNNNNNNNNNNNNNNNNNTCAAAACCAGCGCCCCCCGCCCTCCGTGCCAGCC
CCAGCCGGGACCCCACAAGGCAAAGACCAAGAAGATTGTGTTTGAGGATGAGTTGCTCT
CCCAGGCCCTCCTGGCGnCCAAGAAGCCTATTGGAGCCATCCCTAAGGGGCATAAGCCT
AGGCCCCACCCAGTGCCCGACTATGAGCTTAAGTACCCGCCAGTGAGCAGTGAGAGGGA
ACGGAGCCGCTATGTCGCAGTGTTCCAGGGACCAGTACGGAGAGTTCTTGGGAGCTCCA
GCACGGAGGTGGGGGTGTTGCACAGGCAAAGTTCAGGGCAGCTGGGAGGCCCTGCTTGA
GCTCCCTTGCCCCCACCCCAAAGCCAGAAGGGAGGGCCCAATTTGCAGCCCGGTTTTTG
GAGGGATTTTTAGATTTGAAGnGATTGGTTGAnTTTT
```

Figure 3. An example linked entry for the olfactory tissue. The clusters contributing to the linked entry are displayed together with the ESTs comprising each cluster. A mouse click on a specific clusterID executes the download of the FASTA formatted multi-sequence file. The hyperlinks to the right of the clusterID provides for the display of detailed information pertaining to a cluster such as phrap alignments, consensus sequence and assembly analysis information. UniGene links to each EST are provided if they exist.

(DRES) home page at the Telethon Institute of Genetics and Medicine (http://www.tigem.it).

Alternatively, all clustered data for a specific STACK tissue category can be accessed via WebProbe (http://www.sanbi.ac.za/stackpack/webprobe.html; Fig. 2). A query from this page returns a summary report with links to detailed information for all clusters and linked clusters contained within the specified tissue category. For example, Figure 3 illustrates a linked cluster for the olfactory tissue data set. Each cluster contributing to the linked entry is displayed with its constituent EST accession numbers and hyperlinks to additional information such as PHRAP alignments, consensus view and assembly analysis data. A FASTA formatted file containing all the sequences for a specified cluster can be downloaded by clicking on the clusterID. The WebReport link on the menu bar allows for downloading of cumulative data for a specific tissue category. These data files include all FASTA input files, consensus sequences, alignment files, alignments for all identified isoforms and assembly reports.

There are plans underway to integrate the STACK database under the sequence retrieval system (SRS) at the European Bioinformatics Institute.

ARCHITECTURAL OVERVIEW AND FUTURE DIRECTIONS

The future development of STACK focuses on linking the underlying data more firmly to biological processes and making the resultant information accessible to a widening range of users. Inclusion of genomic information will be used to map clusters and expression state to genome location, as well as to confirm the quality of the indices. Genome context also allows reorganisation of the index into exons and transcript isoforms. Prediction of proteins from transcript isoforms and cross-references to known protein records follow, opening the door for association with standardised annotations, such as Gene Ontology (http://www.geneontology.org).

Organisationally, STACK will make increasing use of the relational database architecture to enhance data access. Maintenance of clusterIDs, or links to new IDs, from release to release are planned. Entrez-styled querying capabilities are being planned for: (i) access to specialised subsets of the database; (ii) identification of isoforms based on physical or developmental expression states; or (iii) locating entries based on physical location within the genome. The above-mentioned functionality is intended to accelerate gene candidate discovery.

AVAILABILITY

STACK is freely available to academia and is distributed via the web site at http://www.sanbi.ac.za/CODES.

The STACK_PACK tool set performs clustering, clustering management, alignment processing and analysis and is freely available to academic institutions and is distributed from http://www.sanbi.ac.za/CODES.

SUPPLEMENTARY MATERIAL

Supplementary Material, available at NAR Online, includes the following information: a description of STACK, the architectural design and how to effectively search the STACK database. A detailed description of the STACK data format and STACK sequence retrieval can be obtained at http://www.sanbi.ac.za/STACK_description.html.

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