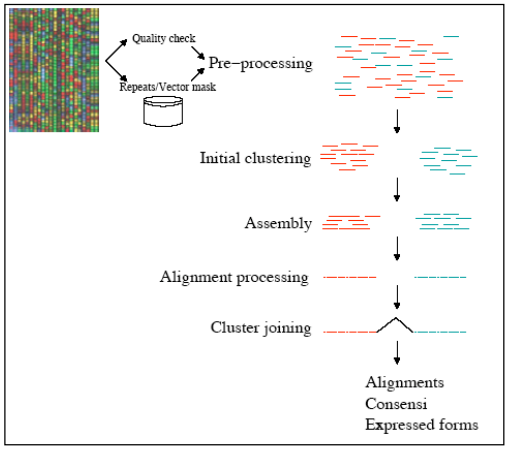
Expressed Sequence Tag (EST)



Vassilos Ioannidis - 2004
(modified from Lorenzo Cerutti, Victor Jongeneel, Anne Estreicher, …)

ESTs - outline

- **Introduction**
- **Improving ESTs**
  - pre-processing
  - clustering
  - assembling
- **Gene indices / UniGene & TIGR db**
- **Practical example**
- **Concluding Remarks**

Transcriptome sequencing

« Traditional » sequencing

cDNA clones isolated on the basis of some functional property of interest to a group

EST sequencing

Large-scale sampling of end sequences of all cDNA clones present in a library

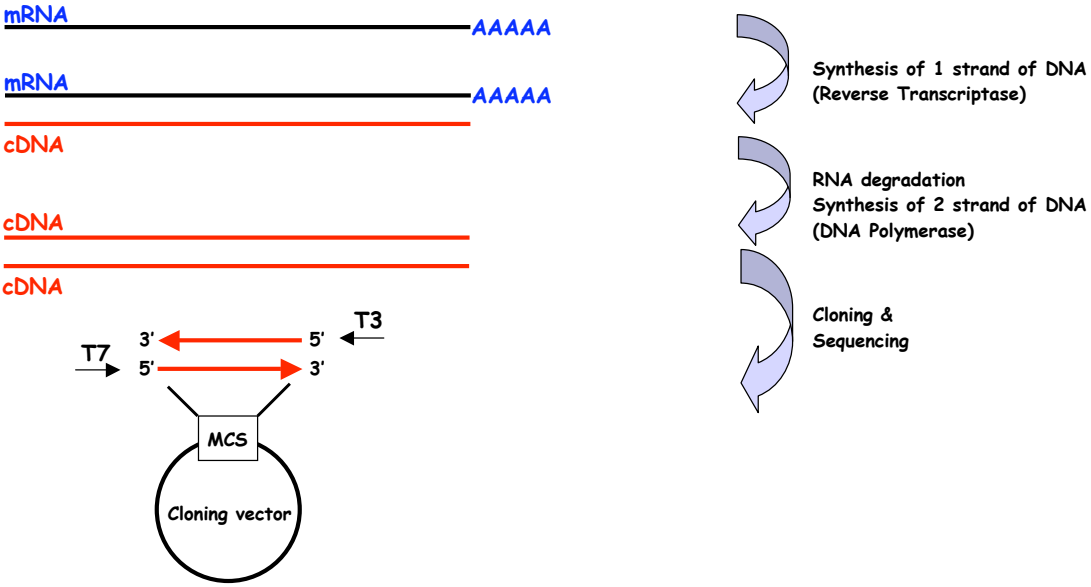
« Full-length » sequencing

Systematic attempts to obtain high-quality sequences of cDNA clones representing all transcribed genes

What are ESTs

- **cDNA libraries prepared from various organisms, tissues and cell lines using directional cloning**
- **Gridding of individual clones using robots**
- **For each clone, single-pass sequencing of both ends** (5' and/or 3') **of insert**
- **Deposit readable part of sequence in database**
- **ESTs represent partial sequences of cDNA clones (300 bp -> 700 bp)**

What are ESTs



Why EST sequencing?

- **Fast & cheap** (almost all steps are automated)
- **They represent the most extensive available survey of the transcribed portion of genomes.**
- **There are indispensable for gene structure prediction, gene discovery and genome mapping:**
  - -> provide experimental evidence for the position of exons
  - -> provide regions coding for potentially new proteins
  - -> characterization of splice variants and alternative polyadenilation
- **Provide an alternative to library screening**
  - -> short tag can lead to a cDNA clone
- **Provide an alternative to full-length cDNA sequencing**
  - -> sequences of multiple ESTs can reconstitute a full-length cDNA
- **Single Nucleotide Polymorphism** (SNP) **data mining**

cDNA libraries

- Most are "native", meaning that clone frequency reflects mRNA abundance
- Most are primed with oligo(dT), meaning that 3' ends are heavily represented
- The complexity of libraries is extremely variable
- "Normalized" libraries are used to enrich for rare mRNAs

cDNA libraries used

- Large number of libraries represented
- Most libraries managed by the IMAGE consortium (http://image.llnl.gov/)
- Human & mouse libraries are the most abundantly represented:

NCBI

dbEST: database of "Expressed Sequence Tags"

dbEST release 090304

Summary by Organism - September 3, 2004

Number of public entries: 23,416,084

Homo sapiens (human)	5,679,423
Mus musculus + domesticus (mouse)	4,246,846
Ciona intestinalis	684,280
Rattus sp. (rat)	683,238
Danio rerio (zebrafish)	575,250
Triticum aestivum (wheat)	561,713
Gallus gallus (chicken)	495,092
Bos taurus (cattle)	493,329
Xenopus laevis (African clawed frog)	432,424

- Many tissues still not sampled
- Quality very uneven

The data sources for clustering can be in-house, proprietary, public database or a hybrid of this (chromatograms and/or sequence files).

Each EST must have the following information:

- A sequence ID (ex. sequence-run ID)
- Location in respect of the poly A (3' or 5')
- The CLONE ID from which the EST has been generated
- Organism
- Tissue and/or conditions
- The sequence

The EST can be stored in FASTA format:

>T27784 EST16067 Human Endothelial cells Homo sapiens cDNA 5'
CCCCCGTCTCTTTAAAAATATATATATTTTAAATATACTTAAATATATATTTCTAATATC
TTTAAATATATATATATATTTNAAAGACCAATTTATGGGAGANTTGCACACAGATGTGAA
ATGAATGTAATCTAATAGANGCCTAATCAGCCCACCATGTTCTCCACTGAAAAATCCTCT
TTCTTTGGGGTTTTTCTTTCTTTCTTTTT.....

Public EST databases

- **EMBL/GenBank have separate sections for EST sequences**
- **ESTs are the most abundant entries in the databases** (>60%)
- **ESTs are now separated by division in the databases:**
  -> human, mouse, plant, prokaryote, ... (EMBL)
- **ESTs sequences are submitted in bulk, but do have to meet minimal quality criteria** ("Phred" score >20%, ie <1% error)

Private EST databases

(producing and selling access to EST data has proven to be a lucrative business...)

- **Human Genome Sciences** (http://www.hgsi.com/) **exploit the data itself, and get patents on promising genes found in its databases**

EST / EST databases quality

- **ESTs represent partial sequences of cDNA clones** (300 bp -> 700 bp)
  -> No attempt to obtain the complete sequence (no overlap necessary)
  -> A single EST represents only a partial gene sequence
  -> Not a defined gene/protein product

- **Single, unverified runs from the 5' and/or 3' ends of cDNA clones**
  -> high error rates (~1/100)
  -> frequent sequence compression and frame-shift errors

- **Trivial contaminants are common** (vector, rRNA, mitRNA, …)

- **Not curated in a highly annotated form**

- **High redundancy in the data** ("native" databases: clone frequency reflects mRNA abundance)

- **Databases are skewed for sequences near 3'-end of mRNAs** (normalization)

- **For most ESTs, no indication as to the gene from which they are derived**

Clone availability

- **In principle, all clones produced by IMAGE are publicly available**

Distributors:

- **US: ATCC** (http://www.lgcpromochem.com/atcc/) **and Invitrogen** (http://clones.invitrogen.com/cloneinfo.php?clone=est)
- **UK: HGMP** (http://www.hgmp.mrc.ac.uk/geneservice/reagents/index.shtml)
- **D: RZPD** (http://www.rzpd.de/products/clones/)

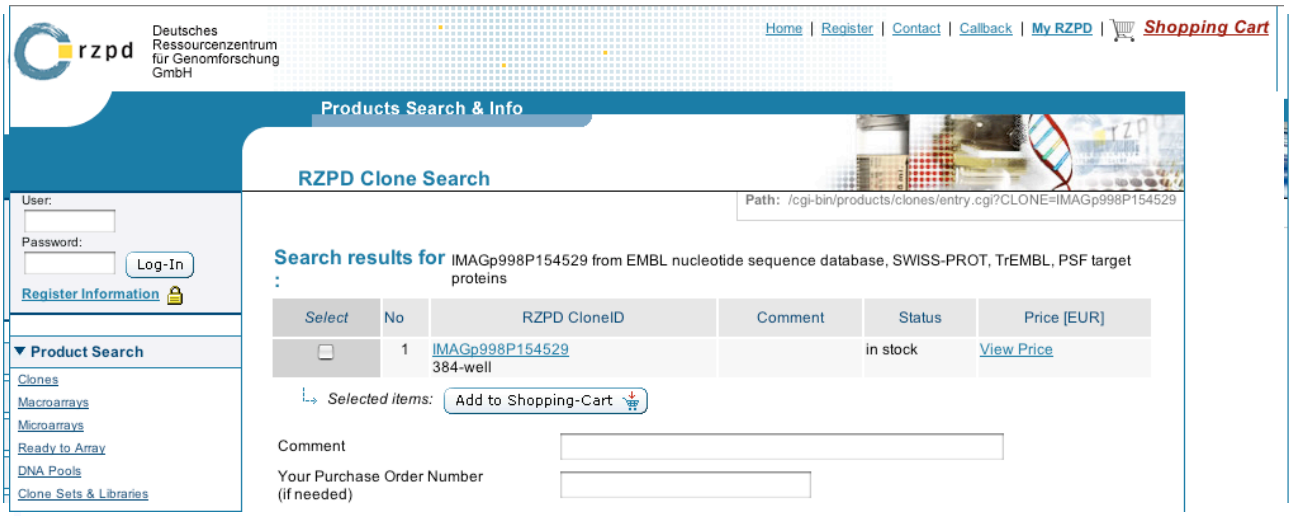
Notice:

- **Error rate is high: ~30% chance that clone doesn't have expected sequence**
- **Invitrogen sells sets of sequence verified clones**

ID AI242177 standard; RNA; EST; 581 BP.
AC AI242177;
SV AI242177.1
DT 05-NOV-1998 (Rel. 57, Created)
DT 03-MAR-2000 (Rel. 63, Last updated, Version 3)
DE qh81g08.x1 Soares_fetal_liver_spleen_1NFLS_S1 Homo sapiens cDNA
DE clone IMAGE:1851134 3' similar to gb:M10988 TUMOR NECROSIS FACTOR
DE PRECURSOR (HUMAN);, mRNA sequence.
RN [1]
RP 1-581
RA NCI-CGAP;
RT National Cancer Institute, Cancer Genome Anatomy Project (CGAP), Tumor
RT Gene Index http://www.ncbi.nlm.nih.gov/ncicgap;
RL Unpublished.
DR RZPD; IMAGp998P154529; IMAGp998P154529.
CC On May 19, 1998 this sequence version replaced gi:2846208.
CC Contact: Robert Strausberg, Ph.D.
CC Tel: (301) 496-1550
CC Email: Robert_Strausberg@nih.gov
CC This clone is available royalty-free through LLNL ; contact the
CC IMAGE Consortium (info@image.llnl.gov) for further information.
CC Insert Length: 1280 Std Error: 0.00
CC Seq primer: -40UP from Gibco
CC High quality sequence stop: 463.

FH Key Location/Qualifiers
FH
FT source 1..581
FT /db_xref=taxon:9606
FT /db_xref=EST1LIB:452
FT /db_xref=RZPD:IMAGp998P154529
FT /note=Organ: Liver and Spleen; Vector: pT7T3D (Pharmacia)
FT with a modified polylinker; Site_1: Pac I; Site_2: Eco RI;
FT This is a subtracted version of the original Soares fetal
FT liver spleen 1NFLS library. 1st strand cDNA was primed
FT with a Pac I - oligo(dT) primer [5'
FT AACTGGAAGAATTAATTAAAGATCTTTTTTTTTTTTTTTTT 3'],
FT double-stranded cDNA was ligated to Eco RI adaptors
FT (Pharmacia), digested with Pac I and cloned into the Pac I
FT and Eco RI sites of the modified pT7T3 vector. Library
FT went through one round of normalization. Library
FT constructed by Bento Soares and M.Fatima Bonaldo.
FT /sex=male
FT /organism=Homo sapiens
FT /clone=IMAGE:1851134
FT /clone_lib=Soares_fetal_liver_spleen_1NFLS_S1
FT /dev_stage=20 week-post conception fetus
FT /lab_host=DH10B (ampicillin resistant)
SQ Sequence 581 BP; 179 A; 130 C; 135 G; 137 T; 0 other;
cttttctaag caaactttat ttctcgccac tgaatagtag ggcgattaca gacacaactc 60
.....

From an EST entry in EMBL to clone shopping



Improving ESTs

Introduction

The value of ESTs can be greatly enhanced by

- **Pre-processing**
  (Steps required to "clean" & prepare ESTs sequences)
- **Clustering**
  (minimization of the chance to cluster unrelated sequences)
- **Assembling**
  (derive consensus sequences from overlapping ESTs belonging to the same cluster)
- **Mapping**
  (associate ESTs or ESTs contigs with exons in genomic sequences)
- **Interpreting**
  (find and correct coding regions)

in order to :

-> solve redundancy & help correcting errors
-> get longer & better annotated sequences
-> allow easier association to mRNAs & proteins
-> allow detection of splice variants
-> fewer sequences to analyze

EST pre-processing consists in a number of essential steps to minimize the chance to cluster unrelated sequences:

- **Screening out low quality regions:**
  - Low quality sequence readings are error prone
- **Screening out contaminations** (rRNA, mitRNA, …)
- **Screening out vector sequences** (vector clipping)
- **Screening out repeat sequences** (repeat masking)
- **Screening out low complexity sequences**

Softwares:

- **Phred** (Ewig et al., 1998)
  - Reads chromatograms and assesses a quality value to each nucleotide
- **VecScreen** (http://www.ncbi.nlm.nih.gov/VecScreen)
- **RepeatMasker** (http://ftp.genome.washington.edu/RM/RepeatMasker.html)
- …

Vector clipping and contaminations

- **Vector sequences can skew clustering even if a small vector fragment remains in each read. Therefore vector sequences must be removed:**
  - Delete 5' and 3' regions corresponding to the vector used for cloning
  - Detection of vector sequences is not a trivial task, because they usually lie in the low quality region of the sequence
  - UniVec is a non-redundant vector database available from the NCBI (http://www.ncbi.nlm.nih.gov/VecScreen/UniVec.html)
- **Contaminations can also skew clustering and therefore must be removed:**
  - Find and delete bacterial DNA, yeast DNA, …

Standard pairwise alignment programs are used for the detection of vector sequences and other contaminants (cross-match, BLASTN, FASTA,…)

Repeats masking

- **Some repetitive elements found in the human genome:**

	Length	Copy number	Fraction of the genome
LINEs (long interspersed elements)	6-8 kb	850'000	21%
SINEs (short interspersed elements)	100-300 bp	1'500'000	13%
LTR (autonomous)	6-11 kb	450'000	8%
LTR (non-autonomous)	1.5-3 kb		
DNA transposons (autonomous)	2-3 kb	300'000	3%
DNA transposons (non-autonomous)	80-3000 bp		
SSRs (simple sequence repeats or micro satellites and mini satellites)			3%

Improving ESTs — Pre-processing

Repeats masking

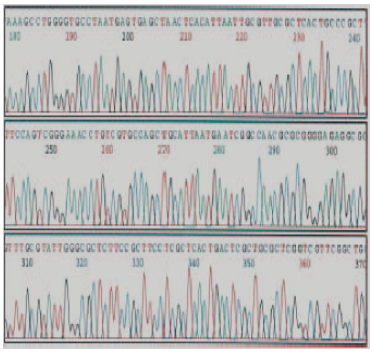
- **Repeated elements:**
  - They represent a big part of the mammalian genome
  - They are found in a number of genomes (plants, …)
  - They induce errors in clustering and assembling
  - They should be MASKED, not deleted, to avoid false sequence assembling
    (also interesting for evolutionary studies. SSRs important for mapping of diseases)

- **Tools to find repeats:**
  - **RepeatMasker** has been developed to find repetitive elements and low-complexity sequences. It uses the cross-match program for the pairwise alignments (http://www.repeatmasker.org/cgi-bin/WEBRepeatMasker)
  - **MaskerAid** improves the speed of RepeatMasker by ~30 folds using WU-BLAST instead of cross-match (http://sapiens.wustl.edu/maskeraid)
  - **RepBase** is a database of prototypic sequences representing repetitive DNA from different eukaryotic species (http://www.girinst.org/Repbase_Update.html)

Low complexity masking

- **Low complexity sequences contain an important bias in their nucleotide compositions** (poly A tracts, AT repeats, etc.)
- **Low complexity regions can provide an artifactual basis for cluster membership**
- **Clustering strategies employing alignable similarity in their first pass are very sensitive to low complexity sequences**
- **Some clustering strategies are insensitive to low complexity sequences, because they weight sequences in respect to their information content (ex. d2-cluster).**
- **Programs as DUST** (NCBI) **can be used to mask low complexity regions**

Base calling
Select high quality reads



ATGAATGTAATCTAATAGANGCCTAATCAGCCCACCATGTTCTCCACTGAAAAATCCTCT
CCCCCGTCTCTTTAAAAATATATATATTTTAAATATACTTAAATATATATTTCTAATATC
TTTAAATATATATATATATTTNAAAGACCAATTTATGGGAGANTTGCACACAGATGTGAA
TTCTTTGGGGTTTTTCTTTCTTTCTTTTTTGATTTTGCACTGGACGGTGACGTCAGCCAT
GTACAGGATCCACAGGGGTGGTGTCAAATGCTATTGAAATTNTGTTGAATTGTATACTTT
TTCACTTTTTGATAATTAACCATGTAAAAAATGAACGCTACTACTATAGTAGAATTGAT

↓ Vector clipping

CCCCCGTCTCTTTAAAAATATATATATTTTAAATATACTTAAATATATATTTCTAATATC
TTTAAATATATATATATATTTNAAAGACCAATTTATGGGAGANTTGCACACAGATGTGAA
ATGAATGTAATCTAATAGANGCCTAATCAGCCCACCATGTTCTCCACTGAAAAATCCTCT
TTCTTTGGGGTTTTTCTTTCTTTCTTTTTTGATTTTGCACTGGACGGTGACGTCAGCCAT
GTACAGGATCCACAGGGGTGGTGTCAAATGCTATTGAAATTNTGTTGAATTGTATACTTT
TTCACTTTTTGATAATTAACCATGTAAAAAATGXXXXXXXXXXXXXXXXXXXXXXXXXXX

↓ Repeat/Low complexity masking

CCCCCGTCTCTTTAAAANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNTTNAAAGACCAATTTATGGGAGANTTGCACACAGATGTGAA
ATGAATGTAATCTAATAGANGCCTAATCAGCCCACCATGTTCTCCACTGAAAAATCCTCT
TTCTTTGGGGTTTTTCTTTCTTTCTTTTTTGATTTTGCACTGGACGGTGACGTCAGCCAT
GTACAGGATCCACAGGGGTGGTGTCAAATGCTATTGAAATTNTGTTGAATTGTATACTTT
TTCACTTTTTGATAATTAACCATGTAAAAAATGXXXXXXXXXXXXXXXXXXXXXXXXXXX

↓ Sequence ready for clustering

CCCCCGTCTCTTTAAAANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNTTNAAAGACCAATTTATGGGAGANTTGCACACAGATGTGAA
ATGAATGTAATCTAATAGANGCCTAATCAGCCCACCATGTTCTCCACTGAAAAATCCTCT
TTCTTTGGGGTTTTTCTTTCTTTCTTTTTTGATTTTGCACTGGACGGTGACGTCAGCCAT
GTACAGGATCCACAGGGGTGGTGTCAAATGCTATTGAAATTNTGTTGAATTGTATACTTT
TTCACTTTTTGATAATTAACCATGTAAAAAATG

EST clustering consists in incorporating overlapping ESTs which tag the same Transcript of the same gene in a single cluster

For clustering, we measure the similarity (distance) **between any 2 sequences. The distance is then reduced to a simple binary value:**

- accept or reject two sequences in the same cluster

Similarity can be measured using different algorithms:

- **Pairwise alignment algorithms:**
  Smith-Waterman is the most sensitive, but time consuming (ex. cross-match);
  Heuristic algorithms, as BLAST and FASTA, trade some sensitivity for speed.

- **Non-alignment based scoring methods:**
  d2-cluster algorithm: based on word comparison and composition (word identity and multiplicity) (Burke et al., 99). No alignments are performed) fast.

Stringent clustering:

- **Greater initial fidelity**
- **One pass**
- **Lower coverage of expressed gene data**
- **Lower cluster inclusion of expressed gene forms**
- **Shorter consensi**

TIGR

Loose clustering:

- **Lower initial fidelity**
- **Multi-pass**
- **Greater coverage of expressed gene data**
- **Greater cluster inclusion of alternate expressed forms**
- **Longer consensi**
- **Risk to include paralogs in the same gene index**

UniGene

Supervised clustering

- **ESTs are classified with respect to known reference sequences or "seeds"** (full length mRNAs, exon constructs from genomic sequences, previously assembled EST cluster consensus)

Unsupervised clustering

- **ESTs are classified without any prior knowledge** ("ab initio")

The two major gene indices use different EST clustering methods:

- **TIGR Gene Index uses a stringent and supervised clustering method, which generates shorter consensus sequences and separates splice variants**
- **A combination of supervised and unsupervised methods with variable levels of stringency is used in UniGene. No consensus sequences are produced**

Assembling, processing and cluster joining

UniGene

- **A multiple alignment for each cluster can be built** (assembly) **and consensus sequences generated** (processing)
- **A number of program are available for assembly and processing:**
  - **PHRAP** (http://www.phrap.org/)
  - **TIGR ASSEMBLER** (Sutton et al., 95)
  - ...

TIGR

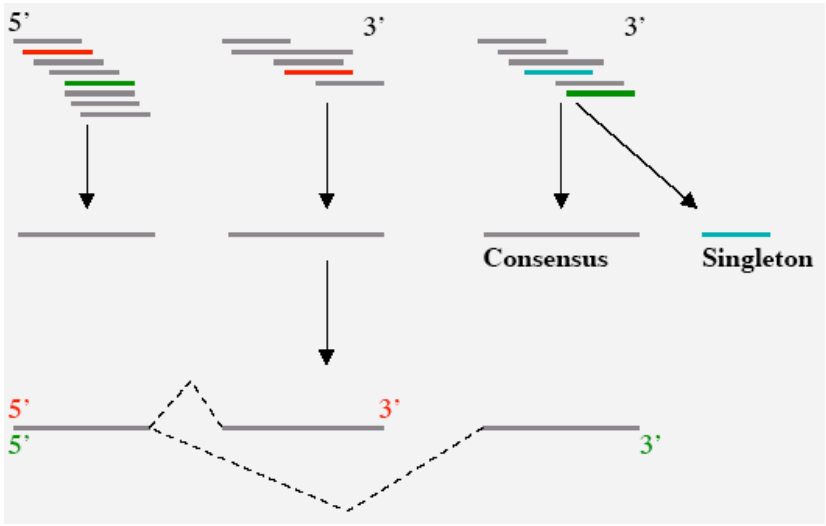
- **Assembly and processing result in the production of consensus sequences and singletons.**
- **Consensus sequences are useful:**
  - **to help visualizing splice variants;**
  - **to reduce the size of data to analyze;**
  - **for gene structure;**
  - ...

Assembling, processing and cluster joining

- All ESTs generated from the same cDNA clone correspond to a single gene
- Generally the original cDNA clone information is available (~90%)
- Using the cDNA clone information and the 5' and 3' reads information, clusters can be joined

Assembly & Processing

Joining



- All high-throughput biology methods require a unique and reliable way to describe the genes they are analyzing
- This index should be stable, unique, extensible, and independent of a system of nomenclature
- The index should document all transcript sequences belonging to the corresponding gene

Some commonly used gene indices

- **EMBL/GenBank/DDBJ accession numbers**
  - Unique and universally accepted **BUT**
  - Highly redundant (many entries per gene)
- **Unigene cluster identifiers (NCBI)**
  - Widely used and non-redundant **BUT**
  - Rely on clustering procedure (unreliable) **AND**
  - Unstable – clusters change with each build
- **RefSeq accession numbers (NCBI)**
  - Stable and non-redundant **BUT**
  - Still very far from comprehensive **AND**
  - Many RefSeq sequences are incomplete **AND**
  - Splice variants are not systematically documented

Indices: The Unigene database

- **Unigene** (http://www.ncbi.nlm.nih.gov/UniGene/) **is an ongoing effort at NCBI to cluster EST sequences with traditional gene sequences**

- **For each cluster, there is a lot of additional information included**
  (Represented organisms comprise animals & plants)

- **Unigene is regularly rebuilt. Therefore:**

cluster identifiers are not stable gene indices !!!

UniGene procedure: (supervised or unsupervised, multipass)

Screen for contaminants, repeats, and low-complexity regions in GenBank:

- **Low-complexity are detected using Dust**
- **Contaminants** (vector, linker, bacterial, mitochondrial, ribosomal sequences) **are detected using pairwise alignment programs**
- **Repeat masking of repeated regions** (RepeatMasker)
- **Only sequences with at least 100 informative bases are accepted**

Clustering procedure:

- **Build clusters of genes and mRNAs** (GenBank)
- **Add ESTs to previous clusters** (megablast)
- **ESTs that join two clusters of genes/mRNAs are discarded**
- **Any resulting cluster without a polyadenilation signal or at least two 3' ESTs is discarded (*)**
- **The resulting clusters are called anchored clusters since their 3' end is supposed known**

(*: UniGene rule**)**

UniGene procedure:

Ensures that the 5' and 3' ESTs from the same cDNA clone belongs to the same cluster

ESTs that have not been clustered, are reprocessed with lower level of stringency

ESTs added during this step are called guest members

Clusters of size 1 (containing a single sequence) are compared against the rest of the clusters with a lower level of stringency and merged with the cluster containing the most similar sequence

For each build of the database, clusters IDs change if clusters are split or merged.

TIGR produces Gene Indices for a number of organisms (http://www.tigr.org/tdb/tgi).

TIGR Gene Indices are produced using stringent supervised clustering methods

Clusters are assembled in consensus sequences, called tentative consensus (TC) sequences, that represent the underlying mRNA transcripts

The TIGR Gene Indices building method tightly groups highly related sequences and discard under-represented, divergent, or noisy sequences

TIGR Gene Indices characteristics:

- separate closely related genes into distinct consensus sequences;
- separate splice variants into separate clusters;
- low level of contamination.

TC sequences can be used for genome annotation, genome mapping, and identification of orthologs/paralogs genes

TIGR procedure: (supervised, stringent)

EST sequences recovered form dbEST (http://www.ncbi.nlm.nih.gov/dbEST);

Sequences are trimmed to remove:

- vectors
- polyA/T tails
- adaptor sequences
- bacterial sequences

Get expressed transcripts (ETs) from EGAD (http://www.tigr.org/tdb/egad/egad.shtml)

- EGAD (Expressed Gene Anatomy Database) is based on mRNA and CDS (coding sequences) from GenBank

Get TCs and singletons from previous database build

Supervised and strict clustering

- Use ETs, TCs, and CDSs as seed;
- Compare cleaned ESTs to the template using FLAST (a rapid pairwise comparison
- program).
- Sequences are grouped in the same cluster if these conditions are true:
  - a minimum of 40 base pair match
  - greater than 94% identity in the overlap region
  - a maximum unmatched overhang of 30 base pairs

TIGR procedure:

Each cluster is assembled using CAP3 assembling program to produce tentative consensus (TC) sequences.

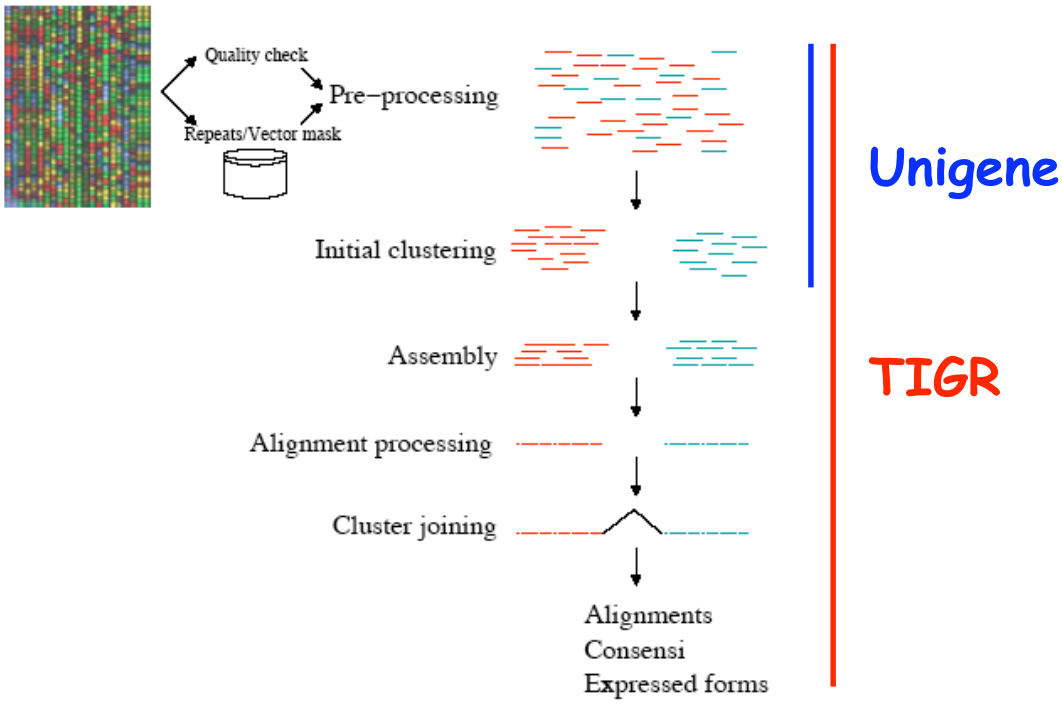
- CAP3 can generate multiple consensus sequences for each cluster
- CAP3 rejects chimeric, low-quality and non-overlapping sequences
- New TCs resulting from the joining or splitting of previous TCs, get a new TC ID

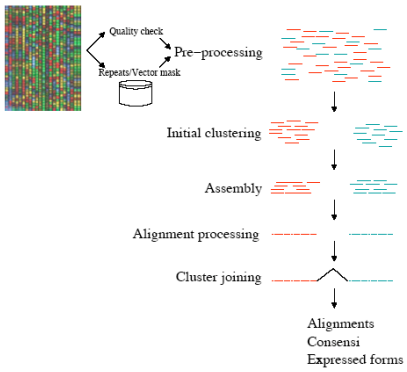
Build TCs are loaded in the TIGR Gene Indices database and annotated using information from GenBank and/or protein homology.

Track of the old TC IDs is maintained through a relational database.

References:

- Quackenbush et al. (2000) Nucleic Acid Research,28, 141-145.
- Quackenbush et al. (2001) Nucleic Acid Research,29, 159-164.





Unigene

TIGR

In house

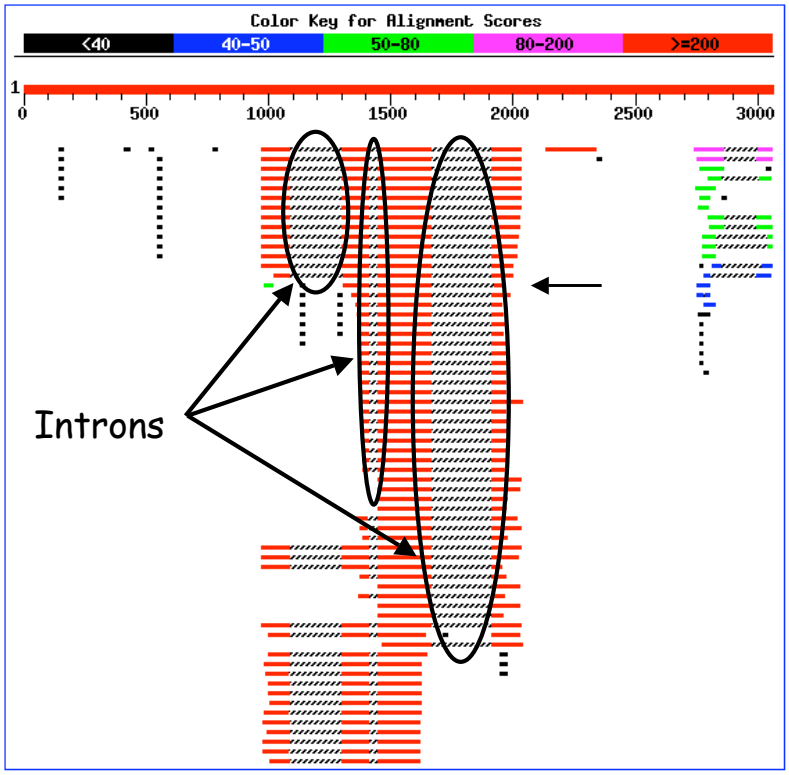
trEST

trEST is an attempt to produce contigs from UniGene clusters and to translate them into proteins. This is a two-step process:

-assembly of contigs from a collection of ESTs
-translation of the assembled contigs into protein

Hence, it must be stressed that **trEST entries are NOT real protein sequences**. They are hypothetical and are known to contain errors. These data are provided because they might help biologists to find which UniGene cluster(s) may be relevant for their work.

BLAST search against EST databases with a genomic *C. Elegans* sequence



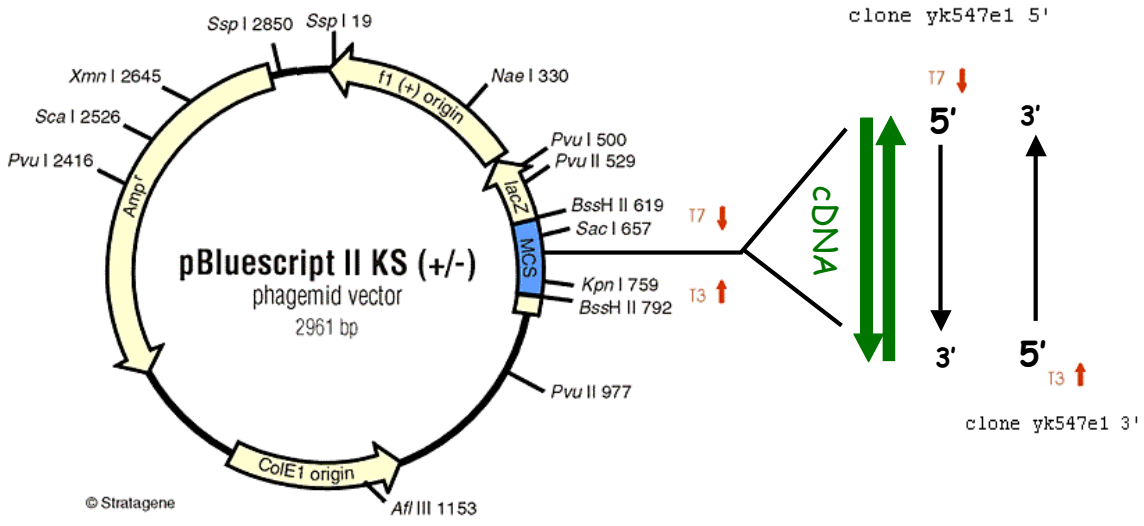
Blast searching EST databases

PE

```
>gi|5585978|dbj|AV202207.1|AV202207 AV202207 Yuji Kohara unpublished cDNA Caenorhabditis elegans cDNA
          clone yk547e1 5'
          Length = 379

 Score =  422 bits (213), Expect = e-117
 Identities = 213/213 (100%)
 Strand = Plus / Plus


Query: 1450 agggaacccacggacaagagcaagtcaccagaaagaagaccaagaagtccgtccaggttg 1509
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 29   agggaacccacggacaagagcaagtcaccagaaagaagaccaagaagtccgtccaggttg 88


Query: 1510 ttaaccgcgccgtcgctggactttcccttgatgctatccttgccaagagaaaccagaccg 1569
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 89   ttaaccgcgccgtcgctggactttcccttgatgctatccttgccaagagaaaccagaccg 148


Query: 1570 aagacttccgtcgccaacagcgtgaacaagccgctaagatcgccaaggatgccaacaagg 1629
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 149  aagacttccgtcgccaacagcgtgaacaagccgctaagatcgccaaggatgccaacaagg 208


Query: 1630 ctgtccgtgccgccaaggctgctgccaacaagg 1662
               ||||||||||||||||||||||||||||||||
Sbjct: 209  ctgtccgtgccgccaaggctgctgccaacaagg 241
```


Blast searching EST databases

PE



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           clone yk547e1 5'.
         Length = 378

 Score =  422 bits (213), Expect = e-117
 Identities = 213/213 (100%)
 Strand = Plus / Plus


Query: 1450 agggaacccacggacaagagcaagtcaccagaaagaagaccaagaagtccgtccaggttg 1509
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Sbjct: 29   agggaacccacggacaagagcaagtcaccagaaagaagaccaagaagtccgtccaggttg 88


Query: 1510 ttaaccgcgccgtcgctggactttcccttgatgctatccttgccaagagaaaccagaccg 1569
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Query: 1570 aagacttccgtcgccaacagcgtgaacaagccgctaagatcgccaaggatgccaacaagg 1629
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Sbjct: 149  aagacttccgtcgccaacagcgtgaacaagccgctaagatcgccaaggatgccaacaagg 208


Query: 1630 ctgtccgtgccgccaaggctgctgccaacaagg 1662
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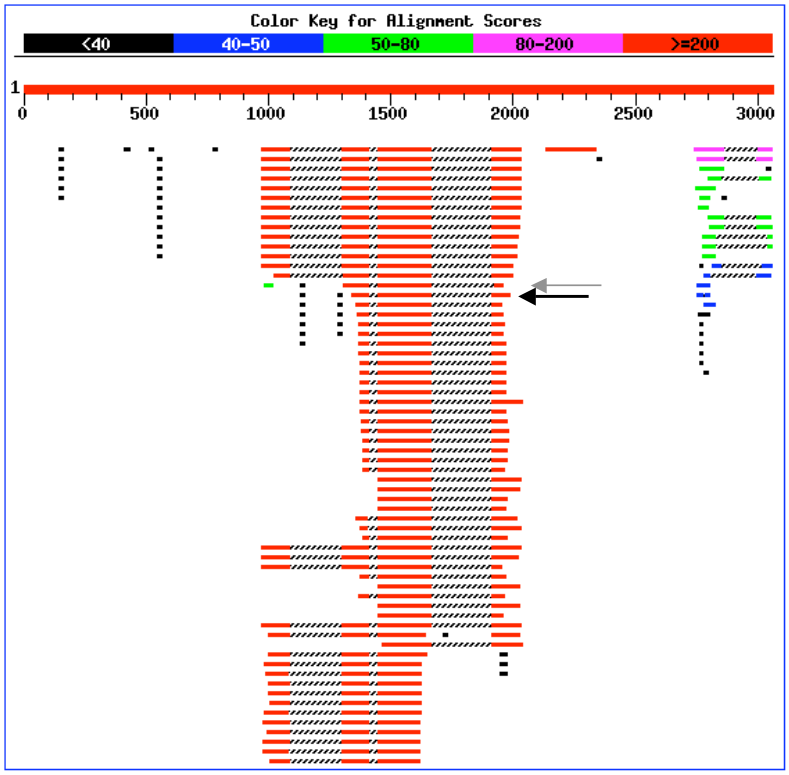
BLAST search against EST databases with a C. Elegans sequence



Blast searching EST databases

PE

Same clone

Sequenced on the reverse strand

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>gi|5583282|dbj|AV199511.1|AV199511 AV199511 Yuji Kohara unpublished cDNA Caenorhabditis elegans cDNA
          clone yk547e1 3'.
          Length = 300

 Score =  422 bits (213), Expect = e-117
 Identities = 213/213 (100%)
 Strand = Plus / Minus


Query: 1450 agggaacccacggacaagagcaagtcaccagaaagaagaccaagaagtccgtccaggttg 1509
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Query: 1510 ttaaccgcgccgtcgctggactttcccttgatgctatccttgccaagagaaaccagaccg 1569
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Sbjct: 211  ttaaccgcgccgtcgctggactttcccttgatgctatccttgccaagagaaaccagaccg 152


Query: 1570 aagacttccgtcgccaacagcgtgaacaagccgctaagatcgccaaggatgccaacaagg 1629
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 151  aagacttccgtcgccaacagcgtgaacaagccgctaagatcgccaaggatgccaacaagg 92


Query: 1630 ctgtccgtgccgccaaggctgctgccaacaagg 1662
          ||||||||||||||||||||||||||||||||
Sbjct: 91   ctgtccgtgccgccaaggctgctgccaacaagg 59
```


Blast searching EST databases

PE

☐ 1: AV199511. AV199511 Yuji Koh...[gi:5583282]

Contact with the authors

```
LOCUS       AV199511      300 bp    mRNA            EST       26-JUL-1999
DEFINITION  AV199511 Yuji Kohara unpublished cDNA Caenorhabditis elegans cDNA
            clone yk547e1 3', mRNA sequence.
ACCESSION   AV199511
VERSION     AV199511.1  GI:5583282
KEYWORDS    EST.
SOURCE      Caenorhabditis elegans.
  ORGANISM  Caenorhabditis elegans
            Eukaryota; Metazoa; Nematoda; Chromadorea; Rhabditida; Rhabditoidea
            ; Rhabditidae; Peloderinae; Caenorhabditis.
REFERENCE   1  (bases 1 to 300)
  AUTHORS   Kohara,Y., Shin-i,T., Thierry-Mieg,J., Thierry-Mieg,D., Mitsuki,H.,
            Nishigaki,A., Motohashi,T., Zeng,Q., Watanabe,H., Sugimoto,A., Sano
            ,M., Miyata,A., Mitani,Y., Iida,K., Uesugi,H., Sugiyama,Y. and
            Nomoto,H.
  TITLE     Expressed genes in C.elegans
  JOURNAL   Unpublished (1999)
COMMENT     Contact: Yuji Kohara
            Genome Biology Lab.
            National Institute of Genetics
            Yata 1111, Mishima, Shizuoka 411, Japan
            Tel: 81-559-81-6854
            Fax: 81-559-81-6855
            Email: ykohara@lab.nig.ac.jp.
FEATURES             Location/Qualifiers
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                     /db_xref="taxon:6239"
                     /clone="yk547e1"
                     /clone_lib="Yuji Kohara unpublished cDNA"
                     /sex="hermaphrodite, male"
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BASE COUNT        41 a     73 c     88 g     98 t
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       61 ttgttggcag cagccttggc ggcacggaca gccttgttgg catccttggc gatcttagcg
      121 gcttgttcac gctgttggcg acggaagtct tcggtctggt ttctcttggc aaggatagca
      181 tcaagggaaa gtccagcgac ggcgcggtta acaacctgga cggacttctt ggtcttcttt
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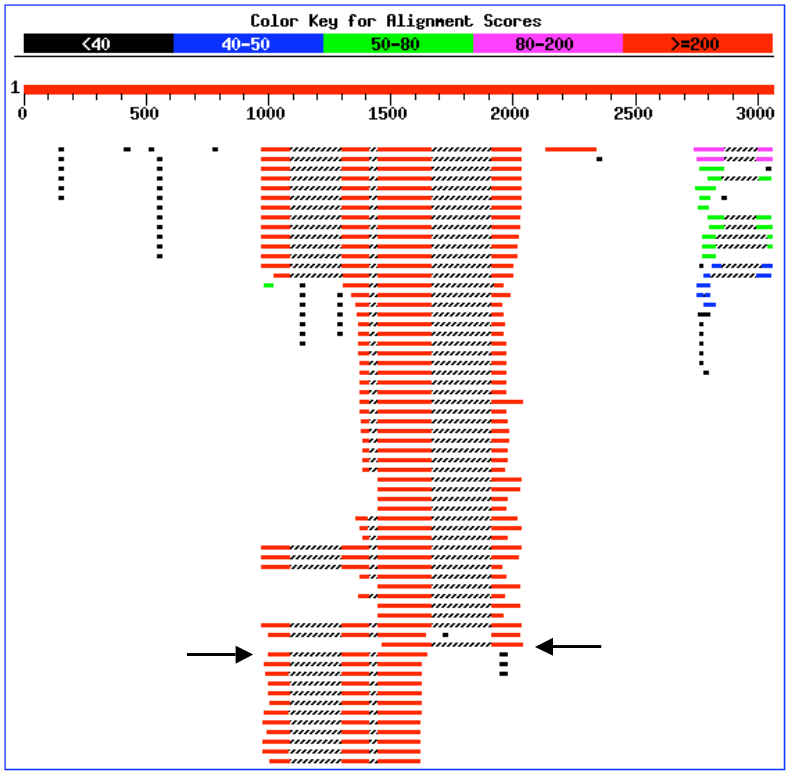
//
```

EST assembly to reconstruct a complete sequence



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EST assembly to reconstruct a complete sequence

```
EST5'.+     CGANGGCCTATCAACAATGAAAGGTCGAAACCTGCGTTTACTCCGGATACAAGATCCACC

EST5'.+     CAGGACACGGNAAAGAGACTTGTCCGTACTGACGGAAAGGTCCAAATCTTCCTCAGTGGA

EST5'.+     AAGGCACTCAAGGGAGCCAAGCTTCGCCGTAACCCACGTGACATCAGATGGACTGTCCTC

EST5'.+     TACAGAATCAAGAACAAGAAGGGAACCCACGGACAAGAGCAAGTCACCAGAAAGAAGACC
EST3'.-                                          AAGAGCAAGTCACCAGAAAGAAGACC

EST5'.+     AAGAAGTCCGTCCAGGTTGTTAACCGCGCCGTCGCTGGACTTTCCCTTGATGCTATCCTT
EST3'.-     AAGAAGTCCGTCCAGGTTGTTAACCGCGCCGTCGCTGGACTTTCCCTTGATGCTATCCTT

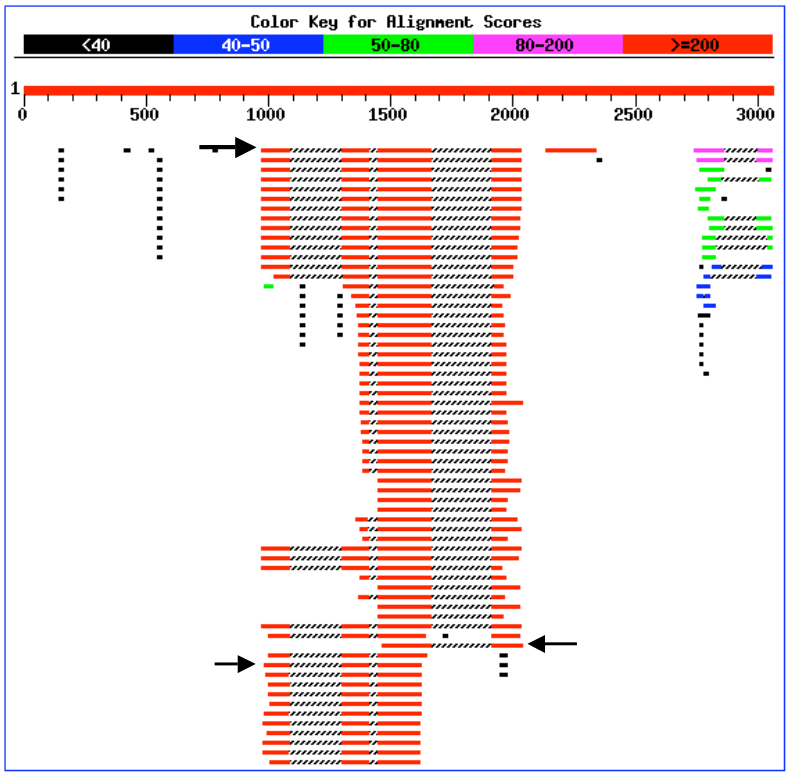
EST5'.+     GCCAAGAGAAACCAGACCGAAGACTTCCGTCGCCAACAGCGTGAACAAGCCGCTAAGATC
EST3'.-     GCCAAGAGAAACCAGACCGAAGACTTCCGTCGCCAACAGCGTGAACAAGCCGCTAAGATC

EST5'.+     GCCAAGGATGCCAACAA
EST3'.-     GCCAAGGATGCCAANAAGGCTGTCCGTGCCGCCAAGGCTGCTNCCAACAAGGNAAAGAAG

EST3'.-     GCCTCTCAGCCAAAGACCCAGCAAAAGACCGCCAAGAATNTNAAGACTGCTGCTCCNCGT

EST3'.-     GTCGGNGGAAANCGATAAACGTTCTCGGNCCCGTTATTGTAATAAATTTTGTTGACC
```

EST assembly to reconstruct a complete sequence

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EST1.+      GTTTAATTACCCAAGTTTGAGATTCGTCAAGCGAGGGCCTATCAACAATGAA-GGTCGAA
EST5'.+                                   CGANGGCCTATCAACAATGAAAGGTCGAA

EST1.+      ACCTGCGTTTACTCCGGATACAAGATCCACCCAGGACACGG-AAAGAGACTTGTCCGTAC
EST5'.+     ACCTGCGTTTACTCCGGATACAAGATCCACCCAGGACACGGnAAAGAGACTTGTCCGTAC

EST1.+      TGACGGAAAGGTCCAAATCTTCCTCAGTGGAAAGGCACTCAAGGGAGCCAAGCTTCGCCG
EST5'.+     TGACGGAAAGGTCCAAATCTTCCTCAGTGGAAAGGCACTCAAGGGAGCCAAGCTTCGCCG

EST1.+      TAACCCACGTGACATCAGATGGACTGTCCTCTACAGAATCAAGAACAAGAAGGGAACCCA
EST5'.+     TAACCCACGTGACATCAGATGGACTGTCCTCTACAGAATCAAGAACAAGAAGGGAACCCA

EST1.+      CGGACAAGAGCAAGTCACCAGAAAGAAGACCAAGAAGTCCGTCCAGGTTGTTAACCGCGC
EST5'.+     CGGACAAGAGCAAGTCACCAGAAAGAAGACCAAGAAGTCCGTCCAGGTTGTTAACCGCGC
EST3'.-           AAGAGCAAGTCACCAGAAAGAAGACCAAGAAGTCCGTCCAGGTTGTTAACCGCGC

EST1.+      CGTCGCTGGACTTTCCCTTGATGCTATCCTTGCCAAGAGAAACCAGACCGAAGACTTCCG
EST5'.+     CGTCGCTGGACTTTCCCTTGATGCTATCCTTGCCAAGAGAAACCAGACCGAAGACTTCCG
EST3'.-     CGTCGCTGGACTTTCCCTTGATGCTATCCTTGCCAAGAGAAACCAGACCGAAGACTTCCG

EST1.+      TCGCCAACAGCGTGAACAAGCCGCTAAGATCGCCAAGGATGCCAACAAGGCTGTCCGTGC
EST5'.+     TCGCCAACAGCGTGAACAAGCCGCTAAGATCGCCAAGGATGCCAACAA
EST3'.-     TCGCCAACAGCGTGAACAAGCCGCTAAGATCGCCAAGGATGCCAAnAAGGCTGTCCGTGC

EST1.+      CGCCAAGGCTGCTGCCAACAAGGAAAAGAAGGCCTCTCAGCCAAAGACCCAGCAAAAGAC
EST3'.-     CGCCAAGGCTGCTNCCAACAAGGNAAAGAAGGCCTCTCAGCCAAAGACCCAGCAAAAGAC

EST1.+      CGCCAAGAATGTGAAGACTGCTGCTCCACGTGTCGGAGGAAAGCGATTAAACGTTCTCGG
EST3'.-   CGCCAAGAATN TNAAGACTGCTGCTCCNCGTGTCGGNGGAAANCGA-TAAACGTTCTCGG
```
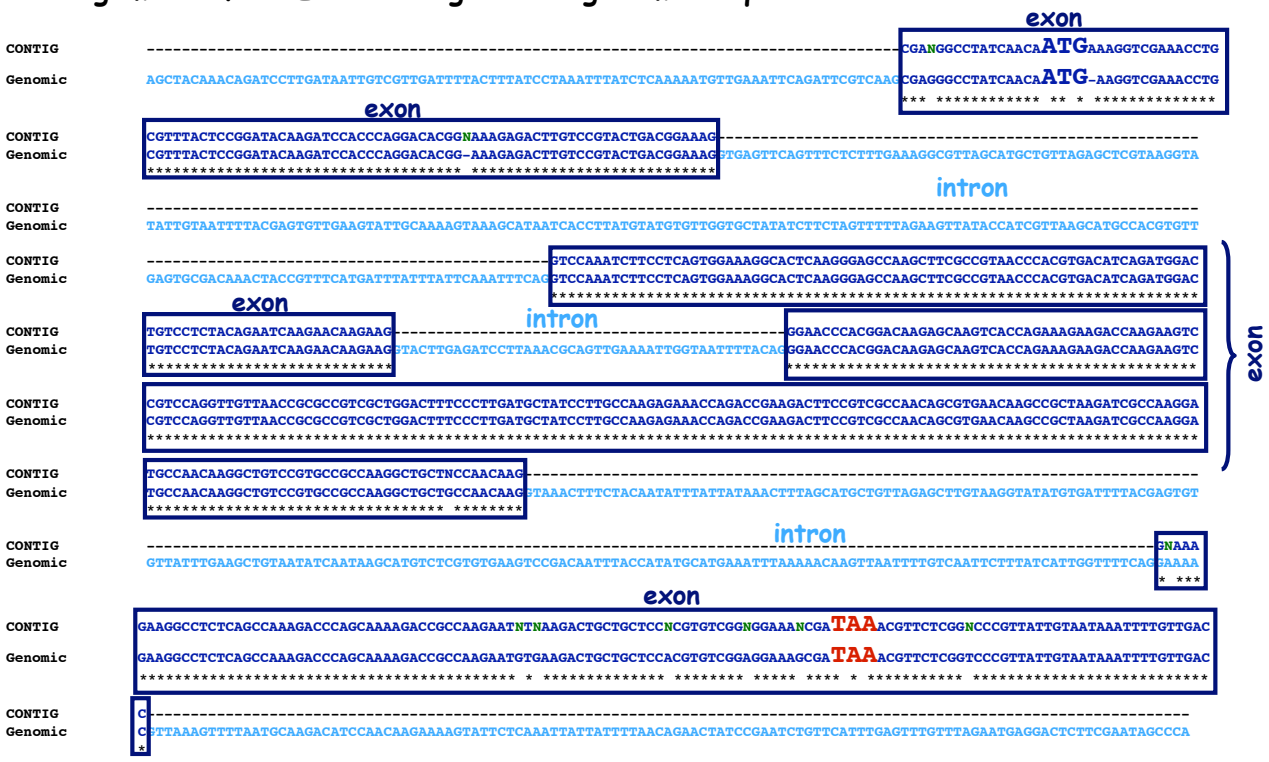

Blast searching EST databases

PE

Alignment of an EST "contig" and a genomic sequence



Concluding remarks

conclusion

Cons:
- low quality data
- native databases
- 3' ends are heavily represented
- bad/no annotation
- Gene Indices
- … (see course)

Futur of ESTs:
- In human and mouse, most will come as byproducts of full-length projects,
- There are good arguments for trying to reach saturation on selected tissues
- ESTs are still the tool of choice for rapid exploration of the transcriptomes of various species, especially with large genomes
- ESTs could form a very solid basis for evolutionary studies

Pros:
- fast & cheap (automated techniques)

- indispensable for **gene structure prediction**, **gene discovery** and **genome mapping** (large / small scale)

- efforts:
  - normalized databases
  - good annotation
  - improvements (pre-processing, clustering, assembling)
  - ORESTES
  - Emerging Gene indices (HUGO, ENSEMBL)