

Question 3

During a positional cloning project aimed at finding a human disease gene, linkage data have been obtained suggesting that the gene of interest lies between two sequence-tagged site markers. How can all the known and predicted candidate genes in this interval be identified? What BAC clones cover that particular region?

doi:10.1038/ng1191

UCSC

One possible starting point for this search is the UCSC Genome Browser home page, at <http://genome.ucsc.edu>. From this page, select *Human* from the *Organism* pull-down menu in the blue bar at the side of the page, and then click *Browser*. On the Human Genome Browser Gateway page, set the assembly pull-down to *Nov. 2002*. To view a region of the genome between two query terms, enter the terms in the search box, separated by a semicolon. For example, to view the region between STS markers *D10S1676* and *D10S1675*, enter 'D10S1676;D10S1675' in the box marked *position* and press *Submit*. Because both of these markers map to a single position in the genome, the genome browser for the region between those markers is returned (Fig. 3.1).

The *STS Markers* track displays genetically mapped markers in blue and radiation hybrid-mapped markers in black. The markers of interest are called by their alternate names (AFMA232YH9 and AFMA230VA9 in this view) and are at the top and bottom of the interval, respectively (Fig. 3.1, arrows).

The full list of known genes in this display is shown in the *Known Genes based on SWISS-PROT, TrEMBL, mRNA, and RefSeq* track. Scroll down to the middle of the page to view this track (Fig. 3.2). These protein-coding genes are taken from SWISS-PROT, TrEMBL, and TrEMBL-NEW. The genomic position of these proteins is determined by aligning the corresponding GenBank mRNAs to the genome assembly using the BLAT program⁸. To export a list of the genes, or other features, in this region, click the *Tables* link in the top blue bar. For more information about a particular gene (such as *MGMT*), click on the gene symbol to get a list of additional links to resources such as Online Mendelian Inheritance in Man (OMIM), PubMed, GeneCards and Mouse Genome Informatics (MGI; Fig. 3.3). Many tracks, including *Acemby Genes*, *Ensembl Genes* and *GenScan Genes*, indicate predicted genes (see Question 7). To view the full set of features in any of these categories, click on the title of that track on the left side of the screen in Fig. 3.2. To view brief descriptions of these tracks, as well as others not mentioned, click on the gray box to the left of the track or scroll down to the track controls and click on the title of a feature of interest. Explanations of the gene-prediction programs can be found in Question 7. Reset the browser to its default settings by clicking on the *reset all* button below the tracks.

To see the BAC clones used for sequencing, return to the page illustrated in Fig. 3.2. Near the bottom of the page, under the *Mapping and Sequencing Tracks*, change the *Coverage* pulldown from *hide* to *full*, then change the *STS Markers* pulldown from *full* to *hide*, and click the *refresh* button. These actions will make the *Coverage* track visible, while hiding the *STS Markers* track. In the resulting view, BAC clones are listed individually, with finished regions shown in black and draft regions shown in various shades of gray (Fig. 3.4). For details such as size and sequence

coverage of a specific clone, click on the clone accession number (such as *AL355529.21*, arrow). From this screen, click on the accession number (as shown in Fig. 3.5) to link to the NCBI Entrez document summary for the clone. The full GenBank entry can be viewed by clicking on *AL355529* on the Entrez document summary page.

According to NCBI naming conventions, this clone is from the RP11 library and has been named 85C15. RP11 is the NCBI designation for RPCI-11, a commonly used human BAC library produced at the Roswell Park Cancer Institute. More information on the naming conventions of genomic sequencing libraries can be found at the NCBI's Clone Registry (Fig. 3.6; <http://www.ncbi.nlm.nih.gov/genome/clone/nomenclature.shtml>).

Clone ordering information is also available, at <http://www.ncbi.nlm.nih.gov/genome/clone/ordering.html>.

NCBI

The NCBI MapViewer also allows for direct viewing of the region between two markers. One can search chromosome 22 for the region between band numbers 22q12.1 and 22q13.2, or for the region between two mapped genes.

Access the Map Viewer home page by starting at the NCBI home page (<http://www.ncbi.nlm.nih.gov>) and clicking *Map viewer* in the list on the right-hand side of the page. To view multiple hits on the same chromosome, type in the search terms separated by the word 'OR'. To see the same region in the human genome between the STS markers *D10S1676* and *D10S1675*, for example, select *Homo sapiens* from the *Select Organism* pulldown, type 'D10S1676 OR D10S1675' in the search box, and hit *Go!* At the top of the resulting page (Fig. 3.7), two red tick marks on the chromosome cartoon indicate that the markers map close to each other on chromosome 10. The search results at the bottom of the page show the alternative names for the two markers (AFMA232YH9 and AFMA230VA9) as well as the maps on which they have been placed. To view both markers at the same time, click on the link for chromosome 10 in the chromosome diagram. Fig. 3.8 shows the region around *D10S1676* and *D10S1675*, with the original queries highlighted in pink.

One can also search for a region between two STS markers using the MapView at Ensembl. Start at the Ensembl Human Genome Browser at http://www.ensembl.org/Homo_sapiens/, click on the ideogram of any chromosome to access the MapView, and enter the marker names in the *Jump to Contigview* section. To use Ensembl to obtain a list of genes (or other annotations) in a defined chromosomal region, click on *Export→Gene List* from any ContigView window (Fig. 1.12, center yellow bar).

D10S1675 is not present on the deCODE map. Red lines connect the positions of the marker on the different maps.

The *Maps & Options* link, in the horizontal blue bar near the top of the page, allows the user to customize the maps and region displayed. To view, for example, the known and predicted genes in this region, as well as the BAC clones from which the sequence was derived, click on the link to open the *Maps & Options* window (Fig. 3.9). First remove all the maps except *Gene* and *STS* from the *Maps Displayed* box by highlighting them, and selecting <<REMOVE. Next, add the *Transcript (RNA)*, *GenomeScan*, *Component* and *Contig* maps by selecting them from the *Available Maps* box and selecting ADD>>. Make the STS map the master by highlighting it, then selecting *Make Master/Move to Bottom*. To limit the view such that only the STSs between D10S1676 and D10S1675 are shown, type the marker names in the *Region Shown* boxes. Finally, remove the check next to *Compress Map* to see the maps in wider format. Hit *Apply* to see the aligned maps. In some cases, it may be useful to select a page size larger than the default of 20 to view more data in the browser window.

Fig. 3.10 shows the maps, as specified in the *Maps & Options* window. The green dots to the right of the STS map show all the maps on which the markers appear. This is a fairly long region of chromosome 10, and not every STS marker is shown. In particular, although there are 82 STSs in this region, only 20 are shown by name in this view. For each known gene, the *Genes_Seq* map

shows all the exons that have been mapped to the genome. Exons for individual known mRNAs are shown on the RNA (*Transcript*) map. Unless a gene is alternatively spliced, the *Genes_Seq* and RNA maps will be the same. The *GScan (GenomeScan)* map shows the NCBI's gene predictions. Any of these genes, known or predicted, are candidates for the disease gene.

The NCBI's assembled contigs, also known as the NT contigs, are found in the *Contig* map. Blue segments come from finished sequence, orange from draft. These contigs are constructed from the individual GenBank sequence entries shown in the *Comp (Component)* map. Draft HTG records (phase 1 and 2; see <http://www.ncbi.nlm.nih.gov/HTGS/>) are displayed in orange and finished HTGs in blue. Most of these GenBank entries are derived from BAC clones. The tiling paths of the BAC clones that were assembled into contigs are clearly visible. One can obtain more details about an entry, including the clone name, by clicking on the accession number to link to Entrez. The clone name is visible directly in the *MapView* if the *Comp* map is the master. A map can be quickly made the master map by clicking on the blue arrow next to its name.

Because this is a zoomed-out view of the chromosome, individual genes and GenBank entries are difficult to visualize. Zooming in, using the controls in the blue sidebar, will provide a region in more detail. Alternatively, click on the *Data As Table View* in the left sidebar to retrieve all data, including those hidden in this view, as a text-based table (partially shown in Fig. 3.11).

Figure 3.1

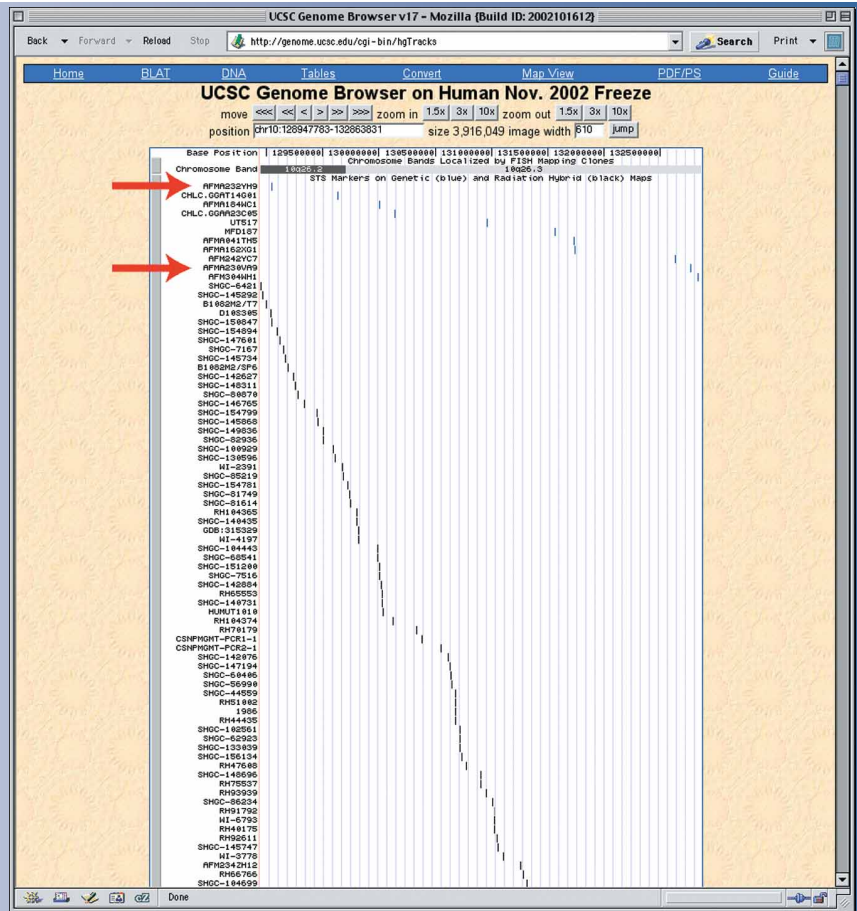
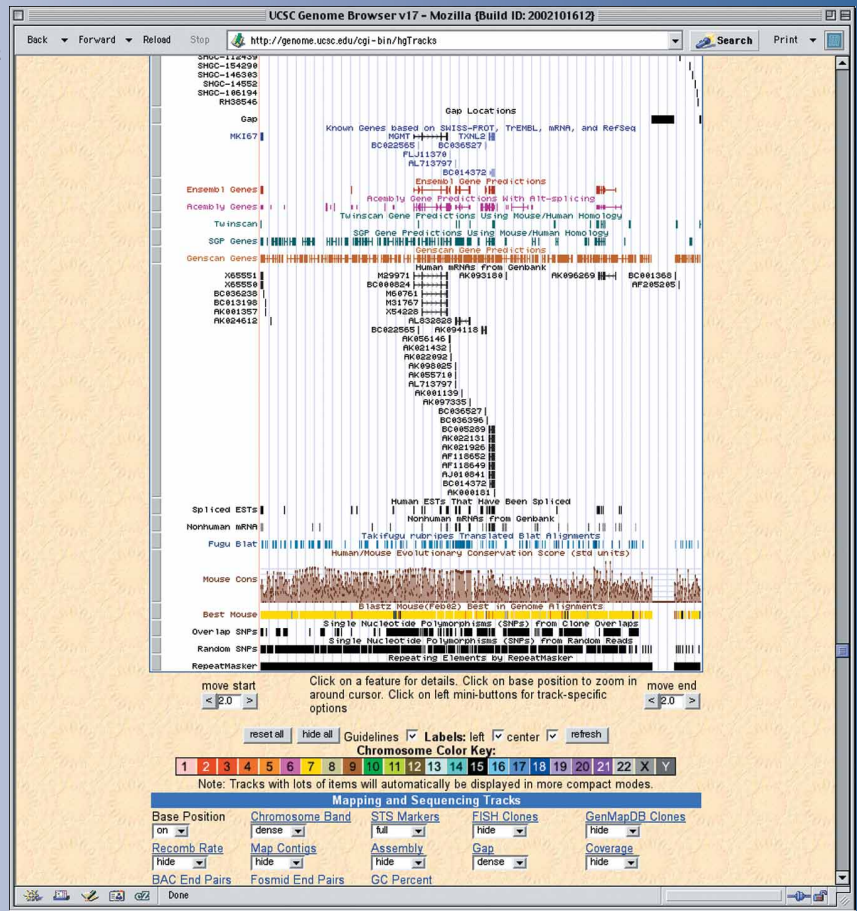


Figure 3.2



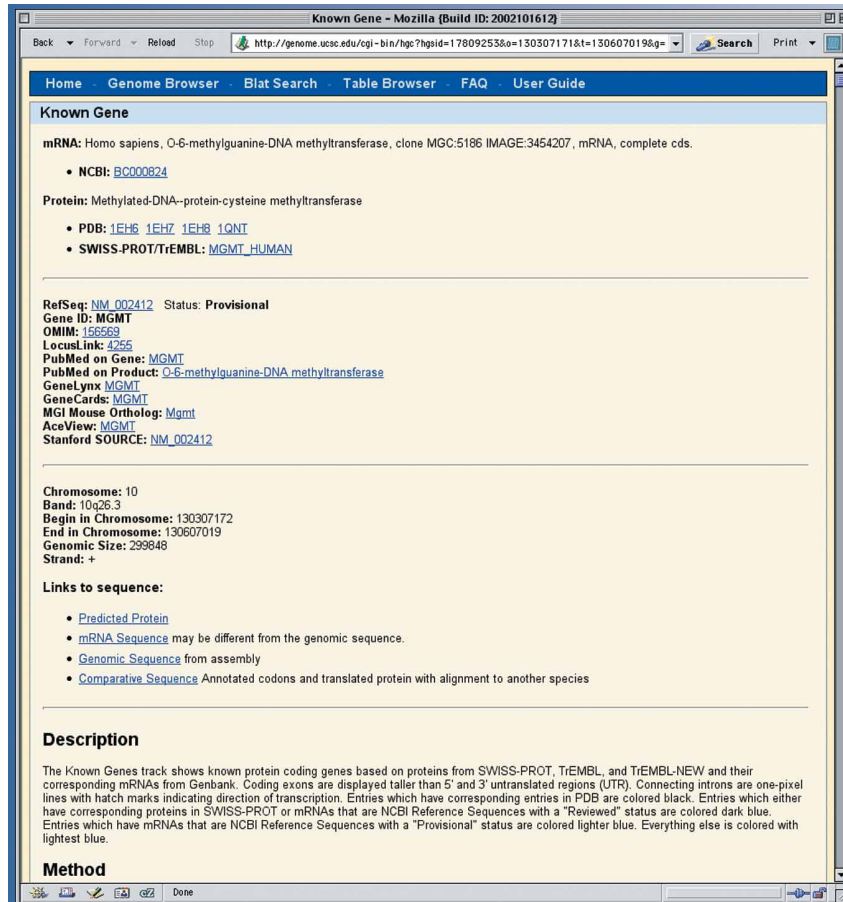


Figure 3.3

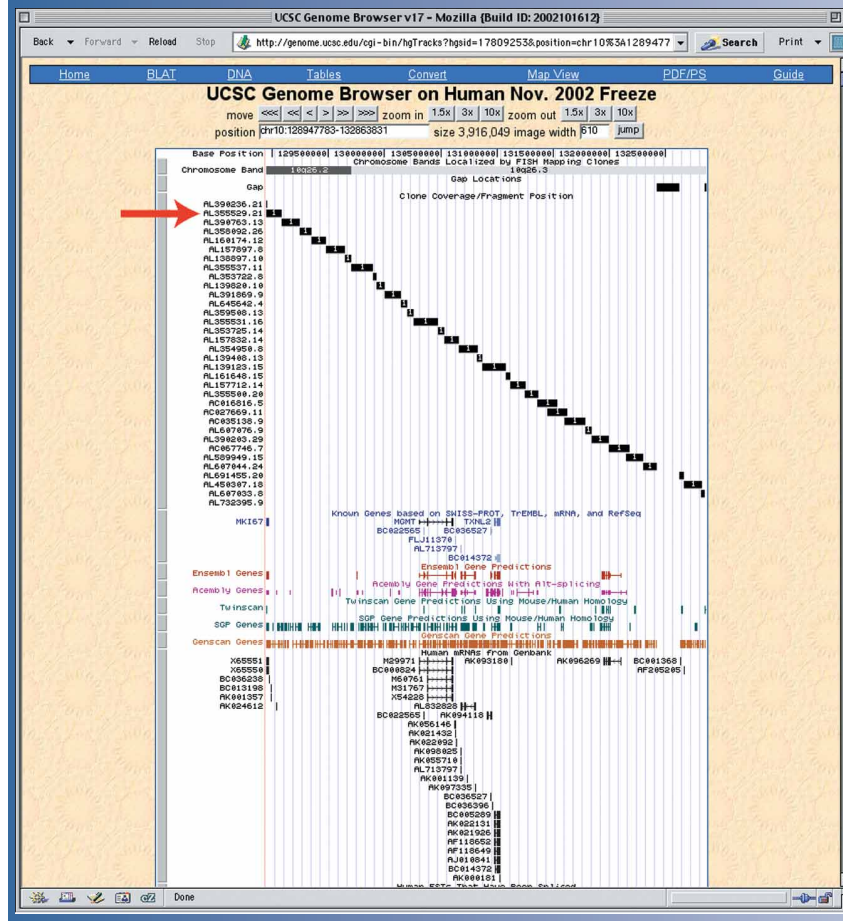


Figure 3.4

Figure 3.5

AL355529.21

Information on AL355529.21

GenBank: [AL355529.21](#)
 status: finished
 fragments: 1
 size: 140466 bases
 chromosome: 10

Description

In dense mode, this track shows the coverage level of the genome. Finished regions are shown in black. Draft regions are shown in various shades of gray that correspond to the coverage.

In full mode, this track shows the position of each contig inside each draft or finished clone (fragment) in the assembly. For some assemblies, clones in the sequencing center tiling path are shown with blue rather than gray backgrounds.

Figure 3.6

NCBI Clone Registry

Standardized clone names

A clone is identified by its microtitre plate address (plate number, row, and column) and prefixed by a library abbreviation to produce a unique name. Standardized names should be used in all Clone Server transactions as well as in GenBank sequence submissions.

RP11-345A12

library: use standardized abbreviations below
 plate: digits, no leading zeros
 row: single upper case letter
 column: digits, no leading zeros

LibAbbr	Type	PlateNo	LibName
CH14	BAC	1-528	CHORI 14 Human male BAC library
CH15	BAC	1-384	CHORI 15 Human female BAC library
CH501	BAC	Any	CHORI-501 Human BAC Library
CH502	BAC	Any	CHORI-502 Human BAC Library
CMB18	BAC	Any	RIKEN human chromosome 18 BAC library
CMB9	BAC	Any	RIKEN human chromosomes 9-12 BAC library
CTA	BAC	1-1000	CalTech human BAC library A
CTB	BAC	1-194	CalTech human BAC library B
CTC	BAC	195-879	CalTech human BAC library C
CTD	BAC	>= 2001	CalTech human BAC library D
CTbac	BAC	Any	CalTech human BAC library
DAAP	BAC	1-408	DNA-Arts.org BAC library APD.1
DADB	BAC	1-408	DNA-Arts.org BAC library DBB.1
DAMA	BAC	1-408	DNA-Arts.org BAC library MANN.1
DAMC	BAC	1-408	DNA-Arts.org BAC library MCF.1
DAQB	BAC	1-357	DNA-Arts.org BAC library QBL.1
DASS	BAC	1-400	DNA-Arts.org BAC library SSTD.1
GS1	BAC	Any	Genome Systems human BAC library 1
GS2	BAC	Any	Genome Systems human BAC library 2
KB	BAC	Any	Keio University human BAC library
RP11	BAC	1-1440	RPCI human BAC library 11
RP13	BAC	Any	RPCI human BAC library 13
SCb	BAC	Any	Research Genetics total human BAC
XXBac	BAC	Any	Unspecified BAC library
CMDC1	BAC	Any	DIV/EN human chromosome 21 D1 library



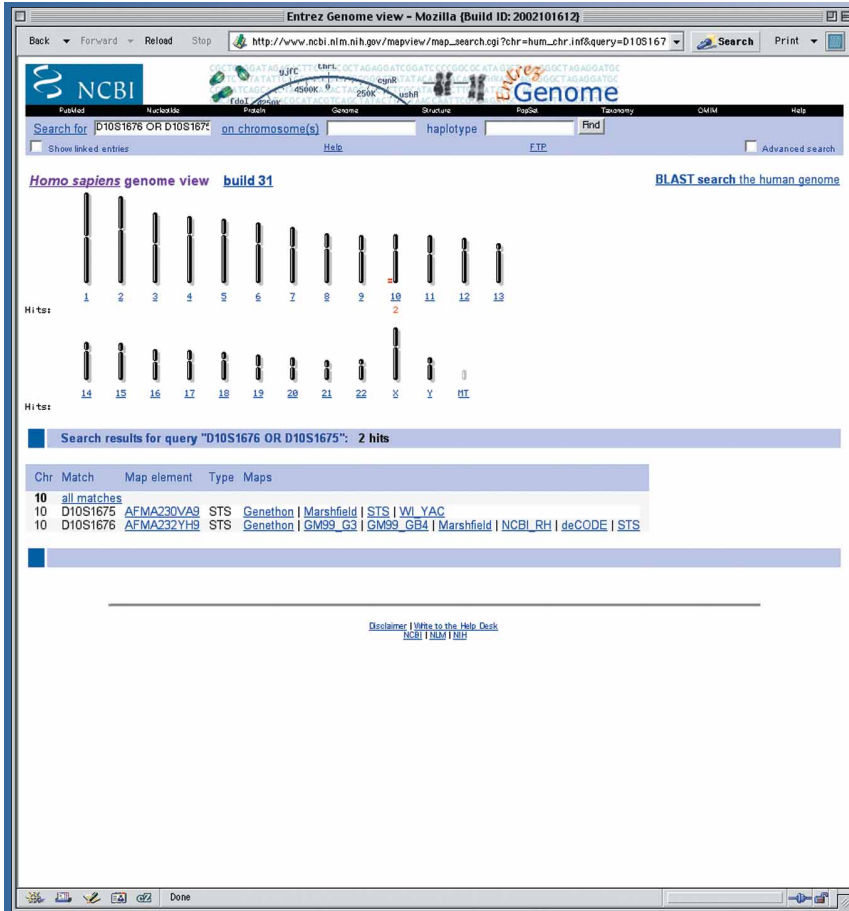


Figure 3.7

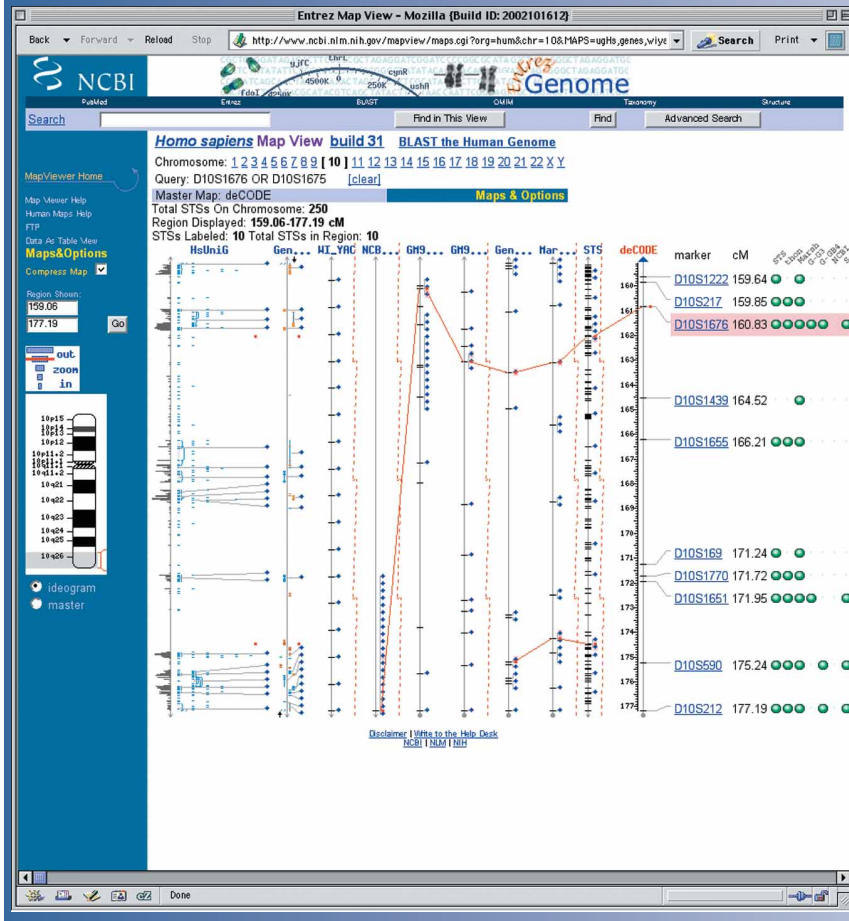


Figure 3.8

Figure 3.9

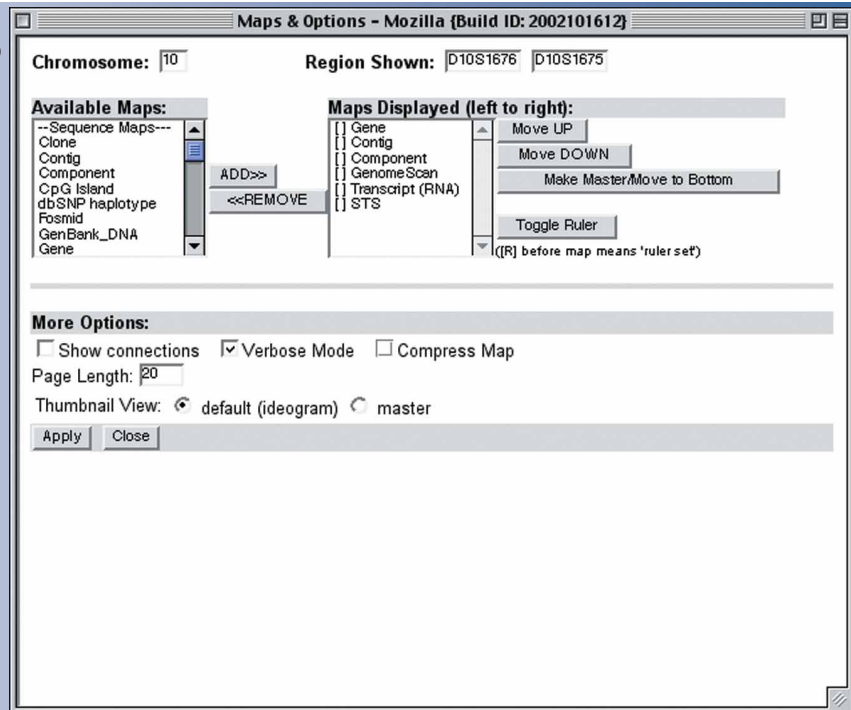
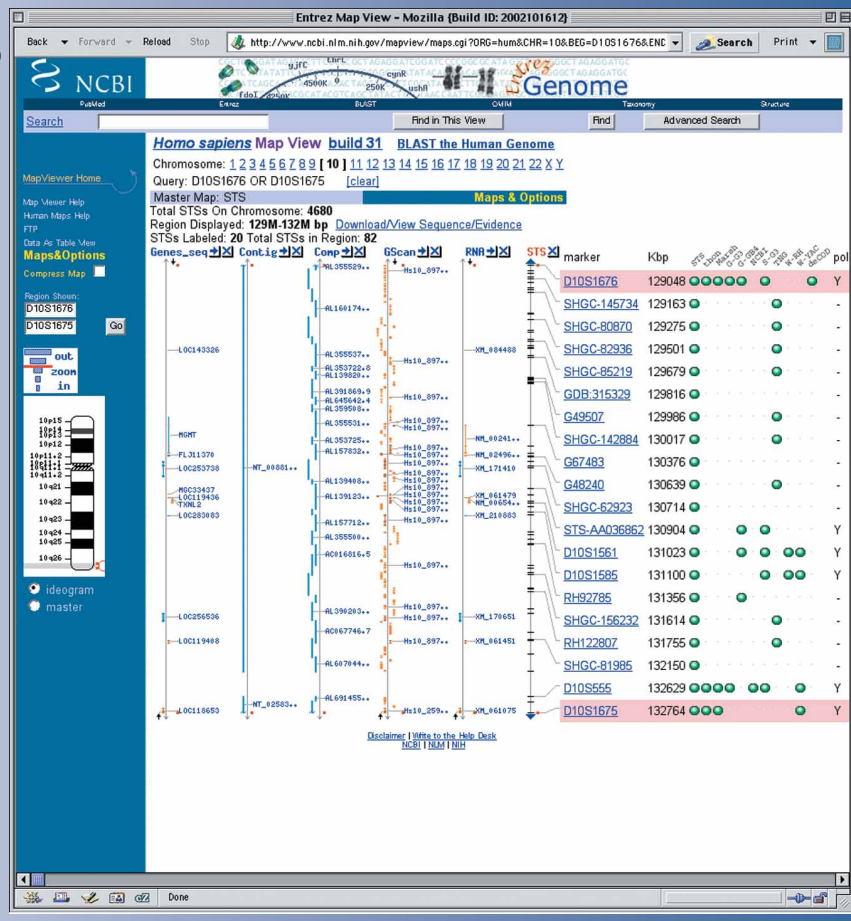


Figure 3.10



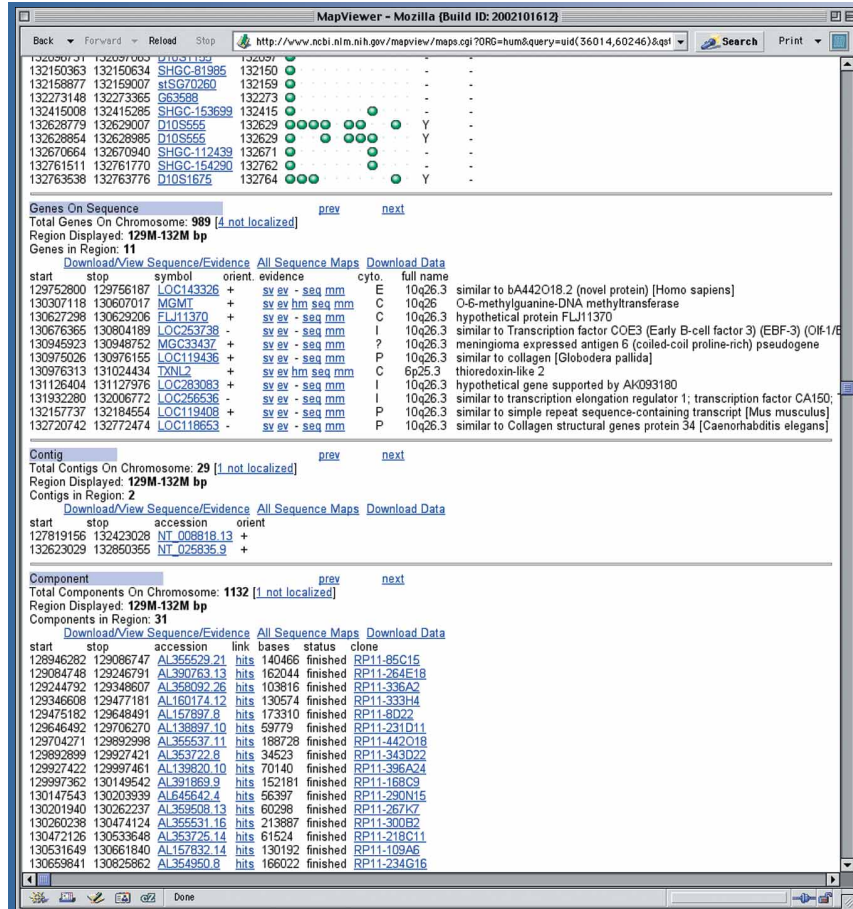


Figure 3.11