GS01 0163
Analysis of Microarray Data

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6 November 2007

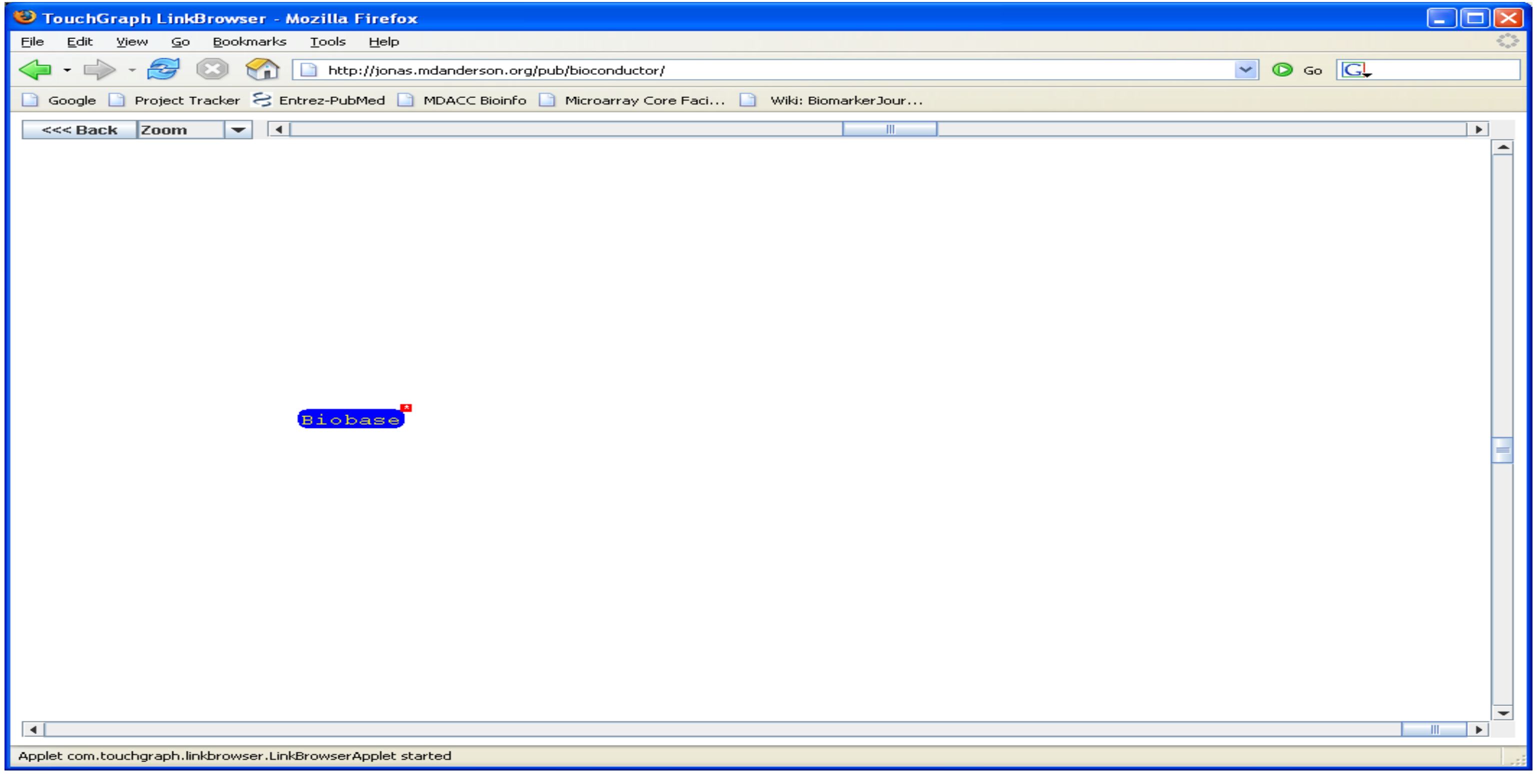
Lecture 20: Genome Browsing

- Learning What BioConductor Contains
- Annotation Environments in R
- AnnBuilder: Rolling Your Own Annotations
- The UCSC Genome Browser
- Chromosome Locations
- Building a Custom Track
- Viewing Your Custom Track

Learning What BioConductor Contains

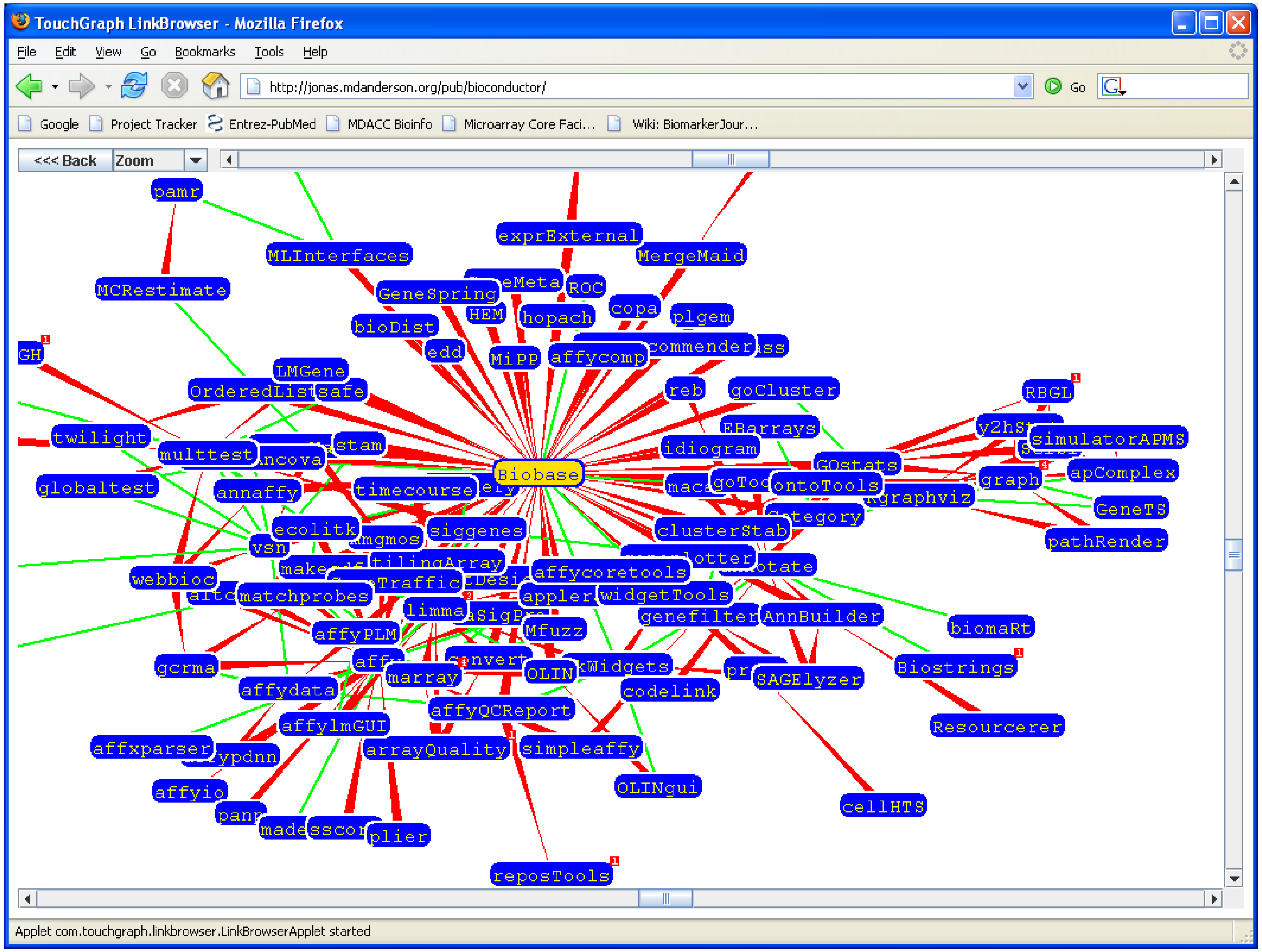
We are developing (i.e., it is not completed, so may behave strangely at times) a graphical tool to browse through the BioConductor documentation.

http://jonas.mdanderson.org/pub/bioconductor/


TouchGraph LinkBrowser - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://jonas.mdanderson.org/pub/bioconductor/
Go
Google Project Tracker Entrez-PubMed MDACC Bioinfo Microarray Core Faci... Wiki: BiomarkerJour...
<<< Back
Zoom
Biobase

Applet com.touchgraph.linkbrowser.LinkBrowserApplet started

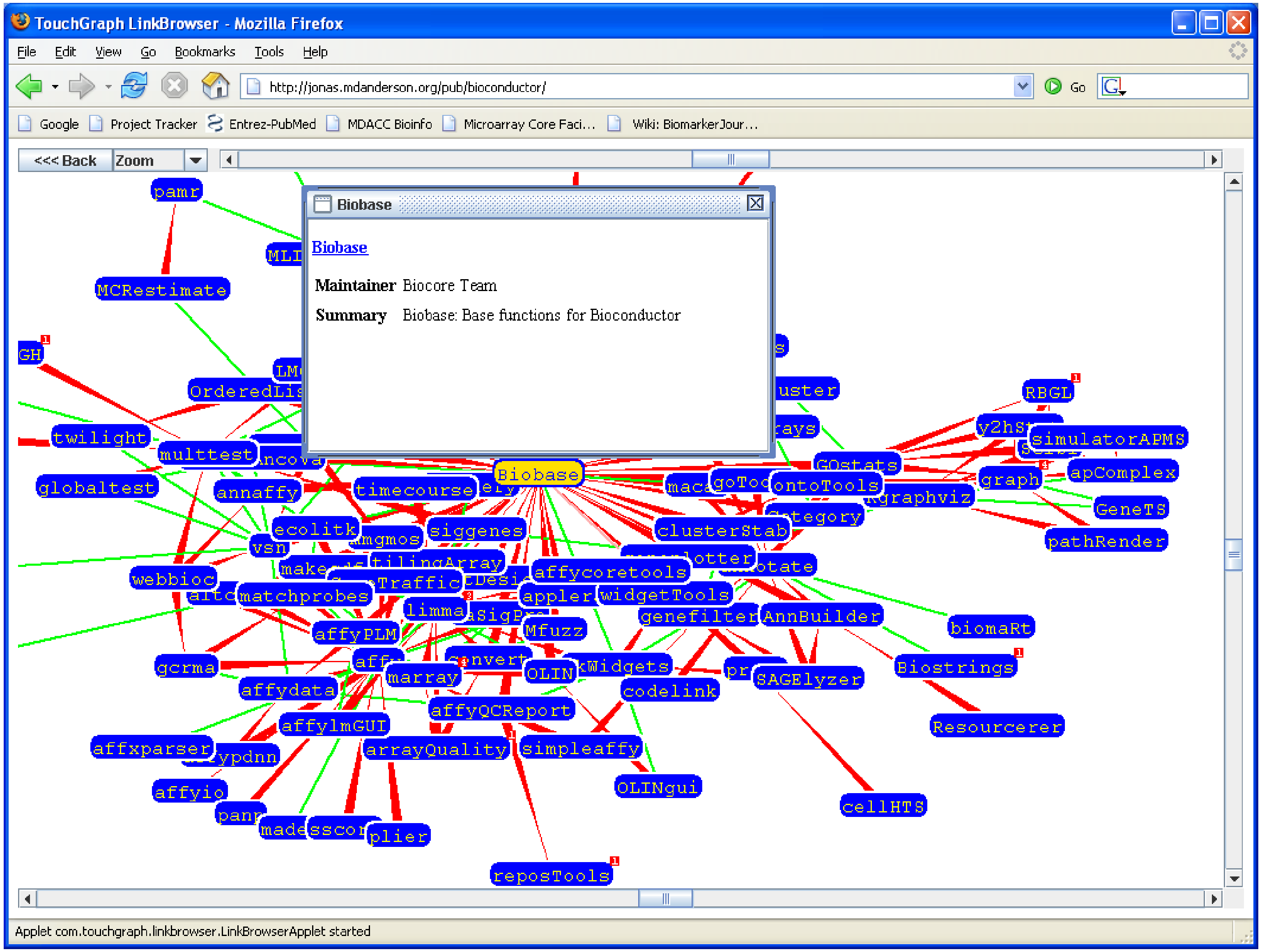
The Documentation Graph



http://jonas.mdanderson.org/pub/bioconductor/

Applet com.touchgraph.linkbrowser.LinkBrowserApplet started

Hovering the Mouse Gives a Summary



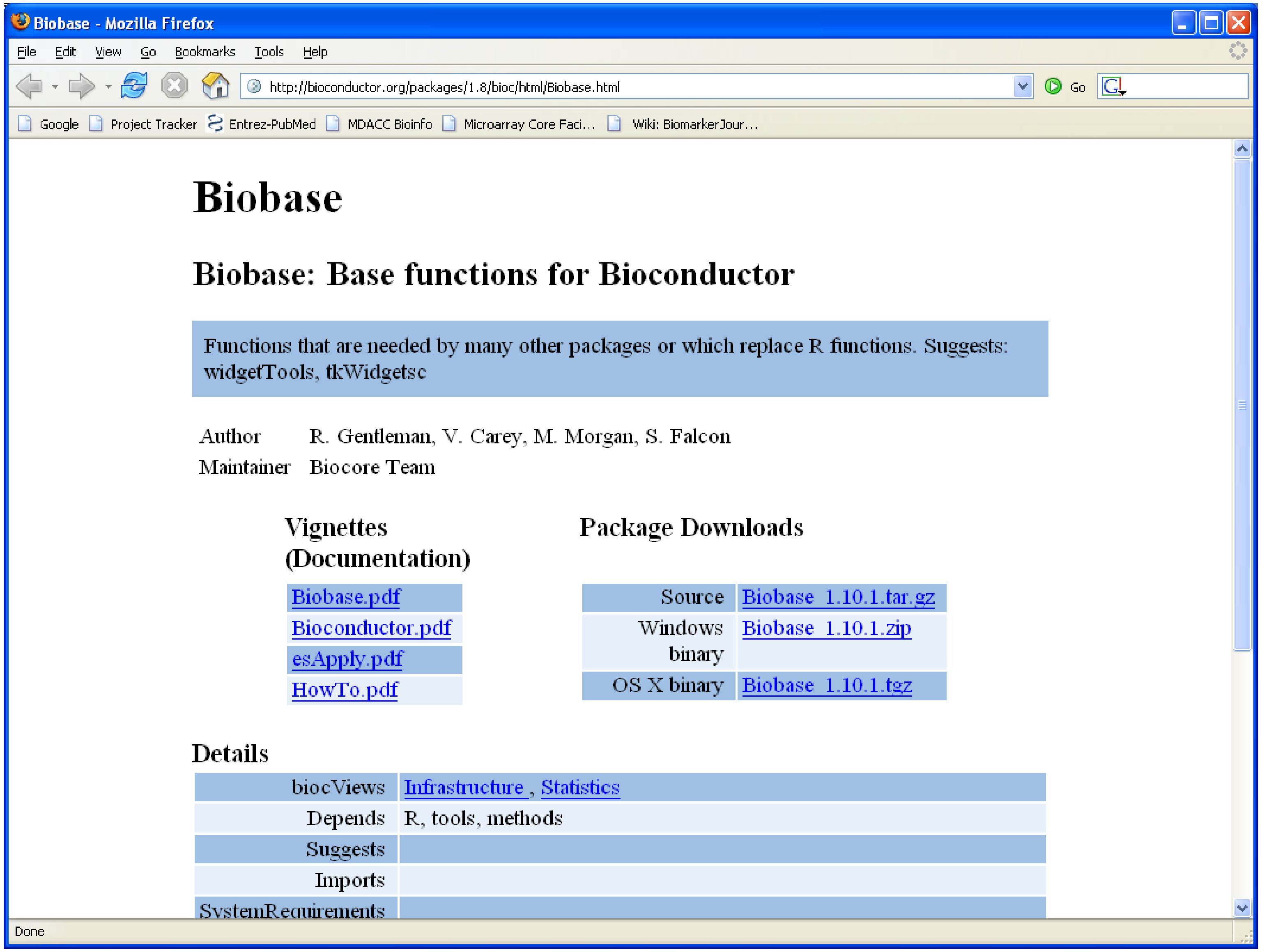
Biobase

Maintainer Biocore Team

Summary Biobase: Base functions for Bioconductor

Applet com.touchgraph.linkbrowser.LinkBrowserApplet started

Left-click Takes You to the Documentation


Biobase - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://bioconductor.org/packages/1.8/bioc/html/Biobase.html
Go
Google Project Tracker Entrez-PubMed MDACC Bioinfo Microarray Core Faci… Wiki: BiomarkerJour…
Done

Biobase

Biobase: Base functions for Bioconductor

Functions that are needed by many other packages or which replace R functions. Suggests: widgetTools, tkWidgetsc

Author	R. Gentleman, V. Carey, M. Morgan, S. Falcon
Maintainer	Biocore Team

Vignettes (Documentation)

- Biobase.pdf
- Bioconductor.pdf
- esApply.pdf
- HowTo.pdf

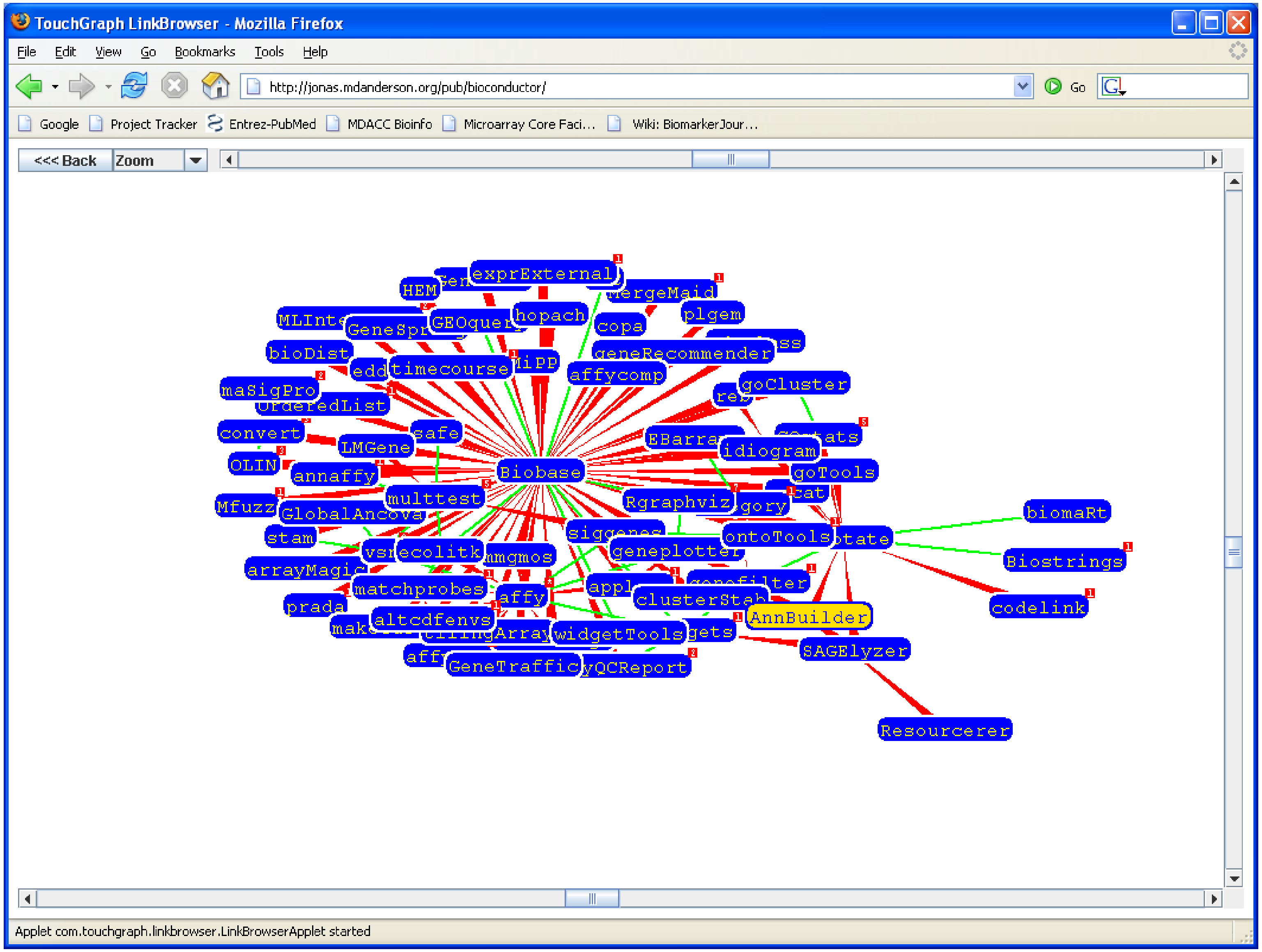
Package Downloads

Source	Biobase_1.10.1.tar.gz
Windows binary	Biobase_1.10.1.zip
OS X binary	Biobase_1.10.1.tgz

Details

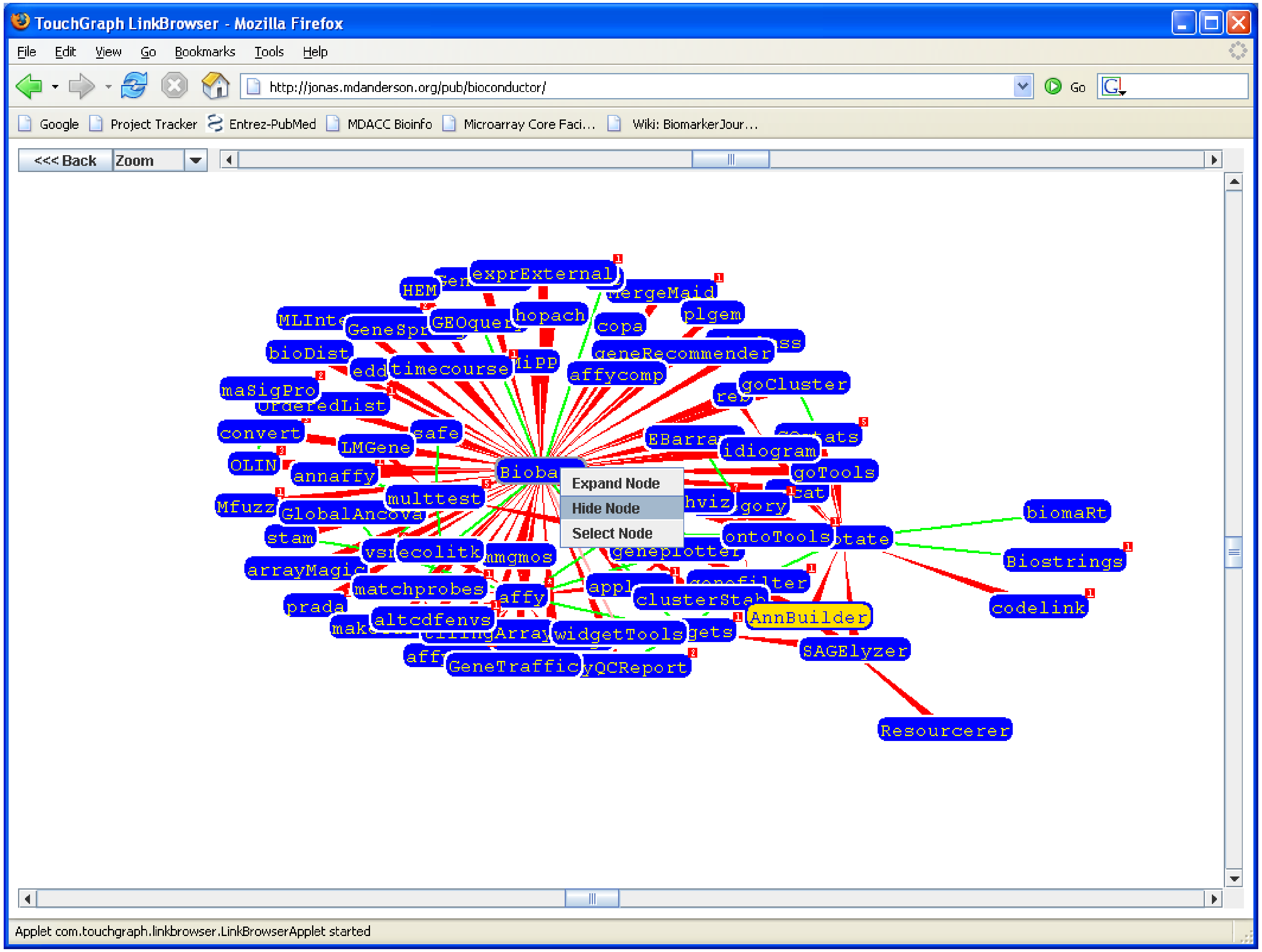
biocViews	Infrastructure , Statistics
Depends	R, tools, methods
Suggests	
Imports	
SystemRequirements	

Left-click Also Recenters on a New Selection



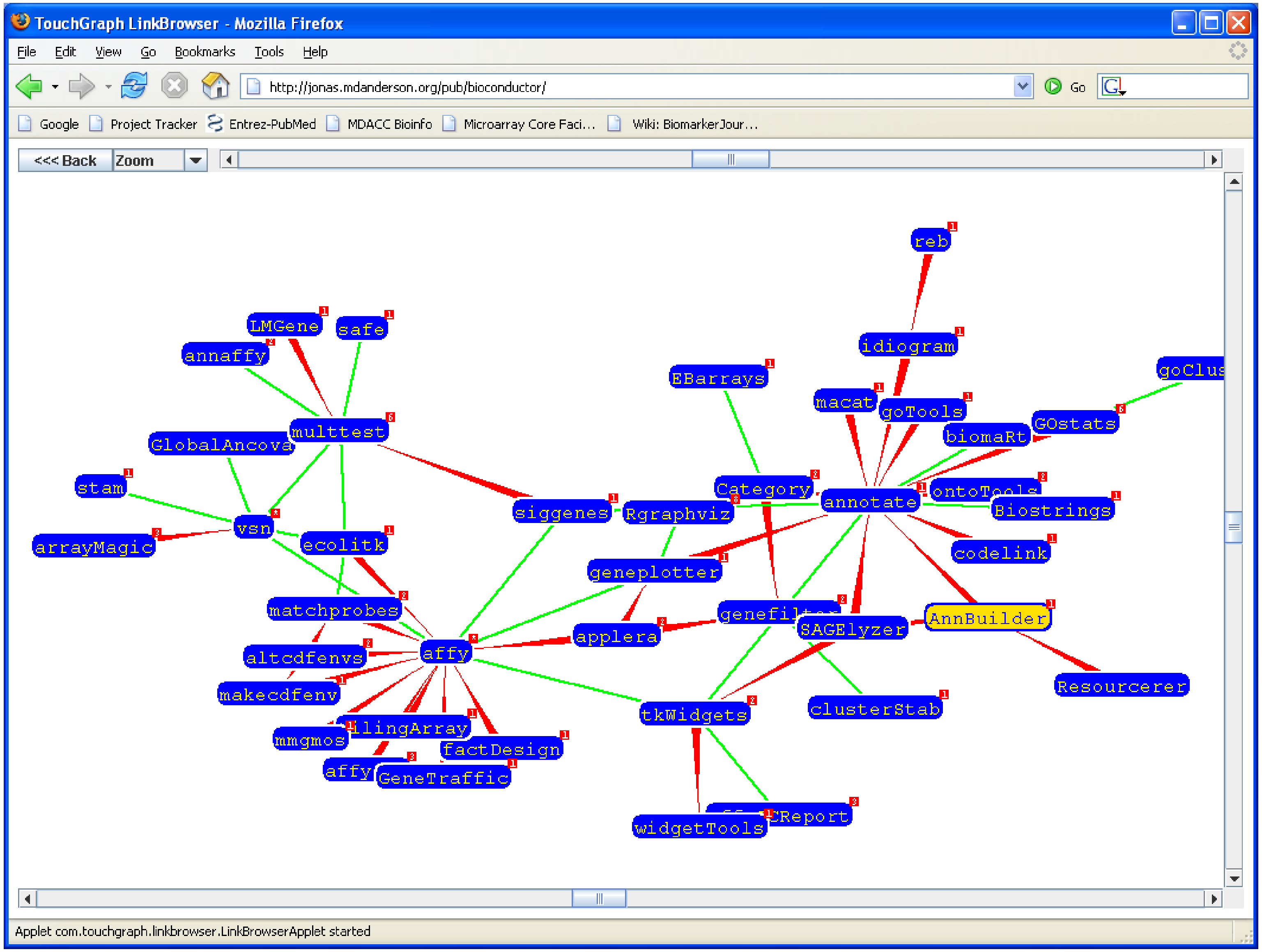
Applet com.touchgraph.linkbrowser.LinkBrowserApplet started

Right-click Lets You Hide Part of the Graph



Applet com.touchgraph.linkbrowser.LinkBrowserApplet started

Hiding BioBase Often Clarifies the Structure



Applet com.touchgraph.linkbrowser.LinkBrowserApplet started

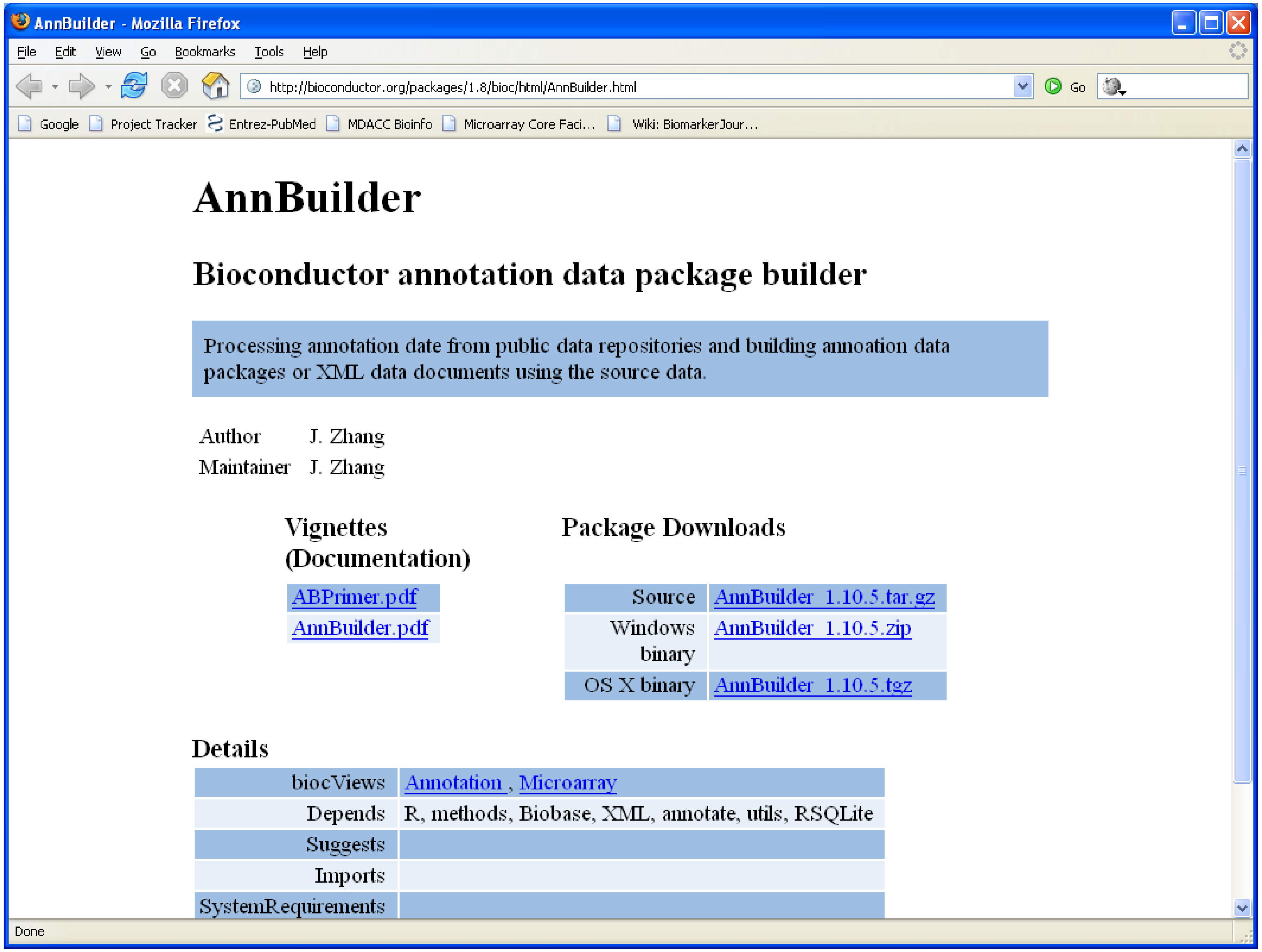
Hubs in the Documentation Graph Are Probably Important

We talked about the `annotate` package previously. It is clear from the graph that this is a central "hub" upon which many of the annotation-related packages depend. (We can also see that `affy` is another hub, defining the basic tools for Affymetrix arrays, and that the `multtest` package for multiple testing is another hub.)

One of the annotation tools that is worth exploring is `biomaRt`, but we are going to leave that for another time. If you want to find out more about the BioMart project, go to `http://www.biomart.org`.

Right now, we want to look at the `AnnBuilder` package.

Documentation for the AnnBuilder Package



AnnBuilder - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://bioconductor.org/packages/1.8/bioc/html/AnnBuilder.html
Go
Google
Project Tracker
Entrez-PubMed
MDACC Bioinfo
Microarray Core Faci…
Wiki: BiomarkerJour…
Done

AnnBuilder

Bioconductor annotation data package builder

Processing annotation date from public data repositories and building annoation data packages or XML data documents using the source data.

Author	J. Zhang
Maintainer	J. Zhang

Vignettes (Documentation)

- ABPrimer.pdf
- AnnBuilder.pdf

Package Downloads

Source	AnnBuilder_1.10.5.tar.gz
Windows binary	AnnBuilder_1.10.5.zip
OS X binary	AnnBuilder_1.10.5.tgz

Details

biocViews	Annotation , Microarray
Depends	R, methods, Biobase, XML, annotate, utils, RSQLite
Suggests	
Imports	
SystemRequirements	

Annotation Environments in R

For most Affymetrix arrays, annotation packages are available directly (and automatically) from BioConductor whenever you need them. These packages were built using `AnnBuilder`.

You can load one of these packages as follows:

```
> require(hgu95av2)

[1] TRUE
```

To see what is in an annotation package, use its name as a function:

```
> hgu95av2()

Quality control information for hgu95av2
Date built: Created: Mon Apr 23 12:21:36 2007
```

```
Number of probes: 12625
Probe number mismatch: None
Probe missmatch: None
Mappings found for probe based rda files:
        hgu95av2ACCNUM found 12625 of 12625
        hgu95av2CHR found 12149 of 12625
        hgu95av2CHRLOC found 11730 of 12625
        hgu95av2ENZYME found 1861 of 12625
        hgu95av2ENTREZID found 12225 of 12625
        hgu95av2GENENAME found 12161 of 12625
        hgu95av2GO found 11421 of 12625
        hgu95av2MAP found 12121 of 12625
        hgu95av2OMIM found 10157 of 12625
        hgu95av2PATH found 4322 of 12625
        hgu95av2PFAM found 12046 of 12625
        hgu95av2PMID found 12120 of 12625
        hgu95av2PROSITE found 12046 of 12625
        hgu95av2REFSEQ found 12004 of 12625
        hgu95av2SYMBOL found 12161 of 12625
        hgu95av2UNIGENE found 11973 of 12625
Mappings found for non-probe based rda files:
        hgu95av2CHRLENGTHS found 25
        hgu95av2ENZYME2PROBE found 677
        hgu95av2GO2ALLPROBES found 7501
        hgu95av2GO2PROBE found 5339
        hgu95av2PATH2PROBE found 189
        hgu95av2PMID2PROBE found 127350
```

Getting Annotations From Environments

Each of the items in the package is an `environment`, which computer scientists may recognize better if we tell them it is a hash table. The key into the probe-based hash table environments is the manufacturers identifier (i.e., an Affymetrix probe set id such as `1854_at`.

```
> get("1854_at", hgu95av2ACCNUM)

[1] "X13293"

> get("1854_at", hgu95av2UNIGENE)

[1] "Hs.179718"
```

```
> get("1854_at", hgu95av2CHR)

[1] "20"

> get("1854_at", hgu95av2MAP)

[1] "20q13.1"

> get("1854_at", hgu95av2CHRLOC)

      20
41729122

> get("1854_at", hgu95av2SYMBOL)

[1] "MYBL2"
```

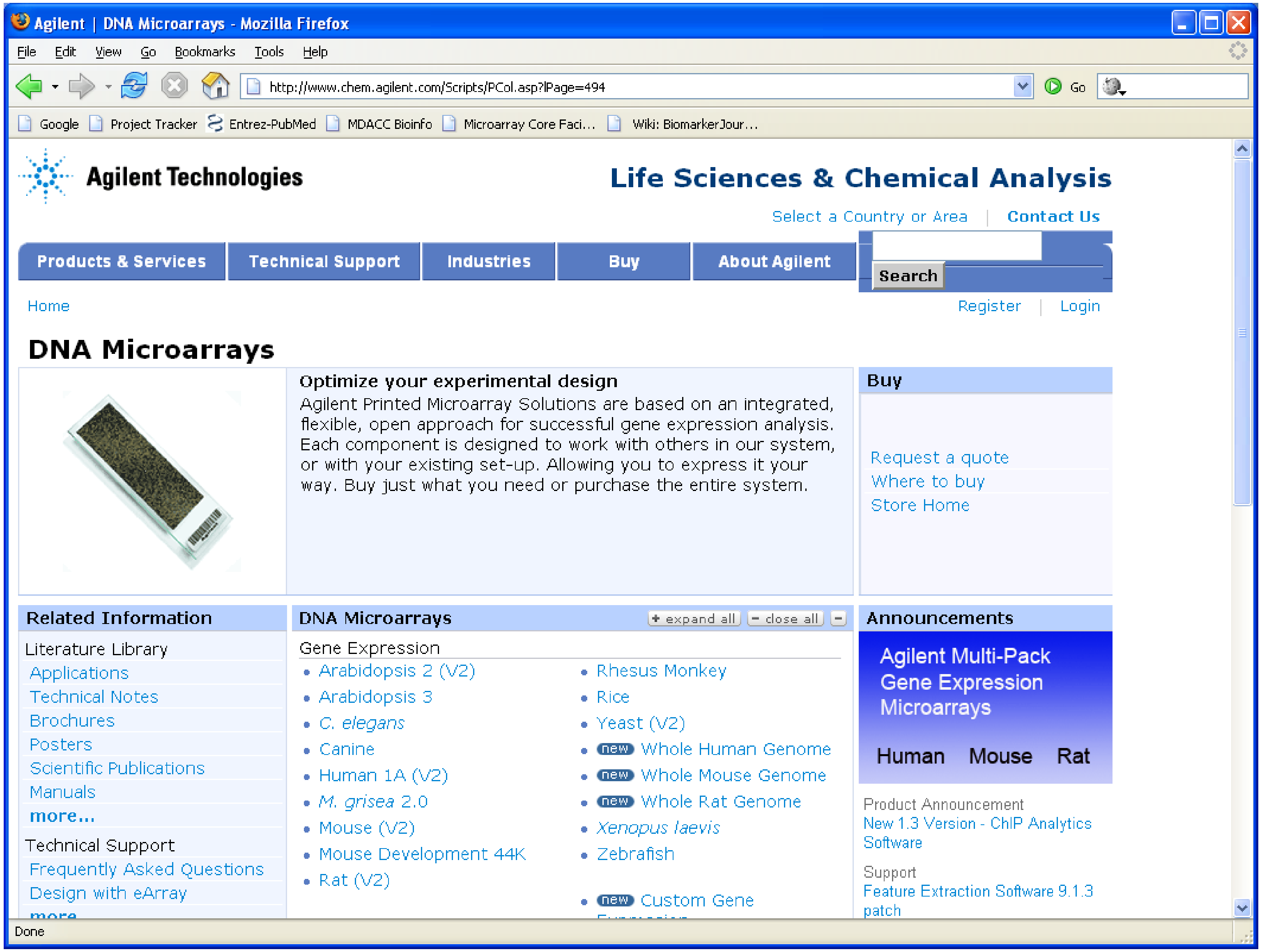
```
> get("1854_at", hgu95av2GENENAME)

[1] "v-myb myeloblastosis viral oncogene homolog (avian)-like

> get("1854_at", hgu95av2ENTREZID)

[1] 4605
```

We have also talked previously about how to find the probe set ids if you start with a gene symbol or a UniGene cluster id.

AnnBuilder: Rolling Your Own Annotations

We recently had to analyze some data from an Agilent 44K two-color glass microarray. The corresponding annotation package was not available, so we had to build our own. Finding the manufacturers basic annotations was a nontrivial task. We started at the web site (`http://www.agilent.com`), then followed the link under "Products and Services" for "Life Sciences" to get to the "DNA Microarrays" page.

Follow the Link for “Whole Human Genome”



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Posters
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Manuals
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Technical Support
Frequently Asked Questions
Design with eArray
more

DNA Microarrays

Gene Expression

- Arabidopsis 2 (V2)
- Arabidopsis 3
- *C. elegans*
- Canine
- Human 1A (V2)
- *M. grisea* 2.0
- Mouse (V2)
- Mouse Development 44K
- Rat (V2)
- Rhesus Monkey
- Rice
- Yeast (V2)
- new Whole Human Genome
- new Whole Mouse Genome
- new Whole Rat Genome
- *Xenopus laevis*
- Zebrafish
- new Custom Gene Expression

Announcements

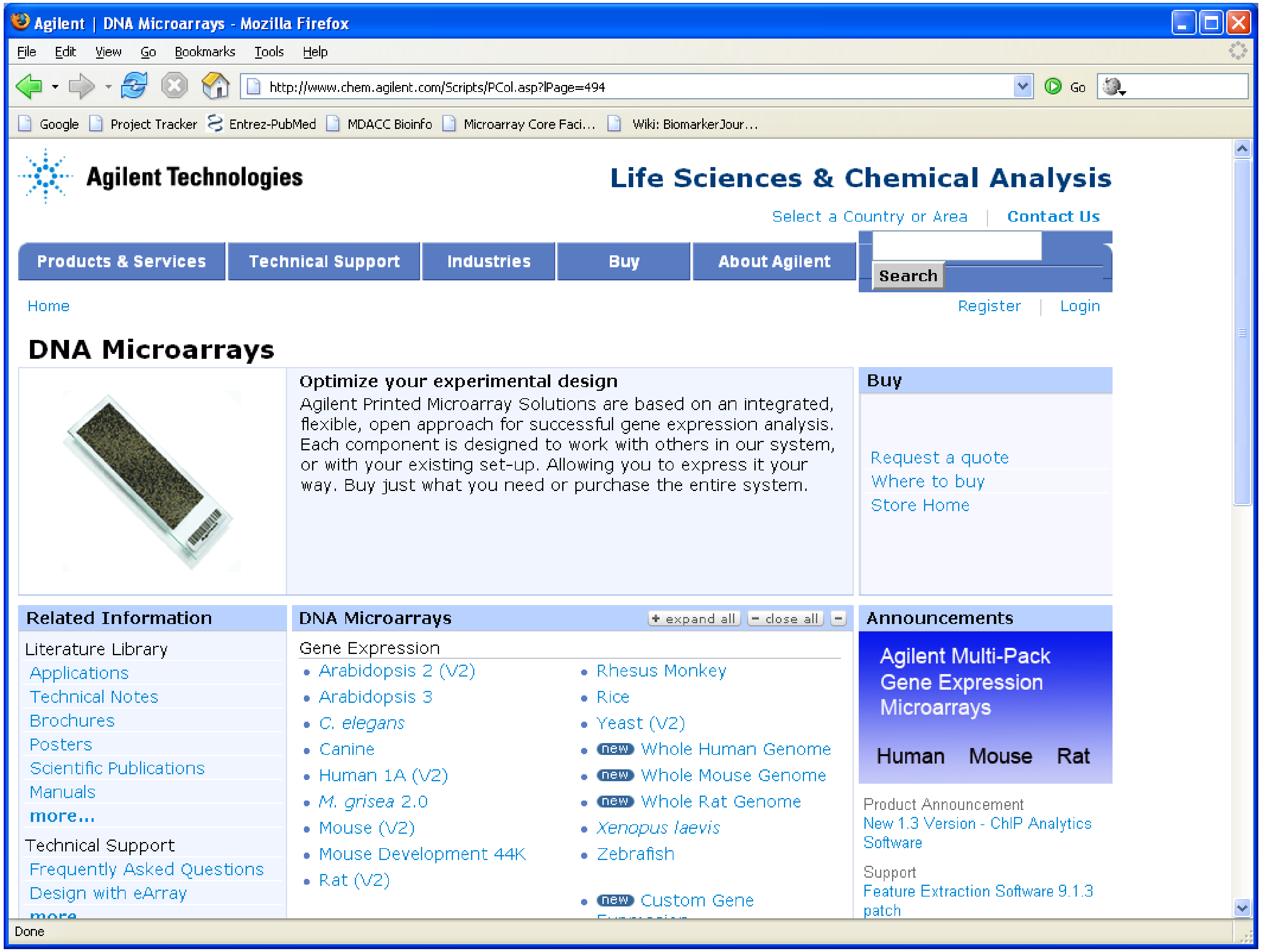
Agilent Multi-Pack Gene Expression Microarrays

Human Mouse Rat

Product Announcement
New 1.3 Version - ChIP Analytics Software

Support
Feature Extraction Software 9.1.3 patch

Follow the Link for “Download Gene Lists”



DNA Microarrays

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- Where to buy
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Related Information

Literature Library

- Applications
- Technical Notes
- Brochures
- Posters
- Scientific Publications
- Manuals
- **more...**

Technical Support

- Frequently Asked Questions
- Design with eArray
- more

DNA Microarrays

Gene Expression

- Arabidopsis 2 (V2)
- Arabidopsis 3
- *C. elegans*
- Canine
- Human 1A (V2)
- *M. grisea* 2.0
- Mouse (V2)
- Mouse Development 44K
- Rat (V2)
- Rhesus Monkey
- Rice
- Yeast (V2)
- new Whole Human Genome
- new Whole Mouse Genome
- new Whole Rat Genome
- *Xenopus laevis*
- Zebrafish
- new Custom Gene Expression

Announcements

Agilent Multi-Pack Gene Expression Microarrays

Human Mouse Rat

Product Announcement
New 1.3 Version - ChIP Analytics Software

Support
Feature Extraction Software 9.1.3 patch

Reading the Feature Info

In any event, we finally obtained a pair of files that contained the mappings from spots to genomic material. (In addition to the "download gene lists", you can also follow the link to "Download design files", but this will only work if you know one of the barcodes on the slides.) We used the `read.table` command to get this file into R:

```
> featureInfo <- read.table("012391_D_DNAFront_BCBottom_2005
+     header = TRUE, row.names = NULL, sep = "\t",
+     quote = "", comment.char = "")
```

Looking at the Feature Info

Here is part of the file:

```
> colnames(featureInfo)

[1] "Column"      "Row"         "Name"        "ID"
[5] "RefNumber"   "ControlType" "GeneName"    "TopHit"
[9] "Description"

> featureInfo[1:5, 1:4]

  Column Row         Name           ID
3    103 426 NM_001003689  A_23_P80353
4    103 424    NM_005503 A_23_P158231
5    103 422    NM_004672 A_32_P223017
```

```
6     103 420 NM_001008727 A_24_P935782
8     103 416    NM_020630 A_24_P343695
```

The critical information is given by the columns that contain the manufaturers identifier (ID) and the GenBank or RefSeq accession number (Name). The function we are going to use to build annotations requires only these two columns (in the reverse order) to be present in a file. So we make them available:

```
> temp <- featureInfo[, c(4, 3)]
> write.table(temp, "agilentGenes.tsv", sep = "\t",
+     quote = FALSE, col.names = NA)
```

Setting Up the Annotation Package

```
> library(AnnBuilder)
> baseName <- "agilentGenes.tsv"
> baseType <- "gb"
> srcUrls <- getSrcUrl("all", organism = "Homo sapiens")
> myDir <- getwd()
```

Building the Annotation Package

The next command takes a **very** long time, since it makes calls to databases all over the internet for every one of the $44,000$ probes on the array. Be prepared to go get lunch while it executes.

```
ABPkgBuilder(baseName = baseName, srcUrls = srcUrls,
    baseMapType = baseType, pkgName = "Agilent44K",
    pkgPath = myDir, organism = "Homo sapiens",
    version = "1.0", author = list(authors = "krc@mdacc.tmc
        maintainer = "krc@mdacc.tmc.edu"), fromWeb = TRUE)
```

Producing the Final Package

This command produces the **source** for a package, which must still be compiled and zipped into a binary package that can be installed easily. This task is most easily accomplished on a UNIX based machine:

```
helios% R CMD build Agilent44K
helios% R CMD build --binary Agilent44K
```

You can then convert the resulting `.tar.gz` file to a `.zip` file, which is the preferred form for distributing a Windows package.

You can check out the results by getting the annotation package from our course web site.

The Agilent 44K Annotations

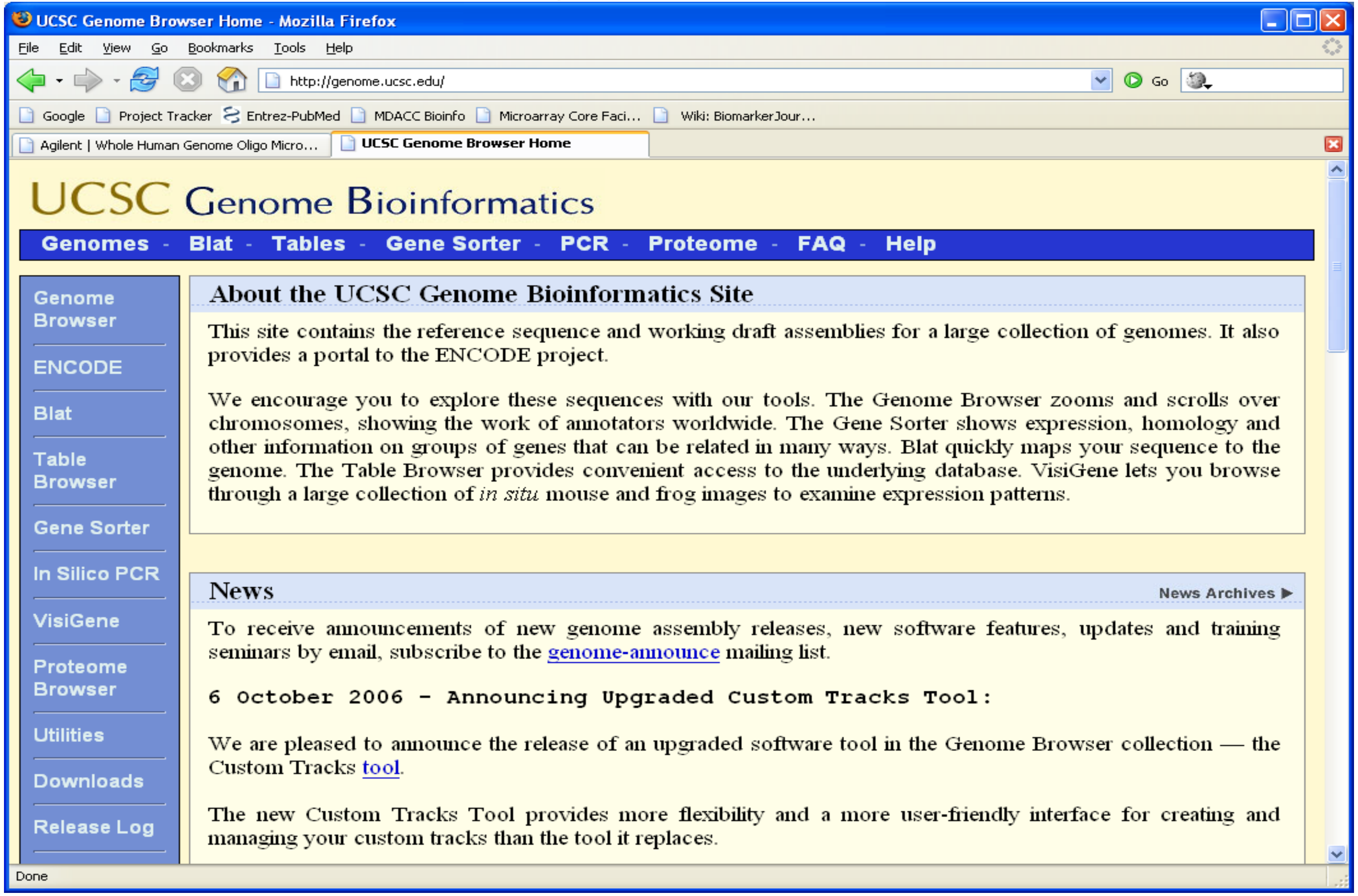
```
> library(Agilent44K)
> Agilent44K()

Quality control information for  Agilent44K
Date built: Created: Sun Sep 03 07:50:38 2006

Number of probes: 41001
Probe number missmatch: None
Probe missmatch: None
Mappings found for probe based rda files:
         Agilent44KACCNUM found 41001 of 41001
         Agilent44KCHR found 31185 of 41001
         Agilent44KCHRLOC found 28795 of 41001
         Agilent44KENZYME found 3056 of 41001
         Agilent44KGENENAME found 27824 of 41001
         Agilent44KGO found 23644 of 41001
         Agilent44KLOCUSID found 31224 of 41001
         Agilent44KMAP found 30939 of 41001
         Agilent44KOMIM found 17942 of 41001
         Agilent44KPATH found 6715 of 41001
         Agilent44KPMID found 30361 of 41001
         Agilent44KREFSEQ found 30057 of 41001
         Agilent44KSUMFUNC found 0 of 41001
         Agilent44KSYMBOL found 31217 of 41001
         Agilent44KUNIGENE found 31010 of 41001
Mappings found for non-probe based rda files:
          Agilent44KCHRLENGTHS found 25
         Agilent44KENZYME2PROBE found 794
         Agilent44KGO2ALLPROBES found 6883
         Agilent44KGO2PROBE found 5117
```

```
Agilent44KORGANISM found 1
Agilent44KPATH2PROBE found 183
Agilent44KPFAM found 21902
Agilent44KPMID2PROBE found 131104
Agilent44KPROSITE found 15055
```

The UCSC Genome Browser

We are going to shift gears slightly:

`http://genome.ucsc.edu/`


UCSC Genome Browser Home - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://genome.ucsc.edu/
Go
Google Project Tracker Entrez-PubMed MDACC Bioinfo Microarray Core Faci… Wiki: BiomarkerJour…
Agilent | Whole Human Genome Oligo Micro…
UCSC Genome Browser Home
UCSC Genome Bioinformatics
Genomes - Blat - Tables - Gene Sorter - PCR - Proteome - FAQ - Help
Genome Browser
ENCODE
Blat
Table Browser
Gene Sorter
In Silico PCR
VisiGene
Proteome Browser
Utilities
Downloads
Release Log
News Archives ▶
Done

About the UCSC Genome Bioinformatics Site

This site contains the reference sequence and working draft assemblies for a large collection of genomes. It also provides a portal to the ENCODE project.

We encourage you to explore these sequences with our tools. The Genome Browser zooms and scrolls over chromosomes, showing the work of annotators worldwide. The Gene Sorter shows expression, homology and other information on groups of genes that can be related in many ways. Blat quickly maps your sequence to the genome. The Table Browser provides convenient access to the underlying database. VisiGene lets you browse through a large collection of *in situ* mouse and frog images to examine expression patterns.

News

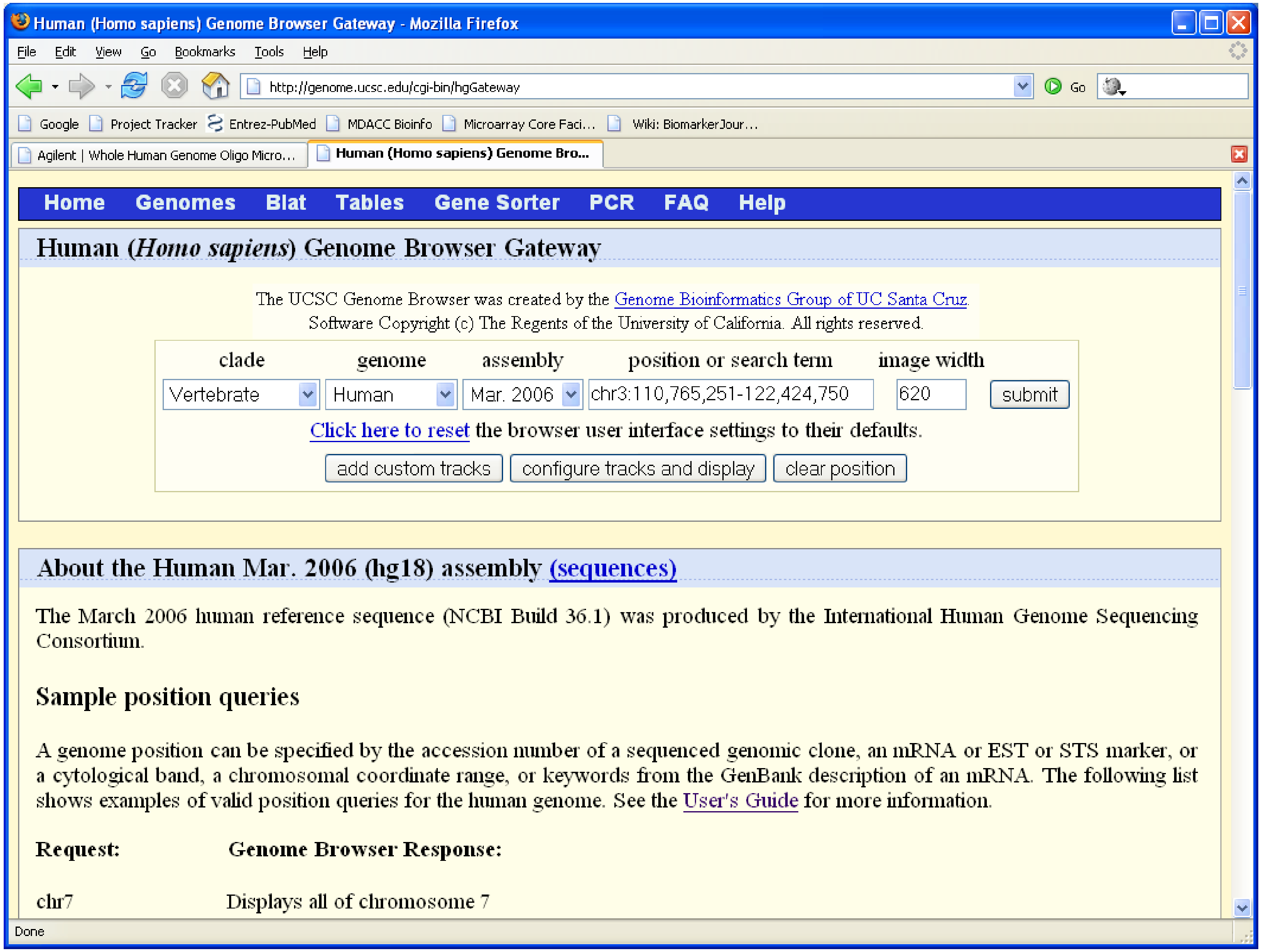
To receive announcements of new genome assembly releases, new software features, updates and training seminars by email, subscribe to the genome-announce mailing list.

6 October 2006 - Announcing Upgraded Custom Tracks Tool:

We are pleased to announce the release of an upgraded software tool in the Genome Browser collection — the Custom Tracks tool.

The new Custom Tracks Tool provides more flexibility and a more user-friendly interface for creating and managing your custom tracks than the tool it replaces.

Follow the Link to “Genome Browser”


Human (Homo sapiens) Genome Browser Gateway - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://genome.ucsc.edu/cgi-bin/hgGateway
Go
Google
Project Tracker
Entrez-PubMed
MDACC Bioinfo
Microarray Core Faci…
Wiki: BiomarkerJour…
Agilent | Whole Human Genome Oligo Micro…
Human (Homo sapiens) Genome Bro…
Home
Genomes
Blat
Tables
Gene Sorter
PCR
FAQ
Help
Done

Human (*Homo sapiens*) Genome Browser Gateway

The UCSC Genome Browser was created by the Genome Bioinformatics Group of UC Santa Cruz.
Software Copyright (c) The Regents of the University of California. All rights reserved.

clade	genome	assembly	position or search term	image width	
Vertebrate	Human	Mar. 2006	chr3:110,765,251-122,424,750	620	submit

Click here to reset the browser user interface settings to their defaults.

add custom tracks | configure tracks and display | clear position

About the Human Mar. 2006 (hg18) assembly (sequences)

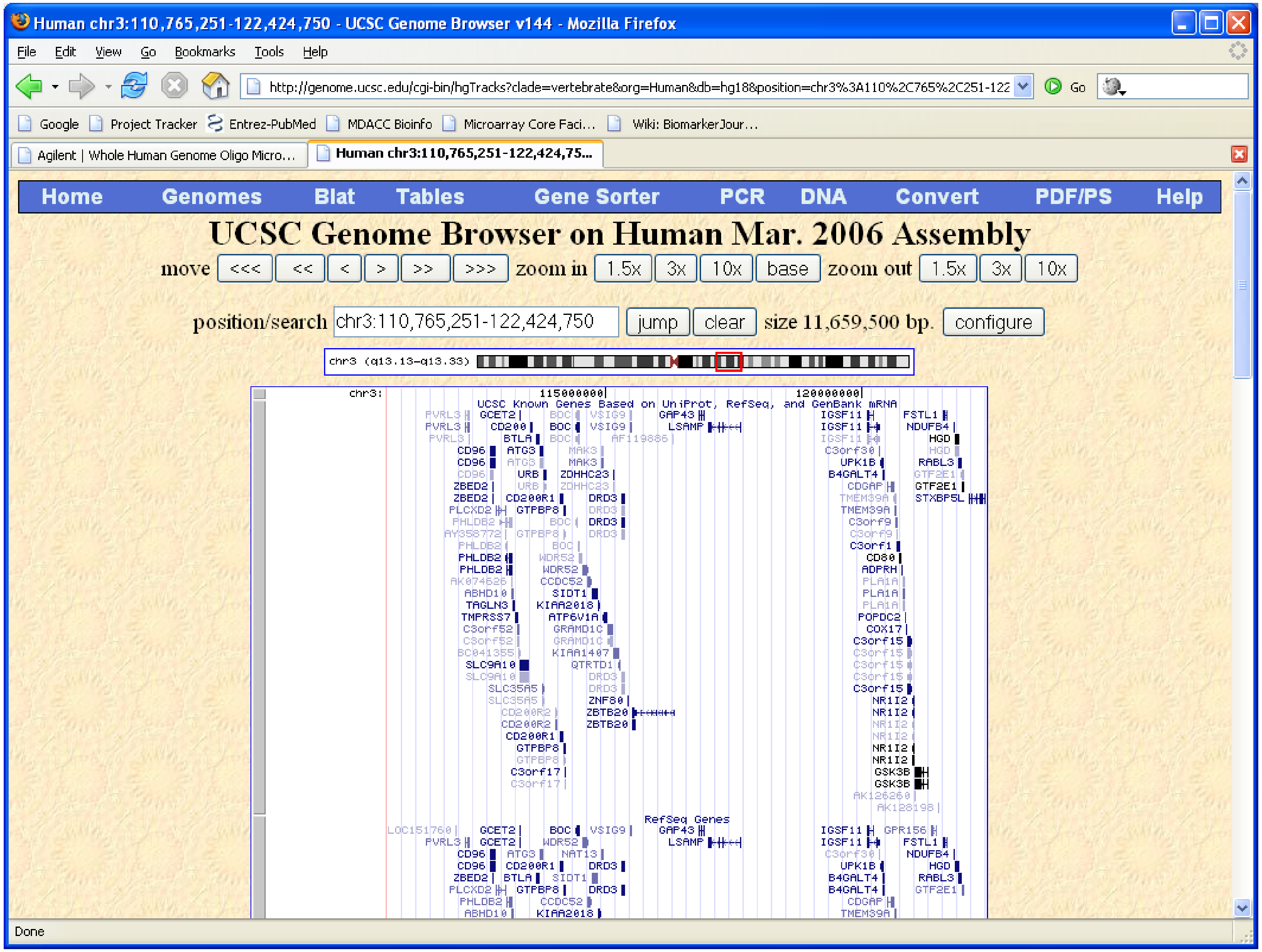
The March 2006 human reference sequence (NCBI Build 36.1) was produced by the International Human Genome Sequencing Consortium.

Sample position queries

A genome position can be specified by the accession number of a sequenced genomic clone, an mRNA or EST or STS marker, or a cytological band, a chromosomal coordinate range, or keywords from the GenBank description of an mRNA. The following list shows examples of valid position queries for the human genome. See the User's Guide for more information.

Request:	Genome Browser Response:
chr7	Displays all of chromosome 7

Press “Submit” to Start Browsing



Human chr3:110,765,251-122,424,750 - UCSC Genome Browser v144 - Mozilla Firefox

http://genome.ucsc.edu/cgi-bin/hgTracks?clade=vertebrate&org=Human&db=hg18&position=chr3%3A110%2C765%2C251-122

UCSC Genome Browser on Human Mar. 2006 Assembly

position/search chr3:110,765,251-122,424,750 size 11,659,500 bp.

Done

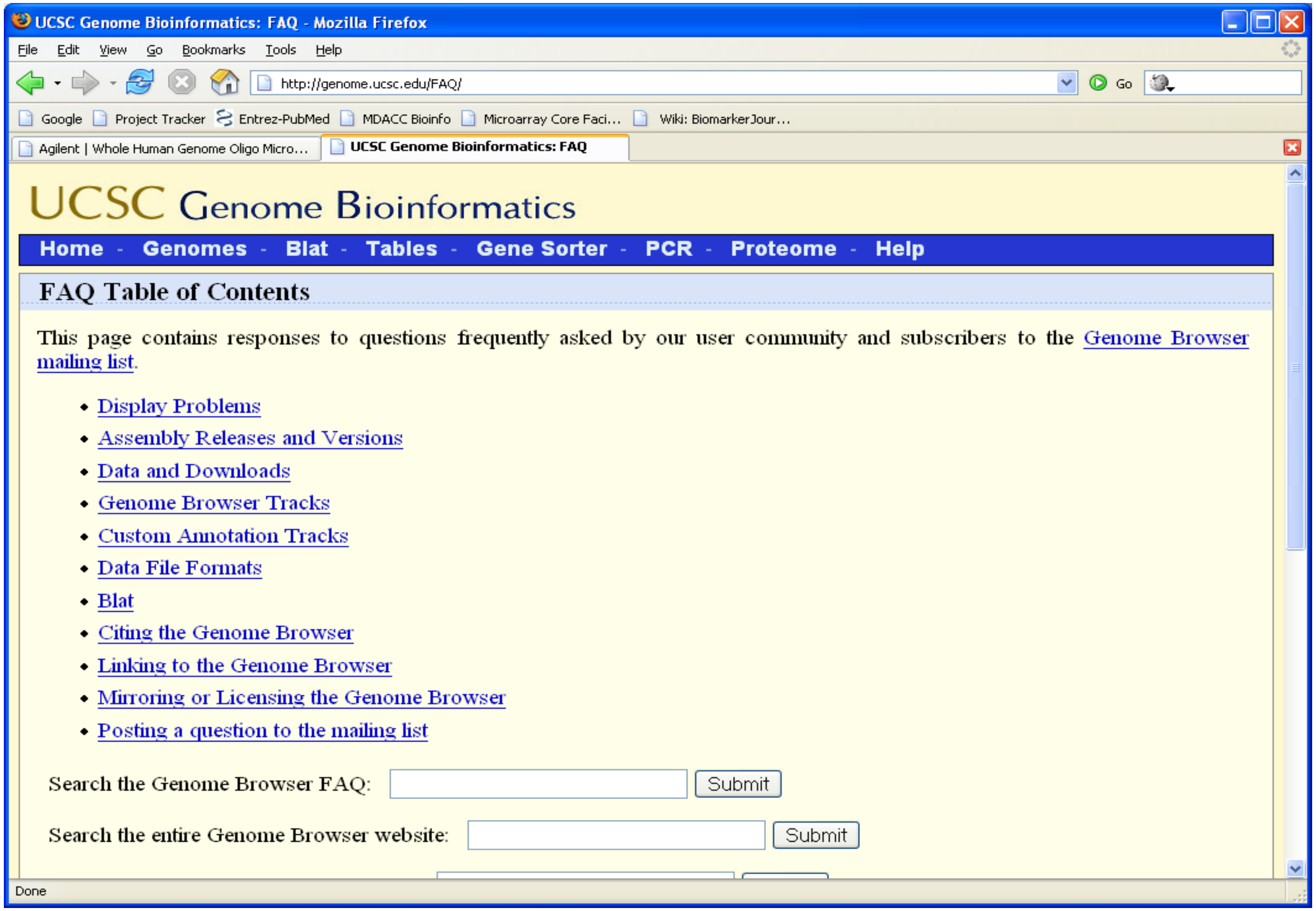
About the Genome Browser

The genome browser lets you see a great deal of information laid out along the latest completed build of the human genome. The most obvious thing to look at are the known genes, which are typically displayed in such a way that you can see the individual introns and exons (provided you zoom in closely).

For our purposes (as people who analyze microarray data), an extremely interesting feature of the Genome Browser is that it lets you add your own "Custom Tracks", which is their name for a set of annotations you can define.

Custom Tracks

To learn about the genome (custom) tracks, go to the FAQ.


UCSC Genome Bioinformatics: FAQ - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://genome.ucsc.edu/FAQ/
Go
Google
Project Tracker
Entrez-PubMed
MDACC Bioinfo
Microarray Core Faci...
Wiki: BiomarkerJour...
Agilent | Whole Human Genome Oligo Micro...
UCSC Genome Bioinformatics: FAQ
UCSC Genome Bioinformatics
Home - Genomes - Blat - Tables - Gene Sorter - PCR - Proteome - Help
Done

FAQ Table of Contents

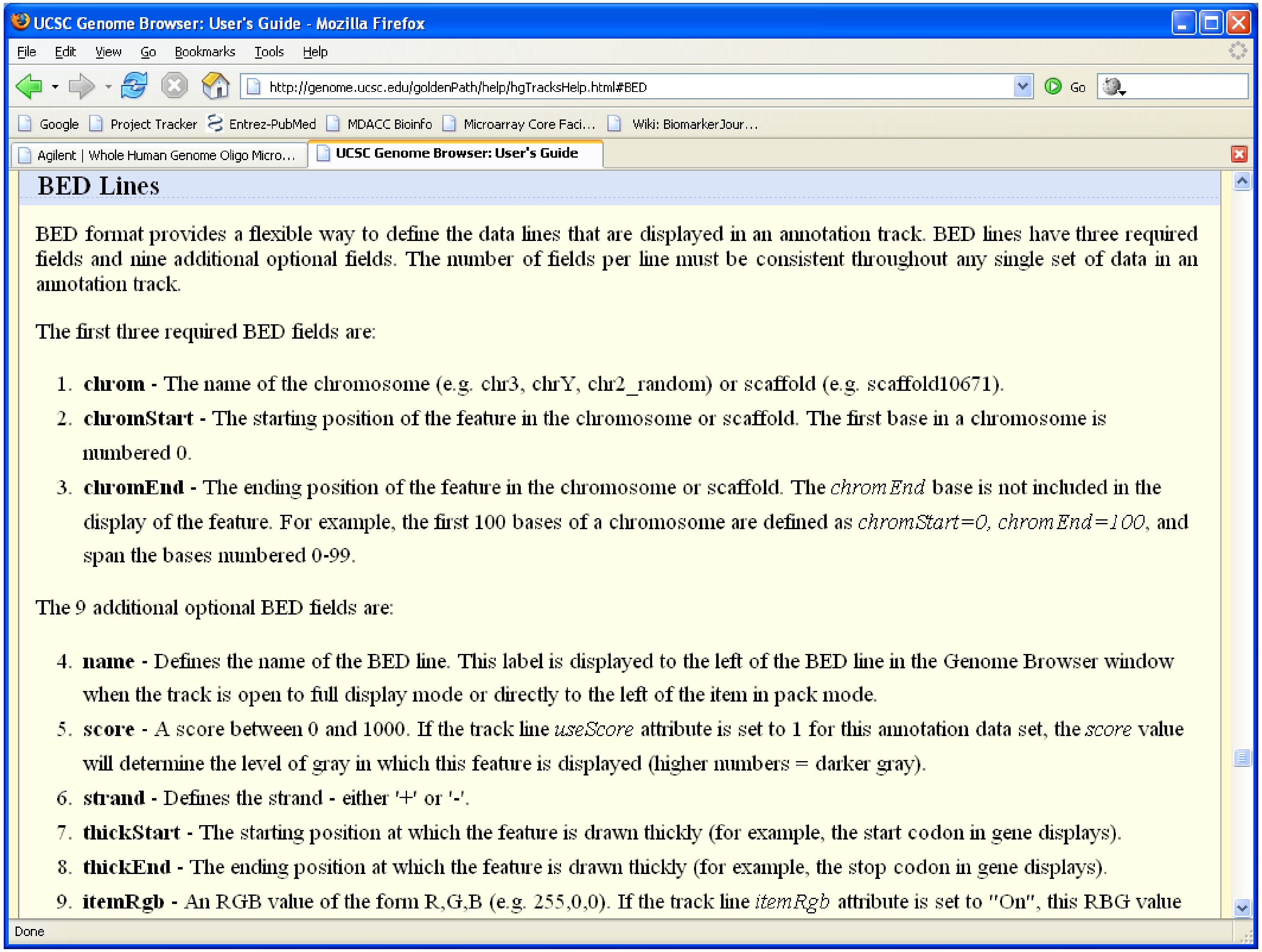
This page contains responses to questions frequently asked by our user community and subscribers to the Genome Browser mailing list.

- Display Problems
- Assembly Releases and Versions
- Data and Downloads
- Genome Browser Tracks
- Custom Annotation Tracks
- Data File Formats
- Blat
- Citing the Genome Browser
- Linking to the Genome Browser
- Mirroring or Licensing the Genome Browser
- Posting a question to the mailing list

Search the Genome Browser FAQ: Submit

Search the entire Genome Browser website: Submit

BED Format


UCSC Genome Browser: User's Guide - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://genome.ucsc.edu/goldenPath/help/hgTracksHelp.html#BED
Go
Google
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Entrez-PubMed
MDACC Bioinfo
Microarray Core Faci...
Wiki: BiomarkerJour...
Agilent | Whole Human Genome Oligo Micro...
UCSC Genome Browser: User's Guide
Done

BED Lines

BED format provides a flexible way to define the data lines that are displayed in an annotation track. BED lines have three required fields and nine additional optional fields. The number of fields per line must be consistent throughout any single set of data in an annotation track.

The first three required BED fields are:

1. **chrom** - The name of the chromosome (e.g. chr3, chrY, chr2_random) or scaffold (e.g. scaffold10671).
2. **chromStart** - The starting position of the feature in the chromosome or scaffold. The first base in a chromosome is numbered 0.
3. **chromEnd** - The ending position of the feature in the chromosome or scaffold. The *chromEnd* base is not included in the display of the feature. For example, the first 100 bases of a chromosome are defined as *chromStart=0, chromEnd=100*, and span the bases numbered 0-99.

The 9 additional optional BED fields are:

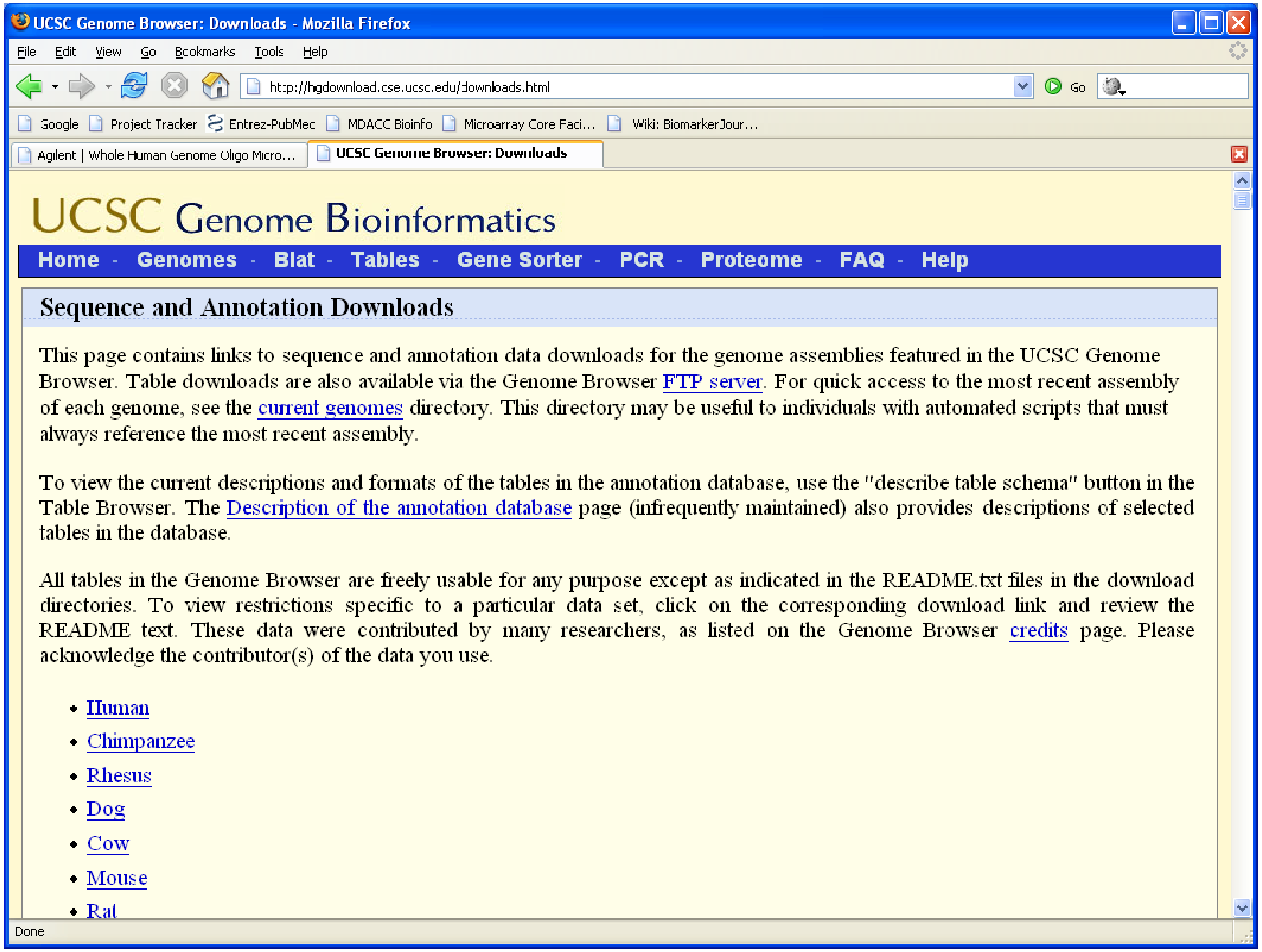
4. **name** - Defines the name of the BED line. This label is displayed to the left of the BED line in the Genome Browser window when the track is open to full display mode or directly to the left of the item in pack mode.
5. **score** - A score between 0 and 1000. If the track line *useScore* attribute is set to 1 for this annotation data set, the *score* value will determine the level of gray in which this feature is displayed (higher numbers = darker gray).
6. **strand** - Defines the strand - either '+' or '-'.
7. **thickStart** - The starting position at which the feature is drawn thickly (for example, the start codon in gene displays).
8. **thickEnd** - The ending position at which the feature is drawn thickly (for example, the stop codon in gene displays).
9. **itemRgb** - An RGB value of the form R,G,B (e.g. 255,0,0). If the track line *itemRgb* attribute is set to "On", this RBG value

Chromosome Locations

You can read more of the custom track documentation on your own; here, we are going to focus on how to build a custom track in R. The first thing we want to point out is that we need to know both the starting base location and the ending base location in order to build a custom track. Thus, the `CHRLOC` annotations that the `AnnBuilder` BioConductor package constructs are not adequate.

Fortunately, we can get start and end points directly from the folks at the UCSC Genome Browser. Go back to the main page, then follow the link for "Downloads".

UCSC Download Page



UCSC Genome Browser: Downloads - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://hgdownload.cse.ucsc.edu/downloads.html
Go
Google Project Tracker Entrez-PubMed MDACC Bioinfo Microarray Core Faci... Wiki: BiomarkerJour...
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UCSC Genome Browser: Downloads
UCSC Genome Bioinformatics
Home - Genomes - Blat - Tables - Gene Sorter - PCR - Proteome - FAQ - Help
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Sequence and Annotation Downloads

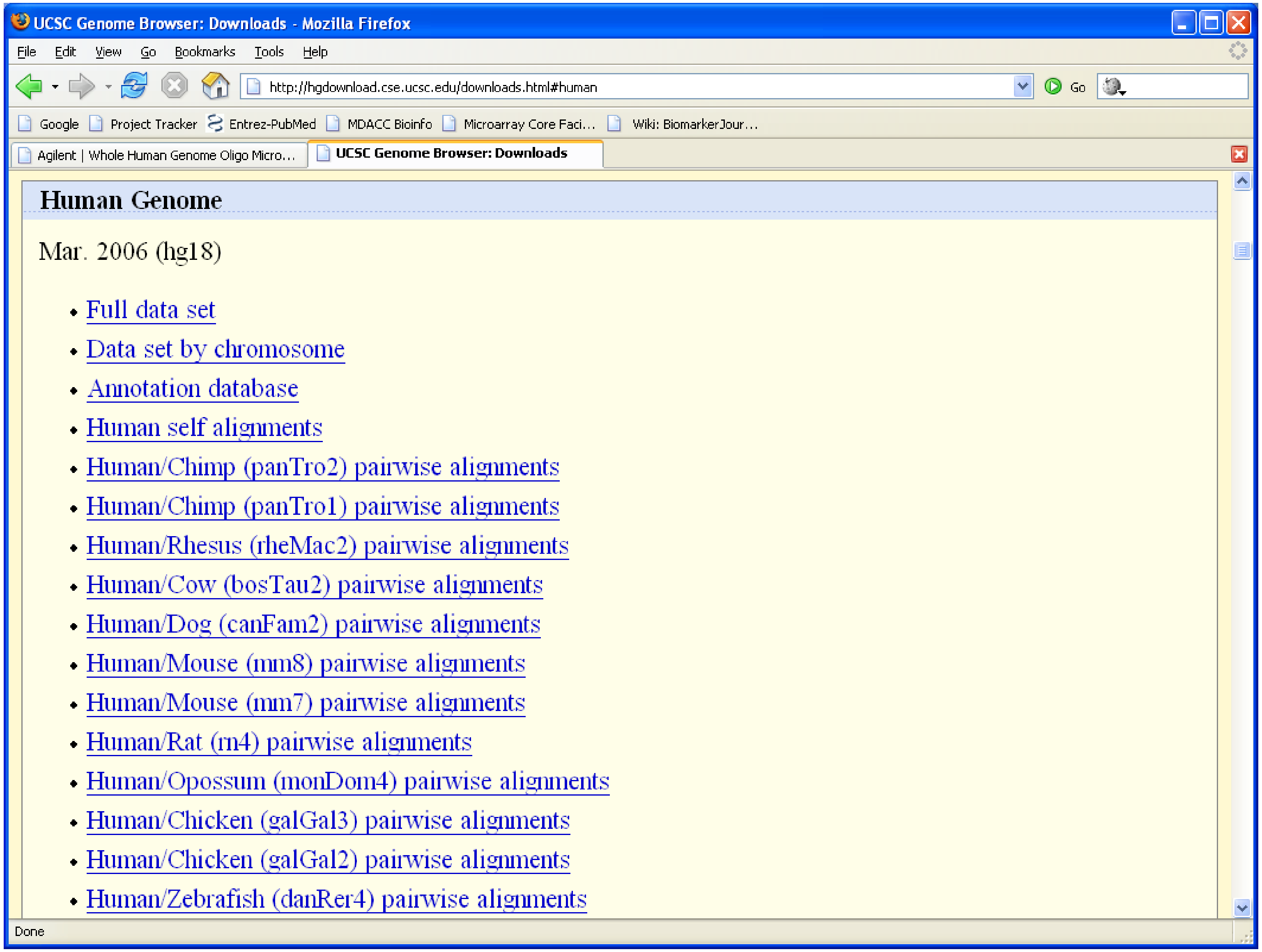
This page contains links to sequence and annotation data downloads for the genome assemblies featured in the UCSC Genome Browser. Table downloads are also available via the Genome Browser FTP server. For quick access to the most recent assembly of each genome, see the current genomes directory. This directory may be useful to individuals with automated scripts that must always reference the most recent assembly.

To view the current descriptions and formats of the tables in the annotation database, use the "describe table schema" button in the Table Browser. The Description of the annotation database page (infrequently maintained) also provides descriptions of selected tables in the database.

All tables in the Genome Browser are freely usable for any purpose except as indicated in the README.txt files in the download directories. To view restrictions specific to a particular data set, click on the corresponding download link and review the README text. These data were contributed by many researchers, as listed on the Genome Browser credits page. Please acknowledge the contributor(s) of the data you use.

- Human
- Chimpanzee
- Rhesus
- Dog
- Cow
- Mouse
- Rat

Follow the link for "Human"



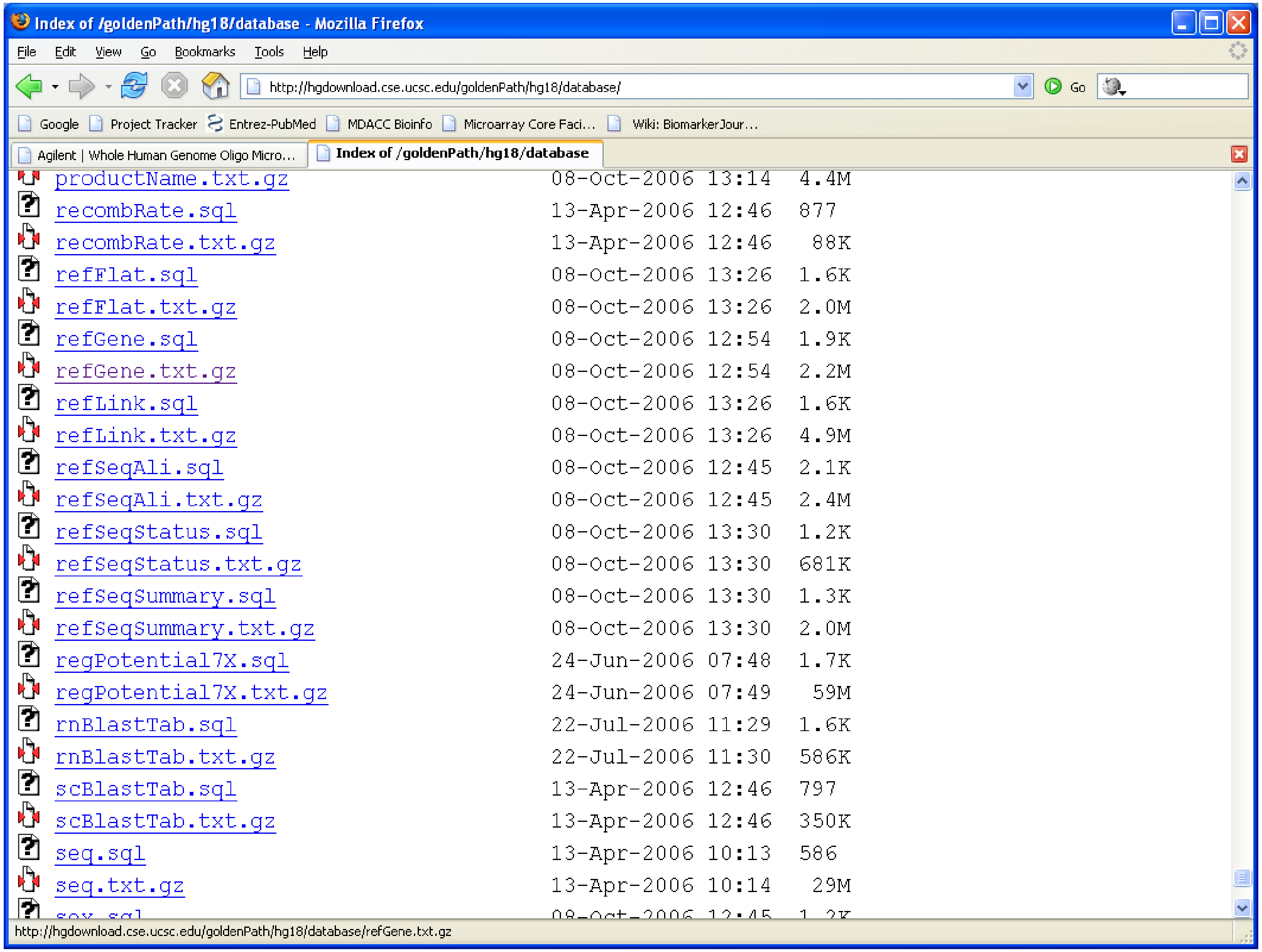
UCSC Genome Browser: Downloads - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://hgdownload.cse.ucsc.edu/downloads.html#human
Go
Google
Project Tracker
Entrez-PubMed
MDACC Bioinfo
Microarray Core Faci…
Wiki: BiomarkerJour…
Agilent | Whole Human Genome Oligo Micro…
UCSC Genome Browser: Downloads
Done

Human Genome

Mar. 2006 (hg18)

- Full data set
- Data set by chromosome
- Annotation database
- Human self alignments
- Human/Chimp (panTro2) pairwise alignments
- Human/Chimp (panTro1) pairwise alignments
- Human/Rhesus (rheMac2) pairwise alignments
- Human/Cow (bosTau2) pairwise alignments
- Human/Dog (canFam2) pairwise alignments
- Human/Mouse (mm8) pairwise alignments
- Human/Mouse (mm7) pairwise alignments
- Human/Rat (rn4) pairwise alignments
- Human/Opossum (monDom4) pairwise alignments
- Human/Chicken (galGal3) pairwise alignments
- Human/Chicken (galGal2) pairwise alignments
- Human/Zebrafish (danRer4) pairwise alignments

In “Annotation Database”, Scroll To “refGene”


Index of /goldenPath/hg18/database - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
Go
Google
Project Tracker
Entrez-PubMed
MDACC Bioinfo
Microarray Core Faci...
Wiki: BiomarkerJour...
Agilent | Whole Human Genome Oligo Micro...
Index of /goldenPath/hg18/database

http://hgdownload.cse.ucsc.edu/goldenPath/hg18/database/

```
productName.txt.gz        08-Oct-2006 13:14  4.4M
recombRate.sql            13-Apr-2006 12:46  877
recombRate.txt.gz         13-Apr-2006 12:46   88K
refFlat.sql               08-Oct-2006 13:26  1.6K
refFlat.txt.gz            08-Oct-2006 13:26  2.0M
refGene.sql               08-Oct-2006 12:54  1.9K
refGene.txt.gz            08-Oct-2006 12:54  2.2M
refLink.sql               08-Oct-2006 13:26  1.6K
refLink.txt.gz            08-Oct-2006 13:26  4.9M
refSeqAli.sql             08-Oct-2006 12:45  2.1K
refSeqAli.txt.gz          08-Oct-2006 12:45  2.4M
refSeqStatus.sql          08-Oct-2006 13:30  1.2K
refSeqStatus.txt.gz       08-Oct-2006 13:30  681K
refSeqSummary.sql         08-Oct-2006 13:30  1.3K
refSeqSummary.txt.gz      08-Oct-2006 13:30  2.0M
regPotential7X.sql        24-Jun-2006 07:48  1.7K
regPotential7X.txt.gz     24-Jun-2006 07:49   59M
rnBlastTab.sql            22-Jul-2006 11:29  1.6K
rnBlastTab.txt.gz         22-Jul-2006 11:30  586K
scBlastTab.sql            13-Apr-2006 12:46  797
scBlastTab.txt.gz         13-Apr-2006 12:46  350K
seq.sql                   13-Apr-2006 10:13  586
seq.txt.gz                13-Apr-2006 10:14   29M
sex.sql                   08-Oct-2006 12:45  1.2K
```

http://hgdownload.cse.ucsc.edu/goldenPath/hg18/database/refGene.txt.gz

Using the RefGene locations in R

Load the file.

```
> refgene <- read.table("refGene.txt", header = FALSE,
+     sep = "\t", comment.char = "", quote = "")
```

Add the column names, which are not included.

```
> colnames(refgene) <- c("bin", "name", "chrom",
+     "strand", "txStart", "txEnd", "cdsStart",
+     "cdsEnd", "exonCount", "exonStarts", "exonEnds",
+     "id", "name2", "cdsStartStat", "cdsEndStat",
+     "exonFrames")
```

We are going to ignore the intron and exon boundaries. We are also going to remove duplicate entries, which seem for some reason to exist;

the search to identify these is time consuming.

```
> temprg <- refgene[, c(1:9, 13:15)]
> omit <- unlist(lapply(levels(temprg$name), function(x,
+     n) {
+     which(n == x)[1]
+ }, as.character(temprg$name)))
> summary(omit)
> refgene <- temprg[omit, ]
> rownames(refgene) <- as.character(refgene[, "name"])
```

Finally, we save this as a binary object that we can load later.

```
> save(refgene, file = "refgene.rda")
```

Linking the Agilent Array to RefGene locations

First, convert the environment in the AnnBuilder package for the Agilent 44K arrays to a list.

```
> temp2 <- as.list(Agilent44KREFSEQ)
```

Next, we produce a list that maps the annotations to the spots. This code works because the `ID` column of the `featureInfo` object contains RefSeq IDs (primarily), which are the names of the rows in the `temp2` object we just created.

```
> ag.annoList <- temp2[as.character(featureInfo[,
+     "ID"])]
```

Alternative Splicing

```
> ag.annoList[1]

$A_23_P80353
[1] "NM_001003689" "NP_001003689" "NM_031488"
[4] "NP_113676"
```

Notice that some probes are associated with more than one RefSeq gene; this happens because different isoforms (produced by alternative splicing) of the same gene have different RefSeq identifiers. That is, the same piece of DNA can give rise to different mRNA molecules. So, we now search through and select just the first annotation for each spot.

```
> agilent.lc <- unlist(lapply(ag.annoList, length))
> agilentREFSEQ <- unlist(lapply(ag.annoList, function(x) {
```

```
+     if (length(x) == 0) {
+         return(NA)
+     }
+     if (length(x) == 1) {
+         return(x)
+     }
+     idx <- 1
+     while (idx <= length(x)) {
+         if (x[[idx]] == "") {
+             idx <- idx + 1
+             next
+         }
+         return(x[[idx]])
+     }
+     return(NA)
+ }))
```

```
> agilentREFSEQ[agilentREFSEQ == ""] <- NA

> length(agilentREFSEQ)

[1] 41675

> sum(!is.na(agilentREFSEQ))

[1] 30612
```

Finally, we use the updated RefSeqs (that we just constructed in the `agilentREFSEQ` object) as indices into the `refgene` chromosome locations above. This computation is also slow, since it uses a search in a list instead of in a hash.

```
> agilent2refgene <- refgene[agilentREFSEQ, ]
> agilent2refgene[1:3, ]

             bin         name chrom strand  txStart
NM_001003689 889 NM_001003689 chr22      + 39931258
NM_005503     98    NM_005503 chr15      + 27001144
NM_004672    795    NM_004672  chr1      - 27554256
                txEnd cdsStart   cdsEnd exonCount   name2
NM_001003689 39957220 39931312 39953547        18 L3MBTL2
NM_005503    27197806 27133379 27196628        14   APBA2
NM_004672    27565924 27554468 27565675        29  MAP3K6
             cdsStartStat cdsEndStat
NM_001003689         cmpl       cmpl
NM_005503            cmpl       cmpl
NM_004672            cmpl       cmpl
```

Building a Custom Track

We analyzed the Agilent 44K microarray data using a linear model. The results are contained in an object called ourResults:

```
> summary(ourResults)

 UntreatedMeanLog      Beta                PValue
 Min.   : 4.870   Min.   :-3.15530   Min.   :2.024e-09
 1st Qu.: 6.907   1st Qu.:-0.19572   1st Qu.:8.142e-02
 Median : 8.058   Median :-0.05431   Median :2.749e-01
 Mean   : 8.742   Mean   :-0.04300   Mean   :3.511e-01
 3rd Qu.: 9.982   3rd Qu.: 0.10075   3rd Qu.:5.823e-01
 Max.   :16.523   Max.   : 3.27672   Max.   :1.000e+00
```

Computing a Displayable Score

We are going to us the p-values to decide which genes to display, and we are going to use the coefficient (`Beta`) to compute a score that shows the amount of differential expression. The allowed scores for a custom track range from 0 to 1000. Since the true values of `Beta` range between -3 and $+3$ (more or less), we are going to multiply by 300 to get a useful score.

```
> score <- 300 * ourResults[, "Beta"]
> score[score > 1000] <- 1000
> score[score < -1000] <- -1000
> score <- abs(score)
```

A Track Data Frame

Now we build a data frame that includes the information we need for a custom track in the desired order:

```
> temp <- data.frame(agilent2refgene[, c("chrom",
+     "txStart", "txEnd", "name2")], score = score,
+     strand = agilent2refgene[, "strand"])
> temp[1:3, 1:5]
```

```
             chrom  txStart    txEnd   name2     score
NM_001003689 chr22 39931258 39957220 L3MBTL2 96.902254
NM_005503    chr15 27001144 27197806   APBA2 74.415391
NM_004672     chr1 27554256 27565924  MAP3K6  2.281971
```

Significant Overexpressed Genes

We built this data frame for all genes; now we are going to select the ones that are significant (p-value < 0.02) and are overexpressed in response to the treatment ($\beta > 0$). We further restrict to those genes that we are able to map to the genome.

```
> trackInfo <- temp[!is.na(temp[, "chrom"]) & ourResults[,
+     "PValue"] < 0.02 & ourResults[, "Beta"] >
+     0, ]
```

We also have to create a header line that tells the browser to make use of the scores.

```
> trackheader <- paste("track name=upNormal",
+     "description=\"Increased in Normal Cells\"",
+     "useScore=1 color=0,60,120")
```

Writing the Track Info to a File

We can now write the header line followed by the track data:

```
> write(trackheader, file = "upNormalRNA.tsv",
+     append = FALSE)
> write.table(trackInfo, file = "upNormalRNA.tsv",
+     append = TRUE, quote = FALSE, sep = "\t",
+     row.names = FALSE, col.names = FALSE)
```

Finally, we do the same thing for the genes that are underexpressed.

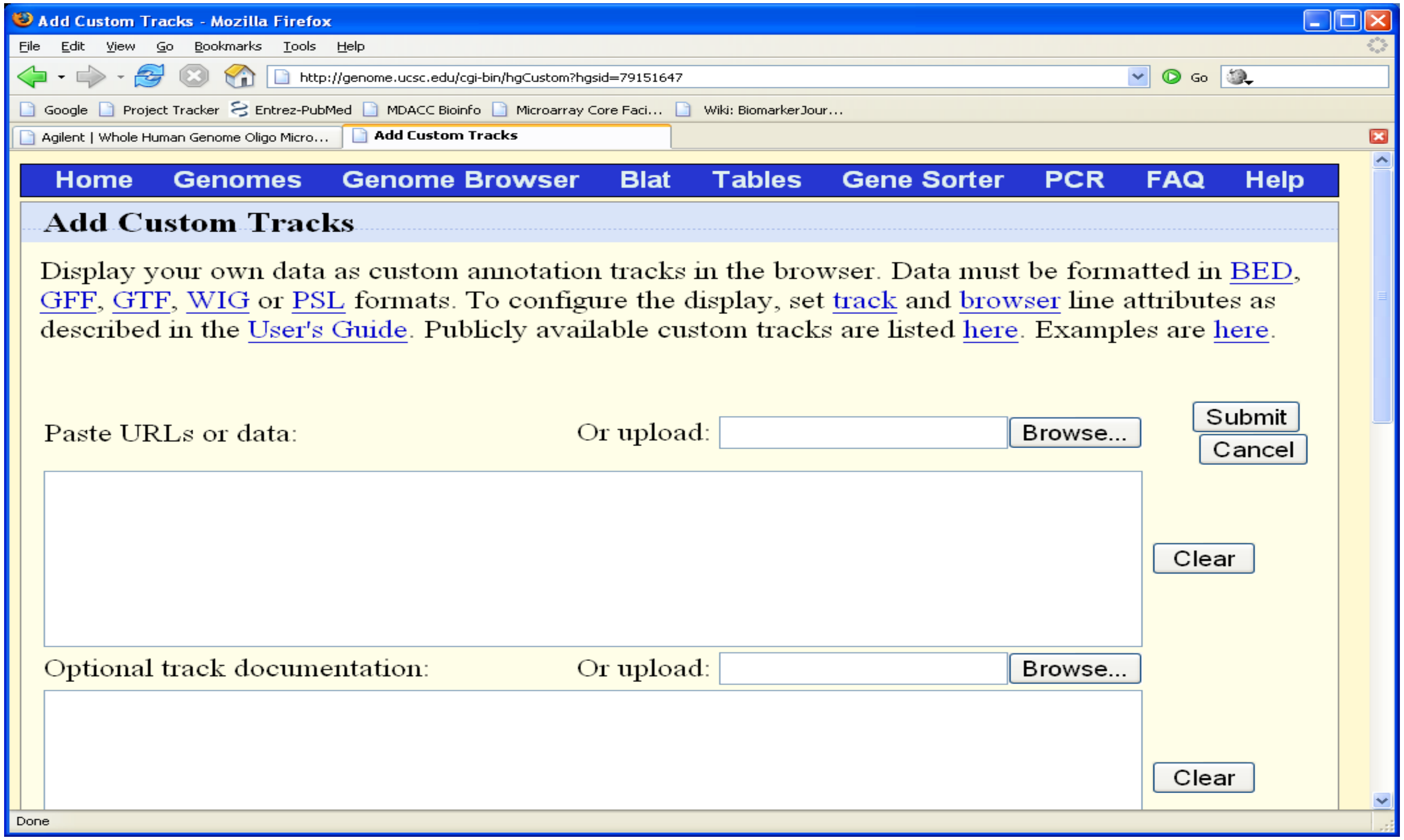
```
> trackInfo <- temp[!is.na(temp[, "chrom"]) & ourResults[,
+     "PValue"] < 0.02 & ourResults[, "Beta"] <
+     0, ]

> trackheader <- paste("track name=downNormal",
+     "description=\"Decreased in Normal Cells\"",
+     "useScore=1 color=100,50,0")

> write(trackheader, file = "dnNormalRNA.tsv",
+     append = FALSE)
> write.table(trackInfo, file = "dnNormalRNA.tsv",
+     append = TRUE, quote = FALSE, sep = "\t",
+     row.names = FALSE, col.names = FALSE)
```

Viewing Your Custom Track

Now we can return to the genome browser and look at our custom tracks. Unfortunately, their web page only lets you attach one at a time unless you can make them available from a web site:


Add Custom Tracks - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://genome.ucsc.edu/cgi-bin/hgCustom?hgsid=79151647
Go
Google
Project Tracker
Entrez-PubMed
MDACC Bioinfo
Microarray Core Faci...
Wiki: BiomarkerJour...
Agilent | Whole Human Genome Oligo Micro...
Add Custom Tracks
Home Genomes Genome Browser Blat Tables Gene Sorter PCR FAQ Help
Submit
Cancel
Browse...
Clear
Browse...
Clear
Done

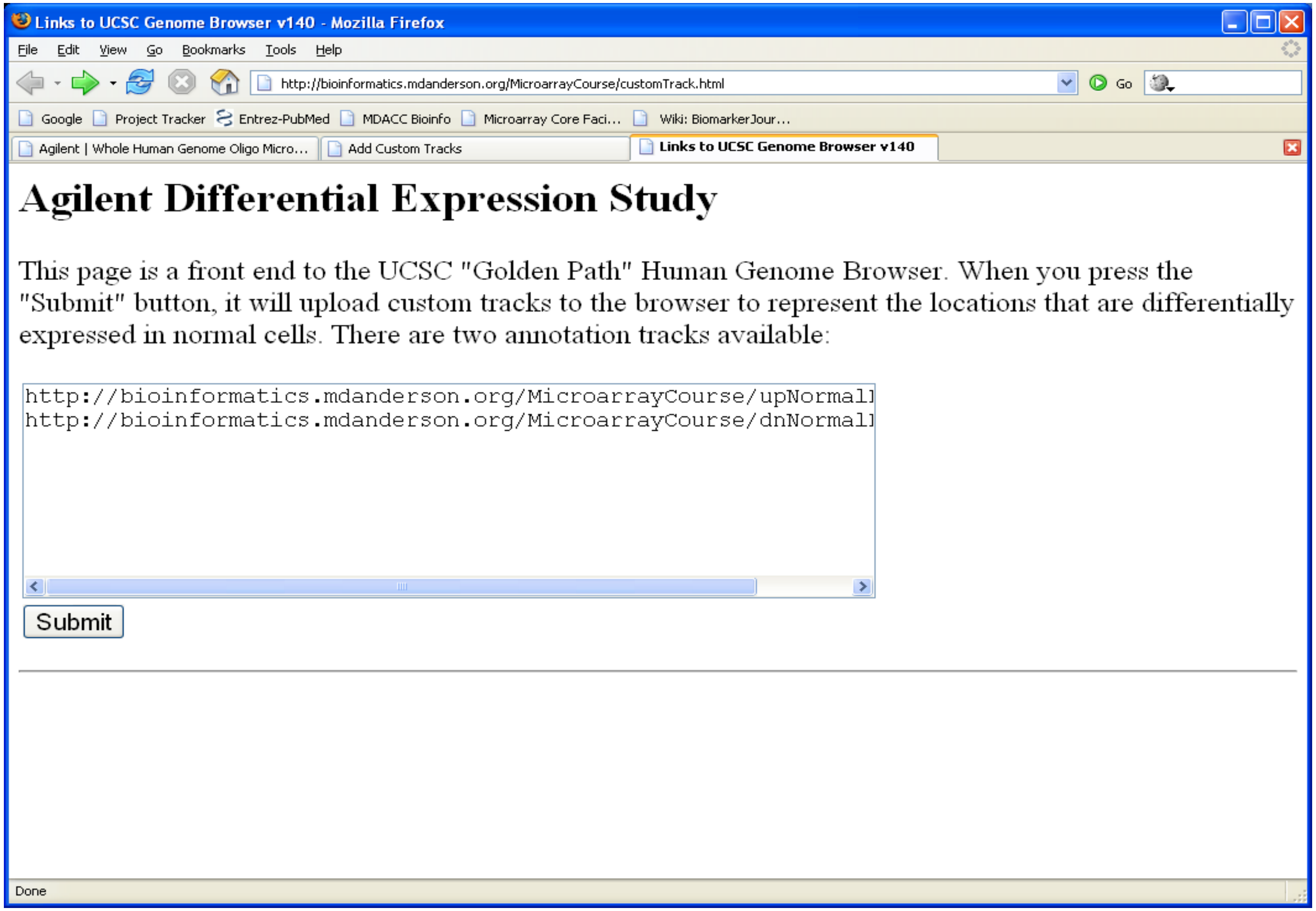
Add Custom Tracks

Display your own data as custom annotation tracks in the browser. Data must be formatted in BED, GFF, GTF, WIG or PSL formats. To configure the display, set track and browser line attributes as described in the User's Guide. Publicly available custom tracks are listed here. Examples are here.

Paste URLs or data: Or upload:

Optional track documentation: Or upload:

http://bioinformatics.mdanderson.org/ MicroarrayCourse/customTrack.html



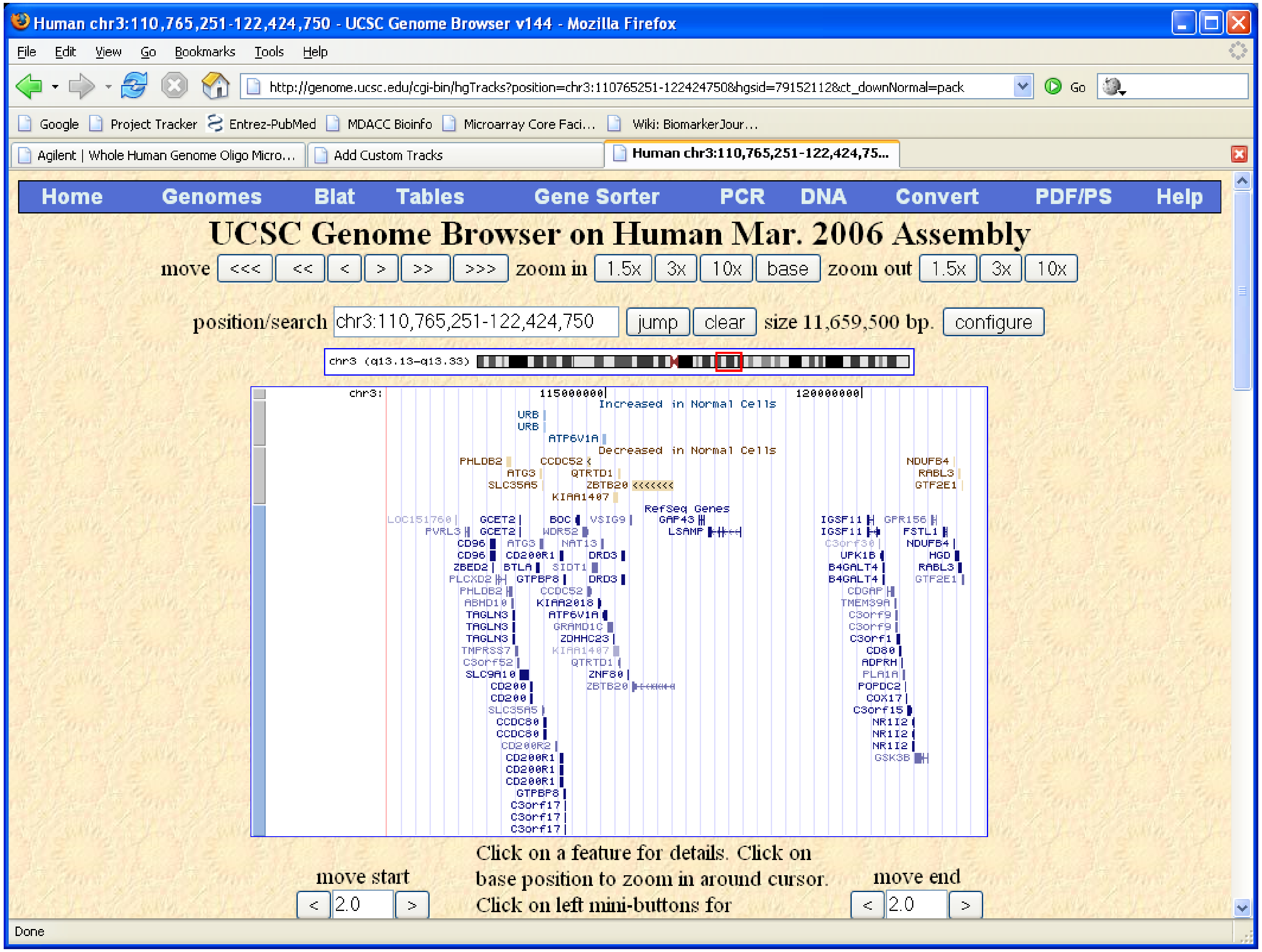
Links to UCSC Genome Browser v140 - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://bioinformatics.mdanderson.org/MicroarrayCourse/customTrack.html
Go
Google Project Tracker Entrez-PubMed MDACC Bioinfo Microarray Core Faci... Wiki: BiomarkerJour...
Agilent | Whole Human Genome Oligo Micro... Add Custom Tracks Links to UCSC Genome Browser v140
Submit
Done

Agilent Differential Expression Study

This page is a front end to the UCSC "Golden Path" Human Genome Browser. When you press the "Submit" button, it will upload custom tracks to the browser to represent the locations that are differentially expressed in normal cells. There are two annotation tracks available:

```
http://bioinformatics.mdanderson.org/MicroarrayCourse/upNormall
http://bioinformatics.mdanderson.org/MicroarrayCourse/dnNormall
```

Displaying Our Tracks



Human chr3:110,765,251-122,424,750 - UCSC Genome Browser v144 - Mozilla Firefox

http://genome.ucsc.edu/cgi-bin/hgTracks?position=chr3:110765251-122424750&hgsid=79152112&ct_downNormal=pack

UCSC Genome Browser on Human Mar. 2006 Assembly

position/search chr3:110,765,251-122,424,750

size 11,659,500 bp.

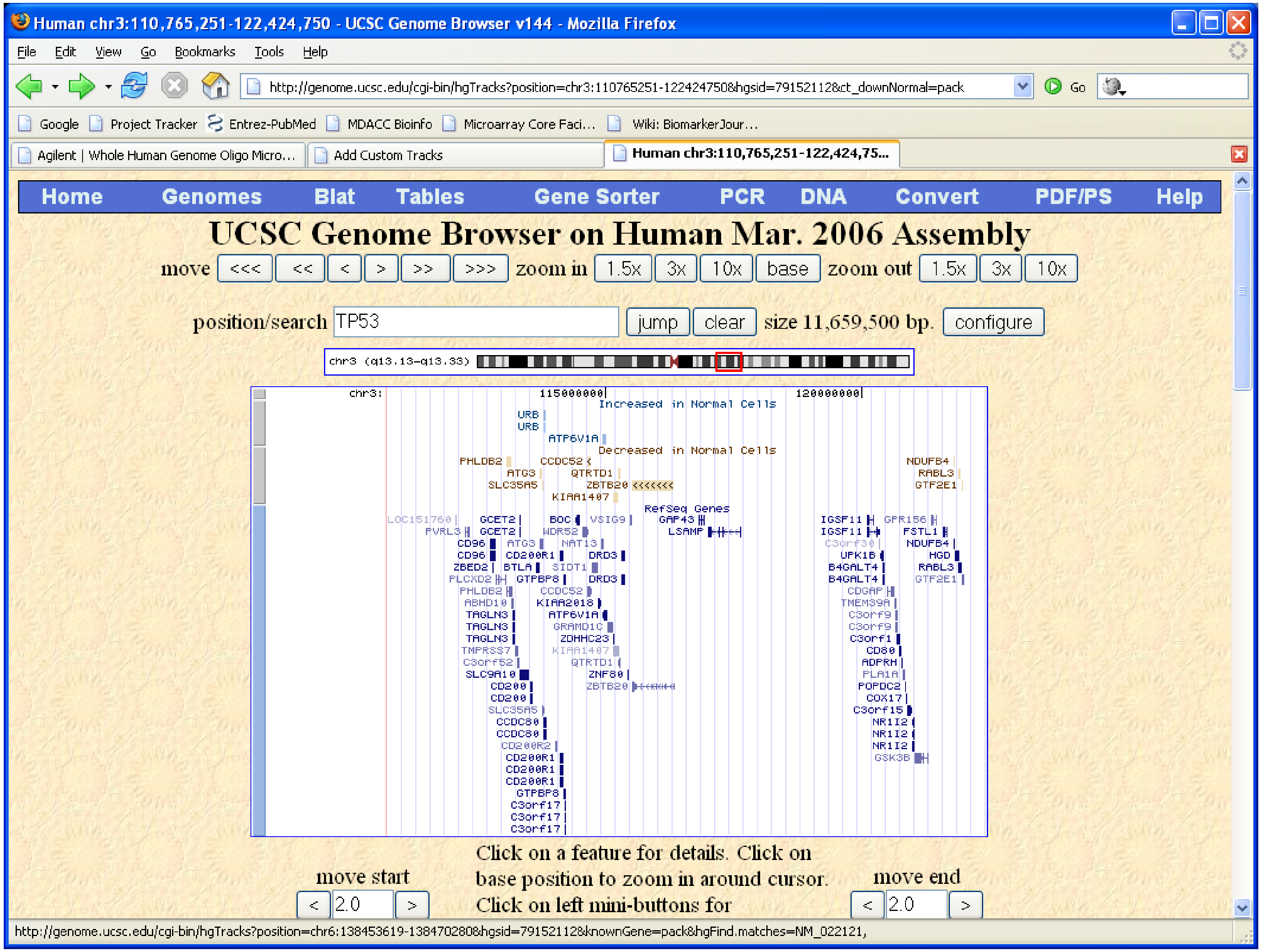
Click on a feature for details. Click on base position to zoom in around cursor. Click on left mini-buttons for

move start 2.0

move end 2.0

Done

Searching for a Gene



Human chr3:110,765,251-122,424,750 - UCSC Genome Browser v144 - Mozilla Firefox

http://genome.ucsc.edu/cgi-bin/hgTracks?position=chr3:110765251-122424750&hgsid=79152112&ct_downNormal=pack

UCSC Genome Browser on Human Mar. 2006 Assembly

position/search: TP53

size 11,659,500 bp.

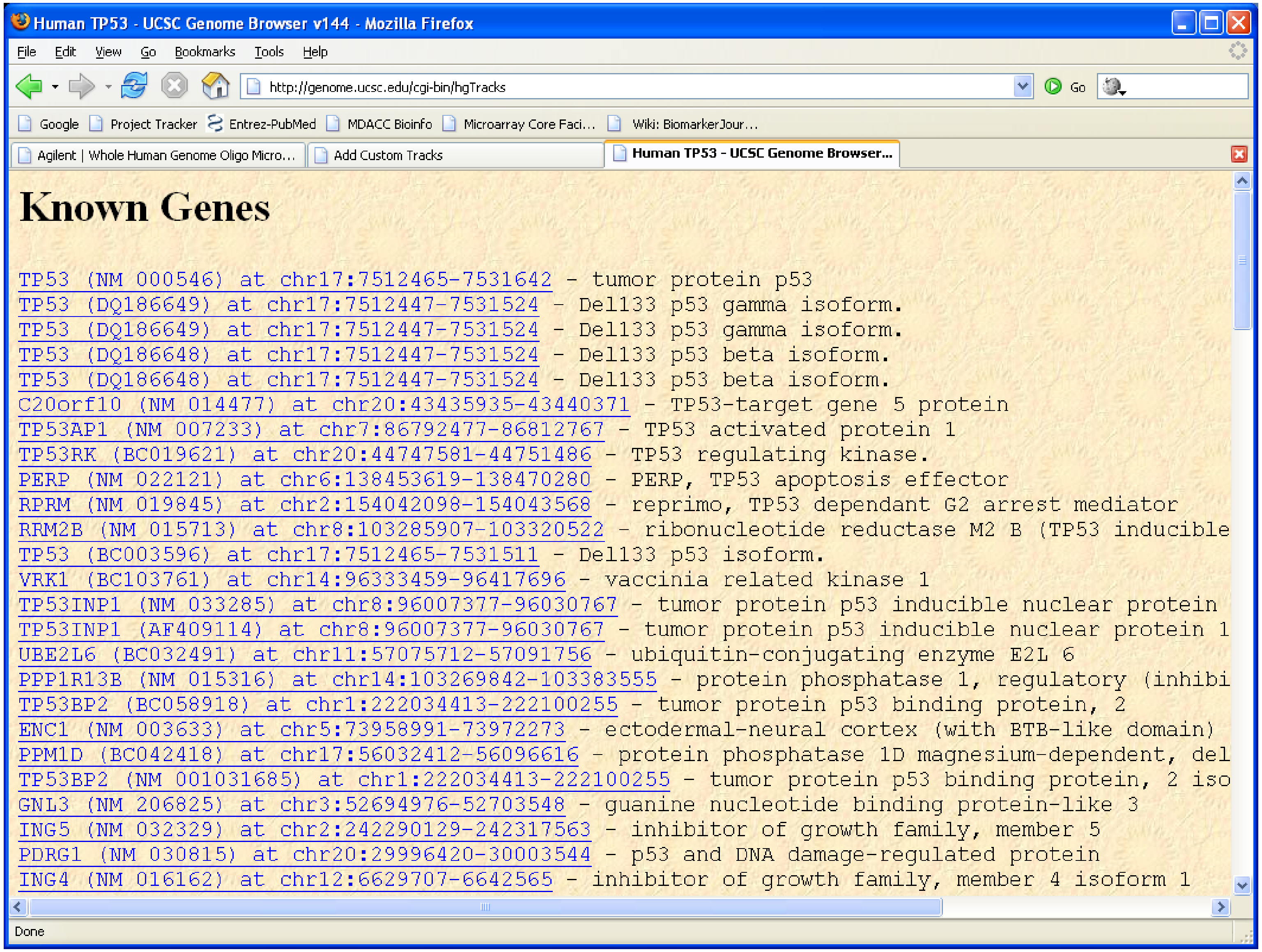
Click on a feature for details. Click on base position to zoom in around cursor. Click on left mini-buttons for

move start: 2.0

move end: 2.0

http://genome.ucsc.edu/cgi-bin/hgTracks?position=chr6:138453619-138470280&hgsid=79152112&knownGene=pack&hgFind.matches=NM_022121,

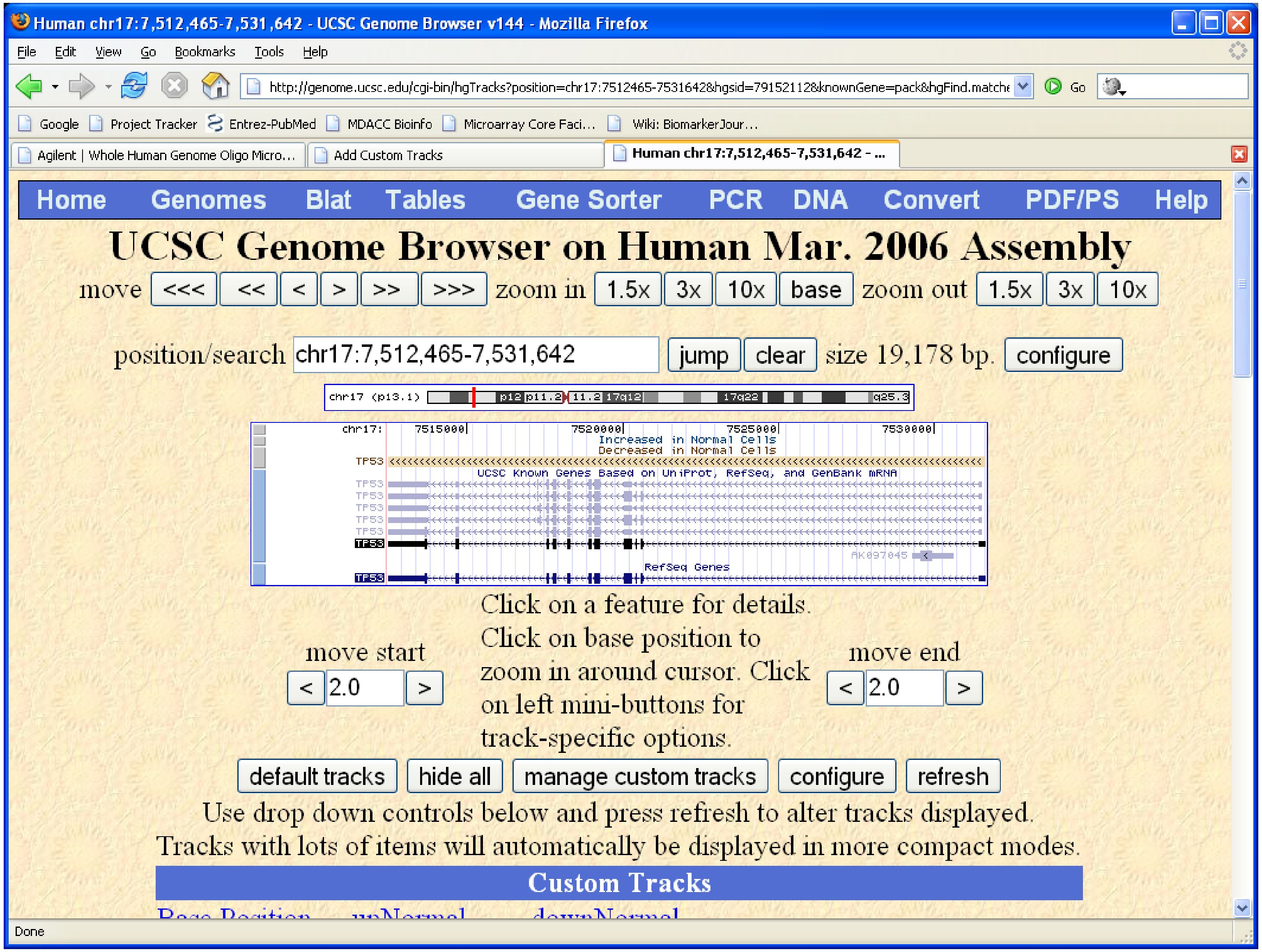
Searching for a Gene


Human TP53 - UCSC Genome Browser v144 - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://genome.ucsc.edu/cgi-bin/hgTracks
Go
Google
Project Tracker
Entrez-PubMed
MDACC Bioinfo
Microarray Core Faci...
Wiki: BiomarkerJour...
Agilent | Whole Human Genome Oligo Micro...
Add Custom Tracks
Human TP53 - UCSC Genome Browser...
Done

Known Genes

```
TP53 (NM 000546) at chr17:7512465-7531642 - tumor protein p53
TP53 (DQ186649) at chr17:7512447-7531524 - Del133 p53 gamma isoform.
TP53 (DQ186649) at chr17:7512447-7531524 - Del133 p53 gamma isoform.
TP53 (DQ186648) at chr17:7512447-7531524 - Del133 p53 beta isoform.
TP53 (DQ186648) at chr17:7512447-7531524 - Del133 p53 beta isoform.
C20orf10 (NM 014477) at chr20:43435935-43440371 - TP53-target gene 5 protein
TP53AP1 (NM 007233) at chr7:86792477-86812767 - TP53 activated protein 1
TP53RK (BC019621) at chr20:44747581-44751486 - TP53 regulating kinase.
PERP (NM 022121) at chr6:138453619-138470280 - PERP, TP53 apoptosis effector
RPRM (NM 019845) at chr2:154042098-154043568 - reprimo, TP53 dependant G2 arrest mediator
RRM2B (NM 015713) at chr8:103285907-103320522 - ribonucleotide reductase M2 B (TP53 inducible
TP53 (BC003596) at chr17:7512465-7531511 - Del133 p53 isoform.
VRK1 (BC103761) at chr14:96333459-96417696 - vaccinia related kinase 1
TP53INP1 (NM 033285) at chr8:96007377-96030767 - tumor protein p53 inducible nuclear protein
TP53INP1 (AF409114) at chr8:96007377-96030767 - tumor protein p53 inducible nuclear protein 1
UBE2L6 (BC032491) at chr11:57075712-57091756 - ubiquitin-conjugating enzyme E2L 6
PPP1R13B (NM 015316) at chr14:103269842-103383555 - protein phosphatase 1, regulatory (inhibi
TP53BP2 (BC058918) at chr1:222034413-222100255 - tumor protein p53 binding protein, 2
ENC1 (NM 003633) at chr5:73958991-73972273 - ectodermal-neural cortex (with BTB-like domain)
PPM1D (BC042418) at chr17:56032412-56096616 - protein phosphatase 1D magnesium-dependent, del
TP53BP2 (NM 001031685) at chr1:222034413-222100255 - tumor protein p53 binding protein, 2 iso
GNL3 (NM 206825) at chr3:52694976-52703548 - guanine nucleotide binding protein-like 3
ING5 (NM 032329) at chr2:242290129-242317563 - inhibitor of growth family, member 5
PDRG1 (NM 030815) at chr20:29996420-30003544 - p53 and DNA damage-regulated protein
ING4 (NM 016162) at chr12:6629707-6642565 - inhibitor of growth family, member 4 isoform 1
```

Searching for a Gene



Human chr17:7,512,465-7,531,642 - UCSC Genome Browser v144 - Mozilla Firefox

http://genome.ucsc.edu/cgi-bin/hgTracks?position=chr17:7512465-7531642&hgsid=79152112&knownGene=pack&hgFind.matche

UCSC Genome Browser on Human Mar. 2006 Assembly

position/search chr17:7,512,465-7,531,642 size 19,178 bp.

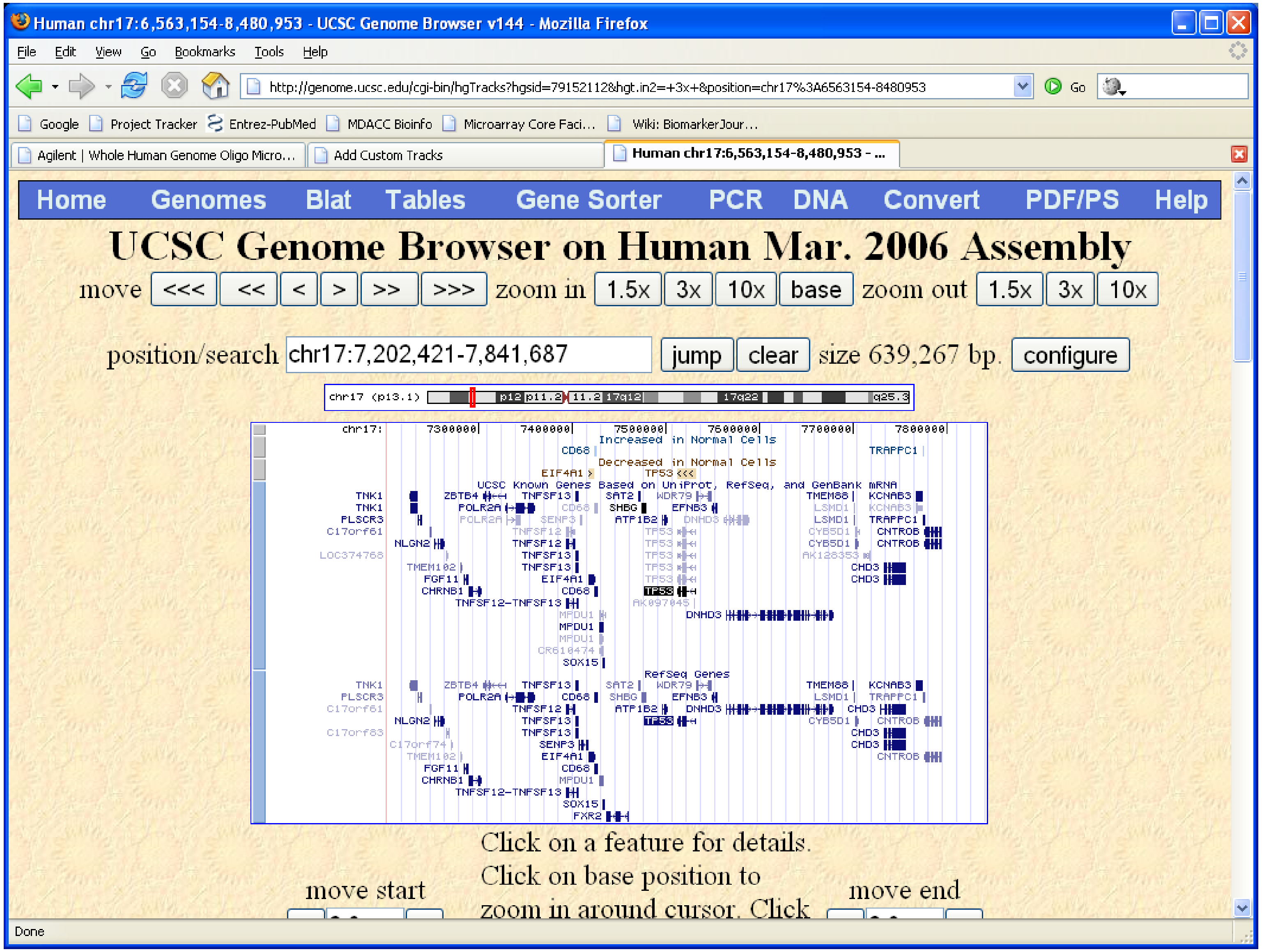
Click on a feature for details. Click on base position to zoom in around cursor. Click on left mini-buttons for track-specific options.

move start 2.0 move end 2.0

Use drop down controls below and press refresh to alter tracks displayed. Tracks with lots of items will automatically be displayed in more compact modes.

Custom Tracks

Searching for a Gene



Human chr17:6,563,154-8,480,953 - UCSC Genome Browser v144 - Mozilla Firefox

http://genome.ucsc.edu/cgi-bin/hgTracks?hgsid=79152112&hgt.in2=+3x+&position=chr17%3A6563154-8480953

UCSC Genome Browser on Human Mar. 2006 Assembly

position/search chr17:7,202,421-7,841,687 size 639,267 bp.

Click on a feature for details. Click on base position to zoom in around cursor. Click

Done