GS01 0163
Analysis of Microarray Data

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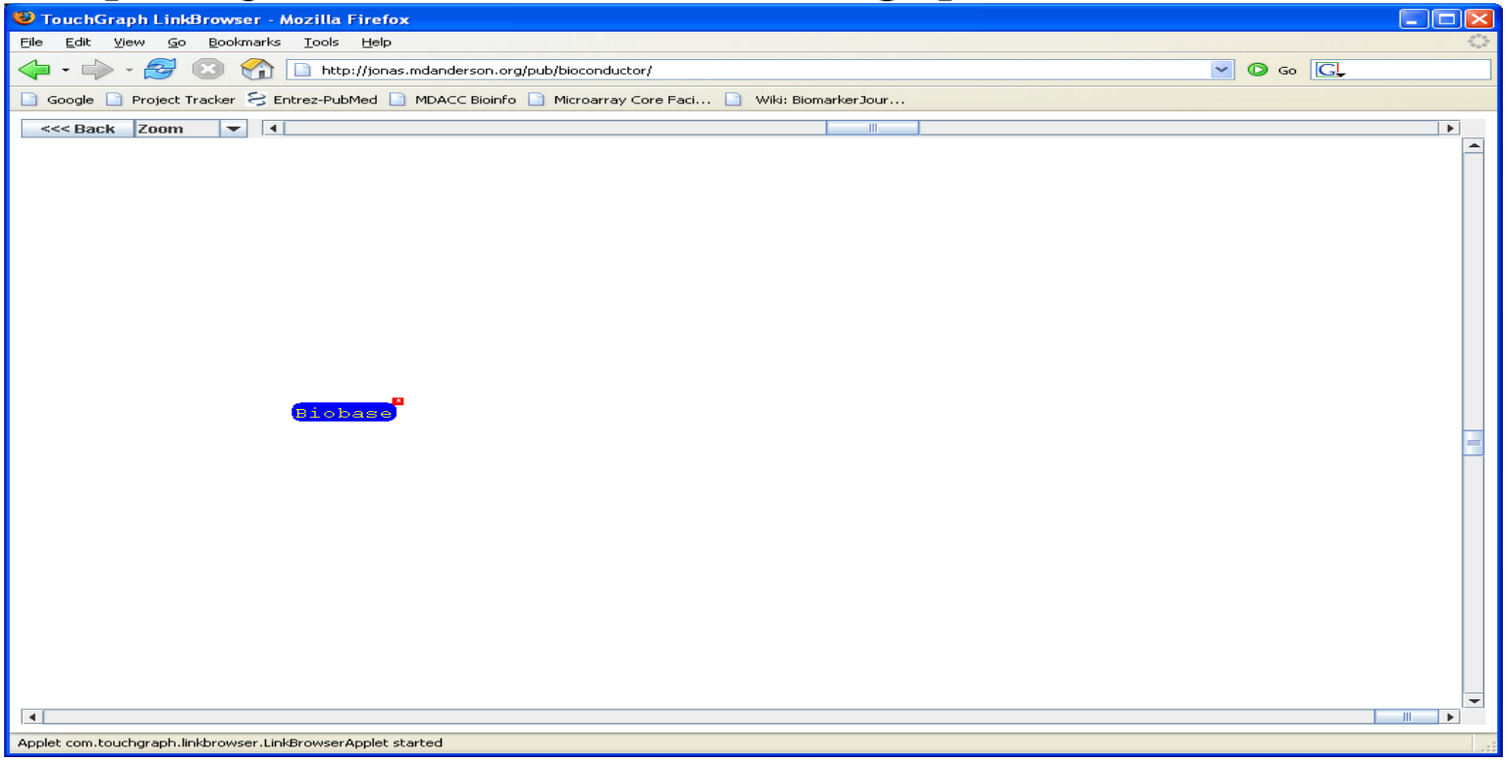
Lecture 14: Genome Browsing

- 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

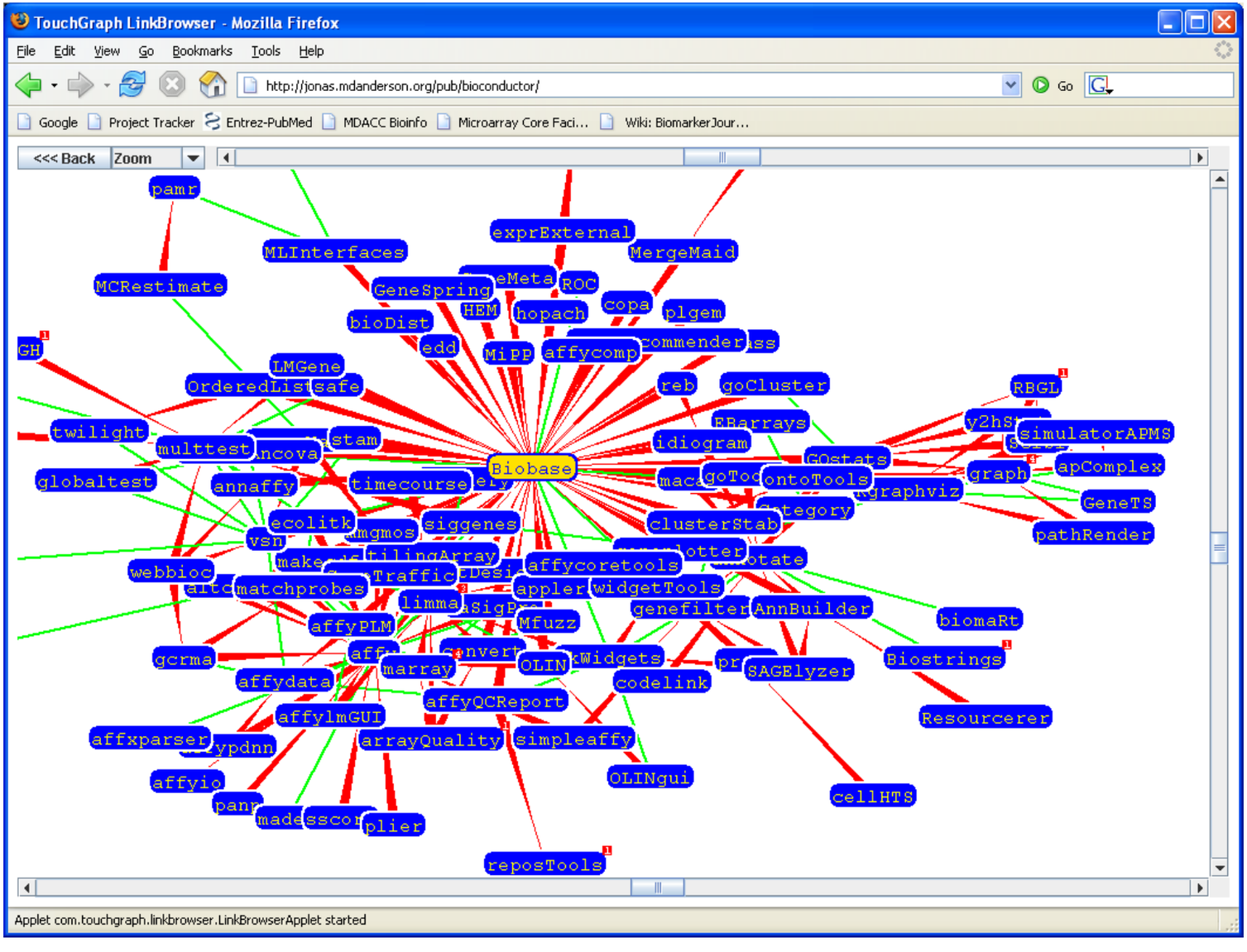
Learning What BioConductor Contains

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

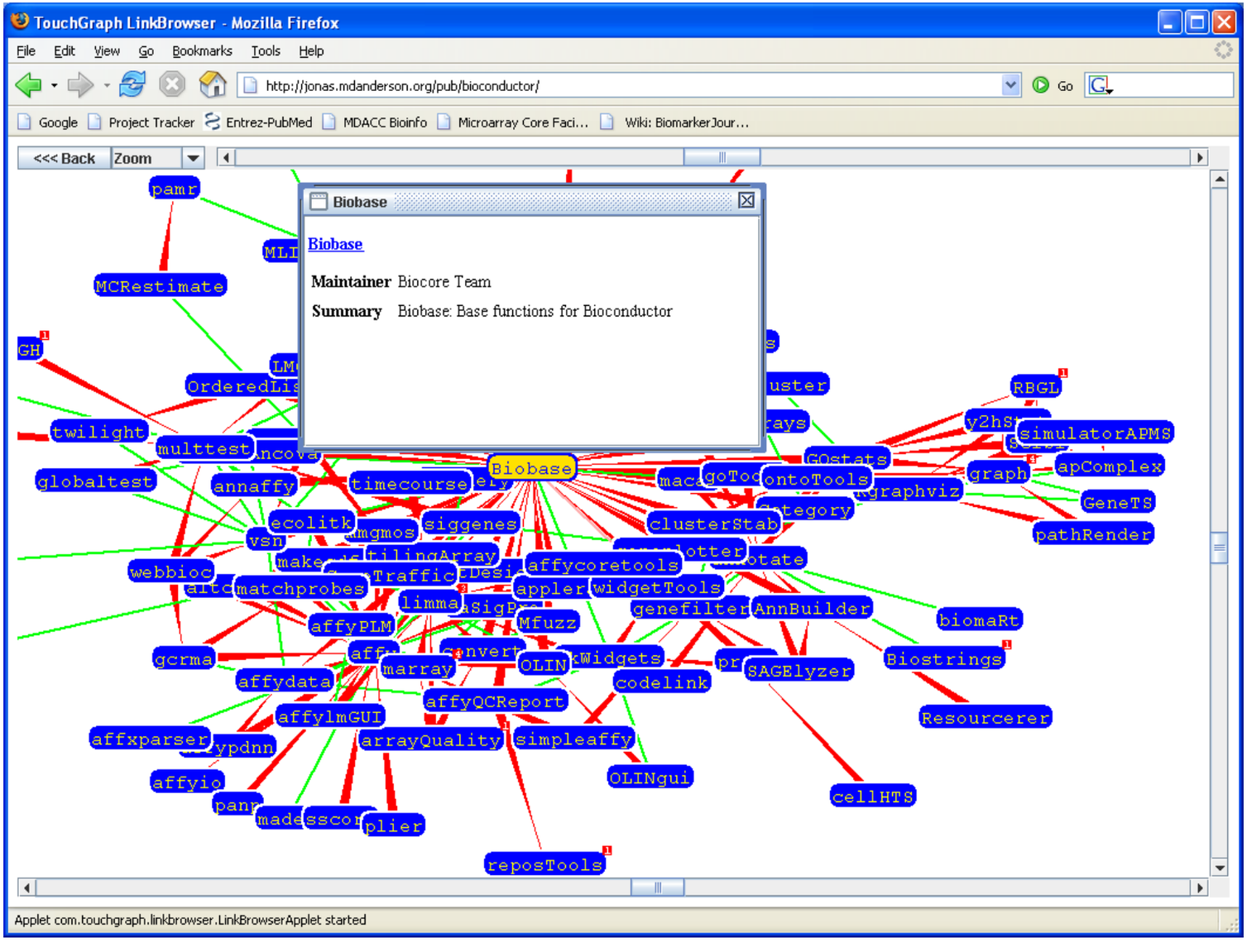
`http://jonas.mdanderson.org/pub/bioconductor/`



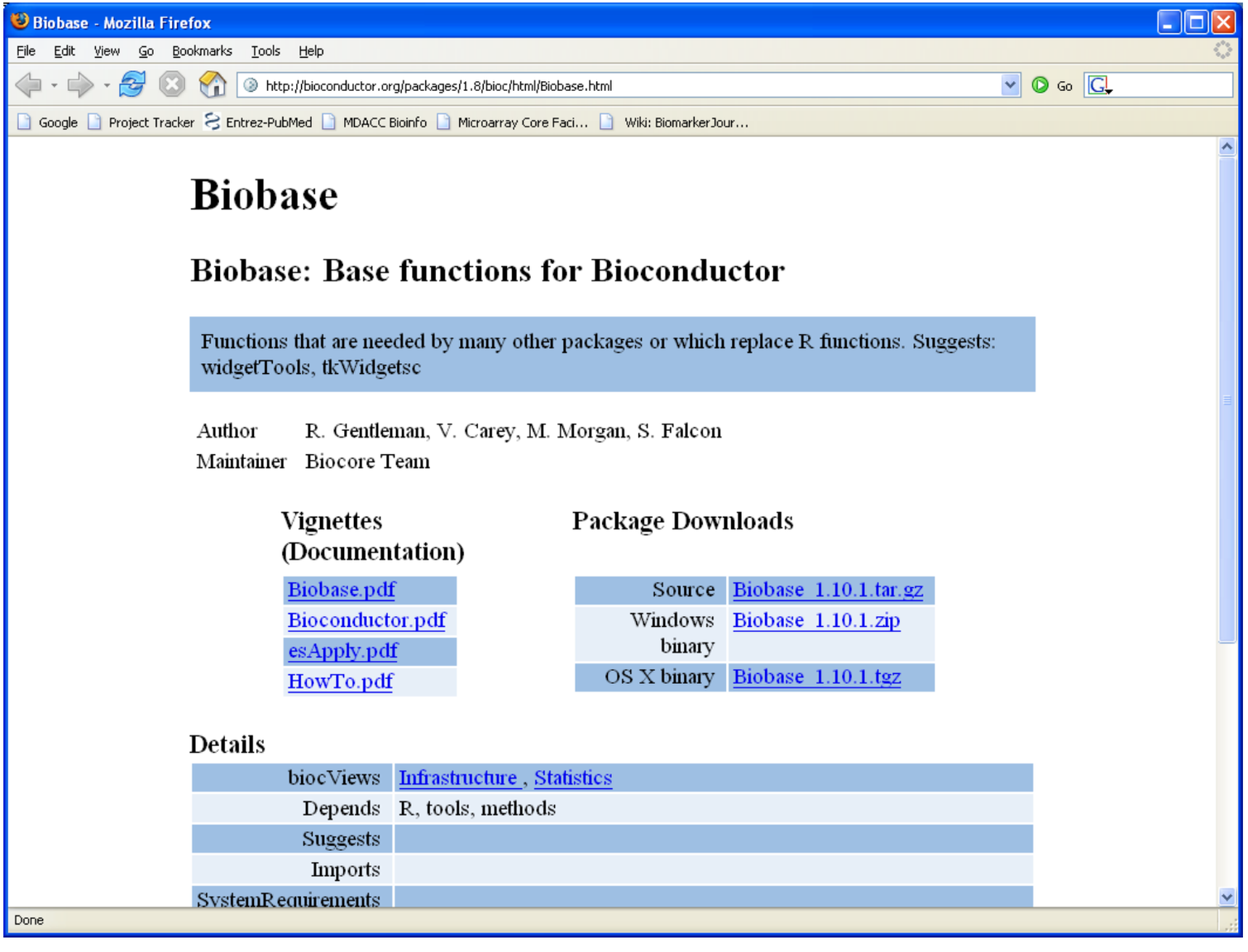
The Documentation Graph



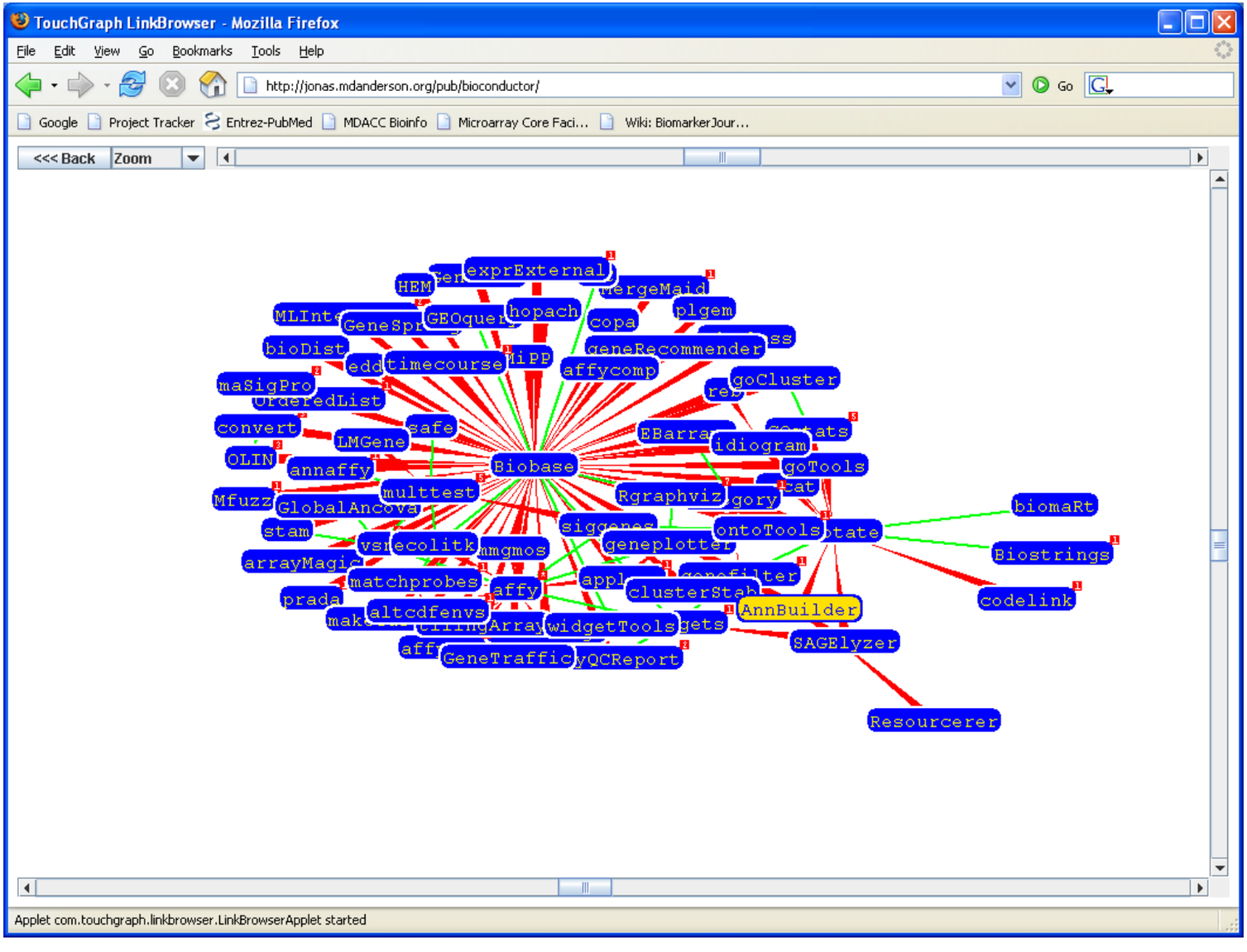
Hovering the Mouse Gives a Summary



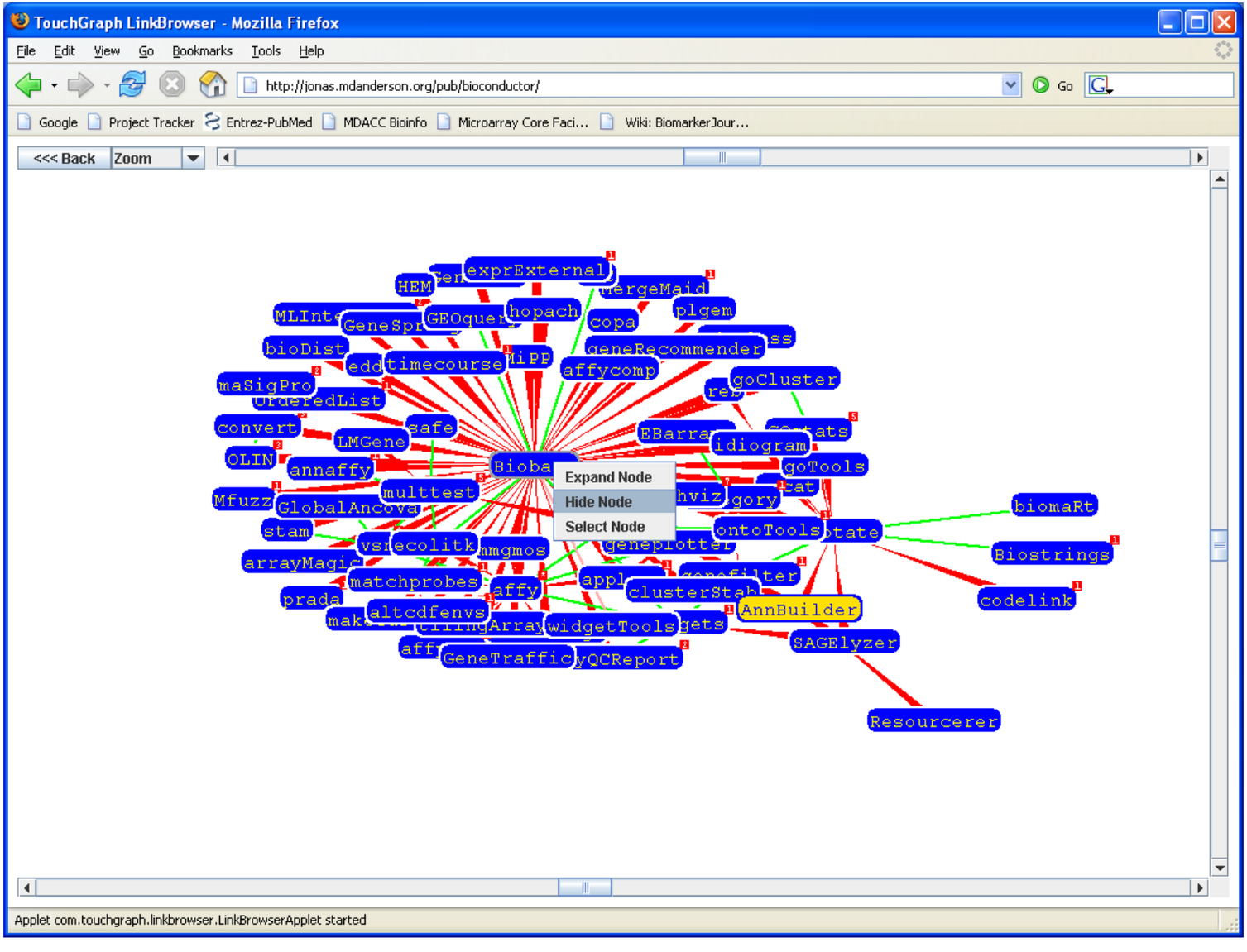
Left-click Takes You to the Documentation



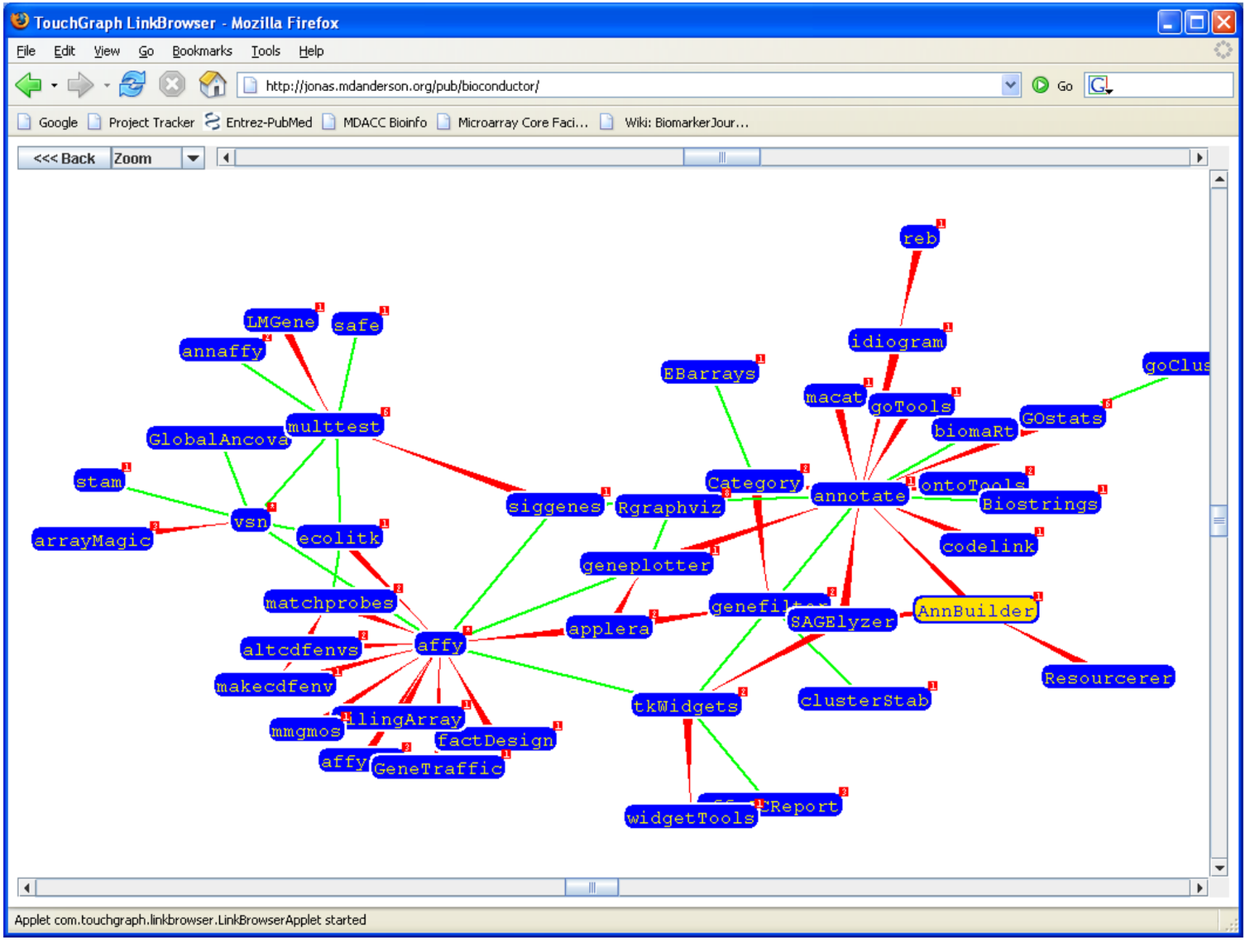
Left-click Also Recenters on a New Selection



Right-click Lets You Hide Part of the Graph



Hiding BioBase Often Clarifies the Structure



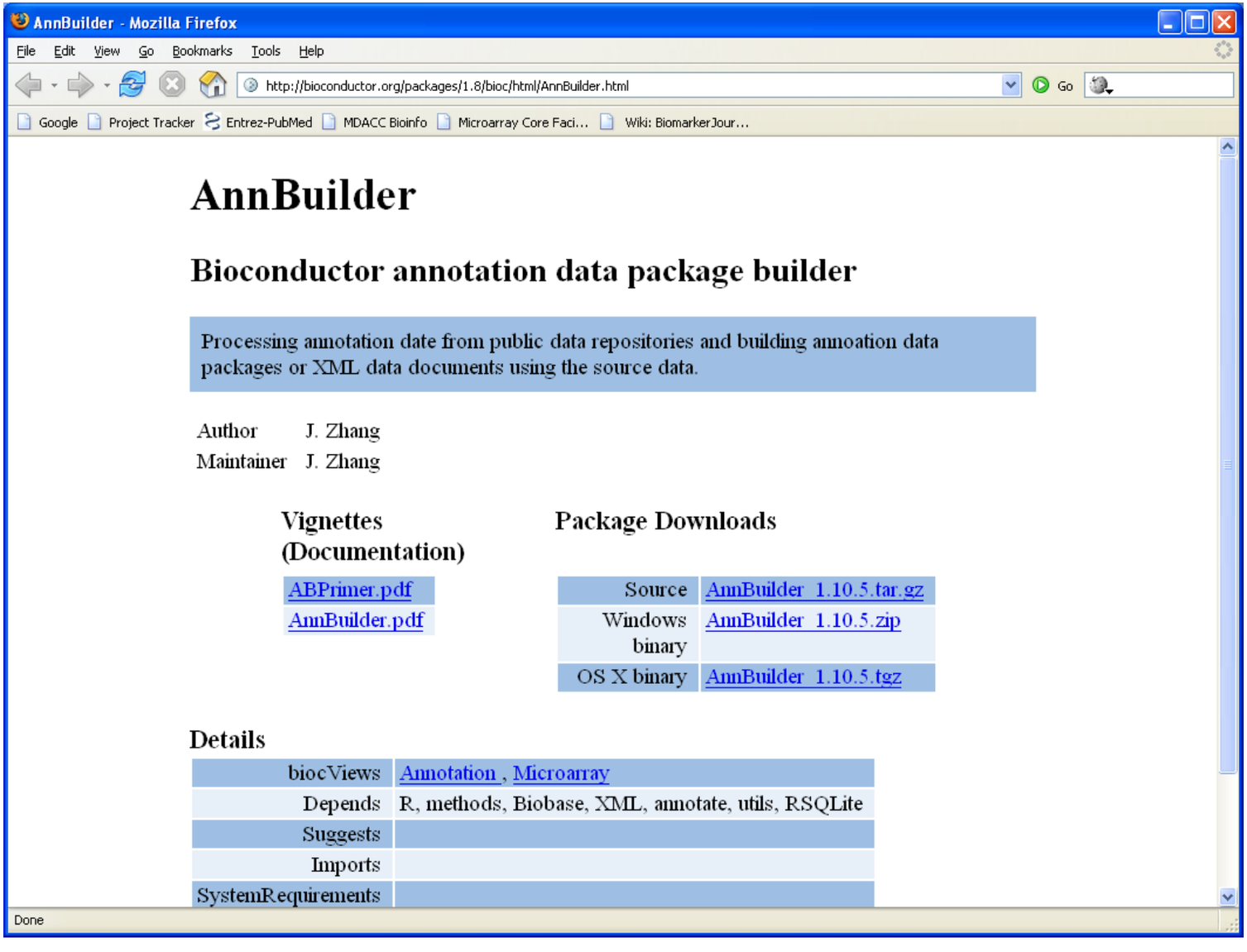
Hubs in the Documentation Graph Are Probably Important

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



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)
```

```
Loading required package: 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: Sun Mar 26 00:53:27 2006

Number of probes: 12625
Probe number missmatch: None
Probe missmatch: None
Mappings found for probe based rda files:
         hgu95av2ACCNUM found 12625 of 12625
         hgu95av2CHRLOC found 11716 of 12625
         hgu95av2CHR found 12171 of 12625
         hgu95av2ENZYME found 1922 of 12625
         hgu95av2GENENAME found 11660 of 12625
         hgu95av2GO found 11101 of 12625
         hgu95av2LOCUSID found 12238 of 12625
         hgu95av2MAP found 12140 of 12625
         hgu95av2OMIM found 9978 of 12625
```



```
          hgu95av2PATH found 4252 of 12625
          hgu95av2PMID found 12136 of 12625
          hgu95av2REFSEQ found 12039 of 12625
          hgu95av2SUMFUNC found 0 of 12625
          hgu95av2SYMBOL found 12184 of 12625
          hgu95av2UNIGENE found 12127 of 12625
Mappings found for non-probe based rda files:
           hgu95av2CHRLENGTHS found 25
          hgu95av2ENZYME2PROBE found 660
          hgu95av2GO2ALLPROBES found 6012
          hgu95av2GO2PROBE found 4274
          hgu95av2ORGANISM found 1
          hgu95av2PATH2PROBE found 173
          hgu95av2PFAM found 10412
          hgu95av2PMID2PROBE found 107253
          hgu95av2PROSITE found 8193
```


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"
```



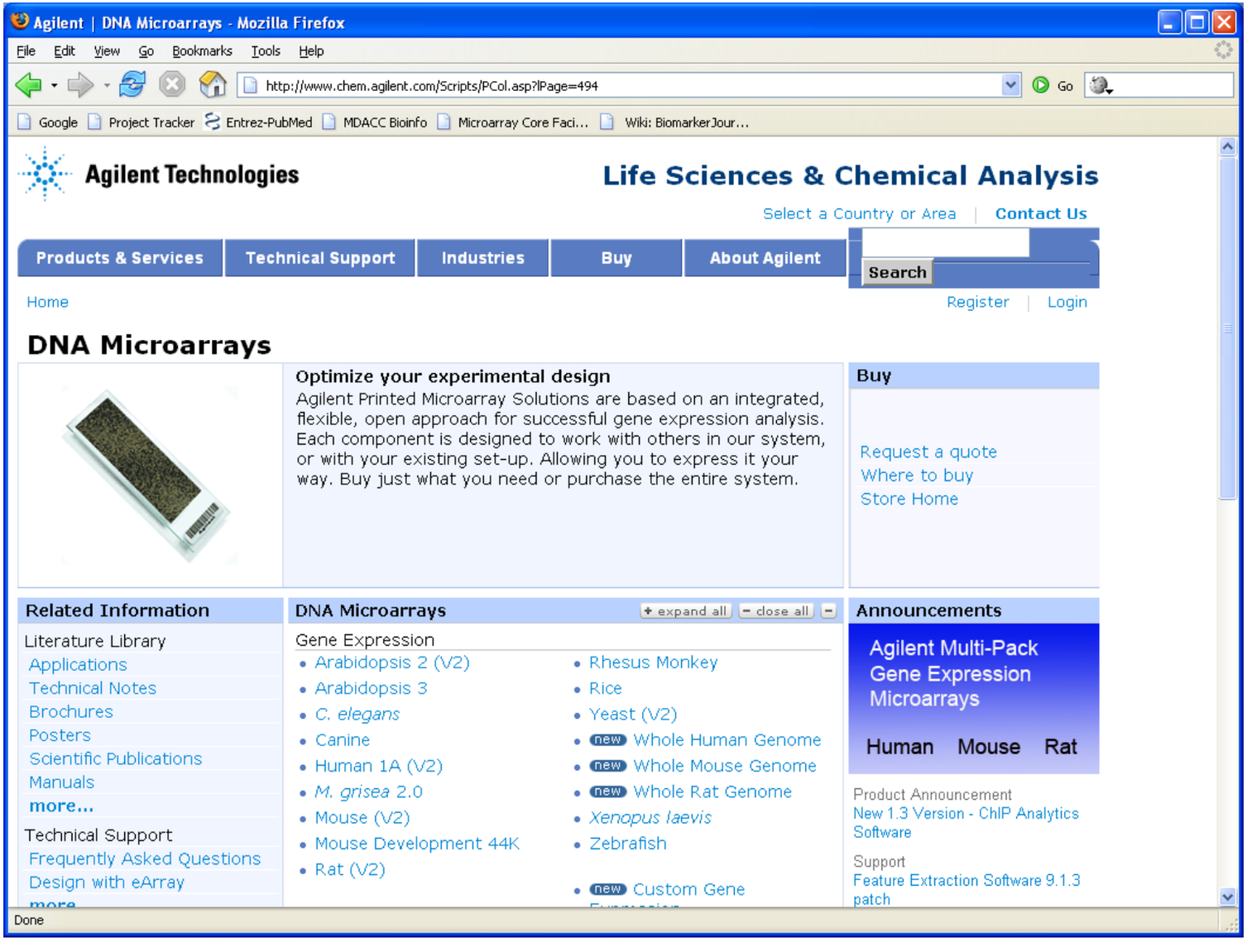
```
> get("1854_at", hgu95av2GENENAME)

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

> get("1854_at", hgu95av2LOCUSID)

[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.

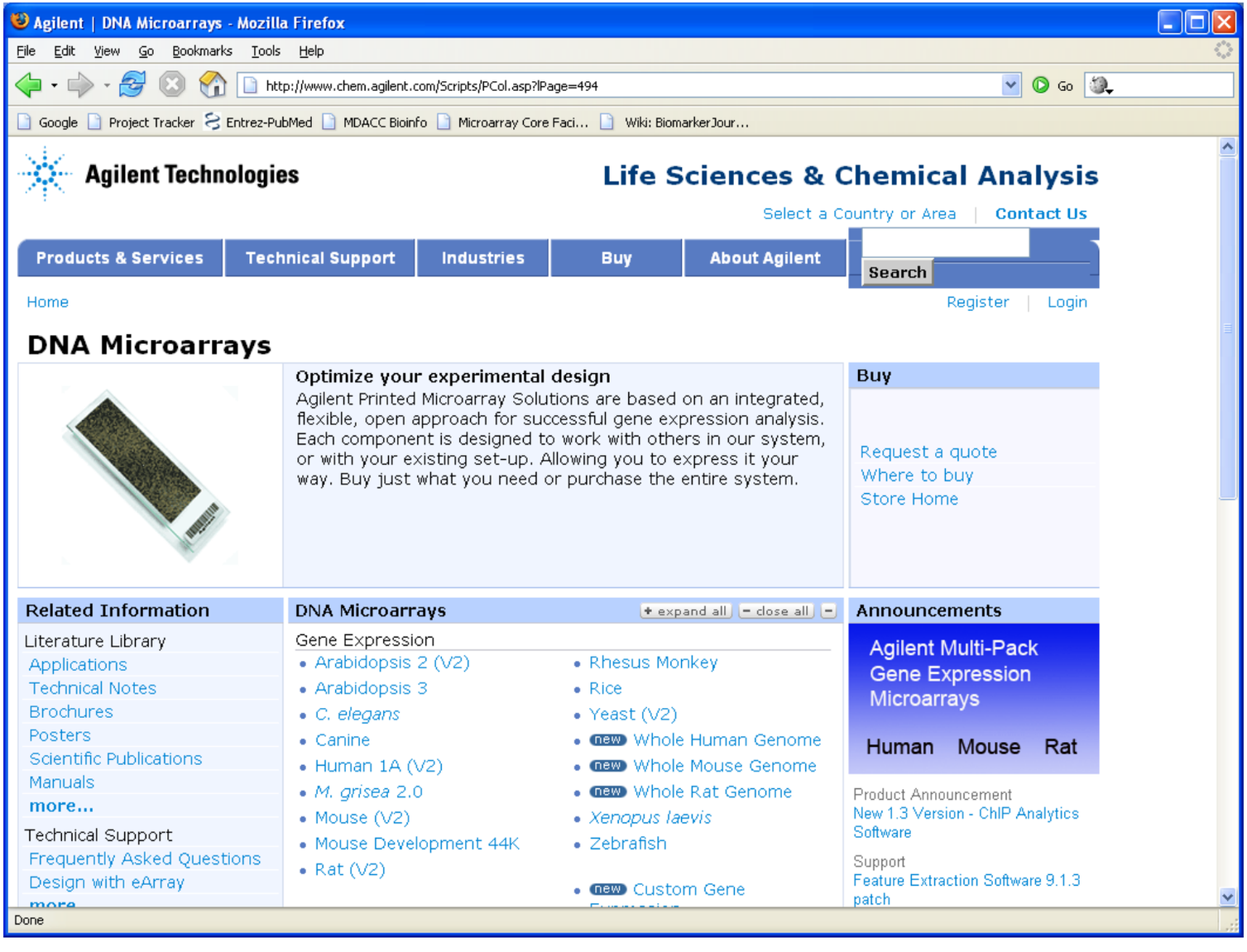
AnnBuilder: Rolling Your Own Annotations

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 folowed the link under "Products and Services" for "Life Sciences" to get to the "DNA Microarrays" page.

Follow the Link for "Whole Human Genome"



Follow the Link for "Download Gene Lists"



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)

Loading required package: Biobase
Loading required package: XML
Loading required package: annotate
Loading required package: RSQLite
Loading required package: DBI

> 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
```

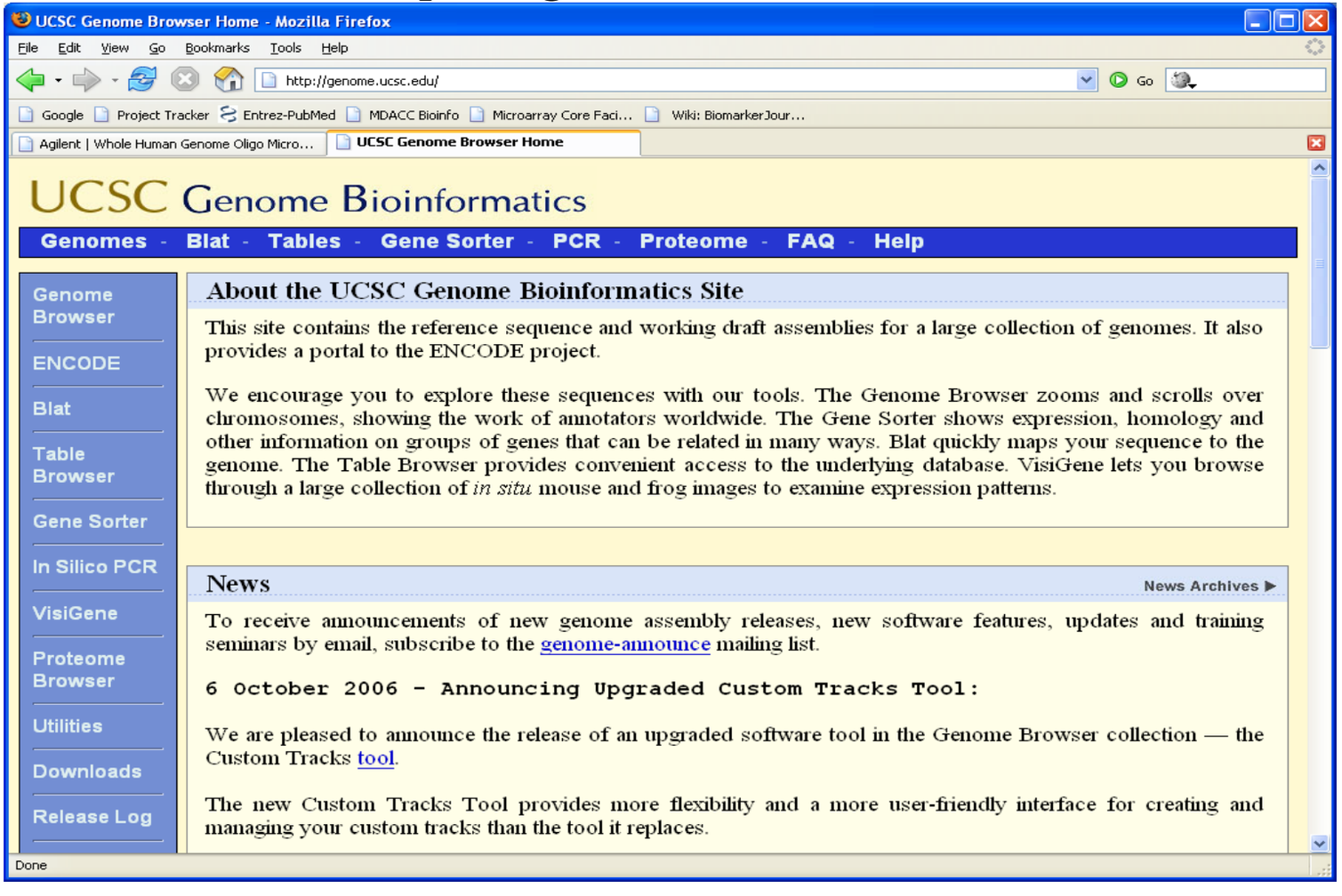


```
Agilent44KORGANISM found 1
Agilent44KPATH2PROBE found 183
Agilent44KPFAM found 21902
Agilent44KPMID2PROBE found 131104
Agilent44KPROSITE found 15055
```

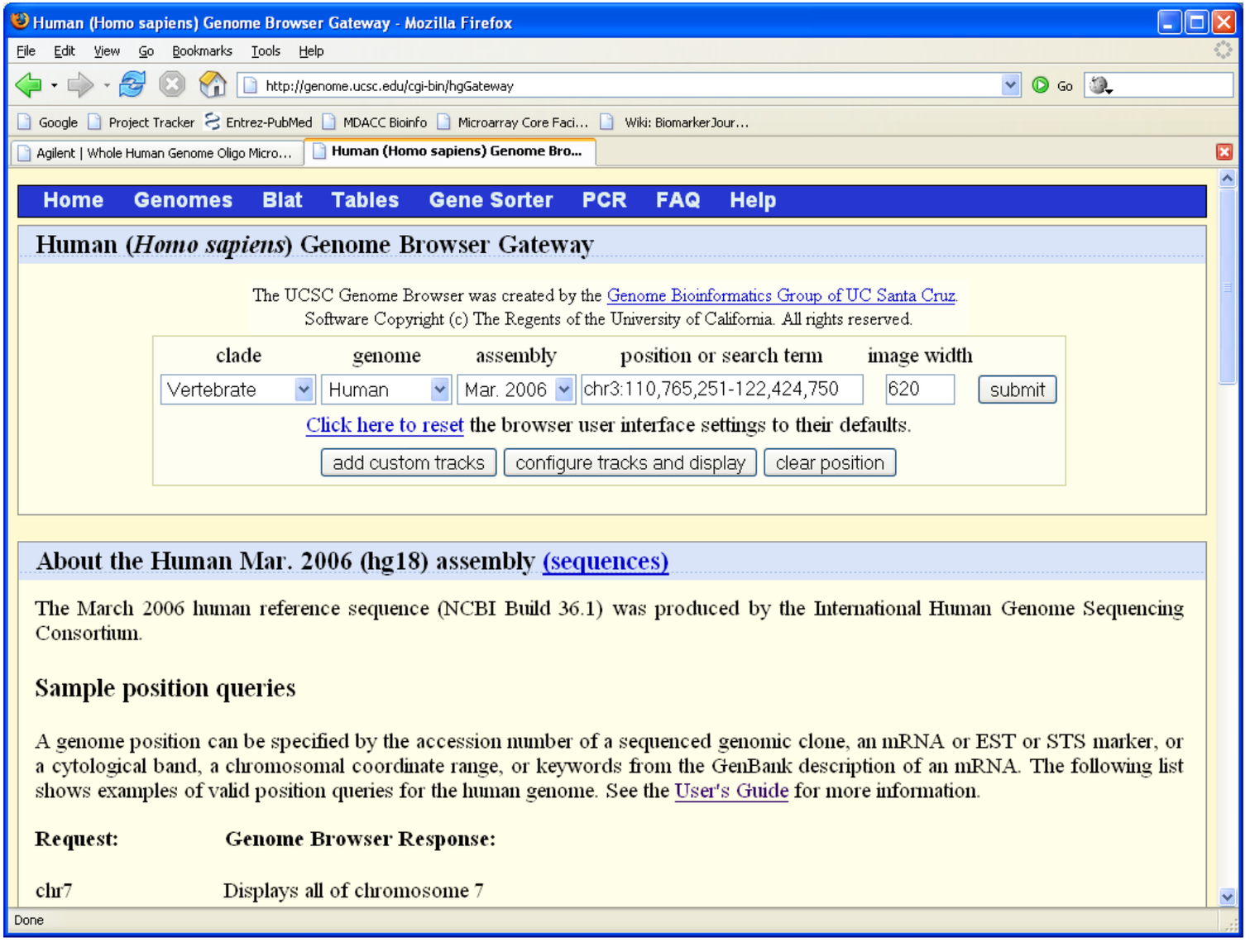

The UCSC Genome Browser

We are going to shift gears slightly:

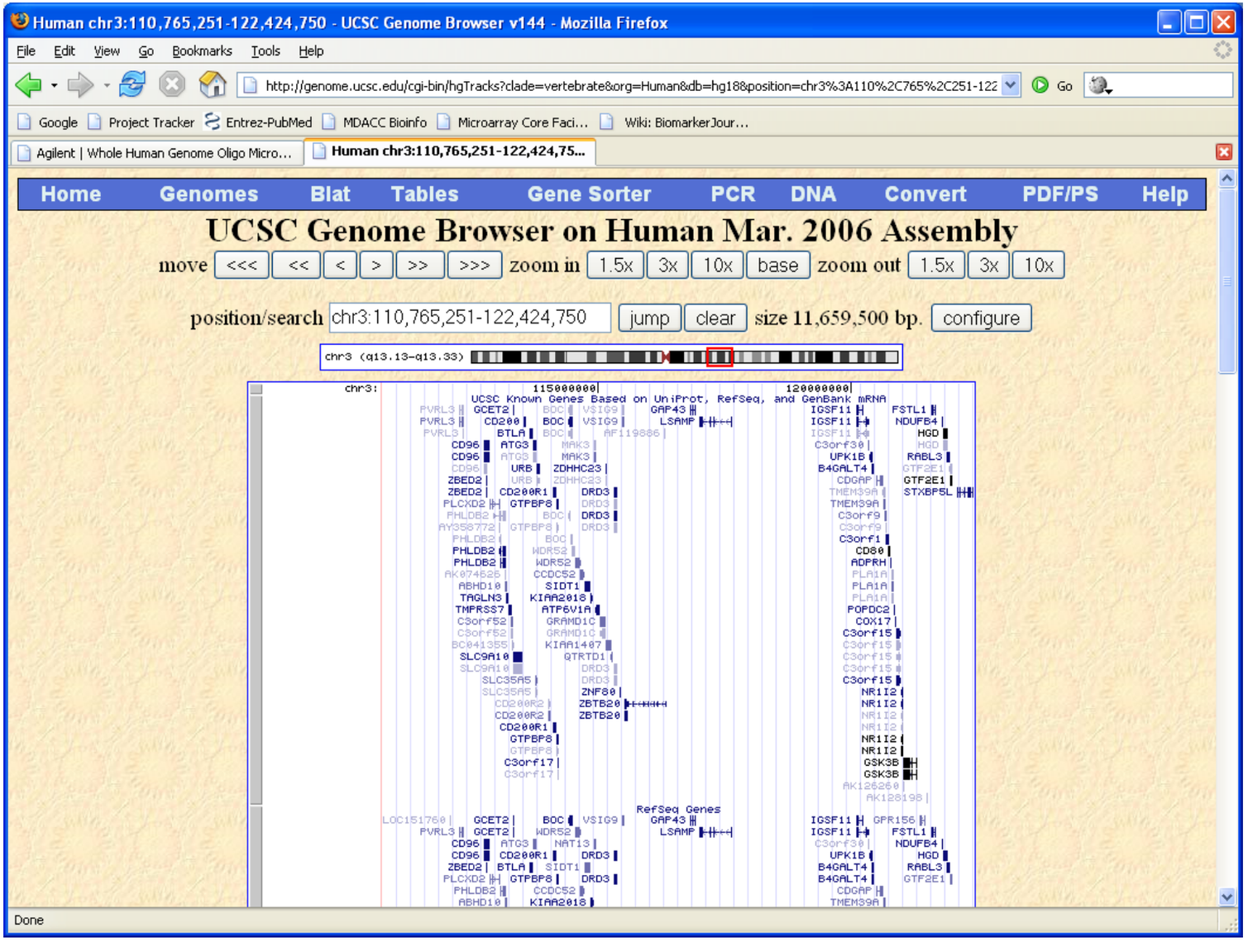
`http://genome.ucsc.edu/`



Follow the Link to "Genome Browser"



Press "Submit" to Start Browsing



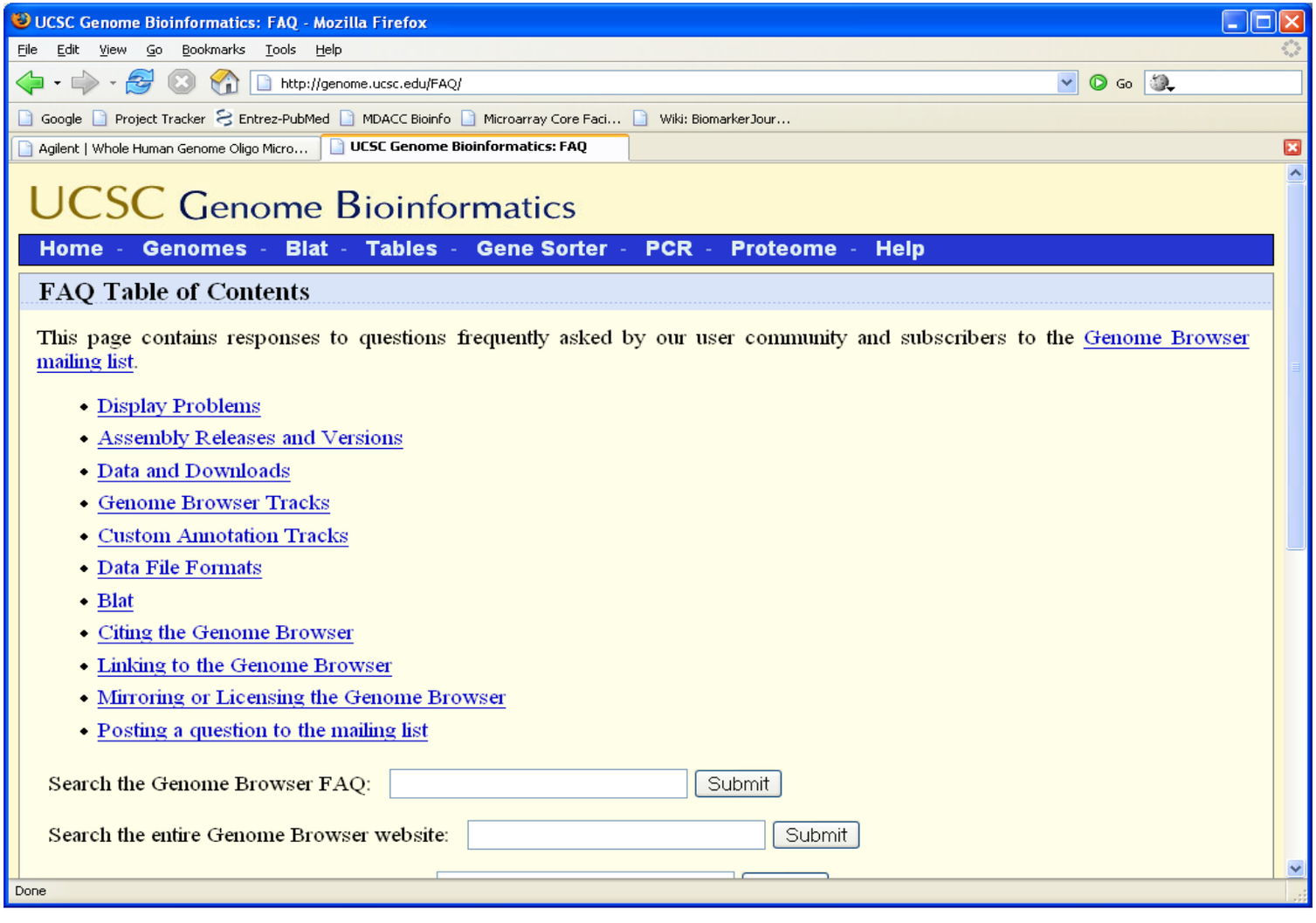
About the Genome Browser

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).

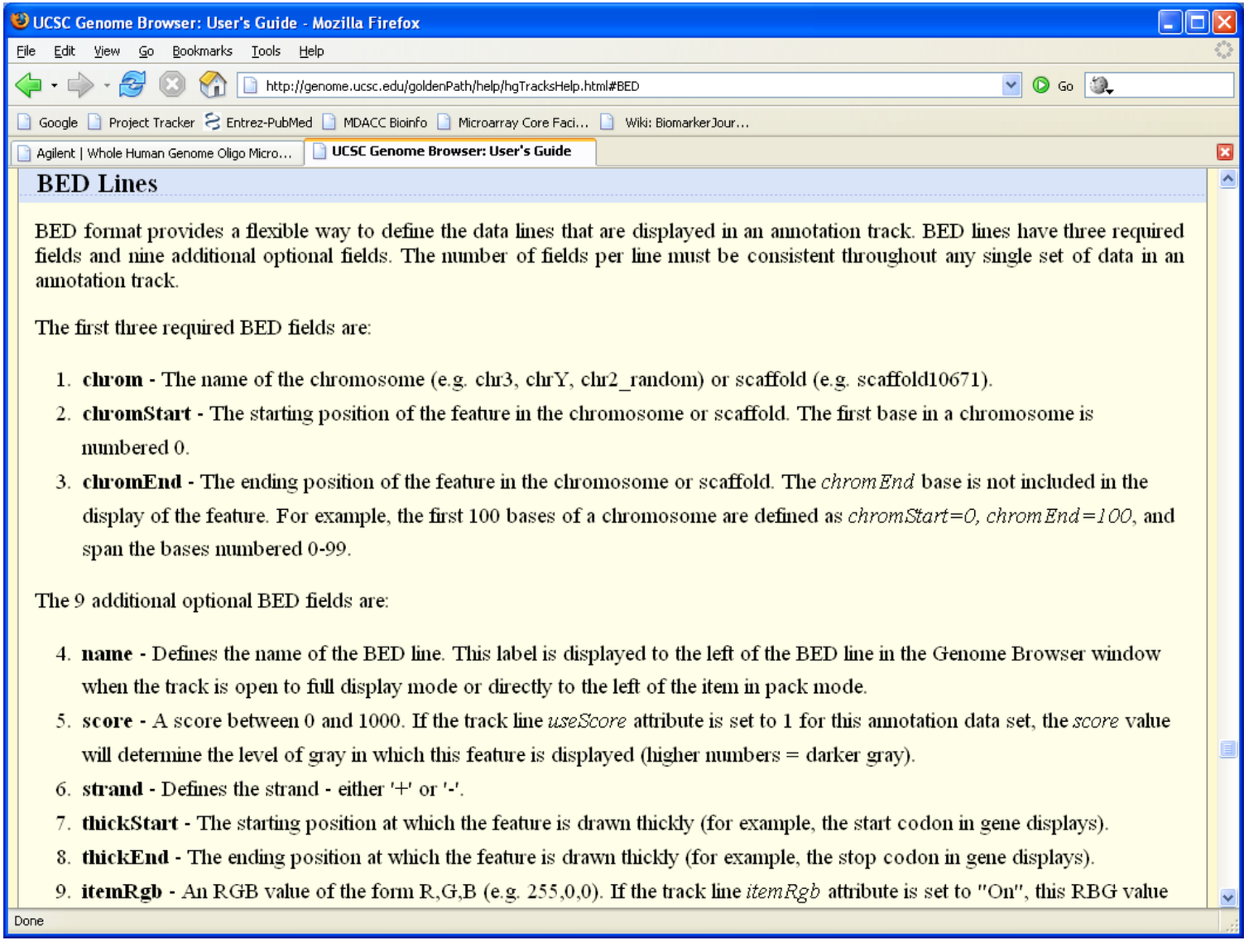
For our purposes (as people who analyze microarray data), an extremely interesting feature of the Genome Browser is that it lets you add your own "Custom Tracks", which is their name for a set of annotations you can define.

Custom Tracks

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



BED Format

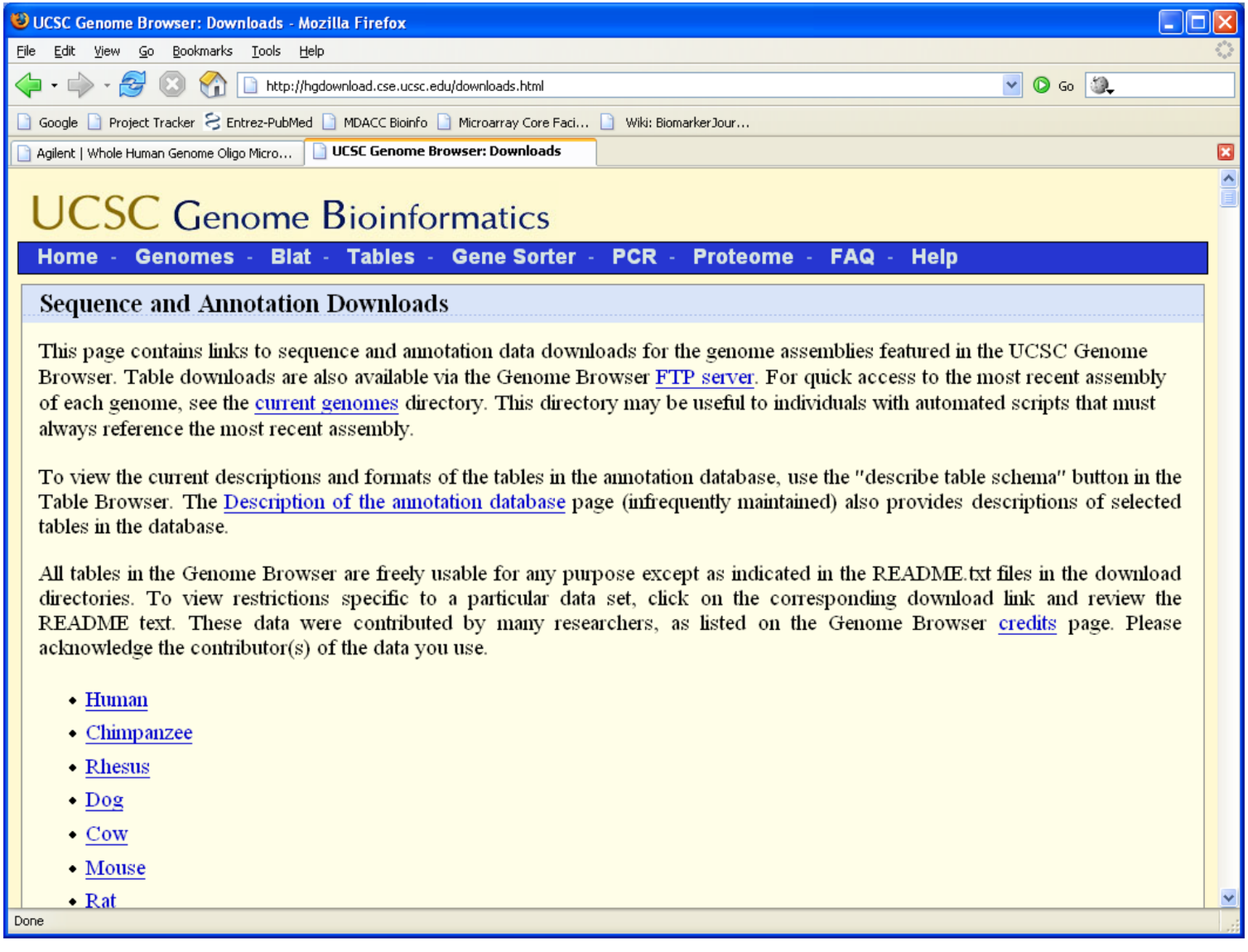


Chromosome Locations

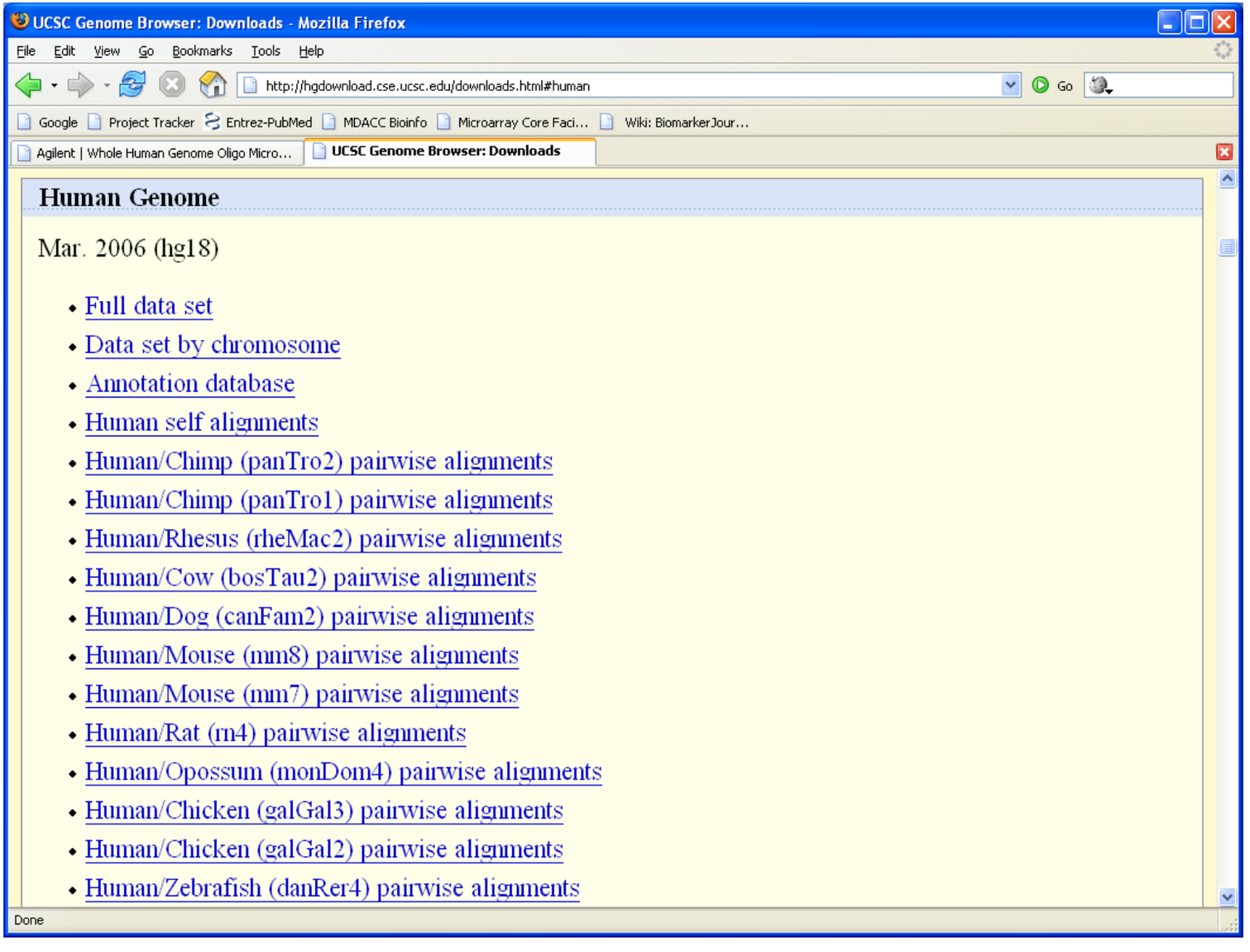
You can read more of the custom track documentation on your own; here, we are going to focus on how to build a custom track in R. The first thing we want to point out is that we need to know both the starting base location and the ending base location in order to build a custom track. Thus, the `CHRLOC` 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".

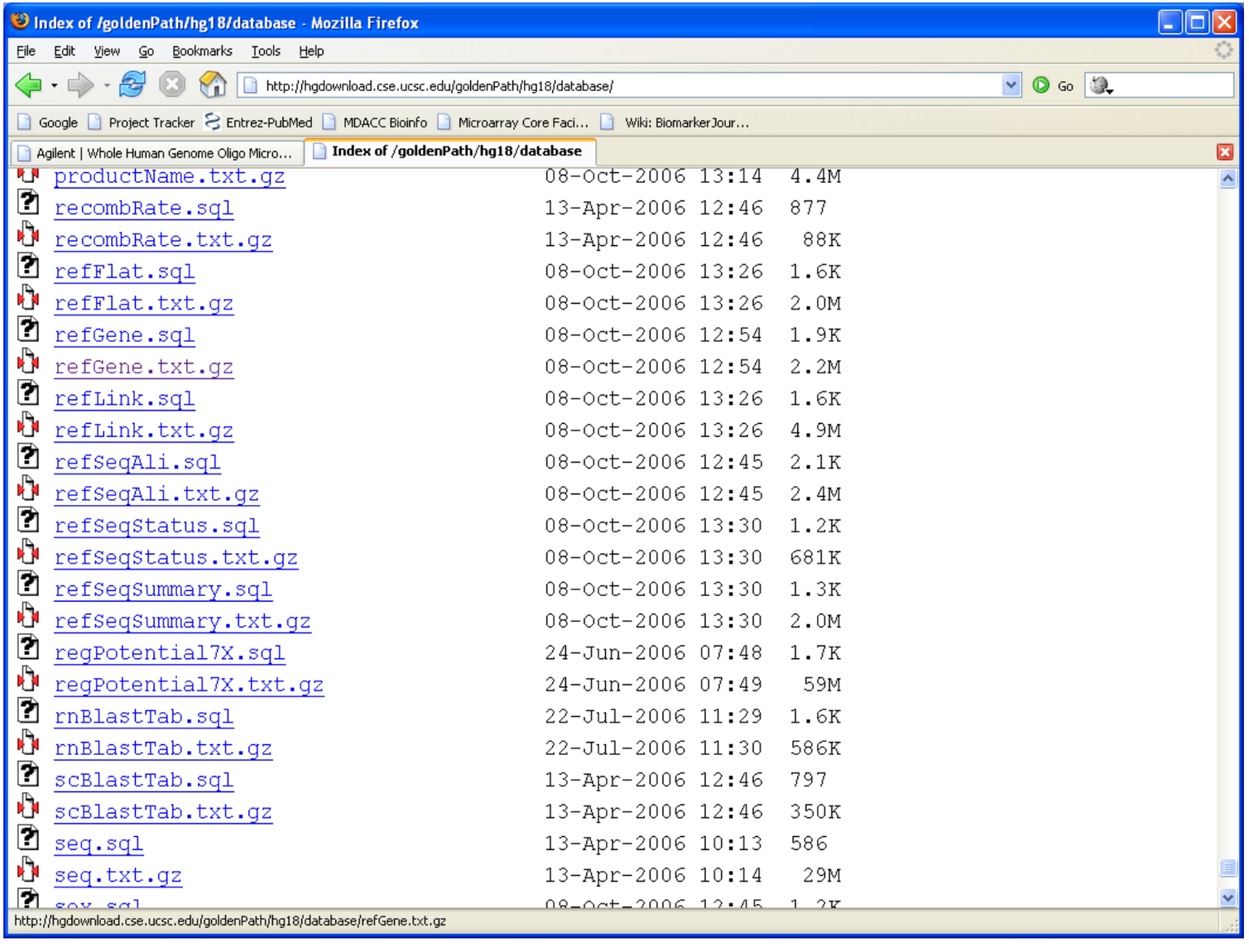
UCSC Download Page



Follow the link for "Human"



In "Annotation Database", Scroll To "refGene"



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:

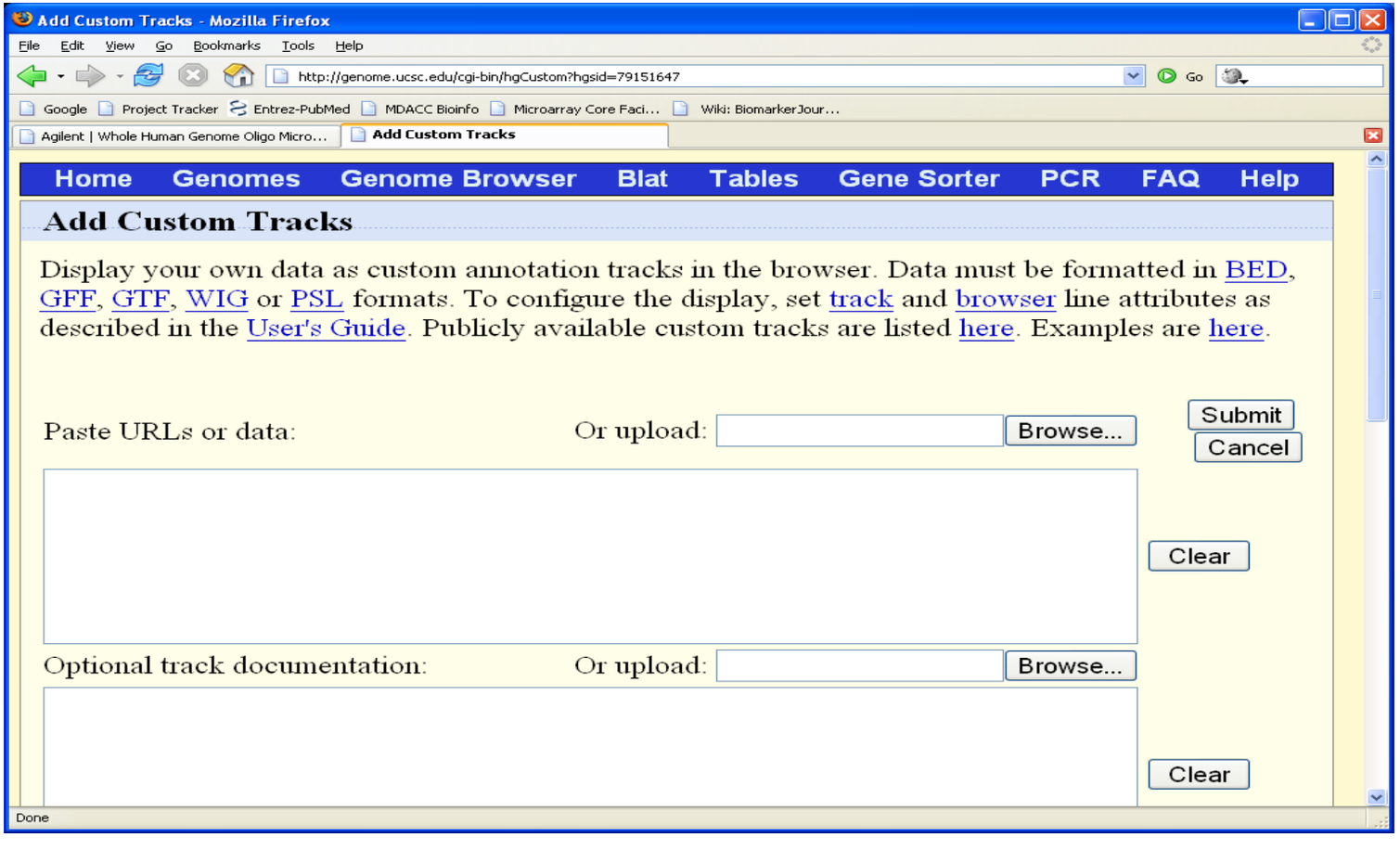
```
> write(trackheader, file = "upNormalRNA.tsv",
+     append = FALSE)
> write.table(trackInfo, file = "upNormalRNA.tsv",
+     append = TRUE, quote = FALSE, sep = "\t",
+     row.names = FALSE, col.names = FALSE)
```


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

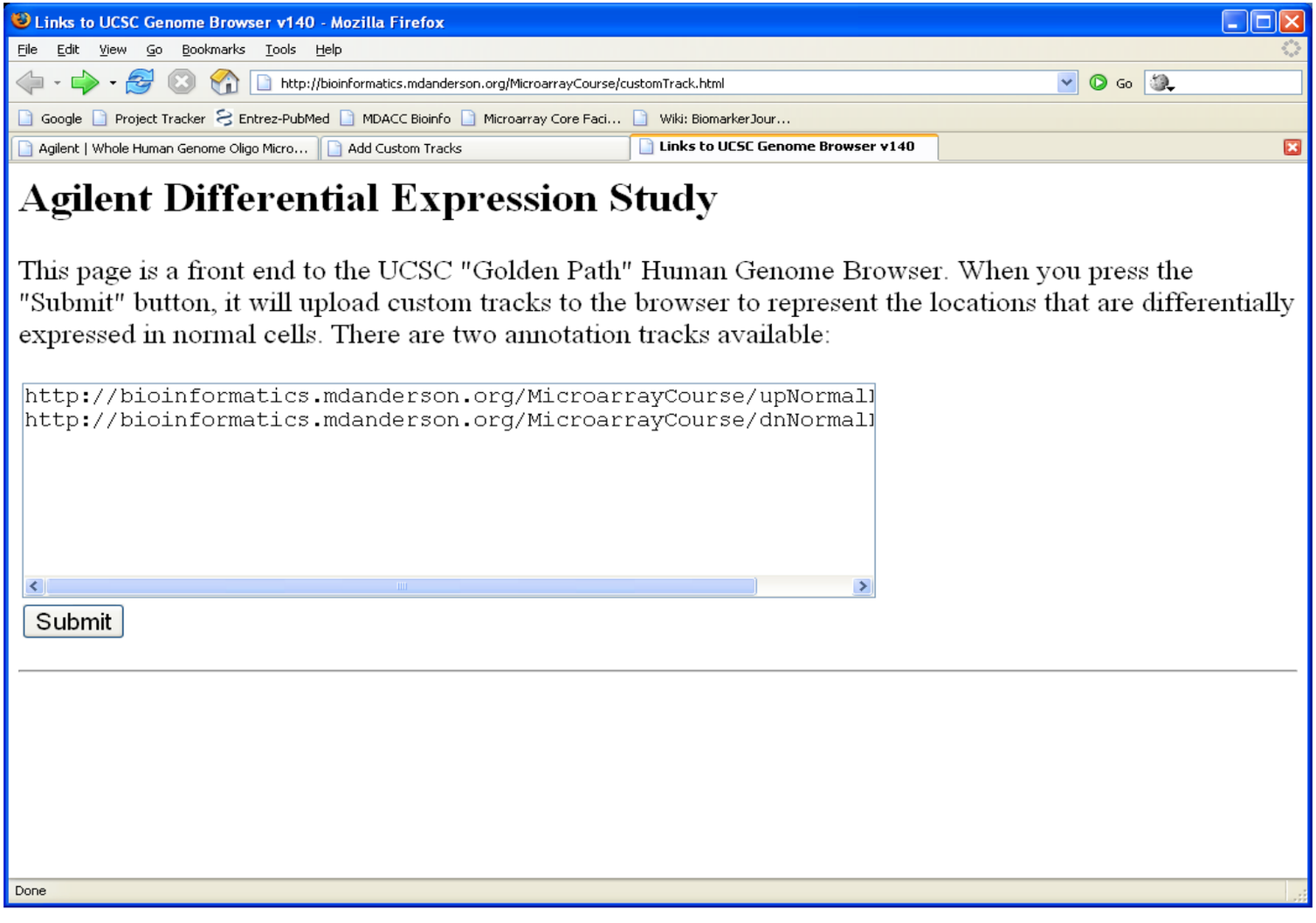
```
> trackInfo <- temp[!is.na(temp[, "chrom"]) & ourResults[,
+     "PValue"] < 0.02 & ourResults[, "Beta"] <
+     0, ]

> 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

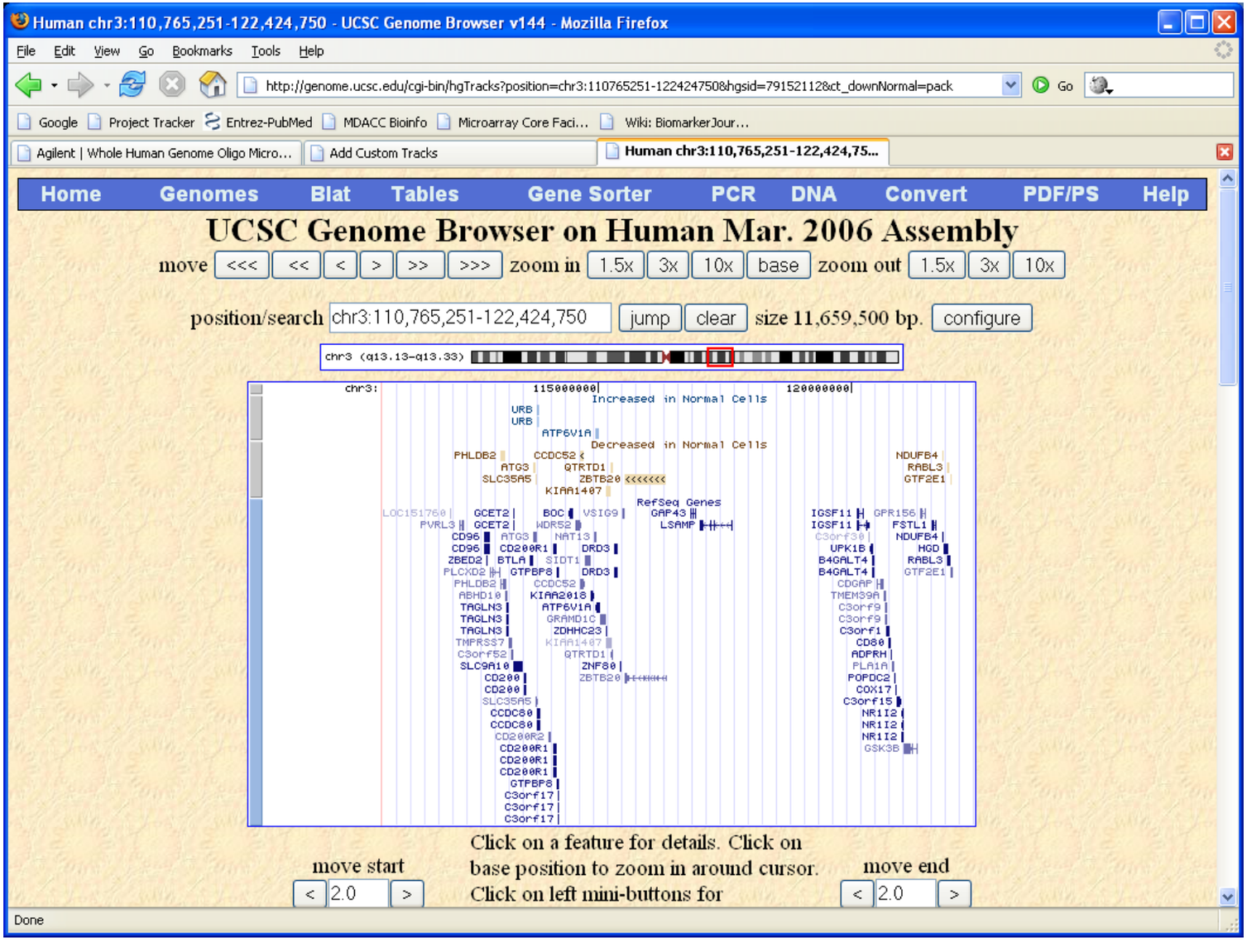
Now we can return to the genome browser and look at our custom tracks. Unfortunately, their web page only lets you attach one at a time unless you can make them available from a web site:



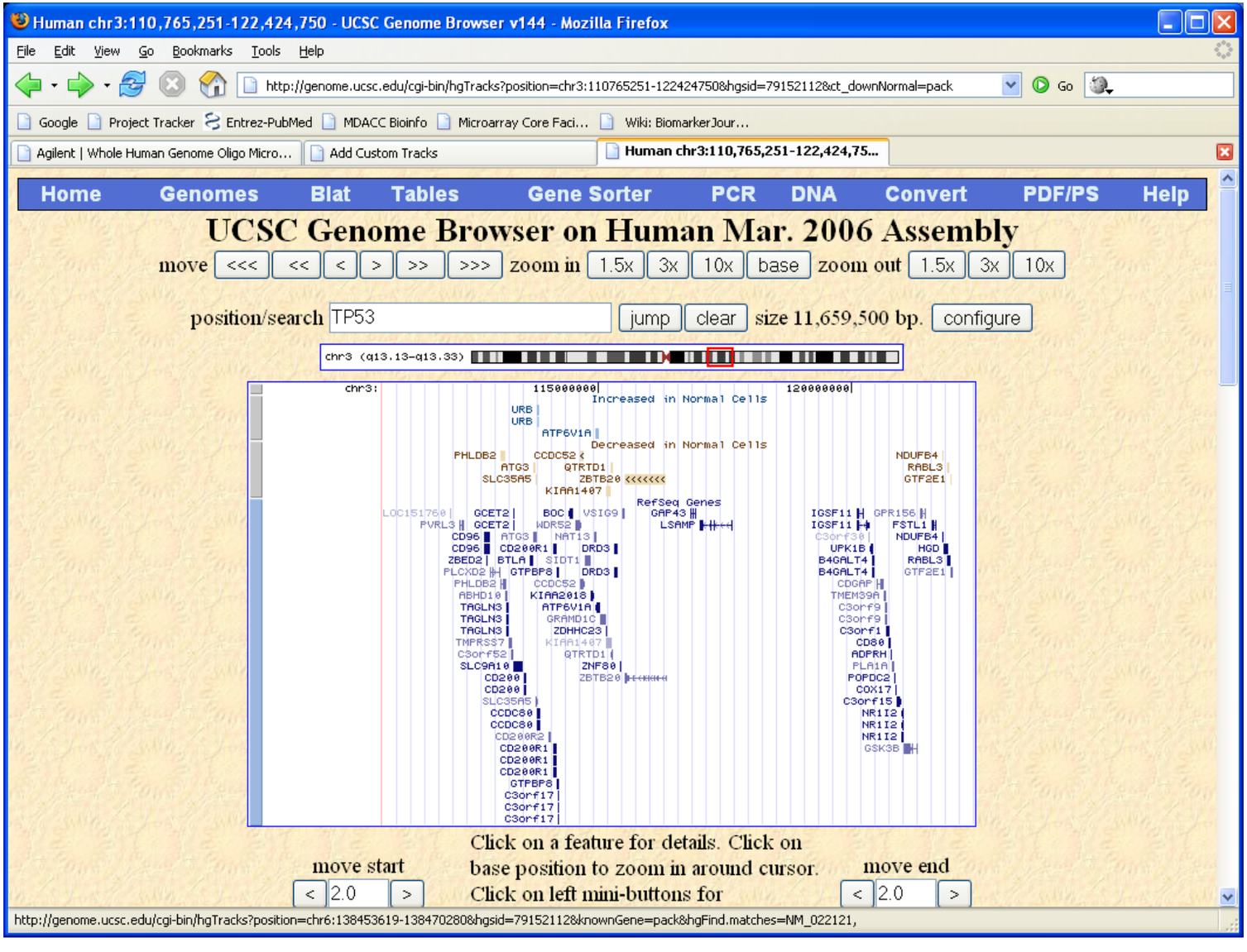
http://bioinformatics.mdanderson.org/MicroarrayCourse/customTrack.html



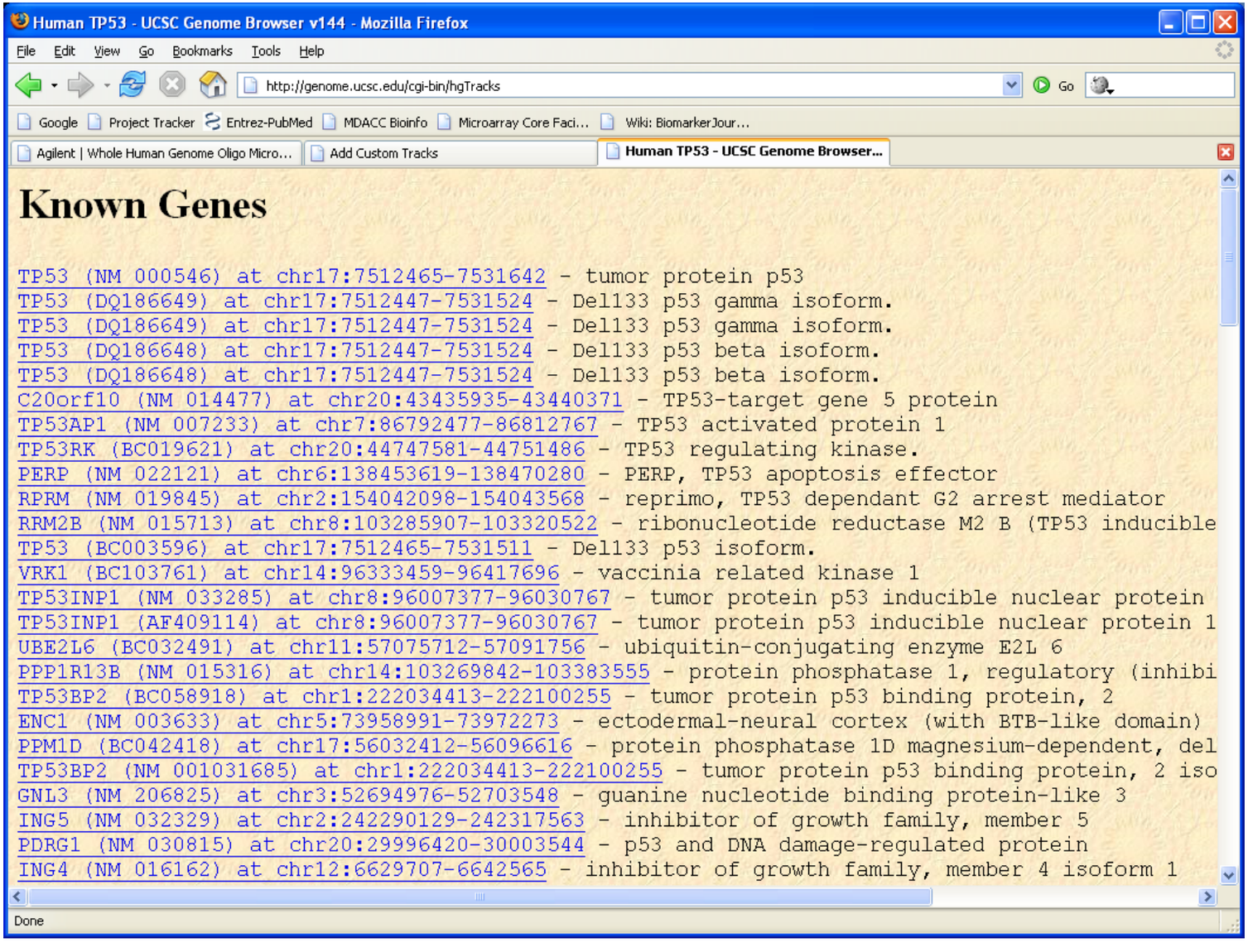
Displaying Our Tracks



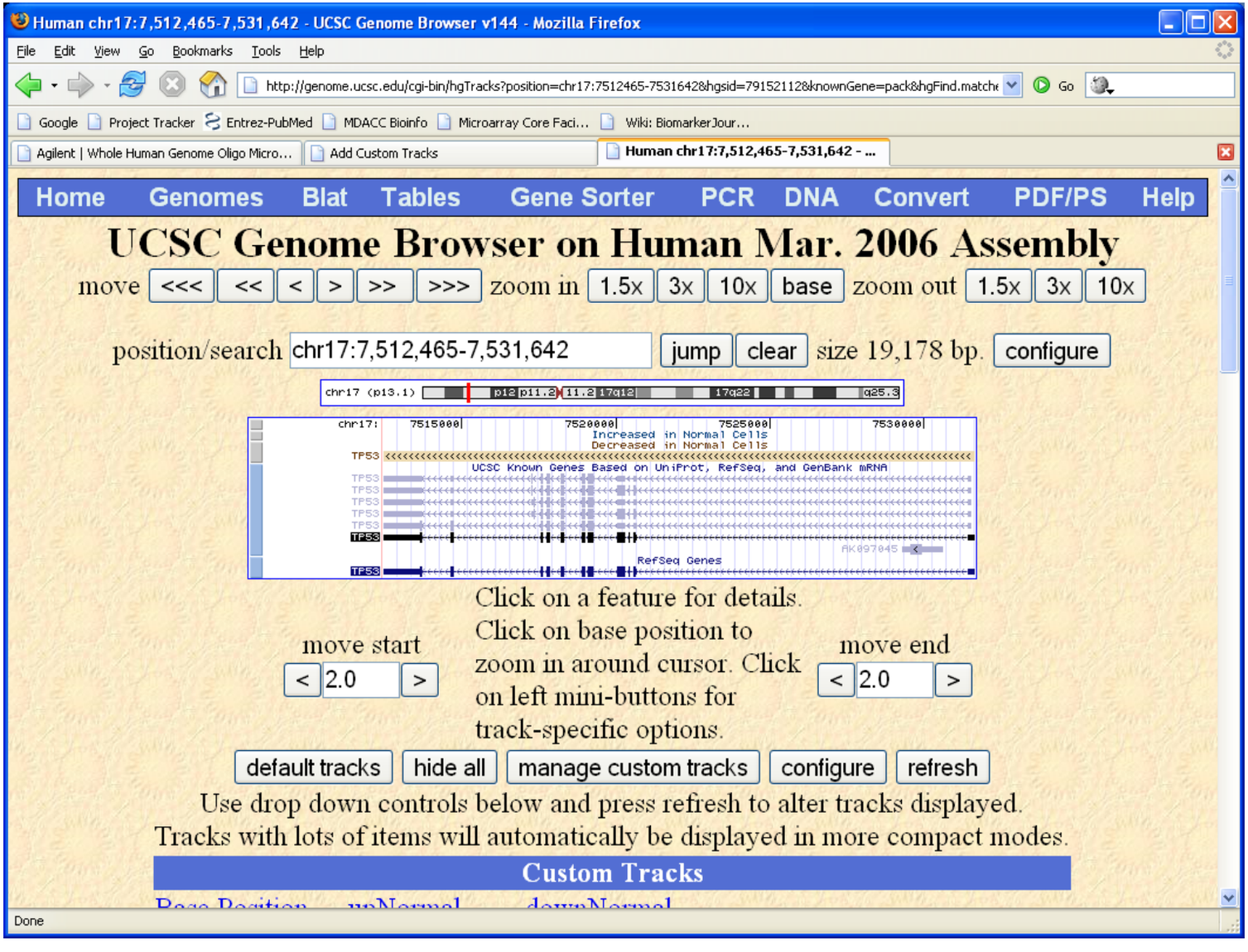
Searching for a Gene



Searching for a Gene



Searching for a Gene



Searching for a Gene