GS01 0163
Analysis of Microarray Data

Keith Baggerly and Brad Broom
Department of Bioinformatics and Computational Biology
UT M. D. Anderson Cancer Center
kabagg@mdanderson.org
bmbroom@mdanderson.org

14 October 2010

Lecture 13: Limma and TCGA

- Linear Models – parallel fits, and borrowing strength
- Design Matrices and Contrast Matrices
- TCGA — What is it?

Looking at Contrasts in R

We talked earlier about incorporating multiple covariates into our modeling, and pointed out that the general statistical extension was the linear model.

Today, I want to introduce `limma`, which is, as you might guess, “linear models for microarrays”.

This takes many standard statistical tests and codes them rather efficiently for (a) massive parallelization and (b) borrowing across arrays.

Much of what follows today is taken straight from the User’s manual.

Example 1: Contrasting Two Groups

Our first case study involves an E. coli knockout experiment, as described in Hughes et al. *J Biol Chem*, **277**:40309-23, 2002. In it, 4 wild-type samples are contrasted with 4 samples from which Lrp has been knocked out. The dataset is available from BioConductor as ecoliLeucine (we also need ecolicdf).

```
> library(ecolicdf)
> library(ecoliLeucine) # loads affy, Biobase
> data(ecoliLeucine) # an AffyBatch
> eLeuRMA <- rma(ecoliLeucine)
```

So, What Do We Know?

```
> pData(eLeuRMA)
/home/laurent/Affymetrix_data/ecoli_sample//nolrp_
/home/laurent/Affymetrix_data/ecoli_sample//nolrp_
/home/laurent/Affymetrix_data/ecoli_sample//nolrp_
/home/laurent/Affymetrix_data/ecoli_sample//nolrp_
/home/laurent/Affymetrix_data/ecoli_sample//wt_1.
/home/laurent/Affymetrix_data/ecoli_sample//wt_2.
/home/laurent/Affymetrix_data/ecoli_sample//wt_3.
/home/laurent/Affymetrix_data/ecoli_sample//wt_4.
rownames(pData(eLeuRMA)) <-
  substr(rownames(pData(eLeuRMA)),45,56)
colnames(exprs(eLeuRMA)) <- rownames(pData(eLeuRM
```

That the data was loaded by Laurent Gautier...

Setting up the Linear Model

In order to use `limma`, we need three things: (1) an expression matrix, (2) a design matrix, and (3) a contrast matrix. The expression matrix we have. What about the other two? The design matrix basically states what treatments were applied to what samples.

```
> library(limma)
> designMatrix <-
    model.matrix(~pData(eLeuRMA$strain))
> # or
> # strain <- rep(c("lrp-","lrp+"),each=4)
> # design <- model.matrix(~factor(strain))
```

What Does This Produce?

```
> designMatrix
  (Intercept) pData(eLeuRMA)$strainlrp+
1           1                         0
2           1                         0
3           1                         0
4           1                         0
5           1                         1
6           1                         1
7           1                         1
8           1                         1
attr(,"assign") [1] 0 1
attr(,"contrasts")
attr(,"contrasts")$`pData(eLeuRMA)$strain`
[1] "contr.treatment"
```

How Do We Fit Things?

```
colnames(designMatrix) <- c("lpr-","lpr+Diff")
fit1 <- lmFit(eLeuRMA, designMatrix)
fit2 <- eBayes(fit1)
summary(fit1)
                 Length Class      Mode
coefficients     14624  -none-     numeric
rank                 1  -none-     numeric
assign               2  -none-     numeric
qr                   5  qr         list
df.residual       7312  -none-     numeric
sigma             7312  -none-     numeric
cov.coefficients     4  -none-     numeric
stdev.unscaled   14624  -none-     numeric
pivot                2  -none-     numeric
```

```
genes                1  data.frame list
Amean             7312  -none-     numeric
method               1  -none-     character
design              16  -none-     numeric
```

How is the second invocation different? What gets added?

How Do We Fit Things? (2)

```
summary(fit2)
                 Length Class       Mode
(as with fit1, plus)
df.prior              1 -none-      numeric
s2.prior              1 -none-      numeric
var.prior             2 -none-      numeric
proportion            1 -none-      numeric
s2.post            7312 -none-      numeric
t                 14624 -none-      numeric
p.value           14624 -none-      numeric
lods              14624 -none-      numeric
F                  7312 -none-      numeric
F.p.value          7312 -none-      numeric
```

How Do We Display Things?

```
options(digits=2)
topTable(fit2,coef=2,n=5,adjust="BH")
                  ID logFC AveExpr   t P.Value
      IG_821_1300838
4282 _1300922_fwd_st  -3.3    12.4 -23 7.2e-09
5365    serA_b2913_st   2.8    12.2  16 1.6e-07
1389    gltD_b3213_st   3.0    10.9  13 6.4e-07
4625     lrp_b0889_st   2.3     9.3  11 2.3e-06
1388    gltB_b3212_st   3.2    10.0  11 2.8e-06
   adj.P.Val   B
     5.3e-05 8.0
     6.0e-04 6.6
```

Double-Checking

```
> t(exprs(eLeuRMA)[c(4282,5365),])
            IG_821_1300838
            _1300922_fwd_st serA_b2913_st
nolrp_1.CEL          13.872        10.403
nolrp_2.CEL          14.253        10.745
nolrp_3.CEL          14.136        10.984
nolrp_4.CEL          13.811        11.195
wt_1.CEL             10.504        13.561
wt_2.CEL             10.960        13.739
wt_3.CEL             10.637        13.415
wt_4.CEL             10.699        13.722
```

That’s most of what there is here.

Example 2: Two Factors

Here, we look at changes over time in MCF7 in response to exposure to estrogen. This involves 8 U95Av2 arrays in the BioConductor package `estrogen`.

```
dataDir <- file.path(.find.package("estrogen"),
  "extdata")
targets <-
  readTargets("phenoData.txt", path=dataDir,
              sep="", row.names="filename")
```

The Sample Info

```
targets
                 filename estrogen time.h
low10-1.cel   low10-1.cel   absent     10
low10-2.cel   low10-2.cel   absent     10
high10-1.cel high10-1.cel  present     10
high10-2.cel high10-2.cel  present     10
low48-1.cel   low48-1.cel   absent     48
low48-2.cel   low48-2.cel   absent     48
high48-1.cel high48-1.cel  present     48
high48-2.cel high48-2.cel  present     48
```

Getting Expression Values

```
library(hgu95av2cdf)
estRMA <- justRMA(celfile.path=dataDir)
dim(estRMA)
Features  Samples
   12625        9
colnames(exprs(estRMA))
[1] "bad.cel"      "high10-1.cel" "high10-2.cel"
[4] "high48-1.cel" "high48-2.cel" "low10-1.cel"
[7] "low10-2.cel"  "low48-1.cel"  "low48-2.cel"
estRMA2 <- estRMA[,c(2:9)]
estRMA <- justRMA(filenames=targets$filename,
            celfile.path=dataDir)
```

Building a Design Matrix

```
treatmentCombos <- factor(rep(1:4,each=2),
  labels=c("e-10h","e+10h","e-48h","e+48h"))
contrasts(treatmentCombos)
      e+10h e-48h e+48h
e-10h     0     0     0
e+10h     1     0     0
e-48h     0     1     0
e+48h     0     0     1
contrasts(treatmentCombos) <- cbind(
  Time=c(0,0,1,1), E10=c(0,1,0,0),
  E48=c(0,0,0,1))
designMatrix <- model.matrix(~treatmentCombos)
```

What Does the Design Matrix Look Like?

```
colnames(designMatrix) <-
    c("Intercept","Time","E10","E48")
designMatrix
  Intercept Time E10 E48
1         1    0   0   0
2         1    0   0   0
3         1    0   1   0
4         1    0   1   0
5         1    1   0   0
6         1    1   0   0
7         1    1   0   1
8         1    1   0   1
```

Properly expanded to cover all samples...

Fit the Model(s)

```
fit1 <- lmFit(estRMA, designMatrix)
fit1EB <- eBayes(fit1)

otherContrasts <- cbind(E10=c(0,0,1,0),
                        E48=c(0,0,0,1))
# note this is with respect to the design!

fit2 <- contrasts.fit(fit1, otherContrasts)
fit2EB <- eBayes(fit2)
```

What’s the Difference?

```
> summary(classifyTestsF(fit1EB,
   p.value=0.0001))
   Intercept  Time   E10   E48
-1          0   136    55   181
0           0 11559 12065 11869
1       12625   930   505   575
> summary(classifyTestsF(fit2EB,
   p.value=0.0001))
     E10   E48
-1     40    76
0  12469 12410
1    116   139
```

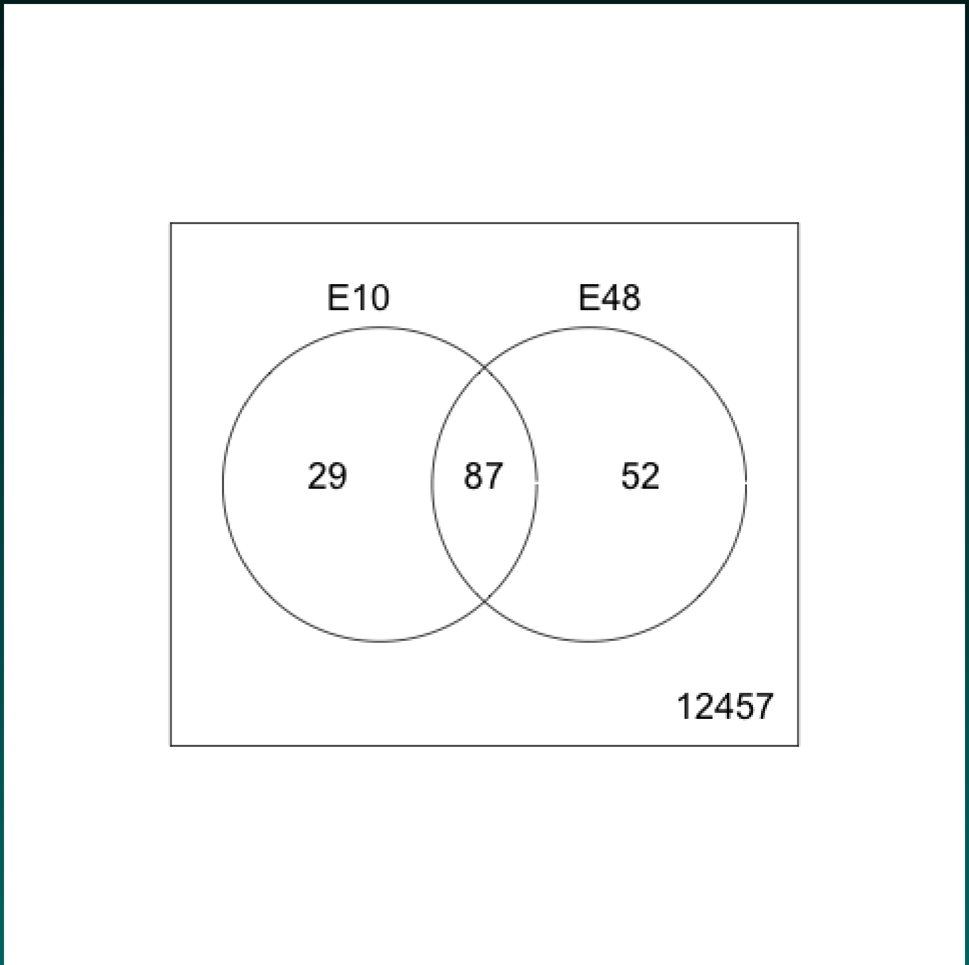
What’s the overall model being tested?

Tabling the Results

```
mod2Results <- classifyTestsF(fit2EB,
   p.value=0.0001)
table(E10=mod2Results[,1], E48=mod2Results[,2])
    E48
E10     -1     0     1
  -1    29    11     0
  0     47 12370    52
  1      0    29    87
```

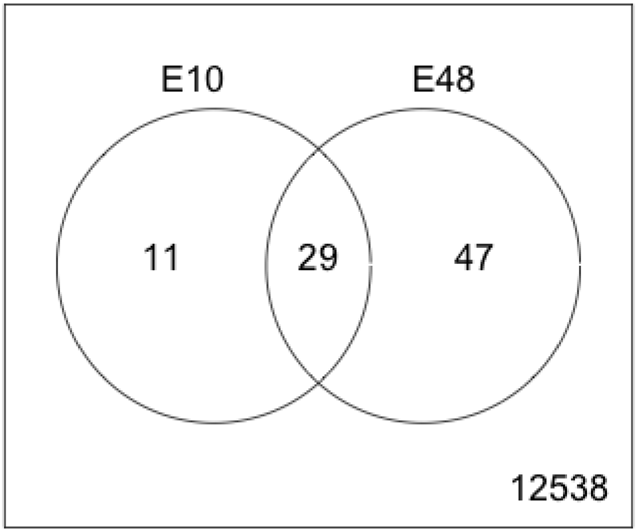
Or, if you prefer (1)

Venn Up

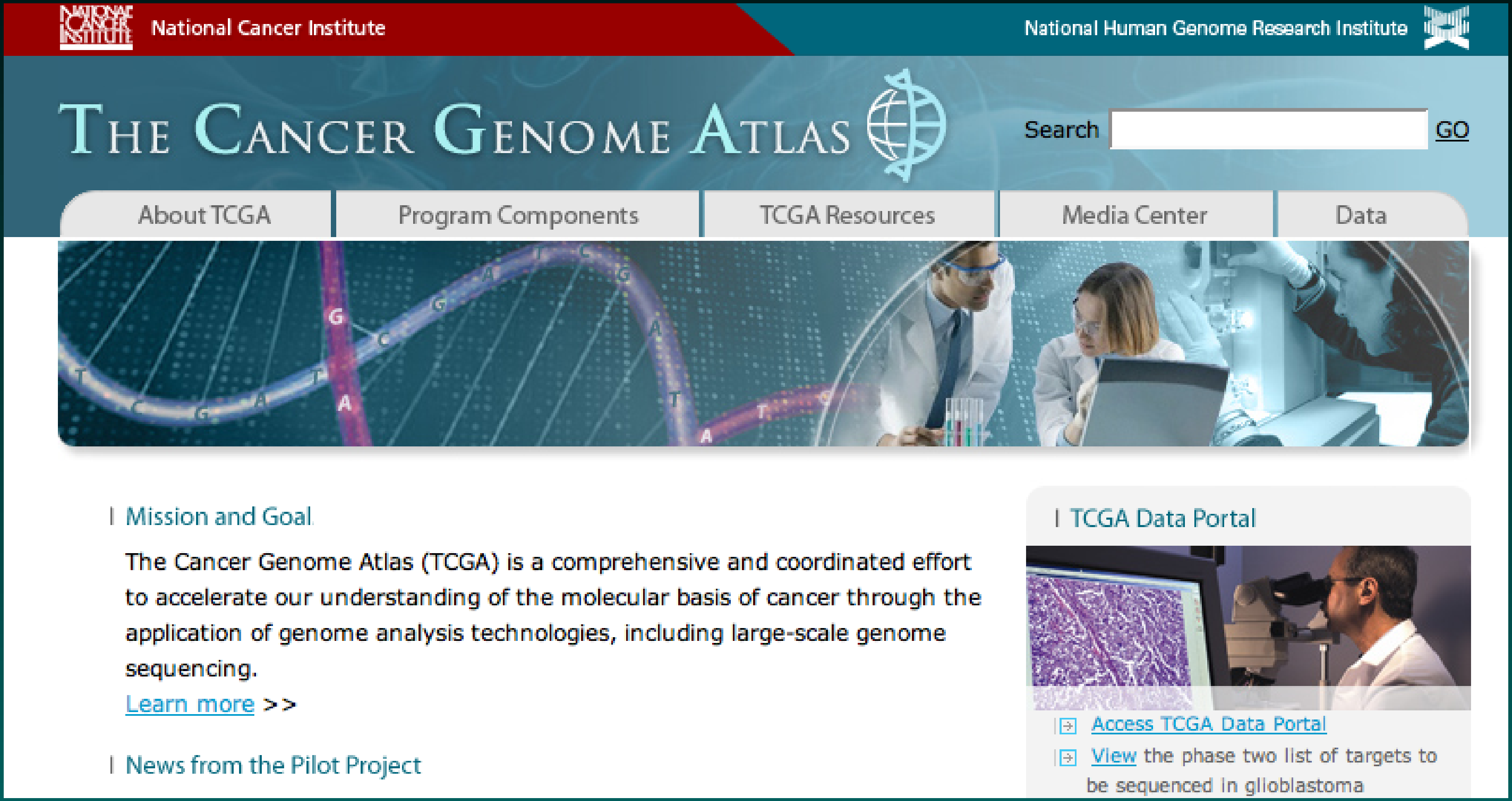


Or, if you prefer (2)

Venn Down (Venn breaks for > 3 sets.)



TCGA: The Cancer Genome Atlas



Mission and Goal

The Cancer Genome Atlas (TCGA) is a comprehensive and coordinated effort to accelerate our understanding of the molecular basis of cancer through the application of genome analysis technologies, including large-scale genome sequencing.

Learn more >>

News from the Pilot Project

TCGA Data Portal

Access TCGA Data Portal

View the phase two list of targets to be sequenced in glioblastoma

http://cancergenome.nih.gov

What is it?

An attempt to do high-throughput studies right.

We’ve run a lot of high-throughput studies, but haven’t always learned as much as we’d hoped. Some common problems:

- small sample sizes
- variable sample quality
- poor clinical information
- batch effects
- looking just at one piece of the puzzle
- (poor experimental design)

A Big Science Pilot

time to think big (it worked for the genome project...)

$100M to start (actually up to a few now, but who’s counting?)

For a small number of tumor types, identify a large number of high-quality samples with good clinical information and some matched normal material. Some prospective collection may be required.

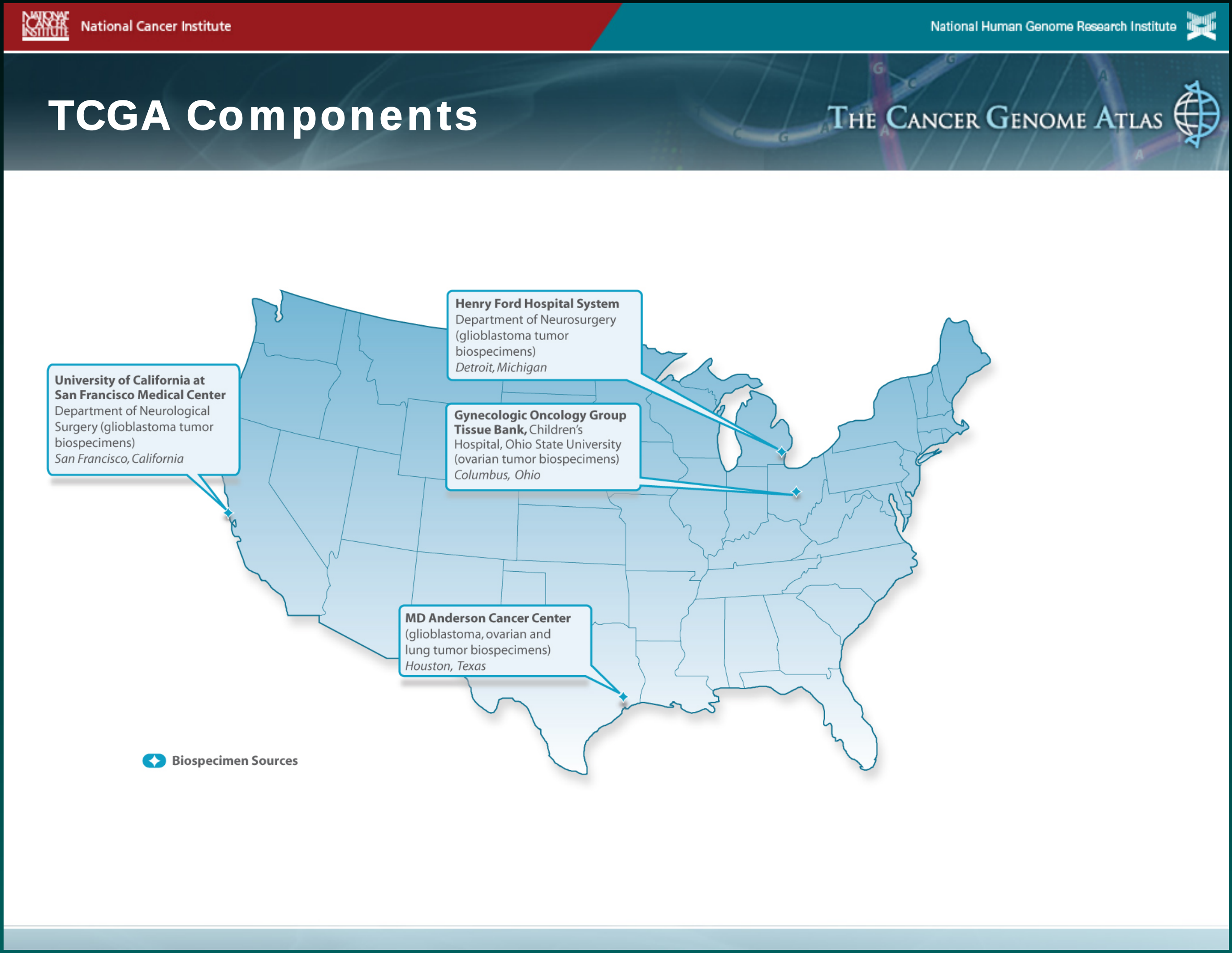
They picked 3 tumor types to start (now 20): brain (glioblastoma, GBM), lung (non-small cell), and ovary (serous adenocarcinoma).

For each, they’re seeking 500 samples, which will then be subjected to a barrage of assays.

The Assays (So Far)

- Sequencing of specific genes
- CGH Arrays (Agilent 244K)
- SNP Arrays (Affy 6/500K, Illumina 550K BeadArray)
- Expression Arrays (Affy U133+2, Agilent 44K)
- Exon Arrays (Affy)
- Methylation Arrays (Illumina)
- micro RNA (miRNA) Arrays (Agilent)

Where MDA Comes In



National Cancer Institute

National Human Genome Research Institute

TCGA Components

The Cancer Genome Atlas

University of California at San Francisco Medical Center
Department of Neurological Surgery (glioblastoma tumor biospecimens)
San Francisco, California

Henry Ford Hospital System
Department of Neurosurgery (glioblastoma tumor biospecimens)
Detroit, Michigan

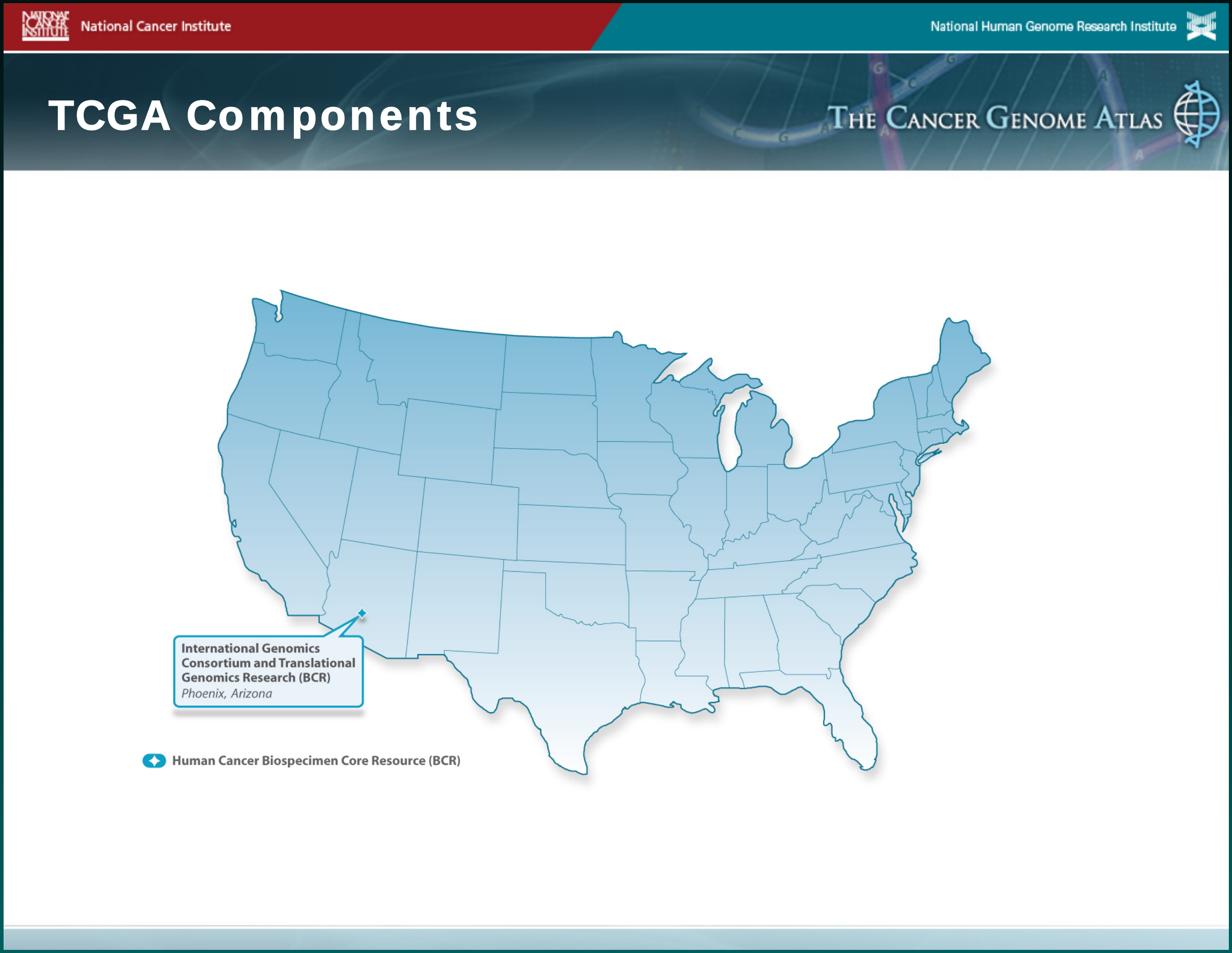
Gynecologic Oncology Group Tissue Bank, Children's Hospital, Ohio State University (ovarian tumor biospecimens)
Columbus, Ohio

MD Anderson Cancer Center
(glioblastoma, ovarian and lung tumor biospecimens)
Houston, Texas

◆ Biospecimen Sources

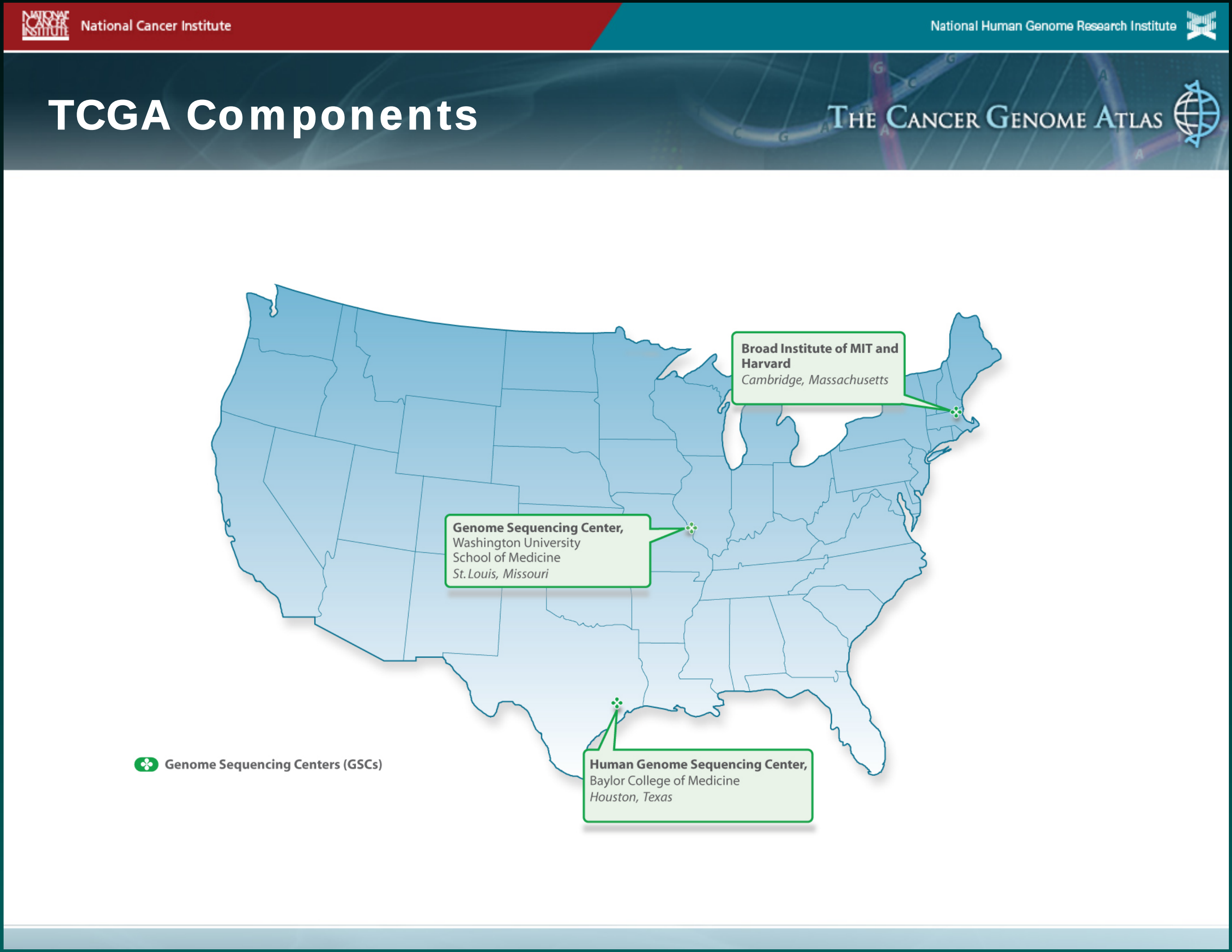
Biospecimen Sources

Where The Samples Go


National Cancer Institute
National Human Genome Research Institute
TCGA Components
THE CANCER GENOME ATLAS
International Genomics Consortium and Translational Genomics Research (BCR)
Phoenix, Arizona
Human Cancer Biospecimen Core Resource (BCR)

Biospecimen Core Resource

Where They Do Sequencing



National Cancer Institute

National Human Genome Research Institute

TCGA Components

THE CANCER GENOME ATLAS

Broad Institute of MIT and Harvard
Cambridge, Massachusetts

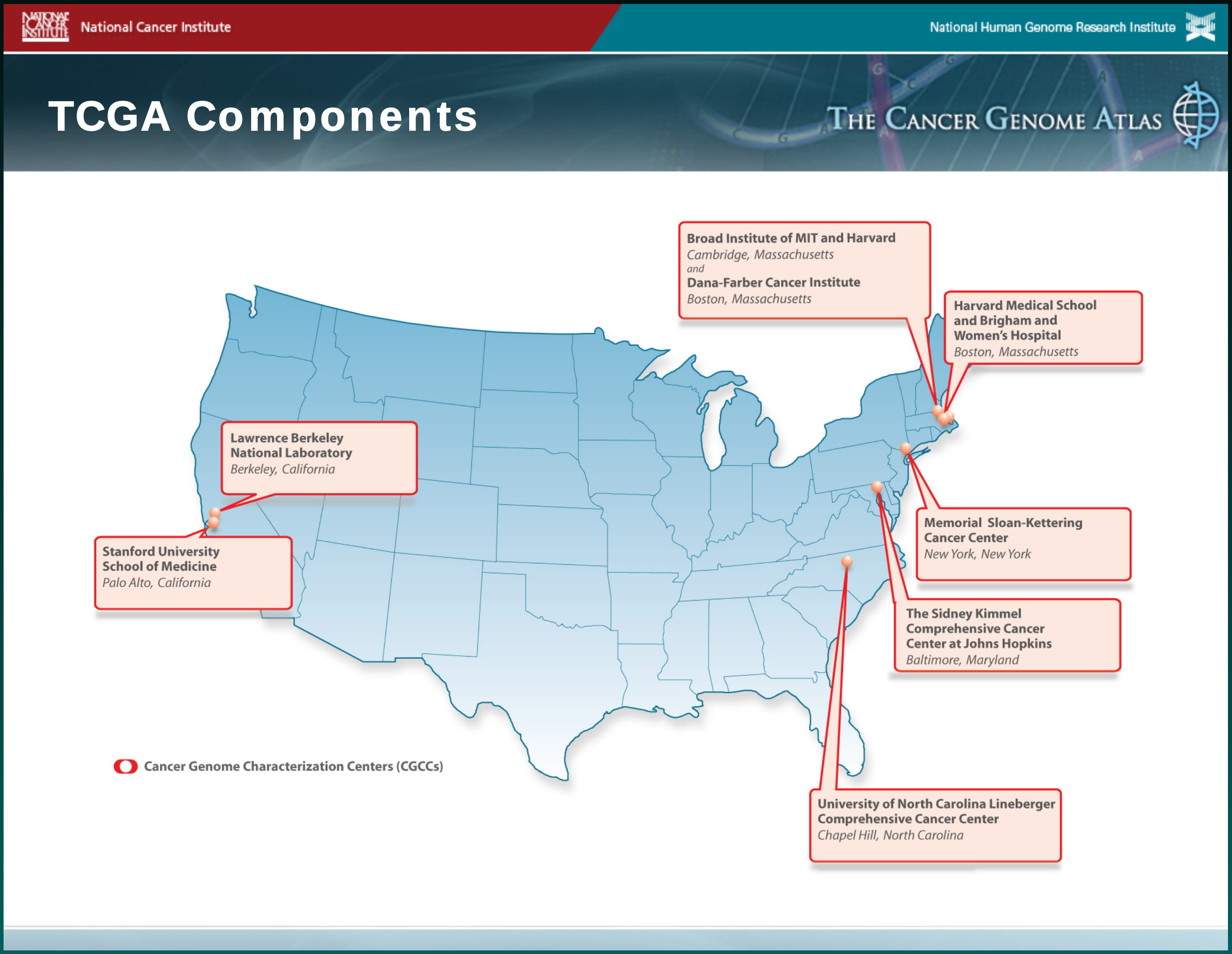
Genome Sequencing Center,
Washington University
School of Medicine
St. Louis, Missouri

Human Genome Sequencing Center,
Baylor College of Medicine
Houston, Texas

✤ Genome Sequencing Centers (GSCs)

Genome Sequencing Centers

Where They Run the Other Assays



National Cancer Institute

National Human Genome Research Institute

TCGA Components

THE CANCER GENOME ATLAS

Broad Institute of MIT and Harvard
Cambridge, Massachusetts
and
Dana-Farber Cancer Institute
Boston, Massachusetts

Harvard Medical School and Brigham and Women's Hospital
Boston, Massachusetts

Lawrence Berkeley National Laboratory
Berkeley, California

Stanford University School of Medicine
Palo Alto, California

Memorial Sloan-Kettering Cancer Center
New York, New York

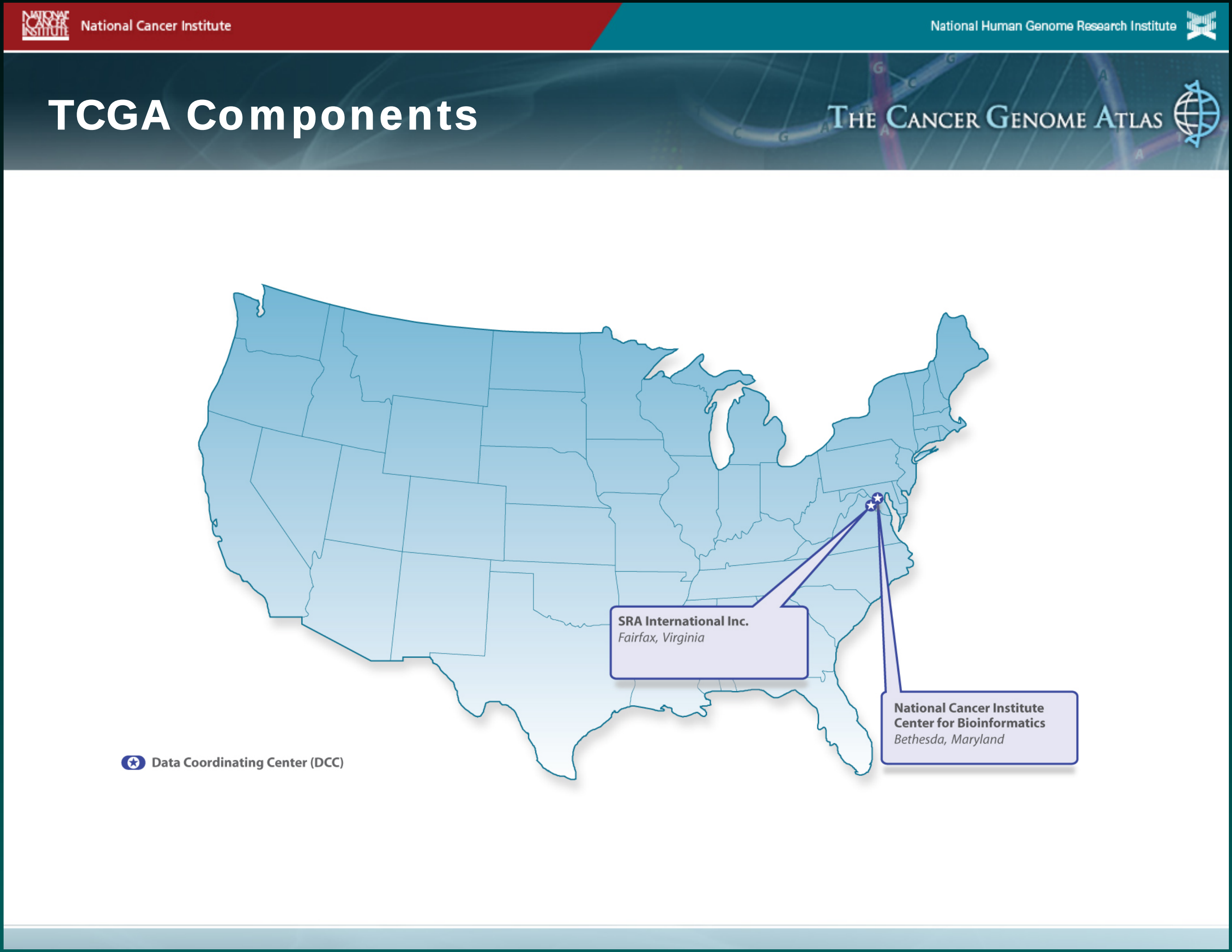
The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins
Baltimore, Maryland

University of North Carolina Lineberger Comprehensive Cancer Center
Chapel Hill, North Carolina

○ Cancer Genome Characterization Centers (CGCCs)

Cancer Genome Characterization Centers

Where They Collect the Data



National Cancer Institute

National Human Genome Research Institute

TCGA Components

THE CANCER GENOME ATLAS

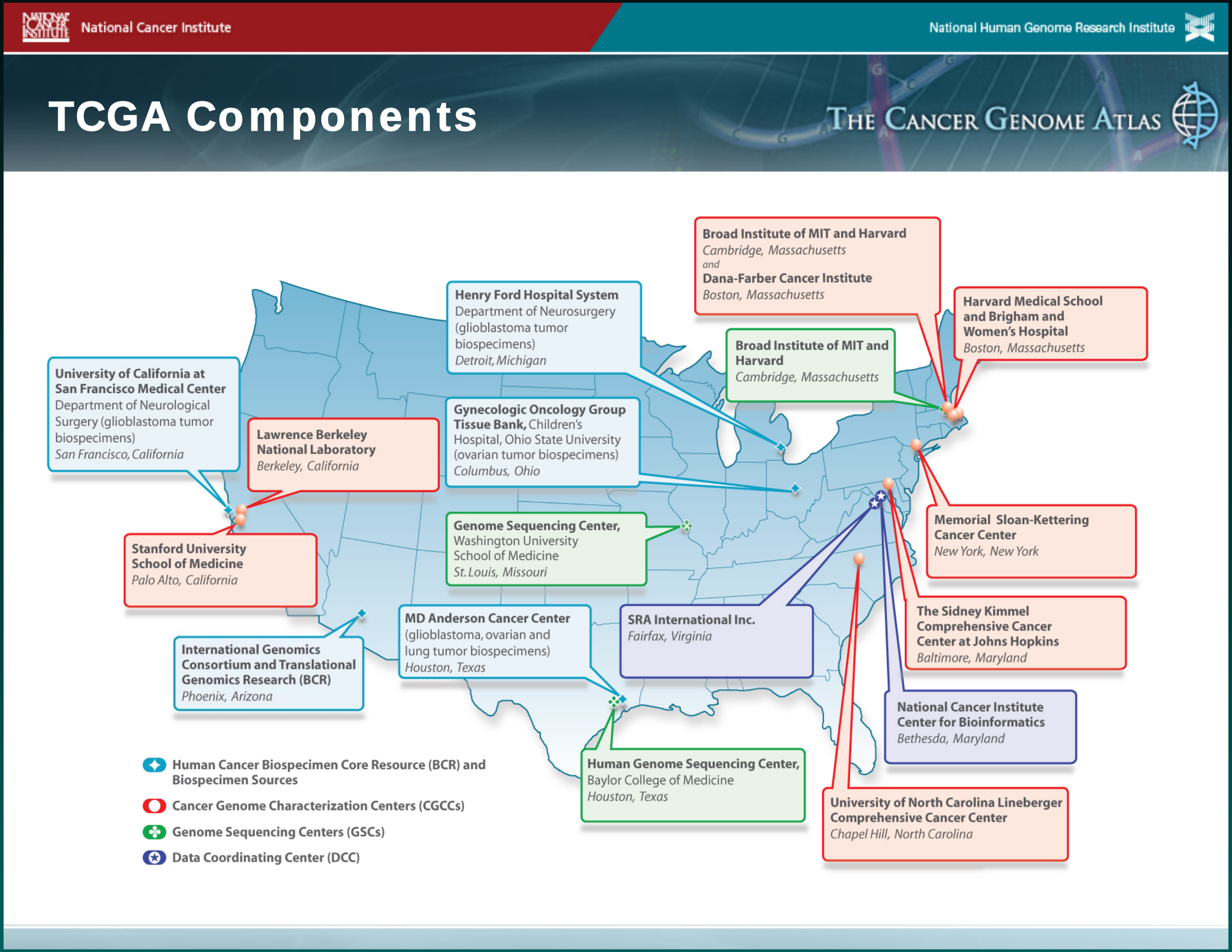
SRA International Inc.
Fairfax, Virginia

National Cancer Institute
Center for Bioinformatics
Bethesda, Maryland

● Data Coordinating Center (DCC)

Data Coordinating Centers

Putting it All Together



National Cancer Institute

National Human Genome Research Institute

TCGA Components

THE CANCER GENOME ATLAS

Broad Institute of MIT and Harvard
Cambridge, Massachusetts
and
Dana-Farber Cancer Institute
Boston, Massachusetts

Henry Ford Hospital System
Department of Neurosurgery (glioblastoma tumor biospecimens)
Detroit, Michigan

Harvard Medical School and Brigham and Women's Hospital
Boston, Massachusetts

Broad Institute of MIT and Harvard
Cambridge, Massachusetts

University of California at San Francisco Medical Center
Department of Neurological Surgery (glioblastoma tumor biospecimens)
San Francisco, California

Lawrence Berkeley National Laboratory
Berkeley, California

Gynecologic Oncology Group Tissue Bank, Children's Hospital, Ohio State University (ovarian tumor biospecimens)
Columbus, Ohio

Memorial Sloan-Kettering Cancer Center
New York, New York

Stanford University School of Medicine
Palo Alto, California

Genome Sequencing Center, Washington University School of Medicine
St. Louis, Missouri

The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins
Baltimore, Maryland

International Genomics Consortium and Translational Genomics Research (BCR)
Phoenix, Arizona

MD Anderson Cancer Center
(glioblastoma, ovarian and lung tumor biospecimens)
Houston, Texas

SRA International Inc.
Fairfax, Virginia

National Cancer Institute Center for Bioinformatics
Bethesda, Maryland

Human Genome Sequencing Center, Baylor College of Medicine
Houston, Texas

University of North Carolina Lineberger Comprehensive Cancer Center
Chapel Hill, North Carolina

- ◆ Human Cancer Biospecimen Core Resource (BCR) and Biospecimen Sources
- ○ Cancer Genome Characterization Centers (CGCCs)
- ✣ Genome Sequencing Centers (GSCs)
- ★ Data Coordinating Center (DCC)

TCGA Map

So, How’s It Going?

Progress 2009

Well, there’s good news and bad news...

Started with GBMs; samples from about 150 patients have been profiled.

They recently (late March) declared a data freeze to allow people to compare results at equal stages.

Concurrently, a “progress meeting” of sorts was held at the NCI. There’s an informative webcast available (`http://cancergenome.nih.gov/media/workshops.asp`).

News From the Front

They'd hoped to be further along.

Sample quality and access to corresponding normal material have been roadblocks.

The standards initially set (e.g., 80% tumor cells, less than 40% necrotic) may be unrealistic, and this may be worse with the other tumor types.

Shove more samples out to assays that may not require matched normal material (e.g., CGH).

More News From the Front

Sequencing and CGH are showing some successes due to sample size.

Gain of chr 7, loss of chr 10, several much more localized alterations.

The main known players (e.g., EGFR) are being found, and a few new ones are showing up as well (NF1, ERBB2). Clustering reveals 4 consistent subtypes.

Limited integration to date (one or two platforms); many studies involve results from other assays.

Progress 2010

GBMs now up to about 380.

Ovarian samples up to about 510.

Lung samples up to about 100, samples for about 10 other tissue types started.

Main GBM paper in Nature, Ovarian paper submitted.

Not too many biological shocks yet.

Much more sequencing data coming.

Where Can We Get the Data?

This has changed quite a bit over time.

`http://tcga-data.nci.nih.gov/datareports`

is good to explore, in particular the latest archive:

`http://tcga-data.nci.nih.gov/datareports/latestArchiveReport.htm`

You can also browse the publicly available data, which contains earlier releases of some of the data.

Controlled Access Data

So, what is “controlled”?

More flattery than is warranted...

Sequence data, SNP data, exon data, clinical data.

Faculty need to sign up to get the data.

We want it, but we need to restrict access to it if we use it here.

Things About the Data

There's a *lot* of it.

Samples were sent out to the characterization centers in batches; roughly 30-45 patients per. The same batch went to each center (mostly).

Data ("raw" and processed) is grouped by Batch into gzipped tarballs, which can be 10s of gigabytes in size. This is why we see only a few files from the archive pulldown.

Descriptions of some of the processing applied can be found at `http://cancergenome.nih.gov/data/types/genomic/description`

Sample mappings are available at `http://tcga-data.nci.nih.gov/tcga/findArchives.htm`

What Does the Data Look Like?

Pretty good, but not perfect.

We did a quick survey of some of the public TCGA data (mostly ovarian) over the past few weeks, identifying some areas where clarification could help.

In brief, these involve code, clinical data, annotation, basics, and bulletins.

What We Did

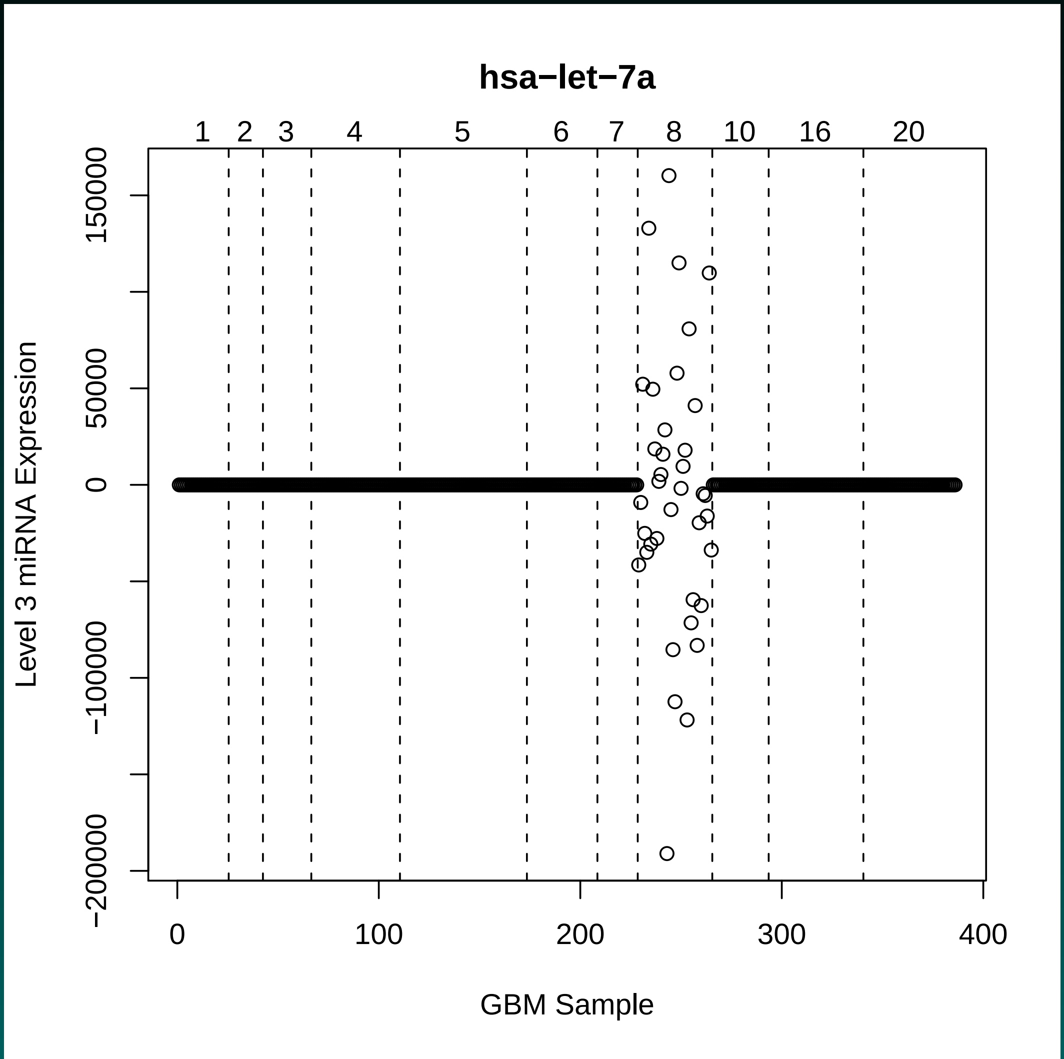
Divvied up data types:

miR (Keith*)
Clinical (Mary Edgerton)
Methylation (Wenyi Wang, Anna Unruh)
Illumina Transcriptome (Peng Qiu)
Affy Expression (Brad Broom)
Agilent Expression (Nianxiang Zhang)

Other (Rehan Akbani, John Weinstein, Chad Creighton, Li Zhang)

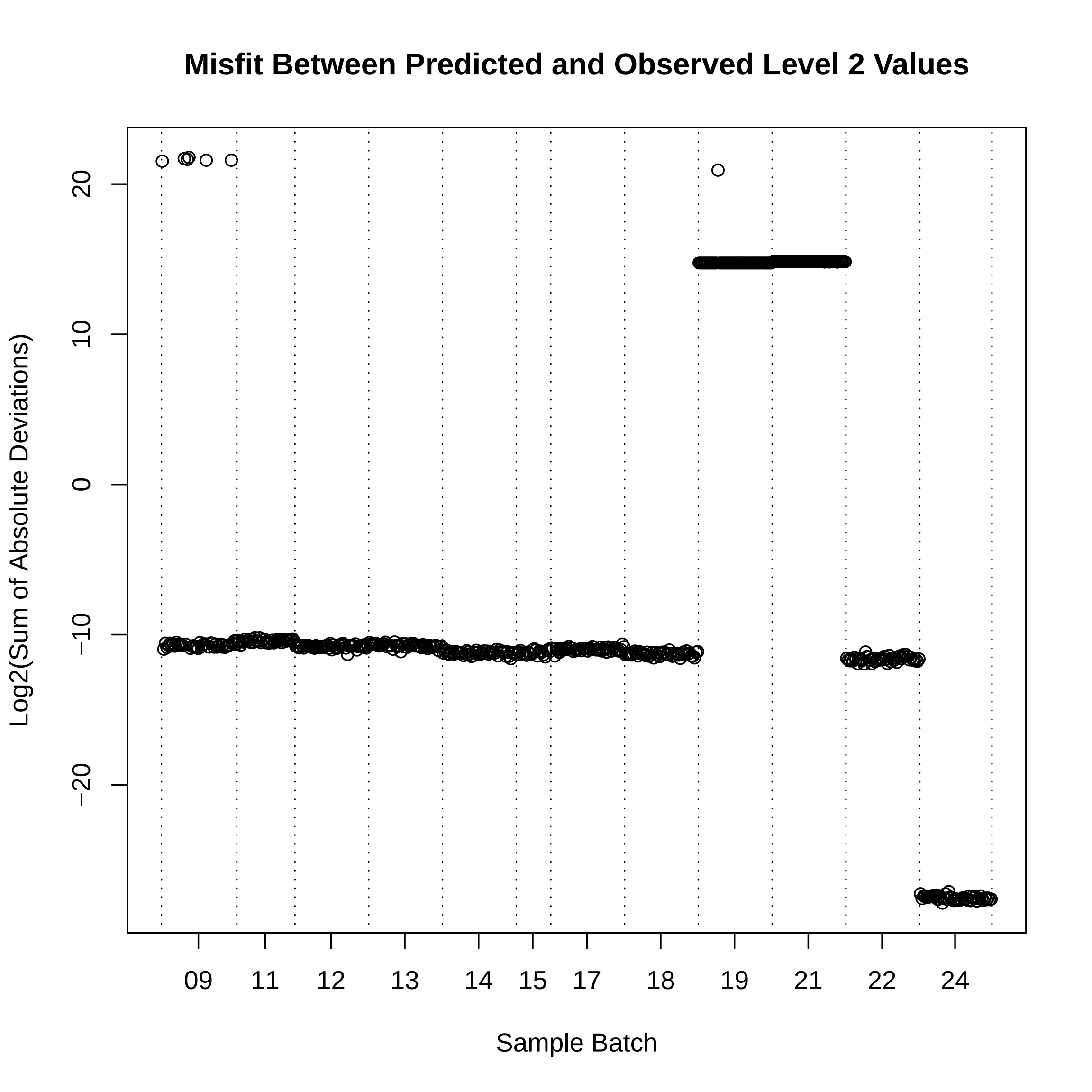
Data acquired through “Browse Public Data” (not Search).

Mimicking Cross-Level Processing is Hard

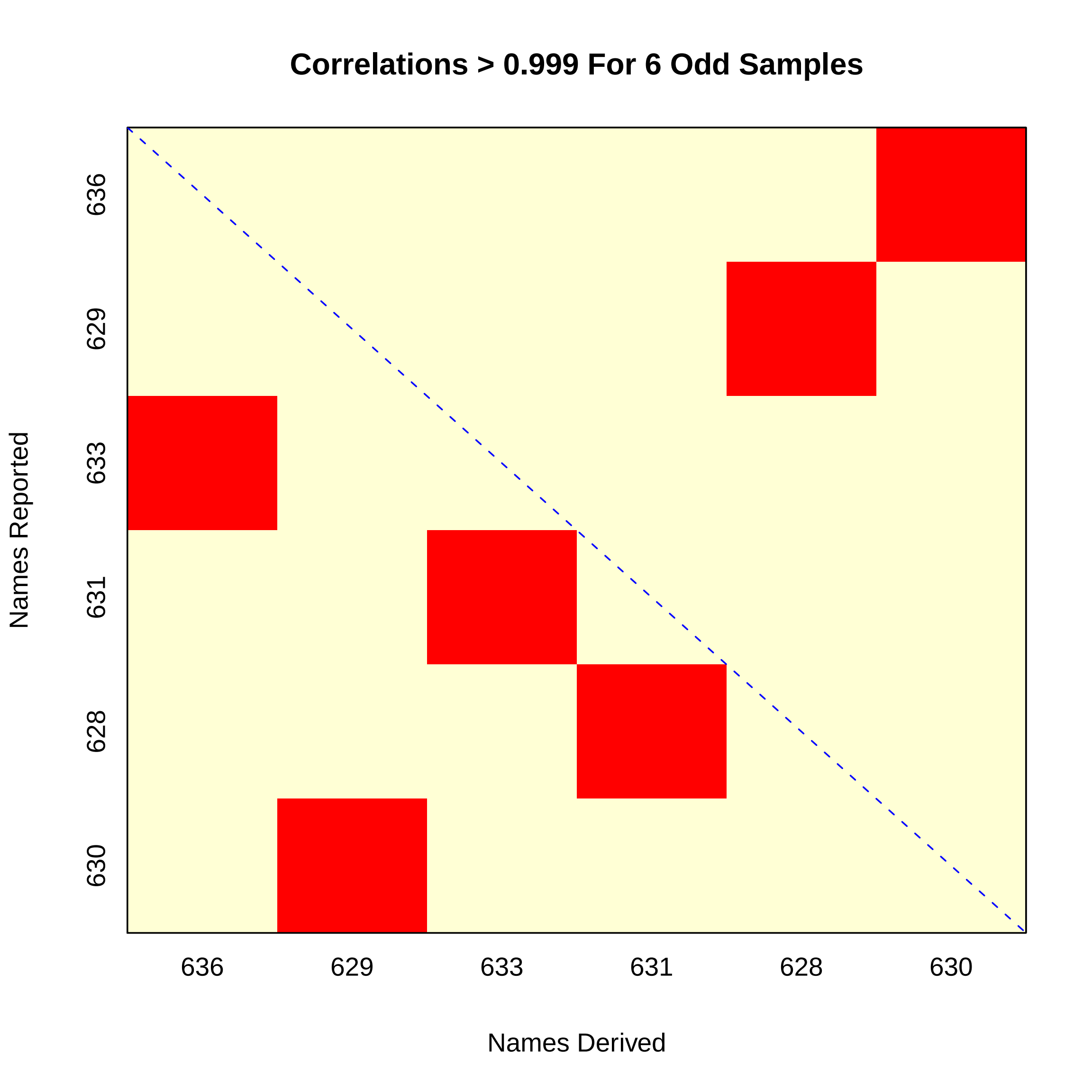


L2 to L3: log2-transform, average, center. *Forgot one*.

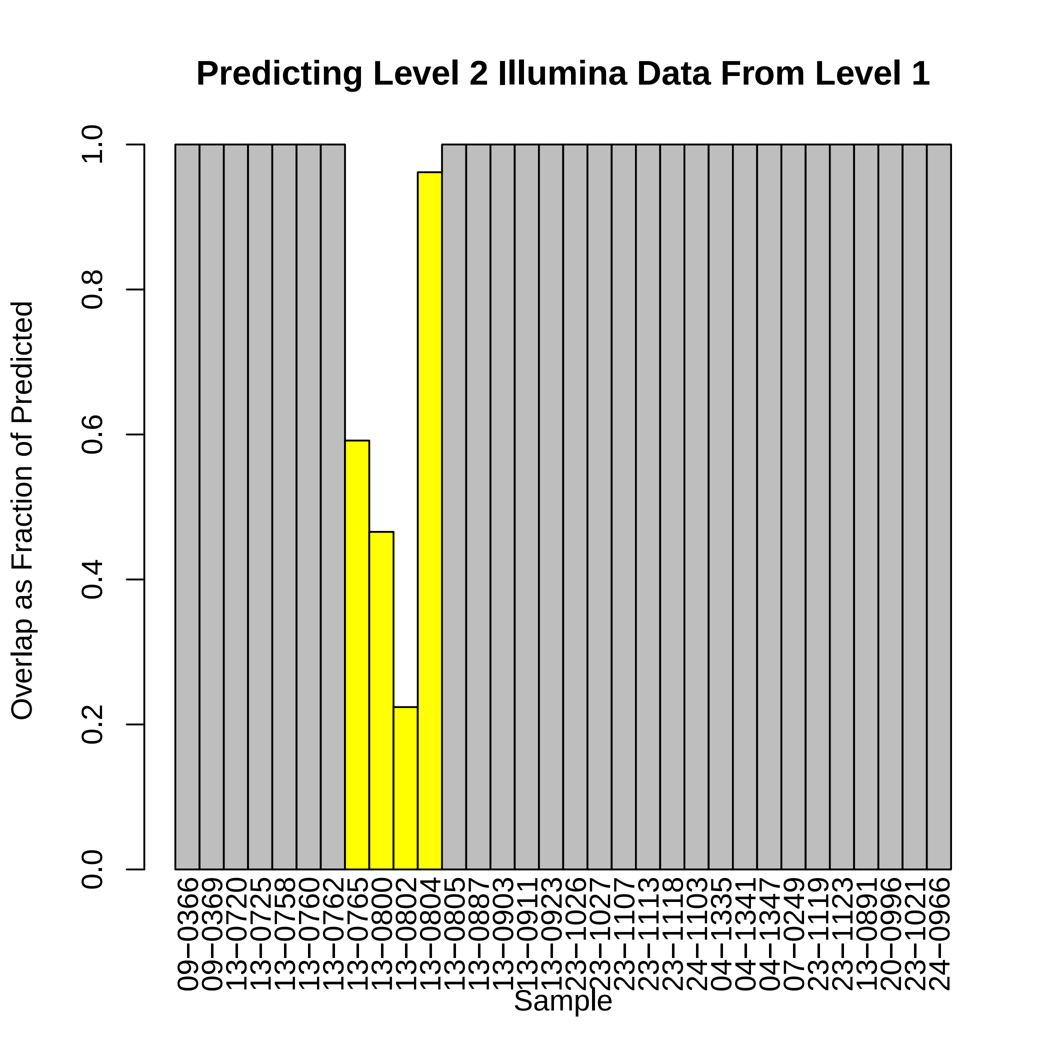
Level 2 Fits: Are We OK?



Checking Some Misfits...

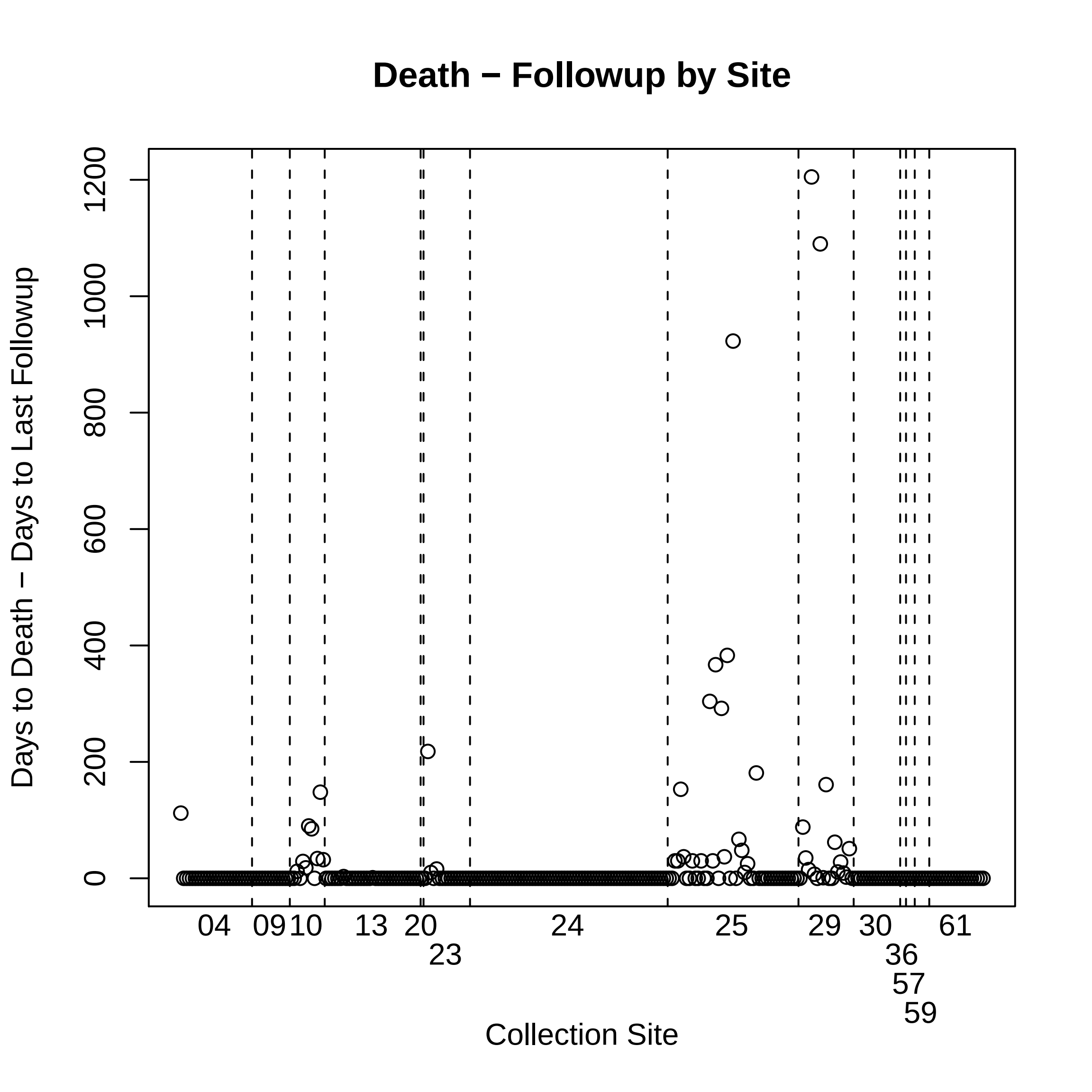


Level 2 Illumina Transcriptome



Note: this uses our reconstruction, not sdrf.

Clinical Definition Varies by Site



Clinical Entries can be Idiosyncratic

One patient with “null” VitalStatus but DaysToDeath

PrimaryTherapyOutcomeSuccess vs TumorResidualDisease

```
         >20mm 11-20mm 1-10mm NoMacDis null
COMP RESP  35      10    136       75   34
PART RESP  17       5     32        2    3
STAB DIS    6       3      8        7    2
PROG DIS   10       5     16        4    2
null       21       6     41       15   31
```

PFS vs RFS?

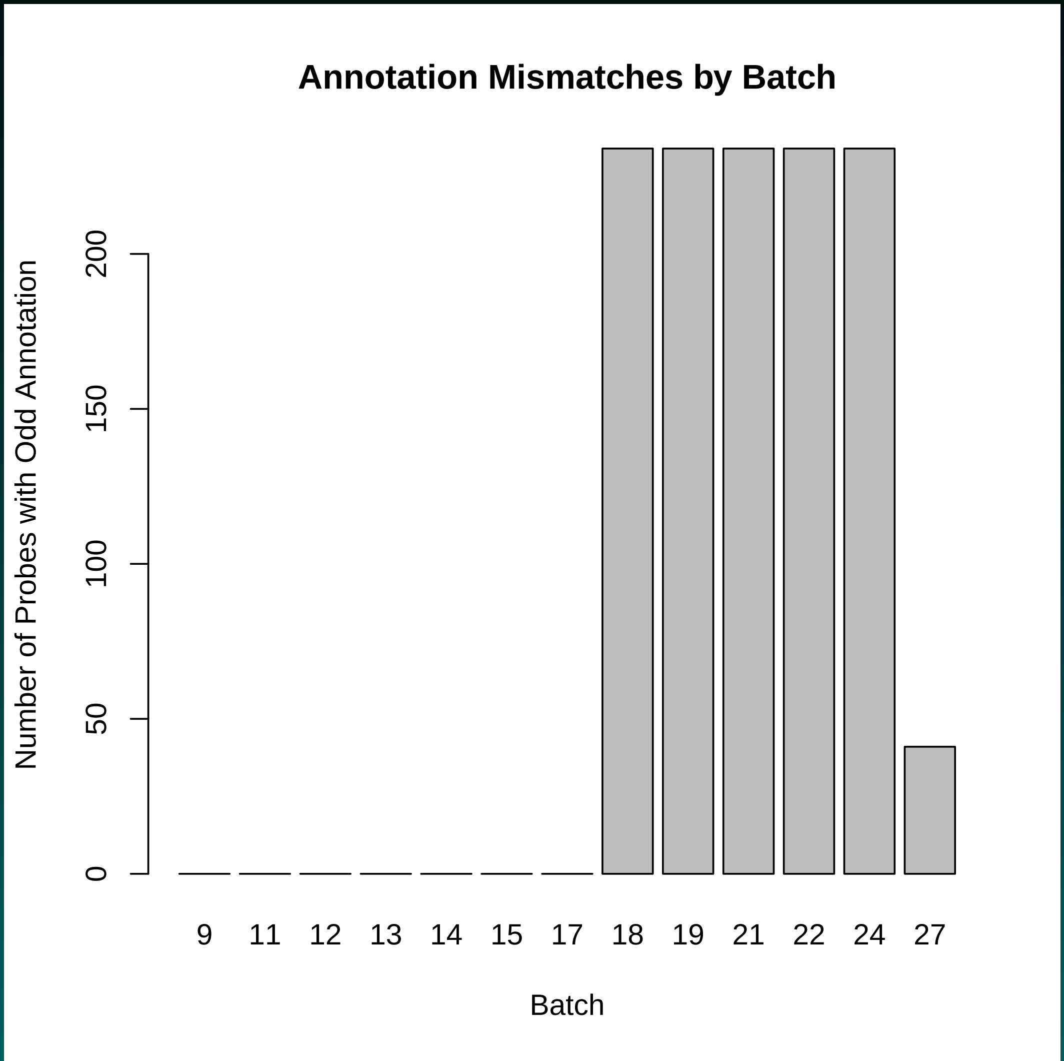
Do we have CA125?

Drug Names and Classes Vary

```
Pacilatxel                  1
Paciltaxal                  1
Paciltaxel                 50
Paciltaxle                  2
Pacitaxel                   1
Pacliatxel                  1
paclitaxel                  4
Paclitaxel                193
Paclitaxel; Albumin-Bount   1
Paclitaxil                  1
taxol                       5
Taxol                     346
```

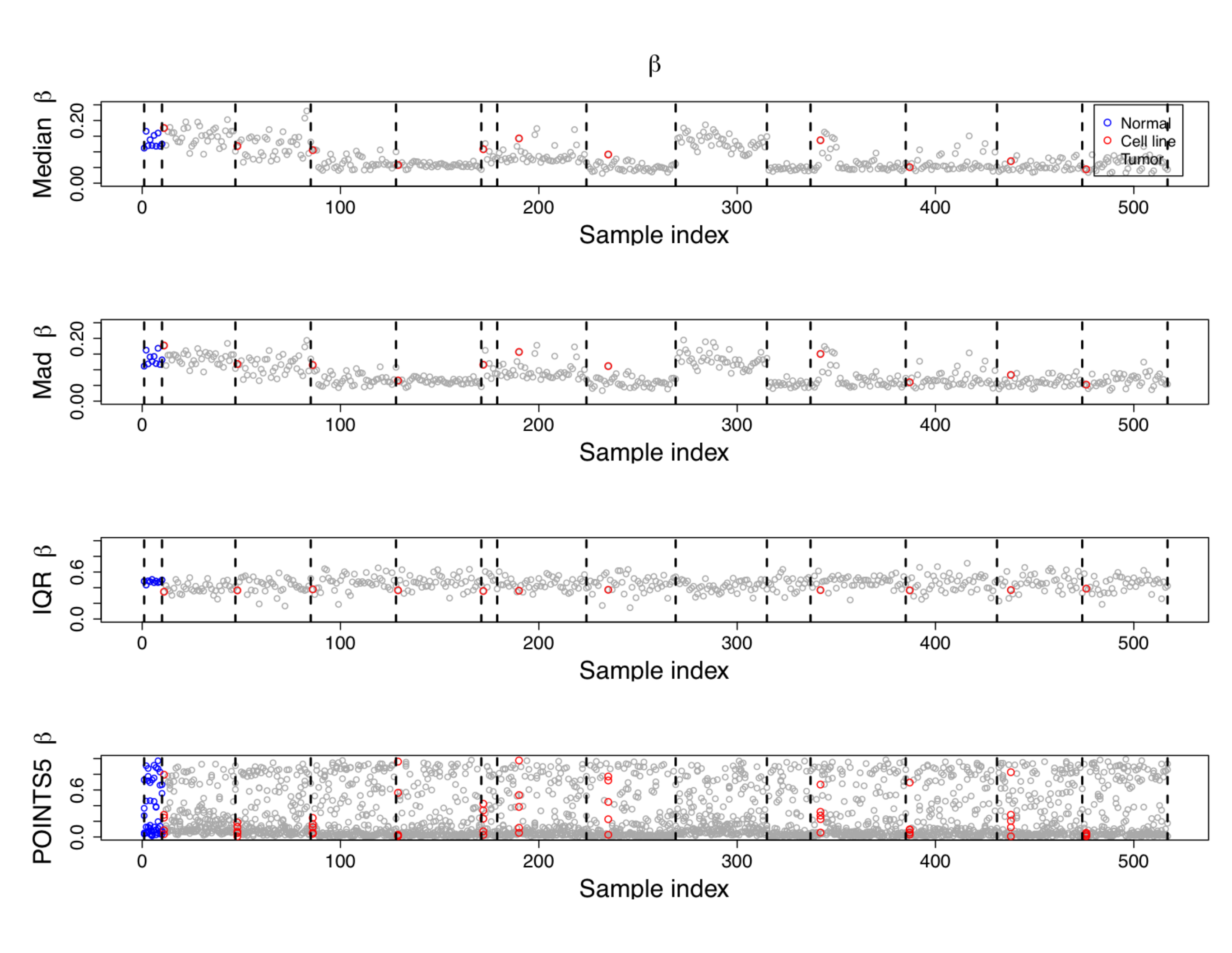
Targeted/Hormonal

What Are Genes Called?



Two annotation files, and Excel.

Interesting Things Within Batch



Can we get run date? Plate? Batch number?

Summary Recommendations

1. Post code for cross-level transitions.
2. Audit the clinical data.
3. Post explicit definitions for column headers.
4. Use common annotation within & across platforms.
5. Include run date/batch number/plate in L1 or SDRF.

Summary Recommendations

1. Post code for cross-level transitions.
2. Audit the clinical data.
3. Post explicit definitions for column headers.
4. Use common annotation within & across platforms.
5. Include run date/batch number/plate in L1 or SDRF.

Ask questions...