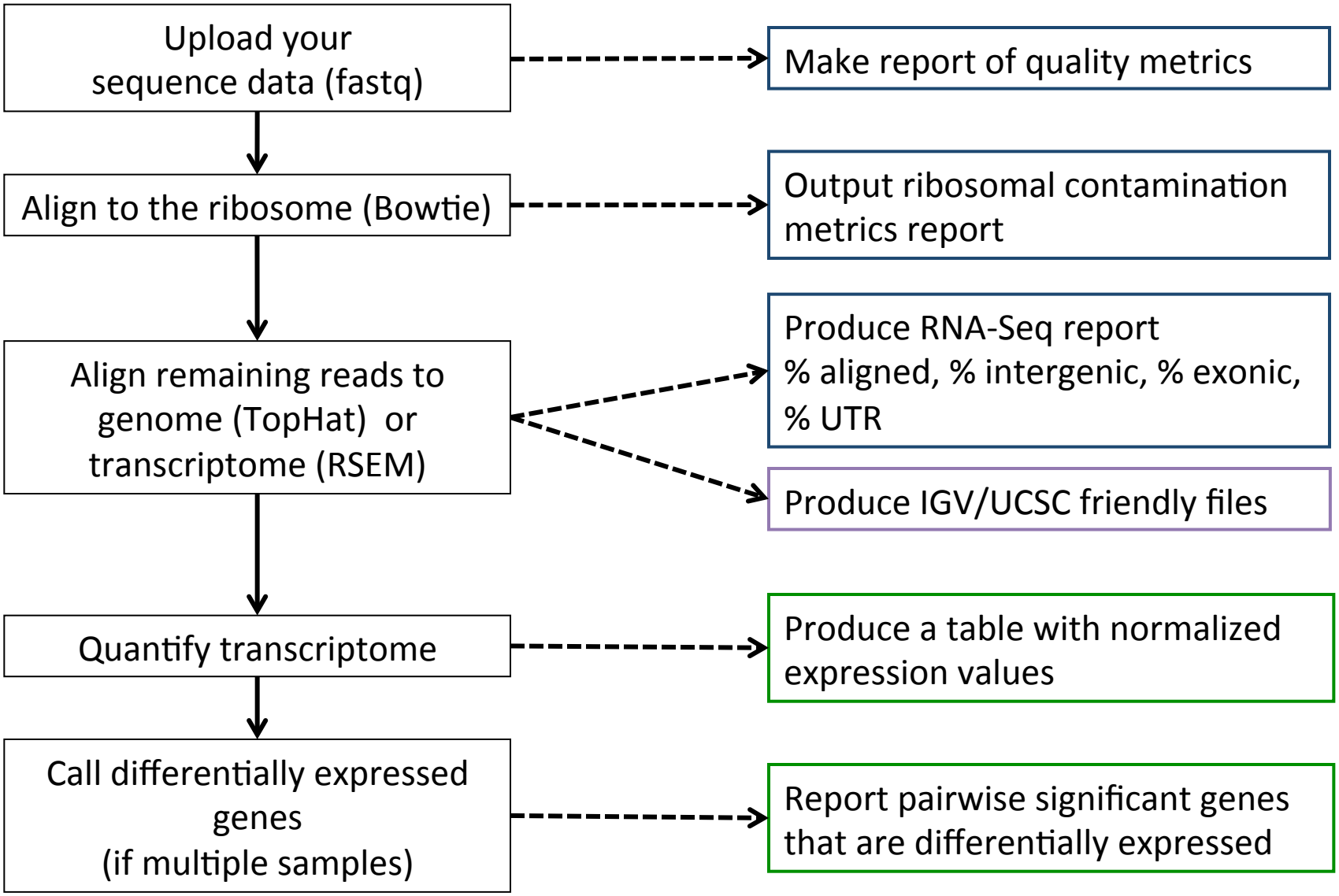
University of Massachusetts Medical School

UMASS

RNA-Seq primer

Our typical RNA quantification pipeline



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Initial analysis

Summary I – Data types, file formats and utilities

- Annotation: Genomic regions
  - Genes
  - Peaks
  - *Bedtools to manipulate them*
- Alignment: Map reads
  - BAM/SAM
  - *Samtools to manipulate them*
- Aggregation: Summary files
  - Wig (UCSC)
  - TDF (IGV)

Summary II – Data process

- Short read alignment (Bowtie, BWA)
  - Making the genome searchable: Hashing/BW
  - Seed an extend (hashing) vs suffix searches (BW)
  - New aligners are mix
- Spliced aligners (TopHat, STAR, GSNAP)
  - Map read fragments then strung them
  - Choosing the fragment size
  - Avoiding biases using information (junctions)
- Quantifying (RSEM/Cufflinks)
  - Read/Isoform assignment
  - Normalization procedures
- Differential expression (DESeq/EdgeR/Cufflinks)

Visualization tricks & Tips

- Viewing normalized data
- Downsampling reads to avoid crashes
- Gene lists
- Sessions

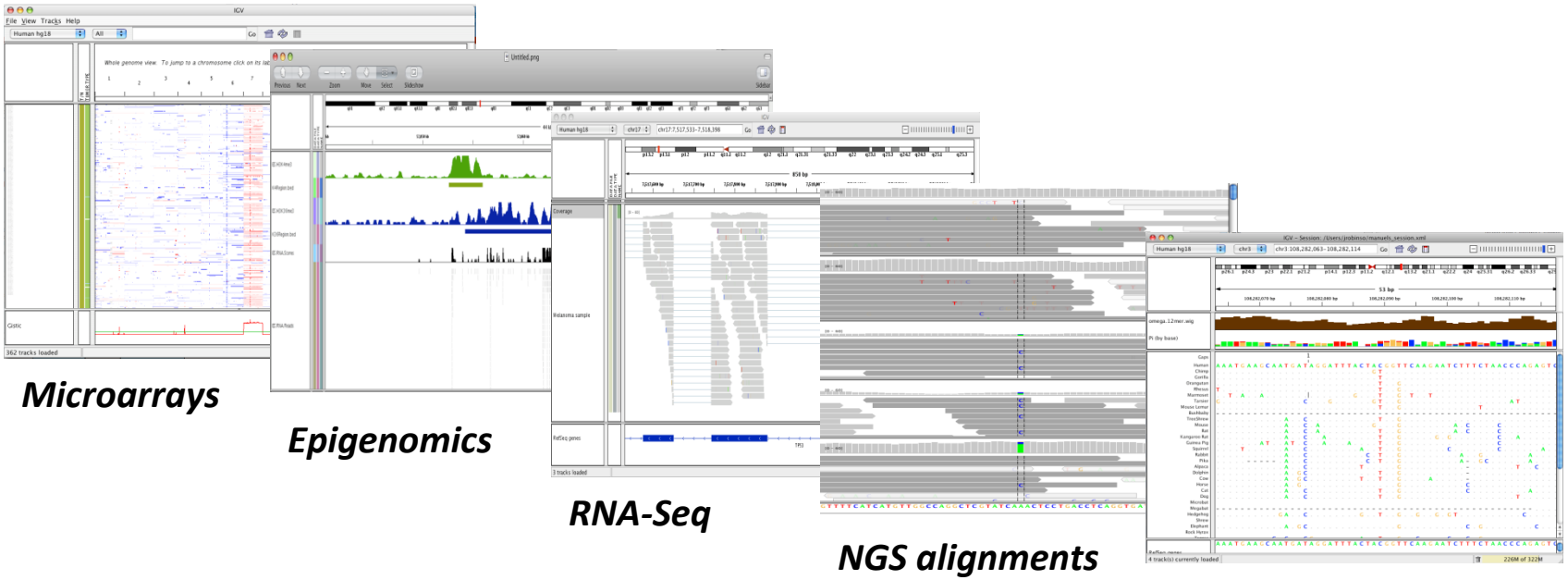
IGV: Integrative Genomics Viewer

Integrative Genomics Viewer

A desktop application

for the visualization and interactive exploration

of genomic data



Microarrays

Epigenomics

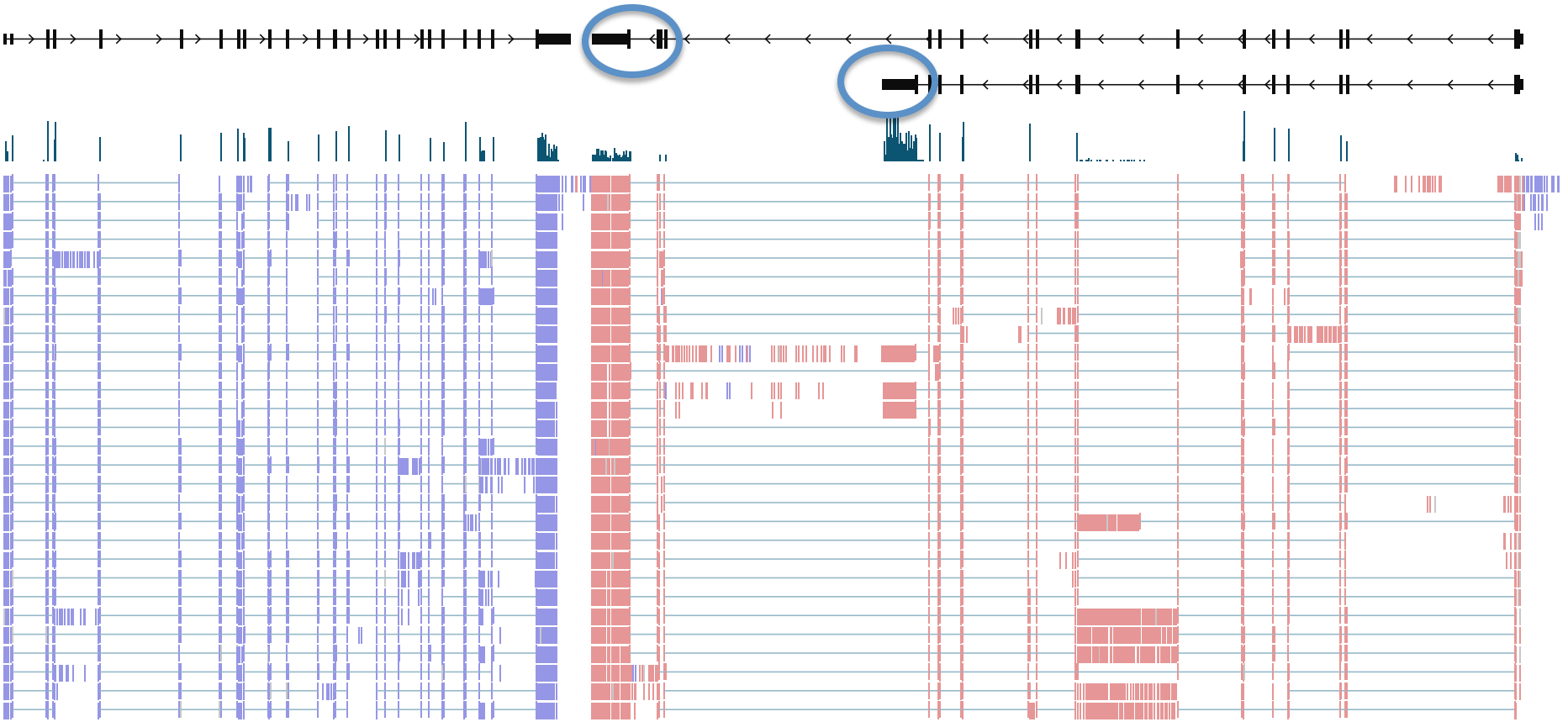
RNA-Seq

NGS alignments

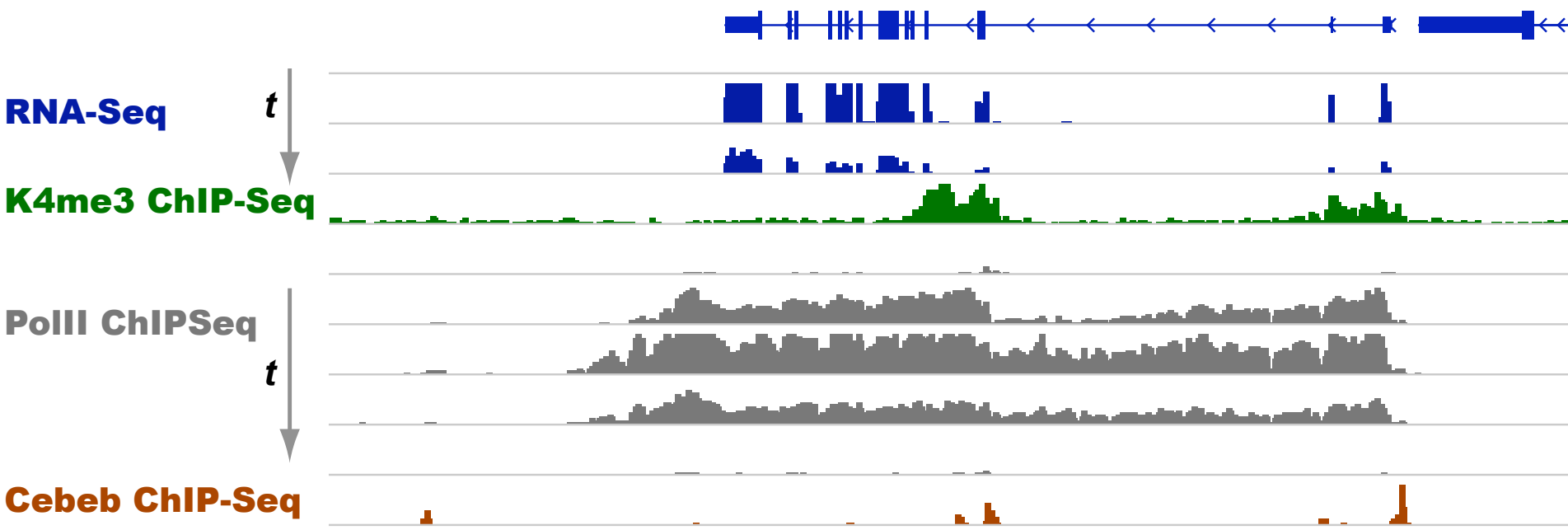
Comparative genomics

BROAD INSTITUTE

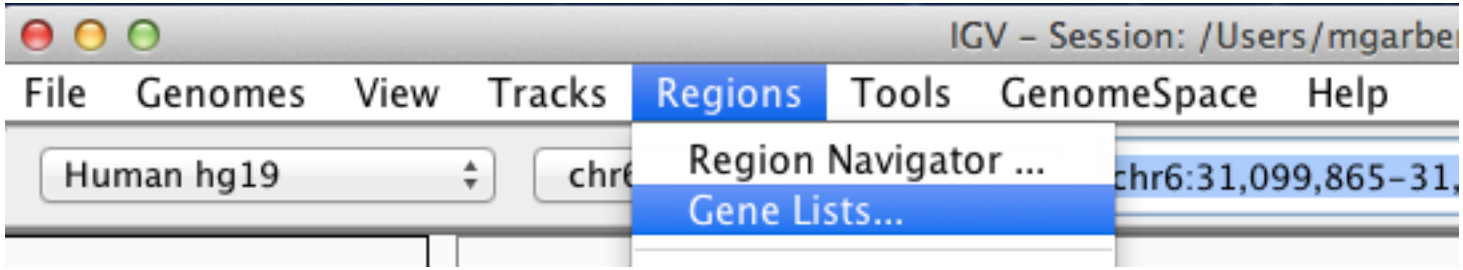
Visualizing read alignments with IGV — RNASeq



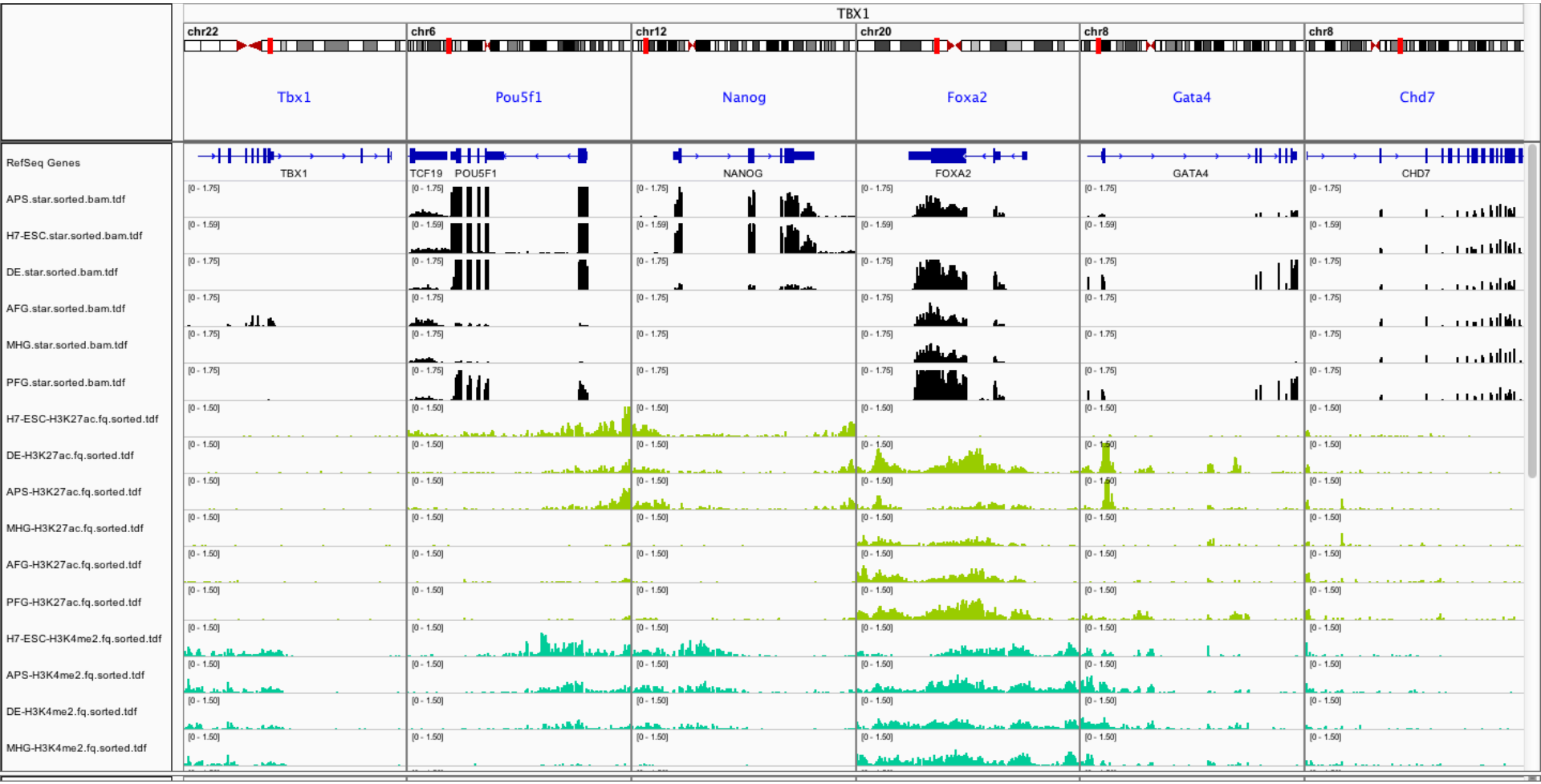
Visualizing read alignments with IGV — zooming out



Viewing several loci simulateneouly: Gene lists



Viewing several loci simultaneously: Gene lists



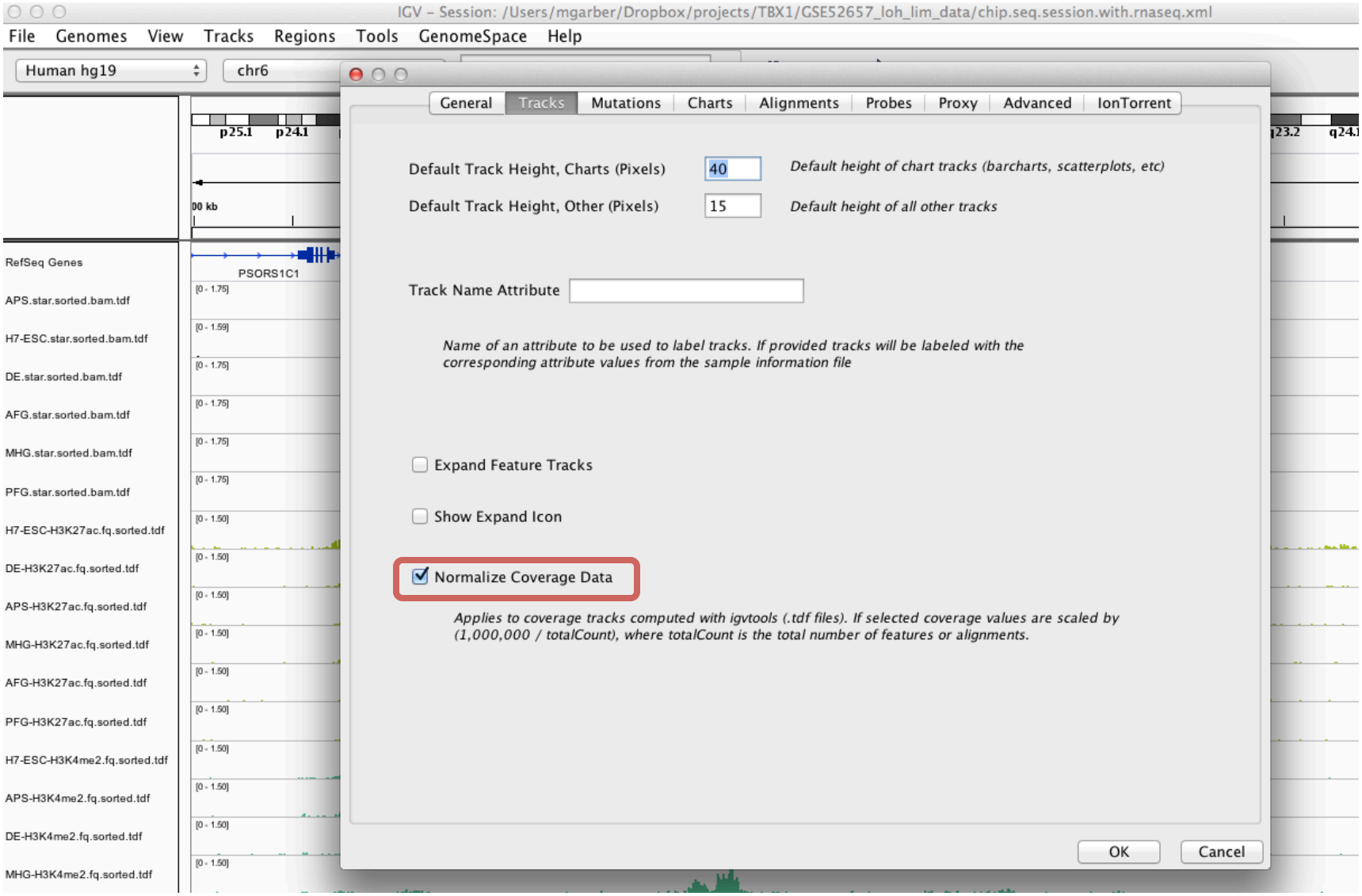
Lets create a list using our small dataset

- Use the following genes
  - Fgf21
  - Bcat2
  - Rasip1
  - Naa60

Which are all within the dataset we selected.

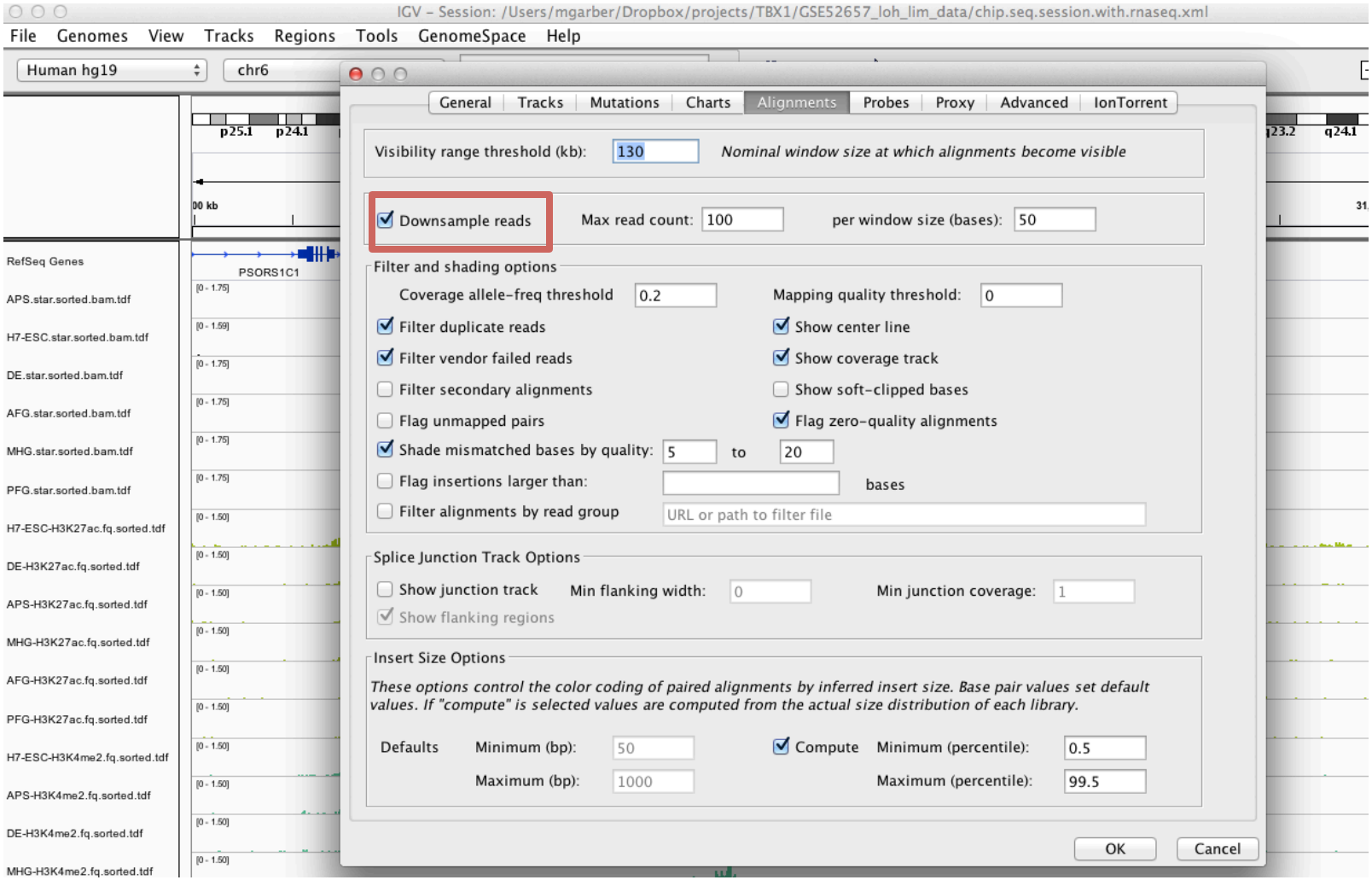
Normalizing tracks

You can use simple read depth normalization for comparison of different tracks



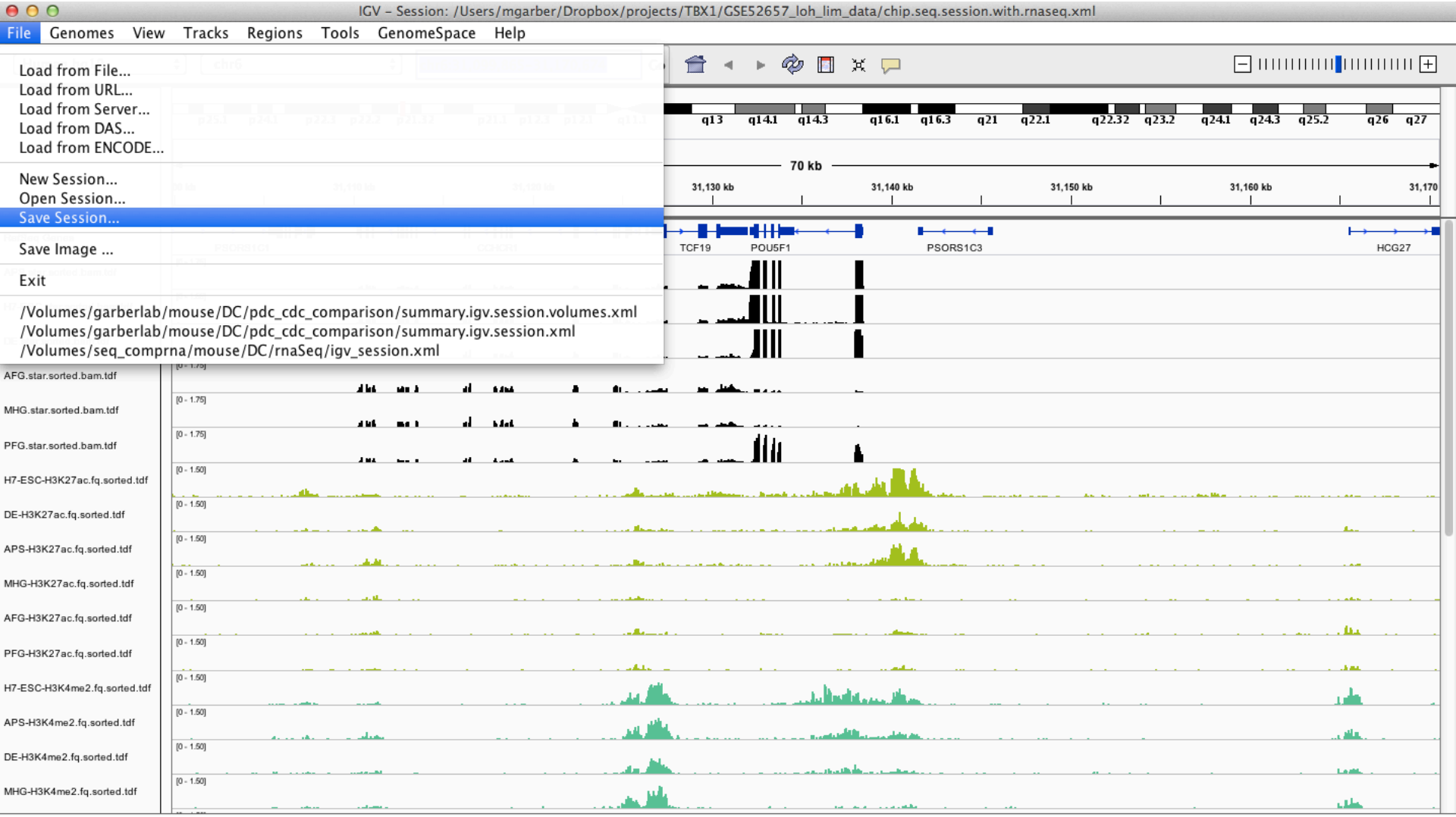
Configure your alignment display

Downsampling reads is critical when loading the full alignments.
When you are loading reads, downsampling ensures that regions with high coverage result in IGV running out of memory.



Saving sessions

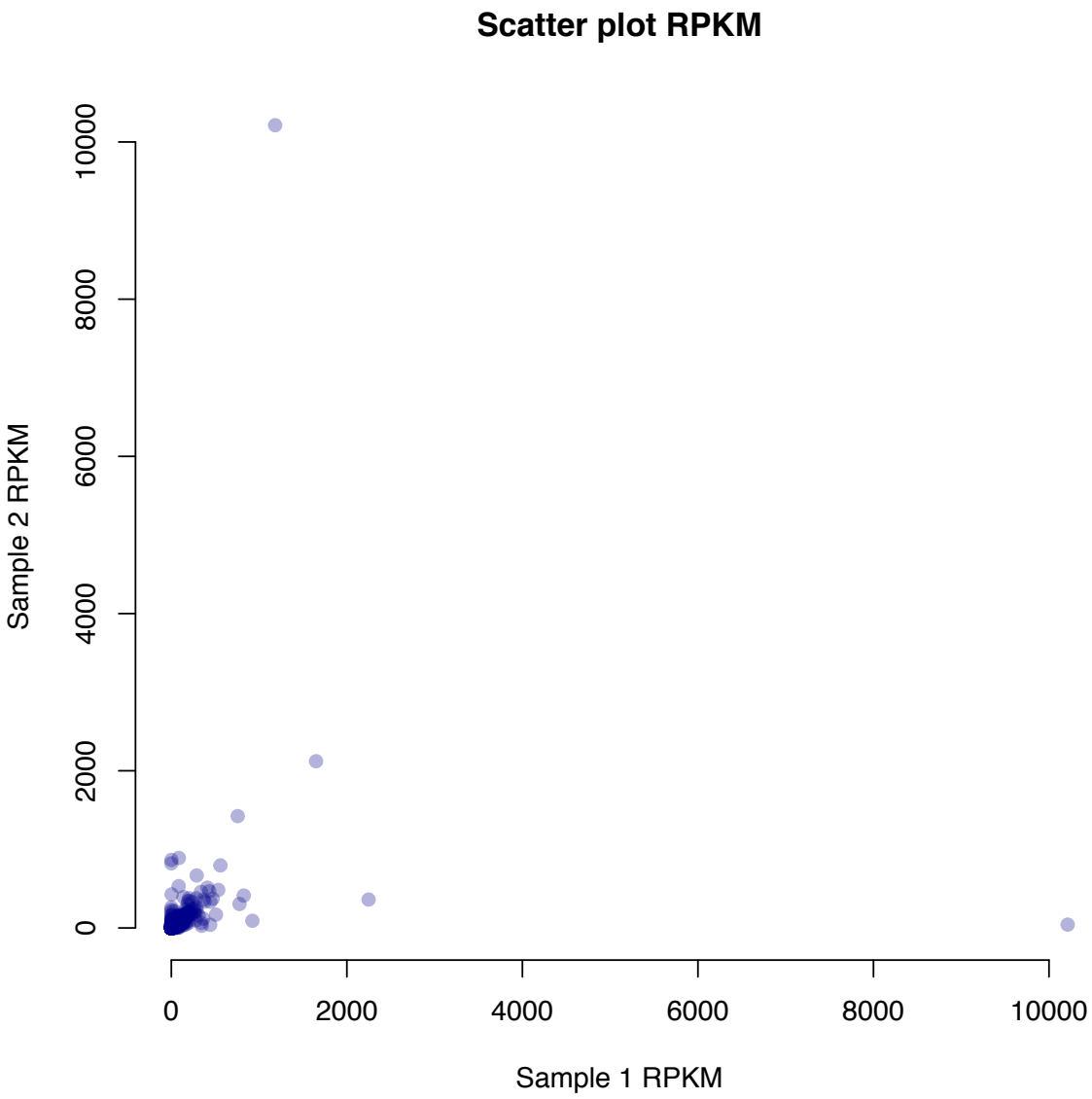
Sessions allows you to store a set of desired tracks along with any setting you want



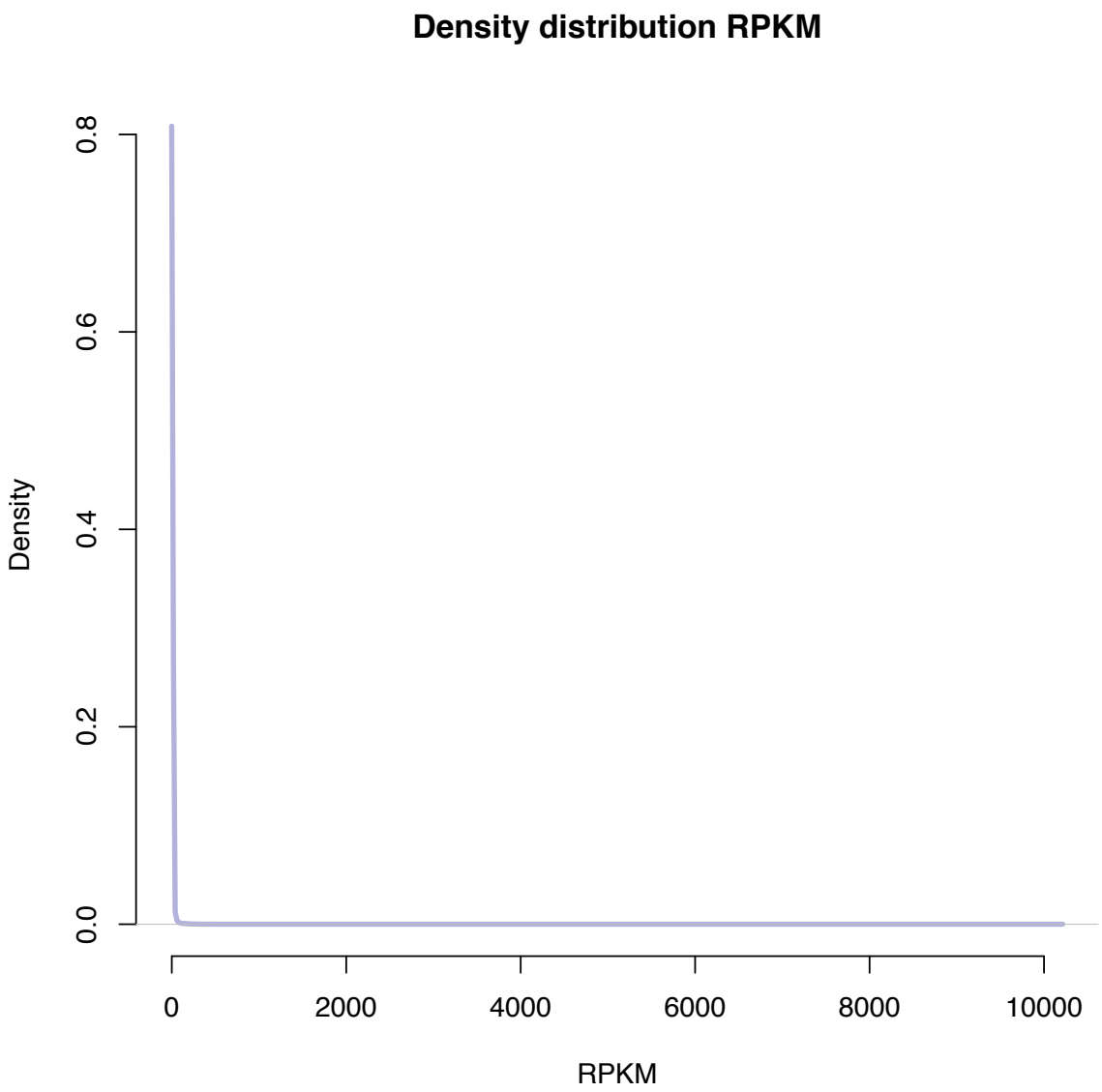
Todays topics

- Looking at ALL of your data

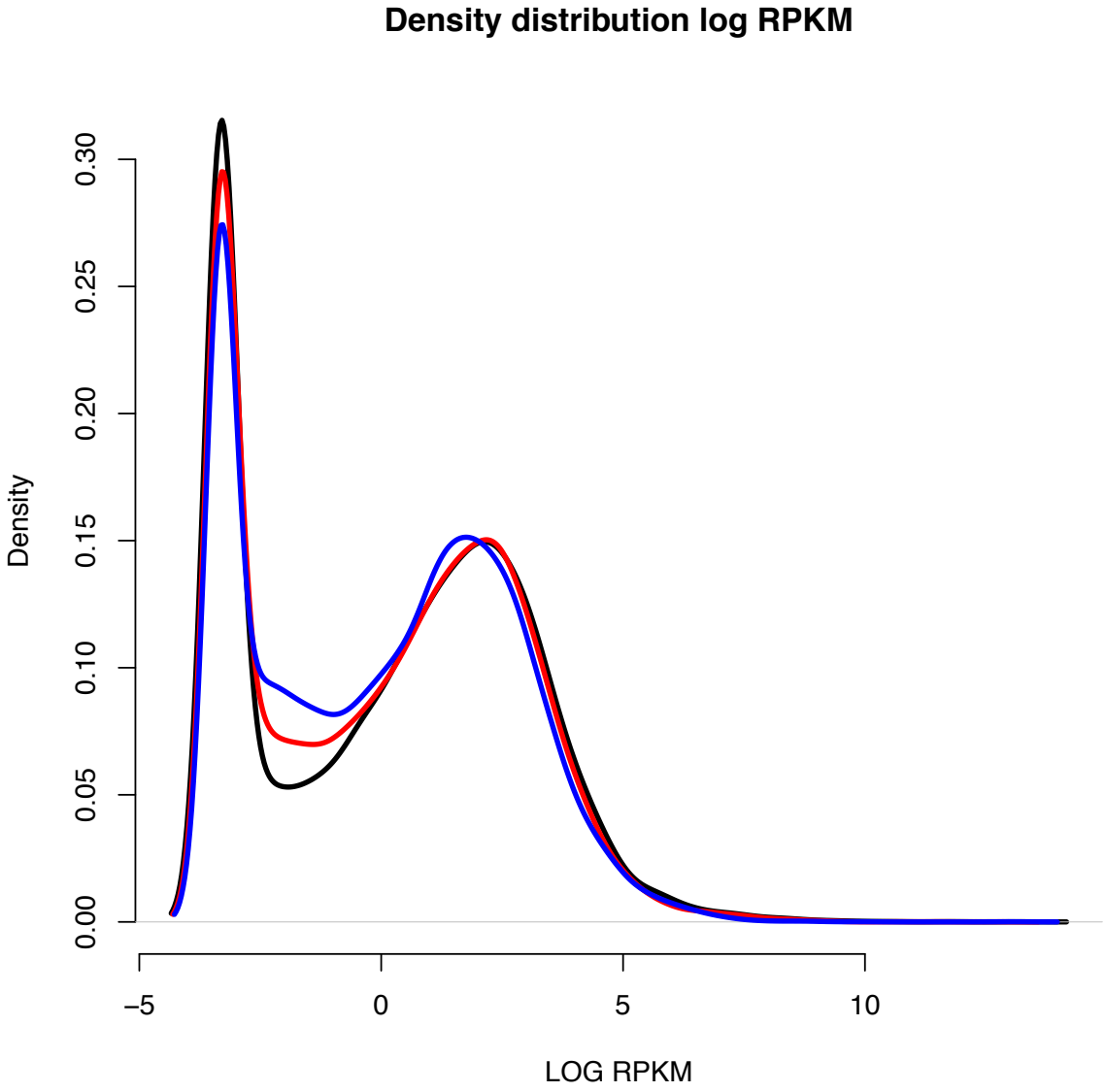
Comparing samples: Scatter plots



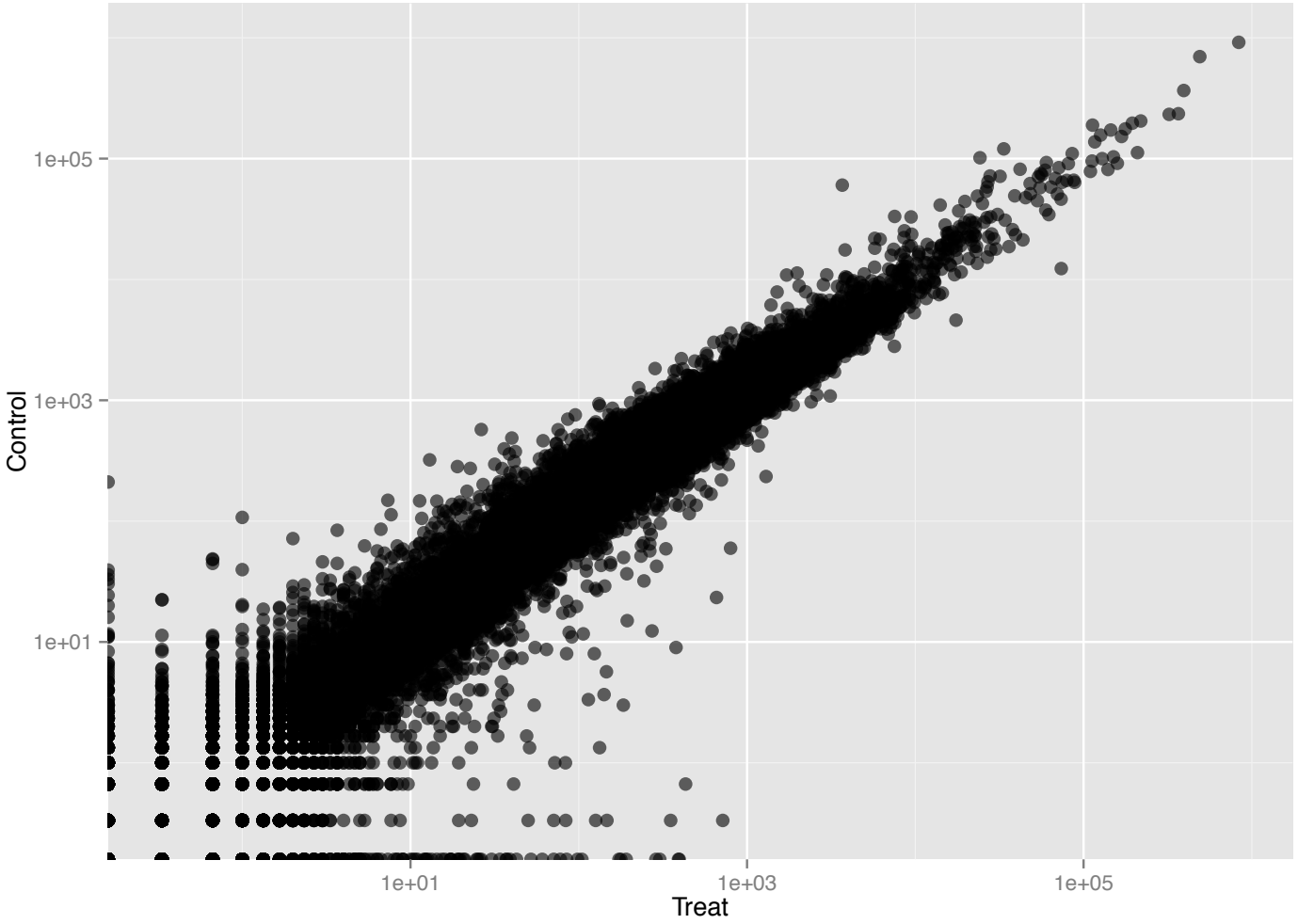
Raw counts/RPKMs are NOT Gaussian



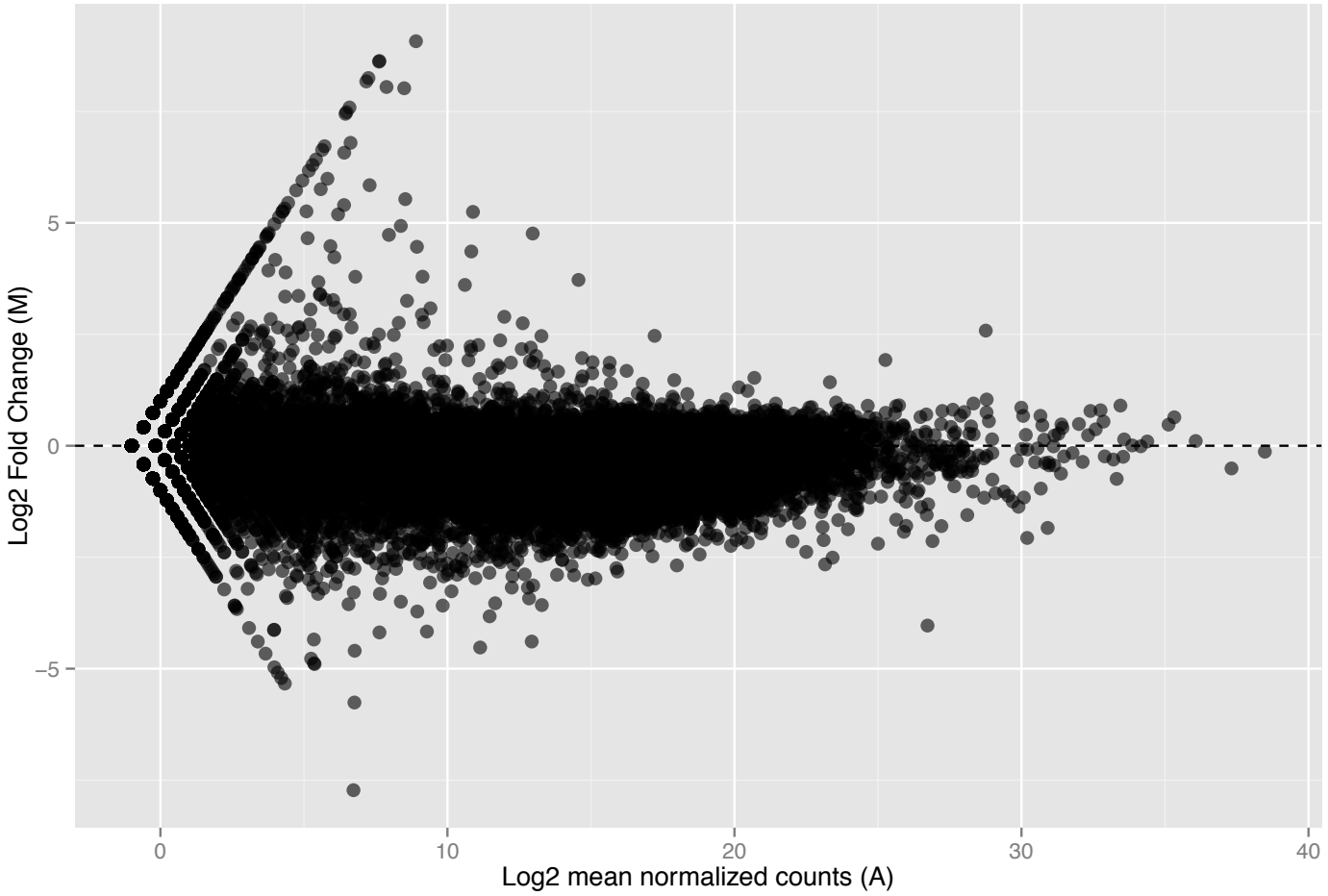
... they are more like Log-Gaussian



And log counts/RPKM can be scatter-plotted



Which can also be looked at as an “MA-Plot”



Hierarchical clustering – vector similarity?

Gene	Cond1	Cond2	Cond3	Cond4
g_1	2.5	5	7.5	10
g_2	0.2	0.5	0.8	1.1
g_3	0.2	0.3	0.4	11
g_4	2.5	8	8	9

Clustering is about similarity:

- Between two rows (specified by a distance function)
- Between two sets of rows (specified by the linkage method)

Common similarity approaches

- Distance between rows (or columns)
  - Correlation: `d(r,s) = (1- cor(r,s))/2`
  - Euclidean: $d(r,s) = \sqrt{\Sigma_i (r_i - s_i)^2}$
- Linkage: Distance betwee two sets (`d(R,S)`)
  - Complete: $max\{d(r,s), s \in S, r \in R\}$
  - Average: $mean\{d(r,s), s \in S, r \in R\}$
  - Single: $min\{d(r,s), s \in S, r \in R\}$

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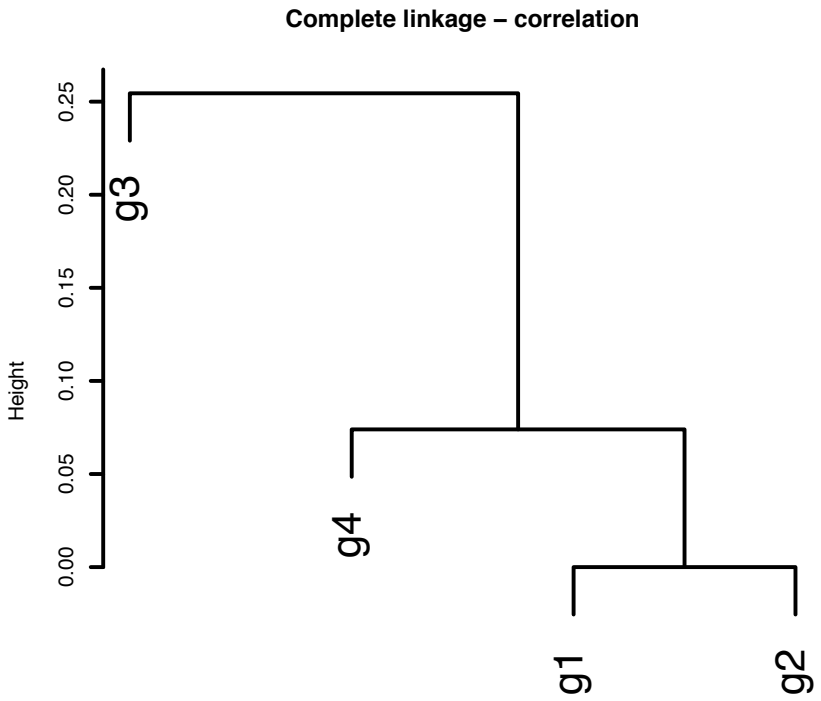
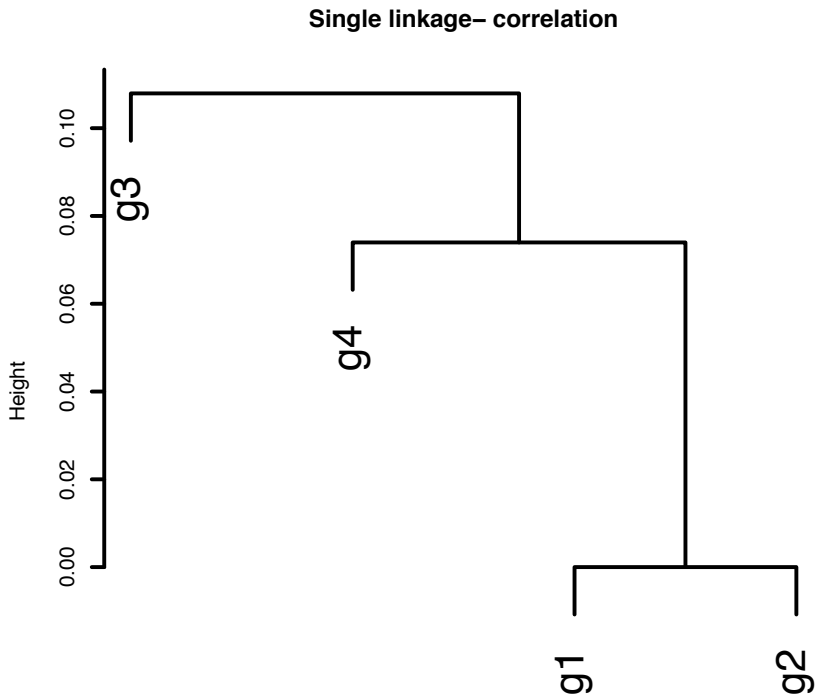
The effect of the linkage method





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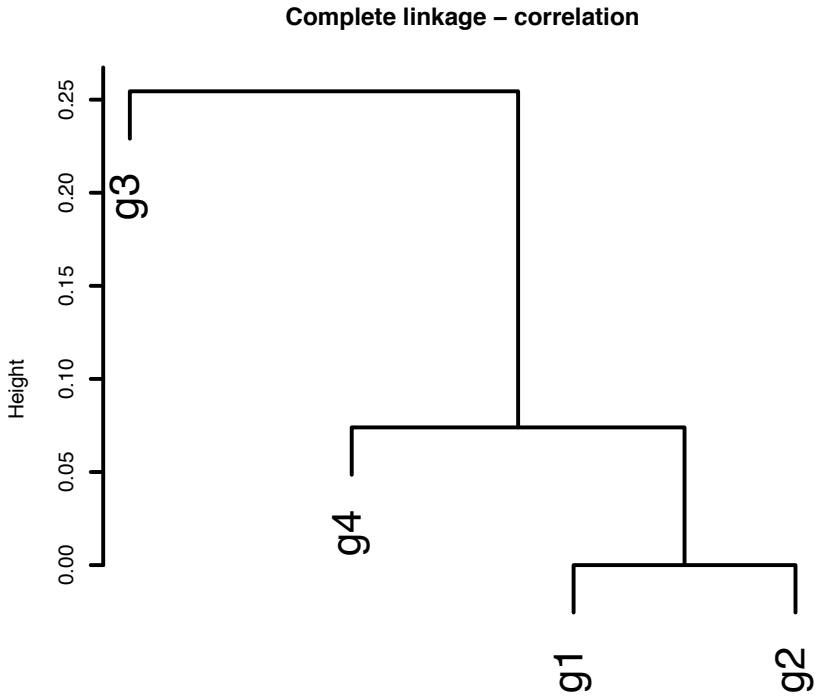
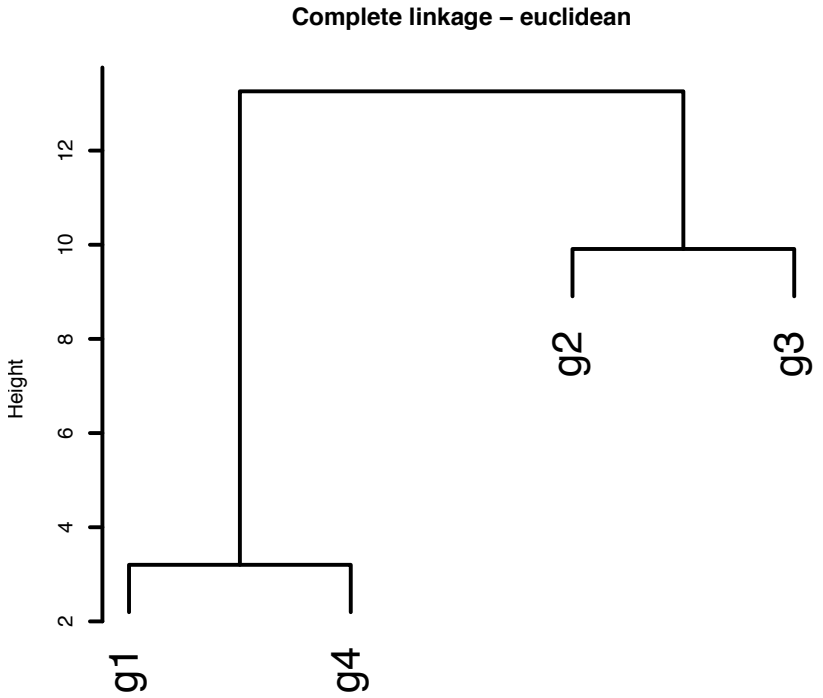
Effect of the distance!





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Playing with clustering

```
#Define the toy matrix#
#######################
m =  rbind (c(2.5,5,7.5,10), c(0.2,0.5,0.8,1.1), c(0.2,0.3,0.4,11), c(2.5,8,8,9))

#Give column and row names#
###########################
rownames(m) = c("g1","g2","g3","g4");
colnames(m) = c("c1","c2","c3","c4");


#Compute the correlation distance matrix#
########################################
submat.dist = as.dist( (1 - cor(t(m)) )  /2 );


#Plot clustering with the three main methods#
#############################################
plot( hclust(submat.dist, method="complete",members=NULL), main="Complete linkeage -
correlation", sub="", xlab="", lwd=3);
plot( hclust(submat.dist, method="average",members=NULL),  main = "Average Linkeage -
correlation", sub="", xlab="", lwd=3);
plot( hclust(submat.dist, method="single",members=NULL),  main = "Single Linkeage- correlation",
sub="", xlab="", lwd=3);

#Plot clustering with the three main methods, using the euclidean distance#
############################################################################
plot( hclust(dist(m), method="complete",members=NULL), main="Complete linkeage - euclidean",
sub="", xlab="", lwd=3);
plot( hclust(dist(m), method="average",members=NULL),  main = "Average Linkeage -  euclidean",
sub="", xlab="", lwd=3);
plot( hclust(dist(m), method="single",members=NULL),  main = "Single Linkeage - euclidean",
sub="", xlab="", lwd=3);
```