STAT540
Lecture 20: March 21th 2016

Resampling: the bootstrap & permutation testing

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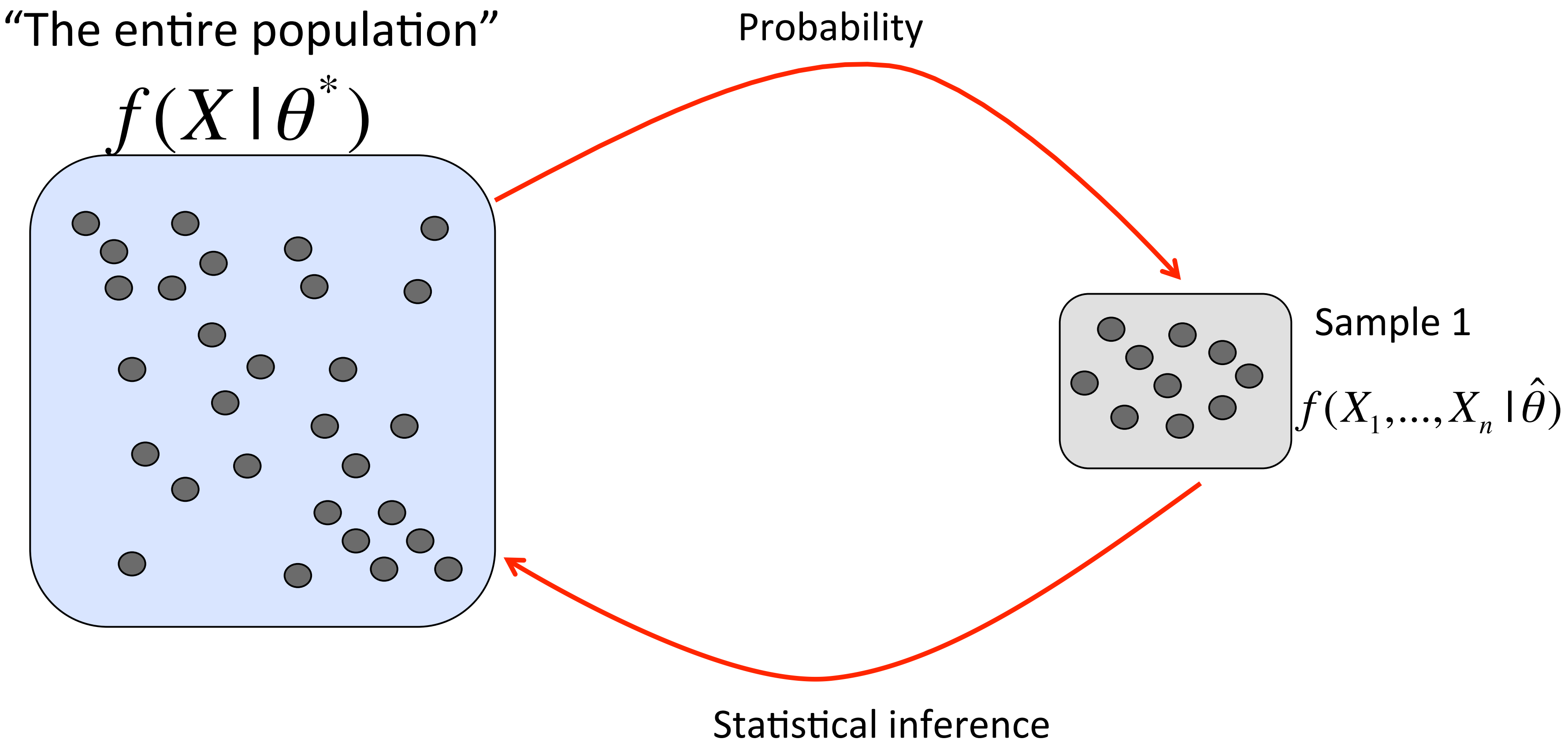
(Based on lecture slides by Jenny Bryan)

Central Dogma of Statistics



Statistical Inference

- We are given a sample (i.e., some data) $X_1,\ldots,X_n$ that are independent draws from an underlying data generating function $f(X \mid \theta^*)$

- We want to known something about *f*, for example we want to know θ^*

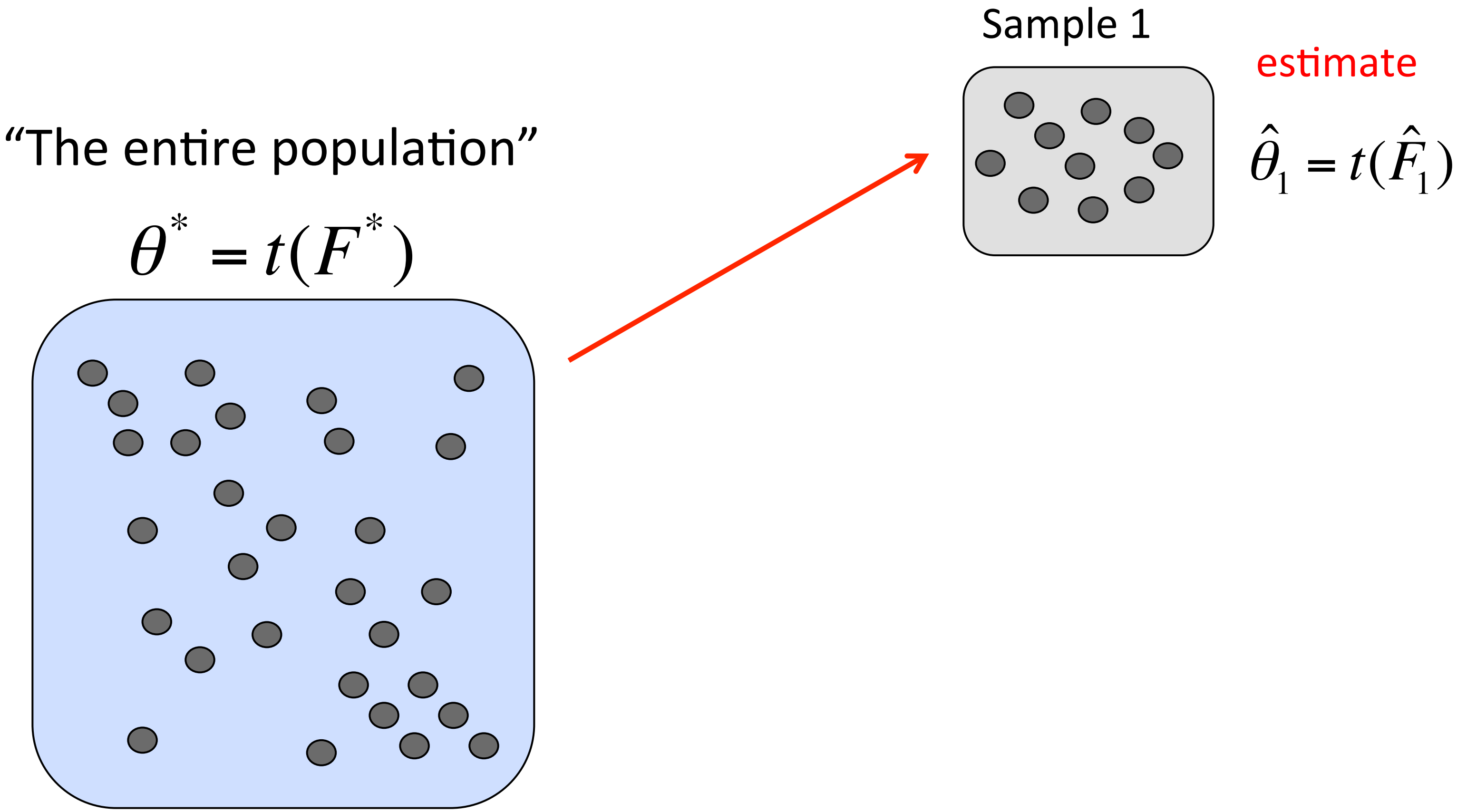
- An estimate $\hat{\theta}$ is just some function of $X_1,\ldots,X_n$, for example you can think of it as $\hat{\theta} = \hat{\theta}(X_1,\ldots.,X_n)$

- If we could repeat our “experiment”, we could get sampling distribution for $\hat{\theta}$

Resampling methods

- Ways of performing statistical inference, and quantify uncertainty in our estimates, that are "internal to the data" under analysis: e.g., you get the necessary knowledge about sampling variability (of parameters/estimates) from the observed data itself.

- Resampling methods:
  - Bootstrap: confidence intervals; standard errors; null distribution/ hypothesis testing
  - Permutation testing: the null distribution/hypothesis testing
  - Cross-validation: generalization error; setting parameters



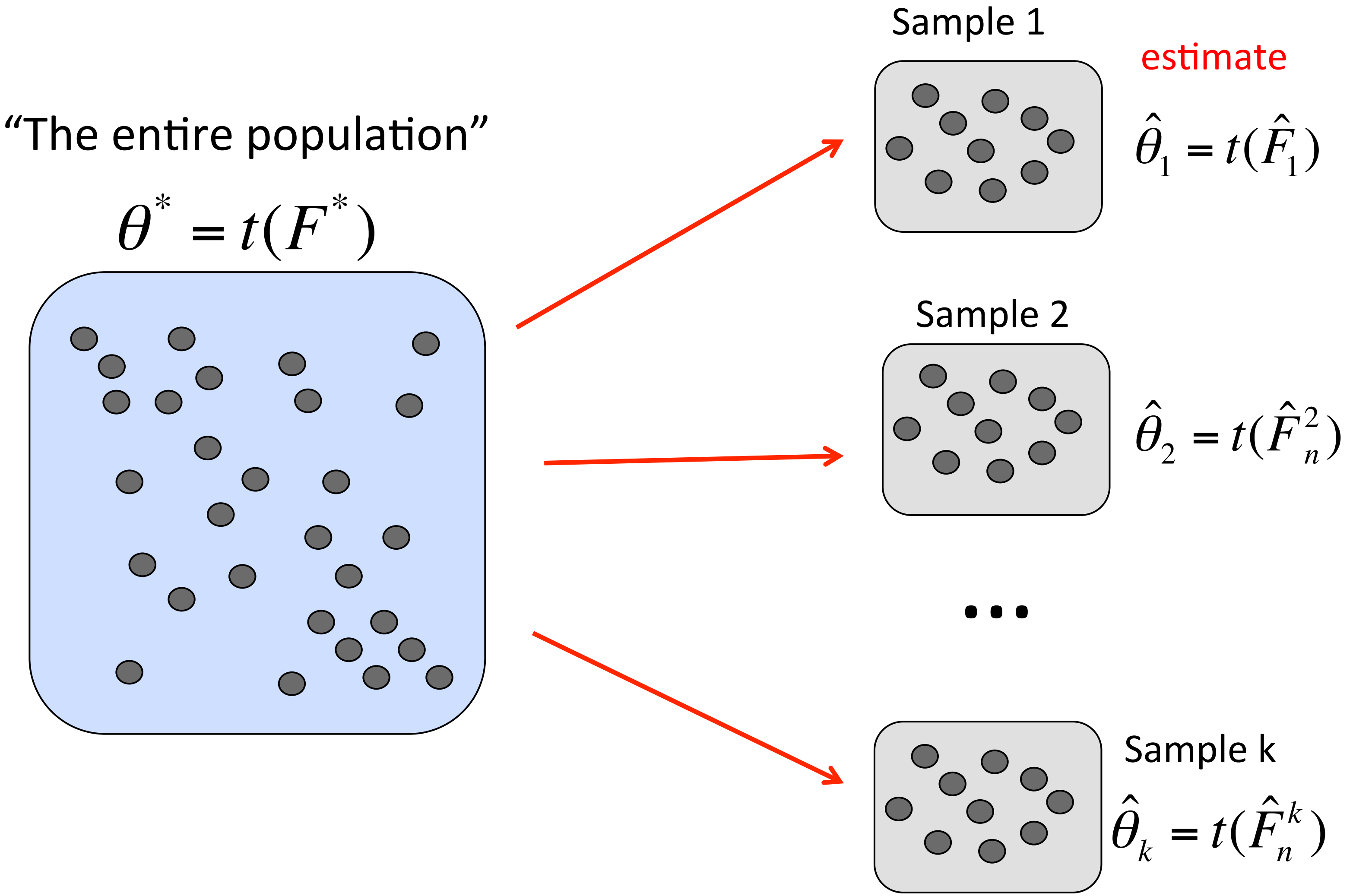
"The entire population"

$$\theta^{*} = t(F^{*})$$

Sample 1

estimate

$$\hat{\theta}_1 = t(\hat{F}_1)$$



“The entire population”

$$\theta^* = t(F^*)$$

Sample 1

estimate

$$\hat{\theta}_1 = t(\hat{F}_1)$$

Sample 2

$$\hat{\theta}_2 = t(\hat{F}_n^2)$$

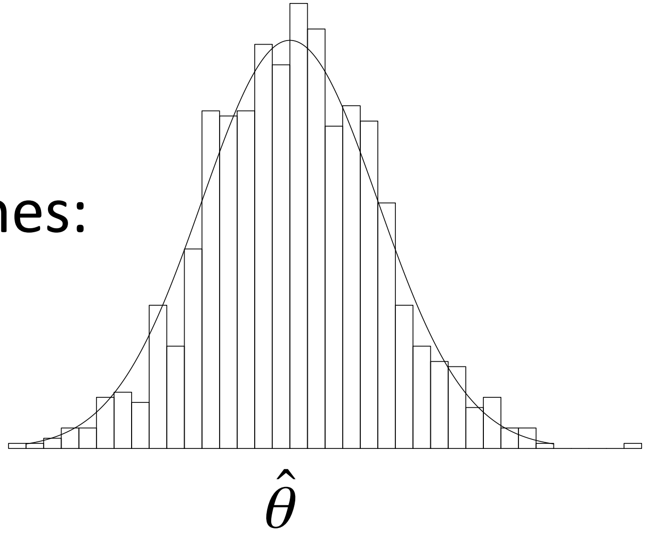
…

Sample k

$$\hat{\theta}_k = t(\hat{F}_n^k)$$

Sampling distribution

- The distribution of the estimates computed from repeating the experiment multiple times: sampling distribution



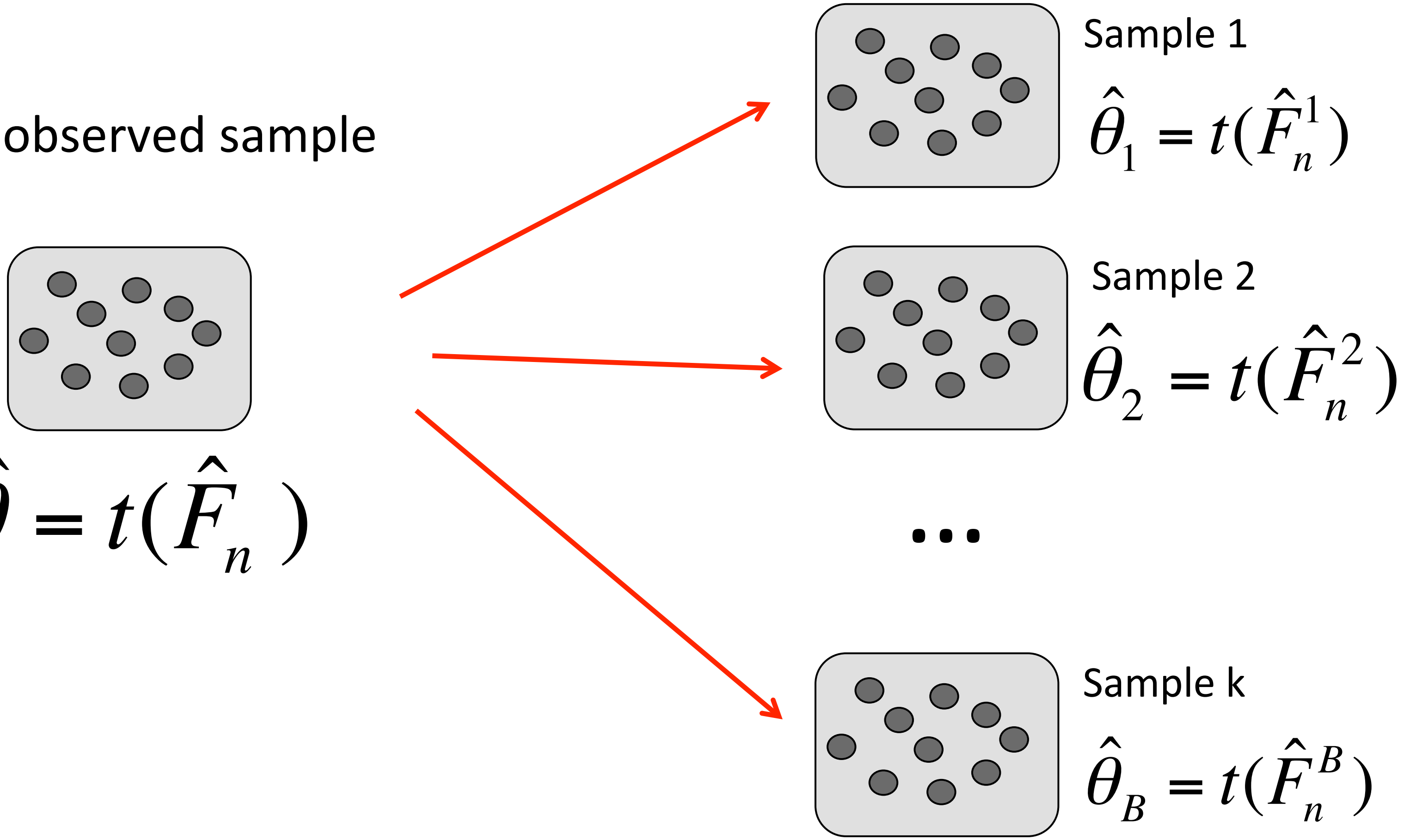
- If we had it, we could assess some properties of our estimate:
  - Standard deviation of the estimate ("standard error")
  - Confidence intervals

Sampling distribution

- The sampling distribution, at the moment, is a theoretical construction—it consists of all possible outcomes for experiments that we could have run.

- In practice, we only ran a single experiment to get all our data. But we still want to assess the properties of our estimate. How?
  - Asymptotic theory
  - Bootstrap

The Bootstrap



Your observed sample

$$\hat{\theta} = t(\hat{F}_n)$$

Sample 1

$$\hat{\theta}_1 = t(\hat{F}_n^1)$$

Sample 2

$$\hat{\theta}_2 = t(\hat{F}_n^2)$$

…

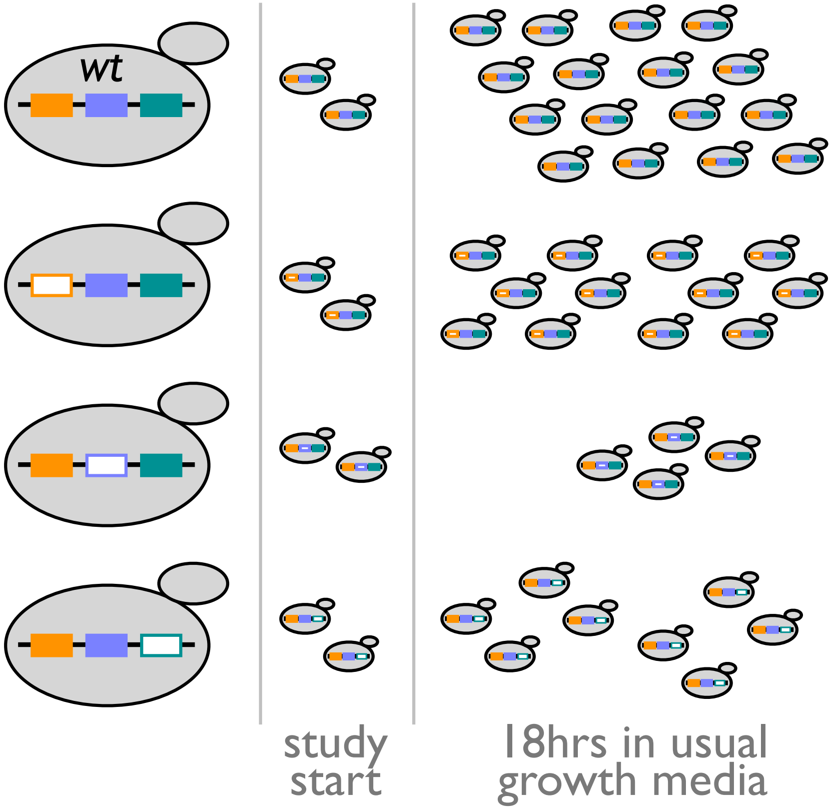
Sample k

$$\hat{\theta}_B = t(\hat{F}_n^B)$$

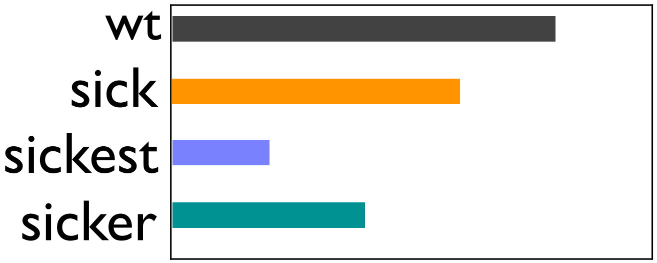
Bootstrap:

Repeat experiment B times (in the bootstrap world) to form b bootstrap replicates of your experiment, then use the B bootstraps to obtain a sampling distribution for your parameter.

Example application of the bootstrap



Study of fitness of yeast deletion mutants



Data source: Giaever G, Flaherty P, Kumm J, Proctor M, Nislow C, et al. (2004) Chemogenomic profiling: identifying the functional interactions of small molecules in yeast. Proc Natl Acad Sci U S A 101: 793-798. Pubmed. DOI: 10.1073/pnas.0307490100

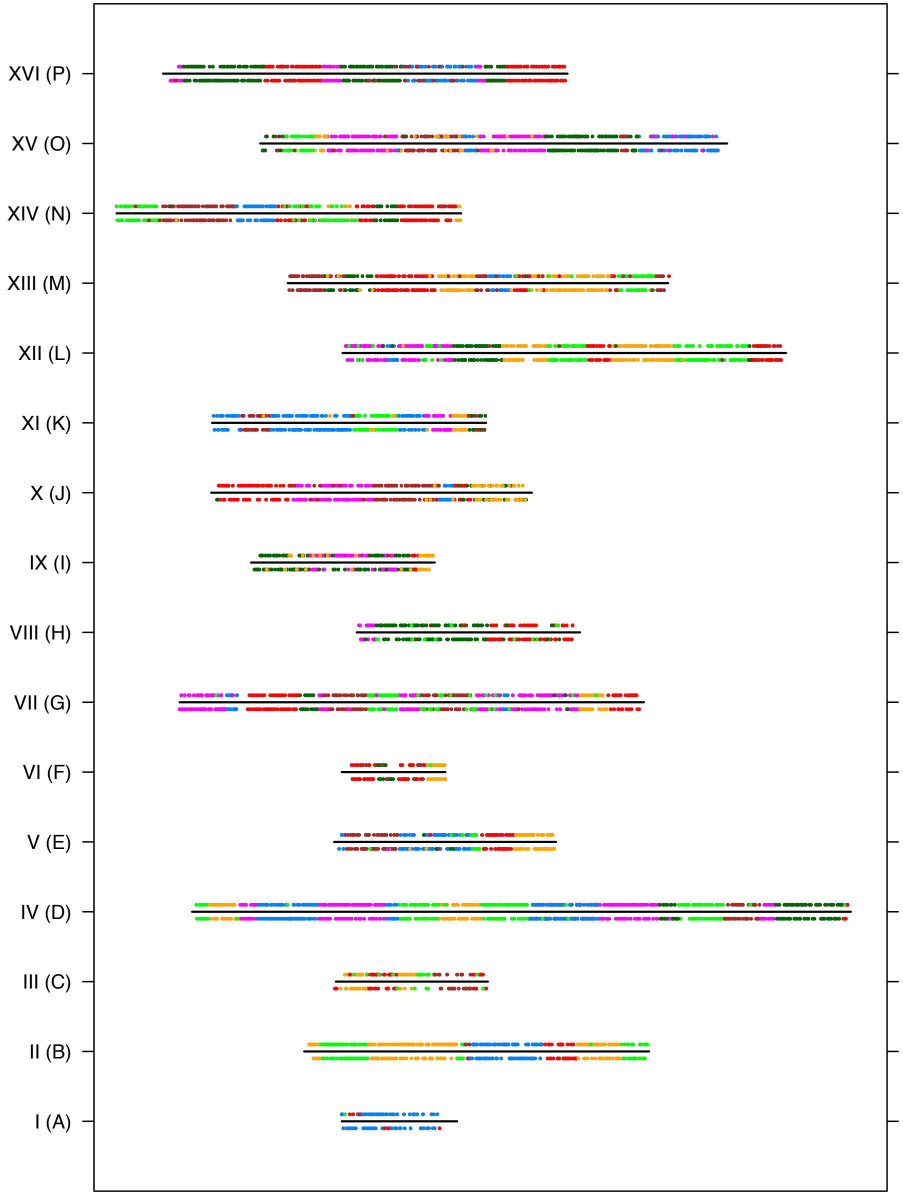
Rationale for growth studies of yeast deletion mutants

- Analogy: flipping circuit breakers in a house to determine which lights and outlets are controlled by each circuit
- If the deletion mutant for gene *g* is defective at some biological activity, that suggests that gene *g* contributes to that activity.
- Growth studies are the 'entry-level' study. In real life, we often measure more complicated phenotypes and subject the mutant to additional challenges, e.g. treatment with drugs or deletion/mutation of additional genes. Also, this type of data is often integrated with from other types of studies.

Yeast genome has 16 chromosomes.

Each gene lives somewhere on one of these chromosomes.

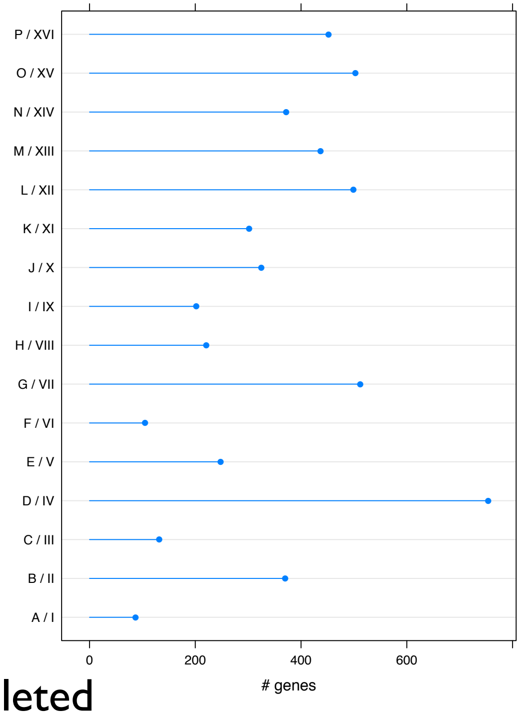
Therefore, each deletion mutant is also associated with one yeast chromosome.

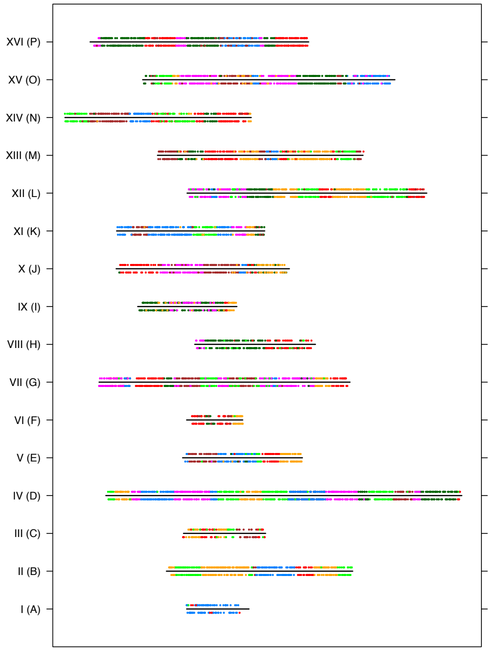


```
> str(hDat)
'data.frame':  5521 obs. of  4 variables:
 $ geneDel     : Factor w/ 5521 levels "YAL001C","YAL002W",..: 1 2 3 4 5 6 7 8..
 $ chromo      : int  1 1 1 1 1 1 1 1 1 1 ...
 $ chromoPretty: Factor w/ 16 levels "A / I","B / II",..: 1 1 1 1 1 1 1 1 1 1 ..
 $ pheno       : num  9.39 9.4 10.38 10.54 8.65 ...
```

```
> peek(hDat)
     geneDel chromo chromoPretty    pheno
190  YBL102W      2       B / II 9.285750
917  YDR089W      4       D / IV 9.528659
1040 YDR185C      4       D / IV 7.079669
1969 YGL201C      7      G / VII 9.754082
2118 YGR046W      7      G / VII 9.262812
3175 YKL085W     11       K / XI 9.479903
3622 YLR176C     12      L / XII 7.359638
```

```
> dotplot(table(hDat$chromoPretty),
+         origin = 0, type = c("p", "h"),
+         xlab = "# genes")
```





Each row consists of

- geneDel = name of the gene that was deleted
- chromo = the associated chromosome (an integer between 1 and 16)
- chromoPretty = a prettier version of the chromosome (more suitable for labeling in tables and figures)
- pheno = a growth phenotype (due to experimental realities and pre-processing, the units are meaningless, i.e. don’t expect to see a cell count here)

Data for our analysis

response = a quantitative measure of growth

e.g. growth rate or # cells at study end

also know the specific yeast gene that was deleted

e.g. YDL133WY = a yeast ORF

and the chromosome on which the gene is found

e.g. “chromosome 4 / D”

Data for our analysis

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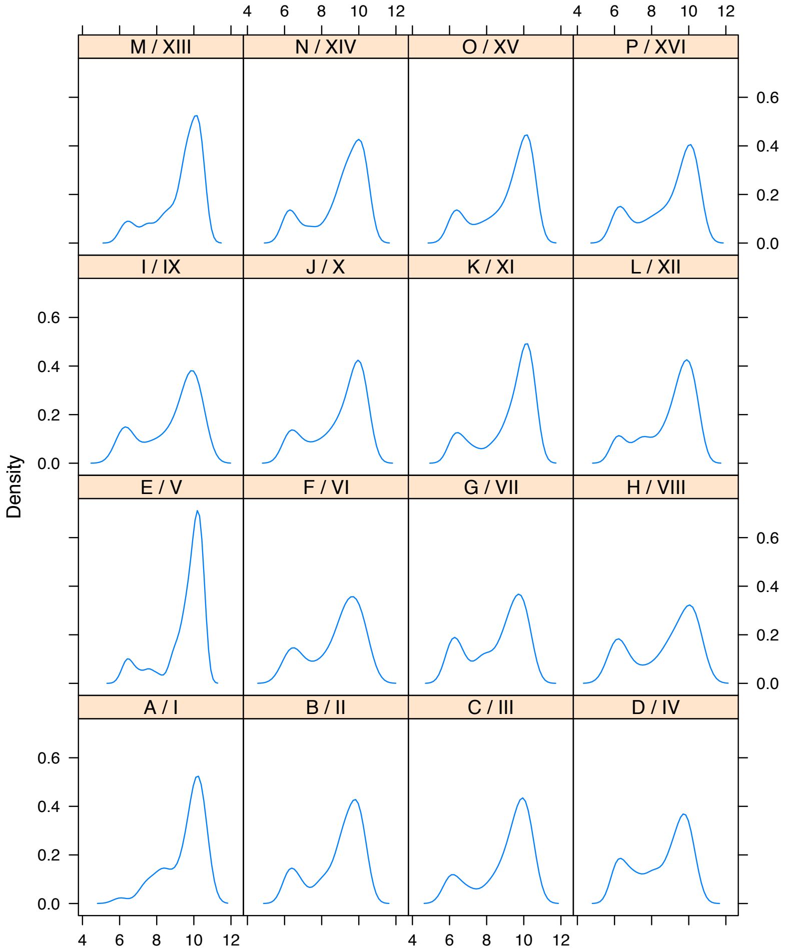
Typical application of bootstrap

Bootstrap

- Estimating key features of the sampling distribution:
  - The standard deviation of the statistics ("standard error")
  - Confidence intervals
  - Assess whether the asymptotic distribution has started to "kick-in"
  - The bias of an estimate
- Hypothesis testing: constructing the null distribution

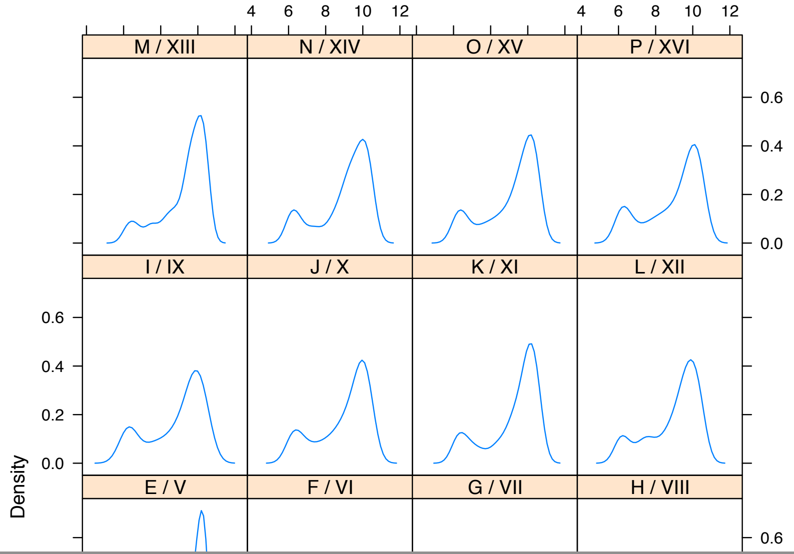
quantitative growth phenotypes for gene deletion mutants

each panel = phenotypes for mutants lacking genes on that chromosome



Data source: Giaever G, Flaherty P, Kumm J, Proctor M, Nislow C, et al. (2004) Chemogenomic profiling: identifying the functional interactions of small molecules in yeast. Proc Natl Acad Sci U S A 101: 793-798. Pubmed. DOI: 10.1073/pnas.0307490100 quantitative growth phenotypes for gene deletion mutants

each panel =

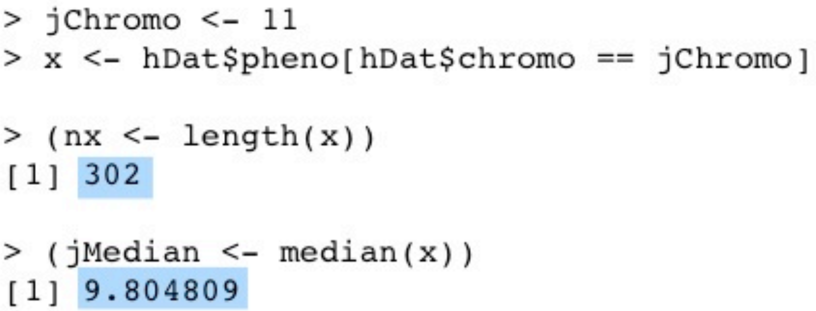


We will use bootstrap to:

a) Assess sampling distribution of the response (quantitative measure of growth)
b) Revisit two-group comparison

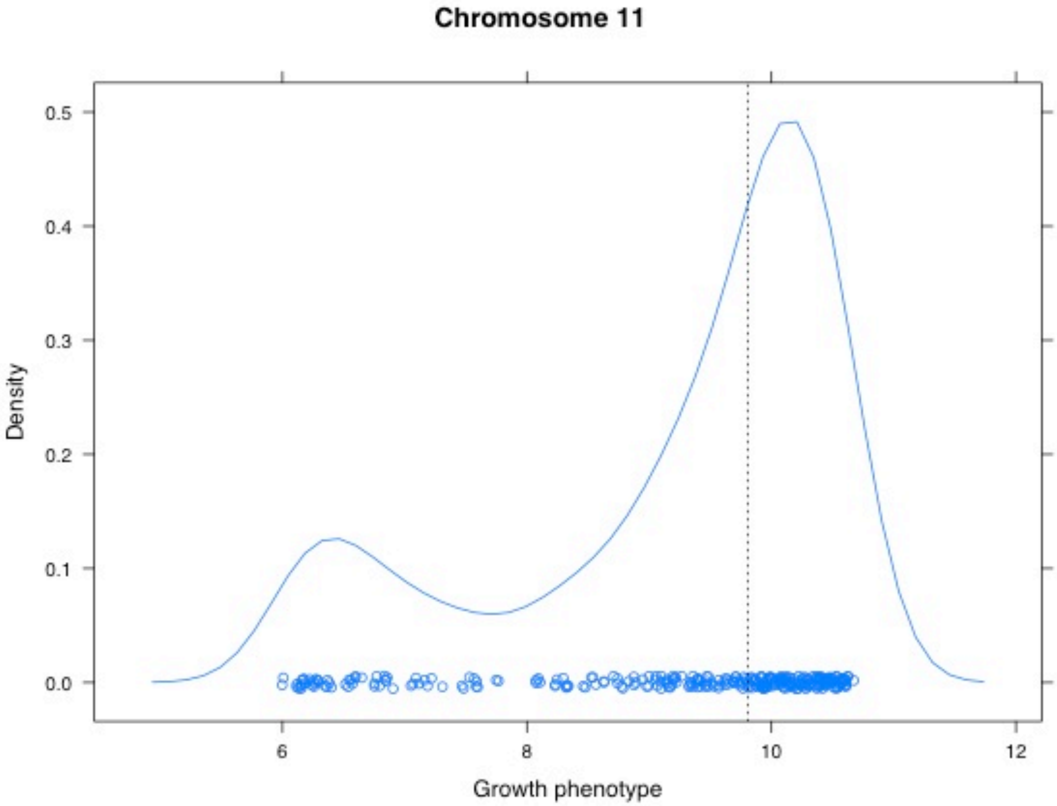
C, et al. (2004) Chemogenomic profiling: identifying the functional interactions of small molecules in yeast. Proc Natl Acad Sci U S A 101: 793-798. Pubmed. DOI: 10.1073/pnas.0307490100

Example: Median for chromosome 11



```
> jChromo <- 11
> x <- hDat$pheno[hDat$chromo == jChromo]

> (nx <- length(x))
[1] 302

> (jMedian <- median(x))
[1] 9.804809
```

- 302 genes on chromosome 11
- Median fitness value if 9.804809



Example: Median of phenotypes for chromosome 11

- Large-sample theory says the sample median is asymptotically normal with mean = true median = *m* and variance 1 / 4n f(*m*)^2
- Good news = asymptotic dist'n is known
- Bad news = depends on the true density at the median*
- Let's compare this theoretical, asymptotic result to the bootstrap result.

*If I knew the density, I'd know the median, wouldn't I?

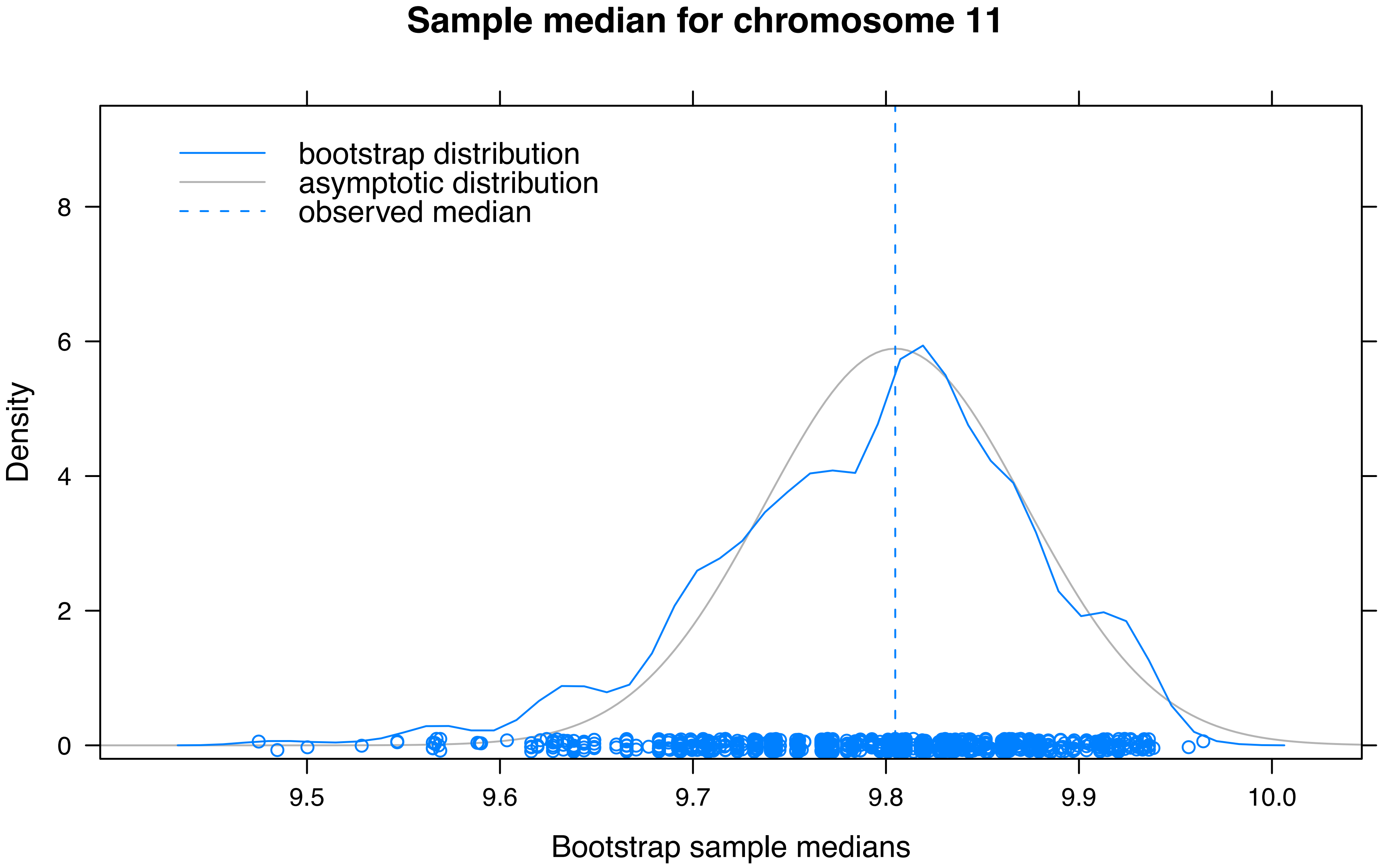
Our bootstrap samples

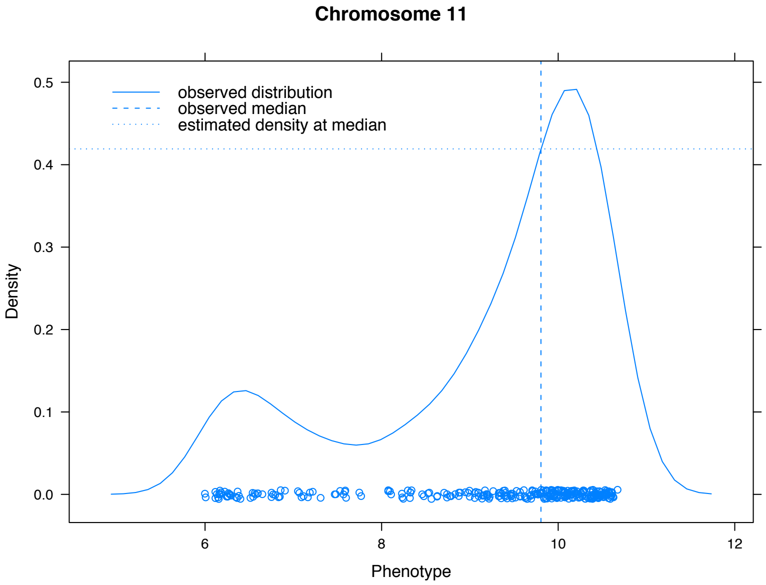
literally I draw a new sample of size n = 302 from the observed data, with replacement

some observations will re-appear ... some once, some twice, etc. .... some don't show up in the bootstrap sample at all

I take the median of the bootstrap sample. That is a bootstrap statistic.

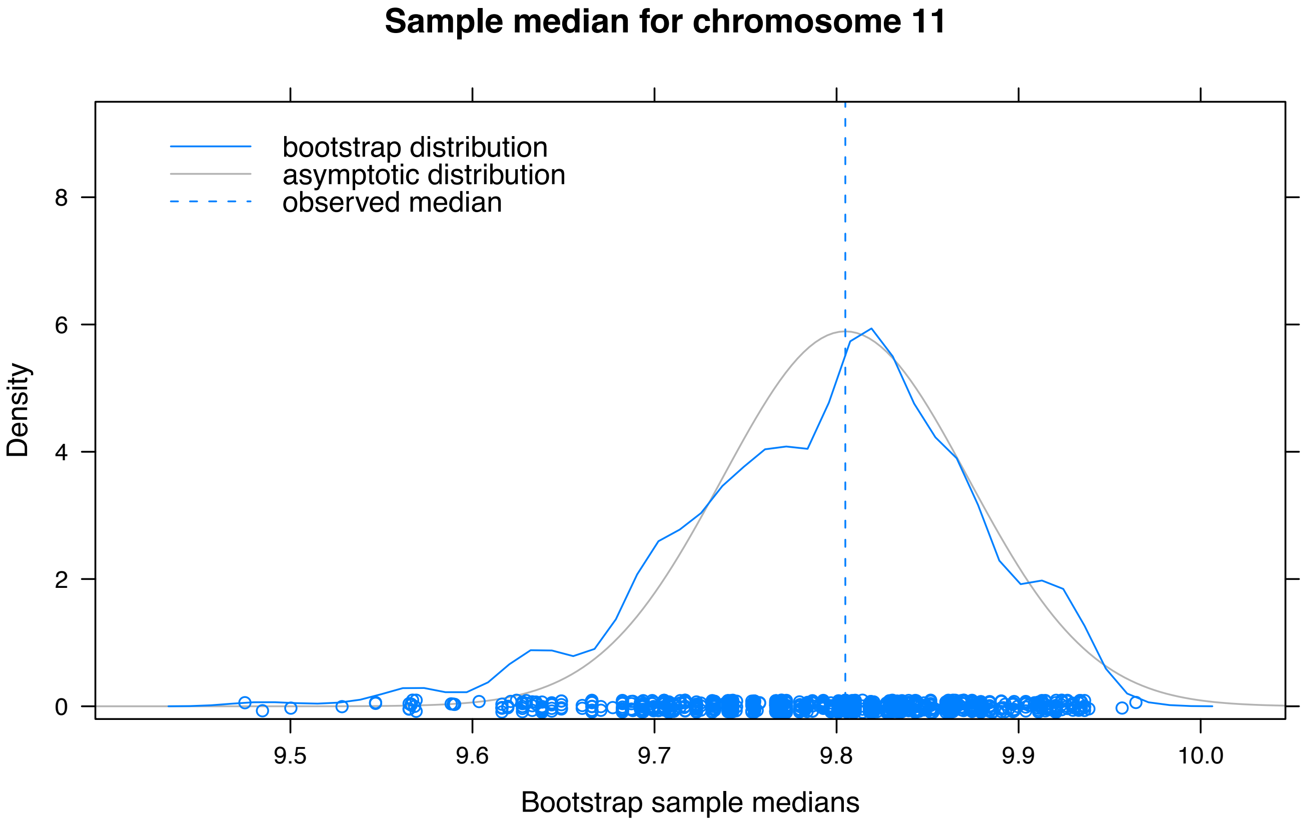
I do that B times. B is a big number.

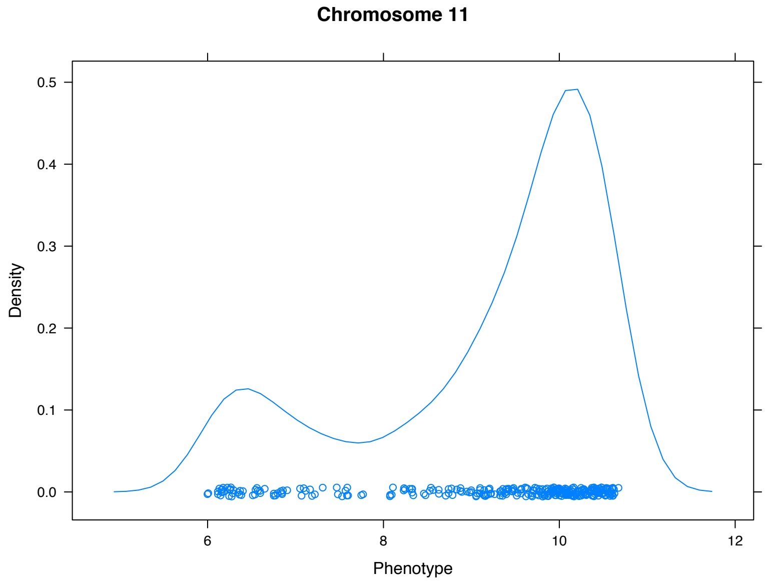

Sample median for chromosome 11
bootstrap distribution
asymptotic distribution
observed median
Density
0
2
4
6
8
9.5
9.6
9.7
9.8
9.9
10.0
Bootstrap sample medians

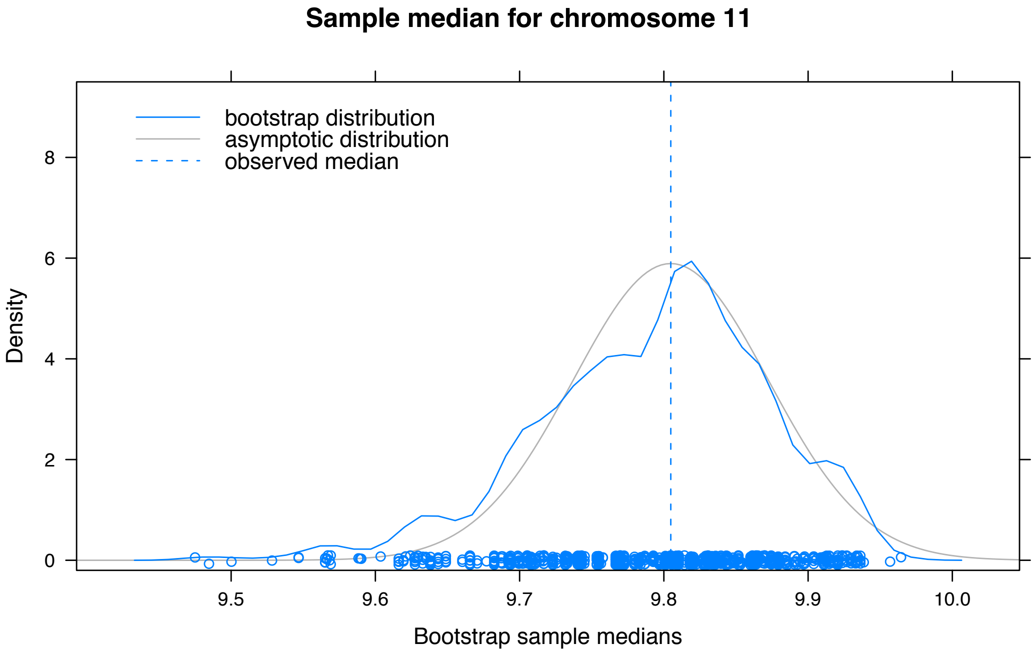


Features we could foresee:

- Both dist'ns have mode @ sample median = 9.8
- Left tail of bootstrap distribution heavier than than that of asymp. norm







```
> B <- 1000

> bootData <-
+   matrix(sample(x, size = B * nx, replace = TRUE),
+          nrow = nx, ncol = B)

> bootTestStat <- apply(bootData, 2, median)

> (bootStdErr <- sqrt(var(bootTestStat)))
[1] 0.07937564

> theorStdErr
[1] 0.0677069

> mean(bootTestStat)
[1] 9.796118

> jMedian
[1] 9.804809
```

I conclude ... for a data-generating distribution as bimodal as this, n = 300 is close to -- but not quite in -- Asymptopia.

Good default template for conducting a bootstrap. Can be adapted for other resampling or random data generation tasks.

```
> B <- 1000
```

make it easy to start w/ small B, then scale up

```
> bootData <-
+   matrix(sample(x, size = B * nx, replace = TRUE),
+          nrow = nx, ncol = B)
```

generate the bootstrap data all at once

```
> bootTestStat <- apply(bootData, 2, median)
```

use data aggregation techniques to compute bootstrap statistics

```
> (bootStdErr <- sqrt(var(bootTestStat)))
[1] 0.08163377

> mean(bootTestStat)
[1] 9.796118

> jMedian
[1] 9.804809

> abs(mean(bootTestStat) - jMedian)/bootStdErr
[1] 0.1094916
```

Typical application of the bootstrap

- The standard deviation of a statistic (“standard error”)
- Assess whether the asymptotic distribution has started to “kick in” at a finite sample size
- Confidence intervals

R packages for bootstrapping

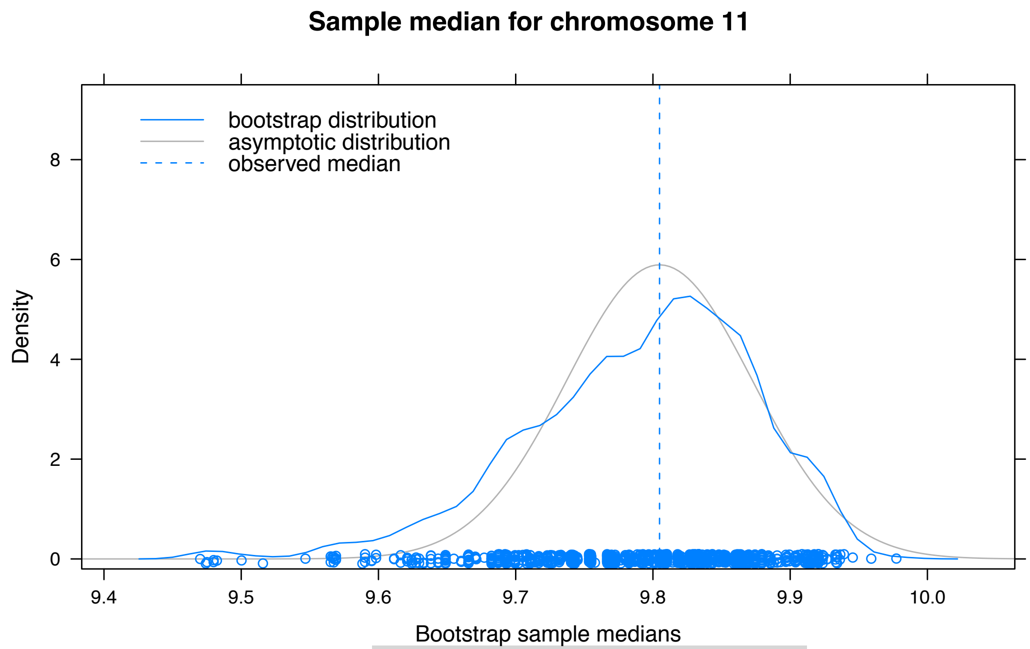
- boot:
  - a companion to the book “Bootstrap Methods and Their Applications” by AC Davison and DV Hinkley – seems to be distributed with R

- bootstrap:
  - Companion to the book “An Introduction to the Bootstrap” by Efron and Tibshirani 1993— seems not to be actively maintained.

Using the boot package

boot output

```
> bootRes <- boot(x, function(z, i) median(z[i]), R = 1000)

> bootRes

ORDINARY NONPARAMETRIC BOOTSTRAP

Call:
boot(data = x, statistic = function(z, i) median(z[i]), R = 1000)

Bootstrap Statistics :
    original       bias    std. error
t1* 9.804809 -0.01221307  0.08194345
```



an interval estimate for the median

```
> boot.ci(bootRes, conf = c(0.90, 0.95), type = "all")
...
Intervals :
Level      Normal              Basic
90%   ( 9.682,  9.952 )   ( 9.692,  9.971 )
95%   ( 9.656,  9.978 )   ( 9.683, 10.019 )

Level     Percentile            BCa
90%   ( 9.638,  9.918 )   ( 9.621,  9.913 )
95%   ( 9.590,  9.927 )   ( 9.579,  9.921 )
```

Bootstrap methods can be used to build CIs. Here showing output from ‘boot’ package, boot.ci() function.

How many bootstrap samples should we generate? (i.e., how large should B be?)

- Efron & Tibshirani recommend B~=200 for the purpose of estimating standard error.
- You will need much more (~1000-10000) for confidence intervals.
- I recommend B=1000 for std error estimation or testing, but why not use much larger B, like B=10,000.

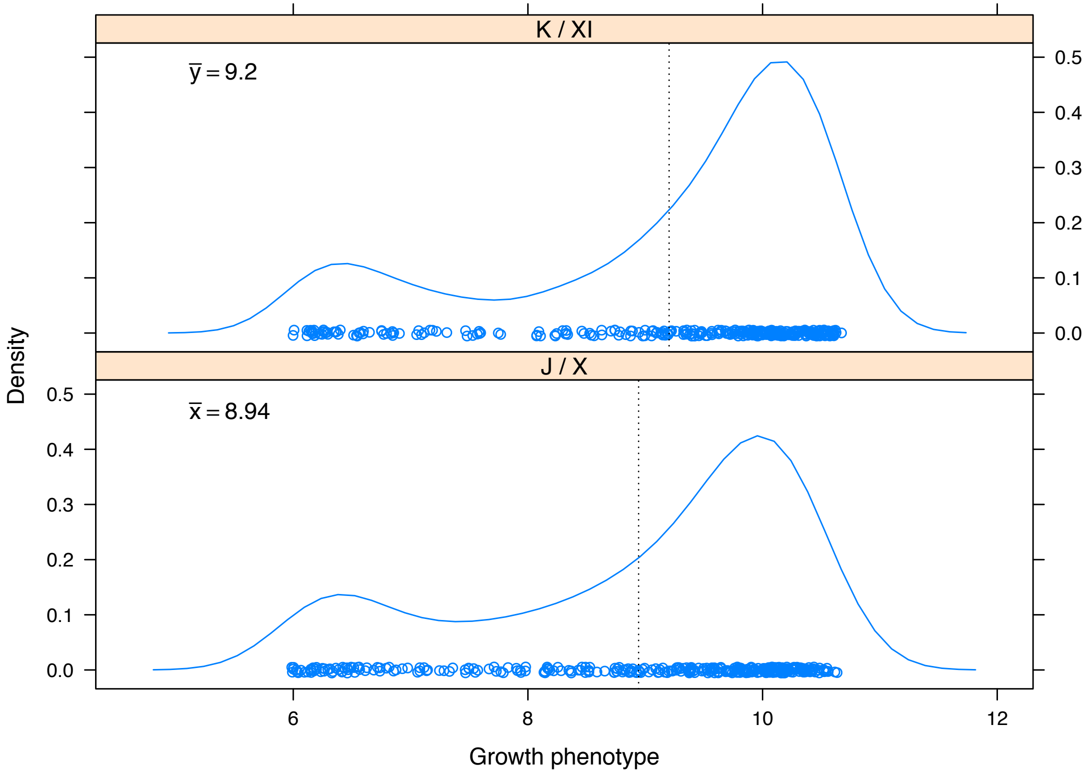
x = data observed from one chromosome, e.g. 10

y = data observed from another chromosome, e.g. 11

Regard x as a realization of X ~ F.

Regard y as a realization of Y ~ G.

F = G?



<u>Specify a null hypothesis</u> *H_0*: F = G (= H)

x = data observed from one chromosome
y = data observed from another chromosome

Regard x as a realization of $X \sim F$.
Regard y as a realization of $Y \sim G$.

$F = G$?

(biological questions: are the genes on different chromosomes equally important to fitness? is there a relationship between gene location and gene function or essentiality?)

Basics of a hypothesis test

- Specify a null hypothesis, H_0
- Choose a test statistic
- Determine the distribution for the test statistic under H_0
- Convert the observed test statistic into a p-value

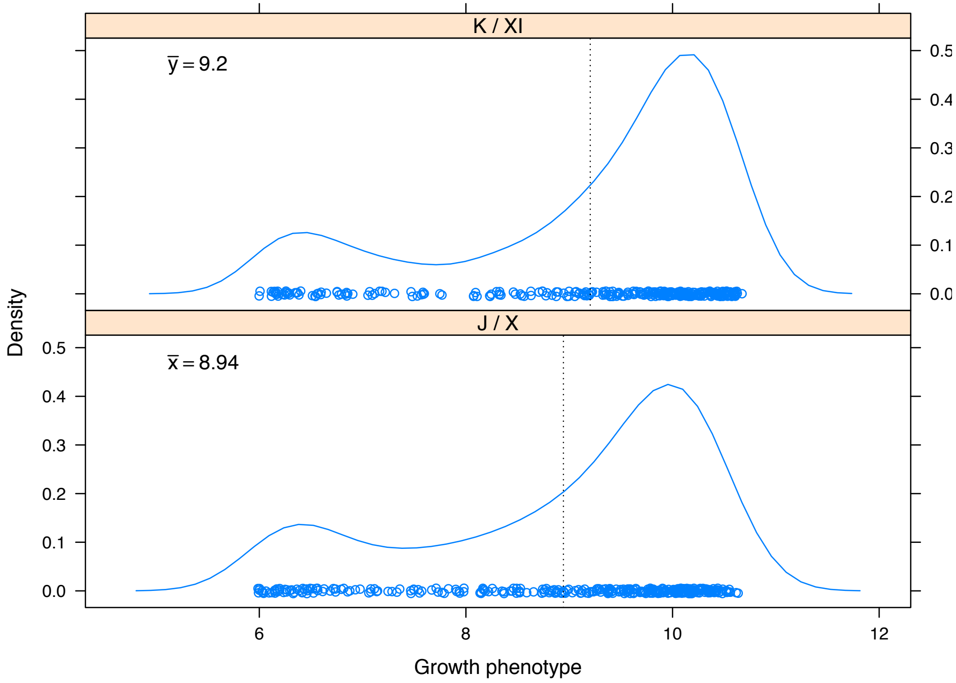
“The p-value is the probability under H_0 of observing a value of the test statistic the same or more extreme than what was actually observed.”

All of Statistics by Larry Wasserman. Springer, 2004. GoogleBooks search. via myilibrary

All of Nonparametric Statistics by Larry Wasserman. Springer, 2006. via SpringerLink | via myilibrary | GoogleBooks search.

Classical tests that address our question

- t test
- Wilcoxon test, aka Mann-Whitney here
- Kolmogorov-Smirnov test, 2 sample version
- Chi-square test of homogeneity
- I'm sure there are others ....



Why you may not want to use a classical testing approach?

- Depending on your test statistics, it may be very difficult, time-consuming, or even impossible, to derive the null distribution of your test statistic.

- In many settings, the classical hypothesis testing may not be asking the exact question that we are interested in.

- If we think about hypothesis testing from first principles and we have a decent computer (and programming skills!), we can often empirically determine, at least approximately well, the null distribution and the p-value.

Null hypothesis: F = G (= H)

Possible test statistic: |avg (x) - avg (y)|

Observed value of test statistic = t

$$t = \left| \overline{x} - \overline{y} \right|$$

How much evidence does t
present against the null hypothesis?

What is the distribution of the test statistic under the null?

Under null, X and Y have same distribution. Let's call it H.

If we knew H, we could draw n_x observations from it -- call this x* -- and another n_y observations from it -- call this y*.

Compute t* = |avg x* - avg y*|.

$$t^* = \left| \overline{x}^* - \overline{y}^* \right|$$

Compute t* = |avg x* - avg y*|.

$$t^* = \left| \bar{x}^* - \bar{y}^* \right|$$

Generate B such observations t* (B large).

What proportion of the t* are as or more extreme as t? That's basically your bootstrap p-value.

Done! Sort of. We don't actually know H, though.

Here we can estimate H with an empirical distribution function.

Amalgamate x and y into one sample. Under the null, they are iid H. Give mass 1/(n_x + n_y) to each observation. That's the empirical distribution function. That's a decent estimate of H.

How to generate data from this estimate of H? Resample with replacement.

Choose a test statistic

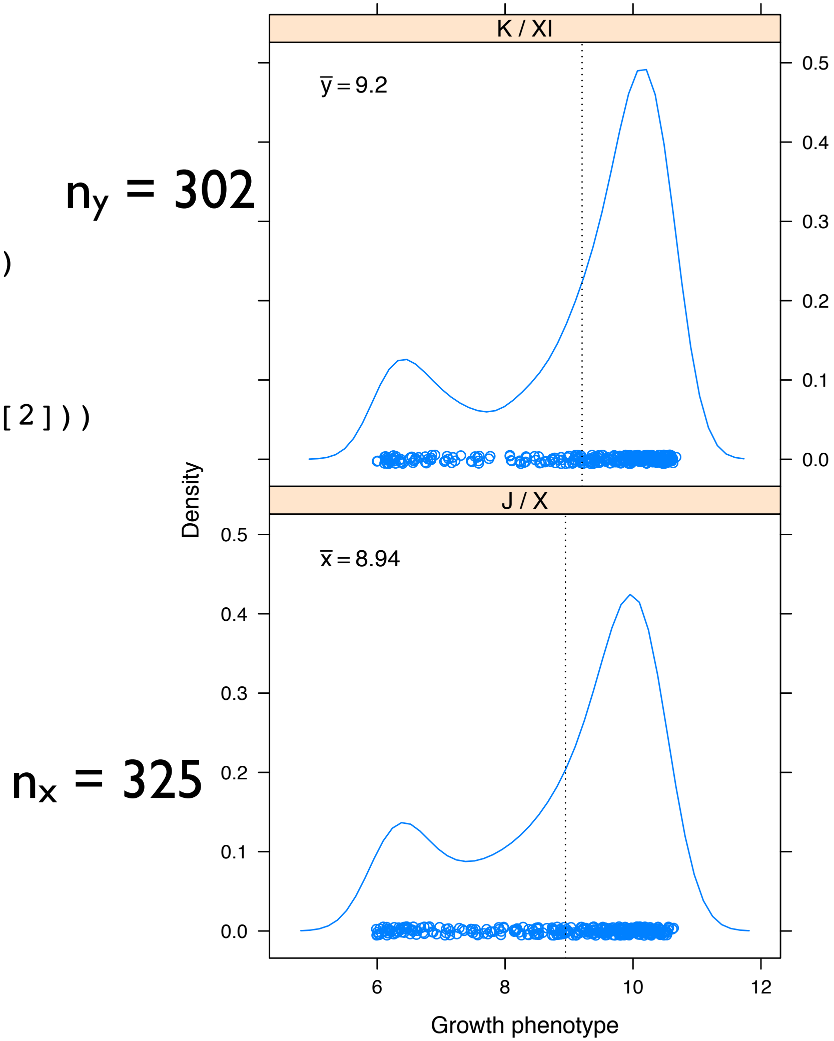
Let's try this: $t = \left|\bar{x} - \bar{y}\right|$

```
> (chromoMeans <- with(kDat,
+                     tapply(pheno, chromo, mean)))
      10        11
8.943558 9.203379

> (obsTestStat <- abs(chromoMeans[1] - chromoMeans[2]))
       10
0.2598215
```

$$\bar{x} = 8.94$$

$$\bar{y} = 9.2$$

$$t = \left|\bar{x} - \bar{y}\right| = 0.26$$



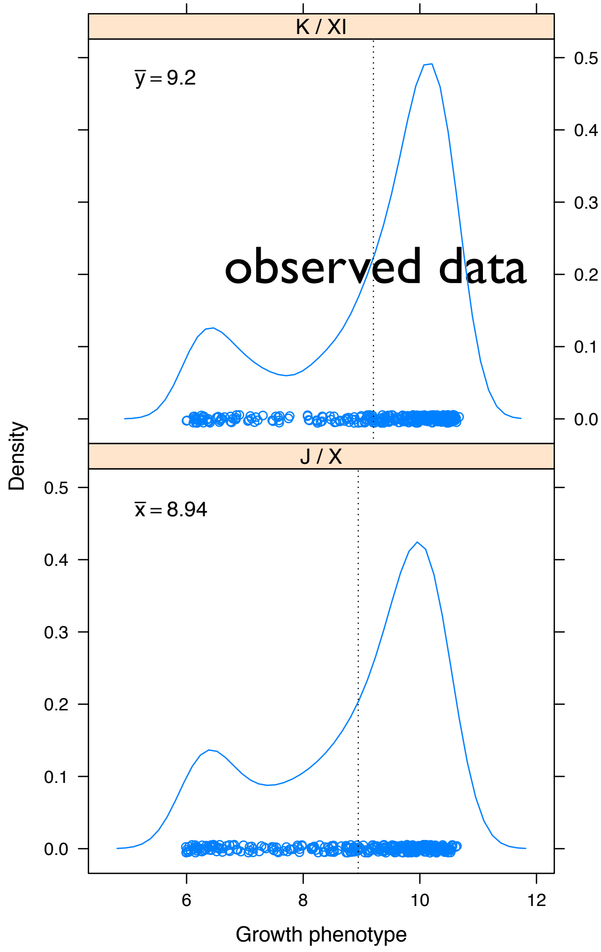
$$t = |\overline{x} - \overline{y}| = 0.26$$

Is this “big” or “extreme” and, therefore, suggests we should reject H_0?

Ideally, we would generate lots of datasets from the (unknown) distribution H and get an empirical null distribution for this test statistic. But we don’t know H ......

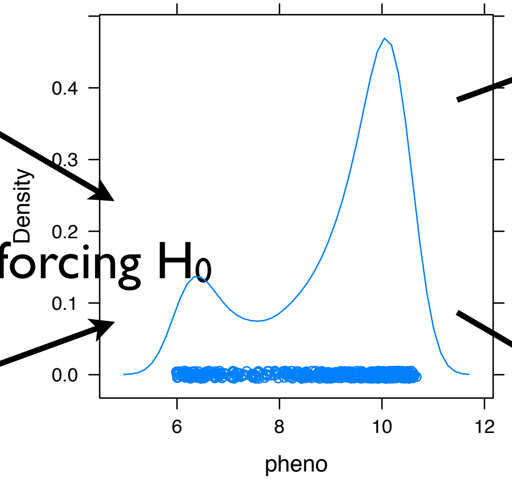
```
> (obsTestStat <- abs(chromoMeans[1] - chromoMeans[2]))
       10
0.2598215

<snip, snip>

> (bootTestStat <- abs(diff(bootMeans)))
        11
0.08155161
```

1 bootstrap sample (“baby steps”)

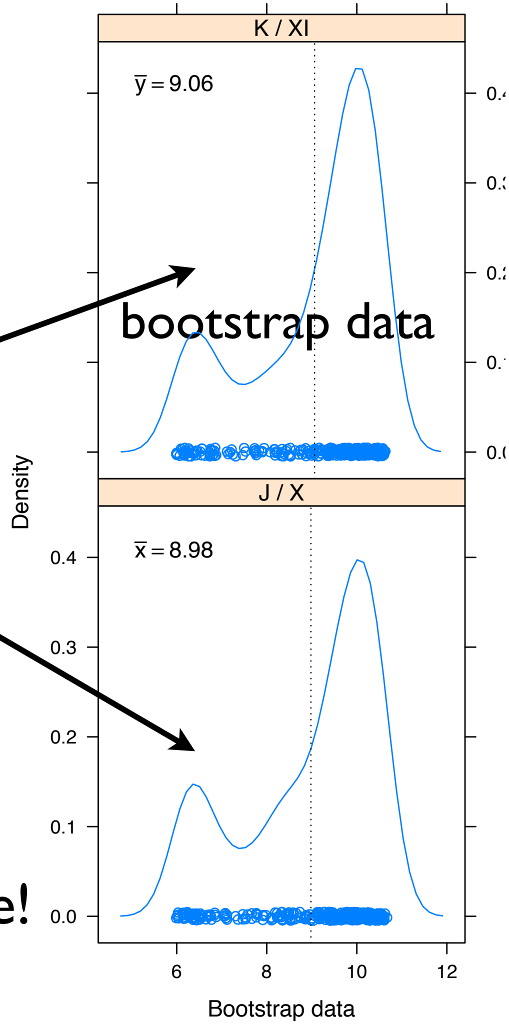


observed data

enforcing H_0

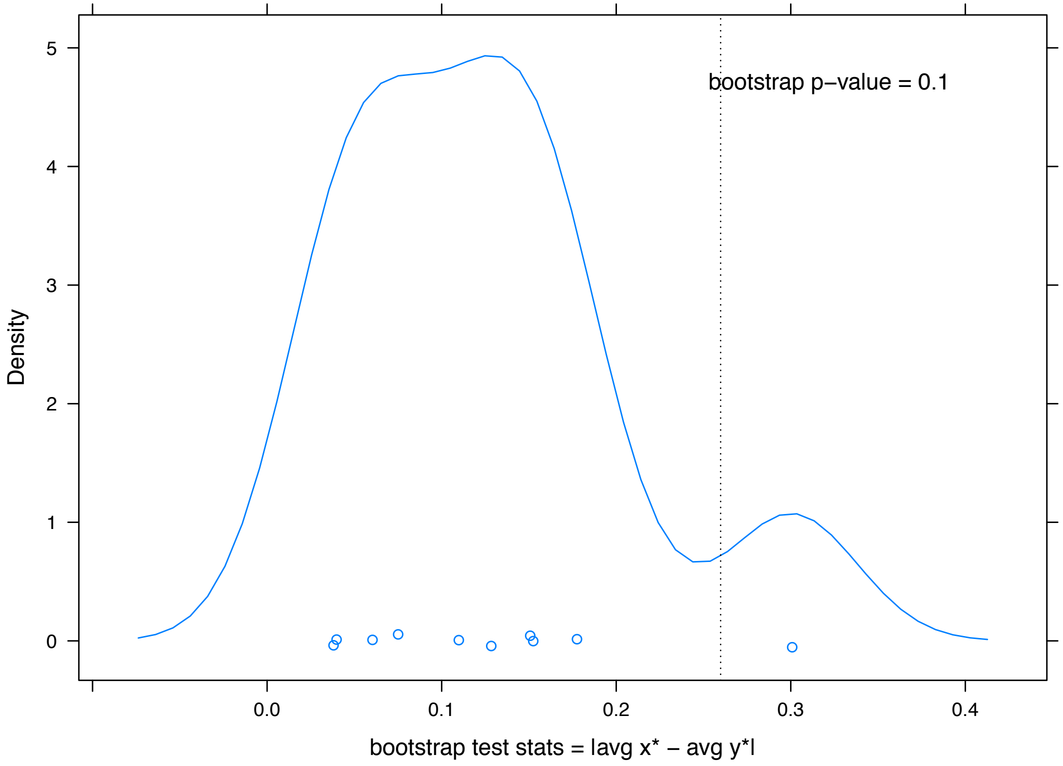


bootstrap data

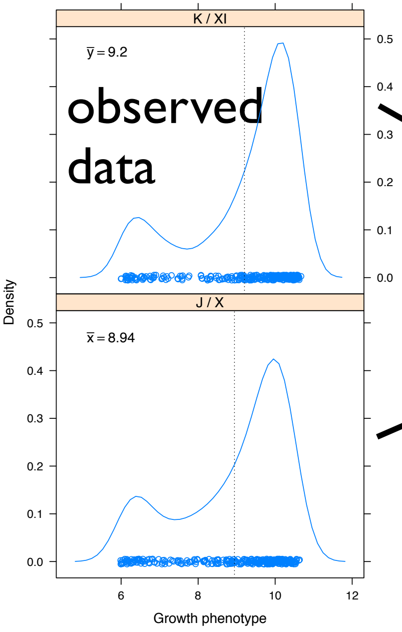


So far, the observed test statistic looks pretty extreme! Let’s scale up a bit ....

```
> (obsTestStat <- abs(chromoMeans[1] - chromoMeans[2]))
       10
0.2598215

...
> bootTestStat
 [1] 0.23677776 0.21074474 0.16380568 0.13258165 0.01663695 0.07176389
 [7] 0.09824504 0.20745668 0.11928580 0.27759690
> mean(bootTestStat >= obsTestStat)
[1] 0.1
```

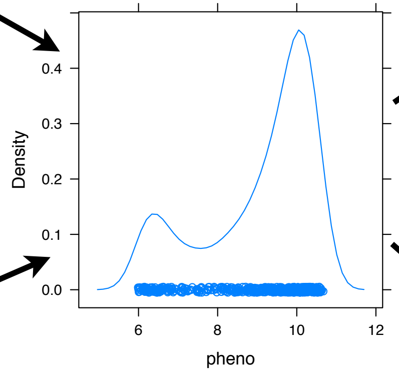


What proportion of the t* are as or more extreme as t? That’s basically your bootstrap p-value.



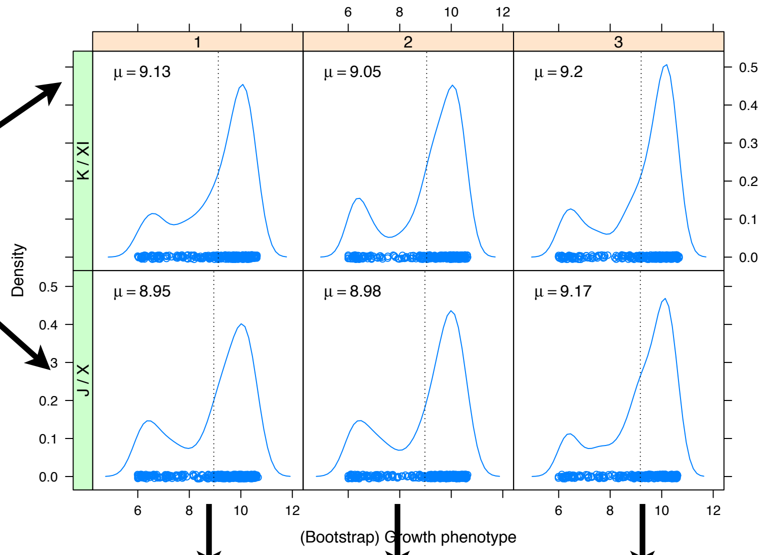
observed data

$$t = |\bar{x} - \bar{y}| = 0.26$$



enforce H0

bootstrap data



and 9,997 more like this

....

$$t^* = |\bar{x}^* - \bar{y}^*| = 0.17$$

$$t^* = |\bar{x}^* - \bar{y}^*| = 0.07$$

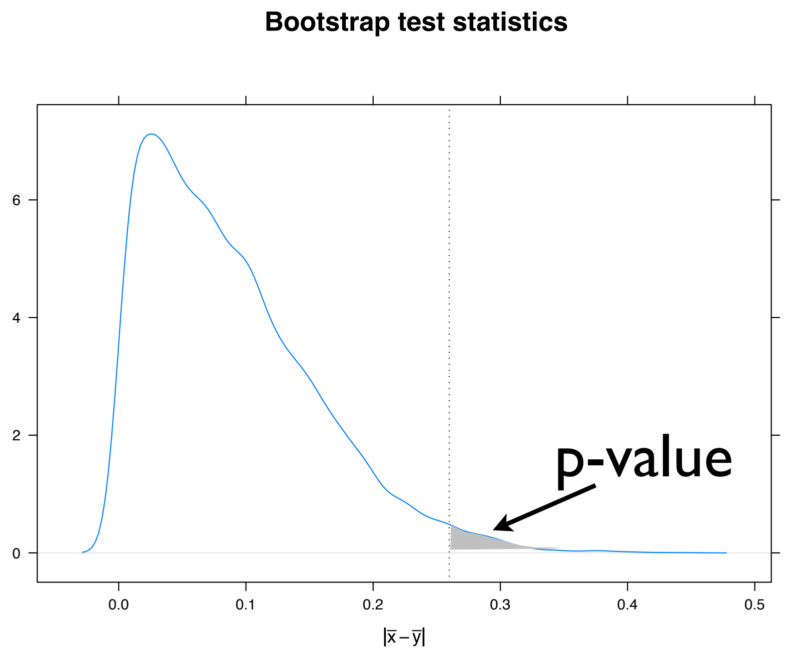
$$t^* = |\bar{x}^* - \bar{y}^*| = 0.03$$

```
(n <- nrow(kDat))                          # 627 obs
B <- 10000
bootDat <-
  matrix(sample(kDat$pheno, size = B * n, replace = TRUE),
         nrow = n, ncol = B)
str(bootDat)
## num [1:627, 1:10000] 10.08 10.07 10.03 9.26 8.28 ...
bootTestStats <-
  apply(bootDat, 2, computeAbsDifferenceOfMeans, jFact = kDat$chromo)
```

No explicit loops!

B = 10,000 bootstrap samples

```
> bootTestStats <-
+         apply(bootDat, 2, computeAbsDifferenceOfMeans, jFact = kDat$chromo)

> densityplot( ~ bootTestStats,
+             xlab = expression(group("|", bar(x) - bar(y),"|")),
+             main = "Bootstrap test statistics",
+             plot.points = FALSE, n = 200, ref = TRUE,
+             panel = function(x, ...) {
+               panel.densityplot(x, ...)
+               panel.abline(v = obsTestStat, lty = 'dotted')
+             })

> ## bootstrap p-value
> mean(bootTestStats >= obsTestStat)
[1] 0.0172

> t.test(pheno ~ chromo, kDat)$p.value
[1] 0.01940612
```



p-value

Bootstrap p-value is very close to Welch’s t-test p-value. That’s comforting!

Permutation test in hypothesis testing

- Most commonly used resampling method for hypothesis testing.
- Sample without replacement your response (and/or group memberships) – e.g., permute the labels.

Simple example: differential gene expression analysis

- Suppose we find to find genes that are differentially expressed between different conditions.
- We compute the test statistic (e.g., t-statistics) for each of the g genes.
- We compute the p-value associated with each test statistic, call it p_g
  - p_g is the probability under null that the test-statistic is at least as extreme as T_g
- We correct p_g's for the number of tests (g tests)
- Declare a significant association if corrected p-values < threshold (0.05)

Standard t-test

- Assume $X_1, X_2, \ldots, X_m$ are from ~ $N(\mu_1 | \sigma^2)$
- Assume $Y_1, Y_2, \ldots, Y_n$ are from ~ $N(\mu_2 | \sigma^2)$

- Compute the pooled variance estimate:

$$s^2 = \frac{1}{m+n-2}(\sum_{i=1}^{m}(X_i - \bar{X})^2 + \sum_{i=1}^{n}(Y_i - \bar{Y})^2).$$

- The t-statistic is given by

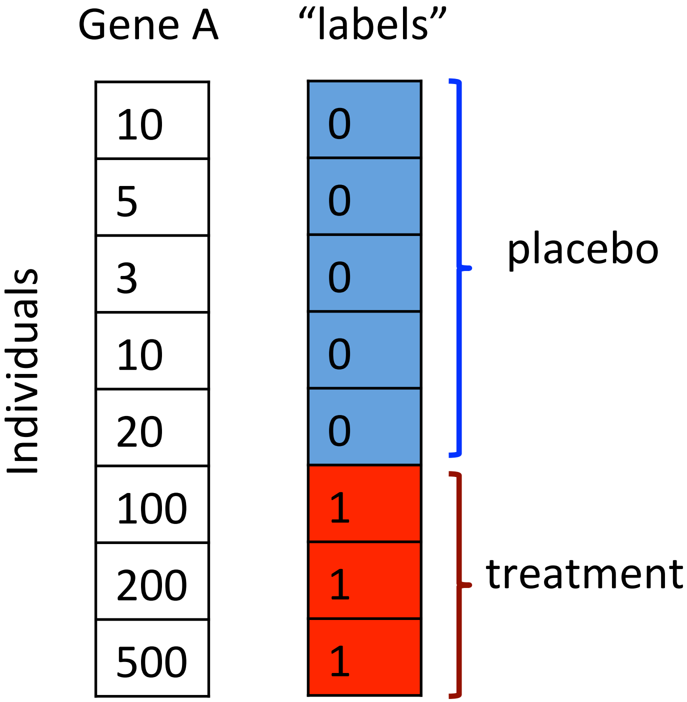
$$T(X,Y) = \frac{\bar{X} - \bar{Y}}{s\sqrt{\frac{1}{m} + \frac{1}{n}}}.$$

Permutation test

- Want to test whether observation in two groups follows the same distribution, without making assumptions about the distributions (e.g., normality)
- Generate a null distribution for the test-statistic:
  - Randomly divide individuals to 'treatment' groups

- For i = 1 …. p, do
  - Permute the group labels, giving new assignment of 'group; to each individual
  - Computer the test statistic from the permutated data

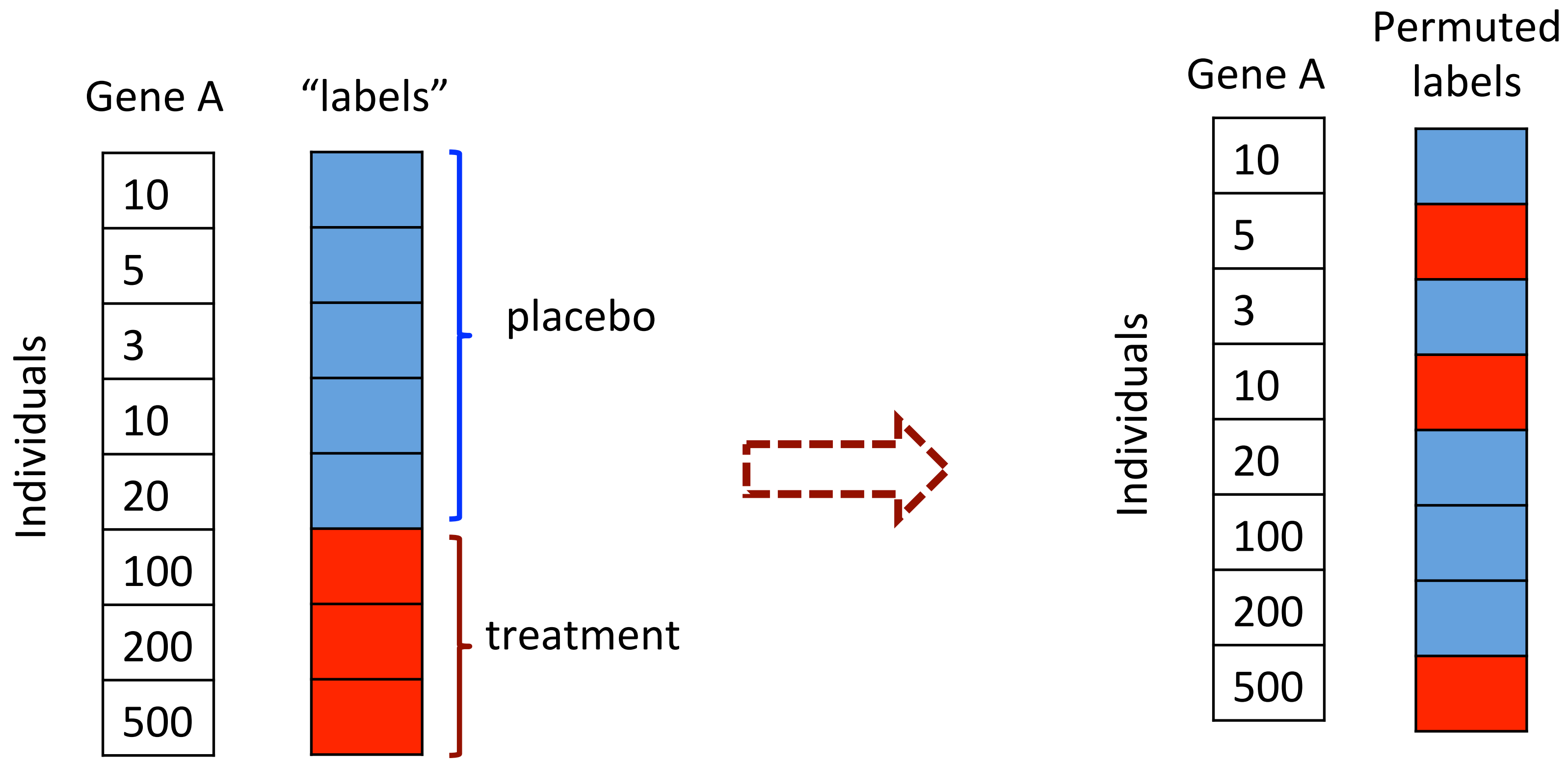
“Real data”



Individuals

Gene A	“labels”	
10	0	placebo
5	0	
3	0	
10	0	
20	0	
100	1	treatment
200	1	
500	1	

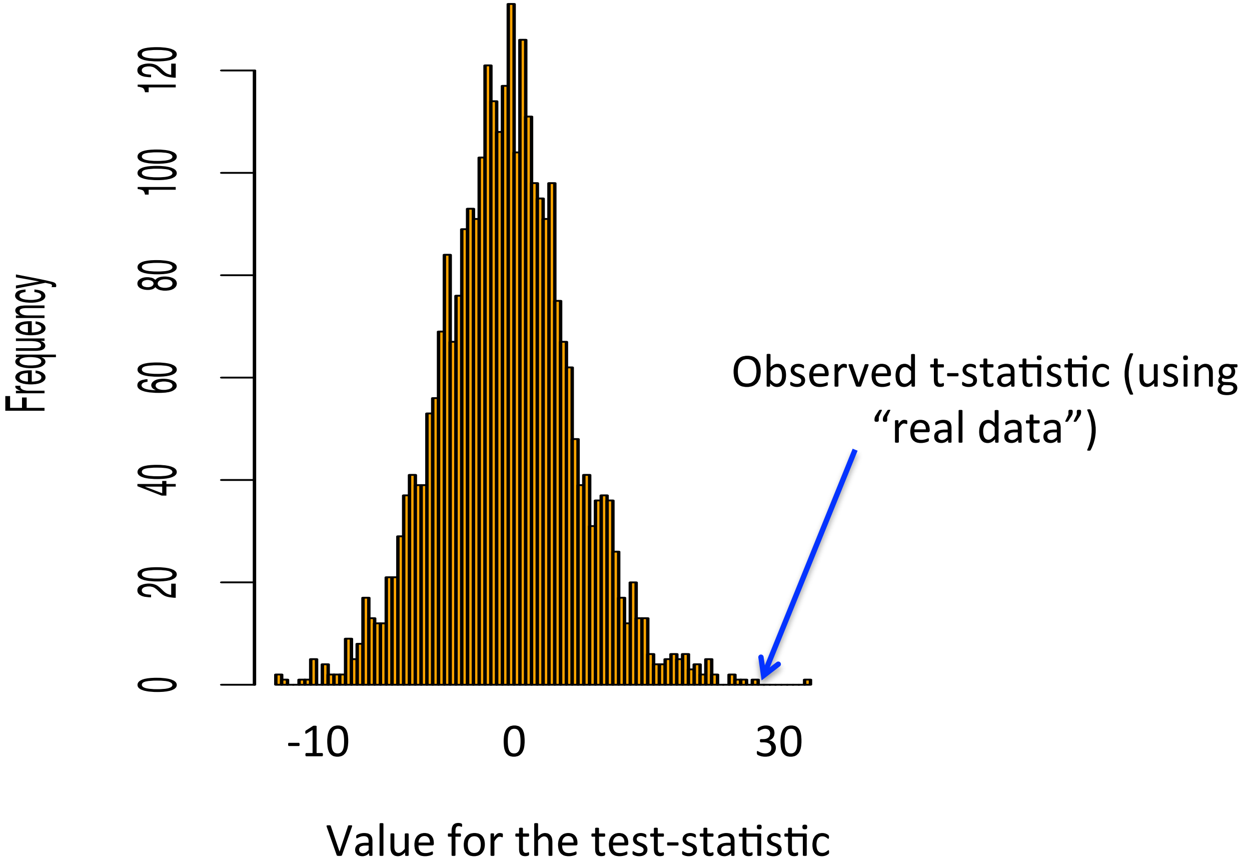
“Real data” **“Permutated data”**



Gene A	“labels”	
10	🟦	placebo
5	🟦	
3	🟦	
10	🟦	
20	🟦	
100	🟥	treatment
200	🟥	
500	🟥	

Gene A	Permuted labels
10	🟦
5	🟥
3	🟦
10	🟥
20	🟦
100	🟦
200	🟦
500	🟥

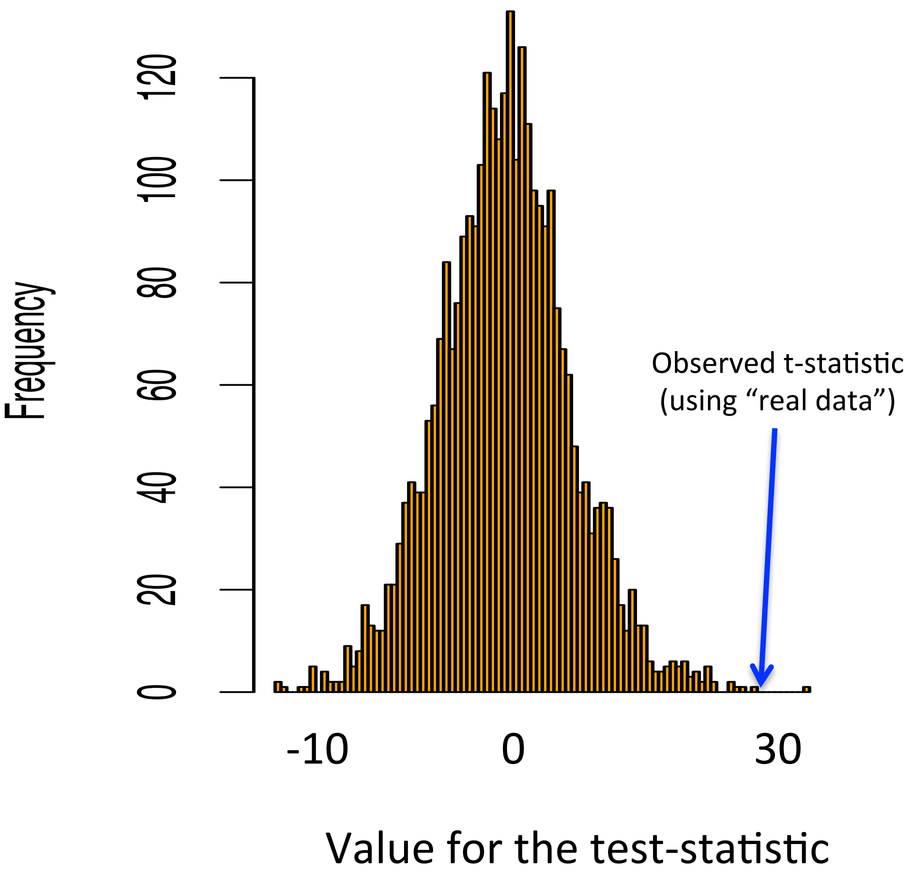
Histogram of test-statistic under null (permutated data)



Observed t-statistic (using "real data")

Histogram of test-statistic under null (permutated data)

The null distribution for $\bar{X} - \bar{Y}$



P-value: $$\frac{\#(T_p > T_r)}{\#\ permutations}$$

Resampling methods

- Ways of performing statistical inference that are "internal to the data" under analysis: e.g., you get the necessary knowledge about sampling variability (of parameters/ estimates) from the observed data itself

- Resampling methods:
  - Bootstrap
  - Permutation testing
  - Cross validation