# Lecture2 : Statistical Test for Genomic Data

2025-03-13

Required Packages

```
#if (!requireNamespace("BiocManager", quietly = TRUE))
#install.packages("BiocManager")
#BiocManager::install("multtest")
#install.packages("nortest")
#install.packages("car")
#install.packages("outliers")
#install.packages("ape")
#install.packages("SNPassoc")
#install.packages("genetics")
```

Statistical Hypothesis Testing

- Sample data 만든 후 그 data에서 sample statistic 계산 조진다.
- 그 다음 우리는 Null huypothesis가 true라 가정한다잉!
- 그 다음 $H_0$의 distribution에서 sample statistic의 위치(?)를 본다.
- 우리는 지금 $H_0$가 true라고 가정을 했기 때문에 sample data에서 나온 ST가 $H_0$의 distribution에 잘 맞아야한다.
- 여기서 잘 맞아야한다라는 뜻은 그 ST가 터무니 없는 확률로 나온다면 안된다는 뜻이다.
- 이때 그 확률을 우리는 p-value라고 한다.
- 그리고 우리는 $H_0$가 true일때 $H_0$를 reject할 허용 오차를 significance level $\alpha$라고 한다.

  p-value가 0.05일 때, 만약 $H_0$가 True일 때 관측된 결과(ST)가 우연히 나타날 확률이 50%이다! 라는 느낌! 즉 $\alpha = 0.05$는 내가 관측한 값이 우연히 나타날 확률이 0.05 보단 작아선 안된다! 라는 threshold를 setting 한 느낌!

- 핵심은 sample statistic의 distribution을 알아야 한다!!!

The Z-test

- 현실에선 쓸 일 없음 ㅋㅋㅋㅋㅋ
- 왜? 모표준편차를 어케아노..
- Under the Null

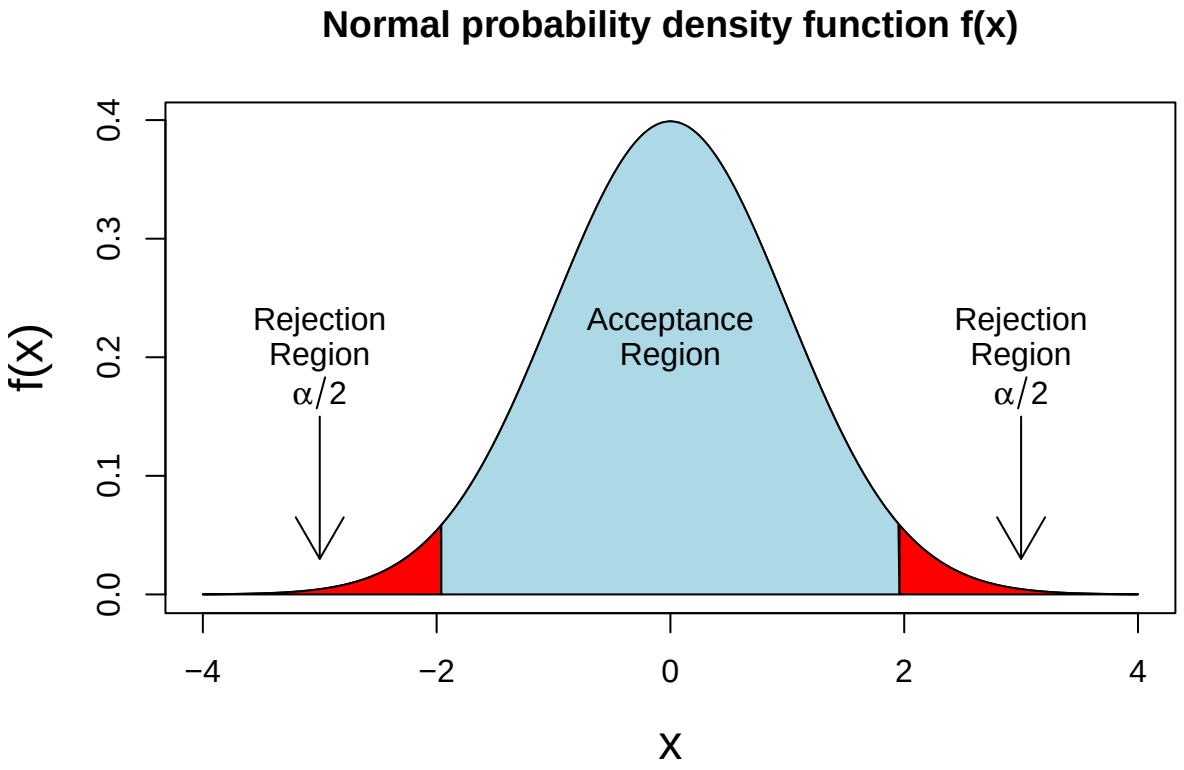
$$H_0 : \mu = 0 \quad \text{vs} \quad H_1 : \mu \neq 0$$

```r
library(multtest)
library(nortest)
library(car)
library(outliers)
library(ape)
library(SNPassoc)
library(genetics)
```

```r
library(multtest)

data(golub, package = "multtest")

golubFactor <- factor(golub.cl, levels=0:1, labels=c("ALL","AML"))

x <- golub[2058, golubFactor=="ALL"]

n <- length(x)

sigma <- 0.25; mu0 <- 0
z.value <- sqrt(n)*(mean(x) - mu0)/sigma

2*pnorm(-abs(z.value)) # p-value
```

```
## [1] 0.9991094
```

```r
f <- function(x) dnorm(x,0,1)
alpha <- 0.05
qz <- qnorm(1-alpha/2)
x1 <- seq(-4, -qz, 0.01); y1 <- dnorm(x1, 0, 1)
x2 <- seq(-qz, qz, 0.01); y2 <- dnorm(x2, 0, 1)
x3 <- seq(qz, 4, 0.01); y3 <- dnorm(x3, 0, 1)
plot(f, -4, 4, cex.lab=1.5, xlab="x", ylab="f(x)",
main="Normal probability density function f(x)")
polygon(c(-4, x1, -qz), c(0, y1, 0), col="red")
polygon(c(-qz, x2, qz), c(0, y2, 0), col="lightblue")
polygon(c(qz, x3, 4), c(0, y3, 0), col="red")
arrows(-3, 0.15, -3, 0.03)
text(-3, 0.23, "Rejection")
text(-3, 0.20, "Region")
text(-3, 0.17, expression(alpha/2))
arrows(3, 0.15, 3, 0.03)
text(3, 0.23, "Rejection")
text(3, 0.20, "Region")
text(3, 0.17, expression(alpha/2))
text(0, 0.23, "Acceptance")
text(0, 0.20, "Region")
```




Confidence Interval

- If we were to repeat the procedure thousands of times, we are 95% certain that the true mean falls in the confidence interval -> 이게 핵심!

```
mean(x) + qnorm(0.025) * sigma/sqrt(n)
```

```
## [1] -0.09424511
```

```
mean(x) + qnorm(0.975) * sigma/sqrt(n)
```

```
## [1] 0.09435251
```

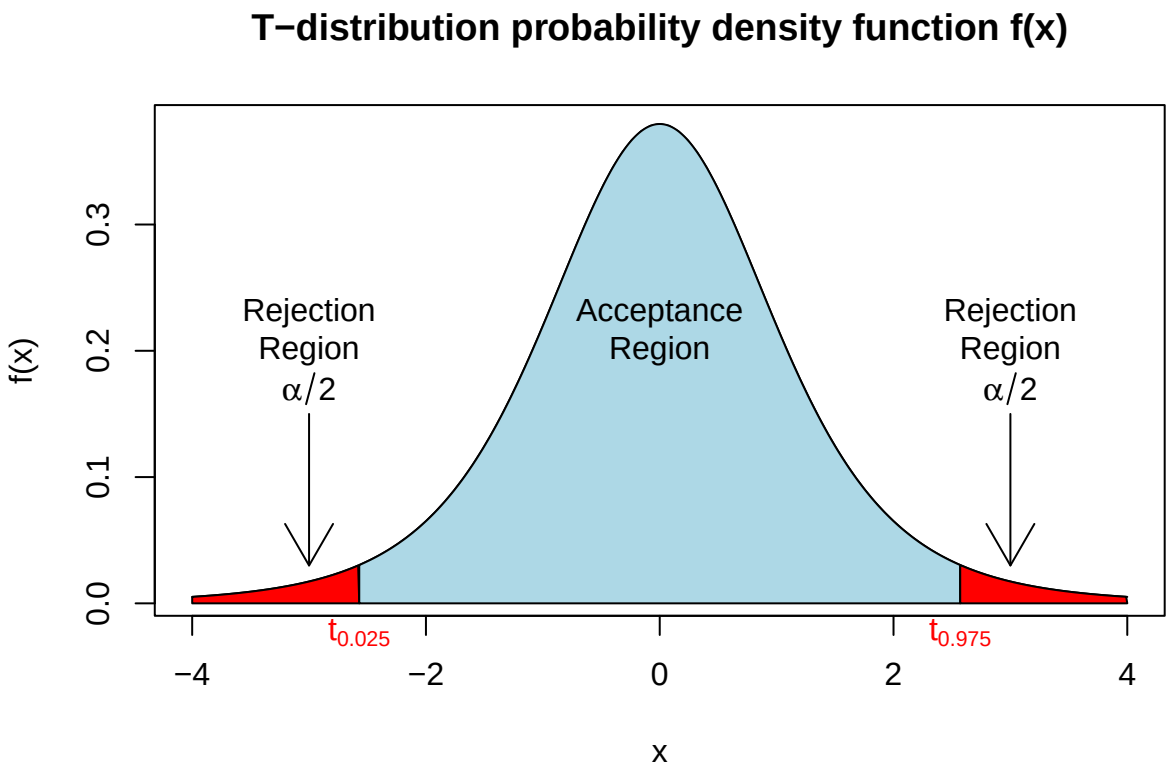
```
mean(x) + c(-1, 1) * qnorm(0.975) * sigma / sqrt(n)
```

```
## [1] -0.09424511  0.09435251
```

One sample T-test

- 모집단의 분산(표준편차)을 모르고, 표본이 정규성을 만족한다고 가정할 때 사용한다.
- 이때 검정 통계량은 t 분포를 따르고 t분포는 degree of freedom에 따라 모양이 달라진다.
- 모집단의 분산 대신 표본의 구한 표본분산을 이용하기 때문에 정규분포가 아닌 t 분포를 따르게 된다.(자세한 증명은 수통 때)

```r
f <- function(x) dt(x, 5)
alpha <- 0.05
qt5 <- qt(1-alpha/2, 5)
x1 <- seq(-4, -qt5, 0.01)
y1 <- f(x1)
x2 <- seq(-qt5, qt5, 0.01)
y2 <- f(x2)
x3 <- seq(qt5, 4, 0.01)
y3 <- f(x3)
plot(f, -4, 4, xlab="x", ylab="f(x)",
main="T-distribution probability density function f(x)")
polygon(c(-4, x1, -qt5), c(0, y1 , 0), col="red")
polygon(c(-qt5, x2, qt5), c(0, y2, 0), col="lightblue")
polygon(c(qt5, x3, 4), c(0, y3, 0), col = "red")

arrows(-3, 0.15, -3, 0.03)
text(-3, 0.23, "Rejection")
text(-3, 0.20, "Region")
text(-3, 0.17, expression(alpha/2))
arrows(3, 0.15, 3, 0.03)
text(3, 0.23, "Rejection")
text(3, 0.20, "Region")
text(3, 0.17, expression(alpha/2))
text(0, 0.23, "Acceptance")
text(0, 0.20, "Region")
mtext(expression(t[0.025]), side=1, at=-qt5, col="red")
mtext(expression(t[0.975]), side=1, at=qt5, col="red")
```



```
#' d :
#'
dnorm(0, mean = 0, sd = 1)
```

R에서 분포와 관련된 함수 정리

```
## [1] 0.3989423
```

```
#' p :
#'          q
pnorm(0, mean = 0, sd = 1)
```

```
## [1] 0.5
```

```
#' q :
#'          ,    p
qnorm(0.975, mean = 0, sd = 1)
```

```
## [1] 1.959964
```

```
qnorm(0.5, mean = 0, sd = 1)
```

```
## [1] 0
```

```
#' r :
#'           n
rnorm(10, mean = 0, sd = 1)
```

```
##  [1] -0.08509947  1.50777218  1.15628894 -1.13062098 -0.54257000 -0.22408926
##  [7]  0.85642403 -0.80121961 -1.57214688  0.59146546
```

```
x <- golub[2058, golubFactor=="ALL"]

mu0 <- 0

n <- 27

t.value <- sqrt(n)*(mean(x) - mu0)/sd(x)

t.value
```

```
## [1] 0.001076867
```

```
qt(0.975, 26)
```

```
## [1] 2.055529
```

```r
2 * pt(-abs(t.value), 26) # p-value
```

```
## [1] 0.999149
```

```r
alpha <- 0.05

mean(x) + c(qt(alpha/2, n-1), qt(1-alpha/2, n-1)) * sd(x)/sqrt(n)
```

```
## [1] -0.1024562  0.1025636
```

```r
t.test(x, mu=0) # default two side test
```

One Sample T-test를 위한 R function : **t.test(data= , mu= , alternative = )**

```
##
##  One Sample t-test
##
## data:  x
## t = 0.0010769, df = 26, p-value = 0.9991
## alternative hypothesis: true mean is not equal to 0
## 95 percent confidence interval:
##  -0.1024562  0.1025636
## sample estimates:
##   mean of x
## 5.37037e-05
```

$$H_1 : \mu > 0$$

```r
#' mu     p-v
t.test(x, mu=0, alternative = "greater") # one side test
```

```
##
##  One Sample t-test
##
## data:  x
## t = 0.0010769, df = 26, p-value = 0.4996
## alternative hypothesis: true mean is greater than 0
## 95 percent confidence interval:
##  -0.085006       Inf
## sample estimates:
##   mean of x
## 5.37037e-05
```

$$H_1 : \mu < 0$$

```r
#' mu     p-v
t.test(x, mu=0, alternative = "less") # one side test
```

```
##
##  One Sample t-test
##
## data:  x
## t = 0.0010769, df = 26, p-value = 0.5004
## alternative hypothesis: true mean is less than 0
## 95 percent confidence interval:
##        -Inf 0.08511341
## sample estimates:
##   mean of x
## 5.37037e-05
```

Example of One sample T-test

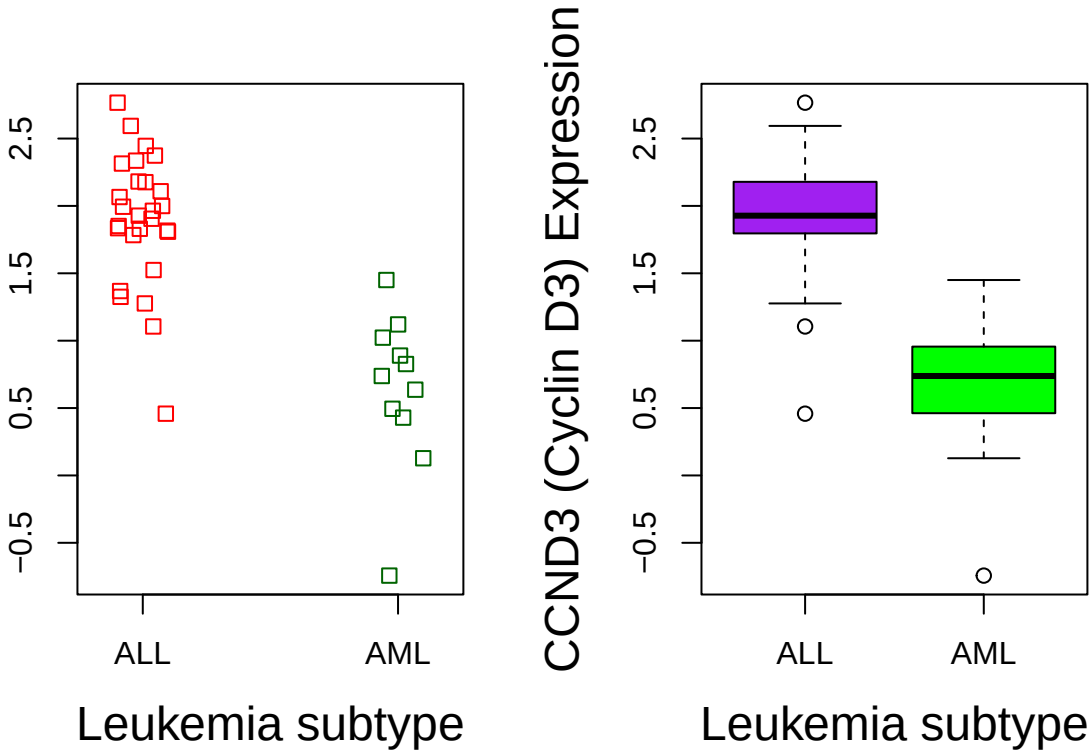
- Using a box-and-whiskers plot and a side-by-side plot, we can investigate the range of the ALL and AML gene expression values of CCND3 (CyclinD3), which are collected in row 1042 of the data matrix golub.

  그니깐 CCND3 gene에서 ALL vs AML을 하자.

```r
# gred()  CCND3 gene  index
ccnd3 <- grep("CCND3", golub.gnames[,2], ignore.case=TRUE)
ccnd3
```

```
## [1] 1042
```

```r
par(mfrow=c(1,2))
stripchart(golub[ccnd3,] ~ golubFactor, method="jitter", cex.lab=1.5, ylab="", vertical = TRUE, col=c("

boxplot(golub[ccnd3,] ~ golubFactor, cex.lab=1.5, main=NULL, xlab="Leukemia subtype", col=c("purple","g
```




```
ALL <- golubFactor=="ALL"

#' CCND3 gene  ALL      ..
#' H_0 = mu
t.test(golub[ccnd3, ALL], mu=0)
```

```
##
##  One Sample t-test
##
## data:  golub[ccnd3, ALL]
## t = 20.06, df = 26, p-value < 2.2e-16
## alternative hypothesis: true mean is not equal to 0
## 95 percent confidence interval:
##  1.699817 2.087948
## sample estimates:
## mean of x
##  1.893883
```

- t = 20.06 존나 큰 값 -> 무조건 기각 즉! ALL의 평균 값이 0이 아니다라고 할 수 있다.

```
t.test(golub[ccnd3, !ALL], mu=0)
```

```
##
##  One Sample t-test
##
## data:  golub[ccnd3, !ALL]
## t = 3.6249, df = 10, p-value = 0.004651
## alternative hypothesis: true mean is not equal to 0
```

```
## 95 percent confidence interval:
##  0.2449118 1.0262701
## sample estimates:
## mean of x
## 0.6355909
```

- 0.004이라서 기각이긴한데 살짝 부족함.

```
t.test(golub[ccnd3, ALL], mu=0, alternative="greater")
```

```
##
##  One Sample t-test
##
## data:  golub[ccnd3, ALL]
## t = 20.06, df = 26, p-value < 2.2e-16
## alternative hypothesis: true mean is greater than 0
## 95 percent confidence interval:
##  1.732853      Inf
## sample estimates:
## mean of x
##  1.893883
```

```
t.test(golub[ccnd3, !ALL], mu=0, alternative="greater") # two side p-v   .
```

```
##
##  One Sample t-test
##
## data:  golub[ccnd3, !ALL]
## t = 3.6249, df = 10, p-value = 0.002326
## alternative hypothesis: true mean is greater than 0
## 95 percent confidence interval:
##  0.3177962       Inf
## sample estimates:
## mean of x
## 0.6355909
```

```
t.test(golub[ccnd3, !ALL], mu=0, alternative="less")
```

```
##
##  One Sample t-test
##
## data:  golub[ccnd3, !ALL]
## t = 3.6249, df = 10, p-value = 0.9977
## alternative hypothesis: true mean is less than 0
## 95 percent confidence interval:
##       -Inf 0.9533856
## sample estimates:
## mean of x
## 0.6355909
```

```
t.test(golub[ccnd3, ALL], mu=0, alternative="less")
```

```
##
##  One Sample t-test
##
## data:  golub[ccnd3, ALL]
## t = 20.06, df = 26, p-value = 1
## alternative hypothesis: true mean is less than 0
## 95 percent confidence interval:
##      -Inf 2.054912
## sample estimates:
## mean of x
##  1.893883
```

Two Sample T-test with Unequal Variances(welch's t-test)

> 예들 들어 CCND3 gene을 이용해서 Two Sample T-test를 한다고 하자..
> 그럼 CCND3 gene에소 ALL vs AML인 그룹의 평균 gene expression을 비교할것이다.
> 만약 귀무가설이 reject되면 이 CCND3 gene은 ALL, AML를 구별하는데 도움을 주는 gene이 될 것이다..!!

- 두그룹의 평균을 비교할 때 사용한다.
- Two Sample T test를 진행하기 위해선

  1. 각 그룹 샘플에 대한 정규성 검정 실시

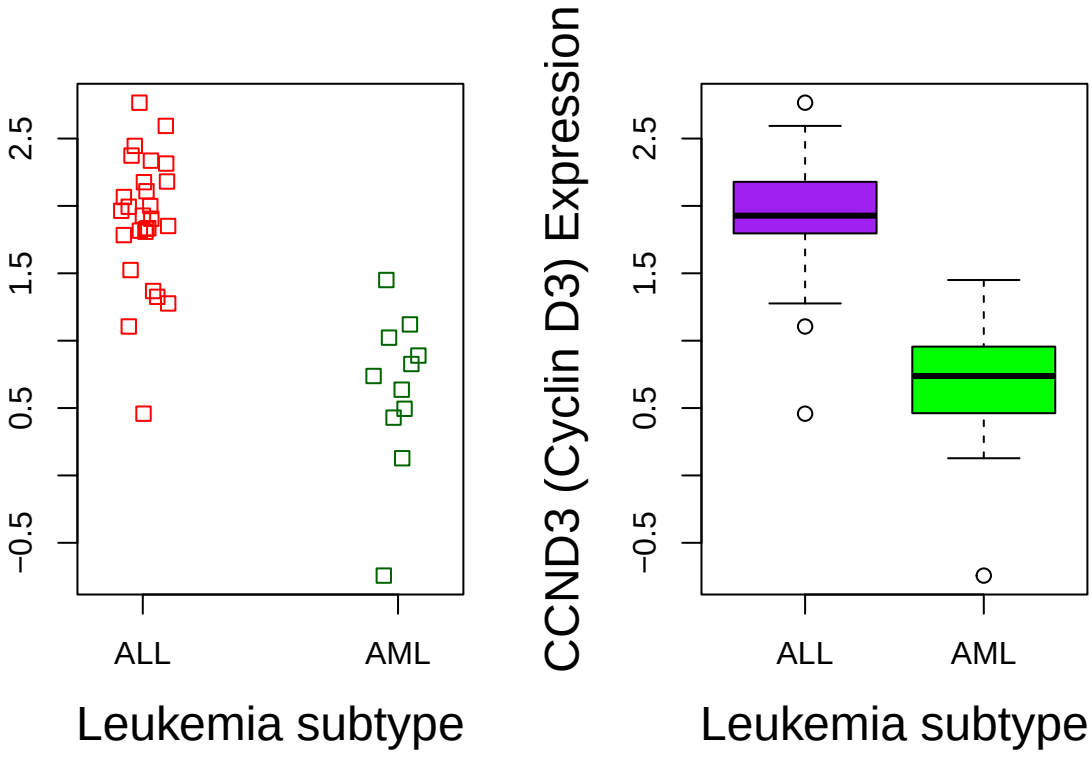
     안 따르면 비모수 검정으로 진행⊠ two sample t-test할려면 무조건 일단은 각 그룹이 정규성을 따라야한다.

  1. Equal variance에 대한 검정 실시
  2. 분산이 같다면 pooled t-test를 실시
  3. 같지 않다면 welch's t-test를 실시(general version)
  4. 그 다음 상황에 맞는 t test를 진행한다.

```
ccnd3 <- grep("CCND3", golub.gnames[,2], ignore.case=TRUE)
ccnd3
```

```
## [1] 1042
```

```
par(mfrow=c(1,2))
stripchart(golub[ccnd3,] ~ golubFactor, method="jitter", cex.lab=1.5, ylab="", vertical = TRUE, col=c("

boxplot(golub[ccnd3,] ~ golubFactor, cex.lab=1.5, main=NULL, xlab="Leukemia subtype", col=c("purple","g
```




```
data.frame(golub[ccnd3,], y =golubFactor)
```

```
##    golub.ccnd3...   y
## 1         2.10892 ALL
## 2         1.52405 ALL
## 3         1.96403 ALL
## 4         2.33597 ALL
## 5         1.85111 ALL
## 6         1.99391 ALL
## 7         2.06597 ALL
## 8         1.81649 ALL
## 9         2.17622 ALL
## 10        1.80861 ALL
## 11        2.44562 ALL
## 12        1.90496 ALL
## 13        2.76610 ALL
## 14        1.32551 ALL
## 15        2.59385 ALL
## 16        1.92776 ALL
## 17        1.10546 ALL
## 18        1.27645 ALL
## 19        1.83051 ALL
## 20        1.78352 ALL
## 21        0.45827 ALL
## 22        2.18119 ALL
## 23        2.31428 ALL
## 24        1.99927 ALL
## 25        1.36844 ALL
## 26        2.37351 ALL
```

```
## 27        1.83485 ALL
## 28        0.88941 AML
## 29        1.45014 AML
## 30        0.42904 AML
## 31        0.82667 AML
## 32        0.63637 AML
## 33        1.02250 AML
## 34        0.12758 AML
## 35       -0.74333 AML
## 36        0.73784 AML
## 37        0.49470 AML
## 38        1.12058 AML
```

```
t.test(golub[ccnd3,] ~ golubFactor, var.equal=FALSE) # result = >       ~
```

R Function for Unequal Variances in two sample t-test : t.test(data ~ groupfactor, var.equal = FALSE)

```
##
##  Welch Two Sample t-test
##
## data:  golub[ccnd3, ] by golubFactor
## t = 6.3186, df = 16.118, p-value = 9.871e-06
## alternative hypothesis: true difference in means between group ALL and group AML is not equal to 0
## 95 percent confidence interval:
##  0.8363826 1.6802008
## sample estimates:
## mean in group ALL mean in group AML
##         1.8938826         0.6355909
```

- From the result, we can say that the data provide strong evidence that the population means do differ.

```
t.test(golub[ccnd3,] ~ golubFactor, var.equal=FALSE, alternative="greater")
```

```
##
##  Welch Two Sample t-test
##
## data:  golub[ccnd3, ] by golubFactor
## t = 6.3186, df = 16.118, p-value = 4.935e-06
## alternative hypothesis: true difference in means between group ALL and group AML is greater than 0
## 95 percent confidence interval:
##  0.9107706       Inf
## sample estimates:
## mean in group ALL mean in group AML
##         1.8938826         0.6355909
```

```
t.test(golub[ccnd3,] ~ golubFactor, var.equal=FALSE, alternative="less")
```

```
##
##  Welch Two Sample t-test
##
## data:  golub[ccnd3, ] by golubFactor
## t = 6.3186, df = 16.118, p-value = 1
## alternative hypothesis: true difference in means between group ALL and group AML is less than 0
## 95 percent confidence interval:
##      -Inf 1.605813
## sample estimates:
## mean in group ALL mean in group AML
##         1.8938826         0.6355909
```

Two Sample T-test with Equal Variances

- 두 집단의 분산에 대한 검정 결과로 $H_0$를 기각하지 못하면 즉, 두 집단의 분산이 같으면 pooled sample variance를 이용한 pooled t-test를 실시.

```
t.test(golub[ccnd3,] ~ golubFactor, var.equal=TRUE)
```

```
t.test(data ~ groupFactor, var.equal=TRUE)
```

```
##
##  Two Sample t-test
##
## data:  golub[ccnd3, ] by golubFactor
## t = 6.7983, df = 36, p-value = 6.046e-08
## alternative hypothesis: true difference in means between group ALL and group AML is not equal to 0
## 95 percent confidence interval:
##  0.8829143 1.6336690
## sample estimates:
## mean in group ALL mean in group AML
##         1.8938826         0.6355909
```

Test for Equal Variances

- F 분포로 조지는 놈!
- Extremely sensitive to non-normality!

```
ccnd3 <- grep("CCND3", golub.gnames[,2], ignore.case=TRUE)
zyxin <- grep("Zyxin", golub.gnames[,2], ignore.case=TRUE)
```

```
#
#' tapply              .
tapply(golub[ccnd3,], golubFactor, var)
```

```
##       ALL       AML
## 0.2406642 0.3381806
```

```
tapply(golub[zyxin,], golubFactor, var)
```

```
##       ALL       AML
## 0.5224983 0.1351442
```

```
var.test(golub[ccnd3, ] ~ golubFactor) #              ->pooled
```

R function for variance test : **var.test(data~groupFactor)**

```
##
##  F test to compare two variances
##
## data:  golub[ccnd3, ] by golubFactor
## F = 0.71164, num df = 26, denom df = 10, p-value = 0.4652
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.2127735 1.8428387
## sample estimates:
## ratio of variances
##          0.7116441
```

```
var.test(golub[zyxin, ] ~ golubFactor) #              ->pooled    .. welch's
```

```
##
##  F test to compare two variances
##
## data:  golub[zyxin, ] by golubFactor
## F = 3.8662, num df = 26, denom df = 10, p-value = 0.02968
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##   1.155958 10.011795
## sample estimates:
## ratio of variances
##           3.866228
```

- 분산에 대한 검정은 F검정 말고도 여러 종류가 있다! 참고!!

```
bartlett.test(golub[ccnd3,] ~ golubFactor)
```

```
##
##  Bartlett test of homogeneity of variances
##
## data:  golub[ccnd3, ] by golubFactor
## Bartlett's K-squared = 0.42236, df = 1, p-value = 0.5158
```

```
bartlett.test(golub[zyxin,] ~ golubFactor)
```

```
##
##  Bartlett test of homogeneity of variances
##
## data:  golub[zyxin, ] by golubFactor
## Bartlett's K-squared = 5.036, df = 1, p-value = 0.02483
```

```
fligner.test(golub[ccnd3,], golubFactor)
```

```
##
##  Fligner-Killeen test of homogeneity of variances
##
## data:  golub[ccnd3, ] and golubFactor
## Fligner-Killeen:med chi-squared = 0.14115, df = 1, p-value = 0.7071
```

```
fligner.test(golub[zyxin,], golubFactor)
```

```
##
##  Fligner-Killeen test of homogeneity of variances
##
## data:  golub[zyxin, ] and golubFactor
## Fligner-Killeen:med chi-squared = 7.2671, df = 1, p-value = 0.007023
```

```
library(car)
leveneTest(golub[ccnd3,], golubFactor)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  1  0.1336 0.7169
##       36
```

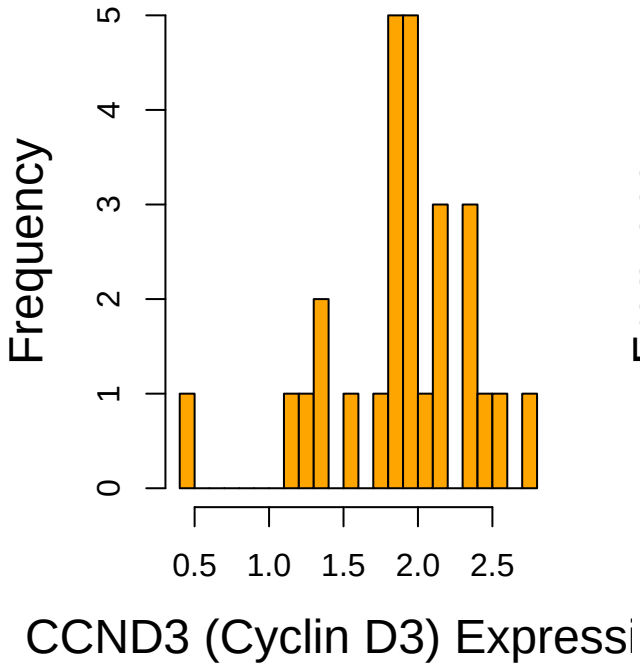
```
leveneTest(golub[zyxin,], golubFactor)
```

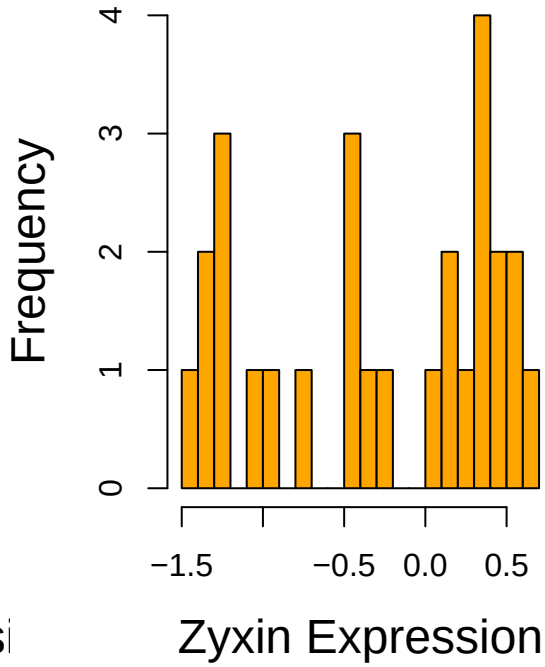
```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value   Pr(>F)
## group  1  9.2119 0.004448 **
##       36
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Histogram and Q-Q plot(정규성 검정)

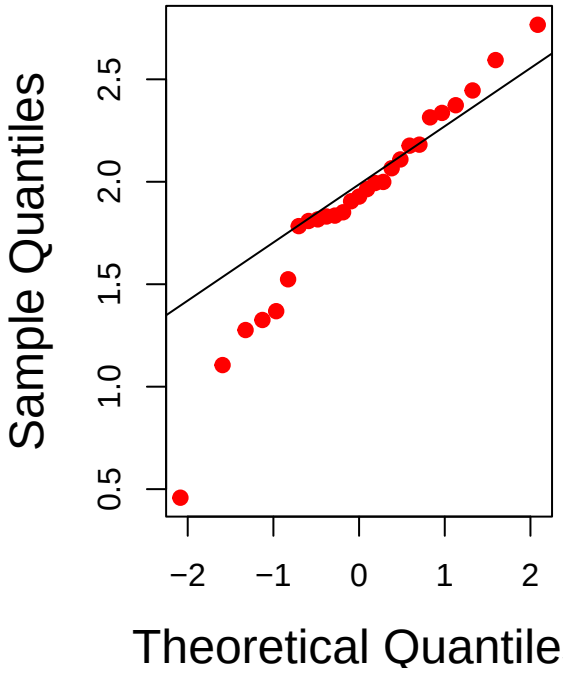
- Various procedures are available to test the hypothesis that a dataset is normally distributed. Many statistical tests including T-test and F-test require normality assumption.

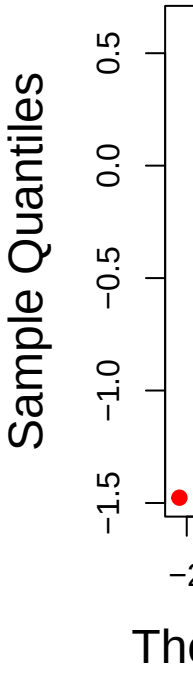
```
# histogram
par(mfrow=c(1,2))
hist(golub[ccnd3, golubFactor=="ALL"], cex.lab=1.5, col="orange",nclass=20, main=NULL, xlab="CCND3 (Cyc
hist(golub[zyxin, golubFactor=="ALL"], cex.lab=1.5, col="orange",nclass=20, main=NULL, xlab="Zyxin Expr
```




```r
# q-q plot
par(mfrow=c(1,2))
qqnorm(golub[ccnd3, golubFactor=="ALL"], pch=19, cex.lab=1.5, col="red", main=NULL)
qqline(golub[ccnd3, golubFactor=="ALL"])

qqnorm(golub[zyxin, golubFactor=="ALL"], pch=19, cex.lab=1.5,col="red", main=NULL)
qqline(golub[zyxin, golubFactor=="ALL"])
```




R function for q-q ploting : `qqnorm() & qqpline()`

Normality Tests

$$H_0 : \text{정규성을 따른다..!}$$

- Normality Tests는 각 집단 별로 다해야한다!!! -> 베리 중요

```
#' Shapiro Wilk test - > based on Q-Q plot
#' Anderson-Darling test -> based on dist of data
#'           !!

shapiro.test(golub[ccnd3, golubFactor == 'ALL'])
```

R function for Normality Test

```
##
##  Shapiro-Wilk normality test
##
## data:  golub[ccnd3, golubFactor == "ALL"]
## W = 0.94663, p-value = 0.1774
```

```
shapiro.test(golub[ccnd3, golubFactor == 'AML'])
```

```
##
##  Shapiro-Wilk normality test
##
## data:  golub[ccnd3, golubFactor == "AML"]
## W = 0.91756, p-value = 0.2989
```

```
shapiro.test(golub[zyxin, golubFactor == 'ALL']) # -> H_0  ! ->    x
```

```
##
##  Shapiro-Wilk normality test
##
## data:  golub[zyxin, golubFactor == "ALL"]
## W = 0.89198, p-value = 0.00881
```

```
shapiro.test(golub[zyxin, golubFactor == 'AML'])
```

```
##
##  Shapiro-Wilk normality test
##
## data:  golub[zyxin, golubFactor == "AML"]
## W = 0.96378, p-value = 0.8178
```

```
#' Anderson-Darling test

library(nortest)

ad.test(golub[ccnd3, golubFactor == 'ALL'])
```

```
##
##  Anderson-Darling normality test
##
## data:  golub[ccnd3, golubFactor == "ALL"]
## A = 0.52154, p-value = 0.1683
```

```
ad.test(golub[ccnd3, golubFactor == 'AML'])
```

```
##
##  Anderson-Darling normality test
##
## data:  golub[ccnd3, golubFactor == "AML"]
## A = 0.39702, p-value = 0.3056
```

```
ad.test(golub[zyxin, golubFactor == 'ALL']) # -> H_0  ! ->   x
```

```
##
##  Anderson-Darling normality test
##
## data:  golub[zyxin, golubFactor == "ALL"]
## A = 1.013, p-value = 0.009583
```

```
ad.test(golub[zyxin, golubFactor == 'AML'])
```

```
##
##  Anderson-Darling normality test
##
## data:  golub[zyxin, golubFactor == "AML"]
## A = 0.1694, p-value = 0.9092
```

Outliers Test

- gene expression values가 not normally distributed 할 때 outlier들이 있을 확률이 매우 높다.

$$H_0$$ : Does not contain an outlier

- 이상치 검정을 위해 Grubbs test를 진행!

```
library(outliers)

#'        alter hypo    !

grubbs.test(golub[ccnd3, golubFactor == 'ALL'])
```

```
##
##  Grubbs test for one outlier
##
## data:  golub[ccnd3, golubFactor == "ALL"]
## G = 2.92639, U = 0.65796, p-value = 0.0183
## alternative hypothesis: lowest value 0.45827 is an outlier
```

```
grubbs.test(golub[ccnd3, golubFactor == 'AML'])
```

```
##
##  Grubbs test for one outlier
##
## data:  golub[ccnd3, golubFactor == "AML"]
## G = 2.37118, U = 0.38152, p-value = 0.02251
## alternative hypothesis: lowest value -0.74333 is an outlier
```

```
grubbs.test(golub[zyxin, golubFactor == 'ALL']) #      !!
```

```
##
##  Grubbs test for one outlier
##
## data:  golub[zyxin, golubFactor == "ALL"]
## G = 1.63480, U = 0.89326, p-value = 1
## alternative hypothesis: lowest value -1.47649 is an outlier
```

```
grubbs.test(golub[zyxin, golubFactor == 'AML'])
```

```
##
##  Grubbs test for one outlier
##
## data:  golub[zyxin, golubFactor == "AML"]
## G = 1.62212, U = 0.71056, p-value = 0.4829
## alternative hypothesis: highest value 2.18299 is an outlier
```

```
golub[ccnd3, golubFactor == 'ALL']
```

```
##  [1] 2.10892 1.52405 1.96403 2.33597 1.85111 1.99391 2.06597 1.81649 2.17622
## [10] 1.80861 2.44562 1.90496 2.76610 1.32551 2.59385 1.92776 1.10546 1.27645
## [19] 1.83051 1.78352 0.45827 2.18119 2.31428 1.99927 1.36844 2.37351 1.83485
```

- 이상값이 있거나 분포가 크게 왜곡된 경우, 순위 기반(비모수) 검정, 강건추정(robust estimation) 기법 등이 대안

Binomial Test

- 이항분포 기반 가설검정은 특정 사건이 일어날 확률 $p$가 이론적으로 가정된 값 $p_0$와 같은지를 통계적으로 검정
- 예 : 어떤 microRNA 서열에서 퓨린이 나타날 확률을 $p_0$라고 가정하고, 실제로 관측된 퓨린 비율이 $p_0$와 다른지 판단.

  어떤 연구에서 길이 22nt의 microRNA가 있다고 하자. 이 microRNA 서열에서 퓨린이 실제로 18개 관측되었다. 나는 "이 microRNA에서 퓨린이 나타날 확률이 $p_0 = 0.7$ 이상일 것이다!" 라는 가설을 세웠다.

$$H_0 : p = 0.7$$

$$H_1 : p > 0.7$$

그럼 p-value는 다음과 같이 계산된다.

$$p\text{-value} \;=\; P(X \geq 18) \;=\; \sum_{x=18}^{22} \binom{22}{x} (0.7)^x \, (0.3)^{22-x}$$

등호 넣는게 매우 중요하다..!

```
sum(dbinom(18:22, 22, 0.7)) # d => pdf value
```

```
## [1] 0.1645488
```

```
1 - pbinom(17, 22, 0.7) # p => cdf
```

```
## [1] 0.1645488
```

```
#' Example : A microRNA of length 22 contains 18 purines.
#' Null hypo - > H_0 : p = 0.7

binom.test(18, 22, p=0.7, alternative="greater", conf.level=0.95)
```

R function for binomtest : **binon.test()**

```
##
##  Exact binomial test
##
## data:  18 and 22
## number of successes = 18, number of trials = 22, p-value = 0.1645
## alternative hypothesis: true probability of success is greater than 0.7
## 95 percent confidence interval:
##  0.6309089 1.0000000
## sample estimates:
## probability of success
##              0.8181818
```

Chi-squared Test

- 카이제곱 검정은 범주형 자료의 빈도분포가 특정 이론적 분포와 일치하는지, 혹인 범주형 변수들이 서로 독립적인지를 평가하기 위한 검정 방법이다.

1. 적합도 검정

- 표본에서 관측된 범주별 빈도 분포가, 연구자가 가정한 분포와 같은지를 검정
- 예: A, C, G, T가 동일 확률(각 1/4)로 나타나는지 여부 확인

$$H_0 : (\pi_1, ..., \pi_m) = (p_1, ..., p_m)$$

2. 독립성 검정

- 두 범주형 변수가 서로 독립인지를 검정
- 질병 유전자형과 임상 그룹이 독립인지, 혹은 연관이 있는지
- 기본 아이디어

1. 관측도수(Observed count) : $o_i$

- 범주 $i$에서 실제로 관측된 빈도(개수)

2. 기대도수(Expected count) : $e_i$

- 귀무가설이 참일 때, 범주 $i$에서 기대되는 또는 이론적으로 예측되는 빈도
- 예를 들어, 총 표본 크기를 $n$이라 하고, 각 범주에 대한 가정 확률(이론 확률 $p_0$ 느낌)을 $p_1, .., p_m$이라 한다면,

$$e_i = np_i$$

3.검정방법

- 카이제곱 검정통계량을 구하고 $q$ 해당하는 p=value가 유의수준보다 작으면 귀무가설을 기각.

```
library(ape)
obs <- table(read.GenBank(c("X94991.1"),as.character=TRUE))
obs
```

```
## X94991.1
##   a   c   g   t
## 410 789 573 394
```

```
e <- rep(sum(obs)/4, 4) # n(   ) * 1/4 (p_i)
e
```

```
## [1] 541.5 541.5 541.5 541.5
```

```
test <- sum((obs-e)^2/e)
test
```

```
## [1] 187.0674
```

```
1-pchisq(test, 3)
```

```
## [1] 0
```

```
qchisq(0.95, 3)
```

```
## [1] 7.814728
```

```r
chisq.test(obs)
```

```
##
##  Chi-squared test for given probabilities
##
## data:  obs
## X-squared = 187.07, df = 3, p-value < 2.2e-16
```

```r
pi <- c(0.75,0.25)
x <- c(5474, 1850)
chisq.test(x, p=pi)
```

```
##
##  Chi-squared test for given probabilities
##
## data:  x
## X-squared = 0.26288, df = 1, p-value = 0.6081
```

```r
#
library(ape)
obs <- table(read.GenBank(c("X94991.1"),as.character=TRUE))
obs
```

```
## X94991.1
##   a   c   g   t
## 410 789 573 394
```

```r
e = rep(sum(obs) / 4, 4) # expected count
test = sum((obs-e)^2/e)

test
```

```
## [1] 187.0674
```

```r
1-pchisq(test,3)
```

```
## [1] 0
```

```r
qchisq(0.95,3)
```

```
## [1] 7.814728
```

```r
chisq.test(obs)
```

```
##
##  Chi-squared test for given probabilities
##
## data:  obs
## X-squared = 187.07, df = 3, p-value < 2.2e-16
```

```r
# observed count     !! table
# pi       default          !

pi = c(0.75, 0.25)
x = c(5474, 1850)
chisq.test(x, p = pi)
```

```
##
##  Chi-squared test for given probabilities
##
## data:  x
## X-squared = 0.26288, df = 1, p-value = 0.6081
```

Confusion Matrix (46쪽 필기)

- 가로축(열, True)에는 실제 참값('진짜' 레이블), 세로축(행, Predict)에는 예측한 레이블
- 예: 백혈병 데이터에서 실제 유형(ALL, AML)과 모델이 예측한 유형(ALL, AML)을 2×2 표로 정리
    - TP (True Positive): 실제가 ALL이고, 예측도 ALL
    - TN (True Negative): 실제가 AML이고, 예측도 AML
    - FP (False Positive): 실제가 AML인데 ALL로 잘못 예측
    - FN (False Negative): 실제가 ALL인데 AML로 잘못 예측

Testing Indepnedence

1. Cutoff 설정: 유전자 발현값이나 어떤 점수 등에 임의의 임계값을 정해, 이를 기준으로 예측
2. Confusion matrix 구성
3. 독립성 검정 수행

$H_0$ : cutoff 쓰레기, 실제 레이블과 예측 레이블이 독립이다.

$H_1$ : 성능 좋노!, 실제 레이블과 예측 레이블이 연관이 있다.

```r
data(golub, package = "multtest")
golubFactor <- factor(golub.cl, levels=0:1, labels=c("ALL","AML"))
gdf5 <- grep("Gdf5", golub.gnames[,2], ignore.case=TRUE)
```

```r
x <- golub[gdf5, ]

# cutoff
cutoff <- 0.1

# cutoff
pred <- ifelse(x < cutoff, "ALL", "AML")
data.frame(predicted=pred, true=golubFactor)
```

```
##    predicted true
## 1        AML  ALL
## 2        ALL  ALL
## 3        AML  ALL
## 4        ALL  ALL
## 5        ALL  ALL
## 6        ALL  ALL
## 7        AML  ALL
## 8        AML  ALL
## 9        ALL  ALL
## 10       ALL  ALL
## 11       ALL  ALL
## 12       ALL  ALL
## 13       ALL  ALL
## 14       ALL  ALL
## 15       ALL  ALL
## 16       AML  ALL
## 17       ALL  ALL
## 18       AML  ALL
## 19       ALL  ALL
## 20       ALL  ALL
## 21       ALL  ALL
## 22       ALL  ALL
## 23       AML  ALL
## 24       ALL  ALL
## 25       AML  ALL
## 26       ALL  ALL
## 27       AML  ALL
## 28       ALL  AML
## 29       ALL  AML
## 30       AML  AML
## 31       ALL  AML
## 32       ALL  AML
## 33       AML  AML
## 34       ALL  AML
## 35       AML  AML
## 36       ALL  AML
## 37       AML  AML
## 38       AML  AML
```

```
table(predicted=pred, true=golubFactor)
```

```
##          true
## predicted ALL AML
##       ALL  18   6
##       AML   9   5
```

```
chisq.test(table(pred, golubFactor))
```

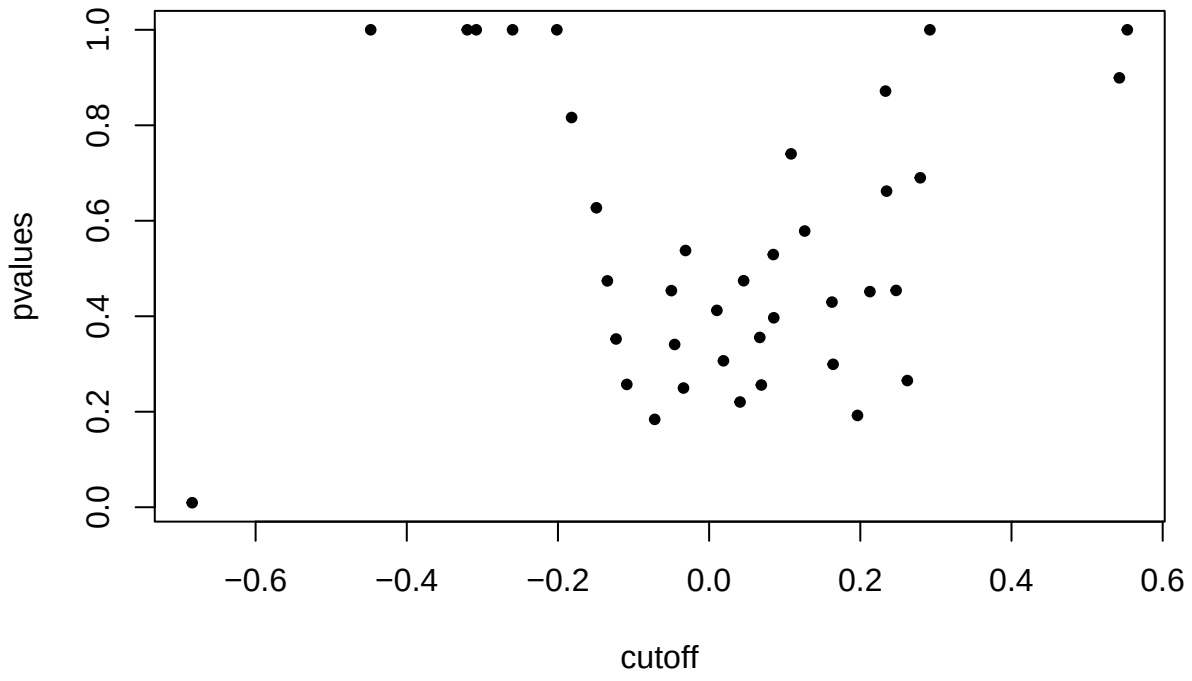
```
##
##  Pearson's Chi-squared test with Yates' continuity correction
##
## data:  table(pred, golubFactor)
```

```
## X-squared = 0.11005, df = 1, p-value = 0.7401
```

```
cutoff <- sort(x)
pval <- 0
```

```
for (i in 1:length(cutoff)) {
  pred <- ifelse(x < cutoff[i], "ALL", "AML")
  pval[i] <- chisq.test(table(pred, golubFactor))$p.val
  }
```

```
plot(cutoff, pval, type="p", pch=20, ylab="pvalues")
```



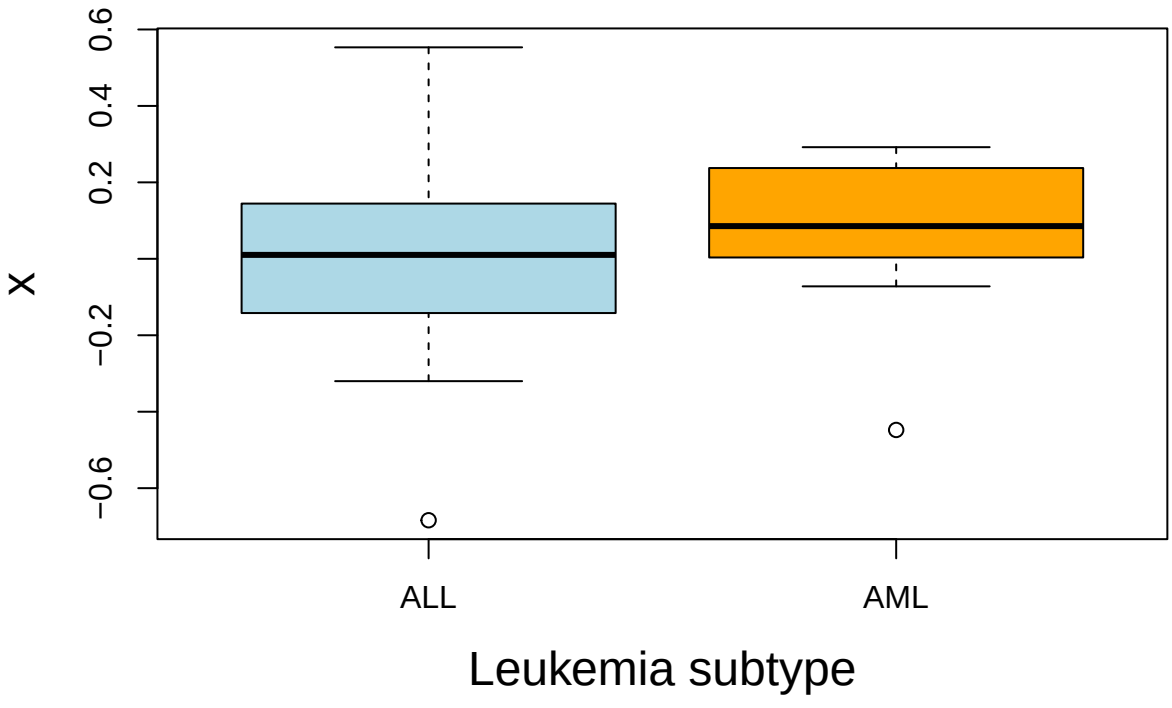

```
cutoff[pval < 0.05]
```

```
## [1] -0.6839
```

```
pred <- ifelse(x < cutoff[1], "ALL", "AML")
table(predicted=pred, true=golubFactor)
```

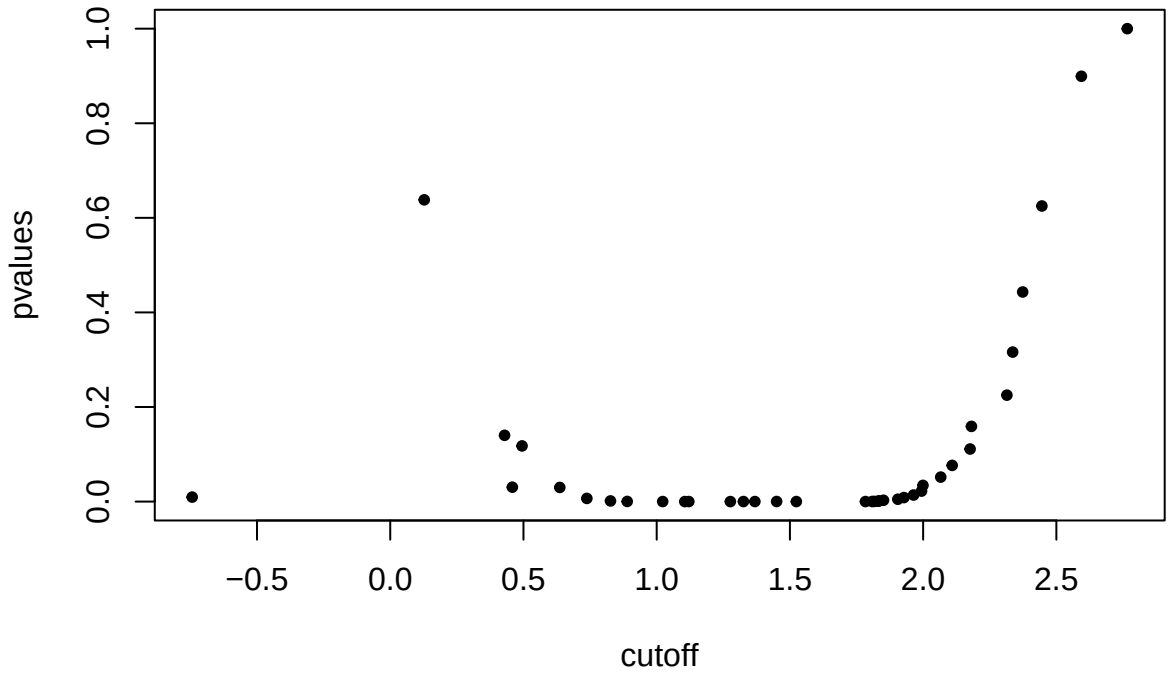
```
##          true
## predicted ALL AML
##       AML  27  11
```

```
boxplot(x ~ golubFactor, cex.lab=1.5, main=NULL,
        xlab="Leukemia subtype", col=c("lightblue", "orange"))
```




```
ccnd3 <- grep("CCND3", golub.gnames[,2], ignore.case=TRUE)
x2 <- golub[ccnd3, ]
```

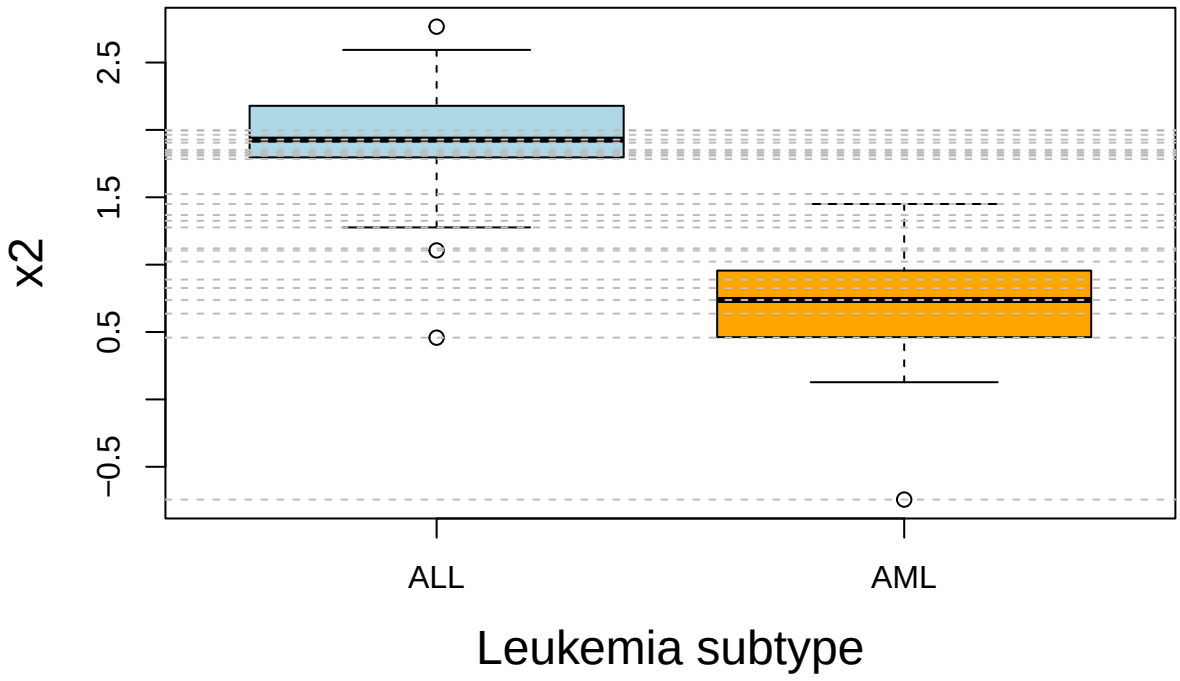
```
cutoff <- sort(x2)
pval2 <- 0
for (i in 1:length(cutoff)) {
  pred2 <- ifelse(x2 < cutoff[i], "ALL", "AML")
  pval2[i] <- chisq.test(table(pred2, golubFactor))$p.val
}

plot(cutoff, pval2, type="p", pch=20, ylab="pvalues")
```




```
cutoff[pval2 < 0.05]
```

```
##  [1] -0.74333  0.45827  0.63637  0.73784  0.82667  0.88941  1.02250  1.10546
##  [9]  1.12058  1.27645  1.32551  1.36844  1.45014  1.52405  1.78352  1.80861
## [17]  1.81649  1.83051  1.83485  1.85111  1.90496  1.92776  1.96403  1.99391
## [25]  1.99927
```

```
boxplot(x2 ~ golubFactor, cex.lab=1.5, main=NULL, xlab="Leukemia subtype",
        col=c("lightblue", "orange"))
abline(h=cutoff[pval2 < 0.05], lty=2, col="gray")
```



Fisher's Exact Test

- Sample이 작을 때 쓰는 Chi-Squared test의 대안.

```
data = matrix(c(100, 1900, 300, 5700), 2, byrow = TRUE)
data
```

```
##      [,1] [,2]
## [1,]  100 1900
## [2,]  300 5700
```

```
odr = (100 * 5700) / (1900 * 300)
```

```
fisher.test(data)
```

```
##
##  Fisher's Exact Test for Count Data
##
## data:  data
## p-value = 1
## alternative hypothesis: true odds ratio is not equal to 1
## 95 percent confidence interval:
##  0.7845883 1.2660152
## sample estimates:
## odds ratio
##          1
```

```
data <- matrix(c(300,500,3000,7000), 2, byrow=TRUE)
data
```

```
##      [,1] [,2]
## [1,]  300  500
## [2,] 3000 7000
```

- odds ratio -≫ 1이면 독립이다.

```
odr = (300 * 7000) / (500 * 3000)
odr
```

```
## [1] 1.4
```

```
fisher.test(data)
```

```
##
##  Fisher's Exact Test for Count Data
##
## data:  data
## p-value = 1.336e-05
## alternative hypothesis: true odds ratio is not equal to 1
## 95 percent confidence interval:
##  1.201492 1.629240
## sample estimates:
## odds ratio
##   1.399977
```

## Fisher's Exact Test

- Example: Oncogenes on chromosome 1. Suppose that the number of significant oncogenes in Chromosome 1 is $n_{11} = 100$ out of a total of 2,000, and the number of significant oncogenes in the whole genome is $n_{21} = 300$ out of a total of 6,000. ⇒Chromo 1 에서 유의한 암유전자 비율이 전체 유전자 genome 에서의 비율과 같은?

| | Significant genes | Non-significant genes |
|---|---|---|
| Chromosome 1 | 100 | 1,900 |
| Genome | 300 | 5,700 |

- Then, the odds ratio

$H_0$ : Odds Ratio = 1 → 같은 비율 가정치
$H_1$ : odds " ≠ 1 → 다른 "

$$\text{OR} = \frac{100 \cdot 5700}{300 \cdot 1900} = 1$$

So, the number of significant oncogenes in Chromosome 1 is exactly proportional to that in the genome.

- The null hypothesis of the Fisher's test is that the odds ratio equals 1 and the alternative hypothesis that it differs from 1.

## Fisher's Exact Test

- Now suppose that the frequencies of significant oncogenes for Chromosome 1 equals $n_{11} = 300$ out of a total of 800, and for the genome $n_{21} = 3{,}000$ out of 10,000:

| | Significant genes | Non-significant |
|---|---|---|
| Chromosome 1 | 300 | 500 |
| Genome | 3,000 | 7,000 |

- With the new values, it's not so clear whether or not oncogenes are significantly over- or under-represented on chromosome 1 compared to the entire genome. However, we can test this hypothesis by testing if the odds ratio is significantly close to 1 as follows:

```
data <- matrix(c(300,500,3000,7000), 2, byrow=TRUE)
fisher.test(data)
```

- There are more significant oncogenes in Chromosome 1 than compared to that in the whole genome. ⇒ p-v < α=0.05 → reject $H_0$
  odds ratio > 1

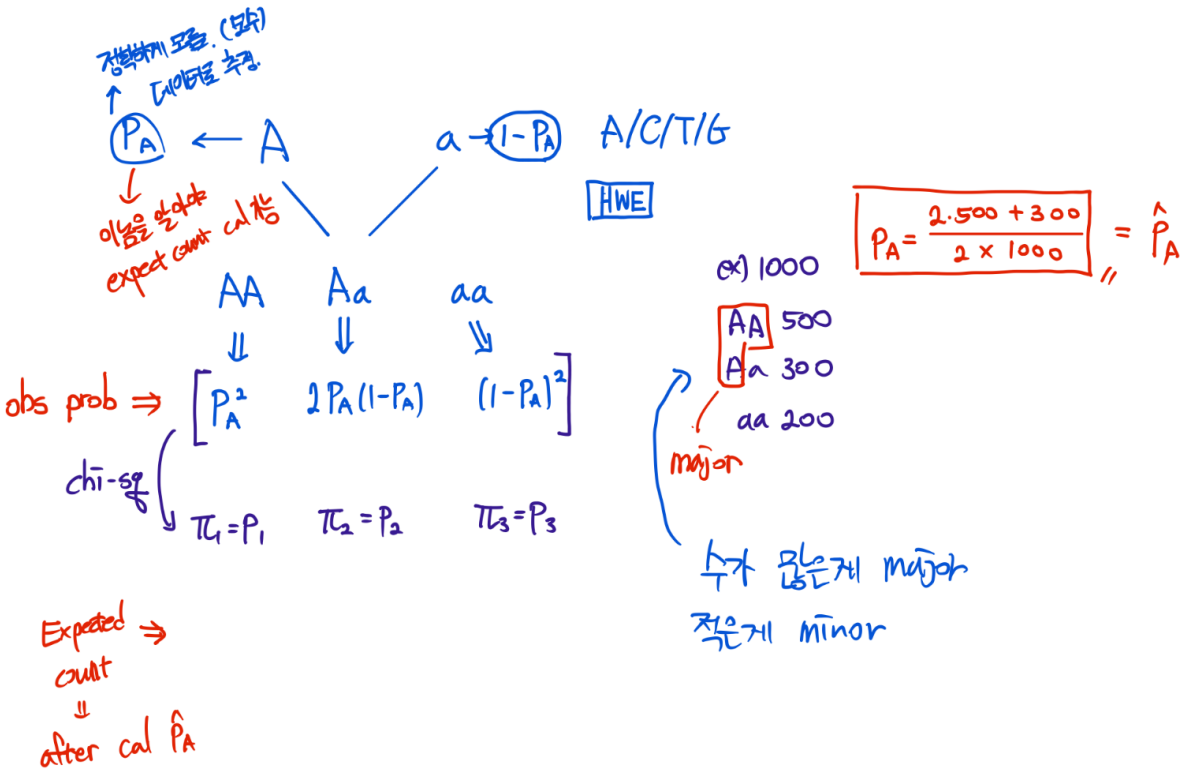
52 / 84

Hardy Weinberg Equilibrium

- Allele frequencies are constant within a popualtion over generations

  Allele 빈도는 세대가 변해도 유지된다..!

- Test of HWE
  - Pearson's $\chi^2$ test
  - Fisher's exact test
- When HWE exists, genotype freq only depend on allele freq.




```
# example
set.seed(202211545)
n <- 10000
F <- runif(1, 0.1, 0.5)
C <- sample(0:1, 2*n, prob=c(F, 1-F), replace=TRUE)
Geno <- apply(matrix(C, ncol=2), 1, sum)

#' Geno denotes the number of major alleles. No major alleles are coded as 0, only 1 major allele as 1
#' Minor allele freq(MAF) is F

#' Estimate MAF - > P_a => minor allele freq, P_A => major allele freq
#' a          -> table()      ?
table(Geno)[1] # aa
```

```
##   0
## 648
```

```
table(Geno)[2] # Aa
```

```
##    1
## 3839
```

```
MAF = (table(Geno)[1] * 2 + table(Geno)[2] * 1) / (n * 2)
round(MAF, 4)
```

```
##      0
## 0.2568
```

```r
#' Chi-squ test gogo
table(Geno)
```

```
## Geno
##    0    1    2
##  648 3839 5513
```

```r
# expect count
Exp = c(n * MAF^2, 2 * n * MAF * (1 - MAF), n * (1 -MAF)^2) # Aa => Aa, aA -> 2

# Chi-sqr test
sum((table(Geno) -  Exp)^2 / Exp )
```

```
## [1] 0.3448113
```

```r
round(sum((table(Geno) -  Exp)^2 / Exp ), 4)
```

```
## [1] 0.3448
```

```r
t = c(MAF^2, 2*MAF*(1-MAF), (1-MAF)^2)
chisq.test(table(Geno), p = t)
```

```
##
##  Chi-squared test for given probabilities
##
## data:  table(Geno)
## X-squared = 0.34481, df = 2, p-value = 0.8416
```

```r
pi = c(F^2, 2*F*(1-F), (1-F)^2)
chisq.test(table(Geno), p = pi)
```

```
##
##  Chi-squared test for given probabilities
##
## data:  table(Geno)
## X-squared = 1.4844, df = 2, p-value = 0.4761
```

```r
table(Geno)
```

```
## Geno
##    0    1    2
##  648 3839 5513
```

```r
#########################################################################################################

library(genetics)

# Geno
# 0 = aa, 1 = Aa, 2 = AA
```

```r
geno_vec = rep(NA, length(Geno))
geno_vec[Geno == 0] = "aa"
geno_vec[Geno == 1] = "Aa"
geno_vec[Geno == 2] = "AA"

geno_factor = genotype(geno_vec, sep = "")

# Fisher's exact HWE test (genetics   )
HWE.exact(geno_factor)
```

```
##
##  Exact Test for Hardy-Weinberg Equilibrium
##
## data:  geno_factor
## N11 = 5513, N12 = 3839, N22 = 648, N1 = 14865, N2 = 5135, p-value =
## 0.5644
```

```r
HWE.chisq(geno_factor)
```

```
##
##  Pearson's Chi-squared test with simulated p-value (based on 10000
##  replicates)
##
## data:  tab
## X-squared = 0.34481, df = NA, p-value = 0.5529
```

Asthma SNP Data

```r
library(SNPassoc)
data(asthma, package = "SNPassoc")
```

```r
dim(asthma)
```

```
## [1] 1578   57
```

```r
#str(asthma)
#lapply(asthma[,-c(1:6)], table)
```

```r
snp1 <- asthma$rs2274276
class(snp1)
```

```
## [1] "factor"
```

```r
summary(snp1)
```

```
##   CC   GC   GG NA's
##  296  758  514   10
```

```r
table(snp1) # c is major and g is minor
```

```
## snp1
##  CC  GC  GG
## 296 758 514
```

```r
ObsCount <- table(snp1)
Nobs <- sum(ObsCount)
FreqG <- (2*ObsCount[3] + ObsCount[2])/(2*Nobs) # P_G
ExpCount <- c(Nobs*(1-FreqG)^2, 2*Nobs*FreqG*(1-FreqG), Nobs*FreqG^2)
rbind(ObsCount, ExpCount)
```

```
##                CC       GC       GG
## ObsCount 296.0000 758.0000 514.0000
## ExpCount 290.5772 768.8457 508.5772
```

```r
ChiSqStat <- sum((ObsCount - ExpCount)^2/ExpCount)
ChiSqStat
```

```
## [1] 0.3120182
```

```r
pchisq(ChiSqStat, df=2, lower.tail=FALSE) # Lower.tail = FALSE = >
```

```
## [1] 0.8555514
```

```r
library(genetics)
Snp1 <- genotype(snp1, sep="")
summary(Snp1)
```

```
##
## Number of samples typed: 1568 (99.4%)
##
## Allele Frequency: (2 alleles)
##    Count Proportion
## G   1786       0.57
## C   1350       0.43
## NA    20         NA
##
##
## Genotype Frequency:
##     Count Proportion
## G/G   514       0.33
## G/C   758       0.48
## C/C   296       0.19
## NA     10         NA
##
## Heterozygosity (Hu)  = 0.4904917
## Poly. Inf. Content   = 0.3701209
```

```
HWE.chisq(Snp1)
```

```
##
##  Pearson's Chi-squared test with simulated p-value (based on 10000
##  replicates)
##
## data:  tab
## X-squared = 0.31202, df = NA, p-value = 0.5998
```

```
country <- asthma$country
table(country)
```

```
## country
##   Australia     Belgium     Estonia      France     Germany      Norway
##         127          14           6         219         154         177
##       Spain      Sweden Switzerland          UK
##         377         281         100         123
```

```
Snp1bg <- genotype(snp1[country=="Belgium"], sep="")
summary(Snp1bg)
```

```
##
## Number of samples typed: 14 (100%)
##
## Allele Frequency: (2 alleles)
##   Count Proportion
## C    17       0.61
## G    11       0.39
##
##
## Genotype Frequency:
##     Count Proportion
## C/C     4       0.29
## C/G     9       0.64
## G/G     1       0.07
##
## Heterozygosity (Hu)  = 0.494709
## Poly. Inf. Content   = 0.3632568
```

```
HWE.chisq(Snp1bg)
```

```
##
##  Pearson's Chi-squared test with simulated p-value (based on 10000
##  replicates)
##
## data:  tab
## X-squared = 1.6915, df = NA, p-value = 0.07109
```

```
HWE.exact(Snp1bg)
```

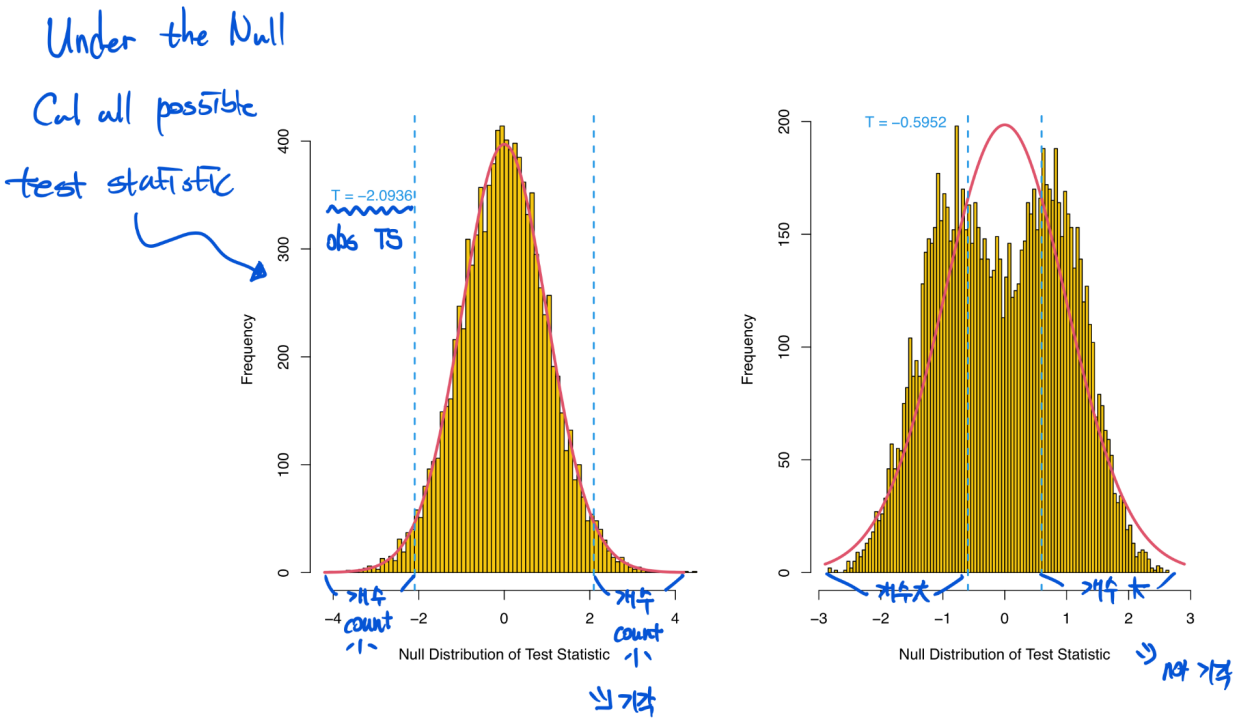
```
##
##  Exact Test for Hardy-Weinberg Equilibrium
##
## data:  Snp1bg
## N11 = 4, N12 = 9, N22 = 1, N1 = 17, N2 = 11, p-value = 0.3198
```

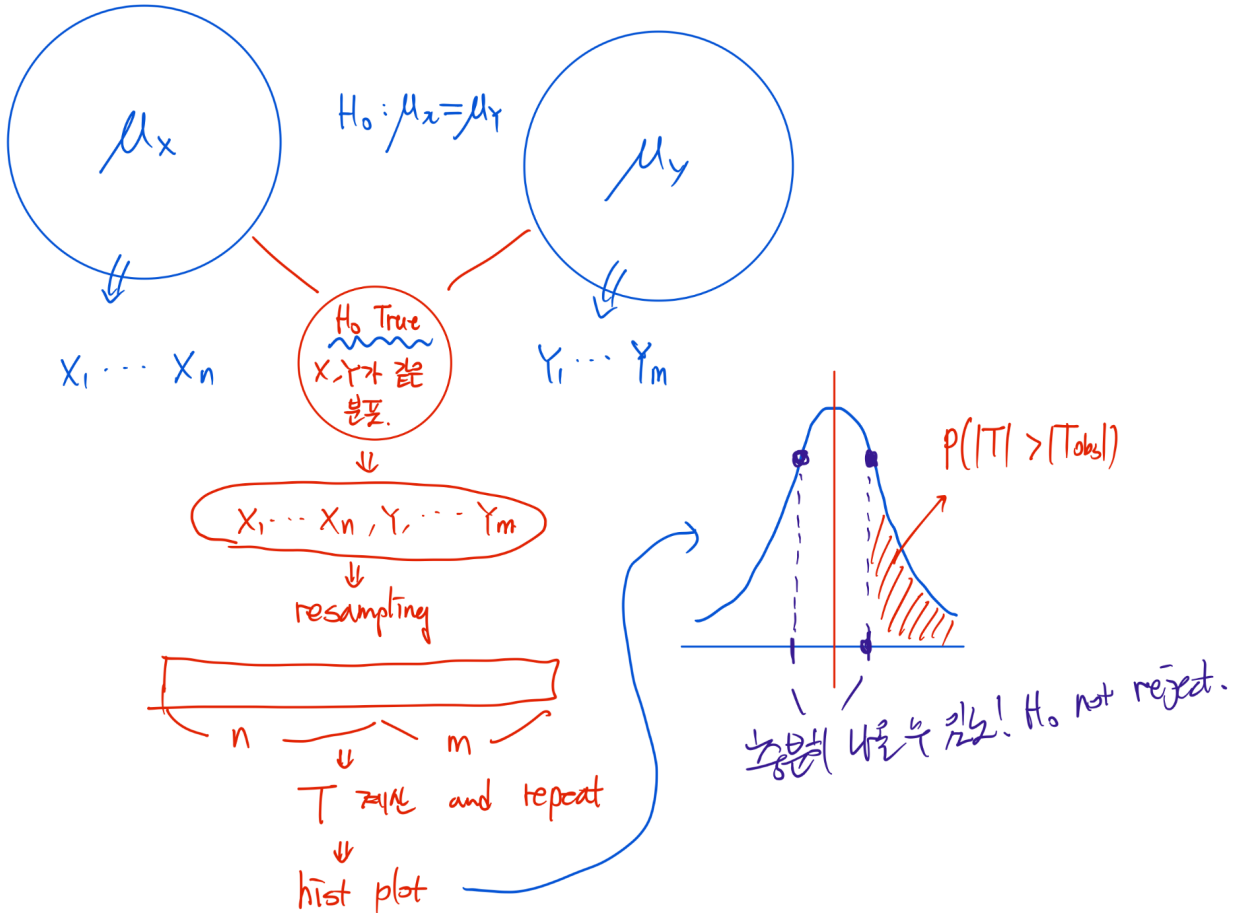
Permutation Test

Two sample t test를 못 쓸때 대안!

- Permutation tests is a resampling based test.
- We can compute a permu test p-v if
  - Null hypo $H_0$ or test statistic $T$ is somewhat unusual like median.
  - 각종 가설(정규성 등)이 기각되었을때 사용.
- Permutation test is popularly used for testion genomic data since population distribution of data is usually unkown and sample size is limited.
- Under the Null that $\mu_x = \mu_y$ , compute all possible test statistic $T$.

그니깐 뮤 엑스 뮤 와이가 같다는 가정하에 가능한 모든 T를 구해서 그 T로 분포를 만듦






저 위의 그림에서 보면

우리는 일단 under the H_0이므로 x ,y가 같은 분포를 지닌다는 가정을 두고 있음..

왜? -> H_0가 x와 y의 평균이 같으니깐 같은 분포에서 나온다!

이제 x, y의 표본들을 다 묶어서 새로 랜덤하게 뽑는다…

그리고 뽑힌 데이터에서 TS 구하고 반복해서 under the Null에서 나올 수 있는 통계량에 대한 분포를 만들어서 p value 계산.

- resampling된 데이터의 개수는 n+m으로 기존과 같다.

```
set.seed(123)
x <- c(1, 3, 4)
y <- c(2, 2, 1, 2)
sample(c(x, y))
```

```
## [1] 2 4 1 3 2 2 1
```

- For the $k$-th permuted data set, a null test statistics $T_k$ is

$$T_k = \frac{\bar{X}^* - \bar{Y}^*}{\sqrt{\frac{\hat{\sigma}^2_{X^*}}{n} + \frac{\hat{\sigma}^2_{Y^*}}{m}}},$$

  where $(X_1^*, \ldots, X_n^*)$ and $(Y_1^*, \ldots, Y_m^*)$ are permuted sets.

- The two-sided permutation $p$-value is then simply

$$\frac{\sum_{k=1}^{K} I\left(|T_k| > |T_{\text{obs}}|\right) + 1}{K + 1}$$

  where $K$ is the total number of permutations and $I(\cdot)$ is the indicator function.

```
library(multtest)
data(golub)

golubFactor <- factor(golub.cl, levels=0:1, labels=c("ALL","AML"))
golubFactor
```

```
##  [1] ALL ALL ALL ALL ALL ALL ALL ALL ALL ALL ALL ALL ALL ALL ALL ALL ALL ALL ALL ALL
## [20] ALL ALL ALL ALL ALL ALL ALL ALL AML AML AML AML AML AML AML AML AML AML AML AML
## Levels: ALL AML
```

```
sample(golubFactor)
```

```
##  [1] AML ALL ALL ALL ALL ALL ALL ALL AML AML AML AML ALL ALL ALL ALL ALL ALL AML AML ALL
## [20] AML ALL ALL ALL ALL AML ALL ALL ALL ALL ALL ALL ALL AML ALL ALL ALL ALL AML ALL ALL ALL
## Levels: ALL AML
```

```
sample(as.numeric(golubFactor))  # resampling...
```

```
##  [1] 2 1 1 1 1 1 1 1 1 2 1 1 1 1 1 1 1 2 1 2 1 1 2 2 1 1 1 1 2 1 2 1 2 1 2 2 1 1
```

```
sample(as.numeric(golubFactor))
```

```
##  [1] 1 1 2 2 1 2 2 1 2 1 2 1 2 2 1 1 1 1 1 1 1 1 2 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 2
```

```
set.seed(12345)
sample(as.numeric(golubFactor))
```

```
##  [1] 1 1 1 2 1 2 1 2 1 1 2 1 1 1 1 1 2 1 1 1 1 1 1 1 2 1 1 1 2 2 2 2 1 1 1 1 1 2
```

- `t.test(data ~ group)` : data를 group이란 그룹 기준으로 비교해 t 검정을 하라.!!

```
y <- as.numeric(golubFactor)  # origin values
K <- 10000 # 10000 rsampling  !
mat.y <- matrix(y, length(y), K) #   y         (n+m),
mat.y[,1:5]
```

```
##       [,1] [,2] [,3] [,4] [,5]
##  [1,]    1    1    1    1    1
##  [2,]    1    1    1    1    1
##  [3,]    1    1    1    1    1
##  [4,]    1    1    1    1    1
##  [5,]    1    1    1    1    1
##  [6,]    1    1    1    1    1
##  [7,]    1    1    1    1    1
##  [8,]    1    1    1    1    1
##  [9,]    1    1    1    1    1
## [10,]    1    1    1    1    1
## [11,]    1    1    1    1    1
## [12,]    1    1    1    1    1
## [13,]    1    1    1    1    1
## [14,]    1    1    1    1    1
## [15,]    1    1    1    1    1
## [16,]    1    1    1    1    1
## [17,]    1    1    1    1    1
## [18,]    1    1    1    1    1
## [19,]    1    1    1    1    1
## [20,]    1    1    1    1    1
## [21,]    1    1    1    1    1
## [22,]    1    1    1    1    1
## [23,]    1    1    1    1    1
## [24,]    1    1    1    1    1
## [25,]    1    1    1    1    1
## [26,]    1    1    1    1    1
## [27,]    1    1    1    1    1
## [28,]    2    2    2    2    2
## [29,]    2    2    2    2    2
## [30,]    2    2    2    2    2
## [31,]    2    2    2    2    2
## [32,]    2    2    2    2    2
## [33,]    2    2    2    2    2
## [34,]    2    2    2    2    2
## [35,]    2    2    2    2    2
## [36,]    2    2    2    2    2
## [37,]    2    2    2    2    2
## [38,]    2    2    2    2    2
```

```
per.y <- apply(mat.y, 2, sample)
per.y[,1:5]
```

```
##       [,1] [,2] [,3] [,4] [,5]
```

```
##  [1,]    1    1    2    1    2
##  [2,]    1    2    2    2    1
##  [3,]    1    1    2    1    1
##  [4,]    1    1    2    1    2
##  [5,]    1    1    1    2    1
##  [6,]    1    1    2    2    2
##  [7,]    2    1    2    2    1
##  [8,]    2    1    1    1    1
##  [9,]    2    1    2    2    2
## [10,]    1    2    1    1    1
## [11,]    1    1    1    2    1
## [12,]    1    1    1    2    1
## [13,]    2    2    1    1    1
## [14,]    1    2    1    1    1
## [15,]    1    2    1    2    2
## [16,]    2    1    1    1    1
## [17,]    2    1    1    2    1
## [18,]    2    1    1    1    2
## [19,]    1    1    1    1    1
## [20,]    1    1    1    1    1
## [21,]    1    1    1    1    1
## [22,]    1    1    2    1    1
## [23,]    1    2    2    1    1
## [24,]    1    1    1    1    1
## [25,]    1    2    1    1    2
## [26,]    1    1    2    1    1
## [27,]    1    1    1    1    1
## [28,]    1    2    1    1    1
## [29,]    1    1    2    1    2
## [30,]    1    2    1    1    1
## [31,]    2    1    1    2    2
## [32,]    1    2    1    1    1
## [33,]    2    1    1    1    2
## [34,]    1    1    1    2    2
## [35,]    2    2    1    1    1
## [36,]    2    1    1    1    1
## [37,]    1    1    1    1    1
## [38,]    1    1    1    1    1
```

```
ccnd3 <- grep("CCND3", golub.gnames[,2], ignore.case=TRUE)
t.test(golub[ccnd3,] ~ per.y[,1], var.equal=FALSE)
```

```
##
##  Welch Two Sample t-test
##
## data:  golub[ccnd3, ] by per.y[, 1]
## t = 0.75, df = 14.632, p-value = 0.4651
## alternative hypothesis: true difference in means between group 1 and group 2 is not equal to 0
## 95 percent confidence interval:
##  -0.4369684  0.9098350
## sample estimates:
## mean in group 1 mean in group 2
##        1.598081        1.361648
```

```
# per.y[,1] ->     resample          ..!
t.test(golub[ccnd3,] ~ per.y[,1], var.equal=FALSE)$stat
```

```
##         t
## 0.7500021
```

```
tobs <- t.test(golub[ccnd3,] ~ golubFactor, var.equal=FALSE)$stat
tobs
```

```
##        t
## 6.318594
```

```
#        10000    ..!
fun <- function(t) t.test(golub[ccnd3,]~t, var.equal=FALSE)$stat
T <- apply(per.y, 2, fun)
T[1:100]
```

```
##   [1]  0.750002075  0.729906717 -4.004972335 -0.848326811 -0.214045573
##   [6] -0.893949582  1.469308283  1.495298270 -0.205403873  0.163761153
##  [11] -1.184941862  0.432700190 -0.853352558  0.374686129 -0.707562241
##  [16]  0.632076881  0.090351758  1.023466276 -1.181556552  0.110726131
##  [21]  0.072785884 -0.018831018  1.180760691  0.851041514 -0.141666430
##  [26] -0.537026868 -1.524128422 -1.092022884 -0.707460317 -0.134482229
##  [31] -0.462554138 -0.884919543  0.309273874 -0.624451996 -1.752288461
##  [36] -1.194563758 -0.173032656  1.549104769 -0.158274988 -0.345184704
##  [41] -0.842889320 -1.515486314 -1.142508699  2.347815023  0.179727292
##  [46] -0.158495995 -0.863011078 -3.297320259 -1.074057289  0.498187397
##  [51] -1.181457977  0.715956073  0.674322785  1.926432263  0.245915984
##  [56] -1.398733482 -0.895901941  1.142328504  1.082408554  1.001011080
##  [61]  0.578050440 -0.498469520  1.223193589 -0.048090449 -0.015820531
##  [66] -0.271203483 -0.219226289 -0.667576824 -2.492837922  1.387870782
##  [71] -1.042095057  0.096681079 -0.326446181  1.357015108  0.269007176
##  [76] -0.453810572  1.402884288 -0.128311274 -2.161289400 -0.423506961
##  [81] -0.483567506 -0.006770731  0.539084479 -0.901801043 -0.825543403
##  [86] -0.154296784  0.352466127 -0.212858357  0.670559329  1.952371758
##  [91] -1.547960482 -0.302994752  0.863762077  1.326009555 -0.414317196
##  [96] -1.326142167  0.824951776  0.833046077 -0.204141118 -0.153654648
```

```
mean(abs(T) > abs(tobs))
```

```
## [1] 0
```

```
(sum(abs(T) > abs(tobs))+1)/(K+1) # p-value
```

```
## [1] 9.999e-05
```

```
1/(K+1)
```

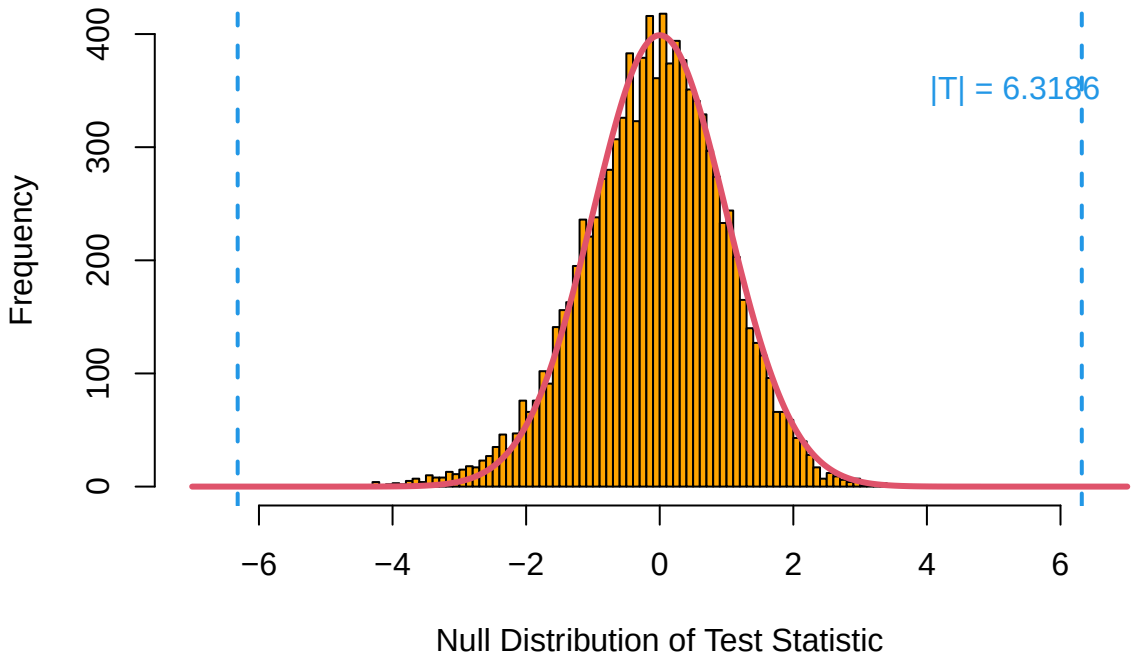
```
## [1] 9.999e-05
```

The two-sided permutation $p$-value is then simply

resampling해서 얻은 TS  resampling 하기 전에 구한 값

$$\frac{\sum_{k=1}^{K} I\left(|T_k| > |T_{\text{obs}}|\right) + 1}{K+1}$$

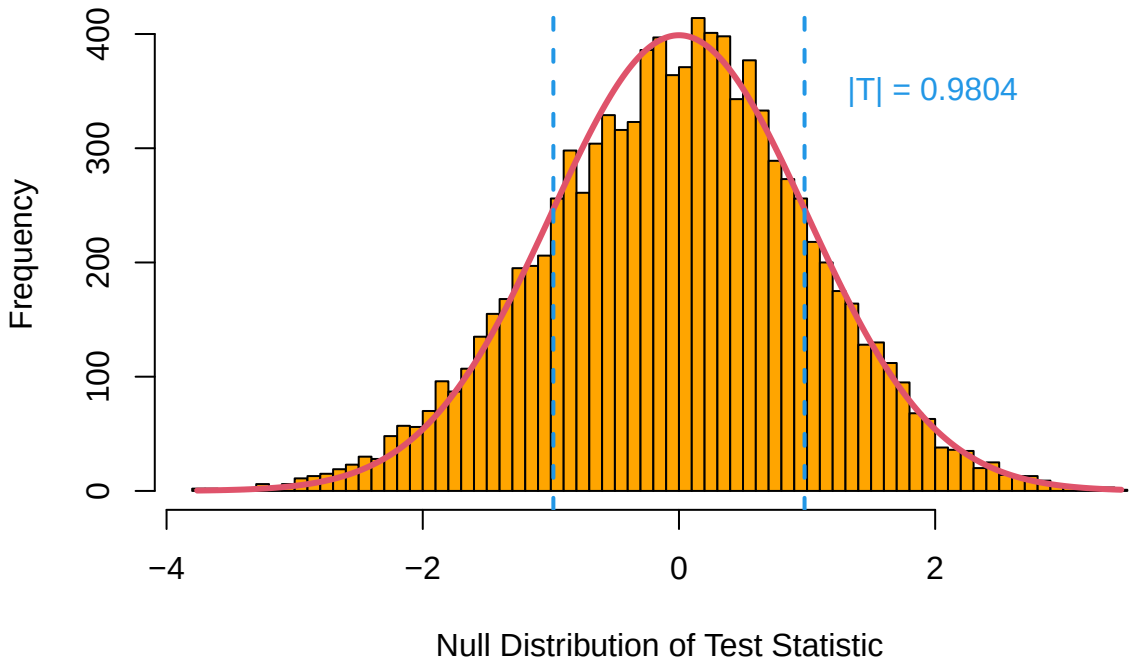
```
hist(T, breaks=100, col="orange", main="", xlim=c(-7, 7), xlab="Null Distribution of Test Statistic")
x0 <- seq(-7, 7, len=1000)
y0 <- dnorm(seq(-7, 7, len=1000))
lines(x0, y0*1000, col=2, lwd=3)
abline(v=-abs(tobs), col=4, lty=2, lwd=2)
abline(v=abs(tobs), col=4, lty=2, lwd=2)
text(abs(tobs)-1, 350, col=4, paste("|T| = ", round(abs(tobs), 4), sep=""))
```




```
gdf5 <- grep("Gdf5", golub.gnames[,2], ignore.case=TRUE)
fun2 <- function(t) t.test(golub[gdf5,]~t, var.equal=FALSE)$stat
T2 <- apply(per.y, 2, fun2)
tobs2 <- t.test(golub[gdf5,] ~ golubFactor, var.equal=FALSE)$stat
(sum(abs(T2) > abs(tobs2))+1)/(K+1)
```

```
## [1] 0.339566
```

```
hist(T2, breaks=100, col="orange", main="", xlab="Null Distribution of Test Statistic")
x0 <- seq(min(T2), max(T2), len=1000)
y0 <- dnorm(seq(min(T2), max(T2), len=1000))
lines(x0, y0*1000, col=2, lwd=3)
abline(v=-abs(tobs2), col=4, lty=2, lwd=2)
abline(v=abs(tobs2), col=4, lty=2, lwd=2)
text(abs(tobs2)+1, 350, col=4, paste("|T| = ", round(abs(tobs2), 4), sep=""))
```




Confidence Interval

- The CI is an interval estimate for a population parameter.
- This interval requires we have a random sample from a normal population.

```
t.test(golub[ccnd3,]) # default mu=0
```

```
##
##  One Sample t-test
##
## data:  golub[ccnd3, ]
## t = 12.225, df = 37, p-value = 1.469e-14
## alternative hypothesis: true mean is not equal to 0
## 95 percent confidence interval:
##  1.276107 1.783174
## sample estimates:
## mean of x
##   1.52964
```

```
# pv    -> H_0  -> mu          x
t.test(golub[ccnd3,])$conf.int
```

```
## [1] 1.276107 1.783174
## attr(,"conf.level")
## [1] 0.95
```

```
t.test(golub[ccnd3,], mu=1.27)$p.value
```

```
## [1] 0.04499534
```

```r
t.test(golub[ccnd3,], mu=1.277)$p.value
```

```
## [1] 0.05077146
```

```r
t.test(golub[ccnd3,], conf.level=0.90)$conf.int
```

```
## [1] 1.318537 1.740743
## attr(,"conf.level")
## [1] 0.9
```

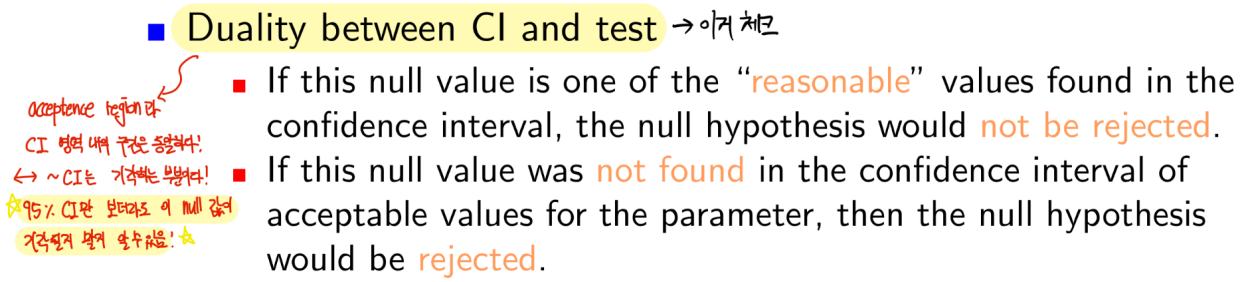
```r
t.test(golub[ccnd3,], mu=1.74)$p.val
```

```
## [1] 0.1011567
```

```r
t.test(golub[ccnd3,], mu=1.741)$p.val
```

```
## [1] 0.09960262
```

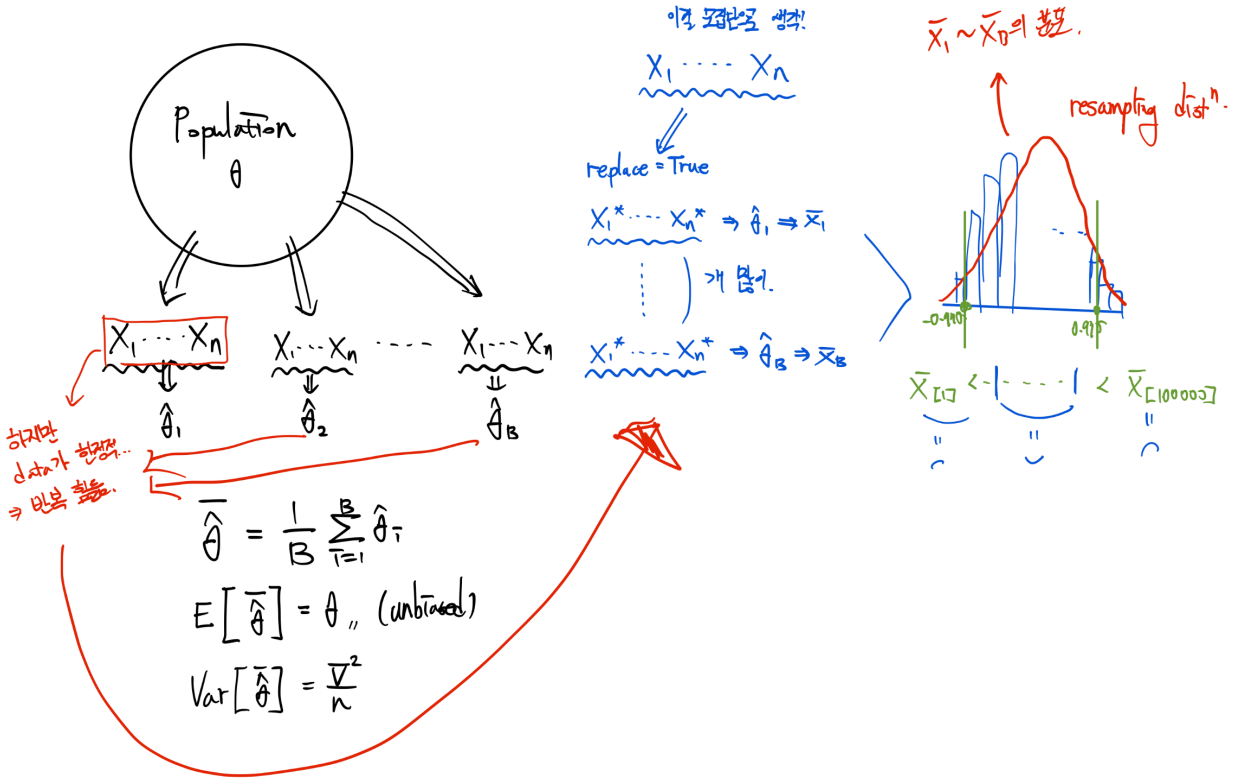
Duality between CI and test




Bootstrap Confidence Interval

- 정규성을 따르지 않을 때! t 분포 이용 못하니깐! Bootstrap 쓰자!

  One sample t test에선 이중성때문에 CI만 알아도 기각할지말지 알 수 있다.




Bootstrap으로 만든 분포는 정확히 말하면

“원래 데이터에서 리샘플링(with replacement)한 샘플들의 표본평균의 분포” 이다!

```
1:10
```

```
##  [1]  1  2  3  4  5  6  7  8  9 10
```

```
sample(1:10, replace=TRUE) # replace = TRUE !!! => Bootstrap
```

```
##  [1] 2 5 7 1 7 2 8 3 4 5
```

```
sample(1:10, replace=TRUE)
```

```
##  [1]  4 10  9  8 10  1  9  5  2  4
```

```
set.seed(1111)
sample(1:10, replace=TRUE)
```

```
##  [1]  6  2 10  4  1  6  6 10  7  1
```

```
K <- 10000
mat <- matrix(golub[ccnd3,], length(golub[ccnd3,]), K)
mat[1:5, 1:5]
```

```
##          [,1]    [,2]    [,3]    [,4]    [,5]
## [1,] 2.10892 2.10892 2.10892 2.10892 2.10892
## [2,] 1.52405 1.52405 1.52405 1.52405 1.52405
## [3,] 1.96403 1.96403 1.96403 1.96403 1.96403
## [4,] 2.33597 2.33597 2.33597 2.33597 2.33597
## [5,] 1.85111 1.85111 1.85111 1.85111 1.85111
```

```
fun3 <- function(t) sample(t, replace=TRUE)
boot <- apply(mat, 2, fun3)
bmean <- apply(boot, 2, mean)
quantile(bmean, c(0.025, 0.975)) # 95%   !
```

```
##     2.5%    97.5%
## 1.275149 1.769409
```

Wilcoxon Signed Rank Test

- one sample t-test의 비모수 버전
- 데이터가 정규성을 따르지 않을 때
- 또는 샘플 크기가 작을 때!

```
x <- c(6003, 6304, 6478, 6245, 6134, 6204, 6150)
wilcox.test(x, mu=6000)
```

```
##
##  Wilcoxon signed rank exact test
##
## data:  x
## V = 28, p-value = 0.01563
## alternative hypothesis: true location is not equal to 6000
```

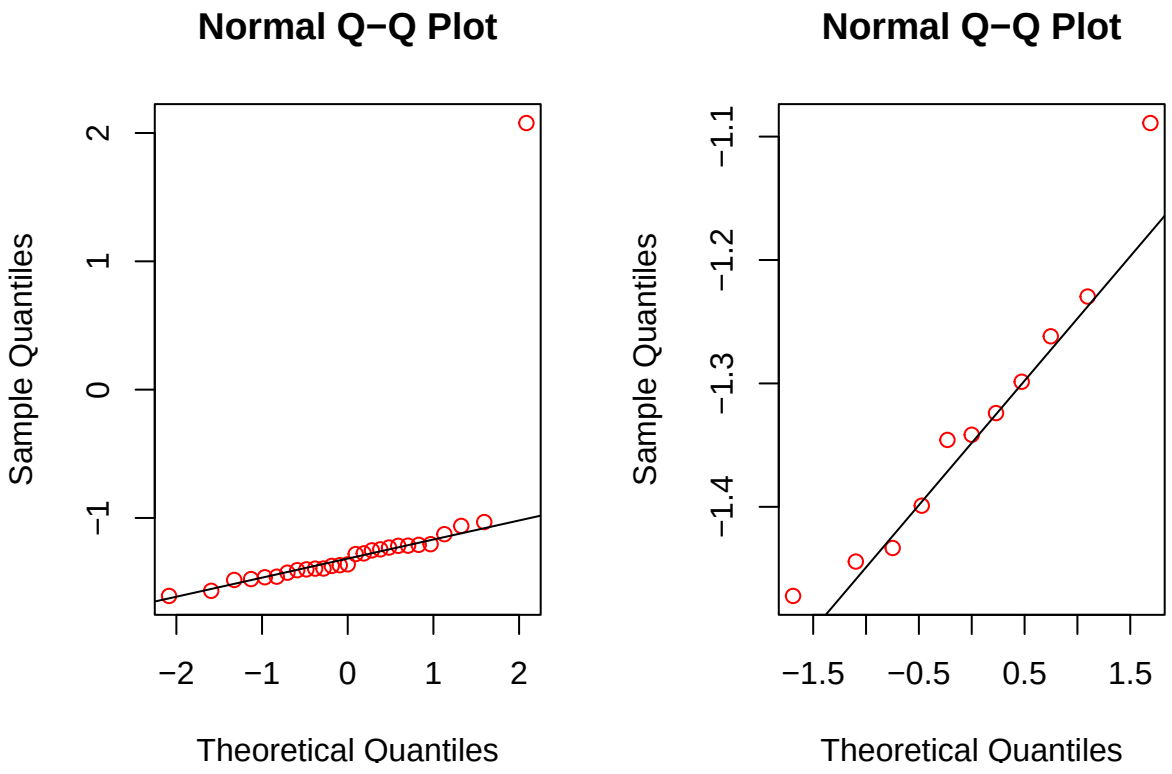
```
nkr <- grep("Nkr", golub.gnames[, 2], ignore.case=TRUE)
shapiro.test(golub[nkr, golubFactor=="ALL"]) #
```

```
##
##  Shapiro-Wilk normality test
##
## data:  golub[nkr, golubFactor == "ALL"]
## W = 0.38118, p-value = 1.268e-09
```

```
# ALL -> t test     !
shapiro.test(golub[nkr, golubFactor=="AML"]) #     .
```

```
##
##  Shapiro-Wilk normality test
##
## data:  golub[nkr, golubFactor == "AML"]
## W = 0.94277, p-value = 0.5535
```

```r
par(mfrow=c(1,2))
qqnorm(golub[nkr, golubFactor=="ALL"], col="red")
qqline(golub[nkr, golubFactor=="ALL"])
qqnorm(golub[nkr, golubFactor=="AML"], col="red")
qqline(golub[nkr, golubFactor=="AML"])
```




```r
t.test(golub[nkr, golubFactor=="ALL"], mu=-1.2)
```

```
##
##  One Sample t-test
##
## data:  golub[nkr, golubFactor == "ALL"]
## t = -0.016947, df = 26, p-value = 0.9866
## alternative hypothesis: true mean is not equal to -1.2
## 95 percent confidence interval:
##  -1.4675900 -0.9367863
## sample estimates:
## mean of x
## -1.202188
```

```r
wilcox.test(golub[nkr, golubFactor=="ALL"], mu=-1.2)
```

```
##
##  Wilcoxon signed rank exact test
##
## data:  golub[nkr, golubFactor == "ALL"]
## V = 59, p-value = 0.001132
## alternative hypothesis: true location is not equal to -1.2
```

```
set.seed(12345)
K <- 100000
nkr.ALL <- golub[nkr, golubFactor=="ALL"]
nkr.ALL
```

Bootstra Confidence Interval in nonparametric test.

```
##  [1] -1.45769 -1.39420 -1.46227 -1.40715 -1.42668 -1.21719 -1.37386 -1.36832
##  [9] -1.47649 -1.21583 -1.28137 -1.03209 -1.36149  2.07770 -1.39503 -1.40095
## [17] -1.56783 -1.20466 -1.24482 -1.60767 -1.06221 -1.12665 -1.20963 -1.48332
## [25] -1.25268 -1.27619 -1.23051
```

```
sample(nkr.ALL, replace=TRUE)
```

```
##  [1]  2.07770 -1.24482 -1.40095 -1.27619 -1.48332 -1.27619 -1.28137 -1.48332
##  [9] -1.39420 -1.12665 -1.28137 -1.21719 -1.37386 -1.21583 -1.56783 -1.36832
## [17] -1.37386 -1.21719 -1.45769 -1.03209 -1.60767 -1.36832 -1.27619 -1.03209
## [25] -1.46227 -1.47649  2.07770
```

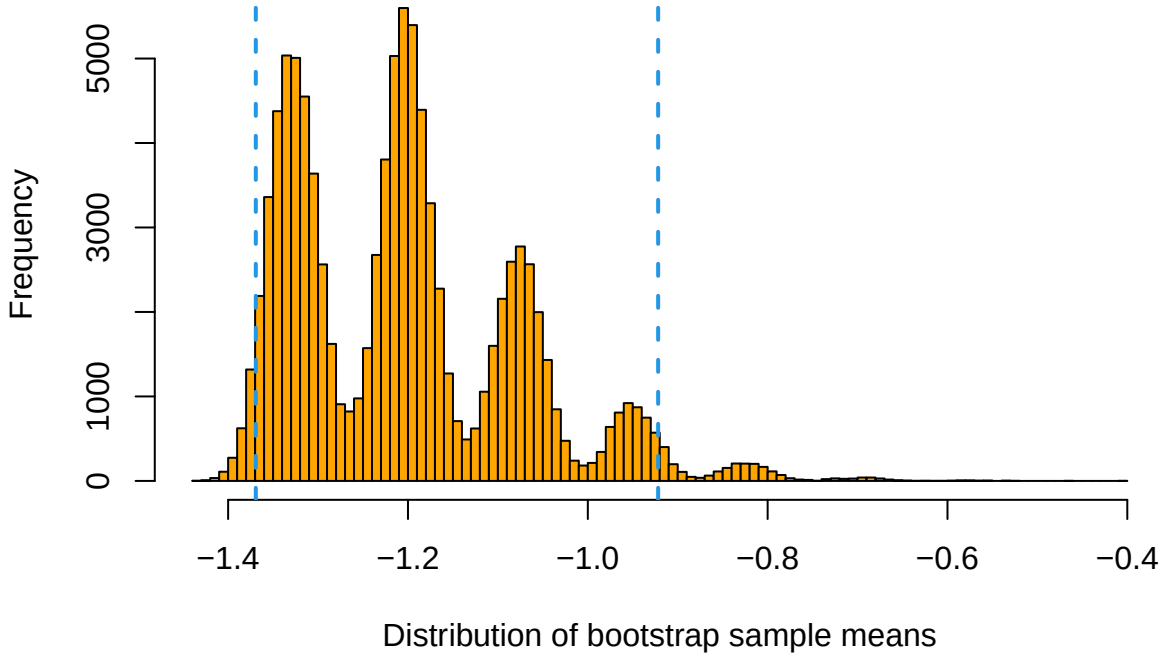
```
mean(sample(nkr.ALL, replace=TRUE))
```

```
## [1] -1.374002
```

```
mat <- matrix(nkr.ALL, length(nkr.ALL), K)
fun3 <- function(t) sample(t, replace=TRUE)
# outlier 2          !
boot <- apply(mat, 2, fun3)
bmean <- apply(boot, 2, mean) # bootstrap
quantile(bmean, c(0.025, 0.975))
```

```
##       2.5%      97.5%
## -1.3692449 -0.9217678
```

```
hist(bmean, breaks=100, col="orange", main="", xlab="Distribution of bootstrap sample means")
abline(v=quantile(bmean, c(0.025, 0.975)), col=4, lty=2, lwd=2)
```




Wilcoxon Rank Sum Test

Mann-Whitney U test라기도 한다.

- two sample t test의 비모수 검정 방법.

```
igf <- grep("IGFBP5",golub.gnames[,2], ignore.case = TRUE)
shapiro.test(golub[igf, golubFactor=="ALL"])
```

```
##
##  Shapiro-Wilk normality test
##
## data:  golub[igf, golubFactor == "ALL"]
## W = 0.58386, p-value = 1.344e-07
```

```
shapiro.test(golub[igf, golubFactor=="AML"])
```

```
##
##  Shapiro-Wilk normality test
##
## data:  golub[igf, golubFactor == "AML"]
## W = 0.66507, p-value = 0.0001673
```

```
wilcox.test(golub[igf,] ~ golubFactor)
```

```
##
##  Wilcoxon rank sum exact test
##
## data:  golub[igf, ] by golubFactor
## W = 151, p-value = 0.9495
## alternative hypothesis: true location shift is not equal to 0
```

Applications to Multiple Genes

- 정규성 통과한 유전자 비율 예제

```
dim(golub)
```

```
## [1] 3051   38
```

```
# 3051      .!
fun <- function(t) shapiro.test(t)$p.value
```

```
all <- apply(golub[, golubFactor=="ALL"], 1, fun)
aml <- apply(golub[, golubFactor=="AML"], 1, fun)

sum(all > 0.05)/nrow(golub)
```

```
## [1] 0.5827598
```

```
sum(aml > 0.05)/nrow(golub)
```

```
## [1] 0.7856441
```

```
pval.t <- function(t) t.test(t ~ golubFactor)$p.value
pval.w <- function(t) wilcox.test(t ~ golubFactor)$p.value

pt <- apply(golub, 1, pval.t)
pw <- apply(golub, 1, pval.w)

pval <- data.frame(cbind(pt, pw))

# t-test   (p<0.05)  wilcox
nrow(pval[pt < 0.05 & pw >= 0.05, ]) #          ..
```

```
## [1] 114
```

```
# wilcox   (p<0.05)  t-test
nrow(pval[pw < 0.05 & pt >= 0.05, ])
```

```
## [1] 91
```

```
#       p-value
apply(pval, 2, function(x) which(x==min(x))) # 2124    signal  ..?
```

```
## $pt
## [1] 2124
##
## $pw
## [1]  896 2124
```

```
#      ranking    gene         .
```

- 통계적으로 p-value가 가장 작다는 건?
- → 그 유전자는 두 그룹(ALL vs AML) 간의 발현량 차이가 가장 극명하게 드러나는 유전자라는 뜻.

chapter 2 end…