

Recap general linear model

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This is part of the online course Statistical Genomics 2022 (SGA)

1 Breast cancer example

- part of study <https://doi.org/10.1093/jnci/djj052>)
- Histologic grade in breast cancer clinically prognostic. Association of histologic grade on expression of KPNA2 gene that is known to be associated with poor BC prognosis.
- Population: all current and future breast cancer patients







EXP. DESIGN









2 Data Exploration

2.1 Import

```
library(tidyverse)
gene <- read.table("https://raw.githubusercontent.com/statOmics/SGA21/master/data/kpna2.txt", header=TRUE)
head(gene)
```

```
##   grade node    gene
## 1     3     1 367.8179
## 2     3     1 590.3576
## 3     1     1 346.6583
## 4     1     1 258.4455
## 5     1     0 153.8416
## 6     3     0 643.6799
```

We will transform the variable grade and node to a factor

```
gene$grade <- as.factor(gene$grade)
gene$node <- as.factor(gene$node)
```

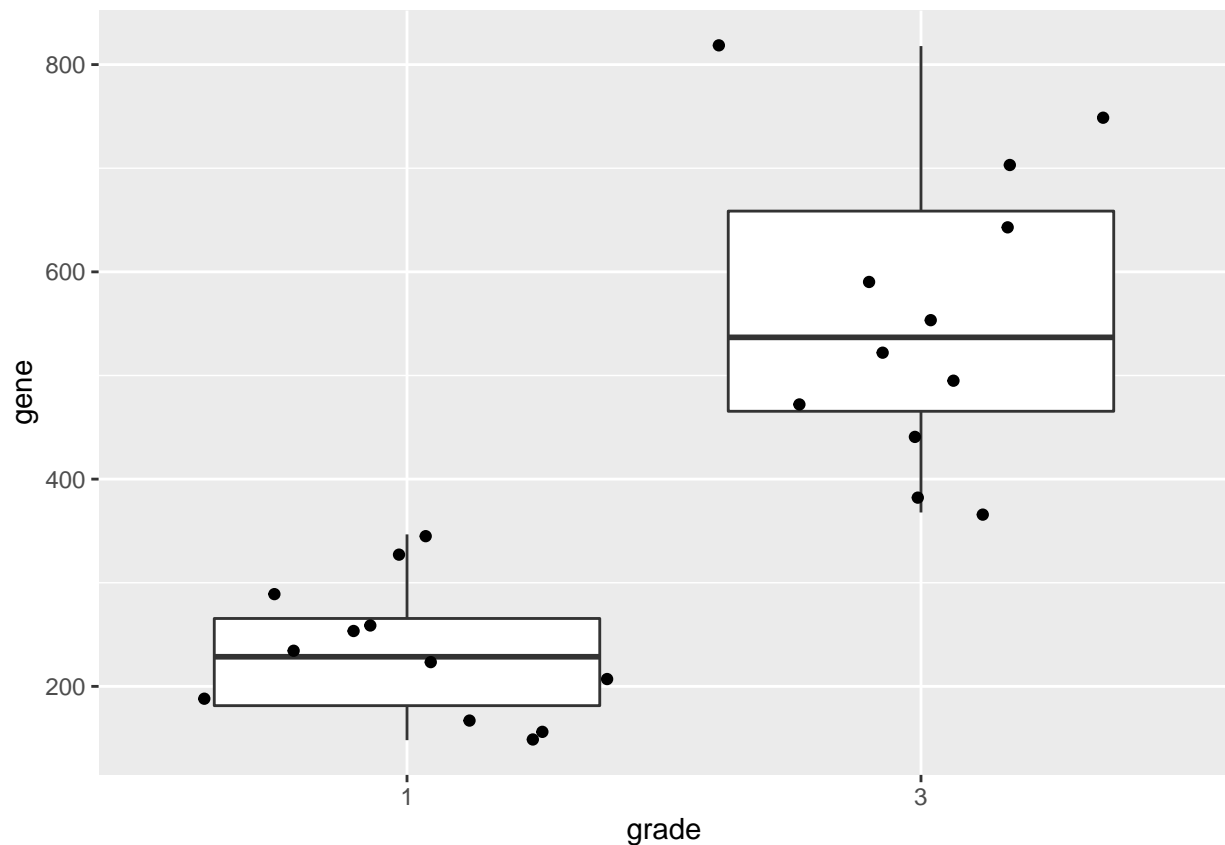
2.2 Summary statistics

```
geneSum <- gene %>%  
  group_by(grade) %>%  
  summarize(mean = mean(gene),  
            sd = sd(gene),  
            n=length(gene)  
            ) %>%  
  mutate(se = sd/sqrt(n))  
geneSum
```

```
## # A tibble: 2 x 5  
##   grade mean    sd     n    se  
##   <fct> <dbl> <dbl> <int> <dbl>  
## 1 1      233.  65.5    12  18.9  
## 2 3      561. 144.     12  41.4
```

2.3 Visualisation

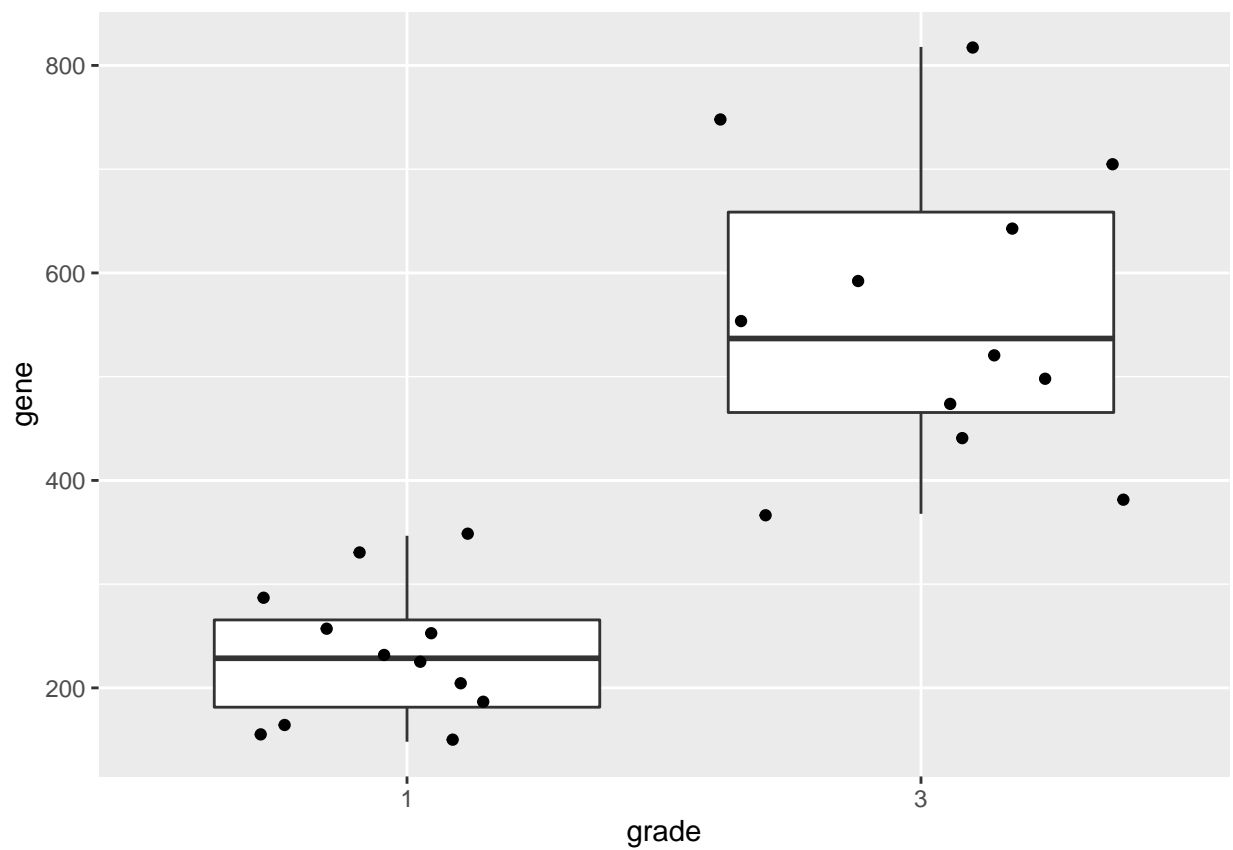
```
gene %>%  
  ggplot(aes(x=grade,y=gene)) +  
  geom_boxplot(outlier.shape=NA) +  
  geom_jitter()
```



We can also save the plots as objects for later use!

```
p1 <- gene %>%  
  ggplot(aes(x=grade,y=gene)) +  
  geom_boxplot(outlier.shape=NA) +  
  geom_jitter()  
  
p2 <- gene %>%  
  ggplot(aes(sample=gene)) +  
  geom_qq() +  
  geom_qq_line() +  
  facet_wrap(~grade)
```

p1



p2



2.4 Research questions

Researchers want to assess the association of the histological grade on KPNA2 gene expression



2.5 Estimation of effect size and standard error

```
effectSize <- tibble(
  delta = geneSum$mean[2] - geneSum$mean[1],
  seDelta = geneSum %>%
    pull(se) %>%
    .^2 %>%
    sum %>%
    sqrt
)
effectSize
```

```
## # A tibble: 1 x 2
##   delta seDelta
##   <dbl>   <dbl>
## 1  329.    45.5
```

3 Statistical Inference

- Researchers want to assess the association of histological grade on KPNA2 gene expression

- Inference?



- Researchers want to assess the association of histological grade on KPNA2 gene expression
 - Inference?
 - testing + CI \rightarrow Assumptions
-

- In general we start from **alternative hypothesis** H_A : we want to show an association
 - Gene expression of grade 1 and grade 3 patients is on average different
 - But, we will assess it by falsifying the opposite:
 - The average KPNA2 gene expression of grade 1 and grade 3 patients is equal
-

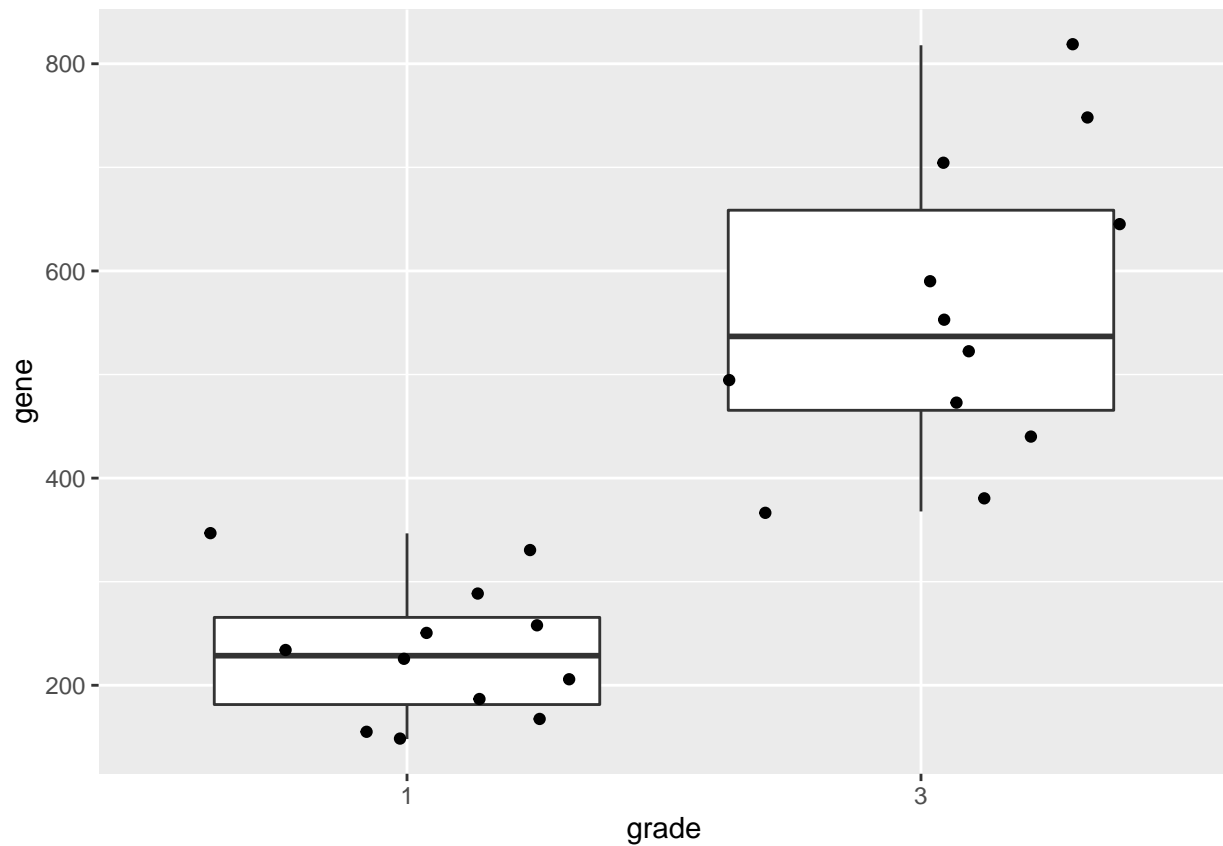
- How likely is it to observe an equal or more extreme association than the one observed in the sample when the null hypothesis is true?

- When we make assumptions about the distribution of our test statistic we can quantify this probability:
p-value.
- If the p-value is below a significance threshold α we reject the null hypothesis

We control the probability on a false positive result at the α -level (type I error)

- The p-value will only be calculated correctly if the underlying assumptions hold!

```
library(gridExtra)
p1
```



```
p2
```



```
t.test(gene~grade,data=gene)
```

```
##
## Welch Two Sample t-test
##
## data: gene by grade
## t = -7.2132, df = 15.384, p-value = 2.598e-06
## alternative hypothesis: true difference in means between group 1 and group 3 is not equal to 0
## 95 percent confidence interval:
## -425.4218 -231.6751
## sample estimates:
## mean in group 1 mean in group 3
## 232.5003 561.0487
```

```
effectSize <- effectSize %>%
  mutate(t.stat=delta/seDelta) %>%
  mutate(p.value= pt(-abs(t.stat),21.352)*2)

effectSize
```

```
## # A tibble: 1 x 4
##   delta seDelta t.stat    p.value
##   <dbl>   <dbl>   <dbl>    <dbl>
## 1  329.    45.5    7.21 0.000000376
```

- Intensities are often not normally distributed and have a mean variance relation
- Commonly log2-transformed
- Differences on log scale:

$$\log_2(B) - \log_2(A) = \log_2 \frac{B}{A} = \log_2 FC_{\frac{B}{A}}$$



3.1 Log transformation

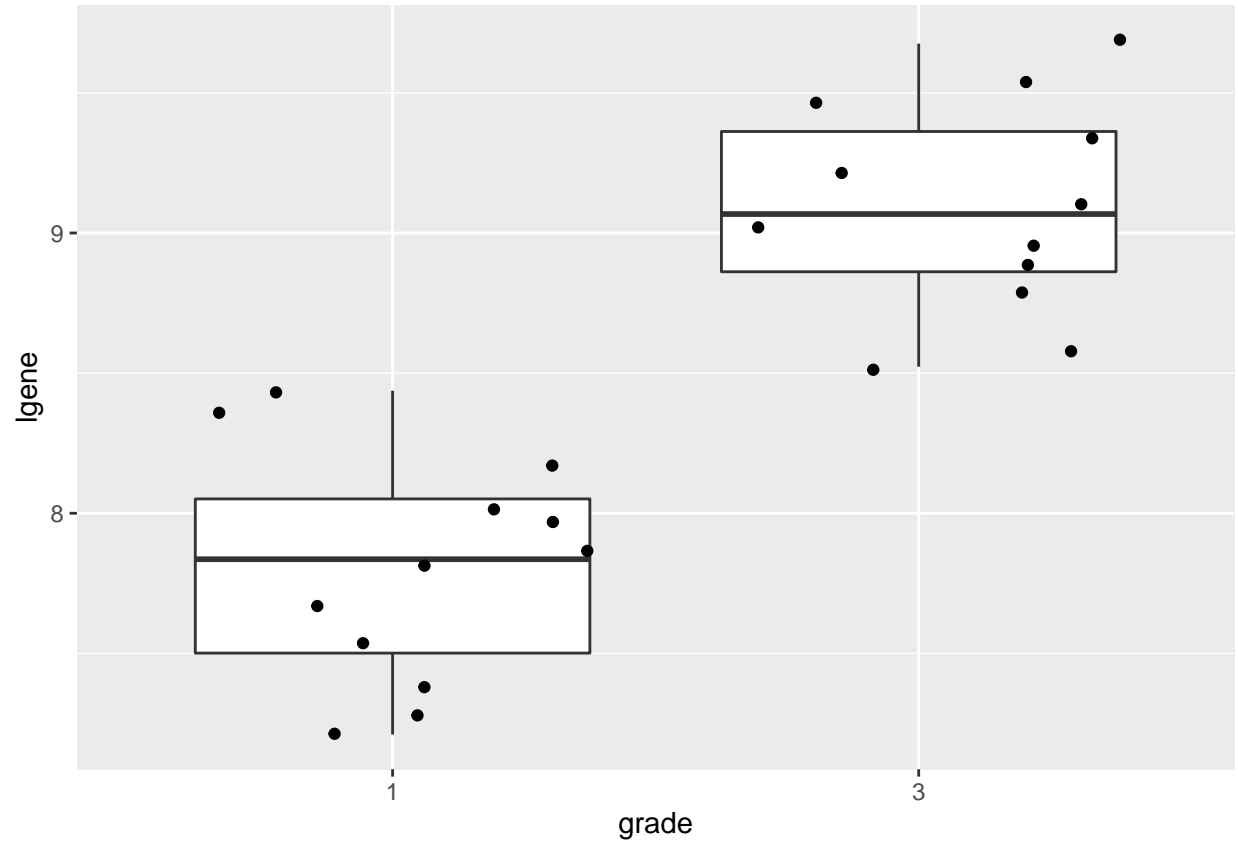
```
gene <- gene %>%
  mutate(lgene = log2(gene))

p1 <- gene %>%
  ggplot(aes(x=grade,y=lgene)) +
  geom_boxplot(outlier.shape=NA) +
  geom_jitter()

p2 <- gene %>%
  ggplot(aes(sample=lgene)) +
  geom_qq() +
```

```
geom_qq_line() +  
facet_wrap(~grade)
```

p1



p2



```
logtest <- t.test(lgene~grade,data=gene,var.equal=TRUE)
logtest
```

```
##
## Two Sample t-test
##
## data: lgene by grade
## t = -8.0455, df = 22, p-value = 5.372e-08
## alternative hypothesis: true difference in means between group 1 and group 3 is not equal to 0
## 95 percent confidence interval:
## -1.610148 -0.950178
## sample estimates:
## mean in group 1 mean in group 3
## 7.808478 9.088641
```

```
log2FC <- logtest$estimate[2]-logtest$estimate[1]
log2FC
```

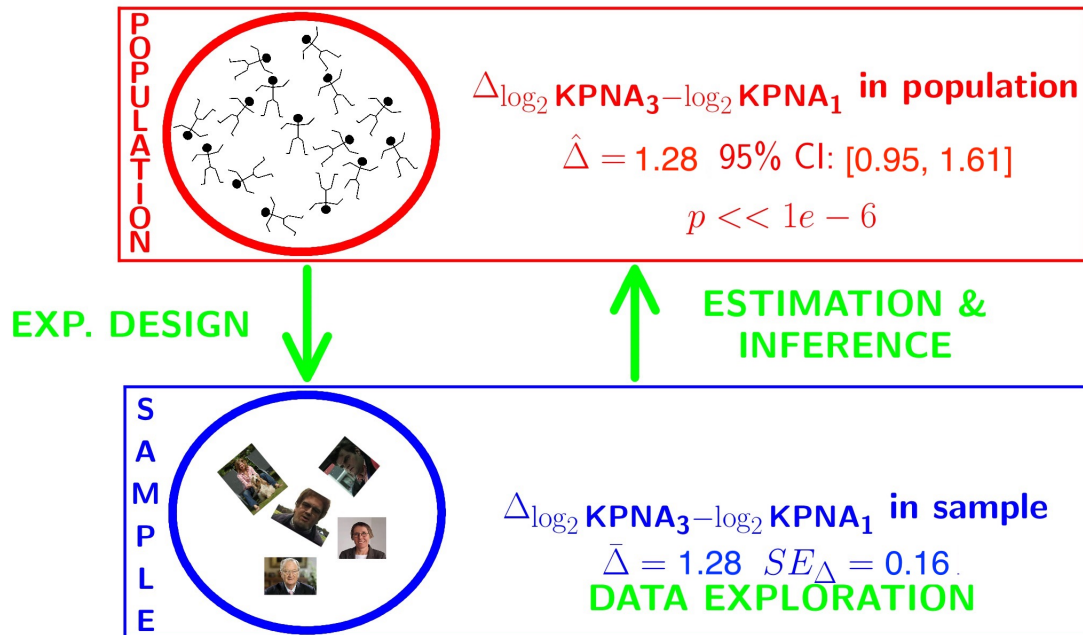
```
## mean in group 3
## 1.280163
```

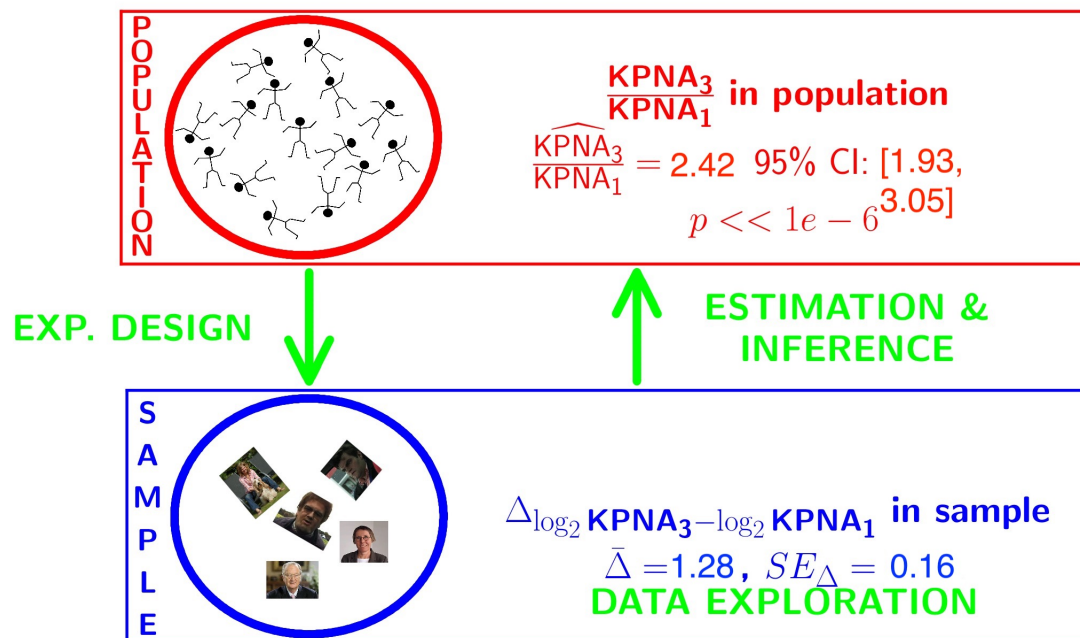
```
names(log2FC) <- "g3-g1"
2^log2FC
```

```
## g3-g1
## 2.428664
```

3.2 Conclusion

There is a extremely significant association of the histological grade on the gene expression in tumor tissue. On average, the gene expression for the grade 3 patients is 2.43 times higher than the gene expression in grade 1 patients (95% CI [1.93, 3.05], $p < 0.001$).





The patients also differ in their lymph node status. Hence, we have a two factorial design: grade x lymph node status!!!

Solution??



4 General Linear Model

How can we integrate multiple factors and continuous covariates in linear model.

$$y_i = \beta_0 + \beta_1 x_{i,1} + \beta_2 x_{i,2} + \beta_{12} x_{i,1} x_{i,2} + \epsilon_i,$$

with

- $x_{i,1}$ a dummy variable for histological grade: $x_{i,1} = \begin{cases} 0 & \text{grade 1} \\ 1 & \text{grade 3} \end{cases}$
- $x_{i,2}$ a dummy variable for : $x_{i,2} = \begin{cases} 0 & \text{lymph nodes were not removed} \\ 1 & \text{lymph nodes were removed} \end{cases}$
- ϵ_i ?

4.1 Implementation in R

```
lm1 <- lm(gene~grade*node,data=gene)
summary(lm1)
```

```
##
## Call:
## lm(formula = gene ~ grade * node, data = gene)
##
## Residuals:
```

	Min	1Q	Median	3Q	Max
	-201.748	-53.294	-6.308	46.216	277.601

```
##
## Coefficients:
```

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	180.51	44.37	4.068	0.0006 ***
grade3	401.33	62.75	6.396	3.07e-06 ***
node1	103.98	62.75	1.657	0.1131
grade3:node1	-145.57	88.74	-1.640	0.1166

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 108.7 on 20 degrees of freedom
## Multiple R-squared:  0.7437, Adjusted R-squared:  0.7052
## F-statistic: 19.34 on 3 and 20 DF,  p-value: 3.971e-06
```

4.2 Assumptions

```
plot(lm1)
```









4.3 Breast cancer example

- Paper: <https://doi.org/10.1093/jnci/djj052>
- Histologic grade in breast cancer provides clinically important prognostic information. Two factors have to be considered: Histologic grade (grade 1 and grade 3) and lymph node status (0 vs 1). The researchers assessed gene expression of the KPNA2 gene a protein-coding gene associated with breast cancer and are mainly interested in the association of histological grade. Note, that the gene variable consists of background corrected normalized intensities obtained with a microarray platform. Upon log-transformation, they are known to be a good proxy for the log transformed concentration of gene expression product of the KPNA2 gene.
- Research questions and translate them towards model parameters (contrasts)?
- Make an R markdown file to answer the research questions

```
library(ExploreModelMatrix)
explMx <- VisualizeDesign(gene, designFormula = ~grade*node)
explMx$plotlist
```

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



You can also explore the model matrix interactively:

```
ExploreModelMatrix(gene, designFormula = ~grade*node)
```

5 Linear regression in matrix form

5.1 Scalar form

- Consider a vector of predictors $\mathbf{x} = (x_1, \dots, x_p)^T$ and
- a real-valued response Y
- then the linear regression model can be written as

$$Y = f(\mathbf{x}) + \epsilon = \beta_0 + \sum_{j=1}^p x_j \beta_j + \epsilon$$

with i.i.d. $\epsilon \sim N(0, \sigma^2)$

5.2 Matrix form

- n observations $(\mathbf{x}_1, y_1) \dots (\mathbf{x}_n, y_n)$

- Regression in matrix notation

$$\mathbf{Y} = \mathbf{X} \boldsymbol{\beta} + \boldsymbol{\epsilon}$$

$$\text{with } \mathbf{Y} = \begin{bmatrix} y_1 \\ \vdots \\ y_n \end{bmatrix}, \mathbf{X} = \begin{bmatrix} 1 & x_{11} & \dots & x_{1p} \\ \vdots & \vdots & & \vdots \\ 1 & x_{n1} & \dots & x_{np} \end{bmatrix}, \boldsymbol{\beta} = \begin{bmatrix} \beta_0 \\ \vdots \\ \beta_p \end{bmatrix} \text{ and } \boldsymbol{\epsilon} = \begin{bmatrix} \epsilon_1 \\ \vdots \\ \epsilon_n \end{bmatrix}$$

5.3 Least Squares (LS)

- Minimize the residual sum of squares

$$\begin{aligned} RSS(\boldsymbol{\beta}) &= \sum_{i=1}^n e_i^2 \\ &= \sum_{i=1}^n \left(y_i - \beta_0 - \sum_{j=1}^p x_{ij} \beta_j \right)^2 \end{aligned}$$

- or in matrix notation

$$\begin{aligned} RSS(\boldsymbol{\beta}) &= (\mathbf{Y} - \mathbf{X}\boldsymbol{\beta})^T (\mathbf{Y} - \mathbf{X}\boldsymbol{\beta}) \\ &= \|\mathbf{Y} - \mathbf{X}\boldsymbol{\beta}\|_2^2 \end{aligned}$$

with the L_2 -norm of a p -dim. vector \mathbf{v} $\|\mathbf{v}\| = \sqrt{v_1^2 + \dots + v_p^2} \rightarrow \hat{\boldsymbol{\beta}} = \underset{\boldsymbol{\beta}}{\operatorname{argmin}} \|\mathbf{Y} - \mathbf{X}\boldsymbol{\beta}\|_2^2$

5.3.1 Minimize RSS

$$\begin{aligned} \frac{\partial RSS}{\partial \boldsymbol{\beta}} &= \mathbf{0} \\ \frac{(\mathbf{Y} - \mathbf{X}\boldsymbol{\beta})^T (\mathbf{Y} - \mathbf{X}\boldsymbol{\beta})}{\partial \boldsymbol{\beta}} &= \mathbf{0} \\ -2\mathbf{X}^T (\mathbf{Y} - \mathbf{X}\boldsymbol{\beta}) &= \mathbf{0} \\ \mathbf{X}^T \mathbf{X} \boldsymbol{\beta} &= \mathbf{X}^T \mathbf{Y} \\ \hat{\boldsymbol{\beta}} &= (\mathbf{X}^T \mathbf{X})^{-1} \mathbf{X}^T \mathbf{Y} \end{aligned}$$

5.3.2 Geometric Interpretation

5.3.2.1 Toy dataset We will illustrate this on a toy dataset

```
library(tidyverse)
data <- data.frame(x=1:3, y=c(1,2,2))
data
```

```
##   x y
## 1 1 1
## 2 2 2
## 3 3 2
```

5.3.2.2 Matrix form for toy dataset We can also write this in matrix form

$$\mathbf{Y} = \mathbf{X}\beta + \epsilon$$

with

$$\mathbf{Y} = \begin{bmatrix} 1 \\ 2 \\ 2 \end{bmatrix}, \quad \mathbf{X} = \begin{bmatrix} 1 & 1 \\ 1 & 2 \\ 1 & 3 \end{bmatrix}, \quad \beta = \begin{bmatrix} \beta_0 \\ \beta_1 \end{bmatrix} \quad \text{and} \quad \epsilon = \begin{bmatrix} \epsilon_1 \\ \epsilon_2 \\ \epsilon_3 \end{bmatrix}$$

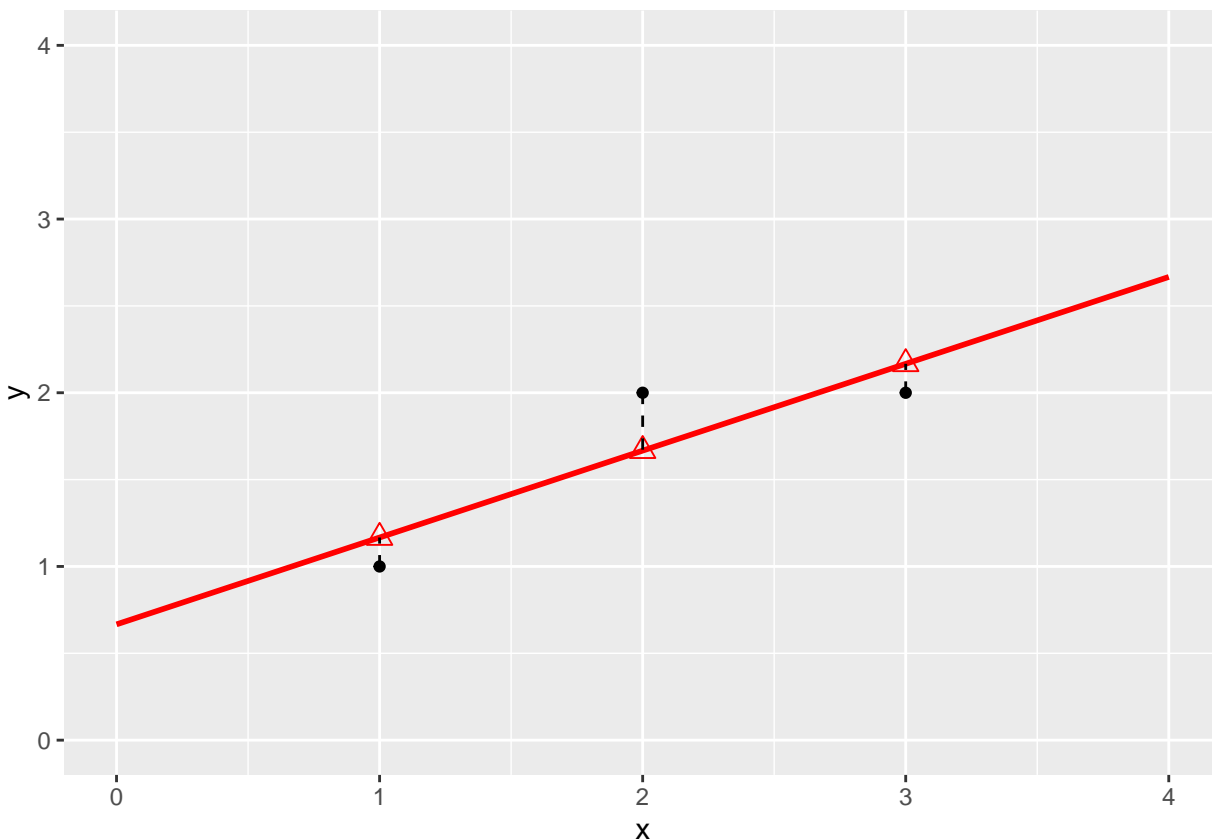
5.3.2.3 Classical interpretation Model fit and predictions based on the toy dataset

```
lm1 <- lm(y~x,data)
data$yhat <- lm1$fitted

data %>%
  ggplot(aes(x,y)) +
  geom_point() +
  ylim(0,4) +
  xlim(0,4) +
  stat_smooth(method = "lm", color = "red", fullrange = TRUE) +
  geom_point(aes(x=x, y =yhat), pch = 2, size = 3, color = "red") +
  geom_segment(data = data, aes(x = x, xend = x, y = y, yend = yhat), lty = 2 )

## `geom_smooth()` using formula 'y ~ x'

## Warning in max(ids, na.rm = TRUE): no non-missing arguments to max; returning
## -Inf
```



5.3.3 Projection

There is also another picture to regression:

- Instead of plotting each observation $i = 1 \dots n$ as a data-point in \mathbb{R}^p with dimensions $1 \dots p$ for every variable/feature that is recorded for each observation
- We can also plot \mathbf{Y} , $\hat{\mathbf{Y}}$ and each column of \mathbf{X} : \mathbf{X}_j with $j = 1 \dots p$ as a vector in \mathbb{R}^n with dimensions $1 \dots n$ for every observation.
- In this representation linear regression can be interpreted as a projection of the vector \mathbf{Y} onto the subspace of \mathbb{R}^n that is spanned by the vectors for the predictors $\mathbf{X}_1 \dots \mathbf{X}_p$.
- The space $\mathbf{X}_1 \dots \mathbf{X}_p$ is also referred to as the column space of \mathbf{X} , the space that consists of all linear combinations of the vectors of the predictors or columns $\mathbf{X}_1 \dots \mathbf{X}_p$.

5.3.3.1 Intermezzo: Projection of vector on X and Y axis

$$\mathbf{e} = \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}, \mathbf{u}_1 = \begin{bmatrix} 1 \\ 0 \end{bmatrix}, \mathbf{u}_2 = \begin{bmatrix} 0 \\ 1 \end{bmatrix}$$



1. Projection of error on x-axis

$$\begin{aligned}
 \mathbf{u}_1^T \mathbf{e} &= \|\mathbf{u}_1\|_2 \|\mathbf{e}_1\|_2 \cos \angle \mathbf{u}_1, \mathbf{e}_1 > \\
 &= \begin{bmatrix} 1 & 0 \end{bmatrix} \begin{bmatrix} e_1 \\ e_2 \end{bmatrix} \\
 &= 1 \times e_1 + 0 \times e_2 \\
 &= e_1
 \end{aligned}$$

2. Projection of error on y-axis

$$\begin{aligned}
 \mathbf{u}_2^T \mathbf{e} &= \begin{bmatrix} 0 & 1 \end{bmatrix} \begin{bmatrix} e_1 \\ e_2 \end{bmatrix} \\
 &= 0 \times e_1 + 1 \times e_2 \\
 &= e_2
 \end{aligned}$$

3. Projection of error on itself

$$\begin{aligned}
\mathbf{e}^T \mathbf{e} &= \begin{bmatrix} e_1 & e_2 \end{bmatrix} \begin{bmatrix} e_1 \\ e_2 \end{bmatrix} \\
&= e_1^2 + e_2^2 \\
&= \|\mathbf{e}\|_2^2 \rightarrow \text{Pythagorean theorem}
\end{aligned}$$

5.3.3.2 Interpretation of least squares as a projection Fitted values:

$$\begin{aligned}
\hat{\mathbf{Y}} &= \mathbf{X} \hat{\boldsymbol{\beta}} \\
&= \mathbf{X}(\mathbf{X}^T \mathbf{X})^{-1} \mathbf{X}^T \mathbf{Y} \\
&= \mathbf{H} \mathbf{Y}
\end{aligned}$$

with \mathbf{H} the projection matrix also referred to as the hat matrix.

```
X <- model.matrix(~x,data)
X
```

```
##      (Intercept) x
## 1             1 1
## 2             1 2
## 3             1 3
## attr(,"assign")
## [1] 0 1
```

```
XtX <- t(X)%*%X
XtX
```

```
##              (Intercept)  x
## (Intercept)           3   6
## x                   6  14
```

```
XtXinv <- solve(t(X)%*%X)
XtXinv
```

```
##              (Intercept)  x
## (Intercept)  2.333333 -1.0
## x           -1.000000  0.5
```

```
H <- X %*% XtXinv %*% t(X)
H
```

```
##              1          2          3
## 1  0.8333333 0.3333333 -0.1666667
## 2  0.3333333 0.3333333  0.3333333
## 3 -0.1666667 0.3333333  0.8333333
```

```
Y <- data$y
Yhat <- H%*%Y
Yhat
```

```
##      [,1]
## 1 1.166667
## 2 1.666667
## 3 2.166667
```

- We can also interpret the fit as the projection of the $n \times 1$ vector \mathbf{Y} on the column space of the matrix \mathbf{X} .
- So each column in \mathbf{X} is also an $n \times 1$ vector.
- For the toy example $n=3$ and $p=2$. The other picture to linear regression is to consider X_0 , X_1 and Y as vectors in the space of the data \mathbb{R}^n , here \mathbb{R}^3 because we have three data points. So the column space of \mathbf{X} is a plane in the three dimensional space.

$$\hat{\mathbf{Y}} = \mathbf{X}(\mathbf{X}^T \mathbf{X})^{-1} \mathbf{X}^T \mathbf{Y}$$

1. Plane spanned by column space:

```
originRn <- data.frame(X1=0,X2=0,X3=0)
data$x0 <- 1
dataRn <- data.frame(t(data))

library(plotly)

p1 <- plot_ly(
  originRn,
  x = ~ X1,
  y = ~ X2,
  z = ~ X3, name="origin") %>%
add_markers(type="scatter3d") %>%
layout(
  scene = list(
    aspectmode="cube",
    xaxis = list(range=c(-4,4)), yaxis = list(range=c(-4,4)), zaxis = list(range=c(-4,4))
  )
)
p1 <- p1 %>%
add_trace(
  x = c(0,1),
  y = c(0,0),
  z = c(0,0),
  mode = "lines",
  line = list(width = 5, color = "grey"),
  type="scatter3d",
  name = "obs1") %>%
add_trace(
  x = c(0,0),
  y = c(0,1),
```



```

    z = c(0,0),
    mode = "lines",
    line = list(width = 5, color = "grey"),
    type="scatter3d",
    name = "obs2") %>%
add_trace(
  x = c(0,0),
  y = c(0,0),
  z = c(0,1),
  mode = "lines",
  line = list(width = 5, color = "grey"),
  type="scatter3d",
  name = "obs3") %>%
add_trace(
  x = c(0,1),
  y = c(0,1),
  z = c(0,1),
  mode = "lines",
  line = list(width = 5, color = "black"),
  type="scatter3d",
  name = "X1") %>%
add_trace(
  x = c(0,1),
  y = c(0,2),
  z = c(0,3),
  mode = "lines",
  line = list(width = 5, color = "black"),
  type="scatter3d",
  name = "X2")
p1

```

2. Vector of Y:

Actual values of **Y**:

```
data$y
```

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

$$\mathbf{Y} = \begin{bmatrix} 1 \\ 2 \\ 2 \end{bmatrix}$$

```

p2 <- p1 %>%
add_trace(
  x = c(0,Y[1]),
  y = c(0,Y[2]),
  z = c(0,Y[3]),
  mode = "lines",
  line = list(width = 5, color = "red"),
  type="scatter3d",

```

```
name = "Y")
p2
```

3. Projection of Y onto column space

Actual values of fitted values $\hat{\mathbf{Y}}$:

```
data$yhat
```

```
## [1] 1.166667 1.666667 2.166667
```

$$\mathbf{Y} = \begin{bmatrix} 1.166667 \\ 1.666667 \\ 2.166667 \end{bmatrix}$$

```
p2 <- p2 %>%
  add_trace(
    x = c(0,Yhat[1]),
    y = c(0,Yhat[2]),
    z = c(0,Yhat[3]),
    mode = "lines",
    line = list(width = 5, color = "orange"),
    type="scatter3d",
    name="Yhat") %>%
  add_trace(
    x = c(Y[1],Yhat[1]),
    y = c(Y[2],Yhat[2]),
    z = c(Y[3],Yhat[3]),
    mode = "lines",
    line = list(width = 5, color = "red", dash="dash"),
    type="scatter3d",
    name="Y -> Yhat"
  )
p2
```

\mathbf{Y} is projected in the column space of \mathbf{X} ! spanned by the columns.

5.3.3.3 How does this projection works?

$$\begin{aligned} \hat{\mathbf{Y}} &= \mathbf{X}(\mathbf{X}^T\mathbf{X})^{-1}\mathbf{X}^T\mathbf{Y} \\ &= \mathbf{X}(\mathbf{X}^T\mathbf{X})^{-1/2}(\mathbf{X}^T\mathbf{X})^{-1/2}\mathbf{X}^T\mathbf{Y} \\ &= \mathbf{U}\mathbf{U}^T\mathbf{Y} \end{aligned}$$

- \mathbf{U} is a new orthonormal basis in \mathbb{R}^2 , a subspace of \mathbb{R}^3
- The space spanned by \mathbf{U} and \mathbf{X} is the column space of \mathbf{X} , e.g. it contains all possible linear combinations of \mathbf{X} . $\mathbf{U}^t\mathbf{Y}$ is the projection of \mathbf{Y} on this new orthonormal basis

```
eigenXtX <- eigen(XtX)
XtXinvSqrt <- eigenXtX$vectors %*%diag(1/eigenXtX$values.5)%*%t(eigenXtX$vectors)
U <- X %*% XtXinvSqrt
```

- U orthonormal basis

U

```
##           [,1]           [,2]
## 1  0.9116067 -0.04802616
## 2  0.3881706  0.42738380
## 3 -0.1352655  0.90279376
```

$t(U) \% \% U$

```
##           [,1]           [,2]
## [1,] 1.000000e+00 4.163336e-16
## [2,] 4.163336e-16 1.000000e+00
```

- UU^T equals projection matrix

$U \% \% t(U)$

```
##           1           2           3
## 1  0.8333333 0.3333333 -0.1666667
## 2  0.3333333 0.3333333  0.3333333
## 3 -0.1666667 0.3333333  0.8333333
```

H

```
##           1           2           3
## 1  0.8333333 0.3333333 -0.1666667
## 2  0.3333333 0.3333333  0.3333333
## 3 -0.1666667 0.3333333  0.8333333
```

```
p3 <- p1 %>%
  add_trace(
    x = c(0,U[1,1]),
    y = c(0,U[2,1]),
    z = c(0,U[3,1]),
    mode = "lines",
    line = list(width = 5, color = "blue"),
    type="scatter3d",
    name = "U1") %>%
  add_trace(
    x = c(0,U[1,2]),
    y = c(0,U[2,2]),
    z = c(0,U[3,2]),
    mode = "lines",
    line = list(width = 5, color = "blue"),
```

```

type="scatter3d",
name = "U2")

```

p3

- $\mathbf{U}^T \mathbf{Y}$ is the projection of \mathbf{Y} in the space spanned by \mathbf{U} .
- Indeed $\mathbf{U}_1^T \mathbf{Y}$

```

p4 <- p3 %>%
  add_trace(
    x = c(0,Y[1]),
    y = c(0,Y[2]),
    z = c(0,Y[3]),
    mode = "lines",
    line = list(width = 5, color = "red"),
    type="scatter3d",
    name = "Y") %>%
  add_trace(
    x = c(0,U[1,1]*(U[,1]%*%Y)),
    y = c(0,U[2,1]*(U[,1]%*%Y)),
    z = c(0,U[3,1]*(U[,1]%*%Y)),
    mode = "lines",
    line = list(width = 5, color = "red",dash="dash"),
    type="scatter3d",
    name="Y -> U1") %>% add_trace(
    x = c(Y[1],U[1,1]*(U[,1]%*%Y)),
    y = c(Y[2],U[2,1]*(U[,1]%*%Y)),
    z = c(Y[3],U[3,1]*(U[,1]%*%Y)),
    mode = "lines",
    line = list(width = 5, color = "red", dash="dash"),
    type="scatter3d",
    name="Y -> U1")

```

p4

- and $\mathbf{U}_2^T \mathbf{Y}$

```

p5 <- p4 %>%
  add_trace(
    x = c(0,U[1,2]*(U[,2]%*%Y)),
    y = c(0,U[2,2]*(U[,2]%*%Y)),
    z = c(0,U[3,2]*(U[,2]%*%Y)),
    mode = "lines",
    line = list(width = 5, color = "red",dash="dash"),
    type="scatter3d",
    name="Y -> U2") %>% add_trace(
    x = c(Y[1],U[1,2]*(U[,2]%*%Y)),
    y = c(Y[2],U[2,2]*(U[,2]%*%Y)),
    z = c(Y[3],U[3,2]*(U[,2]%*%Y)),
    mode = "lines",
    line = list(width = 5, color = "red", dash="dash"),
    type="scatter3d",
    name="Y -> U2")

```

p5

- $\hat{\mathbf{Y}}$ is the resulting vector that lies in the plane spanned by \mathbf{U}_1 and \mathbf{U}_2 and thus also in the column space of \mathbf{X} .

```
p6 <- p5 %>%
  add_trace(
    x = c(0,Yhat[1]),
    y = c(0,Yhat[2]),
    z = c(0,Yhat[3]),
    mode = "lines",
    line = list(width = 5, color = "orange"),
    type="scatter3d",
    name = "Yhat") %>%
  add_trace(
    x = c(Y[1],Yhat[1]),
    y = c(Y[2],Yhat[2]),
    z = c(Y[3],Yhat[3]),
    mode = "lines",
    line = list(width = 5, color = "maroon2"),
    type="scatter3d",
    name = "e") %>%
  add_trace(
    x = c(U[1,1]*(U[,1]%%Y),Yhat[1]),
    y = c(U[2,1]*(U[,1]%%Y),Yhat[2]),
    z = c(U[3,1]*(U[,1]%%Y),Yhat[3]),
    mode = "lines",
    line = list(width = 5, color = "orange", dash="dash"),
    type="scatter3d",
    name = "Y -> U") %>%
  add_trace(
    x = c(U[1,2]*(U[,2]%%Y),Yhat[1]),
    y = c(U[2,2]*(U[,2]%%Y),Yhat[2]),
    z = c(U[3,2]*(U[,2]%%Y),Yhat[3]),
    mode = "lines",
    line = list(width = 5, color = "orange", dash="dash"),
    type="scatter3d",
    name = "Y -> U")
p6
```

5.3.4 Error

Note, that it is also clear from the equation in the derivation of the least squares solution that the residual is orthogonal on the column space:

$$-2\mathbf{X}^T(\mathbf{Y} - \mathbf{X}\beta) = 0$$

5.4 Variance Estimator?

$$\begin{aligned}
 \hat{\Sigma} &= \text{var}[(\mathbf{X}^T \mathbf{X})^{-1} \mathbf{X}^T \mathbf{Y}] \\
 &= (\mathbf{X}^T \mathbf{X})^{-1} \mathbf{X}^T \text{var}[\mathbf{Y}] \mathbf{X} (\mathbf{X}^T \mathbf{X})^{-1} \\
 &= (\mathbf{X}^T \mathbf{X})^{-1} \mathbf{X}^T (\mathbf{I} \sigma^2) \mathbf{X} (\mathbf{X}^T \mathbf{X})^{-1} \\
 &= (\mathbf{X}^T \mathbf{X})^{-1} \mathbf{X}^T \mathbf{I} \mathbf{X} (\mathbf{X}^T \mathbf{X})^{-1} \sigma^2 \\
 &= (\mathbf{X}^T \mathbf{X})^{-1} \mathbf{X}^T \mathbf{X} (\mathbf{X}^T \mathbf{X})^{-1} \sigma^2 \\
 &= (\mathbf{X}^T \mathbf{X})^{-1} \sigma^2
 \end{aligned}$$

5.5 Contrasts

Hypotheses often involve linear combinations of the model parameters!

e.g.

- $H_0 : \log_2 FC_{g3n1-g1n1} = \beta_{g3} + \hat{\beta}_{g3n1} = 0 \rightarrow \text{"grade3+grade3:node1 = 0"}$
- Let

$$\beta = \begin{bmatrix} \beta_0 \\ \beta_{g3} \\ \beta_{n1} \\ \beta_{g3:n1} \end{bmatrix}$$

- we can write that contrast using a contrast matrix:

$$\mathbf{L} = \begin{bmatrix} 0 \\ 1 \\ 0 \\ 1 \end{bmatrix} \rightarrow \mathbf{L}^T \beta$$

- Then the variance becomes:

$$\text{var}_{\mathbf{L}^T \hat{\beta}} = \mathbf{L}^T \Sigma_{\hat{\beta}} \mathbf{L}$$

6 Homework: Adopt the gene analysis on log scale in matrix form!

1. Study the solution of the exercise to understand the analysis in R
 2. Calculate
 - model parameters and contrasts of interest
 - standard errors, standard errors on contrasts
 - t-test statistics on the model parameters and contrasts of interest
 3. Compare your results with the output of the `lm(.)` function
-

6.1 Inspiration

Tip: details on the implementation can be found in the book of Faraway (chapter 2). <https://people.bath.ac.uk/jjf23/book/>

- Design matrix

```
X <- model.matrix(~grade*node, data=gene)
```

- Transpose of a matrix: use function `t(.)`

```
t(X)
```

```
##           1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
## (Intercept) 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
## grade3      1 1 0 0 0 1 1 0 1 1 0 1 0 0 0 1 0 1 0 1 0 1 1 0
## node1       1 1 1 1 0 0 1 1 0 0 0 0 0 0 0 1 1 0 1 1 1 0 1 0
## grade3:node1 1 1 0 0 0 0 1 0 0 0 0 0 0 0 0 1 0 0 0 1 0 0 1 0
## attr("assign")
## [1] 0 1 2 3
## attr("contrasts")
## attr("contrasts")$grade
## [1] "contr.treatment"
##
## attr("contrasts")$node
## [1] "contr.treatment"
```

- Matrix product `%*%` operator

```
t(X)%*%X
```

```
##           (Intercept) grade3 node1 grade3:node1
## (Intercept)         24      12      12          6
## grade3              12      12       6          6
## node1                12       6      12          6
## grade3:node1          6       6       6          6
```

- Degrees of freedom of a model?

$$df = n - p$$

```
summary(lm1)
```

```
##
## Call:
## lm(formula = y ~ x, data = data)
##
## Residuals:
##      1      2      3
```

```
## -0.1667  0.3333 -0.1667
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.6667      0.6236   1.069   0.479
## x            0.5000      0.2887   1.732   0.333
##
## Residual standard error: 0.4082 on 1 degrees of freedom
## Multiple R-squared:  0.75, Adjusted R-squared:  0.5
## F-statistic:      3 on 1 and 1 DF,  p-value: 0.3333
```

```
dfRes <- (nrow(X)-ncol(X))
dfRes
```

```
## [1] 20
```

- Variance estimator: MSE

$$\hat{\sigma}^2 = \frac{\sum_{i=1}^n \epsilon_i^2}{n-p}$$

- Invert matrix: use function solve(.)
- Diagonal elements of a matrix: use function diag(.)

```
t(X)%*%X
```

```
##             (Intercept) grade3 node1 grade3:node1
## (Intercept)           24      12      12           6
## grade3                12      12       6           6
## node1                 12       6      12           6
## grade3:node1           6       6       6           6
```

```
diag(t(X)%*%X)
```

```
## (Intercept)      grade3      node1 grade3:node1
##           24           12           12           6
```