Statistical Methods for High Dimensional Biology

Linear models and ANOVA

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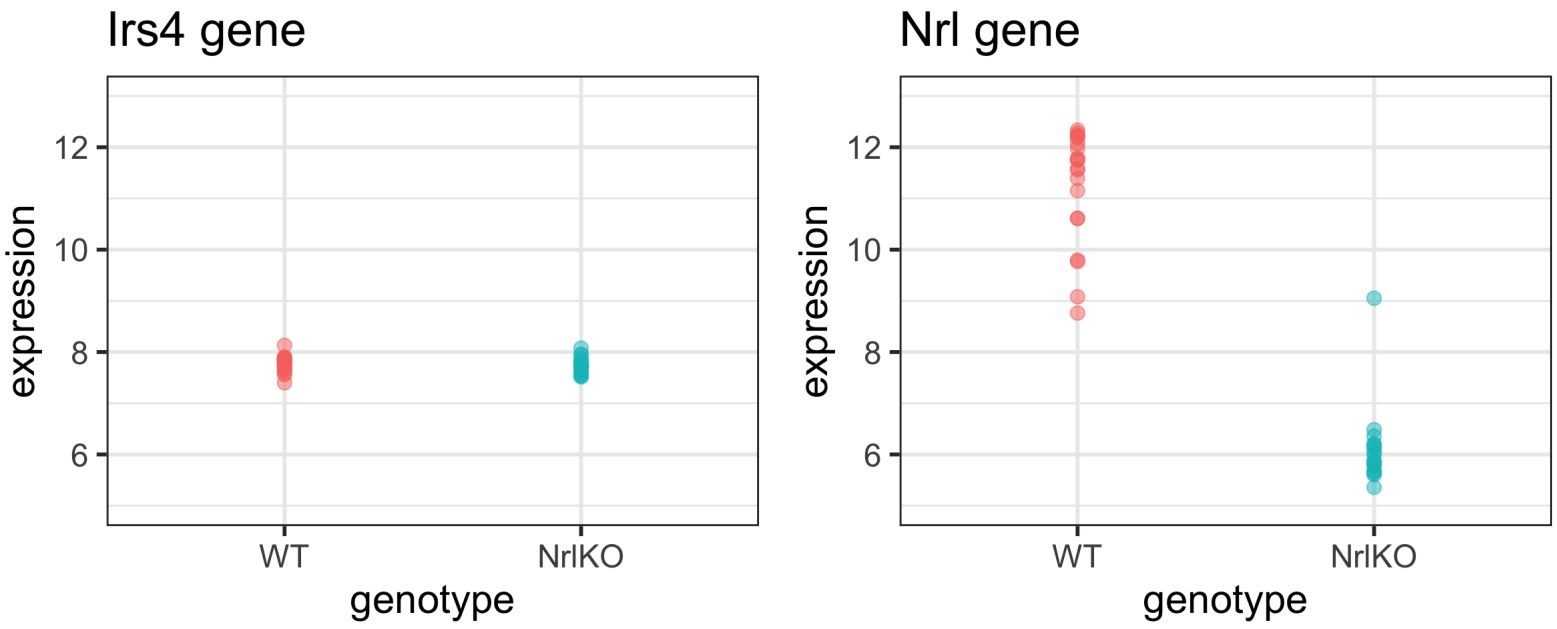
with slide contributions from Gabriela Cohen Freue and Jenny Bryan

Recap: Are these genes truly different in NrlKO compared to WT?

H_0: the expression level of gene g is the same in both conditions.

Is there **enough** evidence in the data to reject H_0?



Statistics: use a random sample to learn about the population

Population (Unknown)

$$Y \sim F$$

$$Z \sim G$$

$$E[Y] = \mu_Y$$

$$E[Z] = \mu_Z$$

$$H_0 : \mu_Y = \mu_Z$$

$$H_A : \mu_Y \neq \mu_Z$$

Sample (Observed, with randomness)

$$Y_1, Y_2, \ldots, Y_{n_Y}$$

$$Z_1, Z_2, \ldots, Z_{n_Z}$$

$$\hat{\mu}_Y = \bar{Y} = \frac{\sum_{i=1}^{n_Y} Y_i}{n_Y}$$

$$T = \frac{\bar{Y} - \bar{Z}}{\sqrt{\hat{Var}(\bar{Y} - \bar{Z})}}$$

($\bar{Y}$ and T are examples of **statistics** computed from the sample)

Summary: Hypothesis testing

1. Formulate scientific hypothesis as a statistical hypothesis $(H_0 \text{ vs } H_A)$

2. Define a test statistic to test H_0 and compute its observed value. For example:

- 2-sample *t*-test
- Welch *t*-test (unequal variance)
- Wilcoxon rank-sum test
- Kolmogorov-Smirnov test

3. Compute the probability of seeing a test statistic as extreme as that observed, under the null sampling distribution (p-value)

4. Make a decision about the significance of the results, based on a pre-specified significance level (α)

We can run these tests in R

Example: use the `t.test` function to test H_0 using a classical 2-sample t-test with equal variance.

```
filter(twoGenes, gene == "Irs4") %>%
  t.test(expression ~ genotype, data = ., var.equal = TRUE)
```

```
##
##     Two Sample t-test
##
## data:  expression by genotype
## t = 0.52854, df = 37, p-value = 0.6003
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.07384018  0.12595821
## sample estimates:
##    mean in group WT mean in group NrlKO
##            7.765671            7.739612
```

Today's Learning Objectives

1. Compare means of different groups (2 or more) using a linear regression model

- Understand how 'dummy' variables represent the levels of a qualitative explanatory variable

2. Write a linear model using matrix notation

- understand which matrix is built by R

3. Distinguish between **single** and **joint** hypothesis tests

- e.g. t-tests vs F-tests

$$H_0 : \mu_1 = \mu_2$$

2-sample t-test (with equal variance)

```
filter(twoGenes, gene == "Irs4") %>%
  t.test(expression ~ genotype, data = ., var.equal = TRUE)
```

(one-way) Analysis of Variance (ANOVA)

```
filter(twoGenes, gene == "Irs4") %>%
  aov(expression ~ genotype, data = .) %>%
  summary()
```

Linear regression model

```
filter(twoGenes, gene == "Irs4") %>%
  lm(expression ~ genotype, data = .) %>%
  summary()
```

All three methods give the same result!*

2-sample t-test (equal variance)

```
##
## Two Sample t-test
##
## data: expression by genotype
## t = 0.52854, df = 37, p-value = 0.6003
## alternative hypothesis: true difference
in means is not equal to 0
## 95 percent confidence interval:
## -0.07384018 0.12595821
## sample estimates:
## mean in group WT mean in group NrlKO
## 7.765671 7.739612
```

*Note differences in sign between t-test & linear regression

(one-way) Analysis of Variance (ANOVA)

```
## Df Sum Sq Mean Sq F value Pr(>F)
## genotype 1 0.0066 0.006617 0.279 0.6
## Residuals 37 0.8764 0.023685
```

Linear regression model

```
## Coefficients:
## Estimate Std. Error t value Pr(>|t|)
## (Intercept) 7.76567 0.03441 225.659 <2e-16 ***
## genotypeNrlKO -0.02606 0.04930 -0.529 0.6
```

These are not coincidences!

2-sample t-test (equal variance)

```
## $t statistic
##         t
## 0.5285386
##
## $p-value
## [1] 0.6002819
##
## $mean difference
## [1] 0.02605902
##
## $(t statistic)^2
##         t
## 0.279353
```

(one-way) Analysis of Variance (ANOVA)

```
## $F statistic
## [1] 0.279353
##
## $p-value
## [1] 0.6002819
```

Linear regression model

```
## $t statistic
## [1] -0.5285386
##
## $p-value
## [1] 0.6002819
##
## $coefficient estimate
## [1] -0.02605902
```

t-test vs linear regression: why the same results?

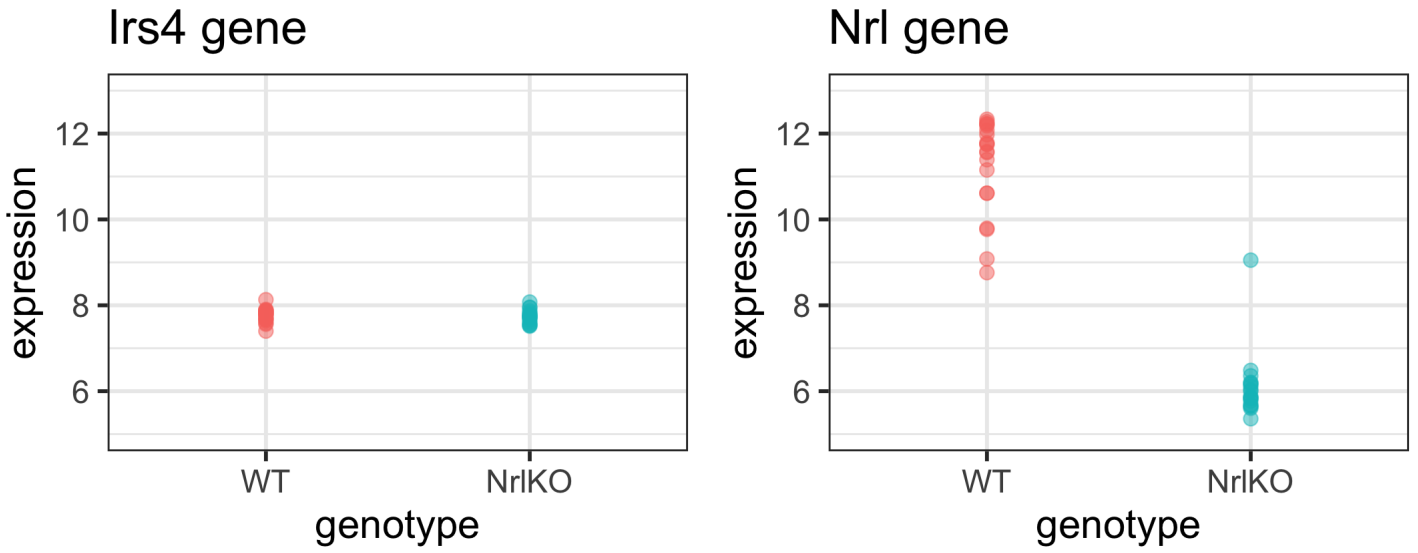
```
list("t statistic" = irs4.ttest$statistic,
     "p-value" = irs4.ttest$p.value)
```

```
## $t statistic
##         t
## 0.5285386
##
## $p-value
## [1] 0.6002819
```

```
list("t statistic" = irs4.lm$coeff[2,3],
     "p-value" = irs4.lm$coeff[2,4])
```

```
## $t statistic
## [1] -0.5285386
##
## $p-value
## [1] 0.6002819
```

t-test vs linear regression: where's the line?



> Note that the x-axis in these plots is not numerical, thus a line in this space does not have any mathematical meaning.

Why can we run a *t*-test with a linear regression model?

From *t*-test to linear regression

Let's change the notation to give a common framework to all methods

$$Y \sim G;\ E[Y] = \mu_Y$$

$$\downarrow$$

$$Y = \mu_Y + \varepsilon_Y;\ \varepsilon_Y \sim G;\ E[\varepsilon_Y] = 0$$

Why is this equivalent?

$$E[Y] = E[\mu_Y + \varepsilon_Y] = \mu_Y + E[\varepsilon_Y] = \mu_Y$$

We are just rewriting Y here

From *t*-test to linear regression

Let's change the notation to give a common framework to all methods

$$Y \sim G;\ E[Y] = \mu_Y$$

$$\downarrow$$

$$Y = \mu_Y + \varepsilon_Y;\ \varepsilon_Y \sim G;\ E[\varepsilon_Y] = 0$$

We can use a indices to accommodate multiple groups, i.e.,

$$Y_{ij} = \mu_j + \varepsilon_{ij};\quad \varepsilon_{ij} \sim G_j;\quad E[\varepsilon_{ij}] = 0;$$

where $j = \{\mathrm{WT}, \mathrm{NrlKO}\}$ (or $j = \{1, 2\}$) identifies the groups; and $i = 1, \ldots, n_j$ identifies the observations within each group

> For example: Y_{11} is the first observation in group 1 or WT

This is called the **cell-means model**

The goal is to test $H_0 : \mu_1 = \mu_2$

using data from the model

$$Y_{ij} = \mu_j + \varepsilon_{ij}; \quad \varepsilon_{ij} \sim G; \quad E[\varepsilon_{ij}] = 0;$$

where j indexes groups (e.g. WT vs NrlKO) and i indexes samples within group

> For simplicity, we assume a common distribution G for all groups

Note that the population means are given by $E[Y_{ij}] = \mu_j$, i.e., the model is written with a cell-means (μ_j) parametrization

Why the name? 'Cell' here refers to a cell of a table - e.g. make a table of means by group, and μ_j represents the population value for each cell j in the table

Recall: sample mean estimator of population mean

Note that for each group, the **population** mean is given by

$$E[Y_{ij}] = \mu_j,$$

- A natural *estimator* of the population mean is the **sample** mean
- Classical hypothesis testing methods use the group sample means as estimators
- See, for example, the `t.test` function in R:

```
irs4.ttest$estimate
```

```
##    mean in group WT mean in group NrlKO
##            7.765671             7.739612
```

However, the `lm` function reports other estimates, why?

```
irs4.ttest$estimate
```

```
##    mean in group WT mean in group NrlKO
##            7.765671             7.739612
```

```
irs4.lm$coefficients[,1]
```

```
##   (Intercept) genotypeNrlKO
##    7.76567142   -0.02605902
```

↓

`(Intercept)` is the sample mean of WT group

but `genotypeNrlKO` is not the sample mean of the NrlKO group - what is it then?

Parameterization: how to write the model?

- By default, the `lm` function does not use the cell-means parameterization
- The goal is to *compare* the means, not to study each in isolation

Let's reformulate from **cell-means** (μ_j):

$$Y_{ij} = \mu_j + \varepsilon_{ij}; \quad \varepsilon_{ij} \sim G; \quad E[\varepsilon_{ij}] = 0;$$

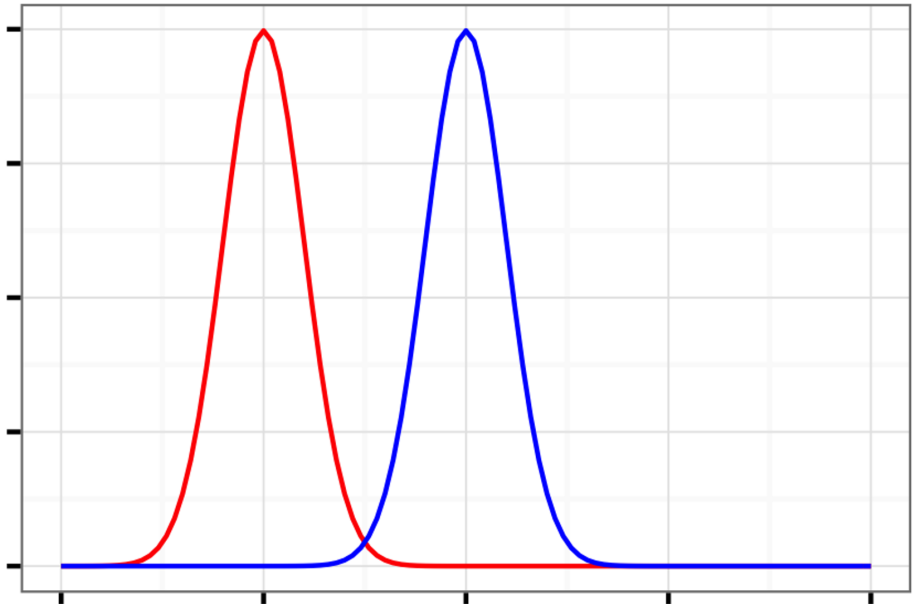
$$\downarrow$$

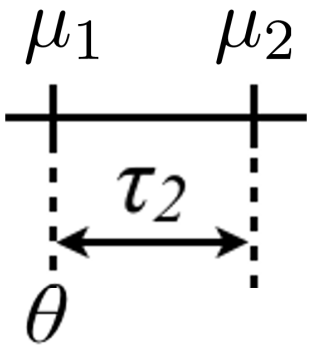
to **reference-treatment effect** (θ, τ_j):

$$Y_{ij} = \theta + \tau_j + \varepsilon_{ij}; \quad \tau_1 = 0, \quad \varepsilon_{ij} \sim G; \quad E[\varepsilon_{ij}] = 0;$$

- Note that for each group, the population mean is given by $E[Y_{ij}] = \theta + \tau_j = \mu_j$, and $\tau_2 = \mu_2 - \mu_1 = E[Y_{i2}] - E[Y_{i1}]$ *compares* the means
- τ_1 must be set to zero, since group 1 is the *reference* group

Relation between parameterization





$$H_0 : \mu_1 = \mu_2$$
$$H_0 : \tau_2 = 0$$

`lm` reports the sample mean of the reference group (WT): $\hat{\theta}$

and the treatment effect, i.e., difference between the sample means of both groups: $\hat{\tau}_2$

For gene Irs4:

```
irs4.lm$coefficients[, 1]
```

```
##   (Intercept) genotypeNrlKO
##    7.76567142   -0.02605902
```

```
irs4.means$meanExpr[irs4.means$genotype == "WT"]
```

```
## [1] 7.765671
```

```
irs4.means$meanExpr[irs4.means$genotype == "NrlKO"] -
    irs4.means$meanExpr[irs4.means$genotype == "WT"]
```

```
## [1] -0.02605902
```

`lm` reports the sample mean of the reference group (WT): $\hat{\theta}$

and the treatment effect, i.e., difference between the sample means of both groups: $\hat{\tau}_2$

For gene Nrl:

```
nrl.lm$coefficients[, 1]
```

```
##   (Intercept) genotypeNrlKO
##     11.244451     -5.154872
```

```
nrl.means$meanExpr[nrl.means$genotype == "WT"]
```

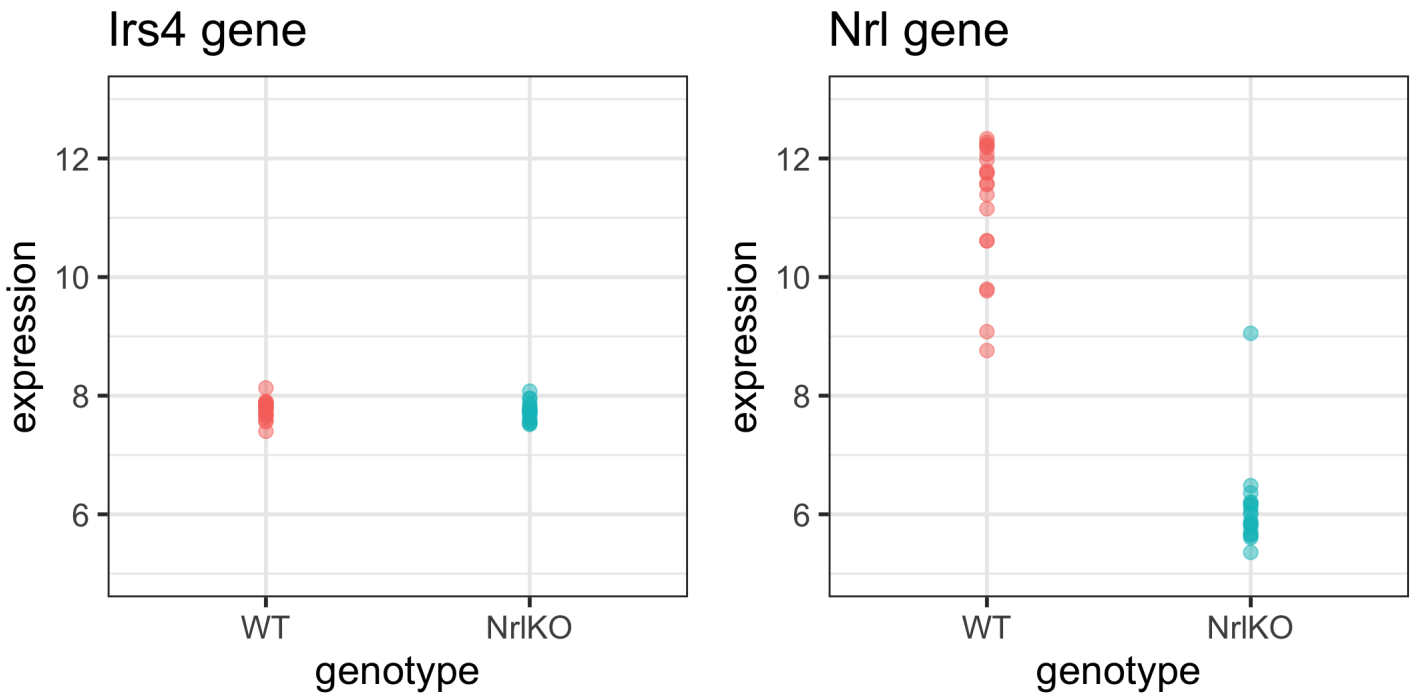
```
## [1] 11.24445
```

```
nrl.means$meanExpr[nrl.means$genotype == "NrlKO"] -
    nrl.means$meanExpr[nrl.means$genotype == "WT"]
```

```
## [1] -5.154872
```

We still haven't answered our question ... where's the line??

$$Y_{ij} = \theta + \tau_j + \varepsilon_{ij}; \quad \tau_1 = 0, \quad \varepsilon_{ij} \sim G; \quad E[\varepsilon_{ij}] = 0;$$



Dummy variables

Let's re-write our model using dummy (or indicator) variables:

$$Y_{ij} = \theta + \tau_j + \varepsilon_{ij} \quad \text{where} \quad \tau_1 = 0; \quad \varepsilon_{ij} \sim G; \quad E[\varepsilon_{ij}] = 0;$$

$$\downarrow$$

$$Y_{ij} = \theta + \tau_2 x_{ij} + \varepsilon_{ij} \quad \text{where} \quad x_{ij} = \begin{cases} 1 \text{ if } j = 2 \\ 0 \text{ otherwise} \end{cases}$$

> Note that $Y_{i1} = \theta + \varepsilon_{i1}$, because $x_{i1} = 0$ and $Y_{i2} = \theta + \tau_2 + \varepsilon_{i2}$, because $x_{i2} = 1$ (for all i)

The second form is written as a *linear* ($y = a + bx + \varepsilon$) regression model, with a special (dummy) explanatory variable x_{ij}

Using dummy variables to model our categorical variable `genotype` we can perform a 2-sample *t*-test with a linear model

$$Y_{ij} = \theta + \tau_2 x_{ij} + \varepsilon_{ij} \text{ where } x_{ij} = \begin{cases} 1 \text{ if } j = 2 \\ 0 \text{ if } j = 1 \end{cases}$$

- Recall that $\tau_2 = \mu_2 - \mu_1$
- The *t*-test in the linear model is carried out on $H_0 : \tau_2 = 0$, where τ_2 is the difference in population means (here NrlKO - WT)

```
list("t statistic"=irs4.ttest$stat,
     "p-value"=irs4.ttest$p.value)
```

```
## $t statistic
##         t
## 0.5285386
##
## $p-value
## [1] 0.6002819
```

```
list("t statistic"=irs4.lm$coeff[2,3],
     "p-value"=irs4.lm$coeff[2,4])
```

```
## $t statistic
## [1] -0.5285386
##
## $p-value
## [1] 0.6002819
```

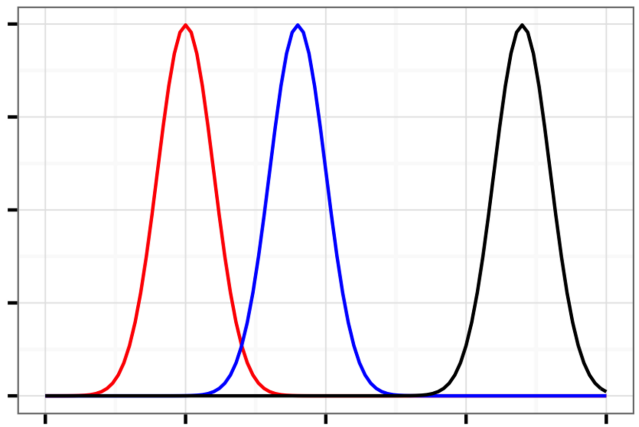
Beyond 2-groups comparisons: difference of means

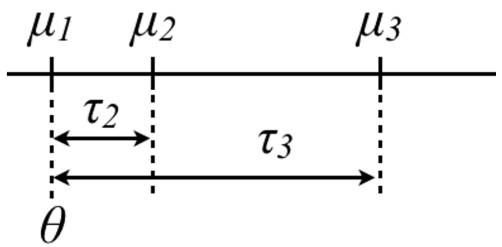
"cell-means"

$$Y_{ij} = \mu_j + \varepsilon_{ij}$$

"reference-treatments"

$$Y_{ij} = \theta + \tau_j + \varepsilon_{ij}, (\tau_1 = 0)$$





More than 2 groups!

Dummy variables can be used to model one *or more* categorical variables with 2 *or more* levels!

2-sample *t*-test using a linear model

$$Y_{ij} = \theta + \tau_2 x_{ij} + \varepsilon_{ij} \quad \text{where} \quad x_{ij} = \begin{cases} 1 \text{ if } j = 2 \\ 0 \text{ if } j = 1 \end{cases}$$

1-way ANOVA with many levels $^{(*)}$ using a linear model

$$Y_{ij} = \theta + \tau_2 x_{ij2} + \tau_3 x_{ij3} + \varepsilon_{ij} \quad \text{where } x_{ij2} = \begin{cases} 1 \text{ if } j = 2 \\ 0 \text{ otherwise} \end{cases} \quad \text{and} \quad x_{ij3} = \begin{cases} 1 \text{ if } j = 3 \\ 0 \text{ otherwise} \end{cases}$$

This is why R can estimate all of them with `lm()`

$^{(*)}$ in general, *yet* another parameterization can be used to present ANOVA

t-test

> Special case of ANOVA, but with ANOVA you can compare **more than two groups** and **more than one factor**.

ANOVA

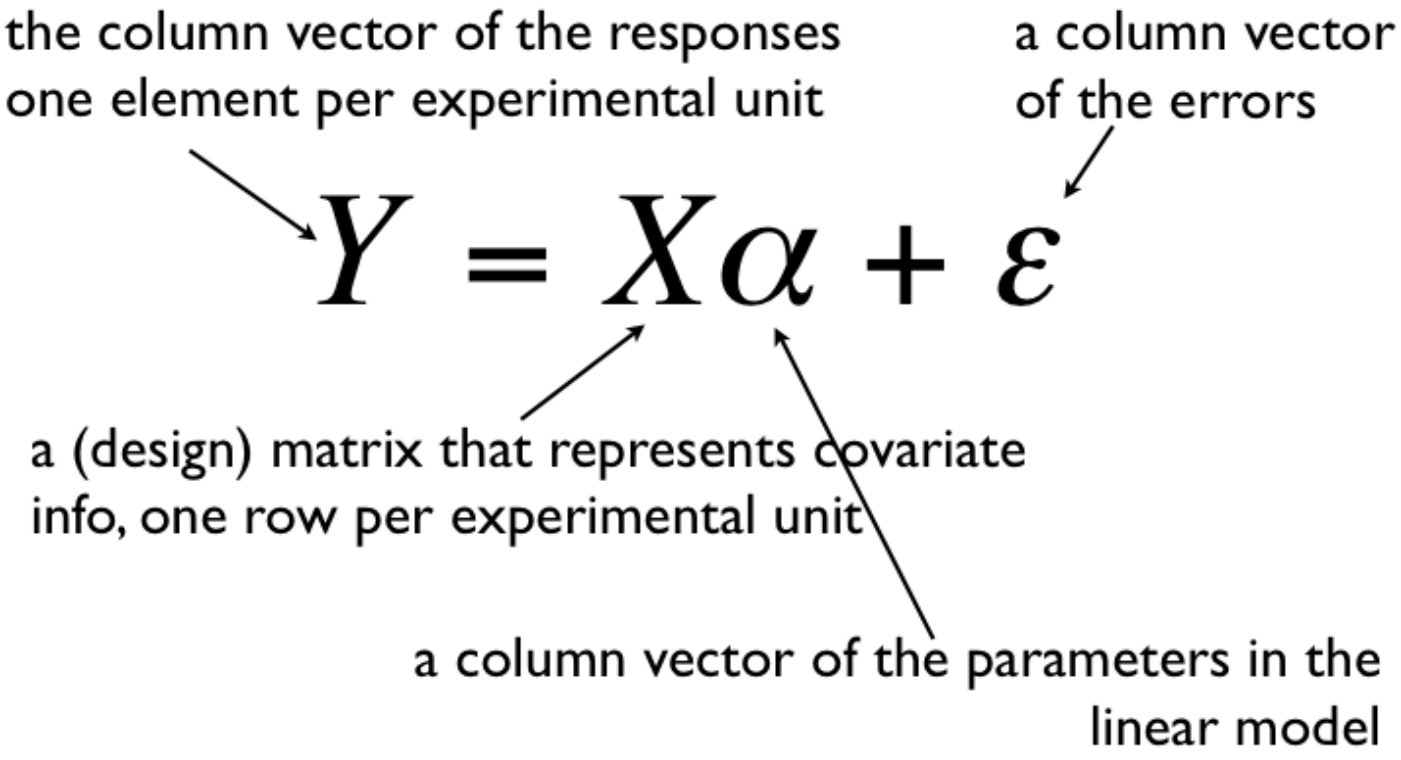
> Special case of linear regression, but with linear regression you can include **quantitative variables** in the model.

Linear regression

> Provides a unifying framework to model the association between a response and **many quantitative and qualitative variables**.

In R: all can be computed using the `lm()` function.

Linear models using matrix notation



It will become handy to write our model using matrix notation

Let's form an X (design) matrix for a 3-group comparison

$$Y_{ij} = \theta + \tau_2 x_{ij2} + \tau_3 x_{ij3} + \varepsilon_{ij}$$

First column in X for reference treatment parameterization is all 1s

Second & third columns contain x_{ij2} and x_{ij3}:

- $x_{i12} = x_{i13} = 0$ for the reference group
- $x_{i22} = 1$ for the 2nd group
- $x_{i33} = 1$ for the 3rd group

$$\begin{bmatrix} Y_{11} \\ \vdots \\ Y_{n_1 1} \\ Y_{12} \\ \vdots \\ Y_{n_2 2} \\ Y_{13} \\ \vdots \\ Y_{n_3 3} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ \vdots & \vdots & \vdots \\ 1 & 0 & 0 \\ 1 & 1 & 0 \\ \vdots & \vdots & \vdots \\ 1 & 1 & 0 \\ 1 & 0 & 1 \\ \vdots & \vdots & \vdots \\ 1 & 0 & 1 \end{bmatrix} \begin{bmatrix} \theta \\ \tau_2 \\ \tau_3 \end{bmatrix} + \begin{bmatrix} \varepsilon_{11} \\ \vdots \\ \varepsilon_{n_1 1} \\ \varepsilon_{12} \\ \vdots \\ \varepsilon_{n_2 2} \\ \varepsilon_{13} \\ \vdots \\ \varepsilon_{n_3 3} \end{bmatrix}$$

$Y_{i1} = 1 \times \theta + 0 \times \tau_2 + 0 \times \tau_3 + \varepsilon_{i1} = \theta + \varepsilon_{i1}$

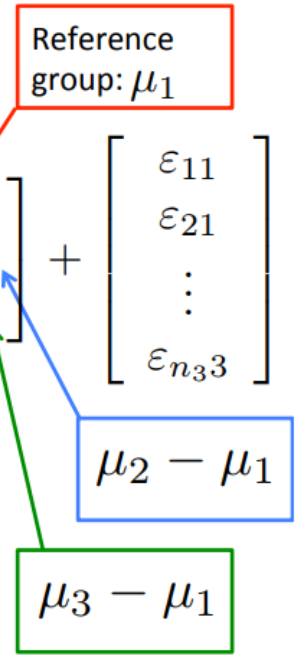
$Y_{i2} = 1 \times \theta + 1 \times \tau_2 + 0 \times \tau_3 + \varepsilon_{i2} = \theta + \tau_2 + \varepsilon_{i2}$

$Y_{i3} = 1 \times \theta + 0 \times \tau_2 + 1 \times \tau_3 + \varepsilon_{i3} = \theta + \tau_3 + \varepsilon_{i3}$

$$Y_{ij} = \theta + \tau_2 x_{ij2} + \tau_3 x_{ij3} + \varepsilon_{ij}$$

$$Y = X\alpha + \varepsilon$$

$$\begin{bmatrix} Y_{11} \\ Y_{21} \\ \vdots \\ Y_{n_3 3} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ \vdots & \vdots & \vdots \\ 1 & 0 & 0 \\ 1 & 1 & 0 \\ \vdots & \vdots & \vdots \\ 1 & 1 & 0 \\ 1 & 0 & 1 \\ \vdots & \vdots & \vdots \\ 1 & 0 & 1 \end{bmatrix} \begin{bmatrix} \theta \\ \tau_2 \\ \tau_3 \end{bmatrix} + \begin{bmatrix} \varepsilon_{11} \\ \varepsilon_{21} \\ \vdots \\ \varepsilon_{n_3 3} \end{bmatrix}$$



The model is still written with a reference-treatment parameterization (difference of means)

$E[Y_{i1}] = \theta$

$E[Y_{i2}] = \theta + \tau_2 \ \rightarrow \tau_2 = E[Y_{i2}] - E[Y_{i1}] = \mu_2 - \mu_1$

$E[Y_{i3}] = \theta + \tau_3 \ \rightarrow \tau_3 = E[Y_{i3}] - E[Y_{i1}] = \mu_3 - \mu_1$

Linear regression can include **quantitative & qualitative covariates.**

$$Y = X\alpha + \varepsilon$$

This gives us a VERY FLEXIBLE framework!!

$$\begin{bmatrix} 1 & 0 & 0 & 0 \\ \vdots & \vdots & \vdots & \\ 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 \\ \vdots & \vdots & \vdots & \\ 1 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ \vdots & \vdots & \vdots & \\ 1 & 0 & 1 & 0 \\ 1 & 0 & 0 & 1 \\ \vdots & \vdots & \vdots & \\ 1 & 0 & 0 & 1 \end{bmatrix}$$

1 categorical covariate

$$\begin{bmatrix} 1 & 0 & 0 & 0 \\ \vdots & \vdots & \vdots & \\ 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 \\ \vdots & \vdots & \vdots & \\ 1 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ \vdots & \vdots & \vdots & \\ 1 & 0 & 1 & 0 \\ 1 & 1 & 1 & 1 \\ \vdots & \vdots & \vdots & \\ 1 & 1 & 1 & 1 \end{bmatrix}$$

2 categorical covariates

$$\begin{bmatrix} 1 & 1.22 \\ 1 & 2.02 \\ 1 & 1.42 \\ \vdots & \vdots \\ 1 & 1.89 \\ 1 & 2.01 \\ \vdots & \vdots \\ 1 & 1.56 \\ 1 & 2.17 \\ 1 & 1.51 \end{bmatrix}$$

1 continuous covariate

$$\begin{bmatrix} 1 & 0 & 1.22 & 0 \\ 1 & 0 & 2.02 & 0 \\ 1 & 0 & 1.42 & 0 \\ \vdots & \vdots & \vdots & \vdots \\ 1 & 0 & 1.89 & 0 \\ 1 & 1 & 2.01 & 2.01 \\ \vdots & \vdots & \vdots & \vdots \\ 1 & 1 & 1.56 & 1.56 \\ 1 & 1 & 2.17 & 2.17 \\ 1 & 1 & 1.51 & 1.51 \end{bmatrix}$$

1 continuous 1 categorical

AND MANY MORE .....

Tip: ?model.matrix

> Linear in the parameters α: X can contain x^2, $log(x)$, etc.

How it works in practice using lm() in R

$$Y = X\alpha + \varepsilon$$

↓

```
lm(y ~ x, data = yourData)
```

y ~ x: formula,
y numeric,
x numeric and/or factor

yourData: data.frame in which x and y are to be found

By default, R uses a ref-tx parametrization but you can control that!

Special `factor` class in R

$$Y = X\alpha + \varepsilon$$

- Mathematically, X is a numeric matrix
- If your data contains categorical variables (e.g., `genotype`), you need to set them as **factors**

> especially important if your categorical variables are encoded numerically!! (`lm` will automatically treat character variables as factors)

- R creates appropriate dummy variables for factors!

```
str(twoGenes$genotype)
```

```
##  Factor w/ 2 levels "WT","NrlKO": 2 2 2 2 2 2 2 2 2 2 ...
```

Under the hood, R creates a numeric X:

```
mm <- model.matrix(~genotype, data = twoGenes)
# show first and last rows of model.matrix
mm[c(1:3, (nrow(mm) - 2):nrow(mm)), ]
```

```
##    (Intercept) genotypeNrlKO
## 1            1             1
## 2            1             1
## 3            1             1
## 76           1             0
## 77           1             0
## 78           1             0
```

```
# show genotypes of first and last samples
twoGenes$genotype[c(1:3, (nrow(mm) - 2):nrow(mm))]
```

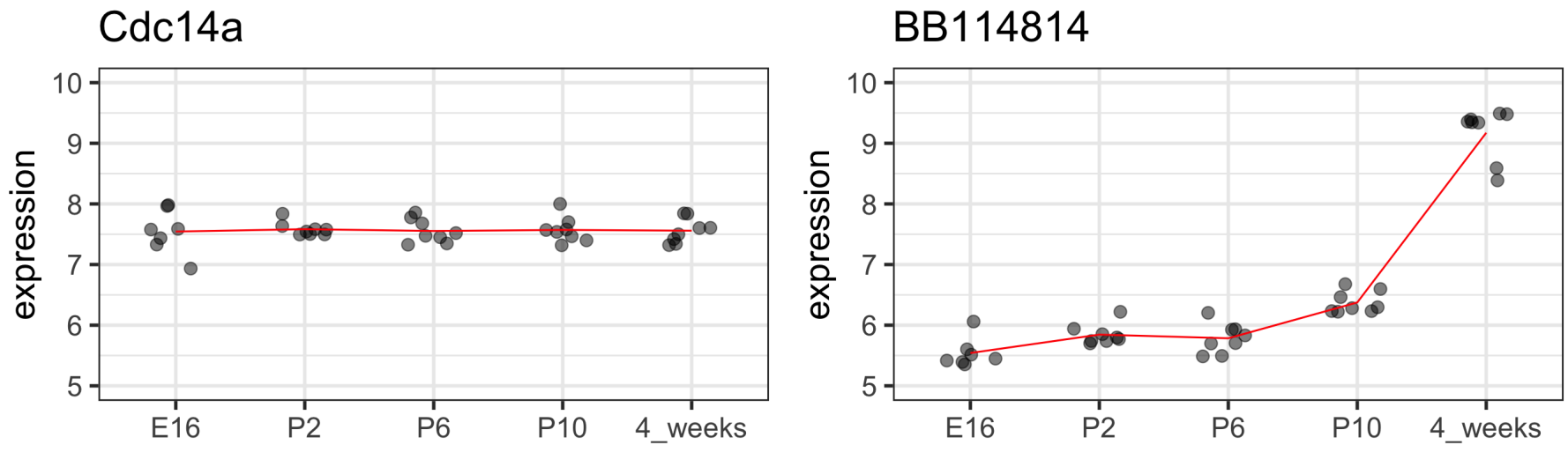
```
## [1] NrlKO NrlKO NrlKO WT    WT    WT
## Levels: WT NrlKO
```

Beyond 2-group comparisons in our case study:

Is the expression of gene X the same at all developmental stages?

$$H_0 : \mu_{E16} = \mu_{P2} = \mu_{P6} = \mu_{P10} = \mu_{4W}$$

Let's look at another two genes for some variety



Note: 4W = 4_weeks

The sample means: $\hat{\mu}_{E16}$, $\hat{\mu}_{P2}$, $\hat{\mu}_{P6}$, $\hat{\mu}_{P10}$, $\hat{\mu}_{4W}$

```
twoGenes %>%
  group_by(gene, dev_stage) %>%
  summarize(meanExpr = mean(expres
  pivot_wider(values_from = meanEx
```

```
## # A tibble: 5 x 3
##   dev_stage BB114814 Cdc14a
##   <fct>        <dbl>  <dbl>
## 1 E16          5.5409 7.5443
## 2 P2           5.8447 7.5836
## 3 P6           5.7842 7.5540
## 4 P10          6.3750 7.5710
## 5 4_weeks      9.1733 7.5590
```

BB114814 gene with notable time effect

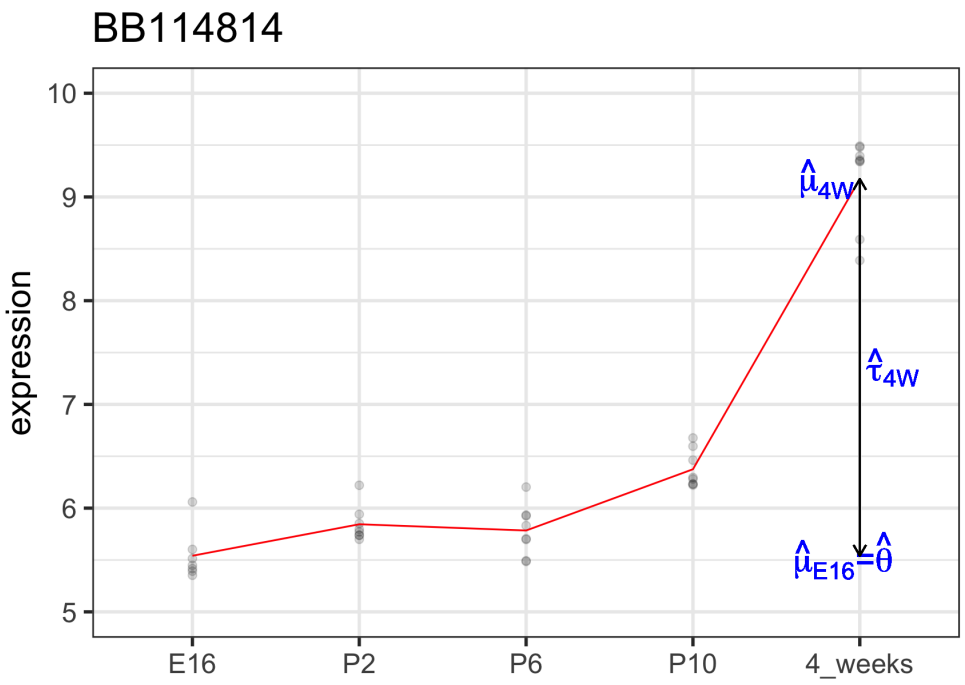
```
twoGenes %>% filter(gene == "BB114814") %>%
  group_by(dev_stage) %>%
  summarize(cellMeans = mean(expression)) %>%
  mutate(timeEffect = cellMeans - cellMeans[1])
```

```
## # A tibble: 5 x 3
##   dev_stage cellMeans timeEffect
## * <fct>          <dbl>      <dbl>
## 1 E16           5.5409    0
## 2 P2            5.8447    0.30379
## 3 P6            5.7842    0.24328
## 4 P10           6.3750    0.83412
## 5 4_weeks       9.1733    3.6324
```

"Effect" here means compared to reference/baseline (E16)

BB114814 gene with notable time effect

```
## # A tibble: 5 x 3
##   dev_stage cellMeans timeEffect
## * <fct>         <dbl>      <dbl>
## 1 E16           5.5409     0
## 2 P2            5.8447     0.30379
## 3 P6            5.7842     0.24328
## 4 P10           6.3750     0.83412
## 5 4_weeks       9.1733     3.6324
```



Can you guess the size of the X matrix??

> How many dummy variables do we need?

Gene BB114814 with notable time effect

We need 4 dummy variables to estimate and test 4 time differences (between 5 time points):

> x_{P2}: P2 vs E16
> x_{P6}: P6 vs E16
> x_{P10}: P10 vs E16
> x_{4W}: 4W vs E16

Mathematically:

$$Y_{ij} = \theta + \tau_{P2}x_{ijP2} + \tau_{P6}x_{ijP6} + \tau_{P10}x_{ijP10} + \tau_{4W}x_{ij4W} + \varepsilon_{ij}$$

Notation: x_{ijk}:

- i indexes for the observation/sample within group
- j indexes the group (here level of `dev_stage`)
- k is the name of the dummy variable

Under the hood, R creates a numeric X:

```
model.matrix(~dev_stage, data = twoGenes) %>% head(19)
```

```
##    (Intercept) dev_stageP2 dev_stageP6 dev_stageP10 dev_stage4_weeks
## 1            1           0           0            0                1
## 2            1           0           0            0                1
## 3            1           0           0            0                1
## 4            1           0           0            0                1
## 5            1           0           0            0                0
## 6            1           0           0            0                0
## 7            1           0           0            0                0
## 8            1           0           0            1                0
## 9            1           0           0            1                0
## 10           1           0           0            1                0
## 11           1           0           0            1                0
## 12           1           1           0            0                0
## 13           1           1           0            0                0
## 14           1           1           0            0                0
## 15           1           1           0            0                0
## 16           1           0           1            0                0
## 17           1           0           1            0                0
## 18           1           0           1            0                0
## 19           1           0           1            0                0
```

Hypothesis tests in `lm` output

```
twoGenes %>% filter(gene == "BB114814") %>%
  lm(expression ~ dev_stage, data = .) %>%
  summary() %>% .$coef
```

```
##                   Estimate Std. Error   t value     Pr(>|t|)
## (Intercept)      5.5409162  0.1021560 54.239748 1.314828e-34
## dev_stageP2      0.3037855  0.1398829  2.171713 3.694652e-02
## dev_stageP6      0.2432795  0.1398829  1.739166 9.105366e-02
## dev_stageP10     0.8341163  0.1398829  5.962962 9.620151e-07
## dev_stage4_weeks 3.6323772  0.1398829 25.967276 5.303201e-24
```

```
##   dev_stage cellMeans timeEffect
## 1 E16          5.5409    0
## 2 P2           5.8447    0.30379
## 3 P6           5.7842    0.24328
## 4 P10          6.3750    0.83412
## 5 4_weeks      9.1733    3.6324
```

$H_0 : \theta = 0$ or $H_0 : \mu_{E16} = 0$

Estimate: $\hat{\theta} = \hat{\mu}_{E16} = \bar{Y}_{\cdot E16}$

> we are not usually interested in testing this hypothesis: baseline mean = 0

Hypothesis tests in `lm` output

```
twoGenes %>% filter(gene == "BB114814") %>%
  lm(expression ~ dev_stage, data = .) %>%
  summary() %>% .$coef
```

```
##                   Estimate Std. Error   t value     Pr(>|t|)
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## dev_stageP10     0.8341163  0.1398829  5.962962 9.620151e-07
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```
##   dev_stage cellMeans timeEffect
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## 4 P10          6.3750    0.83412
## 5 4_weeks      9.1733    3.6324
```

$H_0 : \tau_{P2} = 0$ or $H_0 : \mu_{P2} = \mu_{E16}$

Estimate:
$\hat{\tau}_{P2} = \hat{\mu}_{P2} - \hat{\mu}_{E16} = \bar{Y}_{\cdot P2} - \bar{Y}_{\cdot E16}$

> we *are* usually interested in testing this hypothesis: change from E16 to 2 days old = 0

Hypothesis tests in `lm` output

```
twoGenes %>% filter(gene == "BB114814") %>%
  lm(expression ~ dev_stage, data = .) %>%
  summary() %>% .$coef
```

```
##                   Estimate Std. Error   t value     Pr(>|t|)
## (Intercept)      5.5409162  0.1021560 54.239748 1.314828e-34
## dev_stageP2      0.3037855  0.1398829  2.171713 3.694652e-02
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```
##   dev_stage cellMeans timeEffect
## 1 E16          5.5409    0
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## 3 P6           5.7842    0.24328
## 4 P10          6.3750    0.83412
## 5 4_weeks      9.1733    3.6324
```

$H_0 : \tau_{4W} = 0$ or $H_0 : \mu_{4W} = \mu_{E16}$

Estimate:
$\hat{\tau}_{4W} = \hat{\mu}_{4W} - \hat{\mu}_{E16} = \bar{Y}_{\cdot 4W} - \bar{Y}_{\cdot E16}$

> we *are* usually interested in testing this hypothesis: change from E16 to 4 weeks old = 0

Notice the standard error estimates

```
##                   Estimate Std. Error   t value     Pr(>|t|)
## (Intercept)      5.5409162  0.1021560 54.239748 1.314828e-34
## dev_stageP2      0.3037855  0.1398829  2.171713 3.694652e-02
## dev_stageP6      0.2432795  0.1398829  1.739166 9.105366e-02
## dev_stageP10     0.8341163  0.1398829  5.962962 9.620151e-07
## dev_stage4_weeks 3.6323772  0.1398829 25.967276 5.303201e-24
```

> All data points are used to estimate the variance of the error term for the dummy variables

We generally test two types of null hypotheses:

$$Y = X\alpha + \varepsilon$$

$$\alpha = (\theta, \tau_{P2}, \tau_{P6}, \tau_{P10}, \tau_{4W})$$

$H_0 : \tau_j = 0$

vs

$H_0 : \tau_j \neq 0$

for each *j* individually

e.g., Is gene A differentially expressed 2 days after birth (compared to E16)?

$H_0 : \tau_{P2} = 0$

$H_0 : \tau_j = 0$

vs

$H_0 : \tau_j \neq 0$

for all *j* at the same time

e.g., Is gene A significantly affected by time (`dev_stage`)?

$H_0 : \tau_{P2} = \tau_{P6} = \tau_{P10} = \tau_{4W} = 0$

$H_0 : \tau_j = 0$ vs $H_0 : \tau_j \neq 0$ for each j individually

```
##
## Call:
## lm(formula = expression ~ dev_stage, data = .)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.78553 -0.13324 -0.04796  0.17038  0.51846
##
## Coefficients:
##                  Estimate Std. Error t value Pr(>|t|)
## (Intercept)        5.5409     0.1022  54.240  < 2e-16 ***
## dev_stageP2        0.3038     0.1399   2.172   0.0369 *
## dev_stageP6        0.2433     0.1399   1.739   0.0911 .
## dev_stageP10       0.8341     0.1399   5.963 9.62e-07 ***
## dev_stage4_weeks   3.6324     0.1399  25.967  < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2703 on 34 degrees of freedom
## Multiple R-squared:  0.9662,    Adjusted R-squared:  0.9623
## F-statistic: 243.3 on 4 and 34 DF,  p-value: < 2.2e-16
```

$H_0 : \tau_j = 0$ vs $H_0 : \tau_j \neq 0$ for all j together

```
##
## Call:
## lm(formula = expression ~ dev_stage, data = .)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.78553 -0.13324 -0.04796  0.17038  0.51846
##
## Coefficients:
##                  Estimate Std. Error t value Pr(>|t|)
## (Intercept)        5.5409     0.1022  54.240  < 2e-16 ***
## dev_stageP2        0.3038     0.1399   2.172   0.0369 *
## dev_stageP6        0.2433     0.1399   1.739   0.0911 .
## dev_stageP10       0.8341     0.1399   5.963 9.62e-07 ***
## dev_stage4_weeks   3.6324     0.1399  25.967  < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
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## Multiple R-squared:  0.9662,    Adjusted R-squared:  0.9623
## F-statistic: 243.3 on 4 and 34 DF,  p-value: < 2.2e-16
```

F-test and overall significance of one or more coefficients

- the *t*-test in linear regression allows us to test single hypotheses:

$$H_0 : \tau_j = 0$$

$$H_A : \tau_j \neq 0$$

- but we often like to test multiple hypotheses *simultaneously*:

$$H_0 : \tau_{P2} = \tau_{P6} = \tau_{P10} = \tau_{4W} = 0 \text{ [AND statement]}$$

$$H_A : \tau_j \neq 0 \text{ for some j [OR statement]}$$

  the *F*-test allows us to test such compound tests

To conclude

1. We can use different parametrizations to write statistical models

From **cell-means** (μ_j): $Y_{ij} = \mu_j + \varepsilon_{ij} \quad \text{where } \varepsilon_{ij} \sim G; \quad E[\varepsilon_{ij}] = 0;$

to **reference-treatment effect** (θ, τ_j): (used by default by `lm`)

$$Y_{ij} = \theta + \tau_j + \varepsilon_{ij} \quad \text{where } \tau_1 = 0, \quad \varepsilon_{ij} \sim G; \quad E[\varepsilon_{ij}] = 0;$$

2. We can compare group means (2 or more) using a linear model

- **dummy variables** (e.g., x_{ijP2}) to model the levels of a qualitative explanatory variables

$$Y_{ij} = \theta + \tau_{P2} x_{ijP2} + \tau_{P6} x_{ijP6} + \tau_{P10} x_{ijP10} + \tau_{4W} x_{ij4W} + \varepsilon_{ij}$$

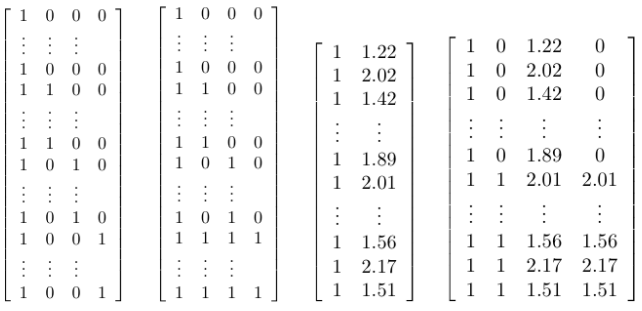
- qualitative variables need to be set as "factors" in the data $\rightarrow$ R creates the dummy variables

3. We can write a linear model using matrix notation:

$$Y = X\alpha + \varepsilon$$

4. Linear models can include **quantitative & qualitative covariates**.

$Y = X\alpha + \varepsilon$ This gives us a VERY FLEXIBLE framework!!



1 categorical covariate | 2 categorical covariates | 1 continuous covariate | 1 continuous 1 categorical

AND MANY MORE .....

Tip: ?model.matrix

5. We use different tests to distinguish between single and joint hypotheses:

- t-tests vs F-tests