Bioinformatics Analysis in R

Advanced Gene Expression: Analysis of Cancer Genome Atlas

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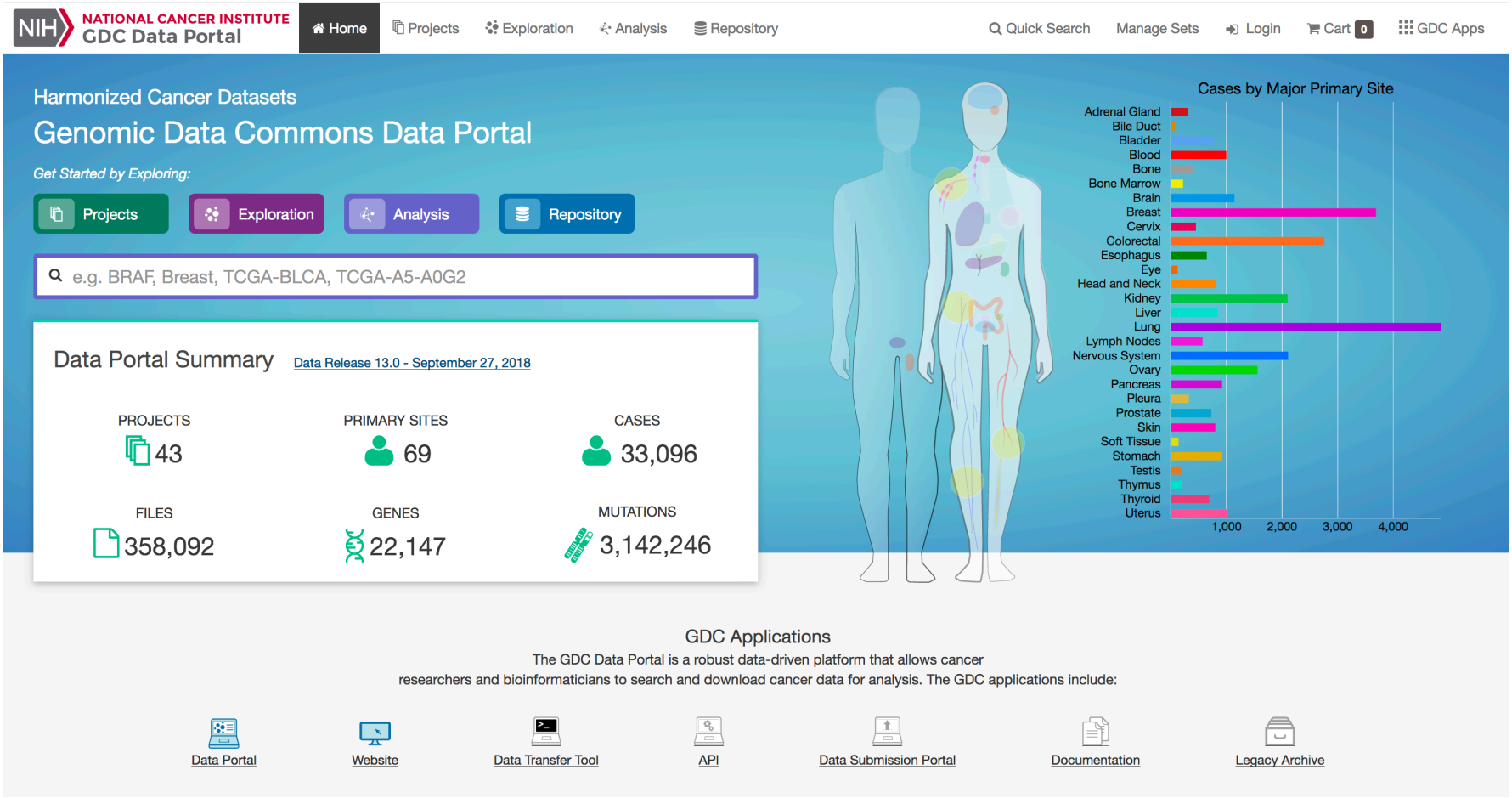
Summary

1. Obtain data from cancer patients from TCGA
2. Pre-process and analysis of RNA-seq data
3. Use machine learning to build a classifier for personalised medicine
4. Use interesting markers for survival analysis

The Cancer Genome Atlas

- TCGA is a NCI (US) funded project to generate cohorts of cancers:
  -Currently 33 cancers with 80-780 patients
- Comprehensive data from tissues:
  - Histology, clinical, gene expression profiling, copy number variation, DNA methylation using arrays or sequencing
- Data is publicly available upon generation and deposited in a portal (portal.gdc.cancer.gov)

The Cancer Genome Atlas - Portal



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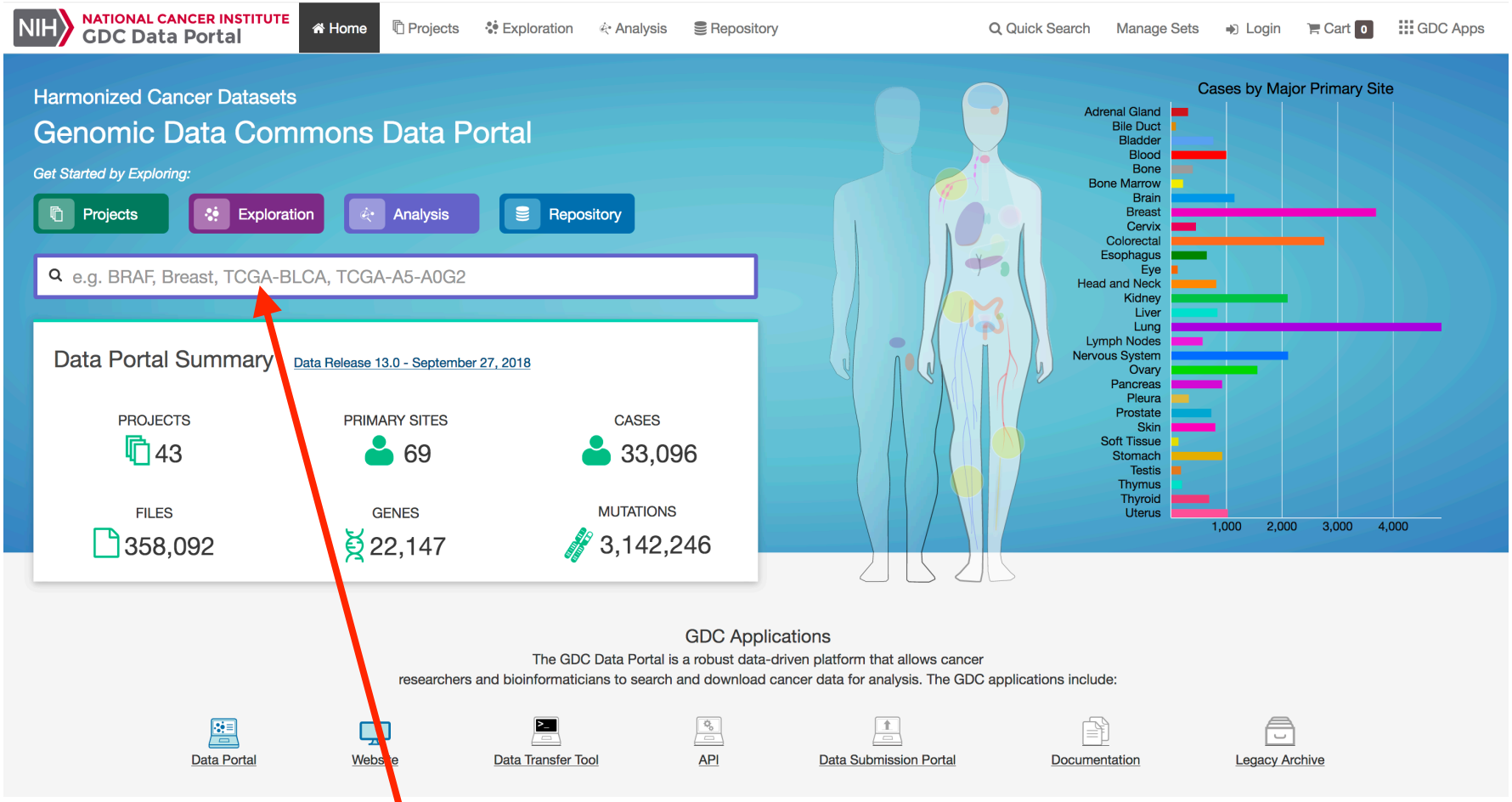

Check a gene or cancer type!

I will try liver ….

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LIHC - Liver Hepatocellular Carcinoma

Explore Project Data | Biospecimen | Clinical | Manifest

Summary

Project ID	TCGA-LIHC
Project Name	Liver Hepatocellular Carcinoma
Disease Type	Adenomas and Adenocarcinomas
Primary Site	Liver and intrahepatic bile ducts
Program	TCGA

CASES 377

FILES 10,814

ANNOTATIONS 28

Cases and File Counts by Data Category

Data Category	Cases (n=377)	Files (n=10,814)
Raw Sequencing Data	377	1,637
Transcriptome Profiling	376	2,122
Simple Nucleotide Variation	375	3,032
Copy Number Variation	376	1,536
DNA Methylation	377	430
Clinical	377	423
Biospecimen	377	1,634

Cases and File Counts by Experimental Strategy

Experimental Strategy	Cases (n=377)	Files (n=10,814)
Diagnostic Slide	365	379
Tissue Slide	377	491
WXS	376	3,820
RNA-Seq	371	1,696
miRNA-Seq	373	1,275
Genotyping Array	376	1,536
Methylation Array	377	430

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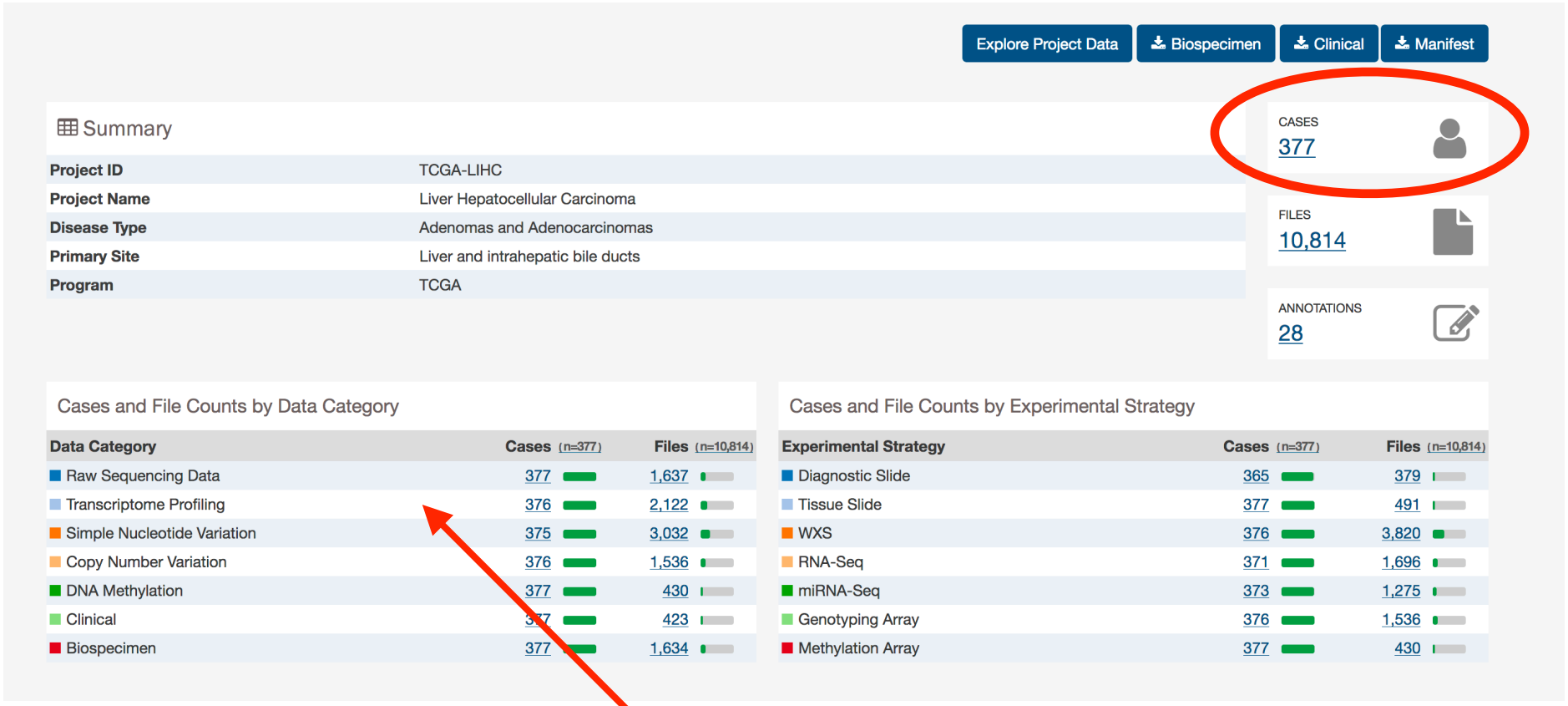
LIHC - Liver Hepatocellular Carcinoma



Gene expression data!

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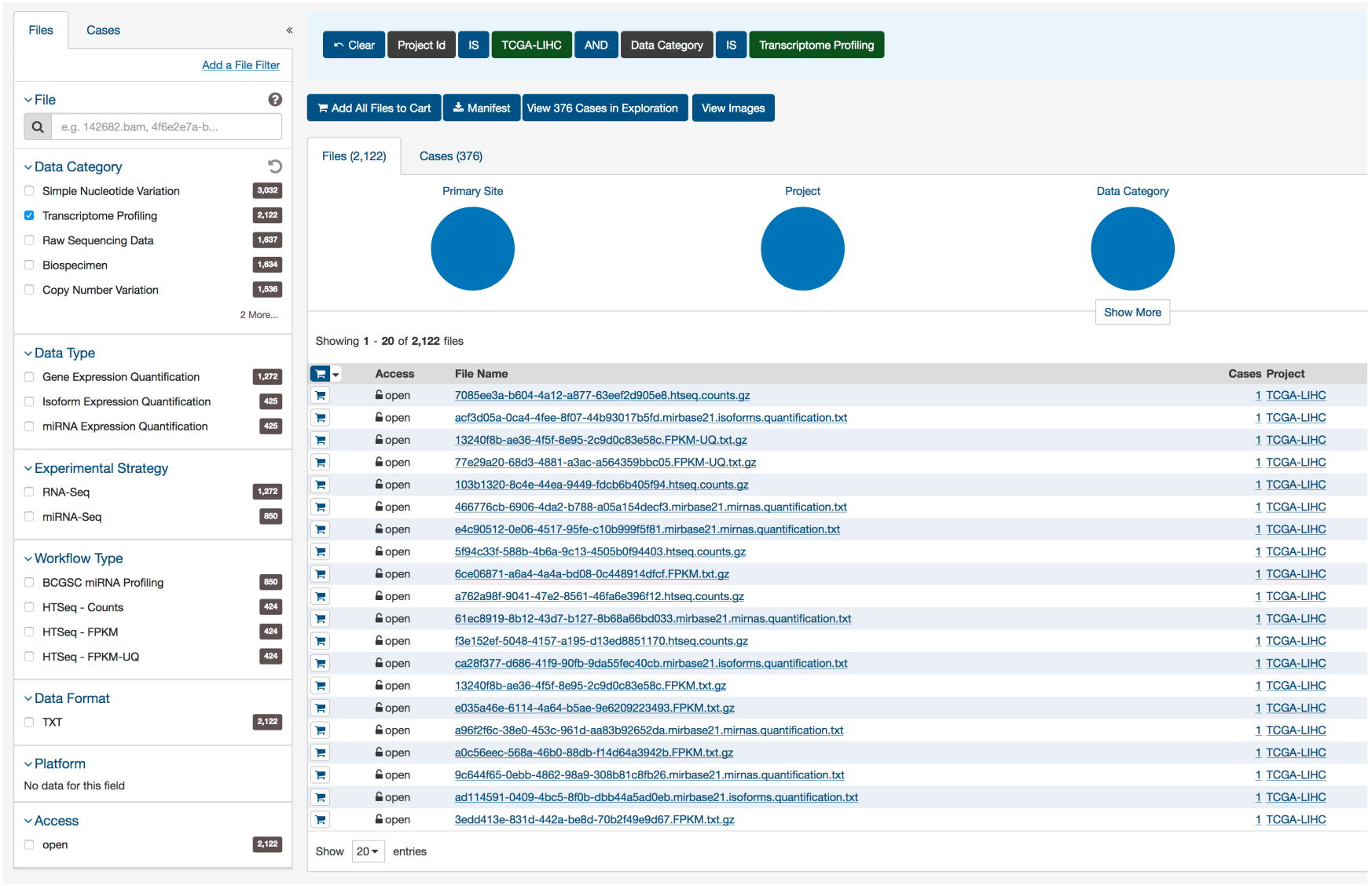
LIHC - Liver Hepatocellular Carcinoma



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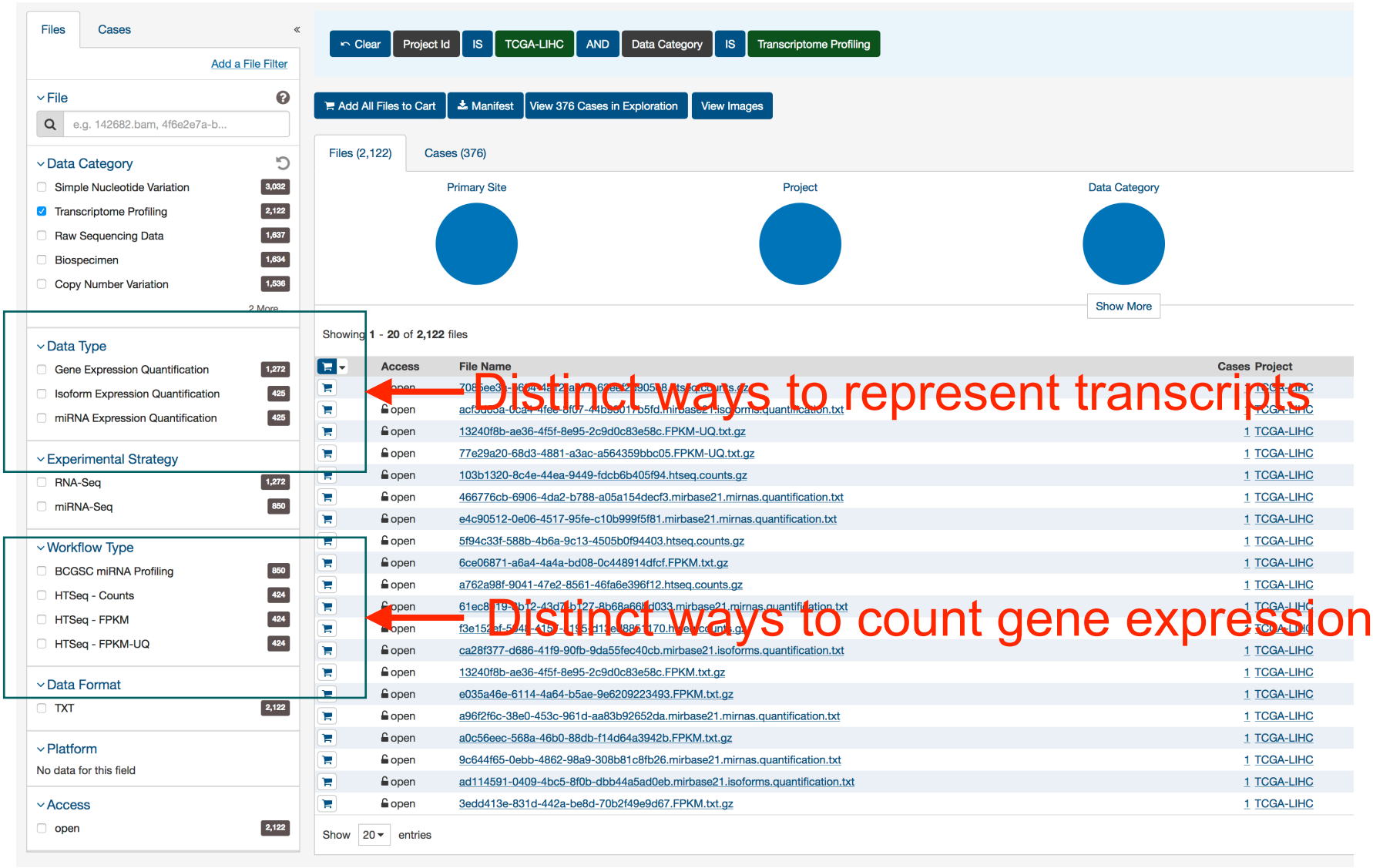
LIHC - Liver Hepatocellular Carcinoma



Distinct ways to represent transcripts

Distinct ways to count gene expression.

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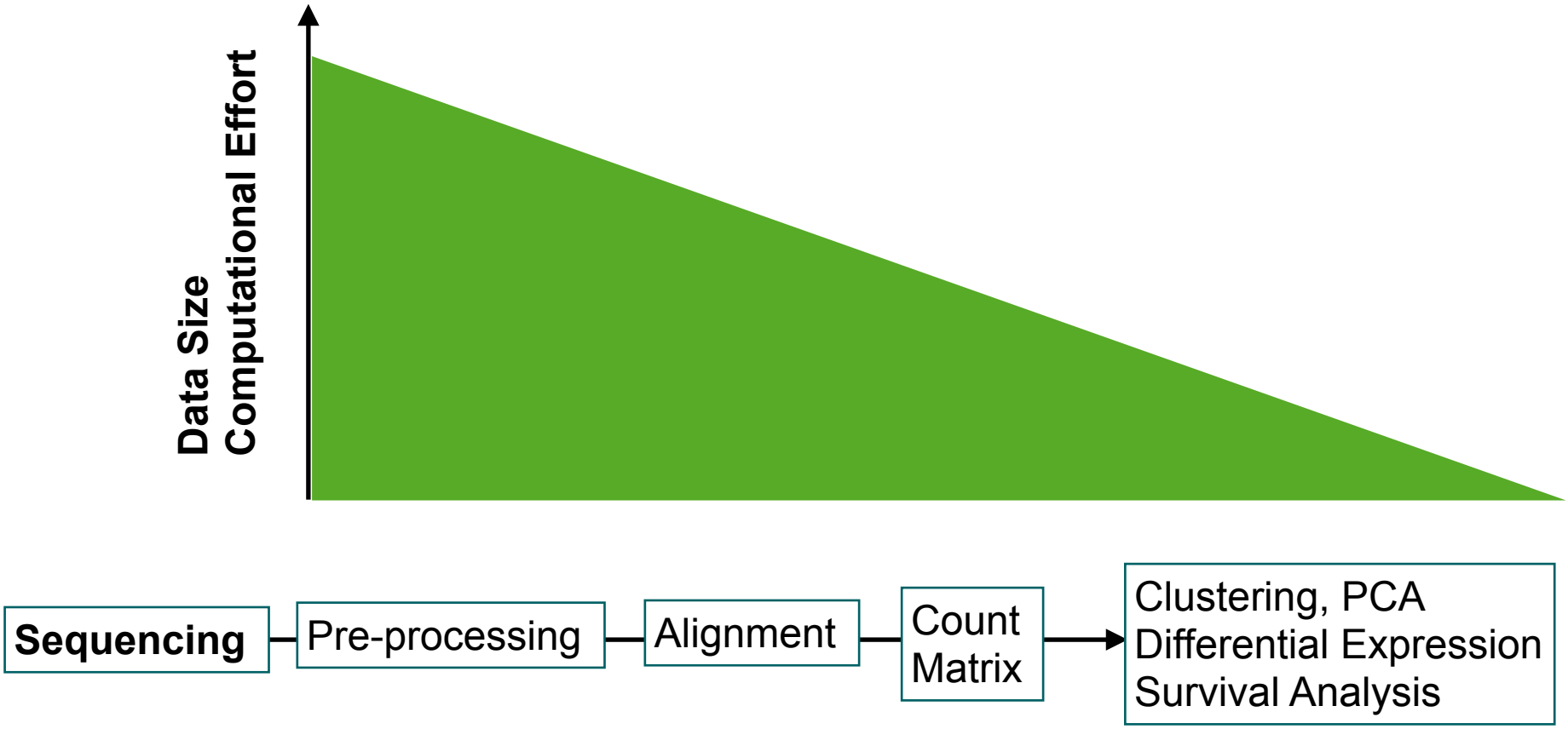
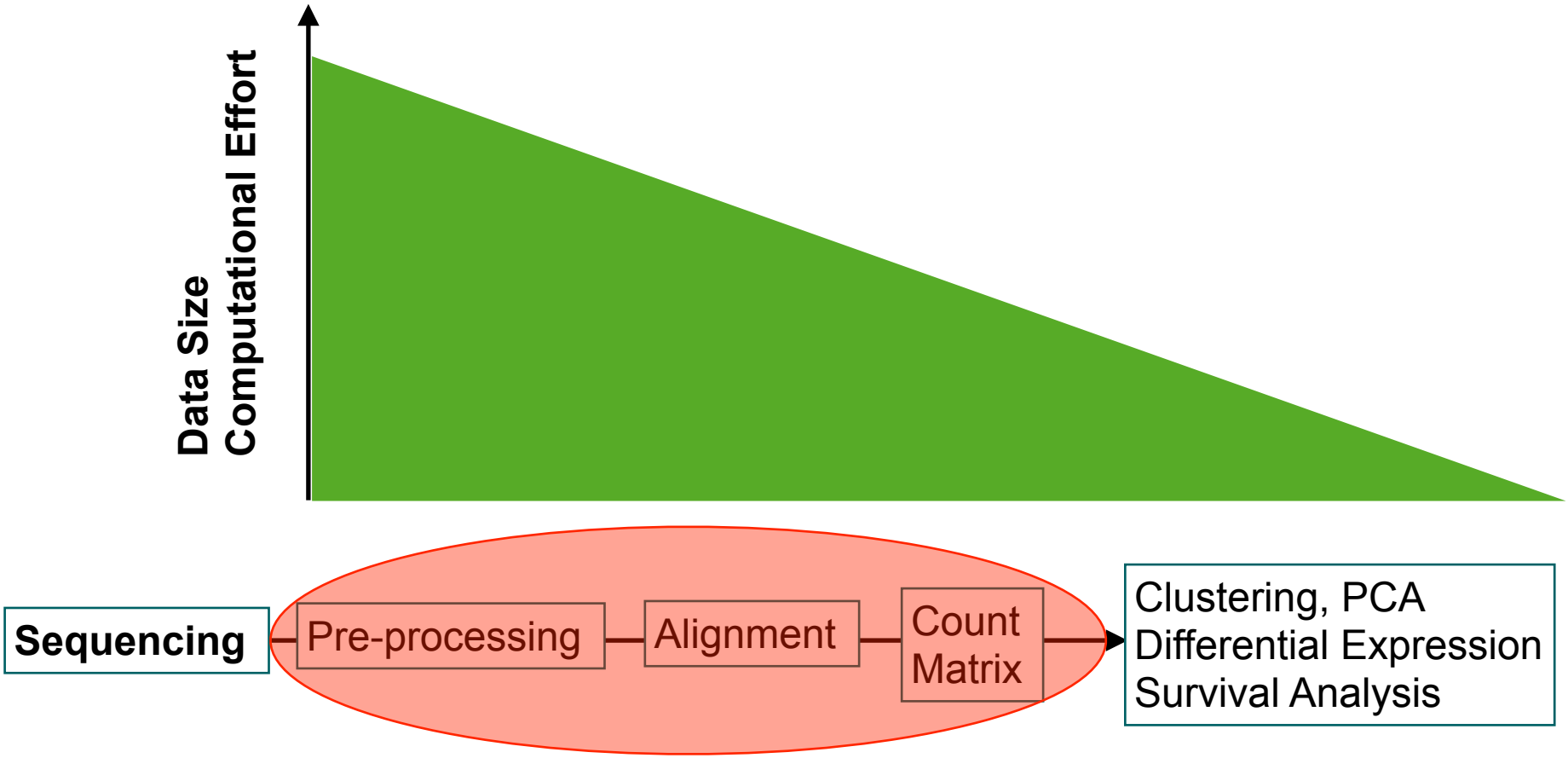
Bioinformatics Pipeline / RNA-seq

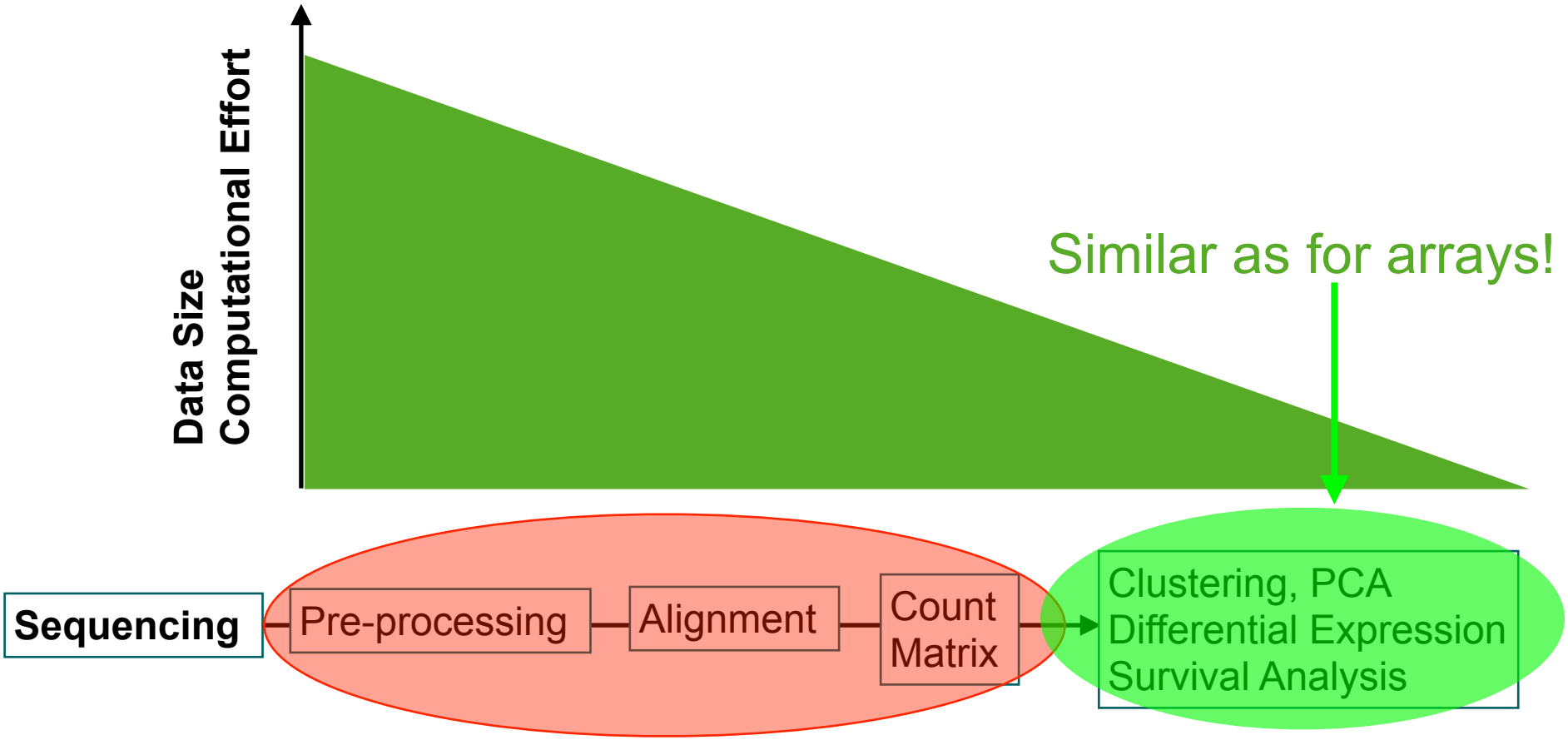


Bioinformatics Pipeline / RNA-seq



Practical part not covered!

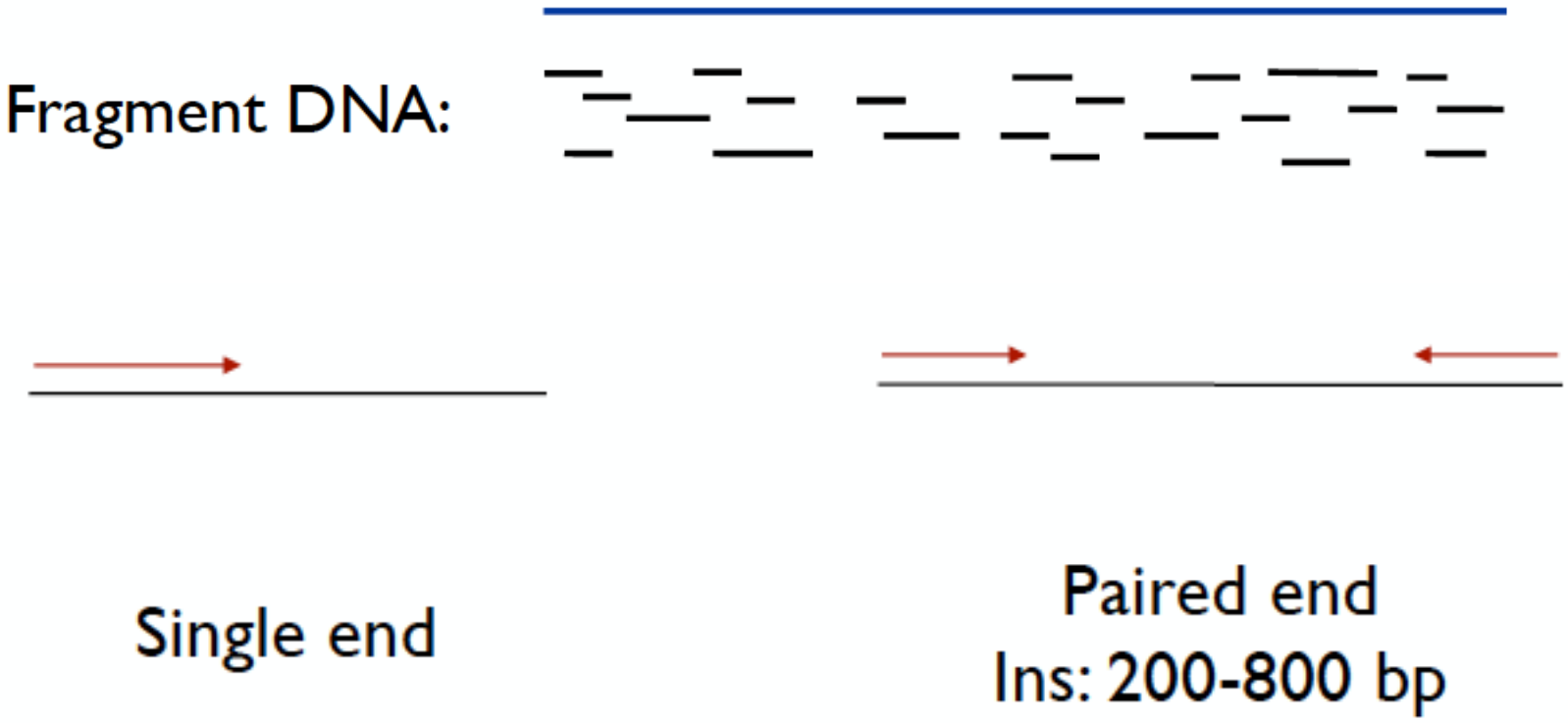
Bioinformatics Pipeline / RNA-seq



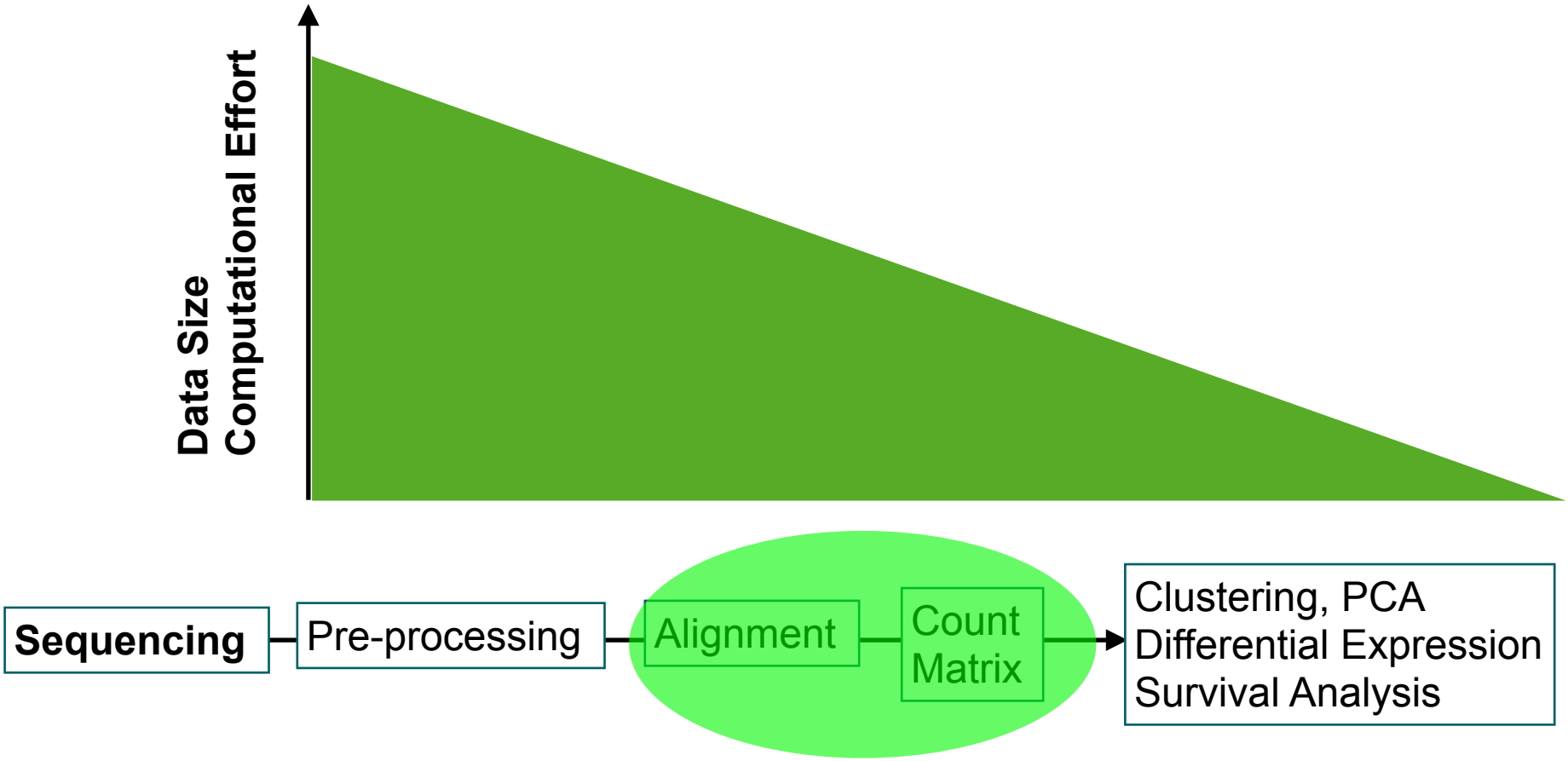
Next Generation Sequencing

- NGS take advantage of parallelization
  - reads millions/billions of reads per run
  - short reads (50-100 bps)
  - error rates (0.1-1%)

Read Types

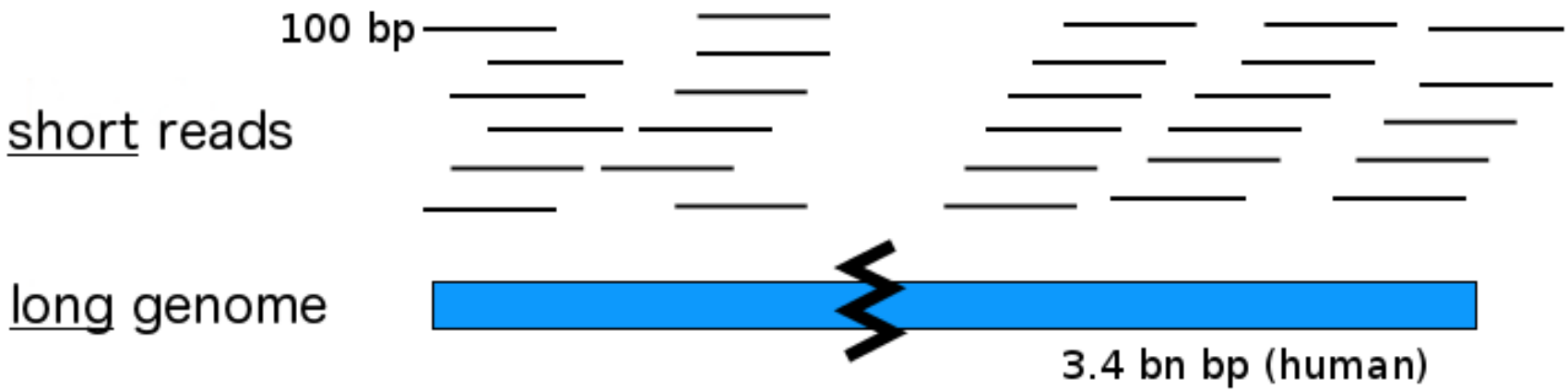


Bioinformatics Pipeline / RNA-seq

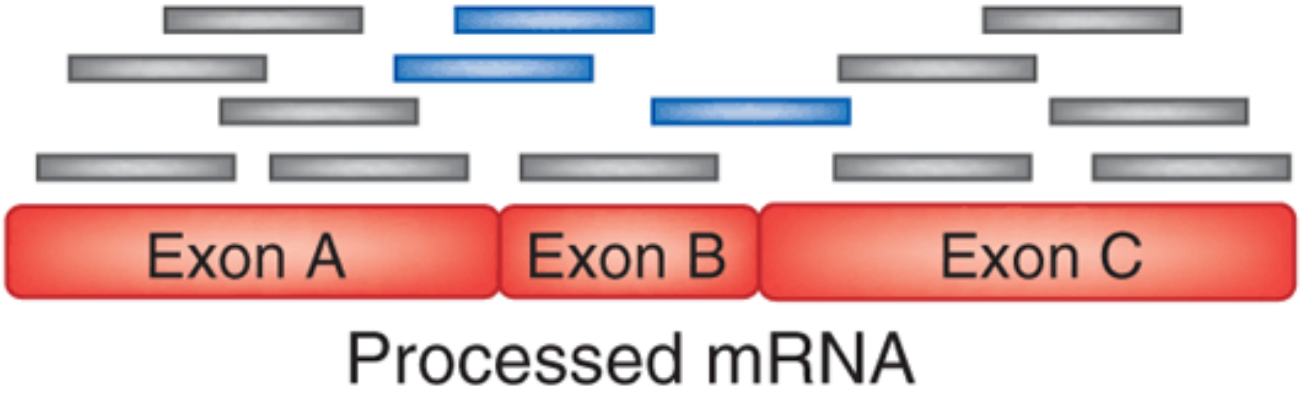


Alignment

- a large reference sequence is given (genome)
    - up to billions of base pairs
- short reads (<200bps)
- find most probable position of the read in the genome (by inexact string matching)



Alignment - Split Read Mapping (RNA-Seq)



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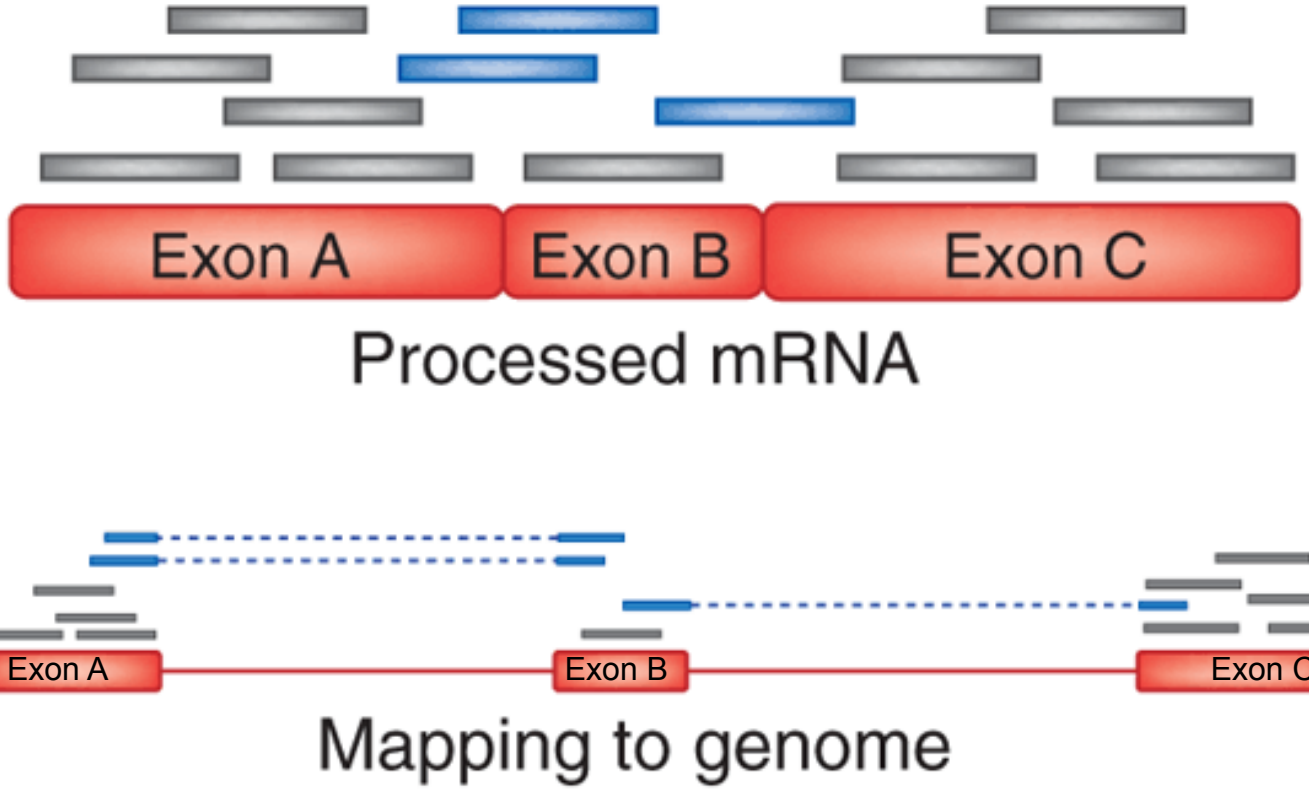


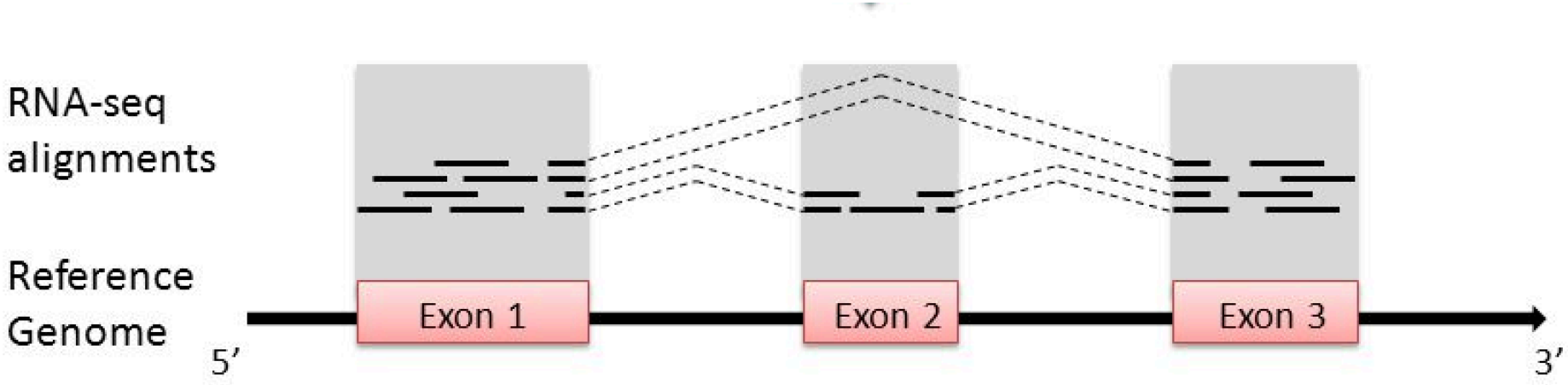
- **reads are split between exons when mapped to genome**
- **aligners use transcript information or try to find splice events (STAR & TOPHAT)**

Reference based aligners - Overview

	Time	Precision	Pairs	GAPs	Phred	Memory	Application (Comments)
BOWTIE	+		+	-	-	5GB	General (max. 3 missmatches)
BWA	+		+	+	+	8GB	General (max of 200bps reads)
NOVOALIGN		+	+	+	+	8GB	General (commercial license)
STAR	+		+	-	+	32GB	RNA-Seq (allow split-maps)
BISMARK	+		+	+	+	10GB	Bisulfite/reduced sequencing

Computers need large memory and a few hours of computation per experiment!

Quantification (Count Matrix)



Simple Counting Approaches

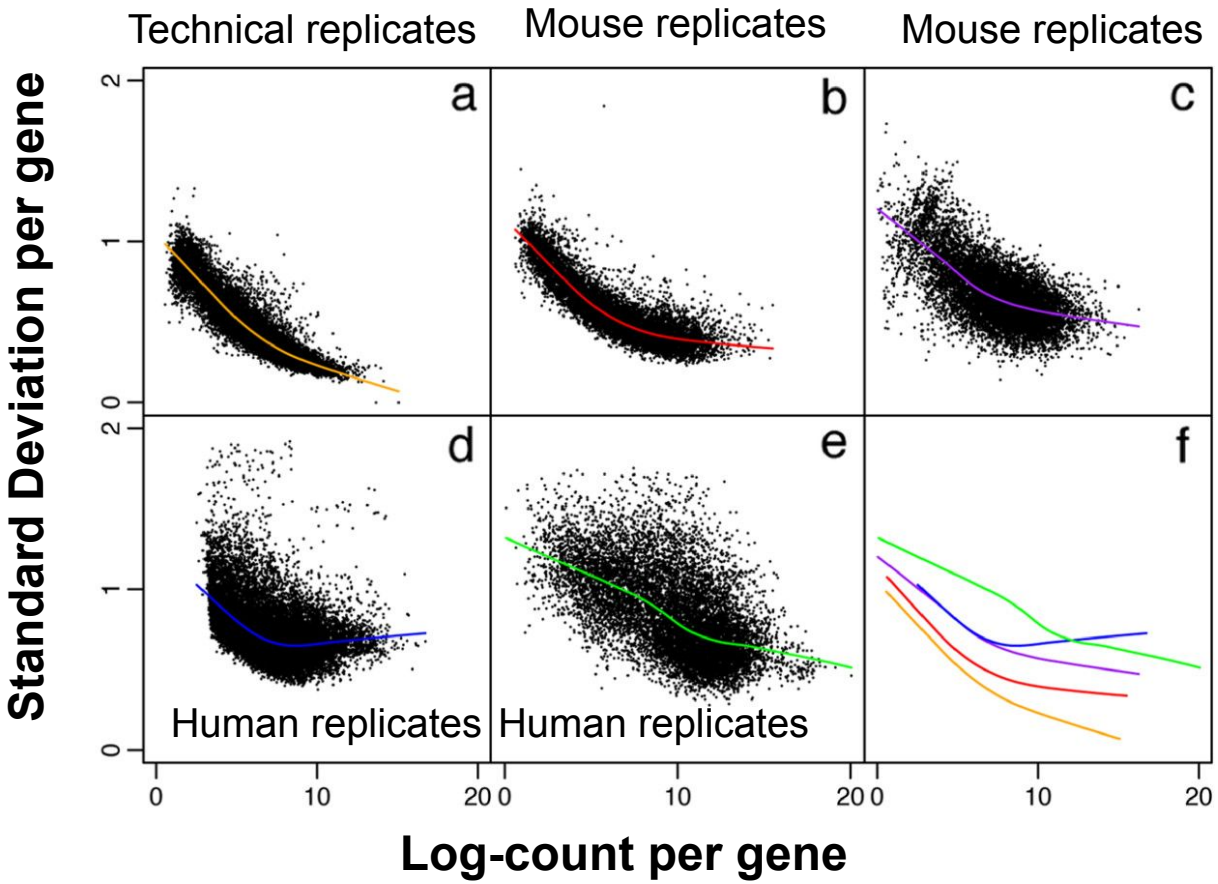
Gene Level - 17 reads
Exon level - exon 1 (8 reads), exon 2 (3 reads), exon 3 (6 reads)
Transcript Level - Exons 1,2 & 3 (10 reads) and exon 1 & 3 (7 reads) *
* complex computational methods required (RSe, or TopHAT needed for this)

Fragments per Kilobase (FPKM)

- normalize counts by read size (kb) and RNA-seq library size (mb)

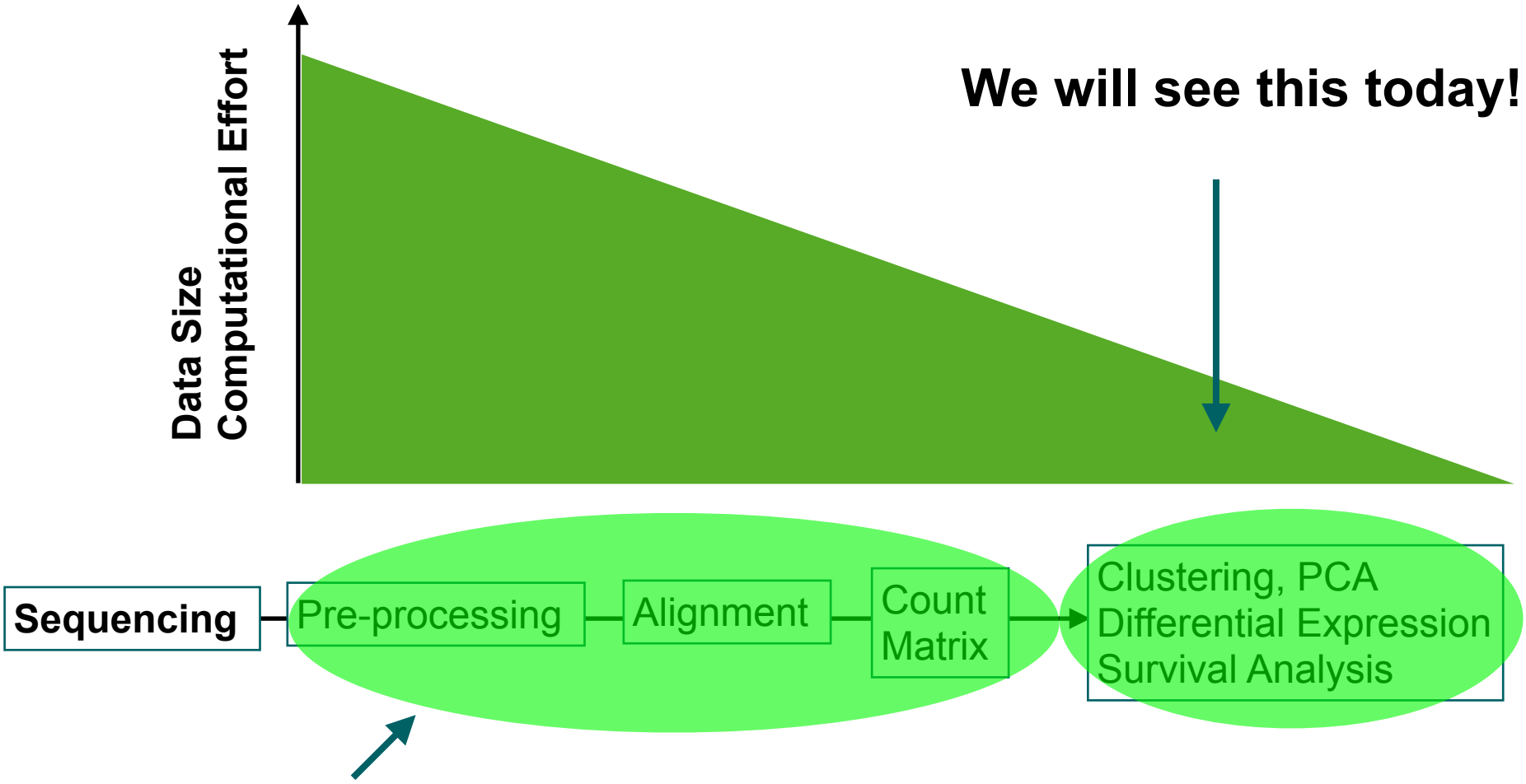
RNA-seq and Differential Analysis

Arrays and RNA-seq have distinct distributions



VOOM analysis is necessary to make variance similar to arrays.

Bioinformatics Pipeline / RNA-seq



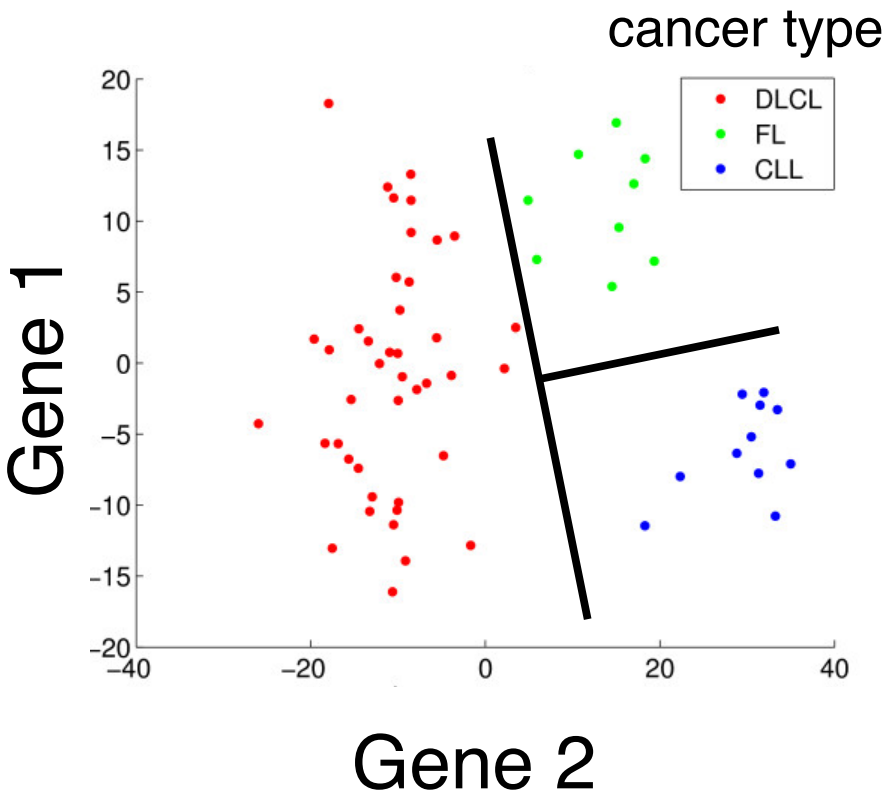
Personalized Medicine

Diagnosis and treatment choices is mostly carried on macromolecular features:

- morphology of tumours (image), symptoms, blood levels

Challenges: use molecular markers (expression or genetics) for diagnosis or treatment selection.

Machine Learning - Classifier



Data

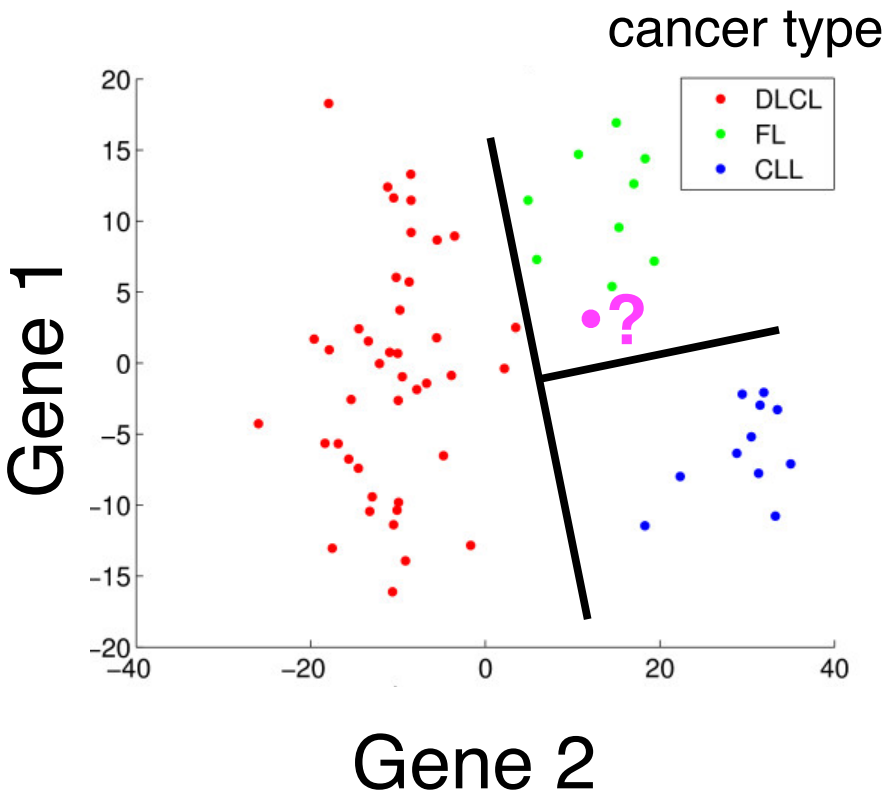
Expression matrix X (genes vs samples)

classification vector *Y* (diagnosis)

Find a function:

f(*x*) → *y*

Machine Learning - Classifier



Data

Expression matrix X (genes vs samples)

classification vector *Y* (diagnosis)

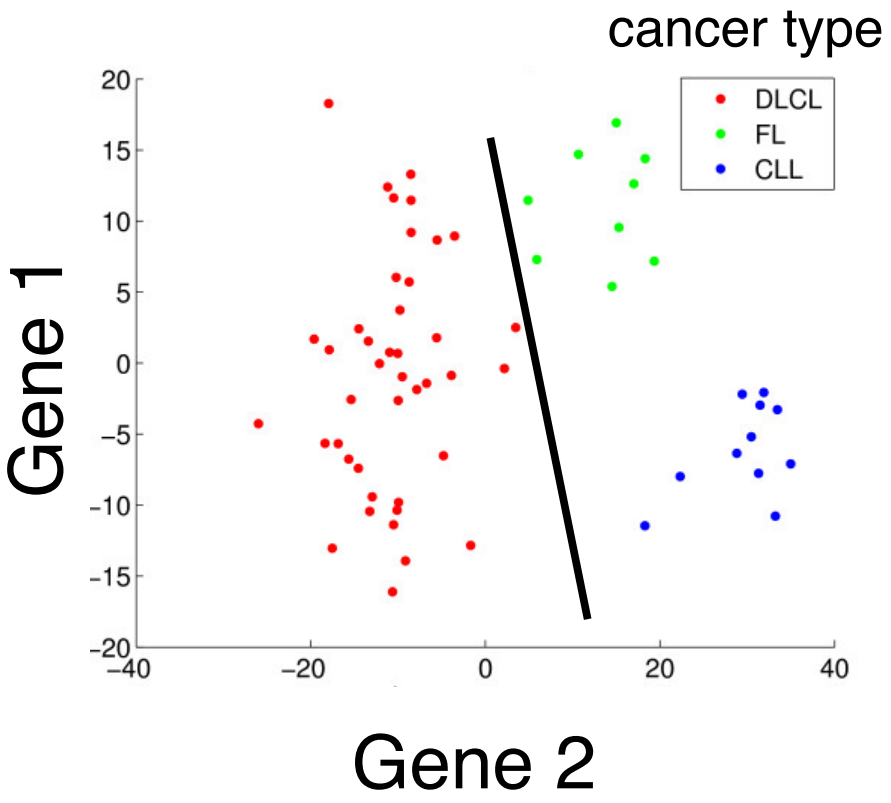
Find a function:

$f(x) \rightarrow y$

For new patients X':

$f(x') \rightarrow y'$

Linear Classifier



Linear Function:

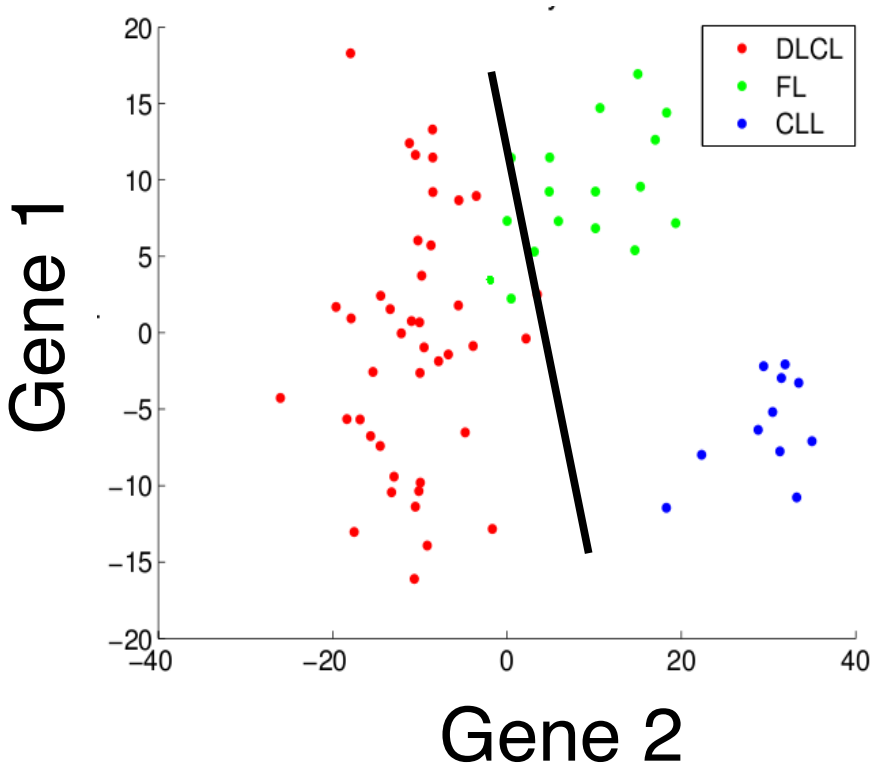
$f(x, A) = a_0 + a_1 x_1 + \ldots + a_L x_L$

$f(x, A) > 0 \Rightarrow$ class A

$f(x, A) \leq 0 \Rightarrow$ class B

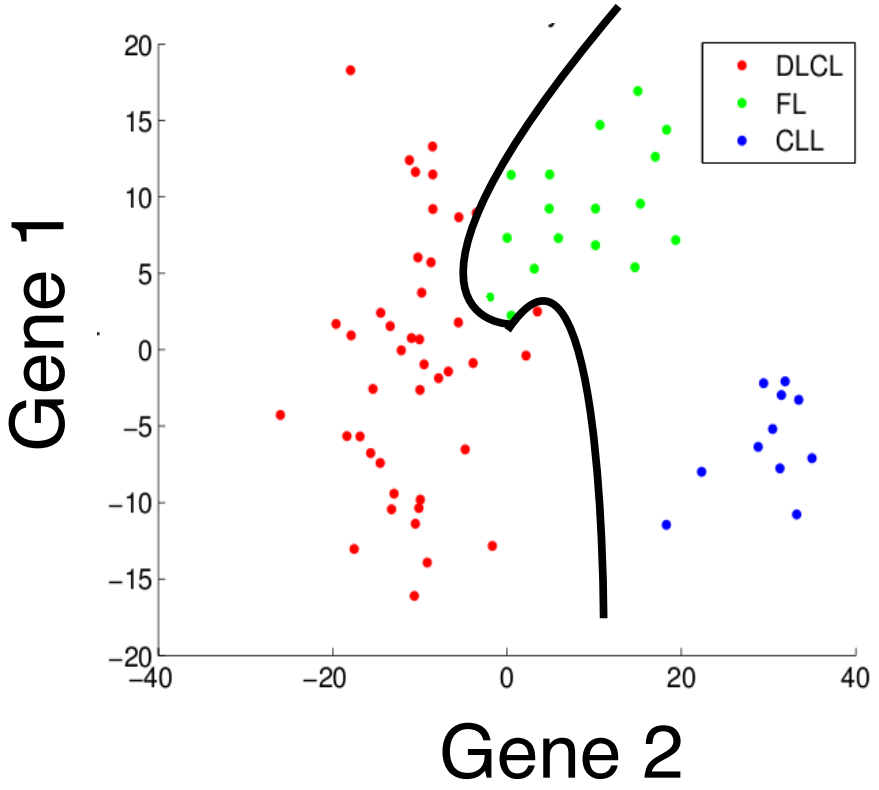
- Works for 2 classes only
  - Train a function for each cancer type
- Find coefficients *A*
  - estimated with neural networks or support vector machines

Linear Classifier - Problems



- Most real world problems are not linearly separable!
- There will be always some error!
- Solution: non-linear functions

Nonlinear Classifier - Problems

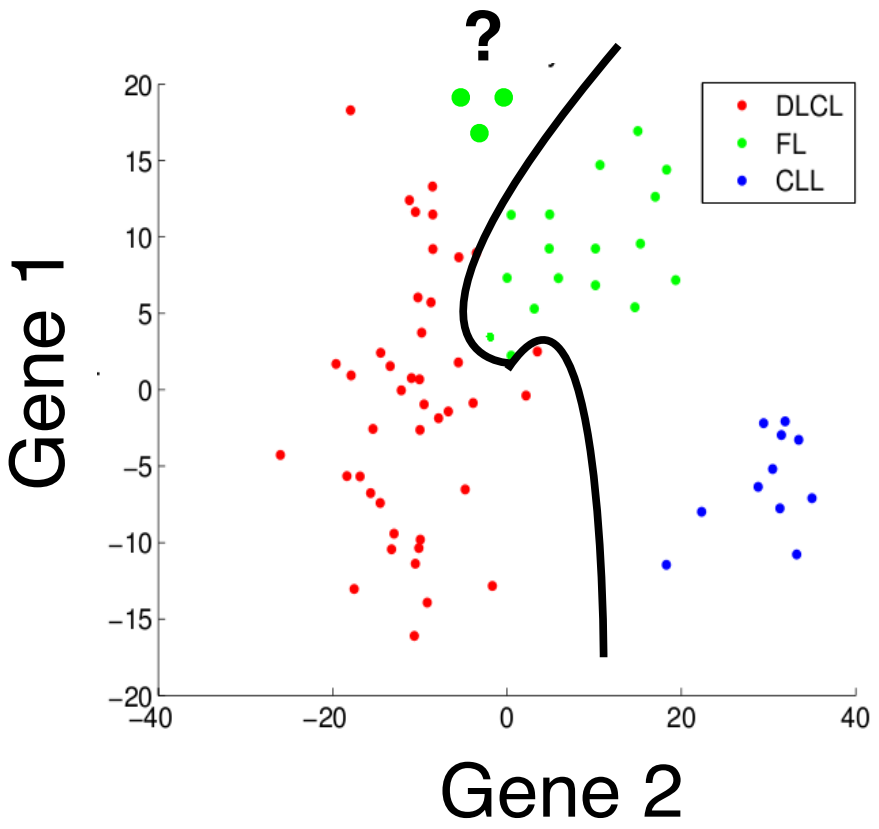


- Polinomial Function
- $f(x, A) = a_0 + a_{11}x^3_1 + \ldots + a_{L1}x^3_L$

  $a_{12}x^2_1 + \ldots + a_{L2}x^2_L$

  $a_{12}x_1 + \ldots + a_{L2}x_L$
- Third order polynomial
- Problem: overfitting

Nonlinear Classifier - Problems



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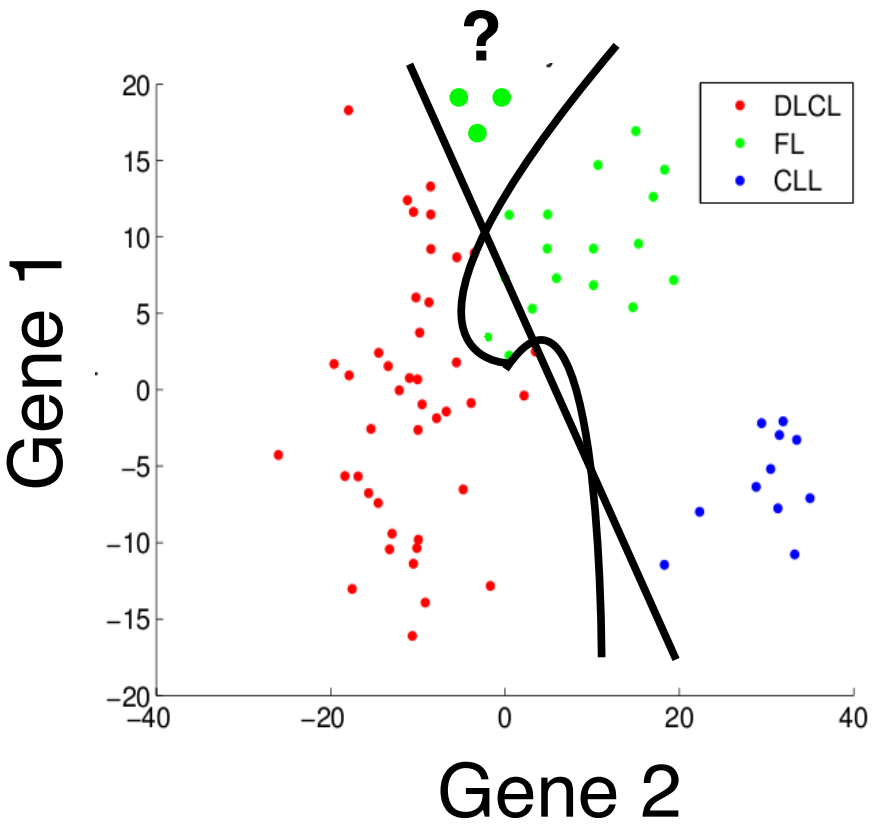
Nonlinear Classifier - Problems



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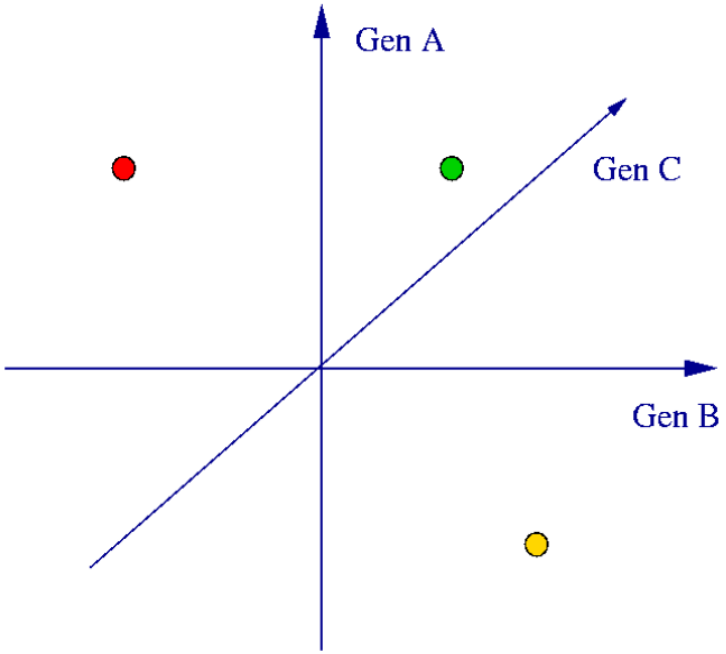
Curse of Dimensionality

Size of a Euclidean space grows with dimension (number of genes)

Dots (patients) are sparsely distributed in space

Curse of Dimensionality : Example



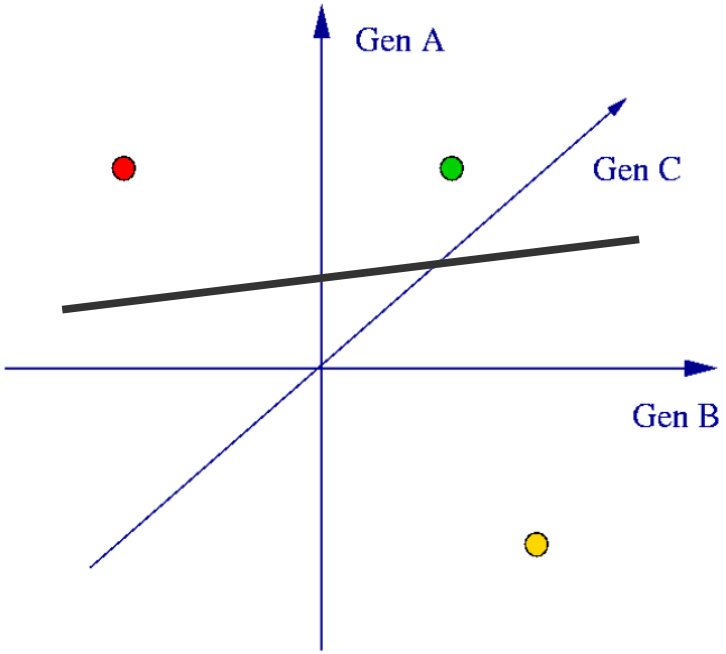
Sparse data

- three genes

- 2 patients with known cancer (red vs yellow)

- 1 unknown (green)

Curse of Dimensionality : Example



- Sparse data
  - three genes
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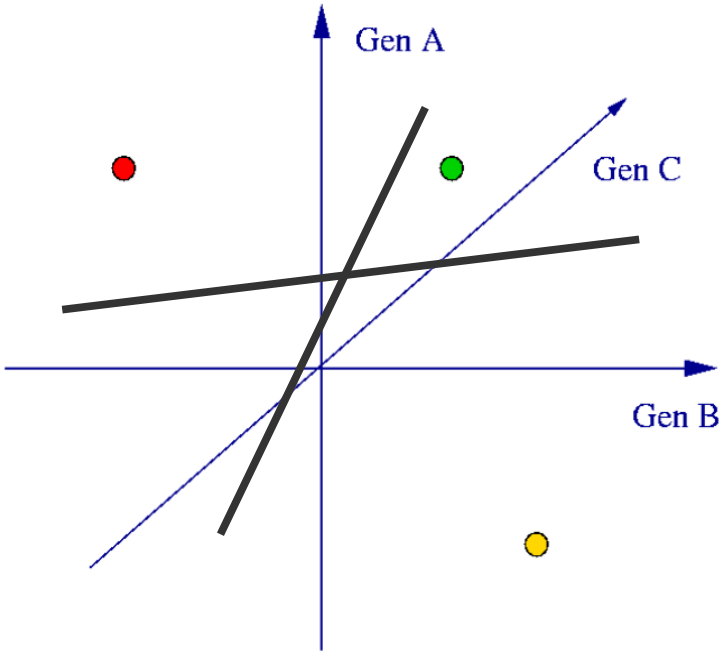
Perfect classifier (on training)

Curse of Dimensionality : Example

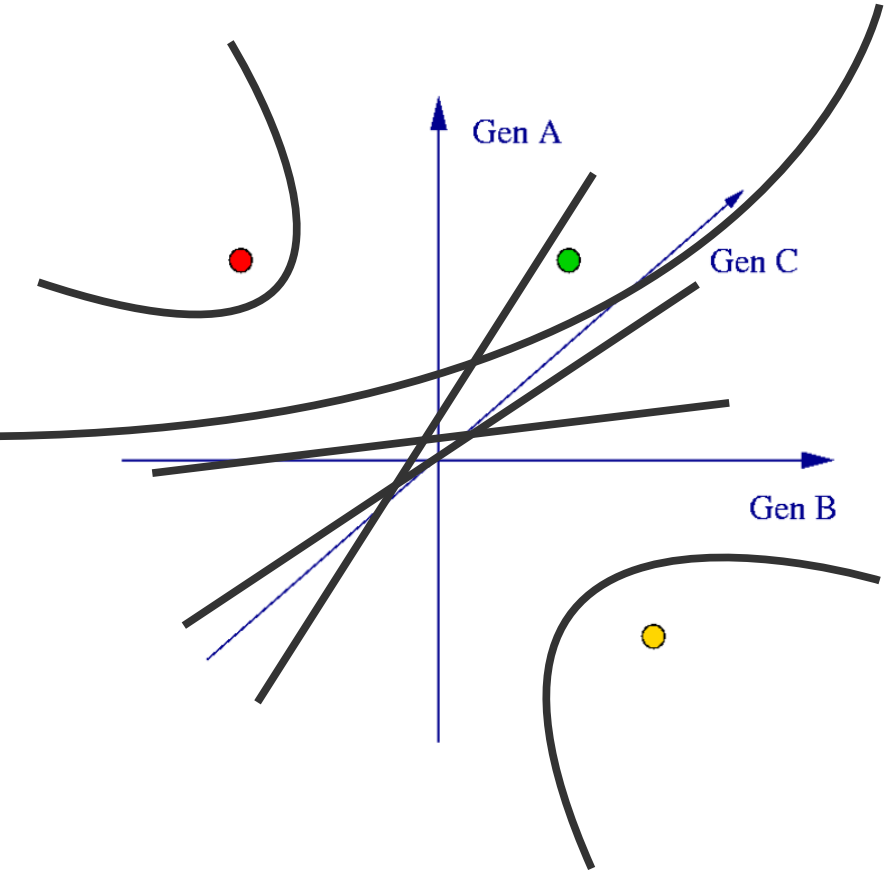


- Sparse data
  - three genes
  - 2 patients with known cancer (red vs yellow)
  - 1 unknown (green)

Both are perfect classifiers (on training)

Hard to generalise!

Curse of Dimensionality : Example



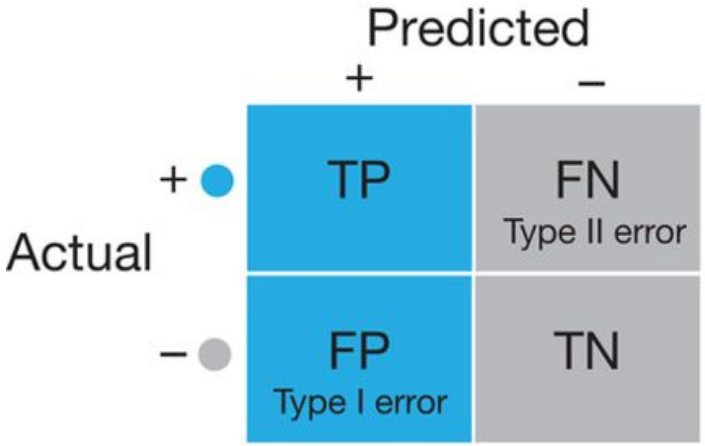
- There are millions of perfect linear classifiers
- And even more non-linear classifiers!

Dealing with Curse of Dimensionality

- Have a proper training / test evaluation procedure
- Use classifiers which are as simple as possible
- Reduce the dimension of your data (feature selection or PCA)

Classifier Evaluation

Measures for a two class problem (cancer + vs. non-cancer -)



Source: Lever et al., Nat. Methods (2016)

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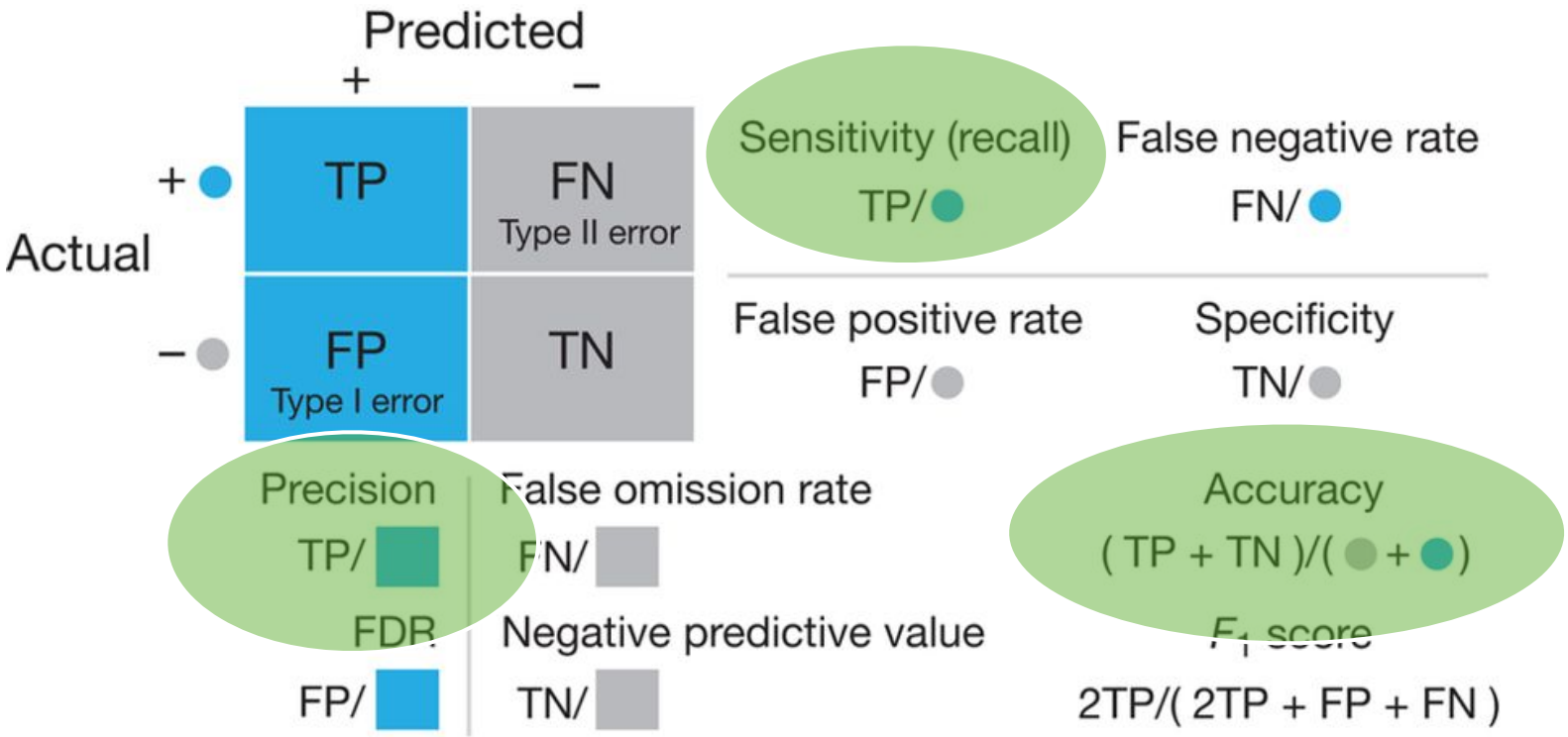

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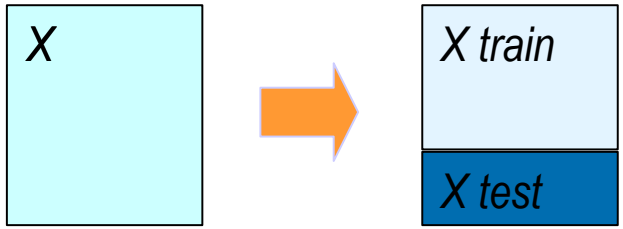
Classifier Evaluation

- The performance of your classifier needs to be evaluated on your test data:
  - an independent "validation cohort"
  - or retain a set of samples (1/3) that has similar distribution of classes of your total data



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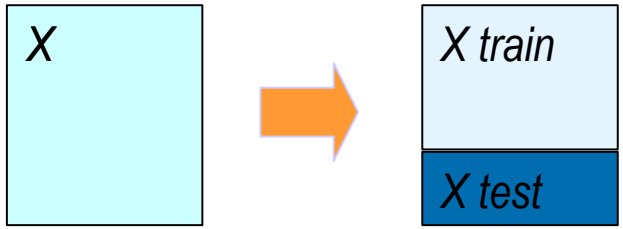
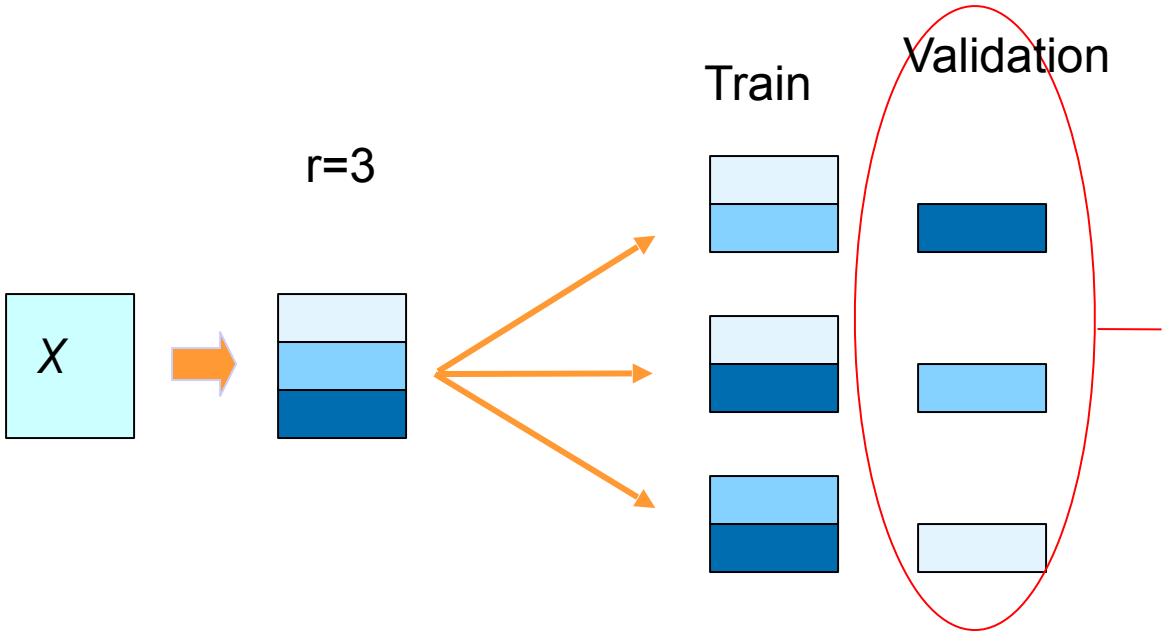


- Never use test data to improve classification (choose a better classifier or marker gene)
  - For this you need to establish validation data (or cross validation)

Cross-validation



mean and std of accuracy,
precision and sensitivity

Elastic Net

Is based on a linear function:

$f(x, A) = a_0 + a_1 x_1 + \ldots + a_L x_L$

$f(x, A) > 0 \Rightarrow$ classe A

$f(x, A) \leq 0 \Rightarrow$ classe B

- Find coefficients *A, while most of then have* 0.
  - A shrinkage factor (λ) controls the number of genes selected.
  - Shrinkage factor can be automatically identified with cross-validation.

Hands on!

Exercise (after the handout)

You should perform clustering of tissues with liver cancer. Tip: use code similar to the one seen in gene expression data (day 3). Since, we are interested in grouping patients, you can transpose the matrix with the function **t**.

1. Can you see nice clusters in the dendrogram?

2. What about genes associated to each group? Are they associated to some particular biological function? Use differential expression analysis and GO enrichment analysis to solve this task.

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Survival Analysis

Can be used to evaluate if characteristics of a patients indicates an increase/decrease risk of survival

- clinical: tumour type, gender
- Molecular: expression of a gene, mutation

Common Survival Tests:

- Cox proportional hazards regression (not seen here)
  - Compares survival with a numeric variable
- Kaplan-Meier graph / Log-rank test
  - compares the survival of groups of individuals

Kaplan-Meier graph / Log-rank test

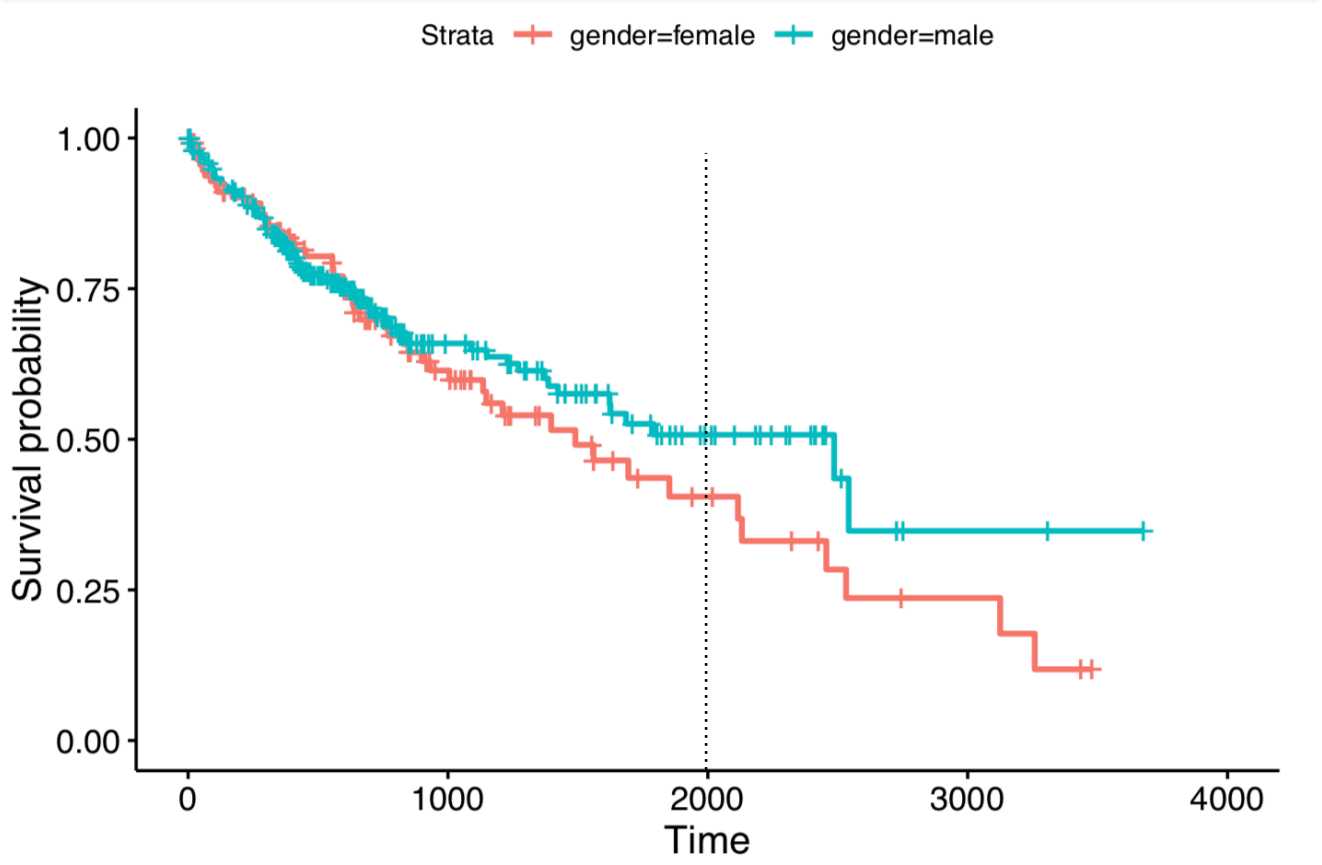
Data:

- **Event**: death / alive
- **Time**: period between first and last observation.
- **Characteristics**: sex, tumor grade

Patient	*Status*	*Time*	*Sex*
1	*Dead*	*343*	*Male*
2	*Alive*	*20*	*Male*
3	*Alive*	*300*	*Female*
4	*Dead*	*200*	*Male*

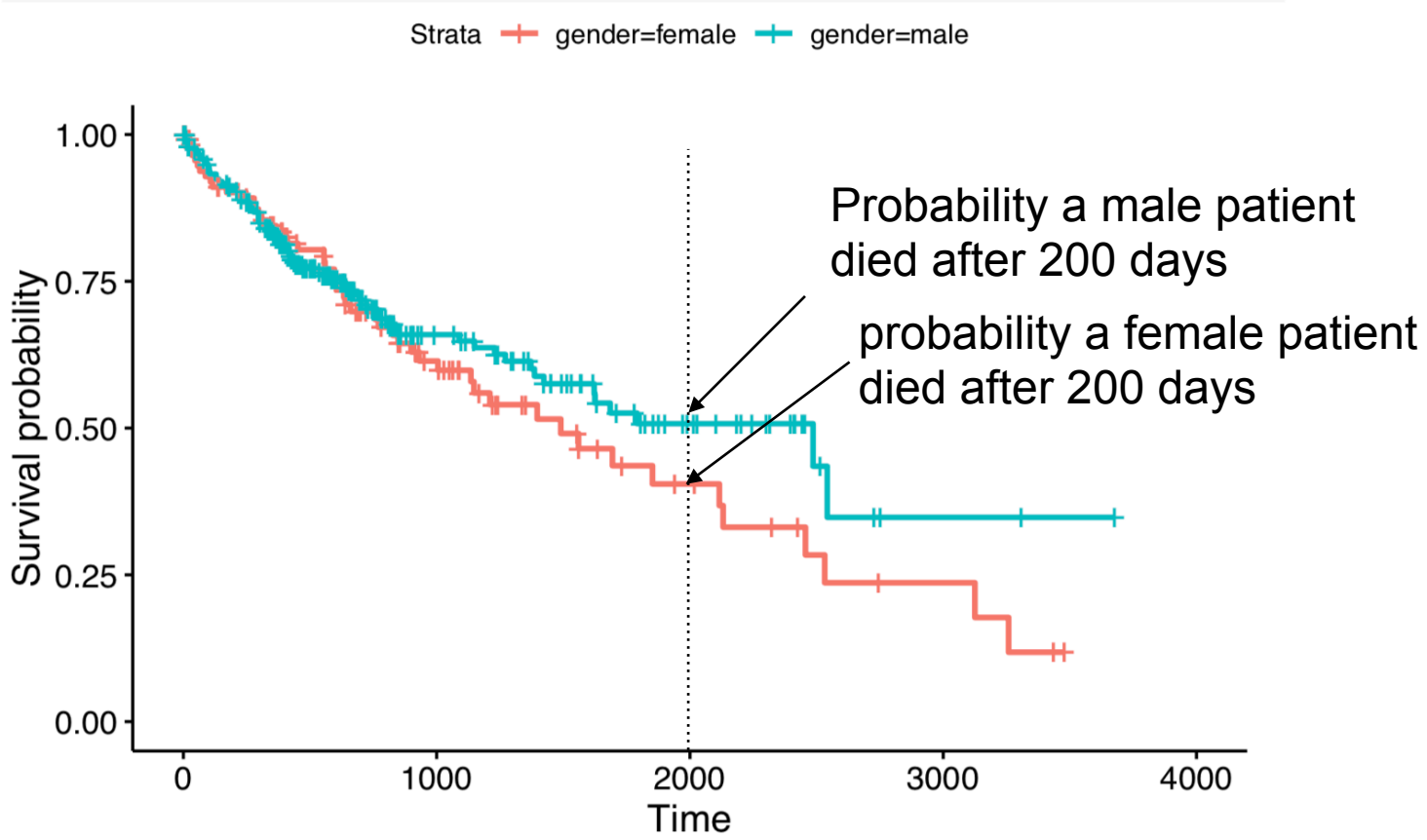
Kaplan-Meier plot

Survival of LIHC patients - male vs. Female



Kaplan-Meier plot

Survival of LIHC patients - male vs. Female



Probability (*X* days) = $\frac{\text{\# cases alive after } X \text{ days}}{\text{\# cases measured after X days}}$

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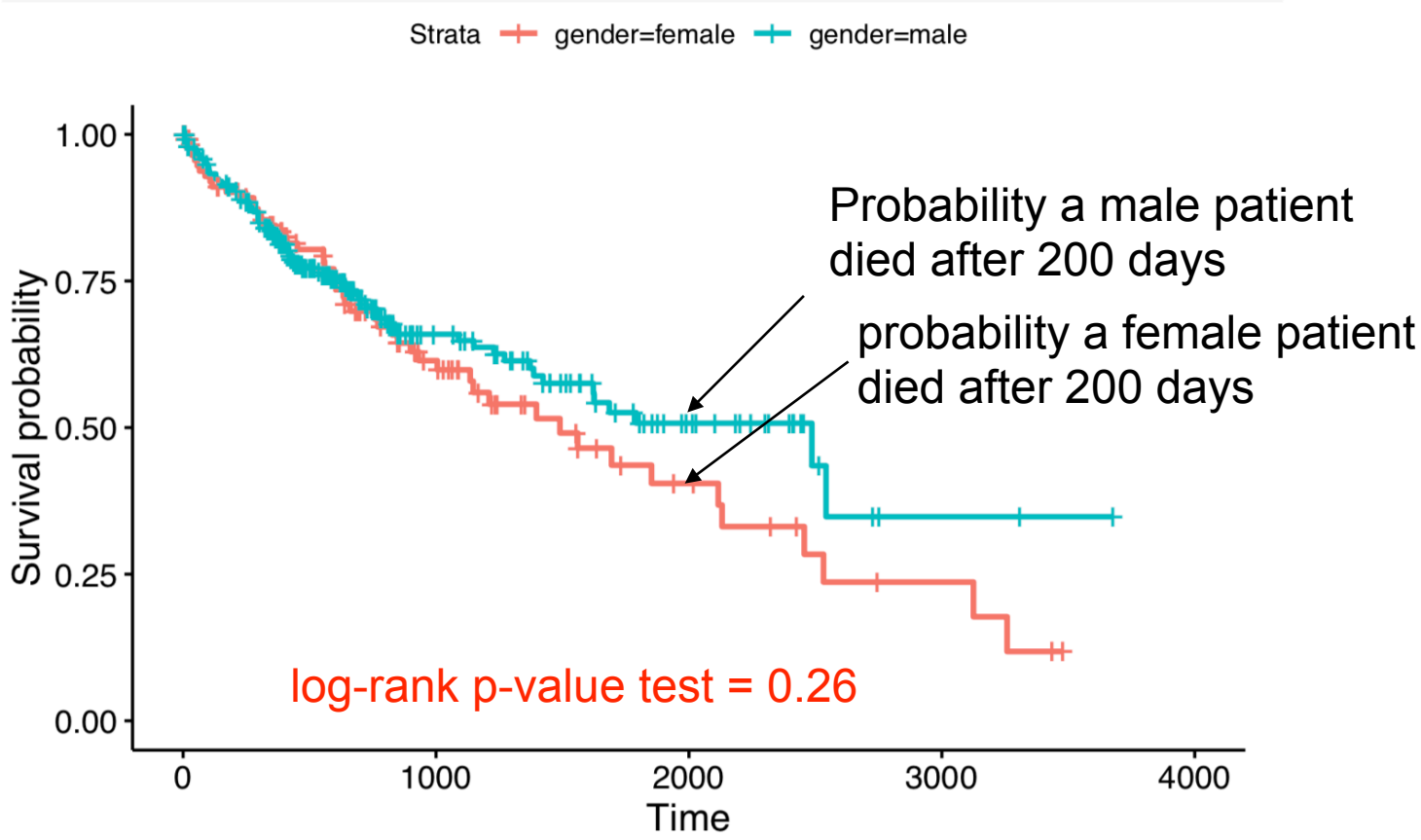
Log-rank test

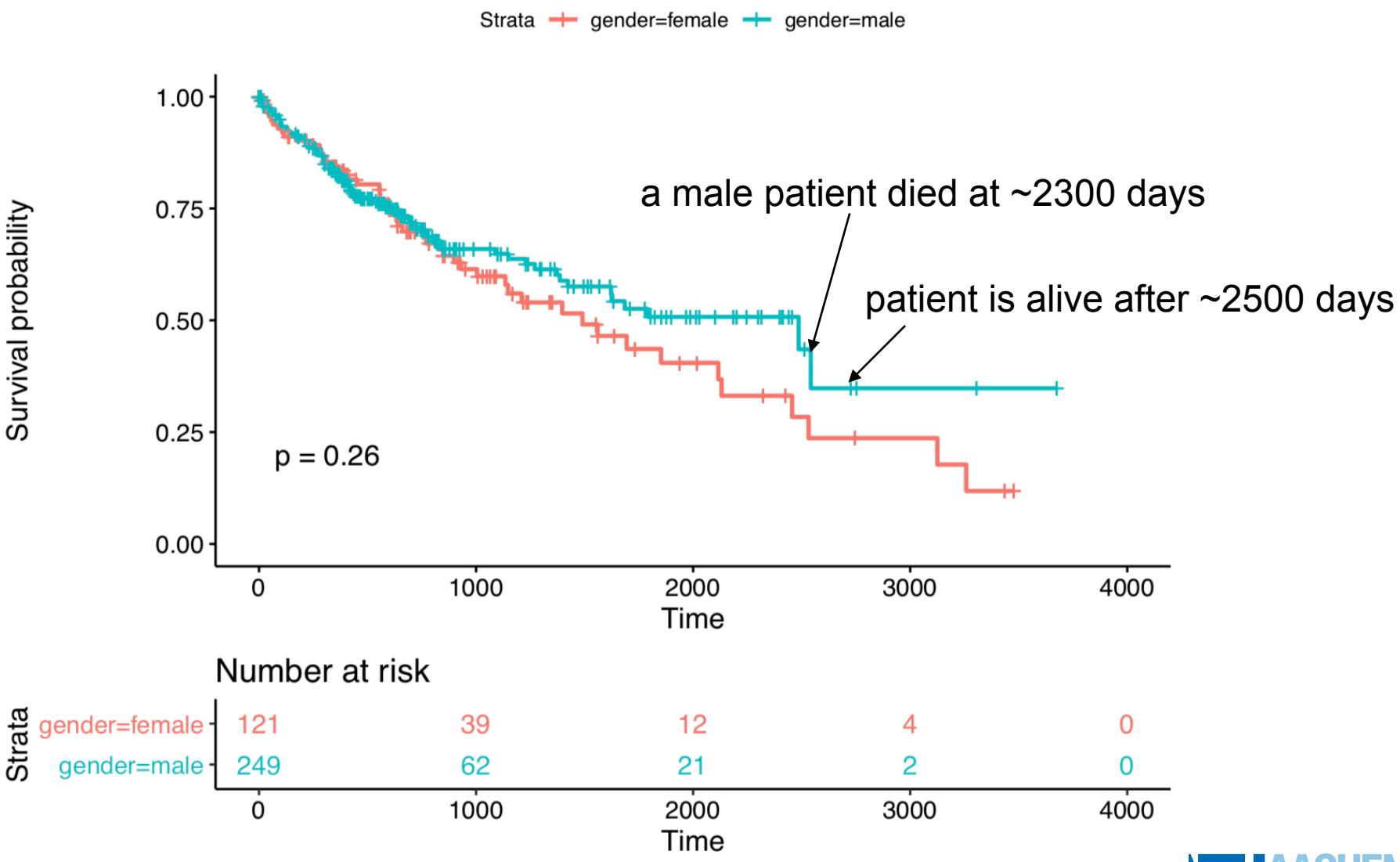
Is the survival difference significant?

Log-rank test

Is the survival difference significant?



Kaplan-Meier plot



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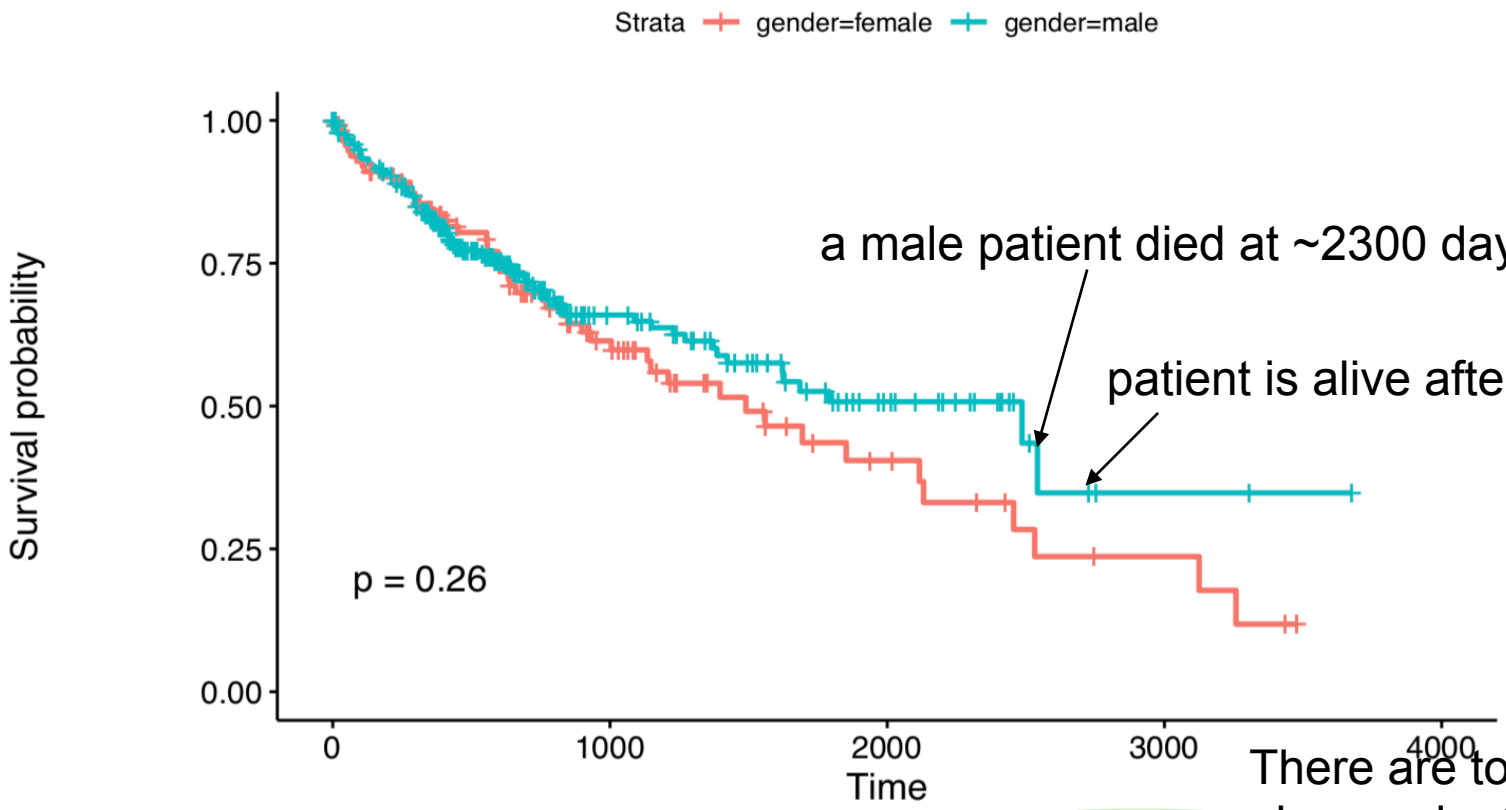
Kaplan-Meier plot



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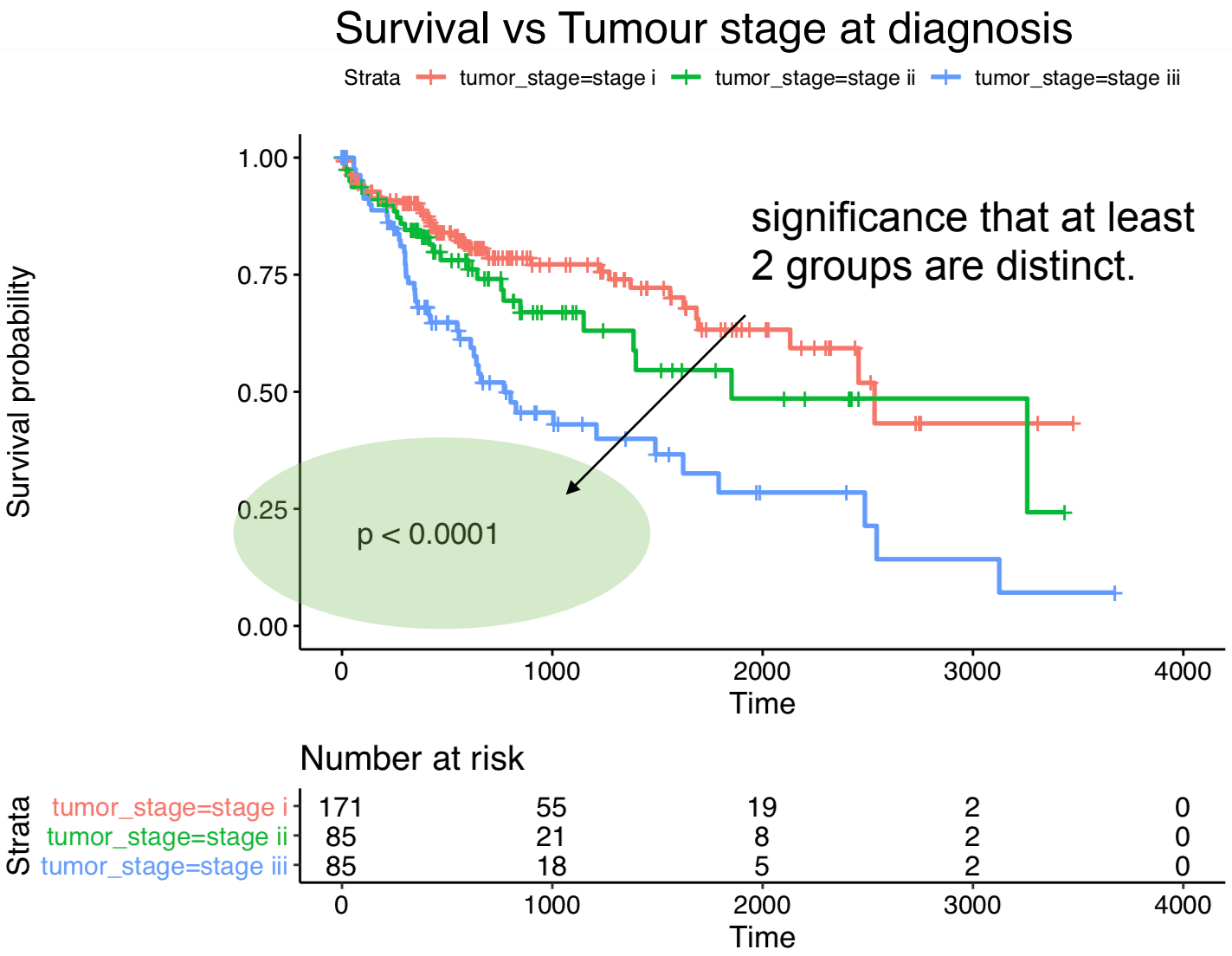
Kaplan-Meier / Log-Rank Test

KM and LRT can compare several groups at a time.



Survival Analysis and Biological Markers

How to perform survival analysis on biological markers?

1. Given their continuous nature of gene expression, Cox hazards test is recommended.

2. An alternative is to group patients by expression of a gene (low/high expression) and use Kaplan-Meyer plots (seen in practical).

Important: if you test several markers you need to correct for multiple testing!!!

Hands on!

Exercise (after the handout)

You should perform clustering of tissues with liver cancer. Tip: use code similar to the one seen in gene expression data (day 3). Since, we are interested in grouping patients, you can transpose the matrix with the function **t**.

1. Can you see nice clusters in the dendrogram?

2. What about genes associated to each group? Are they associated to some particular biological function? Use differential expression analysis and GO enrichment analysis to solve this task.

3. **Check if group of patients are associated to survival, tumour grade or any other clinical variable? You can use the table function for some of these analysis.**