Bioinformatics Lab

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Institute for Computational Genomics

Machine Learning / Classification

Gene expression data imposes challenges to classification:

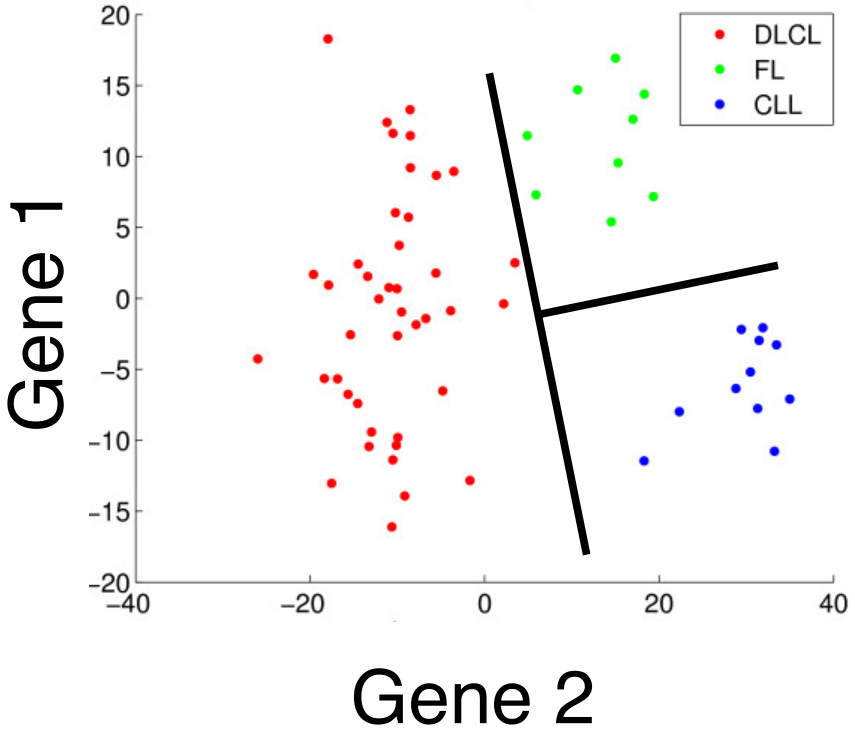
- no. of dimensions is higher (or similar) than number of samples

We need robust experimental approaches for:

- measuring the accuracy of ML methods
- finding best parameters of ML methods
- compare the performance of distinct methods.

Machine Learning - Classifier

cancer type classification



Data:

Expression matrix X (genes vs samples)

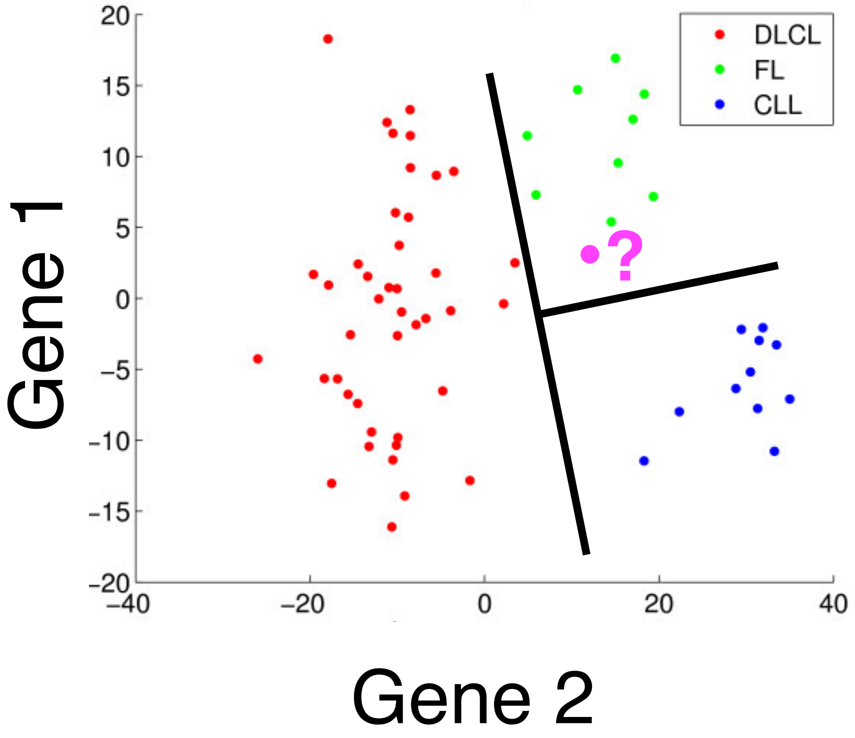
classification vector *Y* (diagnosis)

Find a function:

f(*x*) → *y*

Machine Learning - Classifier

cancer type classification



Data:

Expression matrix X (genes vs samples)

classification vector *Y* (diagnosis)

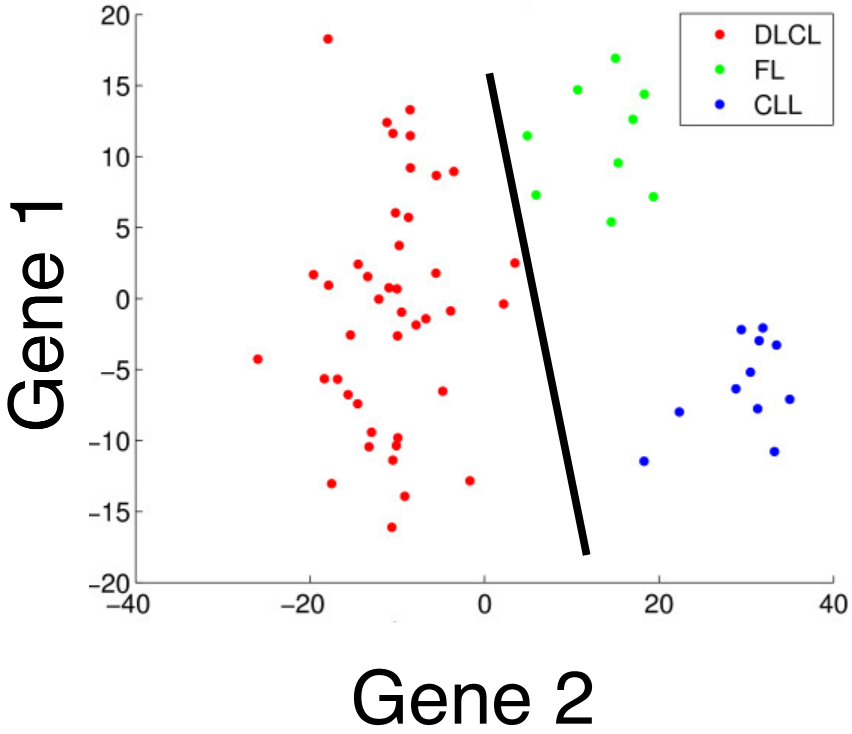
Find a function:

f(x) → y

For new samples X':

f(x') → y'

Linear Classifier



Linear Function:

$$f\,(x\,,A) = a_0 + a_1x_1 + \ldots + a_Lx_L$$

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

$$f\,(\,x, A) \le 0 \Rightarrow \text{classe B}$$

- **Works for 2 classes only**
  - train a function for each cancer type
- **Find coefficients**
  - with linear programming/ neural network

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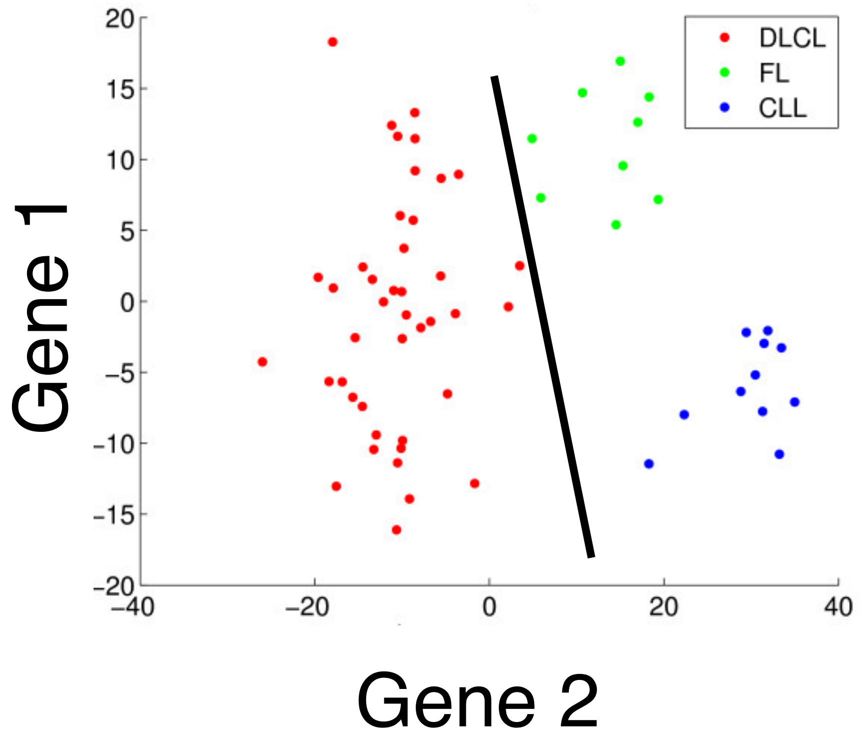
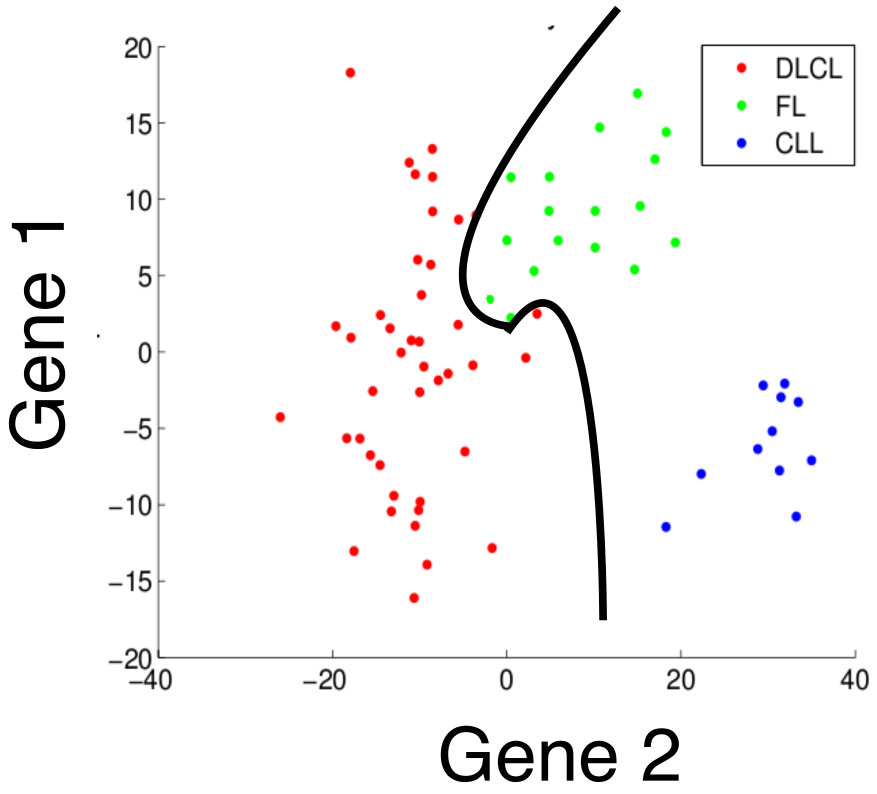
Linear Classifier - Problems



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

Nonlinear Classifier - Problems

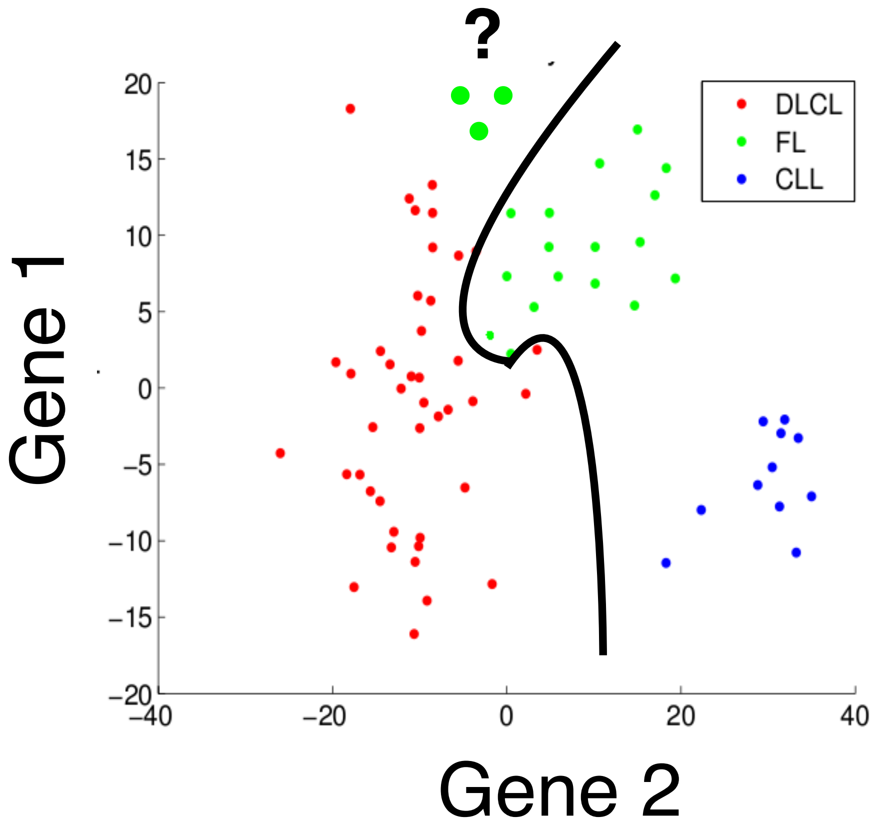


- Polinomial Function
- $f\,(x\,,A) = a_0 + a_{11}x^3{}_1 + \ldots + a_{L1}x^3{}_L$

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

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

Nonlinear Classifier - Problems



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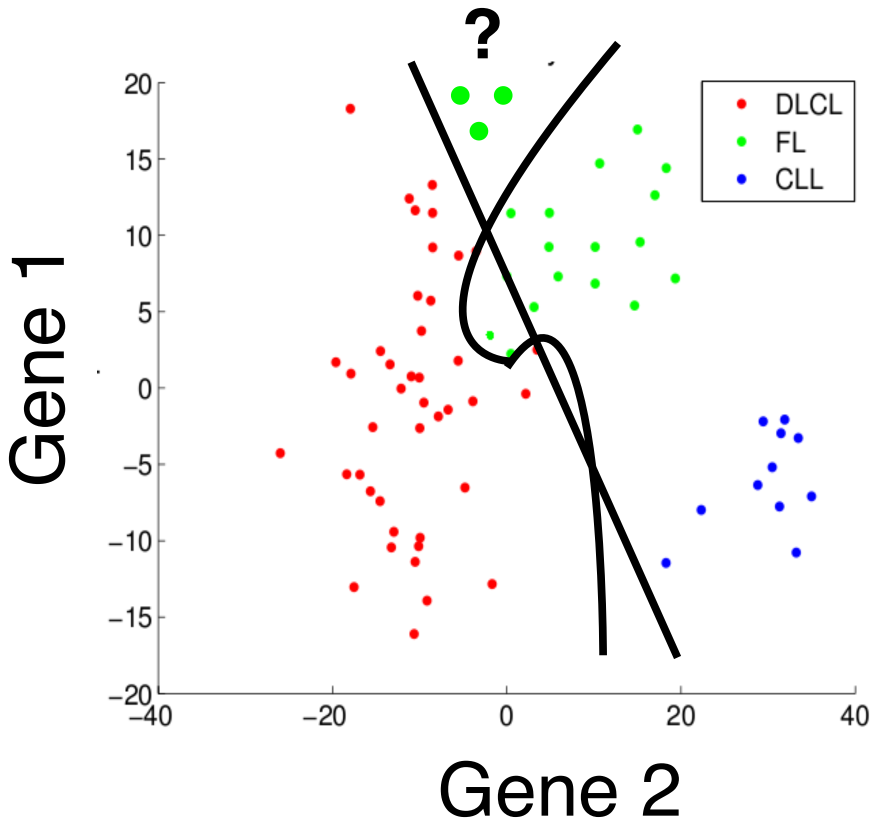
Nonlinear Classifier - Problems



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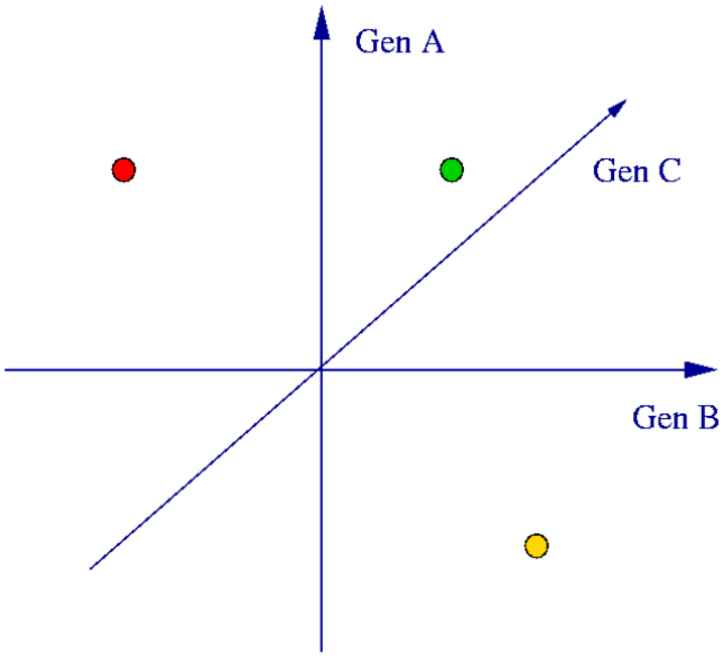
- Third order polynomial
- Problem: overfitting

Curse of Dimensionality

- Size of a Euclidean space grows exponentially with dimension
  - number of genes
- Dots (patients) are sparsely distributed in space

Curse of Dimensionality : Example

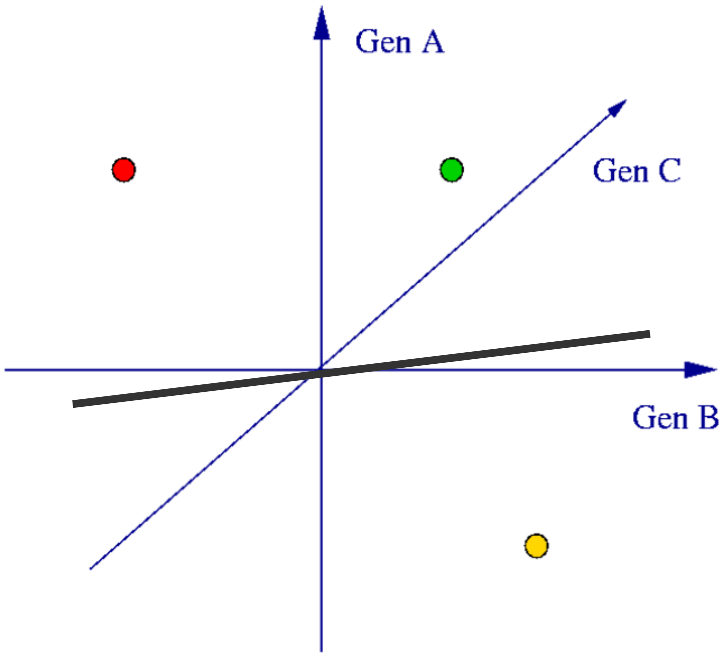
Sparse data: no of samples < no of dimensions



- three genes
- 2 patients with known cancer type(red/yellow)
- 1 unknown cancer type(green)

Curse of Dimensionality : Example

Sparse data: no of samples < no of dimensions

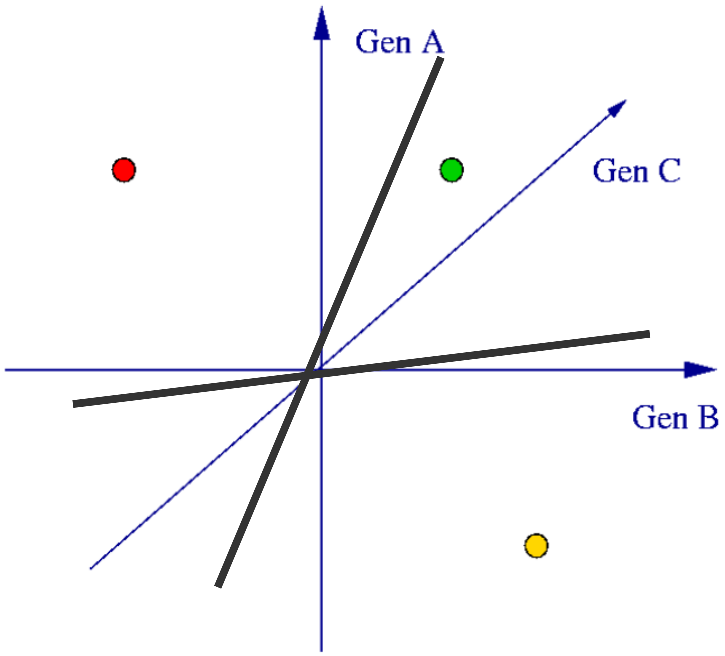


Perfect classifier (on training!)

- three genes
- 2 patients with known cancer type(red/yellow)
- 1 unknown cancer type(green)

Curse of Dimensionality : Example

Sparse data: no of samples < no of dimensions

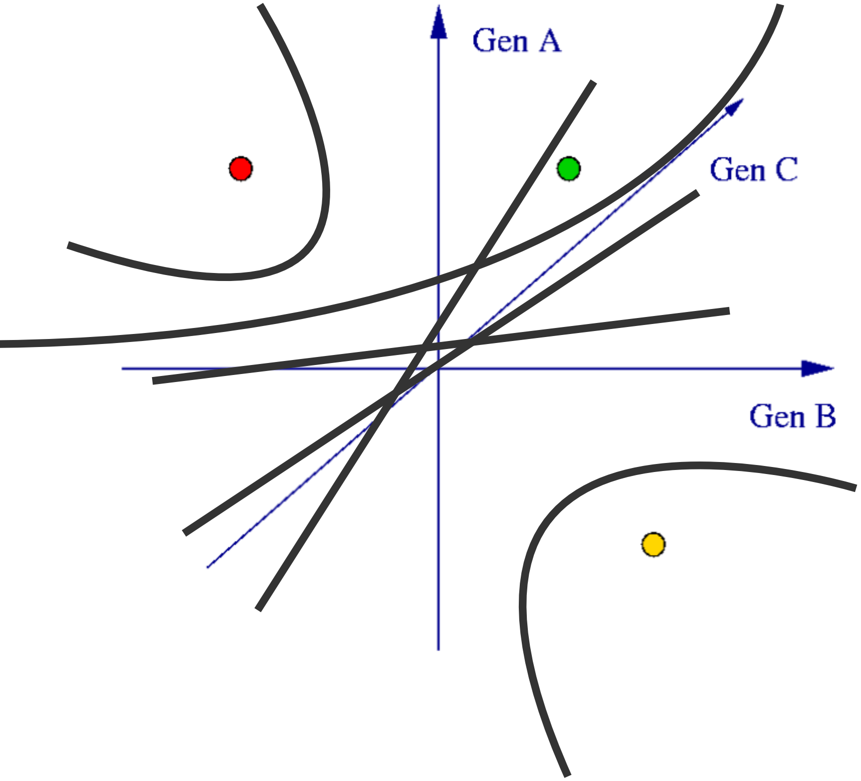


- three genes
- 2 patients with known cancer type(red/yellow)
- 1 unknown cancer type(green)

More perfect classifiers (on training!)
Hard to generalize 1

Curse of Dimensionality : Example

Sparse data: no of samples < no of dimensions



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

Dealing with Curse of Dimensionality

- Have a proper training / test evaluation procedure
- Use simple classifiers
- Reduce the dimension of your data:
    - feature selection
    - PCA or tSNE (black box!)

Classifier Evaluation

1. **Statistics to measure the classification performance**

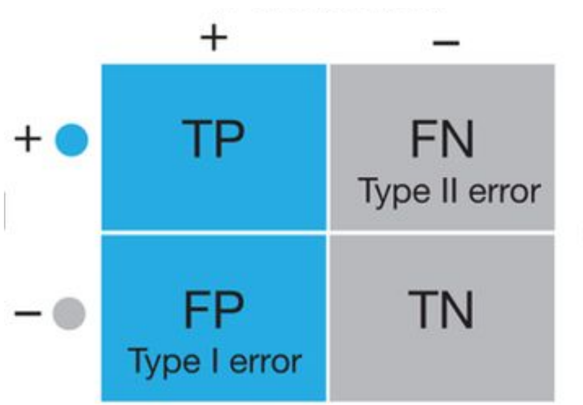
2. Data splitting strategies to avoid overfitting
- ML learns training data but do not generalize to unseen data

Classification Metrics

Measures for two class problem

Predicted Class

Actual Class



	+	–
+ ●	TP	FN Type II error
– ●	FP Type I error	TN

$$\text{Accuracy} = \frac{TP + TN}{TP+FP+FN+TN}$$

$$\text{F1 Score} = \frac{2{*}TP}{2{*}TP+FP+FN}$$

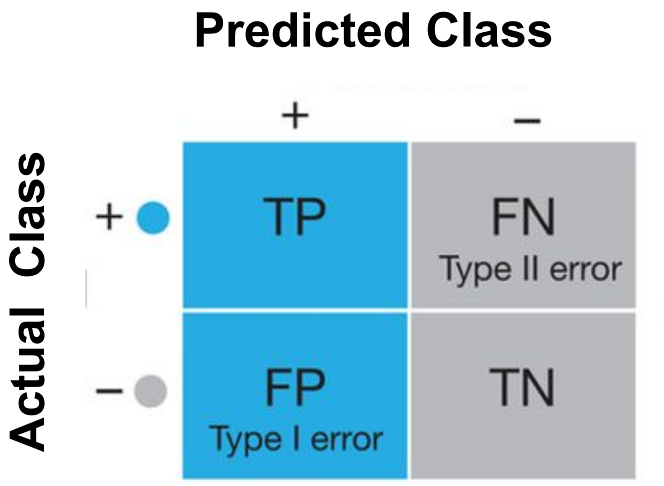
$$\text{Precision} = TP / TP + FP$$

$$\text{Sensitivity/Recall} = TP / TP + FN$$

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

Classification Metrics

Measures for two class problem



Actual Class \ Predicted Class	+	–
+ ●	TP	FN Type II error
– ●	FP Type I error	TN

$$\textbf{Accuracy} = \frac{TP + TN}{TP+FP+FN+TN}$$

$$\textbf{F1 Score} = \frac{2*TP}{2*TP+FP+FN}$$

Precision = TP / TP + FP

Sensitivity/Recall = TP / TP + FN

Extension for multi class:

- evaluate class vs. others / use average accuracy / F1.

Class imbalance:

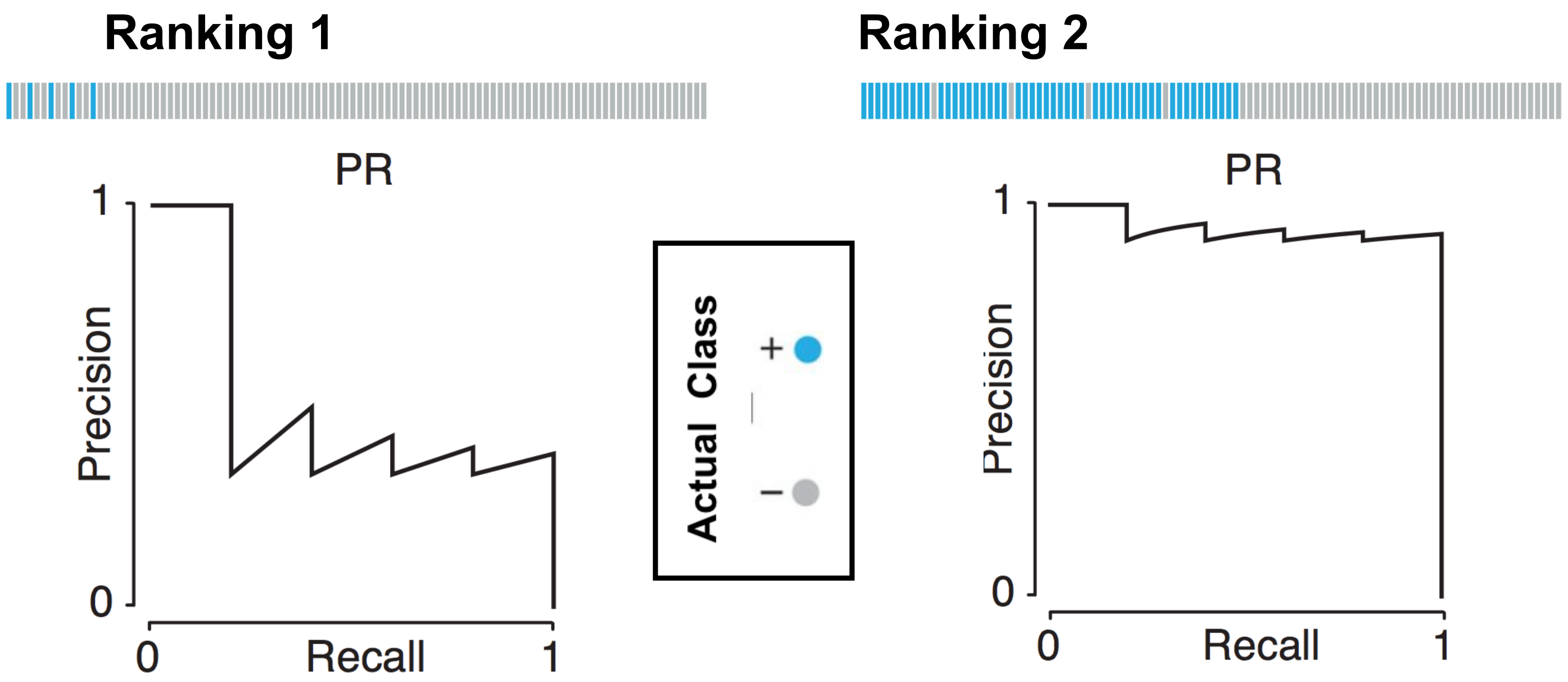
- usually number of negatives is larger / classifiers with low Precision might still have high Acc/Sensitivity

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

Classification Metrics / Class Imbalance

Precision - Recall (PR) curves

- requires ranking of classification, i.e. class probability

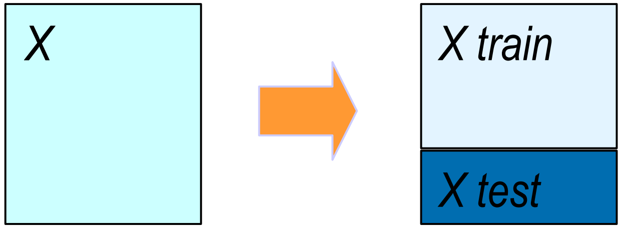


- area under the PR curve -> higher area indicates best classifiers!

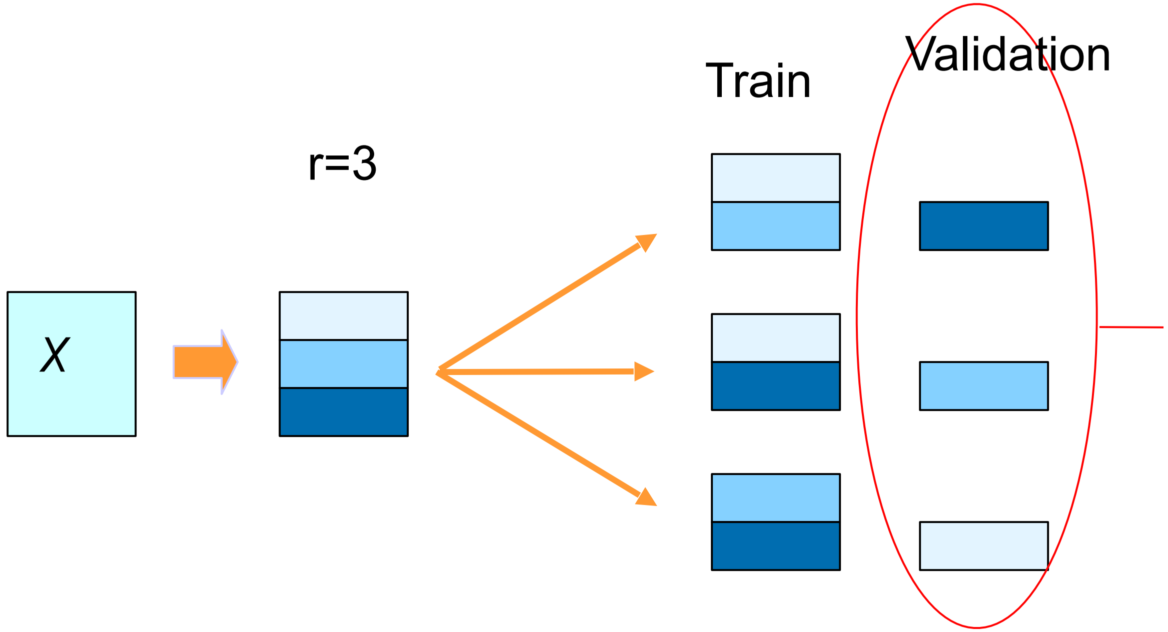
Source: Lever et al., Nat. Methods (2016)

Classifier Evaluation

- The performance of your classifier needs to be evaluated at test data:
  - an independent "test data set"
  - cross-validation



Cross-validation



X

r=3

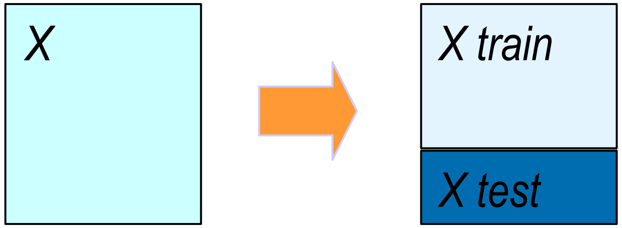
Train

Validation

mean and std of accuracy, precision and sensitivity

Classifier Evaluation

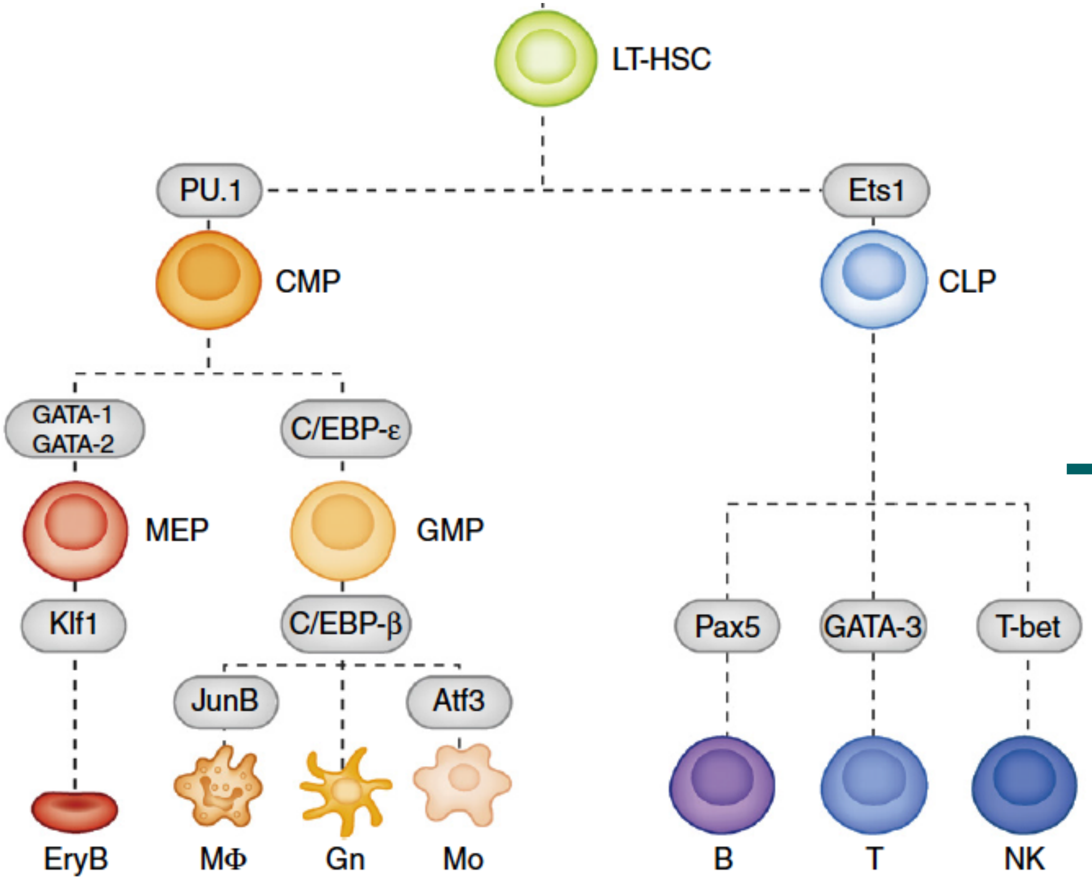
- The performance of your classifier needs to be evaluated at test data:
  - an independent "test data set"
  - cross-validation



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

Problem Definition

Cell Differentiation & Gene Expression

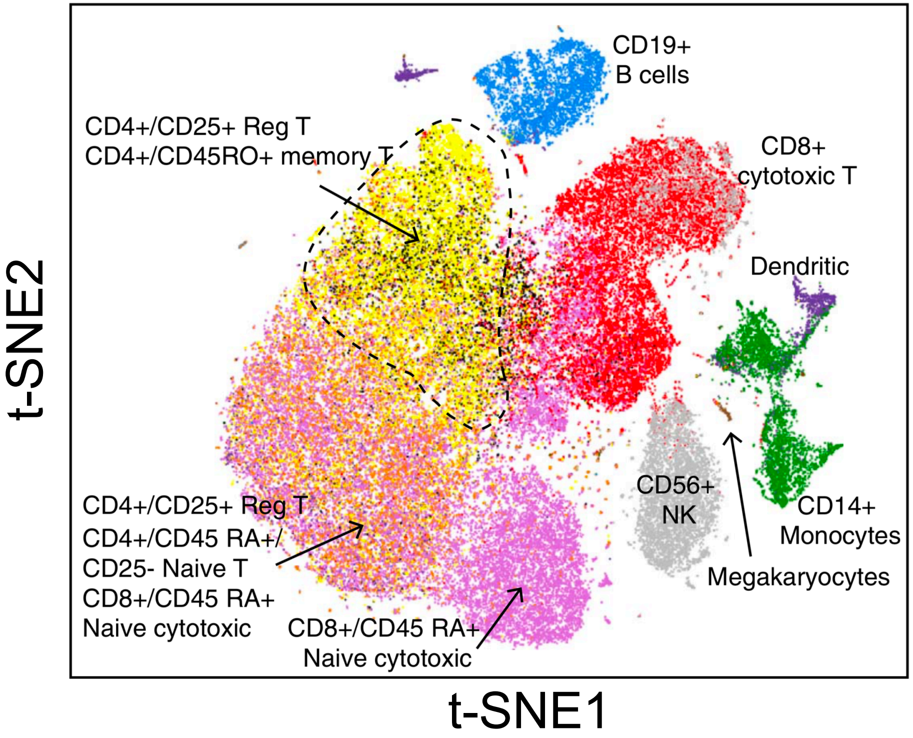


	Cell 1	Cell 2	…
Gene 1	25	918	
Gene 2	0	456	
Gene 3	20	342	
Gene 4	0	214	
…			

Source: Amit (2016), *Nature Immunoloy.*

Gene Expression of Lymphoid Cells

PBMCs from Humans

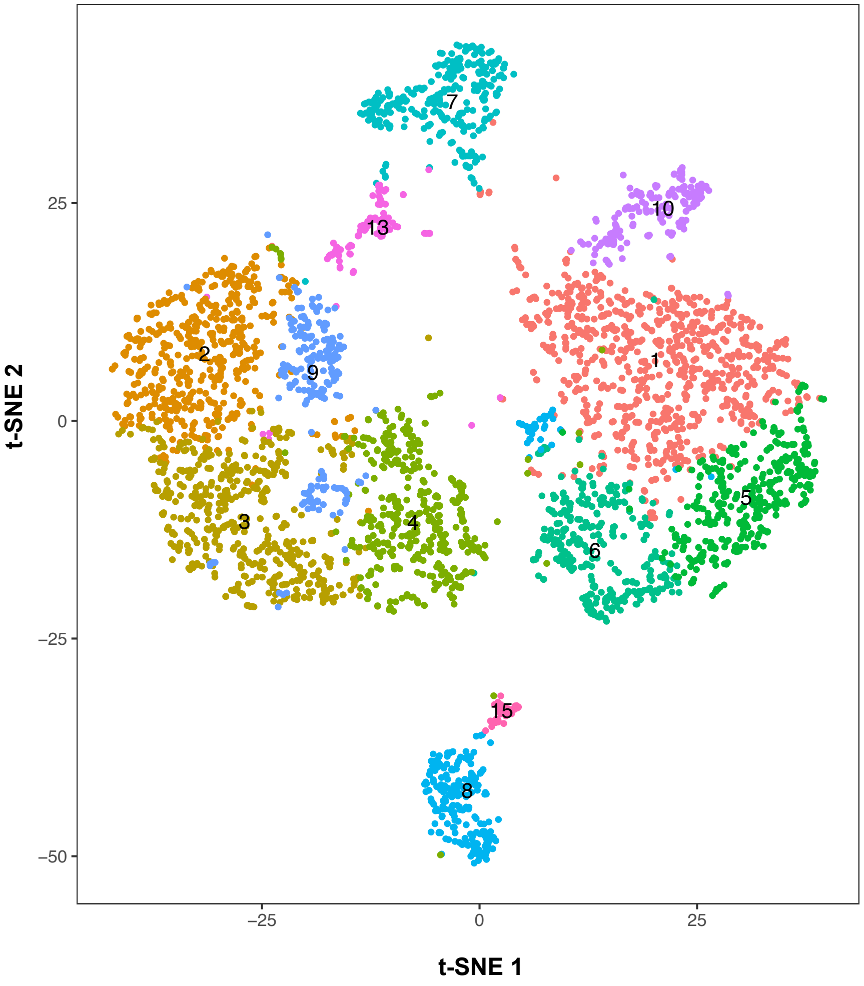


Single cell RNA-seq from 68k cells

Source: Zheng et al. 2017 & Buenrostro et al. 2018

Basics Bioinformatics - Clustering

Gut Immune Cells - 12 groups

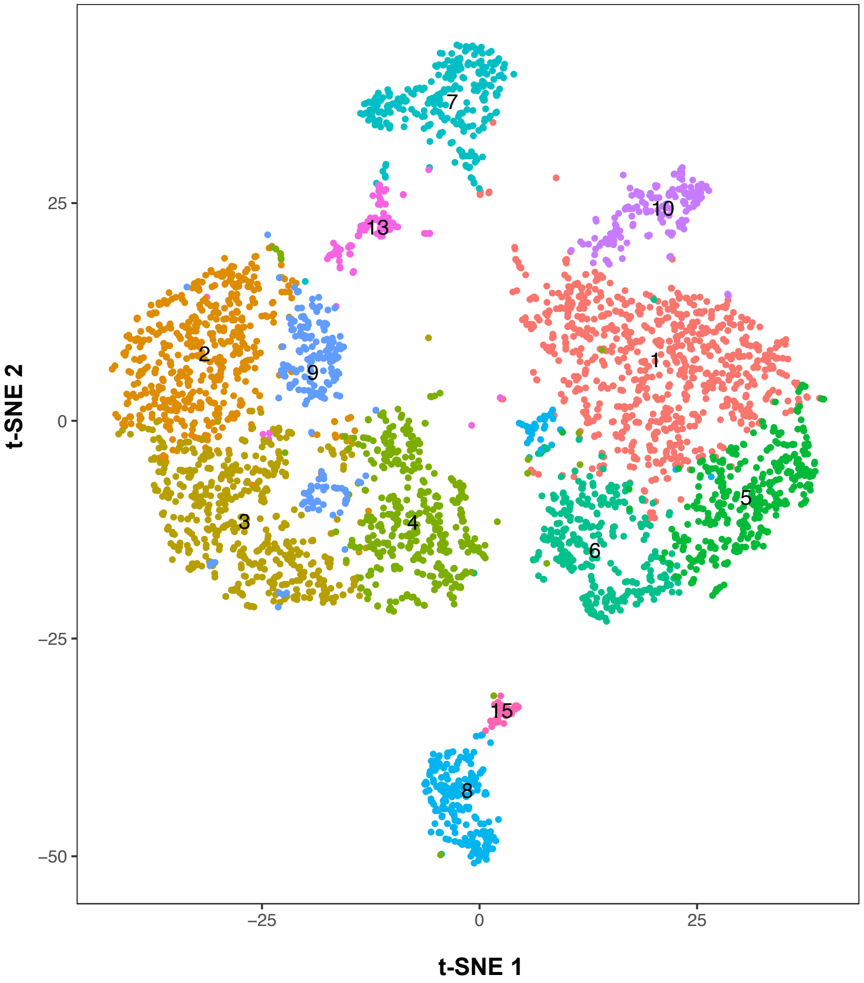


Clustering - identify cells with similar expression patterns
- based on PCA (20 dimension)

How to identify cell types?

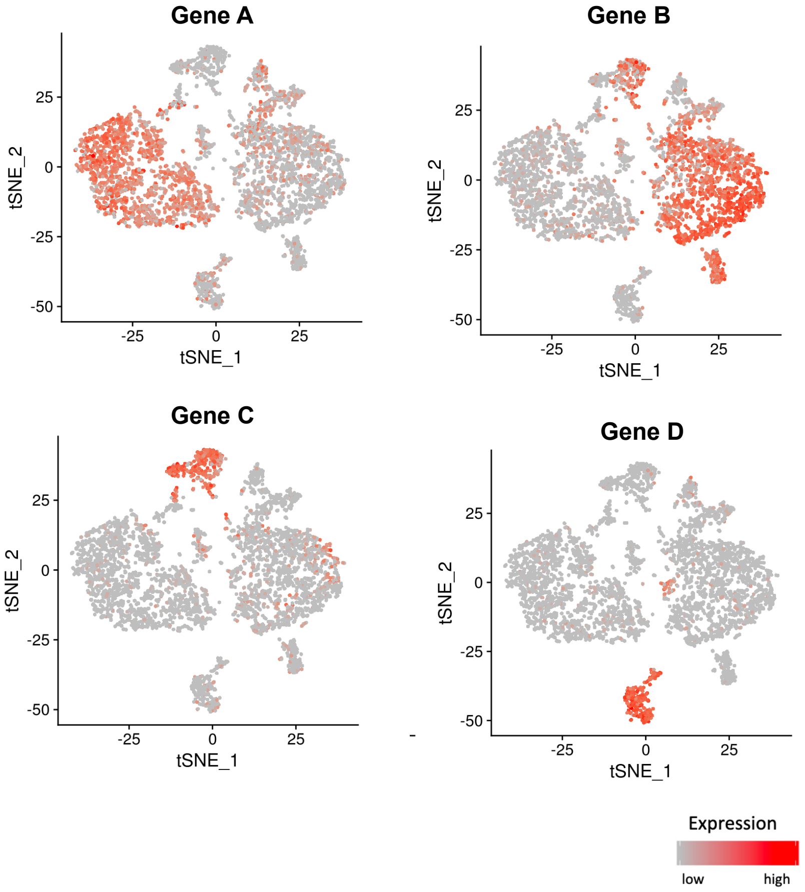
Cell Identity with an Expert

Gut Immune Cells - 12 groups



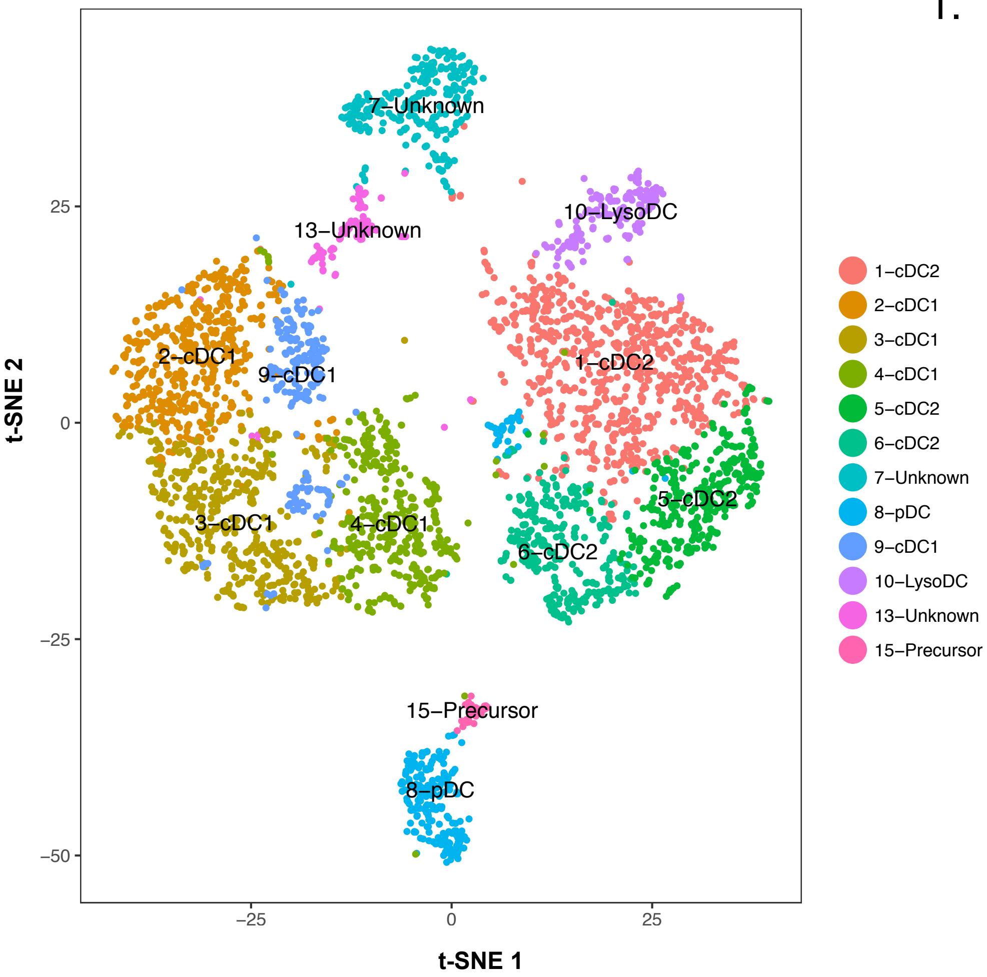
Check expression of:

1. known genes



Cell Identity with an Expert

Gut Immune Cells - 12 groups



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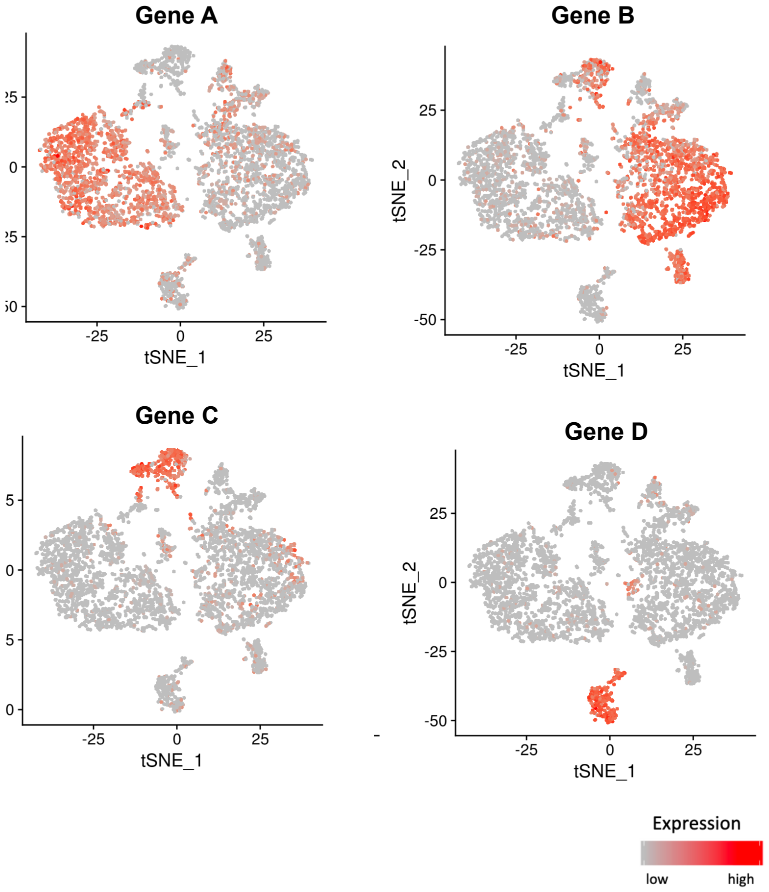

Cell Identity with an Expert

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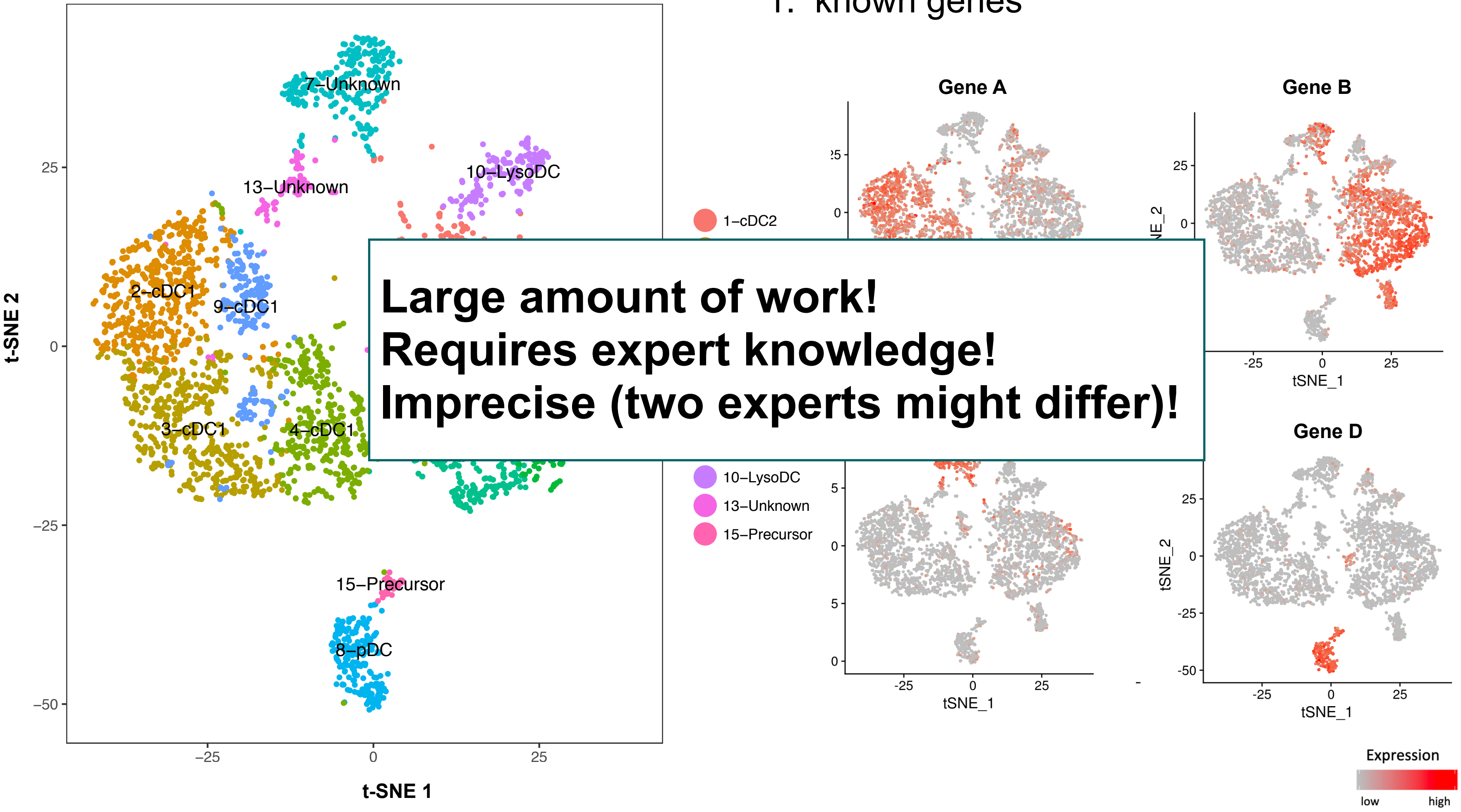

Large amount of work!
Requires expert knowledge!
Imprecise (two experts might differ)!

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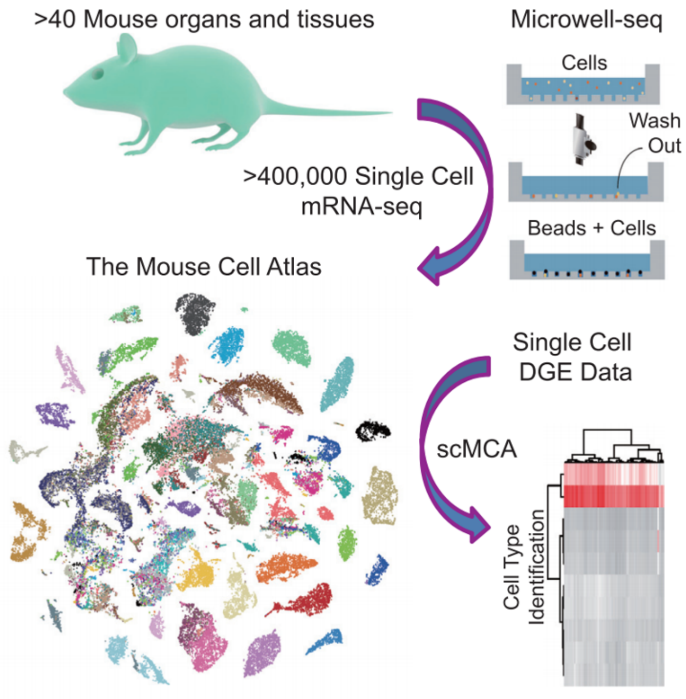
Automatic Cell Identification

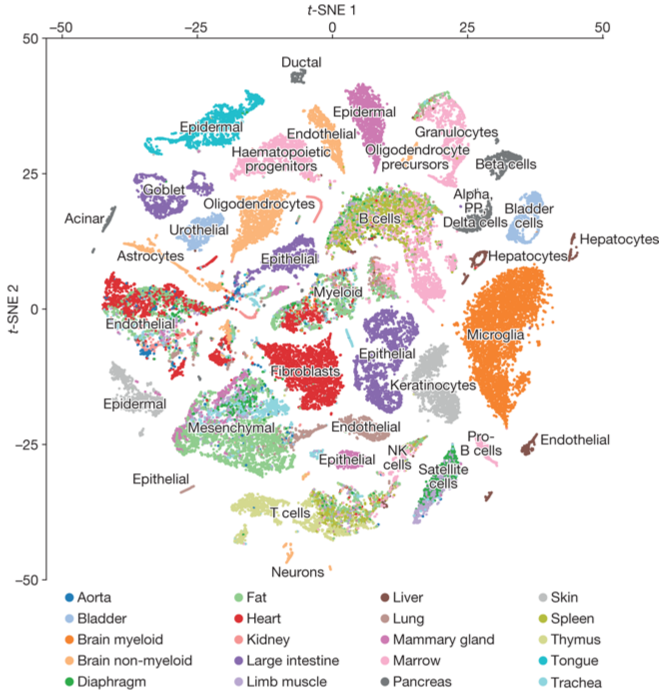
Large consortia provide gene expression and annotation of cells
- annotation is based on *cell ontology*

Mouse cell atlas (MCA)



400.000 cells on 40 tissues

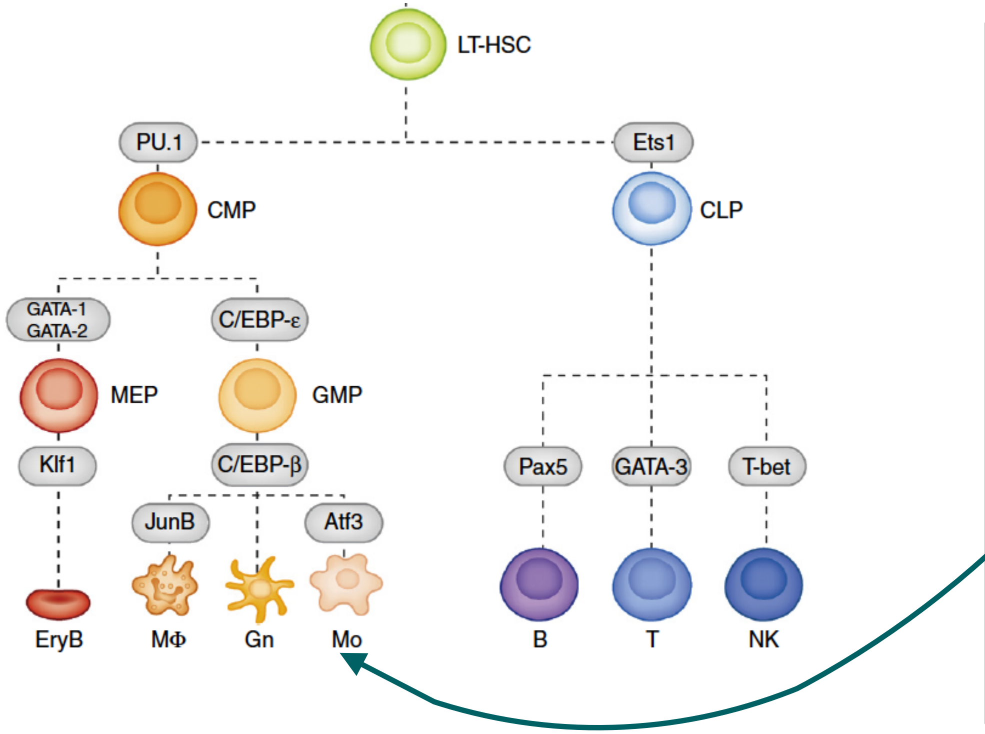
Tabula Muris (TM)

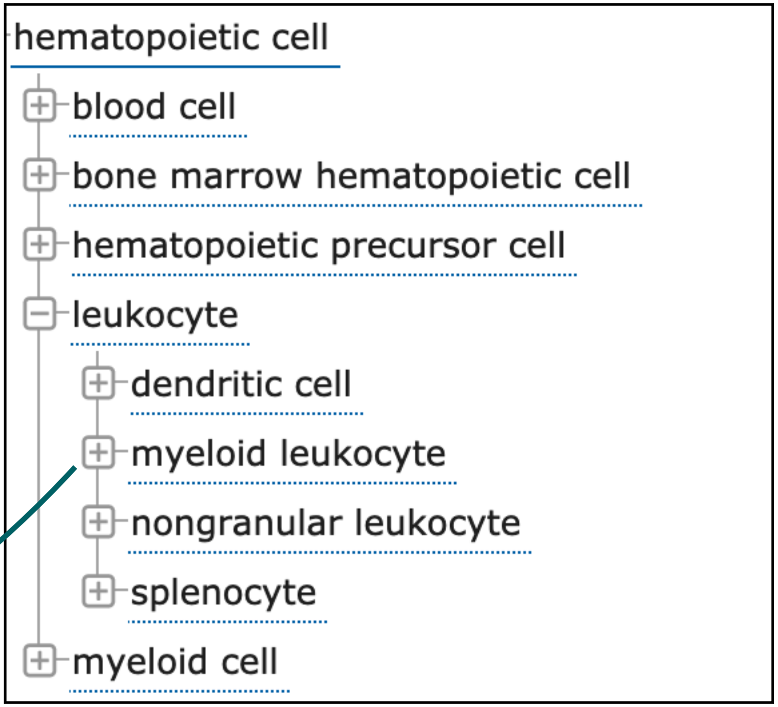


100.000 cells on 20 tissues

Cell Ontology

Controlled vocabulary for cell types in animals




hematopoietic cell
- ⊞ blood cell
- ⊞ bone marrow hematopoietic cell
- ⊞ hematopoietic precursor cell
- ⊟ leukocyte
  - ⊞ dendritic cell
  - ⊞ myeloid leukocyte
  - ⊞ nongranular leukocyte
  - ⊞ splenocyte
- ⊞ myeloid cell

https://www.ebi.ac.uk/ols/ontologies/cl

Available as Json format at:
https://github.com/obophenotype/cell-ontology

Overall Design / Basic Approach

Use machine learning for cell type classifiers:

- elastic net, Neural Networks, Random Forests

For each organ from MCA build a classifier:

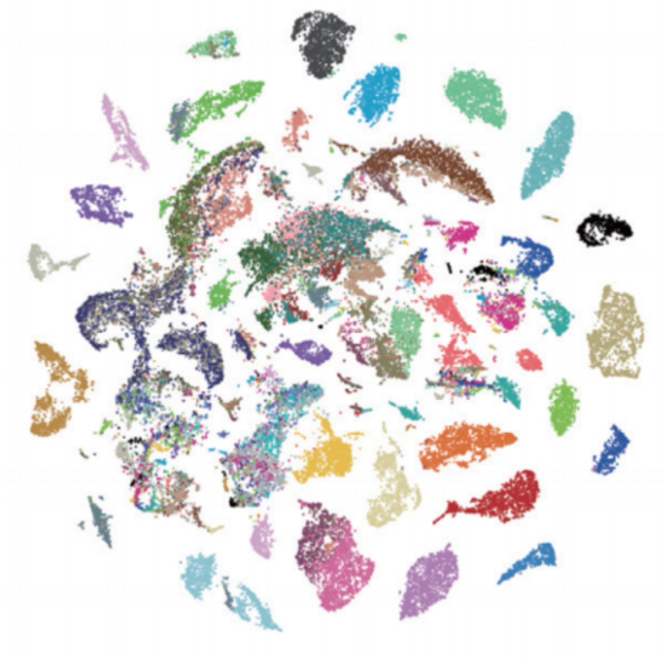
- i.e. Peripheral-Blood from MCA
- check/revise cell annotation (using cell ontology)
- use this data for classifier training/parameter selection with **cross-validation**
- use **area under PR curve** for selection

Test data:

- Find respective organ in TM (i.e. bone marrow)
- Revise cell annotation
- Measure cell type accuracy (PR curve) of MCA model in TM data

Automatic Cell Identification

Mouse cell atlas & Tabula Muris



400.000 cells on 40 tissues

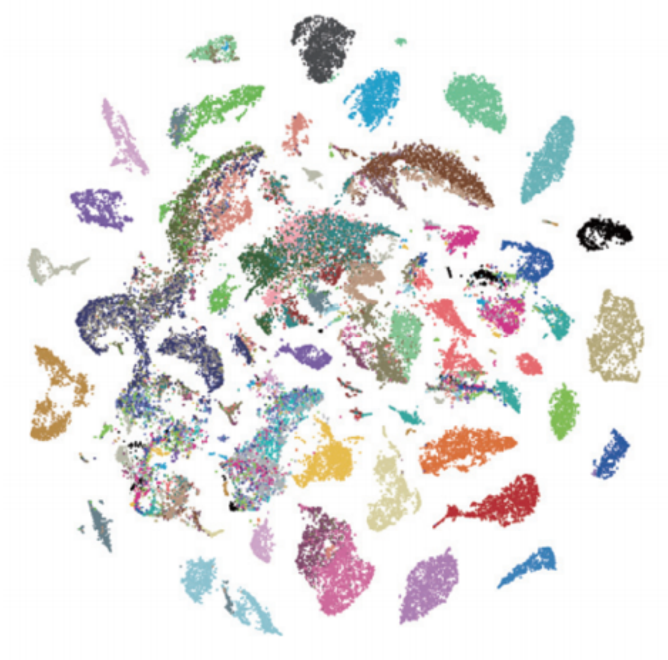
Use pre-annotated cells to build classifiers to annotate novel single cell data (diseases)

Methodological questions:

1. Which machine learning methods to use?
   - Neural networks, statistical methods, ….
2. Feature selection (vs. Blackbox)
   - Find reliable markers from classifiers?
3. Are classifiers robust on sparse data?
   - Evaluate performance when reducing number of reads

Automatic Cell Identification

Mouse cell atlas
& Tabula Muris



400.000 cells on 40 tissues

Challenges:

1. Detect unknown/unseen cells?
   - Detect progenitor cells?

2. Build classifiers across tissues/ whole body?

3. Annotate human samples with mouse trained classifiers?

Challenges: Unseen cells

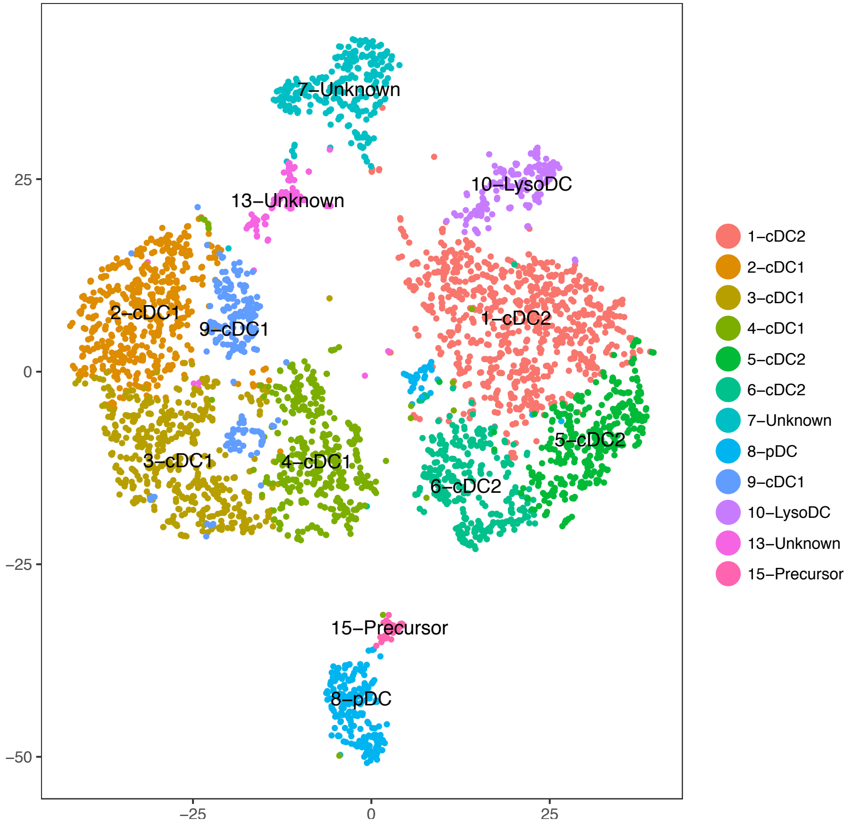
Test data has cell types, which are not included in your classifier.

- You train data did not contained enough cells
- new cell types only found in a disease condition (test data).
- …

Build classifiers that recognise unknown cells

- classifiers have a confidence level
- Indicate that cells with low confidence are unknown

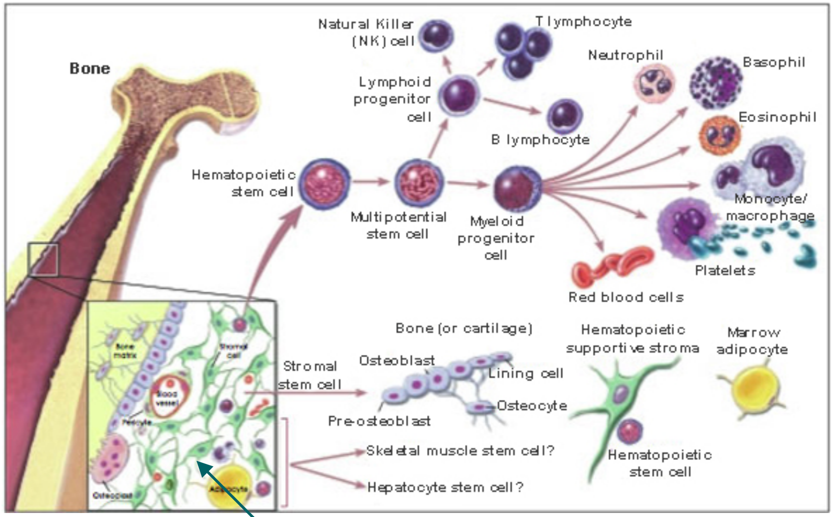
Example: gut immune cells



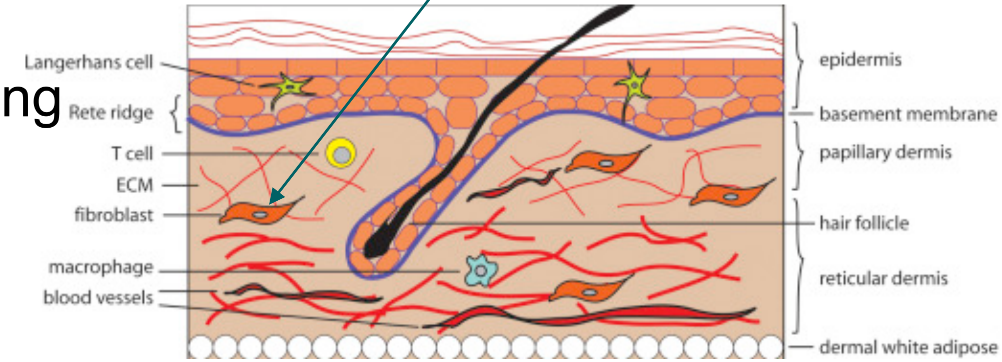
Challenges: Cross organs classification

Most cells are tissue specific
- parenchyma cells
    - aveoli in lungs
    - hepatocytes in liver
    - …

Some cells are in several orgasms:
- stromal cells -> adipose cells, bone cells, fibroblast
- immune cells
- these cells might differ depending of the tissue.



stromal cells

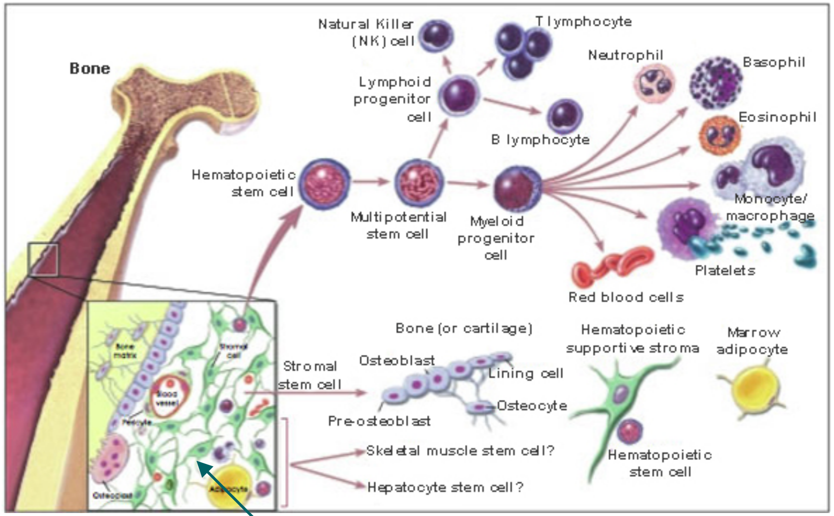


Challenges: Cross organs classification

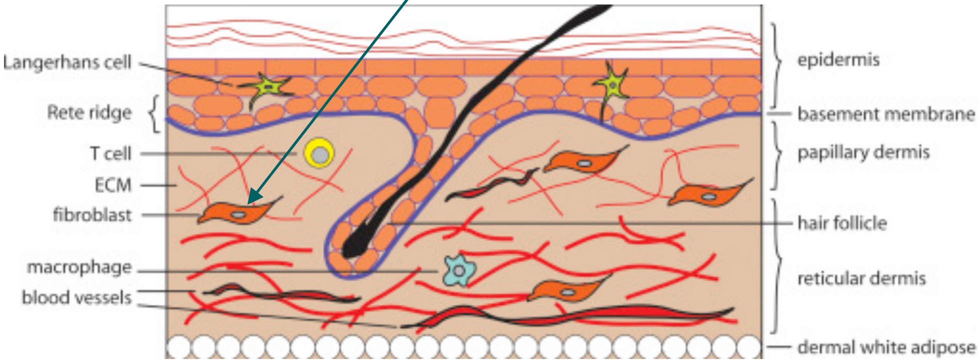
We known the origin/organ of a data.

What is the best strategy to build classifiers?

- a classifier per tissue?
- whole body classifiers?
- combination: per tissue for parenchyma cells and whole body for others?



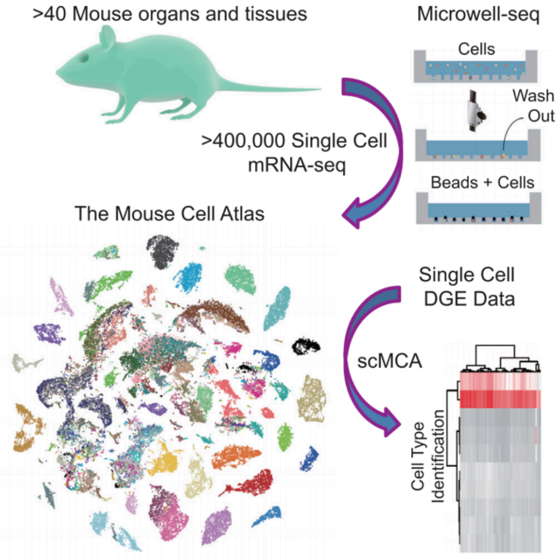
stromal cells



Challenges: Cross organism classification

Use mouse data to classify human samples
- gene names can be mapped but gene function might differ.

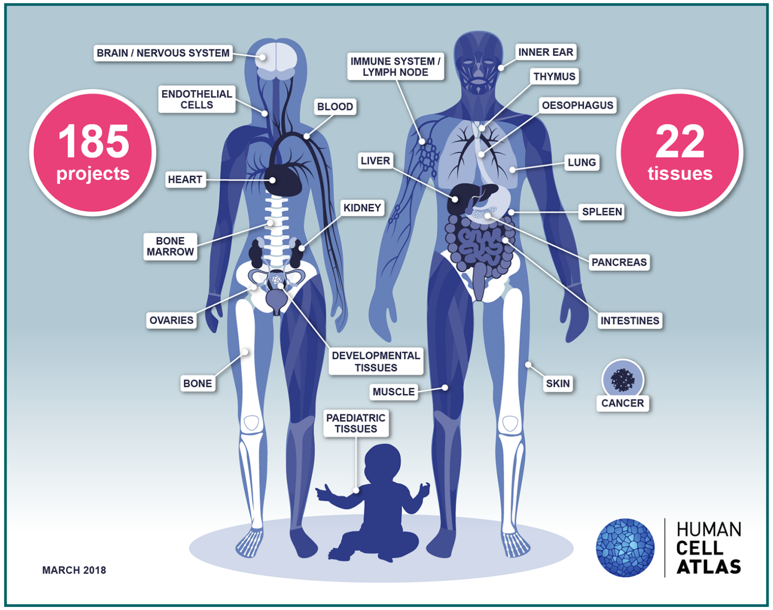
Mouse cell atlas (MCA)



400.000 cells on 40 tissues

Classify

Human Cells



Still being built

Project Proposal

- **Groups: 3-4 participants each**
- **Each group addresses a method problem and challenge**

Method problem

1. Which machine learning methods?
2. Feature selection?
3. Are classifiers robust on sparse data?

Challenges

1. Detect unknown cells
2. Cross tissues/whole body classifiers?
3. Cross organism classifier?

- **Build classifiers and evaluate then on all MCA/TM data**

- additional tasks and data might be defined during the course.

- **Projects code should be deposited in gitlab (git.rwth-aachen.de)**

Calendar

27.05.2019 to 8.07.2019 – Project Development

15.07.2019 – Project Presentation

Links

- Machine learning libraries:
  - python - scikits - https://scikit-learn.org/stable/
  - python & gpu - https://keras.io/
  - R - several individual packages
    - i.e. http://topepo.github.io/caret/index.html
    - seurat / low level single cell and cluster analysis
    - - https://satijalab.org/seurat/

- Cell Ontology:
  - https://github.com/obophenotype/cell-ontology

- Single cell data repositories:
  - Tabua Muris (TM)
    https://figshare.com/articles/MCA_DGE_Data/5435866
  - Mouse cell atlas (MCA)
    https://figshare.com/articles/MCA_DGE_Data/5435866

Relevant data is already at the RWTH Cluster
/hpcwork/nova0028/BioinfoLab/data

Thank you!