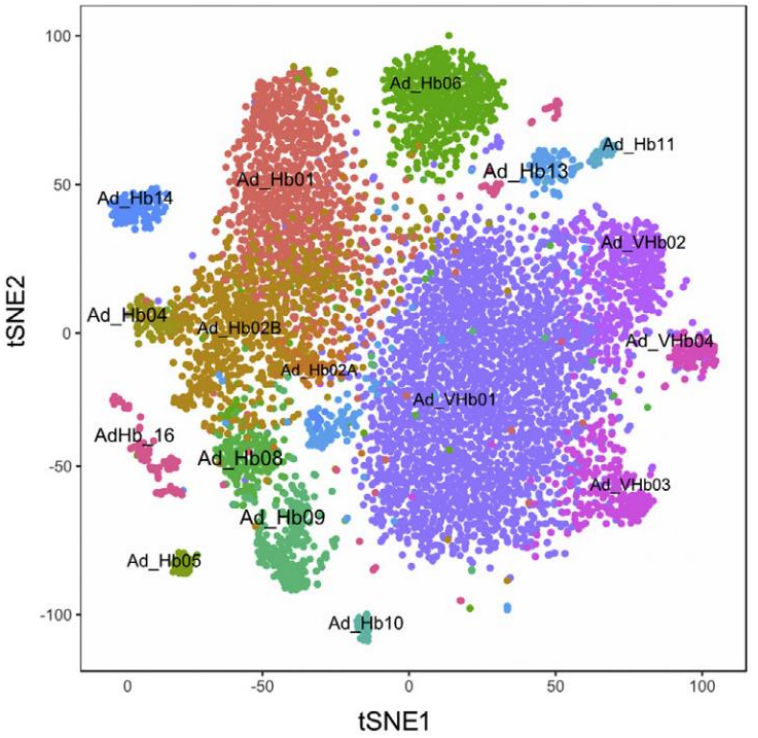
Statistics and data analysis

Dave Zhao
Department of Statistics
University of Illinois Urbana-Champaign



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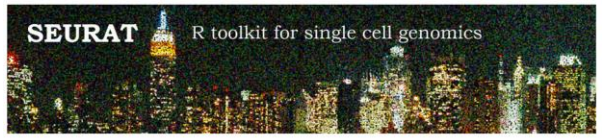
Pandey et al. (2018)

Is statistics really necessary?



Official release of Seurat 4.0

We are excited to release Seurat v4.0! This update brings the following new features and functionality:

- **Integrative multimodal analysis.** The ability to make simultaneous measurements of multiple data types from the same cell, known as multimodal analysis, represents a new and exciting frontier for single-cell genomics. In Seurat v4, we introduce weighted nearest neighbor (WNN) analysis, an unsupervised strategy to learn the information content of each modality in each cell, and to define cellular state based on a weighted combination of both modalities. In our new paper, we generate a CITE-seq dataset featuring paired measurements of the transcriptome and 228 surface proteins, and leverage WNN to define a multimodal reference of human PBMC. You can use WNN to analyze multimodal data from a variety of technologies, including CITE-seq, ASAP-seq, 10X Genomics ATAC + RNA, and SHARE-seq.

Seurat 4.0.6 | Install | Get started | Vignettes | Extensions | FAQ | News | Reference | Archive

  - Dataset: Download here
- **Rapid mapping of query datasets to references.** We introduce Azimuth, a workflow to leverage high-quality reference datasets to rapidly map new scRNA-seq datasets (queries). For example, you can map any scRNA-seq dataset of human PBMC onto our reference, automating the process of visualization, clustering annotation, and differential expression. Azimuth can be run within Seurat, or using a standalone web application that requires no installation or programming experience.
  - Vignette: Mapping scRNA-seq queries onto reference datasets
  - Web app: Automated mapping, visualization, and annotation of scRNA-seq datasets from human PBMC

Additional speed and usability updates: We have made minor changes in v4, primarily to improve the performance of Seurat v4 on large datasets. These changes substantially improve the speed and memory requirements, but do not adversely impact downstream results. We provide a detailed description of key changes here. Users who wish to fully reproduce existing results can continue to do so by continuing to install Seurat v3.

We believe that users who are familiar with Seurat v3 should experience a smooth transition to Seurat v4. While we have introduced extensive new functionality, existing workflows, functions, and syntax are largely unchanged in this update. In addition, Seurat objects that have been previously generated in Seurat v3 can be seamlessly loaded into Seurat v4 for further analysis.

About Seurat

Seurat is an R package designed for QC, analysis, and exploration of single-cell RNA-seq data. Seurat aims to enable users to identify and interpret sources of heterogeneity from single-cell transcriptomic measurements, and to integrate diverse types of single-cell data.

If you use Seurat in your research, please considering citing:

- Hao*, Hao*, et al., Cell 2021 [Seurat V4]
- Stuart*, Butler*, et al., Cell 2019 [Seurat V3]
- Butler* et al., Nat Biotechnol 2018 [Seurat V2]
- Satija*, Farrell*, et al., Nat Biotechnol 2015 [Seurat V1]

All methods emphasize clear, attractive, and interpretable visualizations, and were designed to be easily used by both dry-lab and wet-lab researchers.

Seurat is developed and maintained by the Satija lab and is released under the GNU Public License (GPL 3.0).

Developed by Paul Hoffman, Satija Lab and Collaborators.

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Home » Bioconductor 3.15 » Software Packages » edgeR

edgeR

platforms all | rank 25 / 2140 | support 5 5 / 6 1 | in Bioc 13.5 years
build ok | updated before release | dependencies 10

DOI: 10.18129/B9.bioc.edgeR

Empirical Analysis of Digital Gene Expression Data in R

Bioconductor version: Release (3.15)

Differential expression analysis of RNA-seq expression profiles with biological replication. Implements a range of statistical methodology based on the negative binomial distributions, including empirical Bayes estimation, exact tests, generalized linear models and quasi-likelihood tests. As well as RNA-seq, it be applied to differential signal analysis of other types of genomic data that produce read counts, including ChIP-seq, ATAC-seq, Bisulfite-seq, SAGE and CAGE.

Author: Yunshun Chen, Aaron TL Lun, Davis J McCarthy, Matthew E Ritchie, Belinda Phipson, Yifang Hu, Xiaobei Zhou, Mark D Robinson, Gordon K Smyth

Maintainer: Yunshun Chen <yuchen at wehi.edu.au>, Gordon Smyth <smyth at wehi.edu.au>, Aaron Lun <infinite.monkeys.with.keyboards at gmail.com>, Mark Robinson <mark.robinson at imls.uzh.ch>

Citation (from within R, enter `citation("edgeR")`):

Robinson MD, McCarthy DJ, Smyth GK (2010). "edgeR: a Bioconductor package for differential expression analysis of digital gene expression data." *Bioinformatics*, **26**(1), 139-140. doi: 10.1093/bioinformatics/btp616.

McCarthy DJ, Chen Y, Smyth GK (2012). "Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation." *Nucleic Acids Research*, **40**(10), 4288-4297. doi: 10.1093/nar/gks042.

Chen Y, Lun AAT, Smyth GK (2016). "From reads to genes to pathways: differential expression analysis of RNA-Seq experiments using Rsubread and the edgeR quasi-likelihood pipeline." *F1000Research*, **5**, 1438. doi: 10.12688/f1000research.8987.2.

Installation

To install this package, start R (version "4.2") and enter:

```
if (!require("BiocManager", quietly = TRUE))
    install.packages("BiocManager")

BiocManager::install("edgeR")
```

For older versions of R, please refer to the appropriate Bioconductor release.

Documentation

To view documentation for the version of this package installed in your system, start R and enter:

```
browseVignettes("edgeR")
```

PDF		edgeR Vignette
PDF		edgeRUsersGuide.pdf
PDF		Reference Manual
Text		NEWS

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Home | Install | Help

Home » Bioconductor 3.15 » Software Packages » phyloseq

phyloseq

platforms all | rank 78 / 2140 | support 3 / 7 | in Bioc 10 years
build ok | updated before release | dependencies 76

DOI: 10.18129/B9.bioc.phyloseq

Handling and analysis of high-throughput microbiome census data

Bioconductor version: Release (3.15)

phyloseq provides a set of classes and tools to facilitate the import, storage, analysis, and graphical display of microbiome census data.

Author: Paul J. McMurdie <joey711 at gmail.com>, Susan Holmes <susan at stat.stanford.edu>, with contributions from Gregory Jordan and Scott Chamberlain

Maintainer: Paul J. McMurdie <joey711 at gmail.com>

Citation (from within R, enter `citation("phyloseq")`):

McMurdie PJ, Holmes S (2013). "phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data." *PLoS ONE*, **8**(4), e61217. http://dx.plos.org/10.1371/journal.pone.0061217.

Installation

To install this package, start R (version "4.2") and enter:

```
if (!require("BiocManager", quietly = TRUE))
    install.packages("BiocManager")

BiocManager::install("phyloseq")
```

For older versions of R, please refer to the appropriate Bioconductor release.

Documentation

To view documentation for the version of this package installed in your system, start R and enter:

```
browseVignettes("phyloseq")
```

HTML	R Script	analysis vignette
HTML	R Script	phyloseq and DESeq2 on Colorectal Cancer Data
HTML	R Script	phyloseq basics vignette
HTML	R Script	phyloseq Frequently Asked Questions (FAQ)
PDF		Reference Manual
Text		NEWS

https://satijalab.org/seurat/, https://bioconductor.org/packages/release/bioc/html/edgeR.html, https://bioconductor.org/packages/release/bioc/html/phyloseq.html

Statistics is necessary for more complicated analyses.

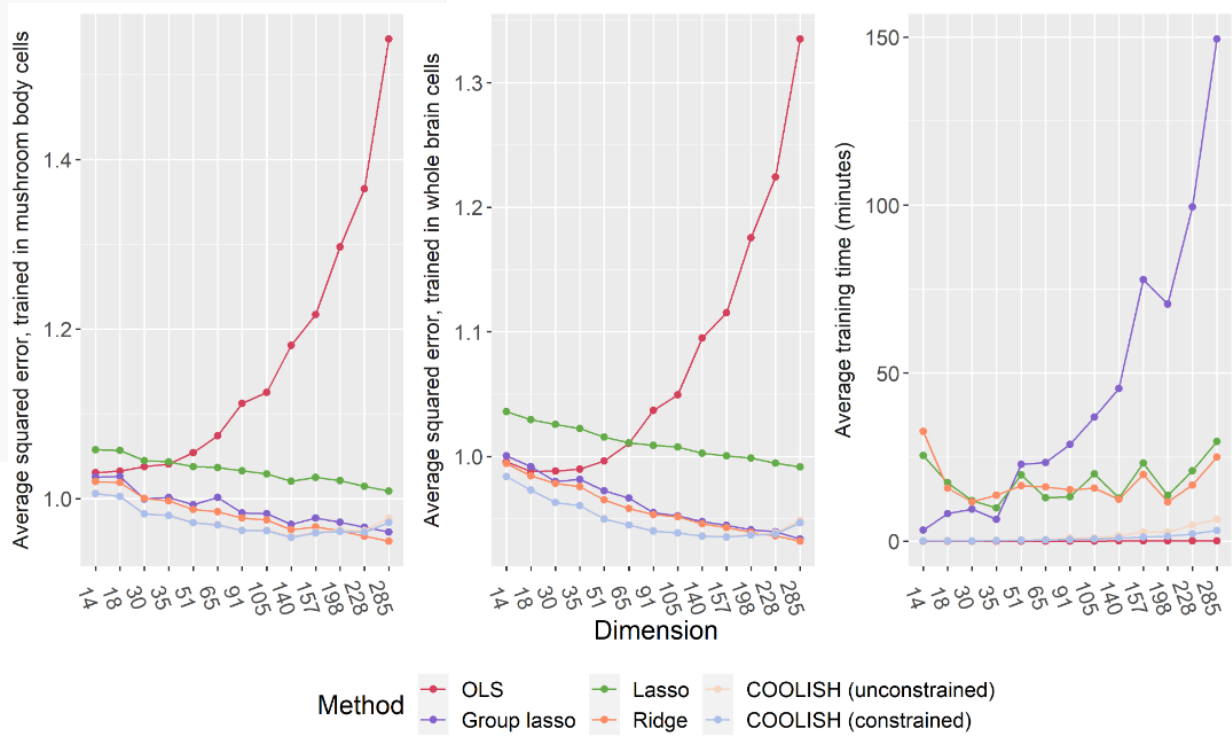
Run Principal Component Analysis

Source: `R/generics.R`, `R/dimensional_reduction.R`

Run a PCA dimensionality reduction. For details about stored PCA calculation parameters, see `PrintPCAParams`.

```
RunPCA(object, ...)

# S3 method for default
RunPCA(
  object,
  assay = NULL,
  npcs = 50,
  rev.pca = FALSE,
  weight.by.var = TRUE,
  verbose = TRUE,
  ndims.print = 1:5,
  nfeatures.print = 30,
  reduction.key = "PC_",
  seed.use = 42,
  approx = TRUE,
  ...
)
```

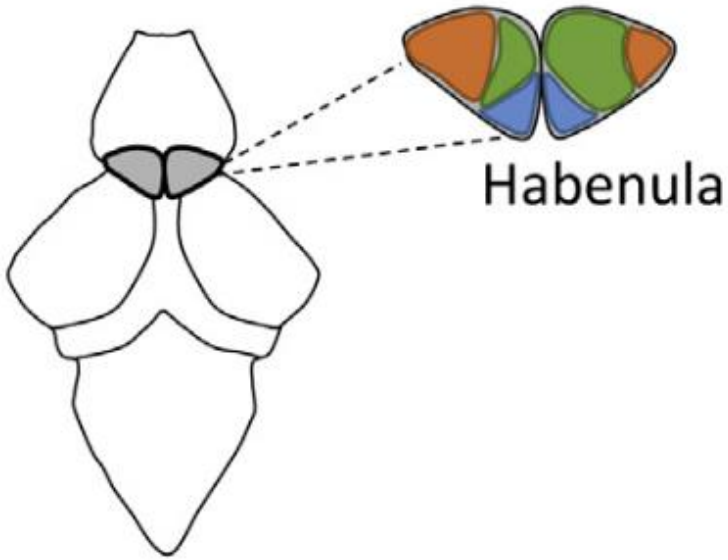


https://satijalab.org/seurat/reference/findmarkers, Wang and Zhao (2021)

Role of statistics

Scientific question

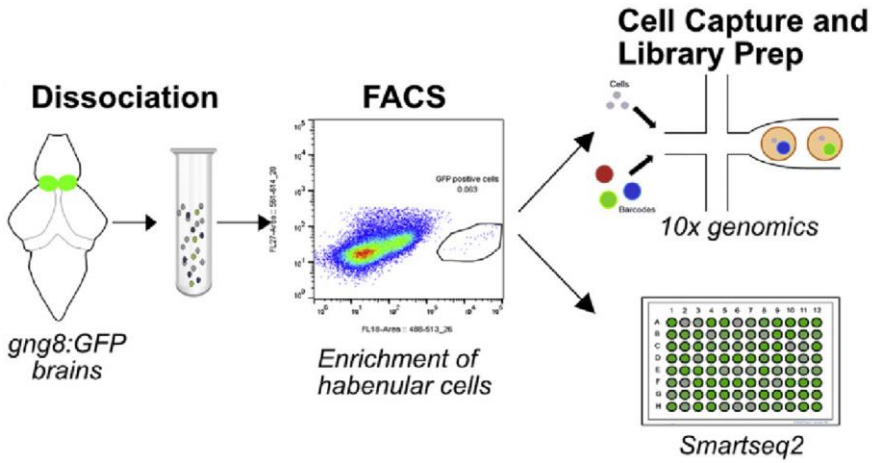
How do cells in the larval zebrafish habenula coordinate their functions?



Pandey et al. (2018)

Experimental data

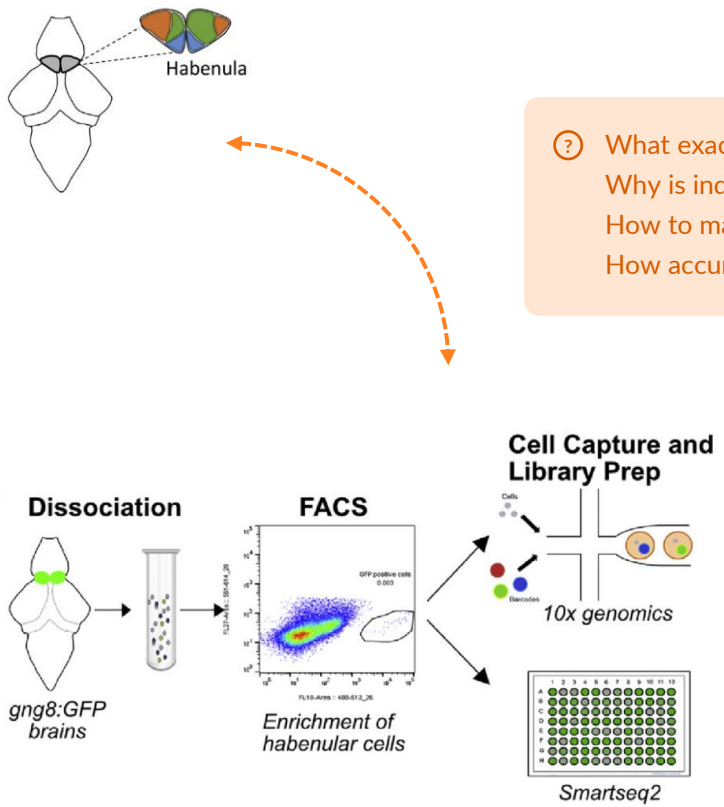
Single-cell RNA-seq on 10 pooled larval zebrafish habenula.



Pandey et al. (2018)

The problem of induction

How can we make general conclusions from specific examples?

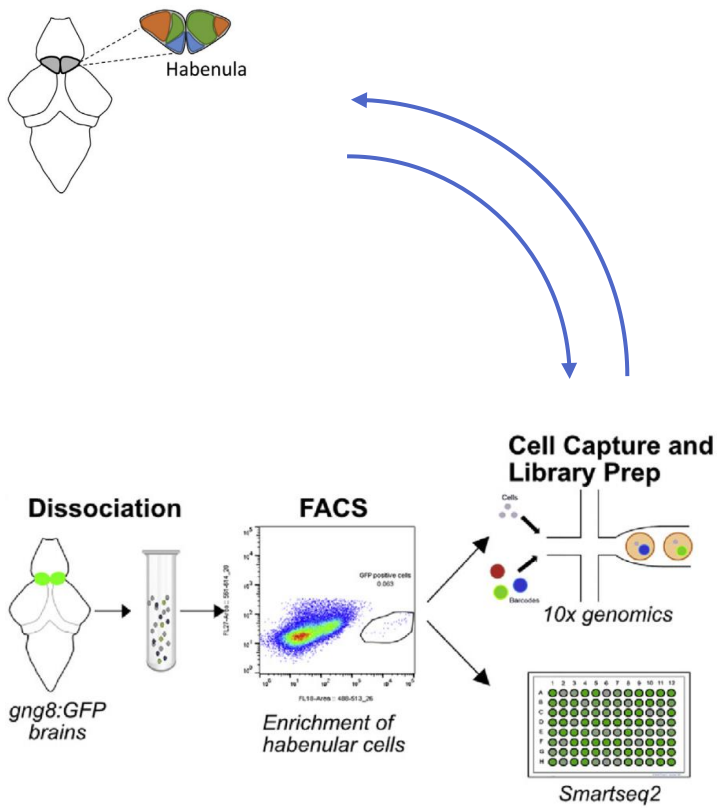


- What exactly is a general conclusion?
- Why is induction justified?
- How to make inferences?
- How accurate are the inferences?

Pandey et al. (2018)

Statistics

Statistics provides a mathematical theory of induction.

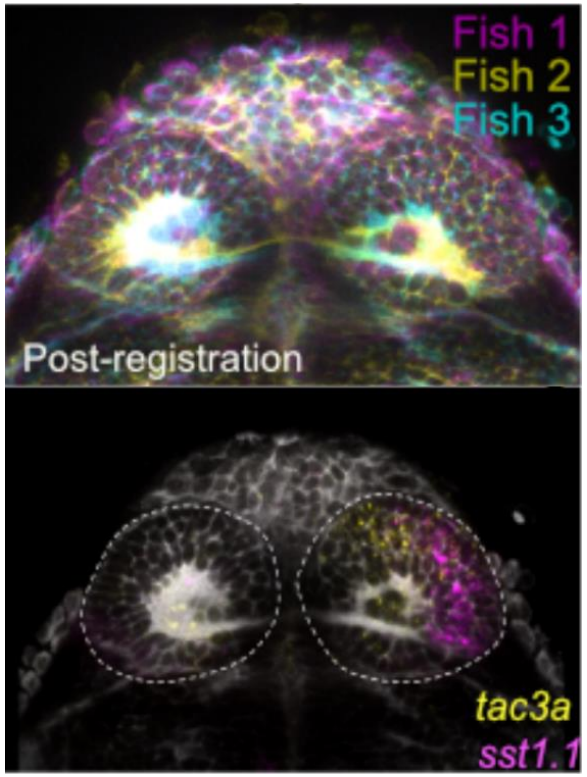


Pandey et al. (2018)

Statistical concepts

A general conclusion is viewed as some statement about a population.

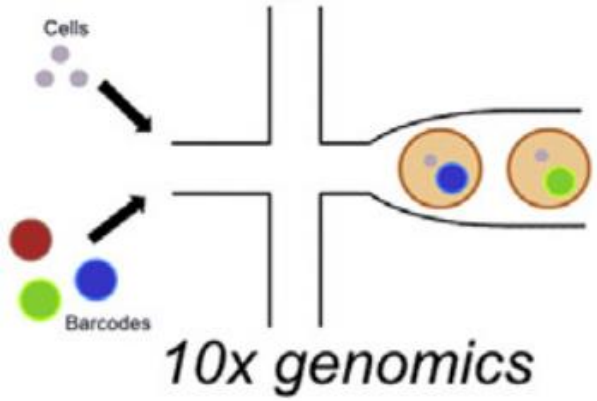
The population is the hypothetical collection of all objects you want to generalize to, e.g., all cells in the larval zebrafish habenula.



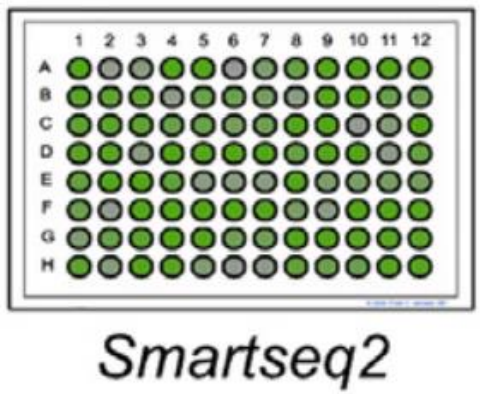
Pandey et al. (2018)

What exactly is a general conclusion?

Each member of the population can be characterized by numerical variables.



Variables need to be operationally defined and may need to be recoded numerically. Some important variables may not be directly observable.

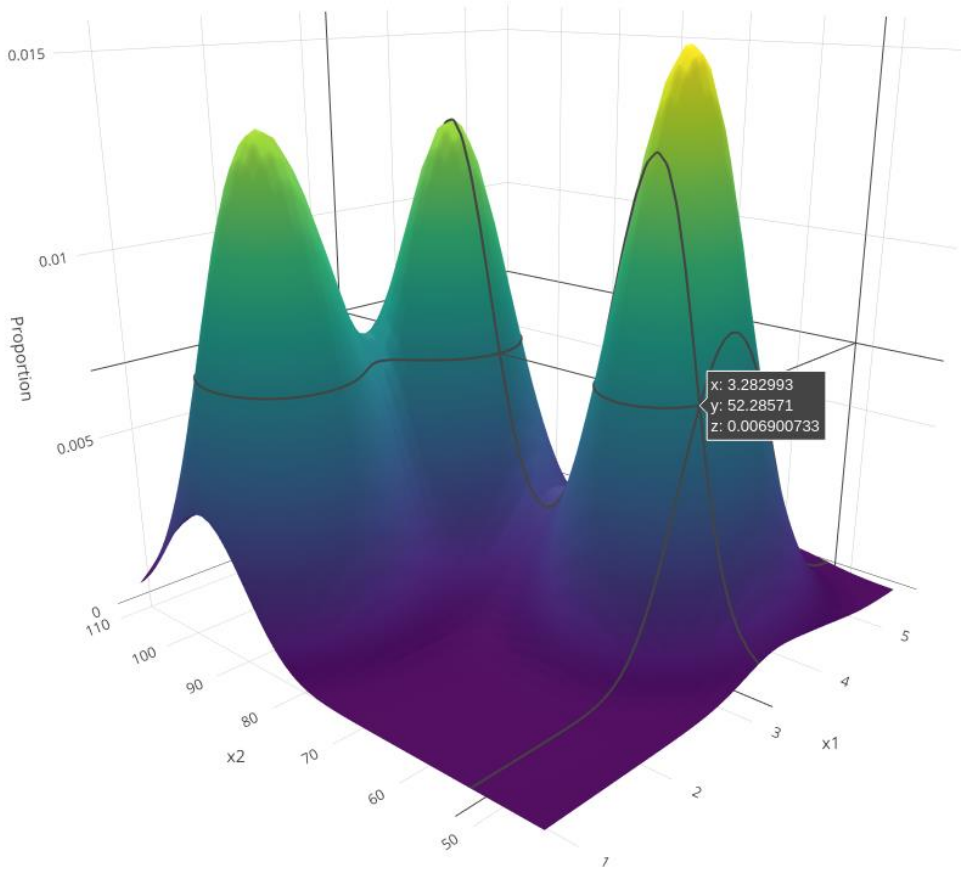


Pandey et al. (2018)

What exactly is a general conclusion?

A general conclusion describes properties of the population distribution function of the variables.

$P(X_1 = x_1, \ldots, X_p = x_p) \approx$ proportion of the population whose first variable has value x_1, second variable has value x_2, …, and pth variable has value x_p.

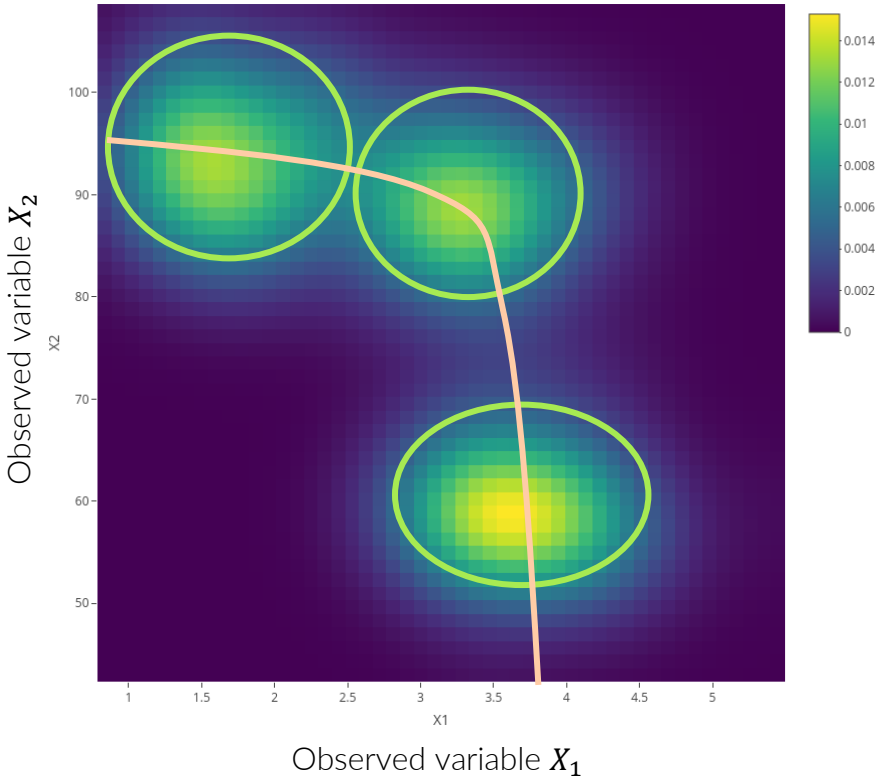


What exactly is a general conclusion?

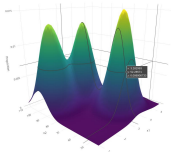
There are three important types of properties:
1. Define latent variables.

These properties define the "best" latent variables, according to some investigator-defined metric.

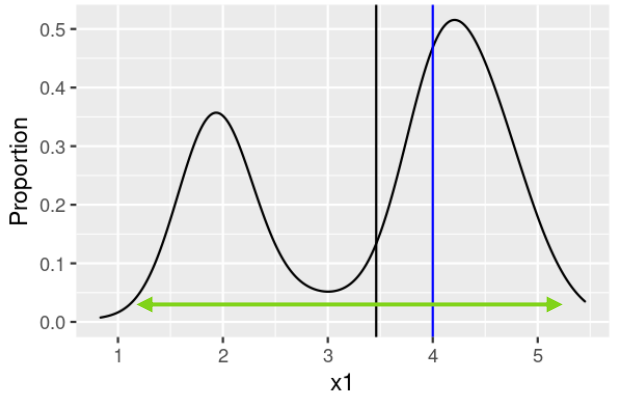
Latent (cluster) variable $Z_1 = g_1(X_1, \ldots, X_p)$
Latent (factor/component) variable
$Z_2 = g_2(X_1, \ldots, X_p)$
g_1 and g_2 depend on pop. dist. function



What exactly is a general conclusion?

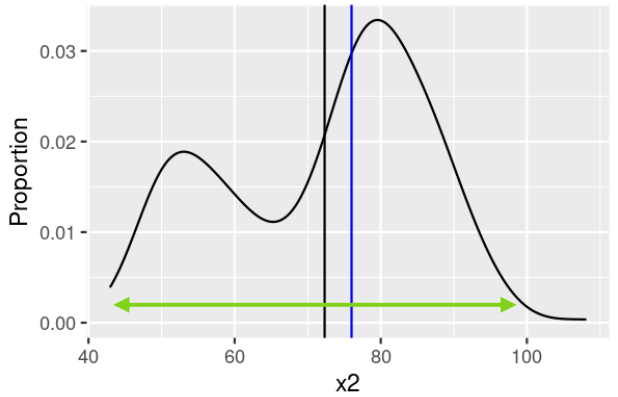


2. Describe univariate characteristics.



Mean, median, and standard deviation of X_1.

These properties include measures of central tendency, variability, etc.



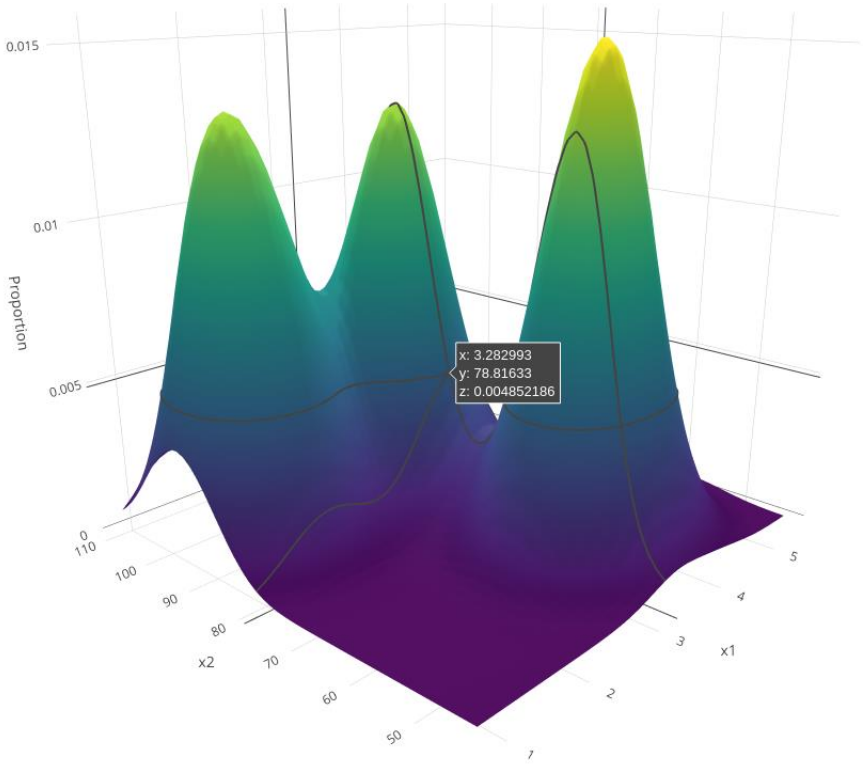
Mean, median, and standard deviation of X_2.

What exactly is a general conclusion?

3. Describe dependence between variables.

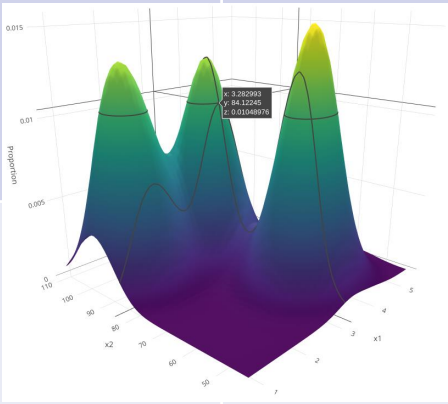
Dependence between independent and dependent variables is the most common type of multivariate characteristic.

$P(X_1 = x_1 \mid X_2 = x_2) \approx$ the population distribution of X_1 in the subgroup of the population with $X_2 = x_2$:



What exactly is a general conclusion?

A general conclusion is a mathematical statement regarding population parameters of interest.

	Univariate	Dependence
Observed variables		
Latent variables		

Mathematical models of probability distributions impose assumptions but are easier to understand.

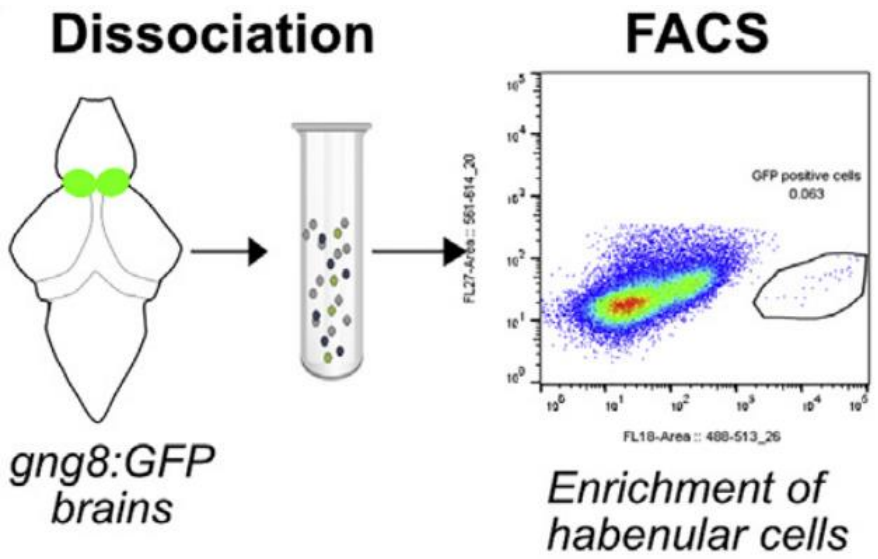
Regression modeling is a common type of mathematical model and trades stronger assumptions for less complexity in expressing dependence between variables.

Model $P(X_1 = x_1 \mid X_2 = x_2)$ using a generalized linear model, e.g.:

- $X_1 \mid X_2 \sim \text{NegBin}(\mu(X_2), \sigma(X_2))$
- $\log \mu(X_2) = \beta_0 + \beta_1 X_2$
- $\sigma(X_2) = \mu(X_2)(1 + \phi\mu(X_2))$

Specific examples are viewed as being sampled from the population.

Sampling must be designed by the experimenter and should maximize information, optimize efficiency, and minimize systematic biases.



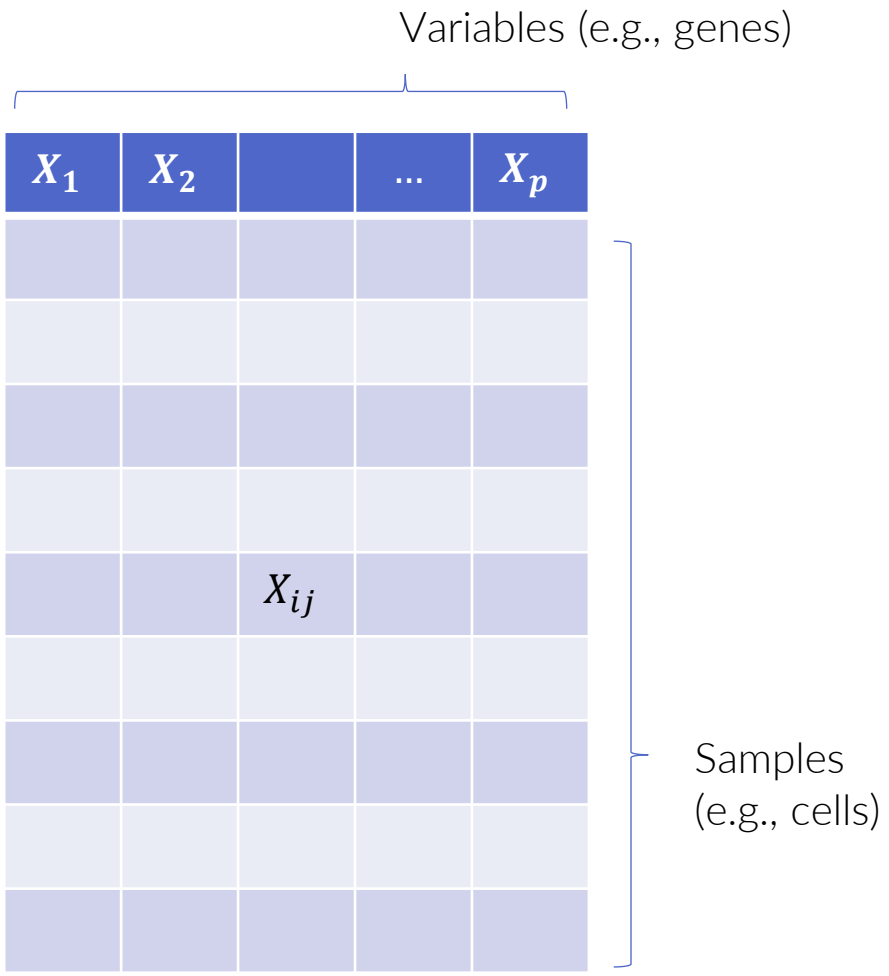
Pandey et al. (2018)

By the laws of probability, a properly chosen sample will be representative of the population.

$$P\left(\left|\frac{1}{n}\sum_{i=1}^{n} X_i - \mu\right| > \epsilon\right) \leq \frac{\mathrm{var}(X_i)}{\epsilon^2 n}$$

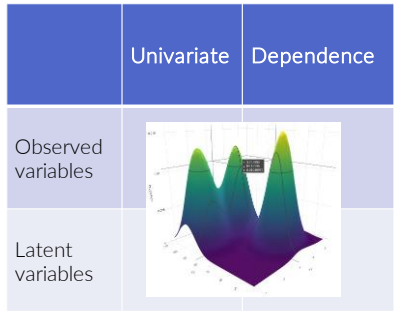
Data are viewed as numerical variables measured on each sample.

The “samples by variables” data table is the fundamental unit of statistical analysis.



How to make inferences?

Inductive processes correspond to functions of the observed data.



$f(X_{11}, \ldots, X_{np})$

X_1	X_2		...	X_p
		X_{ij}		

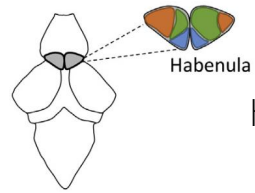
By the laws of probability, it is possible to quantify the uncertainty of inferences from a properly chosen sample.

$$\frac{\sqrt{n}(\bar{X} - \mu)}{\mathrm{var}(X_i)} \xrightarrow{d} N(0,1)$$

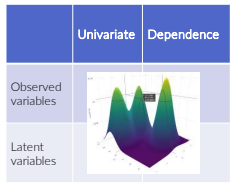
Data analysis concepts

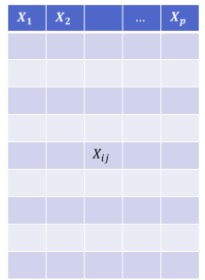
The problem of mathematization

How can the mathematical theory of statistics help answer explanatory scientific questions using biological experimental results?

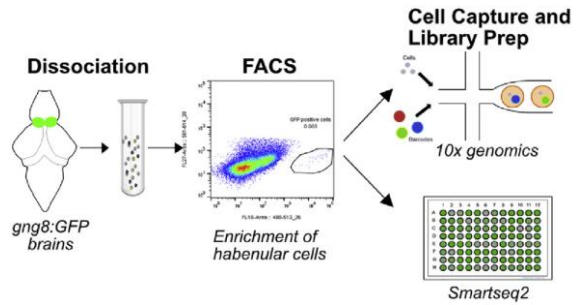


How do cells in the larval zebrafish habenula coordinate their functions?





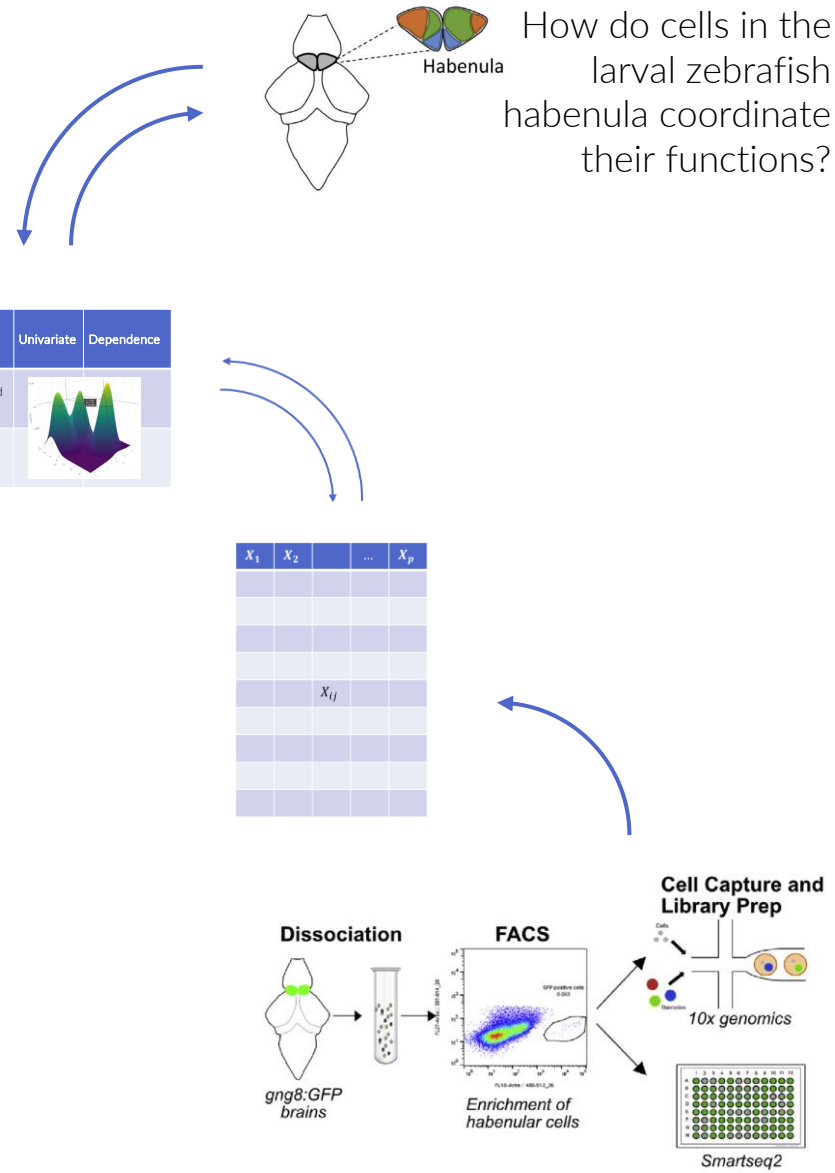
? How to express explanatory questions as math?
How to convert biological results to numerical data table?
How to interpret statistical results?



Pandey et al. (2018)

Data analysis

Data analysis mathematizes scientific questions and experimental data and interprets statistical results.



Pandey et al. (2018)

Pose relevant descriptive questions.

The descriptive questions must be answerable by statistical methods.

Explanatory question	Descriptive question
How do cells work?	??

How to express explanatory questions as math?

Express descriptive question in terms of population parameters.

Determine what types of variables and dependence structures the question is asking about.

	Univariate	Dependence
Observed variables		What genes differentiate larval zebrafish habenula cell types?
Latent variables		

Define latent (cluster) variable $Z_1 = g_1(X_1, \ldots, X_p)$.
For which genes j does $P(X_j \mid Z_1 = z)$ change for different clusters z?

How to convert biological results to numerical data table?

Experimental data must be preprocessed into numerical form.

Preprocessing usually include quantification, quality control, normalization, and additional steps.

Computational Methods for Data Analysis

Alignment and quantification

For the 10X droplet data, raw sequencing data was converted to matrices of expression counts using the cellranger software provided by 10X genomics[1]. Briefly raw BCL files from the Illumina NextSeq or HiSeq were demultiplexed into paired-end, gzip-compressed FASTQ files for each channel using "cellranger mkfastq." Both pairs of FASTQ files were then provided as input to "cellranger count" which partitioned the reads into their cell of origin based on the 16bp cell barcode on the left read. Reads were aligned to a zebrafish reference transcriptome (ENSEMBL Zv10, release 82 reference transcriptome), and transcript counts quantified for each annotated gene within every cell. Here, the 10-base pair unique molecular identifier (UMI) on the left read was used to collapse PCR duplicates, and accurately quantify the number of transcript molecules captured for each gene in every cell. Both cellranger mkfastq and cellranger count were run with default command line options. This resulted in an expression matrix (genes x cells) of UMI counts for each sample.

For SS2 data, raw reads were mapped to a zebrafish transcriptome index (Zv10 Ensembl build) using Bowtie 2 [60], and expression levels of each gene was quantified using RSEM [61]. We also mapped the reads to the Zv10 genome using Tophat2. We only used libraries with genome alignment rate > 90% and transcriptome alignment rate (exonic) > 30%. RSEM yielded an expression matrix (genes x samples) of inferred gene counts, which was converted to TPX (transcripts per 10^4) values and then log-transformed after the addition of 1, consistent with the normalization of the droplet data.

Filtering expression matrix and correcting for batch effects

Cells were first filtered to remove those that contain less than 500 genes detected and those in which > 6% of the transcript counts were derived from mitochondrial-encoded genes (a sign of cellular stress and apoptosis). Genes that were detected in less than 30 cells were also removed. Among the remaining cells, the median number of UMIs per cell was 2,279 and the median number of genes was 1,319 for larval data. The same for adult data was 1,614 UMI/cell and 709 genes/ cell, respectively (Figures S1C, S1D, S5A, and S5B).

We used a linear regression model to correct for batch effects in the gene expression matrix using the RegressOut function in the Seurat R package, and used the residual expression values for further analysis. The residual matrix was then scaled, centered and used for the selection of variable genes, PCA and clustering.

```
file = "/home/user/data/stat530_2022/scrna-seq/GSM2818521_larva_counts_matrix.txt"

larval = read.table(file, header = TRUE)
dim(larval)

library(Seurat)
## set random seed for reproducibility
set.seed(1)

obj = CreateSeuratObject(counts = larval,
                         min.cells = 30, min.features = 500)

## ############################################################
## preprocessing
## ############################################################
## ------------------------------------------------------------
## quality control
## ------------------------------------------------------------
obj[["percent.mt"]] = PercentageFeatureSet(obj, pattern = "^MT-")

VlnPlot(obj,
        features = c("nCount_RNA",
                     "nFeature_RNA",
                     "percent.mt"))

obj = subset(obj, percent.mt <= 6)

## ------------------------------------------------------------
## normalization
## ------------------------------------------------------------
obj = NormalizeData(obj)
obj = FindVariableFeatures(obj)
obj = ScaleData(obj, vars.to.regress = "percent.mt")
```

Pandey et al. (2018)

How to interpret statistical results?

Bioinformatics databases can help annotate results.

Interpreting statements about population distribution functions in their scientific context is challenging.

Descriptive answer	Explanatory answer
Genes … differentiate larval zebrafish habenula cell types.	Cells in the larval zebrafish habenula coordinate their functions by…

https://david.ncifcrf.gov/summary.jsp

Inference methods

Define latent (cluster) variable $Z_1 = g_1(X_1, \dots, X_p)$.
For which genes j does $P(X_j \mid Z_1 = z)$ change for different clusters z?

$f(X_{11}, \dots, X_{np})$

X_1	X_2		...	X_p
		X_{ij}		

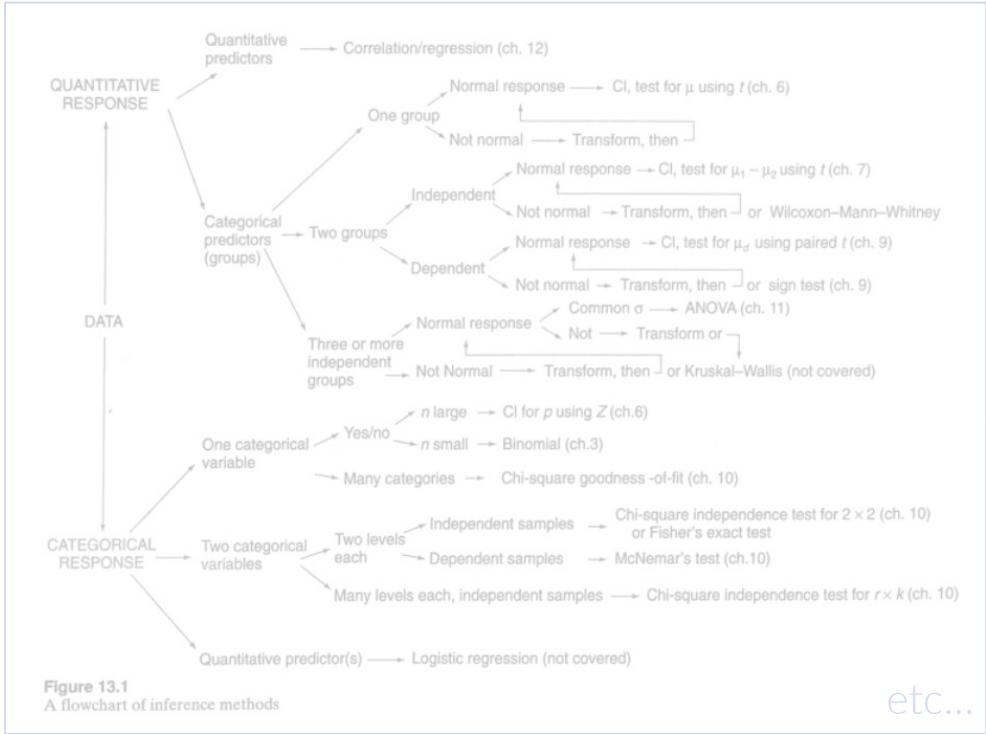


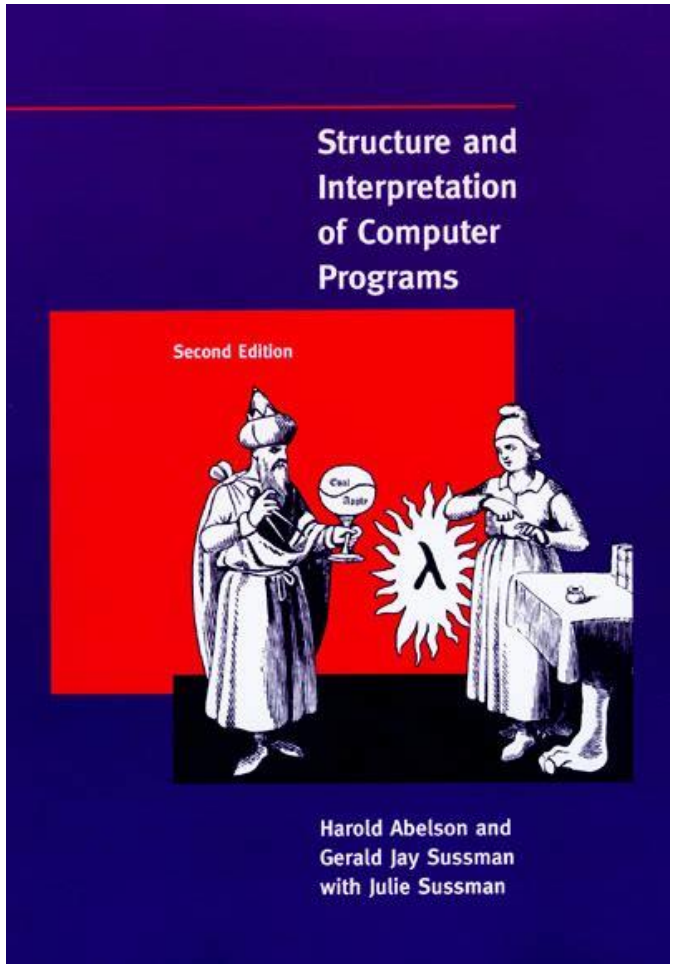
Figure 13.1
A flowchart of inference methods

etc...

https://sciences.ucf.edu/biology/d4lab/methods-1/course-resources/stats-flowchart

“The language in which you'll spend most of your working life hasn't been invented yet, so we can't teach it to you. Instead we have to give you the skills you need to learn new languages as they appear.”

Why Structure and Interpretation of Computer Programs matters (https://people.eecs.berkeley.edu/~bh/sicp.html)



https://mitpress.mit.edu/sites/default/files/sicp/index.html

	Population parameters of interest						
Question to be answered	Mean	Median	Var	GLMs	…	Clusters	Factors
Testing *"Is?"*				Regression and classification			
Estimation *"How much?"*						Clustering	Dimension reduction

	Population parameters of interest						
Question to be answered	Mean	Median	Var	GLMs	…	Clusters	Factors
Testing *"Is?"*							
Estimation *"How much?"*							

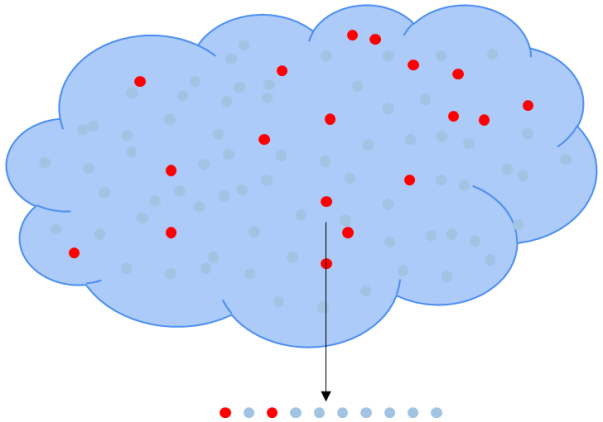
Other considerations:

1. Data structure
   - Variable types
   - Missingness
   - Censoring
   - Etc.

2. Assumptions
   - Parametric
   - Semiparametric
   - Nonparametric

3. Culture
   - Best practices
   - Trends
   - Etc.

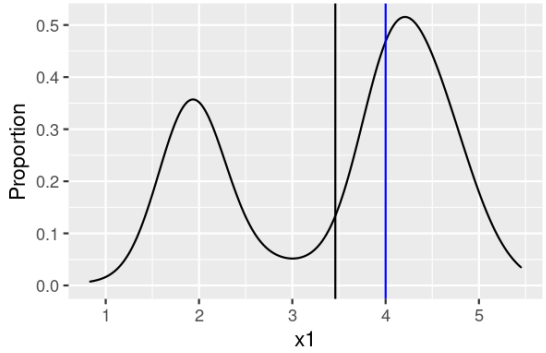
Many statistical methods come in families indexed by tuning parameters.



Tuning parameters generally trade off variability for bias and can be very difficult to choose.

$$\hat{p}_{ab} = \frac{2 + a}{10 + a + b}$$

	Population parameters of interest						
Question to be answered	Mean	Median	Var.	Depend.	…	Clusters	Factors
Testing *"Is?"*	Is $\mu_1 = 0$?						
Estimation *"How much?"*	What is μ_1?						



Mean, median, and standard deviation of X_1.

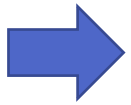
	Population parameters of interest						
Question to be answered	Mean	Median	Var.	Depend.	…	Clusters	Factors
Testing *"Is?"*				Is $\beta_1 = 0$?			
Estimation *"How much?"*				What is β_1?			

Model $P(X_1 = x_1 \mid X_2 = x_2)$ as a generalized linear model, e.g.:

- $X_1 \mid X_2 \sim \text{NegBin}(\mu(X_2), \sigma(X_2))$
- $\log \mu(X_2) = \beta_0 + \beta_1 X_2$
- $\sigma(X_2) = \mu(X_2)(1 + \phi\mu(X_2))$

	Population parameters of interest						
Question to be answered	Mean	Median	Var.	Depend.	…	Clusters	Factors
Testing *"Is?"*							
Estimation *"How much?"*							

	Univariate	Dependence
Observed variables		What genes differentiate adult zebrafish habenula cell types?
Latent variables		



Define latent (cluster) variable $Z_1 = g_1(X_1, \dots, X_p)$.
For which genes j does $P(X_j \mid Z_1 = z)$ change for different clusters z?

Define latent (cluster) variable $Z_1 = g_1(X_1, \ldots, X_p)$.

	Population parameters		
Question	Dep	Clusts	Factors
Testing			
Est.			

Estimate clusters using shared nearest neighbor clustering.

A cluster is a collection of samples that are more closely related to each other than to samples outside the cluster.

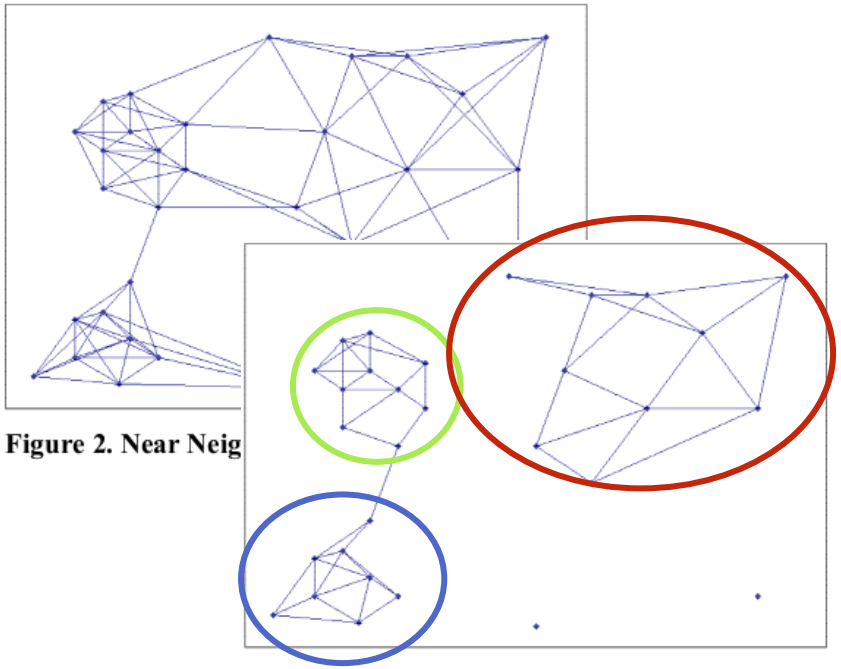


Figure 2. Near Neig

Figure 3. Unweighted Shared Near Neighbor Graph

Ertoz, Steinbach, Kumar (2003), Waltman and van Eck (2013)

Define latent (cluster) variable $Z_1 = g_1(X_1, \ldots, X_p)$.

Construct shared nearest neighbors by estimating principal components.

The kth principal component is $\mathbf{Z_k} = g_k(X_1, \ldots, X_p)$ where $g_k(x) = \alpha_{k1}x_1 + \cdots + a_{kp}x_p$ such that $\mathbf{var}\ g_k(x)$ is maximized for $\|a_k\|_2 = 1$ and the Z_k are uncorrelated. The number of PCs to use is a tuning parameters.

	Population parameters		
Question	Dep	Clusts	Factors
Testing			
Est.			

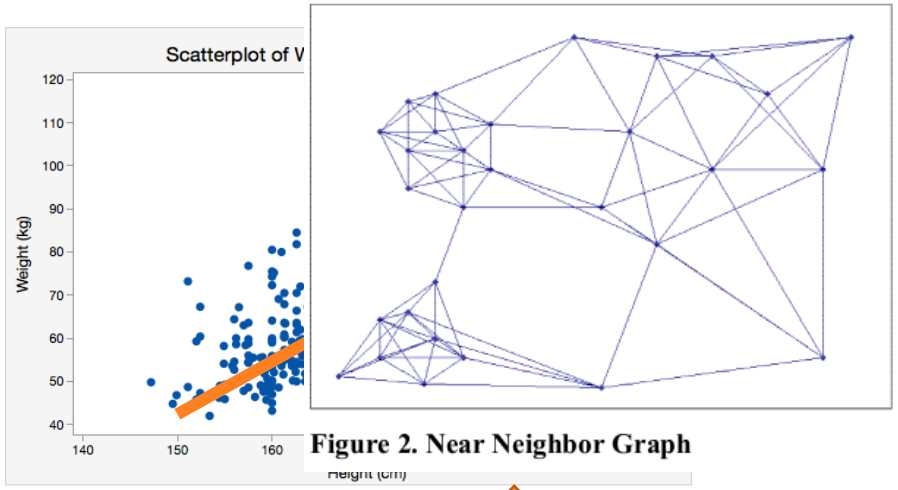


Figure 2. Near Neighbor Graph

X_1	...	X_p	Z_1	...	Z_{10}

```
## dimension reduction
obj = RunPCA(obj)
```

https://online.stat.psu.edu/stat200/lesson/3/3.4/3.4.1

Define latent (cluster) variable $Z_1 = g_1(X_1, \ldots, X_p)$.

	Population parameters		
Question	Dep	Clusts	Factors
Testing			
Est.			

Choose the number of clusters by choosing resolution.

```
## clustering
obj = FindNeighbors(obj)
obj = FindClusters(obj,
                   resolution = 0.5)
```

The resolution is a tuning parameter; there are no “true” clusters.

Define latent (cluster) variable $Z_1 = g_1(X_1, \ldots, X_p)$.

	Population parameters		
Question	Dep	Clusts	Factors
Testing			
Est.			

Visualize clusters by estimating UMAP coordinates.

A UMAP coordinate is another type of latent variable $Z_k = g_k(X_1, \ldots, X_p)$ where g_k is nonlinear. It has many tuning parameters.

```
## dimension reduction
obj = RunUMAP(obj, dims = 1:20)

## visualization
DimPlot(obj)
```

For which genes j does $P(X_j \mid Z_1 = z)$ change for different clusters z?

	Population parameters		
Question	Dep	Clusts	Factors
Testing			
Est.			

Test each gene's association with cluster using a Wilcoxon test.

The tests are then adjusted for multiple comparisons.

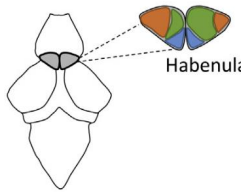
```
markers = FindAllMarkers(obj)

## view top markers for cluster 0
head(markers[markers$cluster == 0,])

## view top markers for cluster 5
head(markers[markers$cluster == 5,])

## visualize markers
FeaturePlot(obj,
            features = c("G0S2",
                         "LRRTM1"))
```

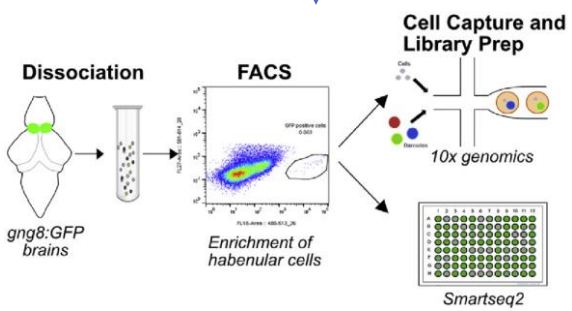
Summary

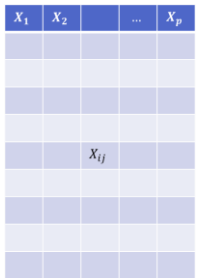


How do cells in the larval zebrafish habenula coordinate their functions?

What genes differentiate larval zebrafish habenula cell types?

Define latent (cluster) variable $Z_1 = g_1(X_1, \ldots, X_p)$.
For which genes j does $P(X_j \mid Z_1 = z)$ change for different clusters z?





	cluster	avg_log2FC	pct.1	pct.2	p_val_adj	gene
1:	0	3.100836	0.946	0.312	0.000000e+00	G0S2
2:	1	2.253668	0.667	0.170	2.114650e-186	TENM3
3:	2	2.110081	0.926	0.226	7.071570e-274	TSPAN18B
4:	3	1.859363	0.524	0.017	0.000000e+00	PTH2R
5:	4	2.233065	0.521	0.014	0.000000e+00	ADRB2A
6:	5	3.356025	0.868	0.029	0.000000e+00	LRRTM1
7:	6	7.112995	1.000	0.060	0.000000e+00	ICN
8:	7	4.561219	0.856	0.061	0.000000e+00	CBLN1
9:	8	3.328361	0.860	0.132	2.878485e-204	TUBB5
10:	9	2.443857	0.781	0.008	0.000000e+00	PPP1R1C
11:	10	1.500814	0.345	0.045	1.549424e-51	MAB21L2
12:	11	3.400962	0.971	0.009	0.000000e+00	GAD2
13:	12	6.452704	0.960	0.010	0.000000e+00	SI:DKEY-117I10.1

Pandey et al. (2018)