# *Neurological Disorders and the Brain*

*Bioinformatics tools and resources to study healthy and diseased brains*

Bioinformatics and Research Computing
Whitehead Institute

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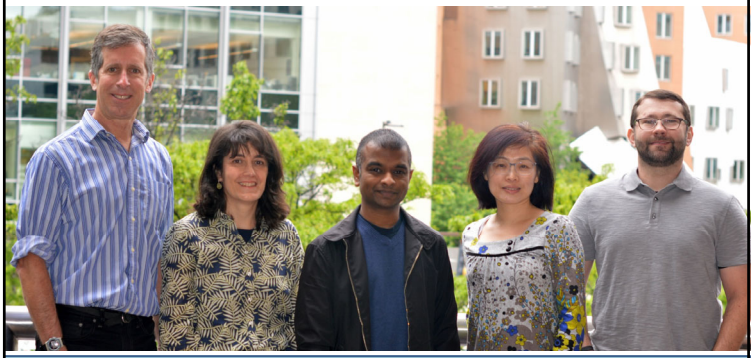
BaRC Hot Topics

Bioinformatics & Research Computing
Consultation and collaboration, training and education, and software in the areas of Bioinformatics and Graphics.
at Whitehead Institute

BaRC Bioinformatics and Research Computing



George Bell | Inma Barrasa | Prat Thiru | Bingbing Yuan | Kris Richardson

## What is bioinformatics?

Bioinformatics is an interdisciplinary field that develops methods and software tools for understanding biological data and interpreting experiments.

It combines

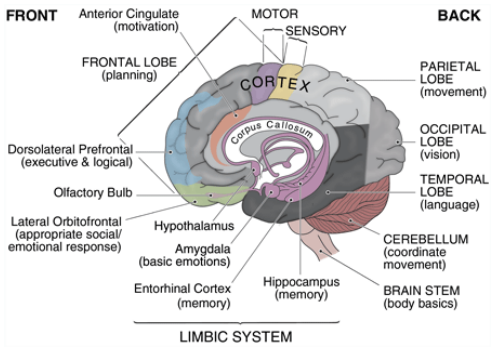
- biology
- computer science
- mathematics
- statistics



## Overview of today's session

- Introduction: brain anatomy and cell types
- Studying gene expression in the brain
  - Allen Brain Atlas. **Ex1** browsing the Aging, Dementia and Traumatic Brain Injury Study.
  - Tabula Muris: Gene expression of individual cells **Ex2**
  - Protein expression: Histology staining **Ex3**
- Brain genetics and brain disease
  - Brain genetics **Ex4**
    - Tay–Sachs disease
    - SNPedia, OMIM
- Models to learn about the human brain
  - Studying human proteins in yeast (Parkinson's Disease)
  - Benefits of iPS cell - derived human cells (Parkinson's Disease)
  - Seizure experiments in zebrafish

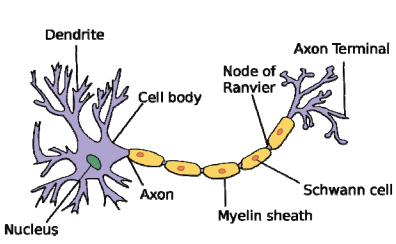
# Brain anatomy and function

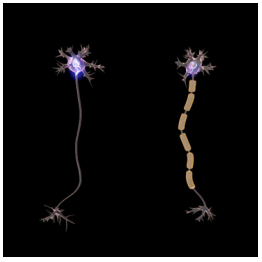



http://www.brainwaves.com/
http://www.brainfacts.org/3d-brain



# Myelin function

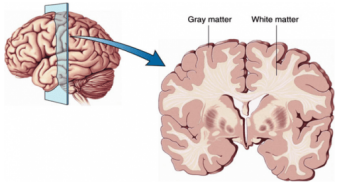


https://en.wikipedia.org/wiki/Myelin



# White vs grey matter




https://difference.guru/

- **Grey matter:**
  - high densities of neuron cell bodies
  - Grey matter undergoes development and growth throughout childhood and adolescence
  - High alcohol consumption has been correlated with significant reductions in grey matter volume
- **White matter:**
  - light color: due to fiber from myelin.
  - continues to develop, and peaks in middle age
  - Alzheimer's disease: amyloid plaques in white matter



## Brain Myeloid Cells

- Microglial cells
- Macrophage

- Brain myeloid cells provide a form of continuous immune surveillance within the brain.
- The population of myeloid cells play an essential role in both health and disease with regard to the brain.

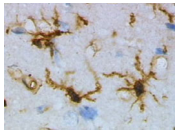
## Brain Non Myeloid Cells

- Astrocytes
- Oligodendrocytes
- Neurons
- Others…

- Non brain myeloid cells are non-immune cells.
- The mammalian brain contains between 100 million and 100 billion neurons, depending on the species.
- These cells carry out essentials roles of the brain – structure, signaling, protection, etc…

## Brain Myeloid Cells

- Microglial cells
- Macrophages

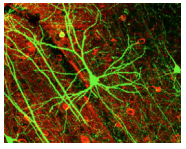


Microglial

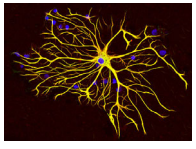
Macrophage

## Brain Non Myeloid Cells

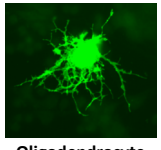
- Astrocytes
- Oligodendrocytes
- Neurons
- Others…



Neuron



Astrocyte

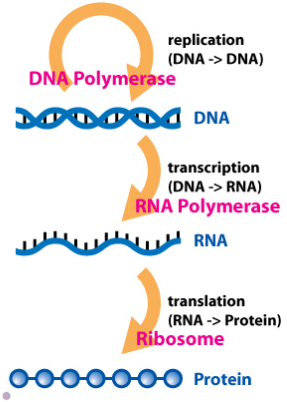


Oligodendrocyte



## The central dogma of biology

"DNA makes RNA and RNA makes protein"

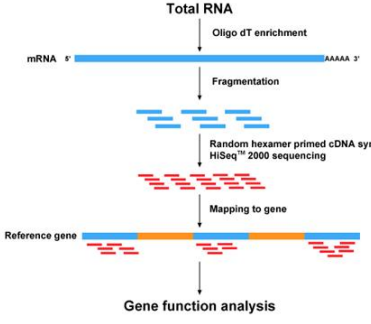



- For most activities in the cell, proteins are what do the actual work
- Looking at changes in amounts of a protein could reveal a lot about changes in cell state
- Problem: It's hard to measure levels of all proteins at once
- Partial solution:
  - Measure RNA levels instead
  - Assumption: Changes in RNA levels tend to match changes in protein levels

Image: https://en.wikipedia.org/wiki/Central_dogma_of_molecular_biology



## Measuring the RNA level of every gene at once




- The technique of **RNA-seq** can measure the amount of RNA from every gene in the genome in any collection of cells

Assumption: For every gene,

↑ RNA ⇒ ↑ protein ⇒ ↑ metabolic activity

↓ RNA ⇒ ↓ protein ⇒ ↓ metabolic activity

Image: http://bio.lundberg.gu.se/courses/vt13/rnaseq.html



## Allen Brain Atlas

- http://portal.brain-map.org/
- The Allen Brain Atlas is a resource containing information about
  - Human and mouse brain
  - Brain cell types
  - Brain development
  - Aging, dementia and traumatic brain
  - Glioblastoma

# **Ex1.a** Browsing the Aging, Dementia and Traumatic Brain

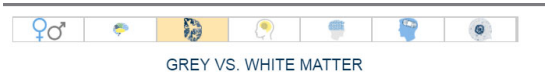
- On go to http://aging.brain-map.org/
- Click on "Data Overview" on the top right of the page
- Click on "Brain regions" and wait for the figures to display.


- Sequentially click on the Brain Region circles and the CABP7(calcium binding protein 7), CUX2(cut-like homeobox 2) and RORB (RAR-related orphan receptor beta) to see genes expressed in hippocampus versus genes expressed in cortex.
- Click on "15 most region-enriched genes in cortex" to see a heatmap of gene expression.



# **Ex1.b** Browsing the Aging, Dementia and Traumatic Brain

- Repeat the exercise by looking at Grey versus White matter and genes markers for excitatory, **NEFH** (neurofilament, heavy polypeptide), or inhibitory, **PVALB** (parvalbumin) neuronal populations.

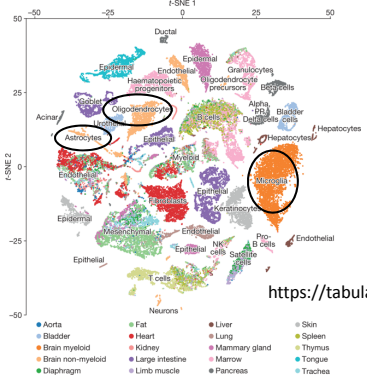



- Explore the Dementia tab 2 of 2




# Tabula Muris

- Sequenced 100,000 cells from 20 tissues/organs from 3 female and 4 male mice.



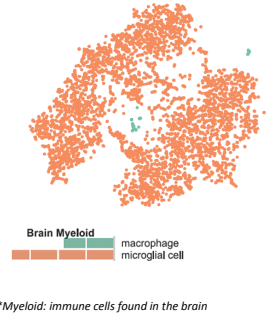

https://tabula-muris.ds.czbiohub.org/

The Tabula Muris Consortium et al. *Single –cell transcriptomics of 20 mouse organs creates a Tabula Muris* Nature 562:362-367 (2018)



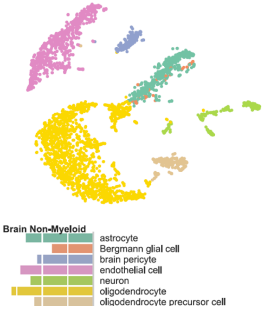
# Tabula Muris: Cell Type Markers

Brain Myeloid*




*Myeloid: immune cells found in the brain

Brain Non–Myeloid



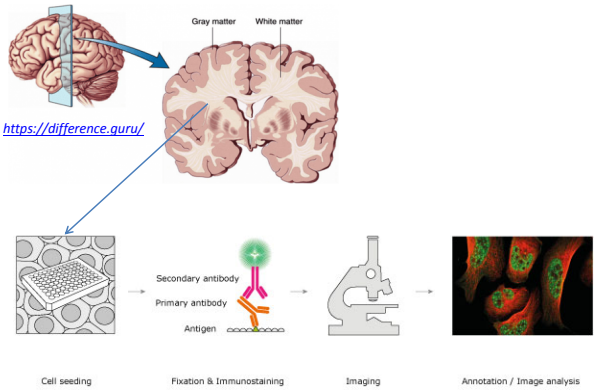

The Tabula Muris Consortium et al. *Single –cell transcriptomics of 20 mouse organs creates a Tabula Muris* Nature 562:362-367 (2018)

## **Ex2** Browsing Tabula Muris to see gene expression in single cells (scRNAseq)

1. Tabula Muris site
   https://tabula-muris.ds.czbiohub.org/
2. Select FACS and Brain Non-Myeloid
3. Type each of the gene names in the blank table, one at a time, and see where they are expressed
4. Does this agree with what we saw before?
5. Are some genes expressed in
   all cells? no cells?



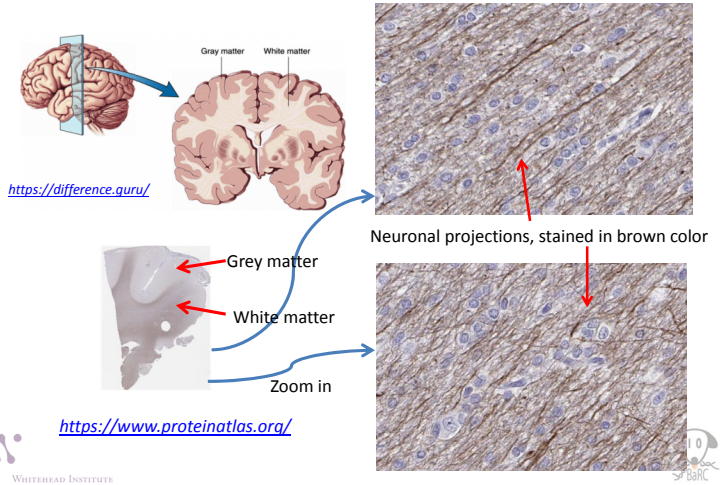
## Measuring the protein level of a gene




https://www.proteinatlas.org/learn/method/immunocytochemistry



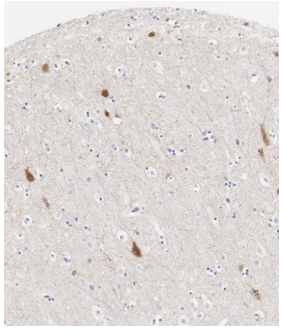
## NEFL (Neurofilament light) located in white matter



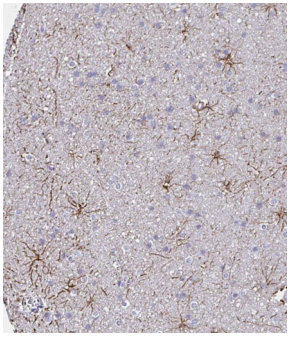

## **Ex3**: Identify Brain neural cells by antibody staining

- Go to Human Protein Atlas website:
  - https://www.proteinatlas.org/humanproteome/tissue/brain
  - Search for either GAD1 (inhibitory GABAergic interneurons glutamate decarboxylase 1 ) or GFAP (Glial fibrillary acidic protein)
  - Click on **TISSUE** from top right
  - Under **RNA and Protein Expression Summary**, click on **Brain** and choose **Cerebral cortex**.
  - Find out the cells express this gene under **Antibody staining** section. Cells expressed this gene were stained with brown color. You can view the cells by clicking on images and zooming in.

# **Ex3**: GAD1 and GFAP in cortex



GAD1



GFAP



# Brain genetics and brain disease



# Tay-Sachs disease (TSD)

- Rare disease affects central nervous system
- Variant forms: infantile, juvenile, adult onset
- Loss of learned skills, smile, crawl
- Paralysis, dementia, death
- No treatment, no way to prevent/reduce the progression of this disorder.

# **Ex4**: Tay-Sachs disease

- **Ex4.a** Which gene is responsible for the disease?
  - Go to OMIM (https://www.omim.org) and search for "Tay-Sachs disease" [Hint: Look under Phenotype-Gene Relationships]
- **Ex4.b** How does the mutation of this gene result in Tay-Sachs disease?
  - Hint: look under "Biochemical features".
- **Ex4.c** What mutation(s) in this gene cause Tay-Sachs disease?
  - Hint: look under "Molecular Genetics".

# **Ex4c:** Mutations causing Tay-Sachs

To look at these mutations in more detail…

- Go to the UCSC Genome Browser: https://genome.ucsc.edu/cgi-bin/hgGateway
- Select human (hg38)
- Under Position/Search Term, type HEXA and then any of the “HEXA” links
- Under "Phenotype and literature", set "ClinVar Variants" to “pack“ and click a “refresh” button.
- The red variants are pathogenic (harmful).



# Models to learn about the human brain

- **Yeast**
- Mouse
- **Zebrafish**
- Induced pluripotent stem cells (also known as iPS cells or **iPSCs**) derived from patients.



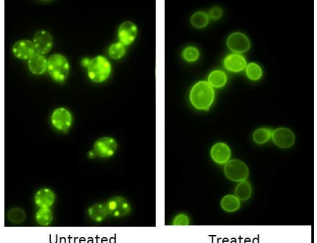
# Parkinson’s disease

- Parkinson's disease occurs when neurons in an area of the brain that controls movement become impaired and/or die. Normally, these neurons produce an important brain chemical known as dopamine.
- Many brain cells of people with Parkinson's contain Lewy bodies, unusual clumps of the protein **alpha-synuclein**.
- Scientists are trying to better understand the normal and abnormal functions of alpha-synuclein and its relationship to genetic mutations that impact Parkinson’s disease and Lewy body dementia.



# Studying Parkinson’s disease in yeast

- Many people with Parkinson’s disease have clumps of a protein (alpha-synuclein) in their brain
- What do these clumps do?
- Can we prevent or treat these clumps?
- One solution: genetically modify yeast cells so they make clumps of this same human protein

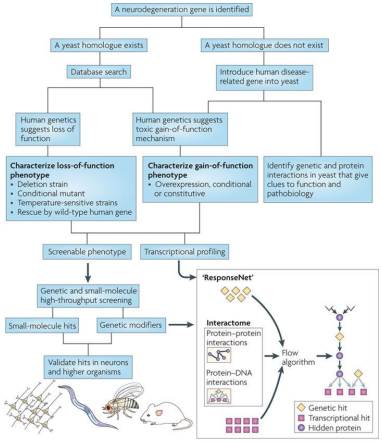


Untreated Treated

Yeast cells with alpha-synuclein attached to a fluorescent marker (bright spots on left) change after treatment with chemical called NAB (on right).

Tardiff et al., 2013

# Modelling neurodegeneration in yeast

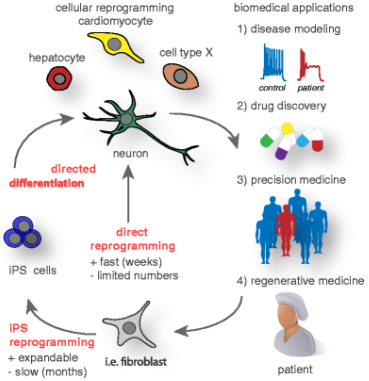



Creating, characterizing and screening a yeast model of neurodegenerative disease

*Khurana V, Lindquist S. 2010*



# Studying Parkinson's disease in human neurons



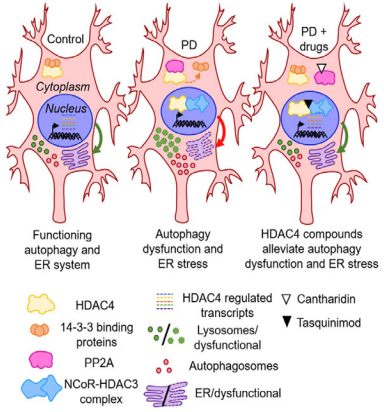

*Mall, M. & Wernig, M. J Mol Med (2017) 95: 695. https://doi.org/10.1007/s00109-017-1550-4*



*Single-Cell Sequencing of iPSC-Dopamine Neurons Reconstructs Disease Progression and Identifies HDAC4 (histone deacetylase 4) as a Regulator of Parkinson Cell Phenotypes*

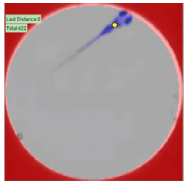
*Charmaine Lang et al.*

*Cell Stem Cell*
Volume 24, Issue 1,
Pages 93-106.e6
(January 2019)
DOI:
10.1016/j.stem.2018.10.023




# Studying seizures and the brain in zebrafish

- What happens in the brain when one has a seizure?
- Can we do anything to change the severity of the seizure?
- One solution: Study zebrafish that have been treated with PTZ to cause seizures



Tracking a normal fish (video)

# Studying seizures and the brain in zebrafish