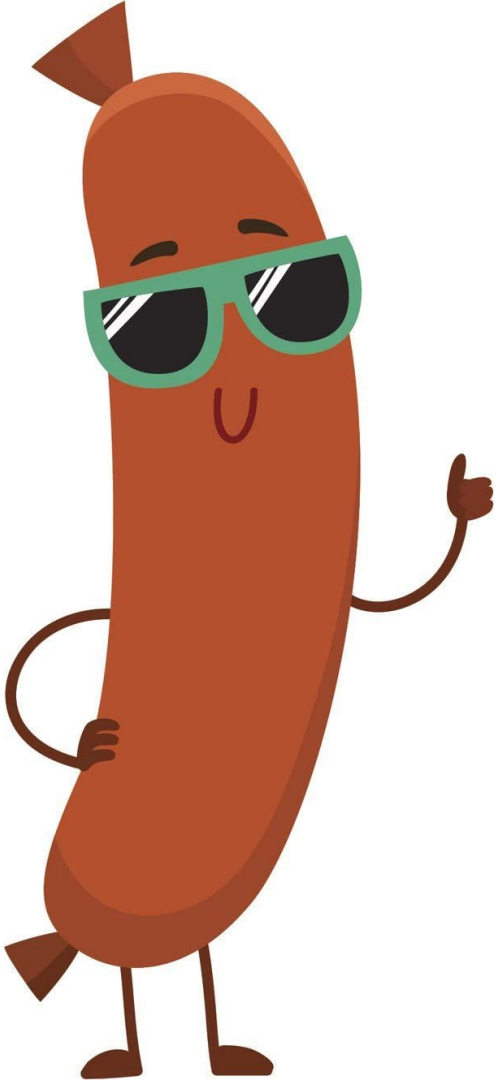


Wet Lab Techniques

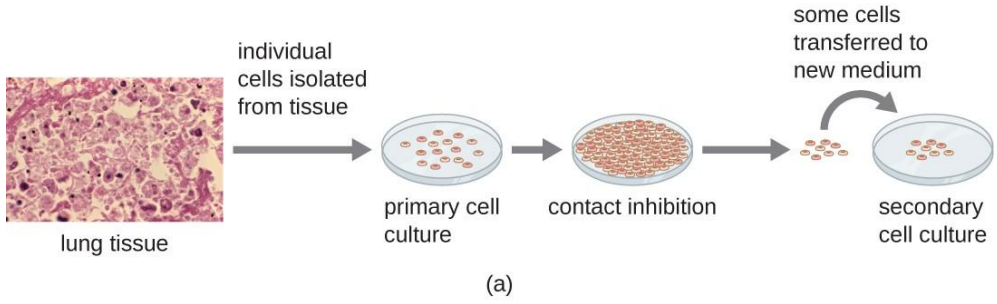
how the sausage is made

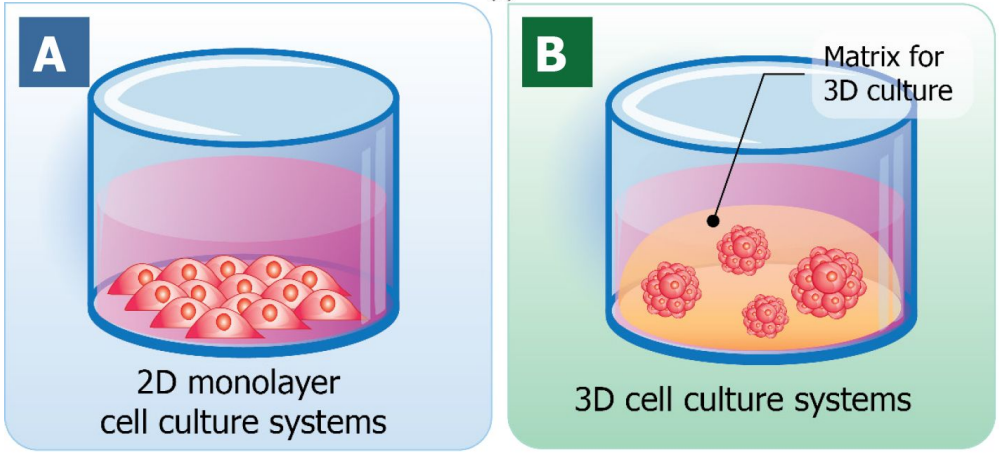
Wet Lab Techniques: Overview

- Tissue culture
- Mouse models
- DNA/RNA Quantification
- qPCR
- Blots
- Mass Spectrometry
- Microscopy and Imaging
- Immunofluorescence
- Flow Cytometry

Tissue Culture

- Primary
  - Cells isolated from animal/plant tissue. Eventually undergo senescence
- Secondary
  - Cells obtained from established primary culture that has been transformed
- 2D
  - Inexpensive, well-established, comparative literature, easy observation, not representative
- 3D
  - Physiologically relevant
  - Organoids - self renewal, organization, organ functionality





Tissue Culture Timeline

-Primary/Secondary

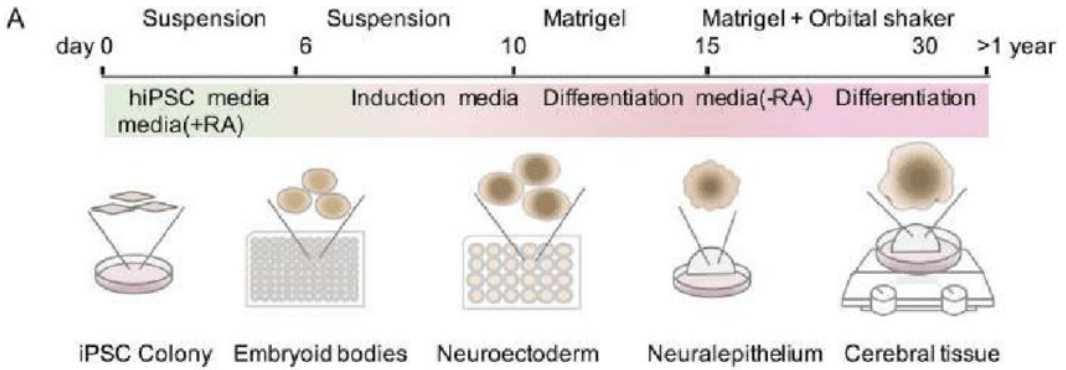
-Usually ready within days or a week

-2D cultures from **human** stem cells (Ex. Motor neurons)

-Usually a month or more

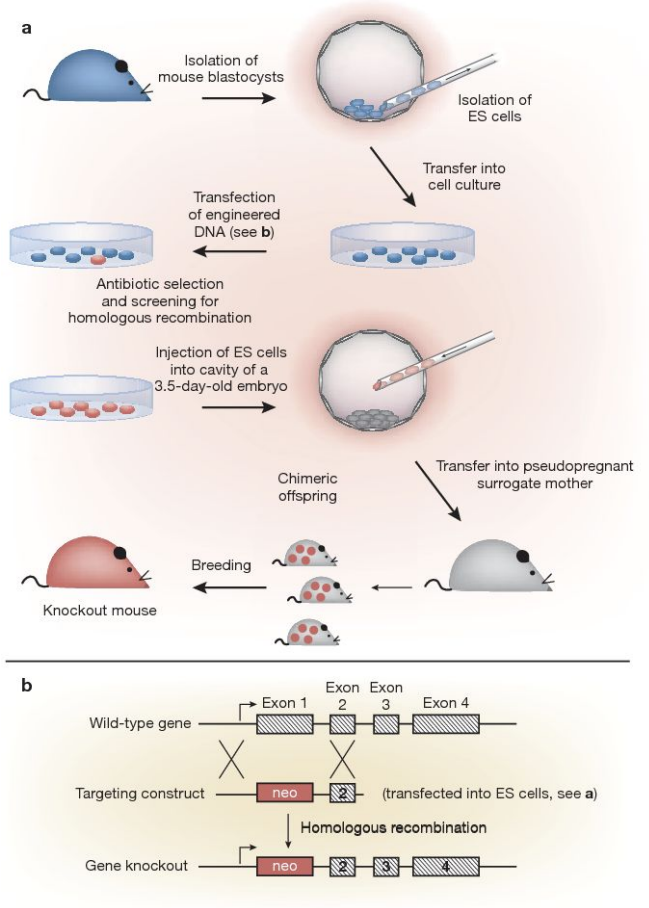
-3D cultures (Ex. Oragnoids)

-Months, earliest use at 30 days but generally used at 4 months (even out to 9 months-1 year!)



Liu et al 2018

Transgenic Mouse Models



What are transgenic mice?

- Mouse models that have their genomes altered through genetic engineering techniques
- Target specific genes
  - Knockout models disrupt and inactivate gene of interest
  - Model SNPs to better understand molecular mechanisms
- Tissue specific analysis
  - Inducible models
- Phenocopy human disease
- Test new potential drugs and therapies
- Can be very time consuming to generate!

The Jackson Laboratory

charles river

ingenious targeting laboratory

"A better model is within reach."

DNA/RNA Quantification Methods

Nanodrop

-Spectrophotometry calculation of nucleic acids

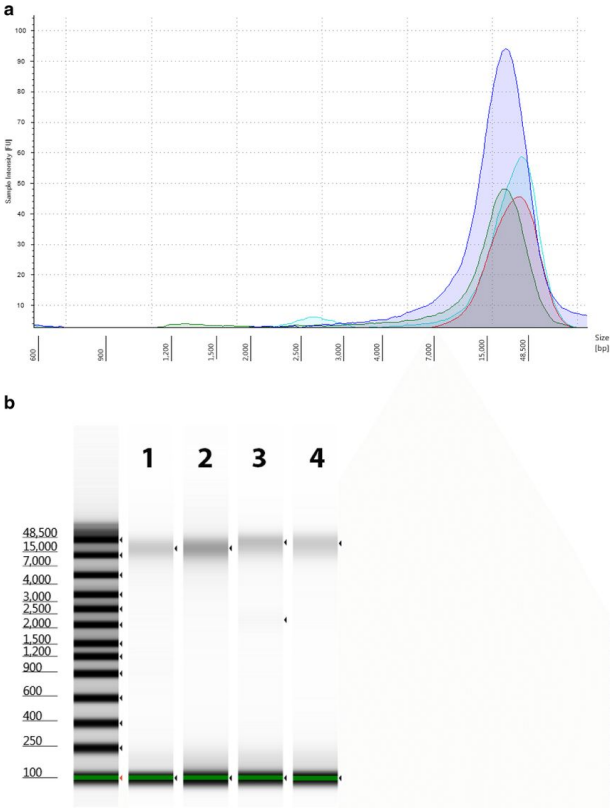
-Relatively cheap

Tapestation

-Electrophoresis “on-chip”

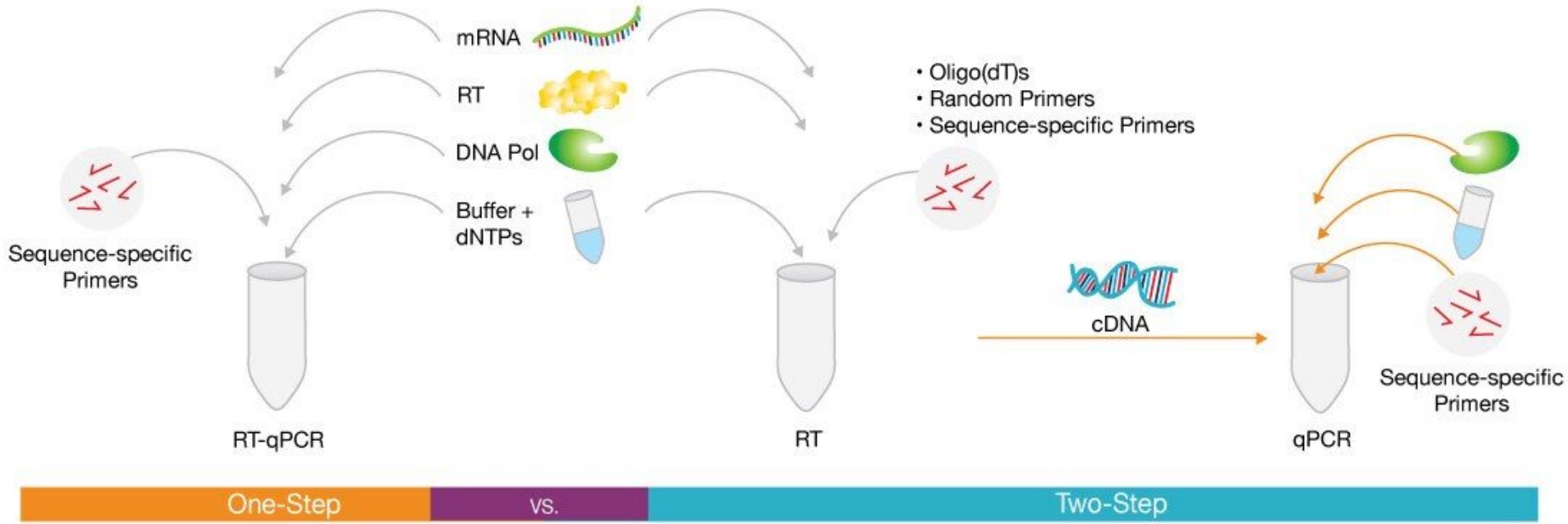
-More sensitive

-Requires additional reagents



Kong et al 2017

Quantitative Reverse Transcription PCR: RT-qPCR

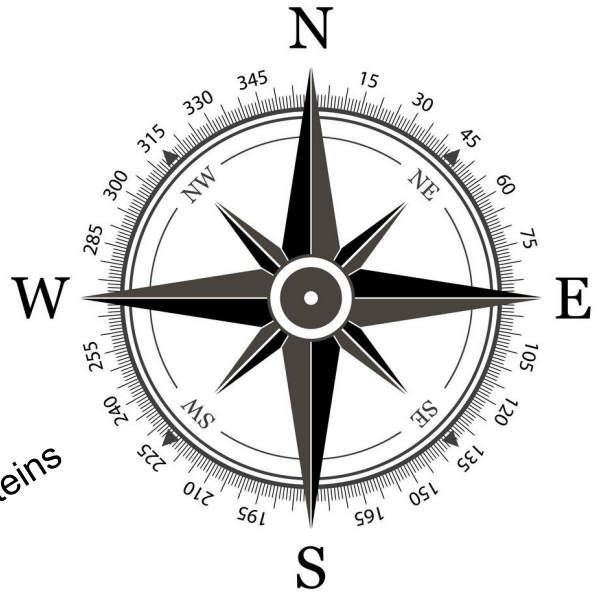


- Starting material is RNA
- RNA is transcribed into complementary DNA (cDNA) by reverse transcriptase (RT)
- cDNA is used as the template for the qPCR reaction

Blots

reverse northern blot: RNA

northern blot: RNA



western blot: proteins

eastern blot: post translational modifications

far western blot: protein-protein interactions

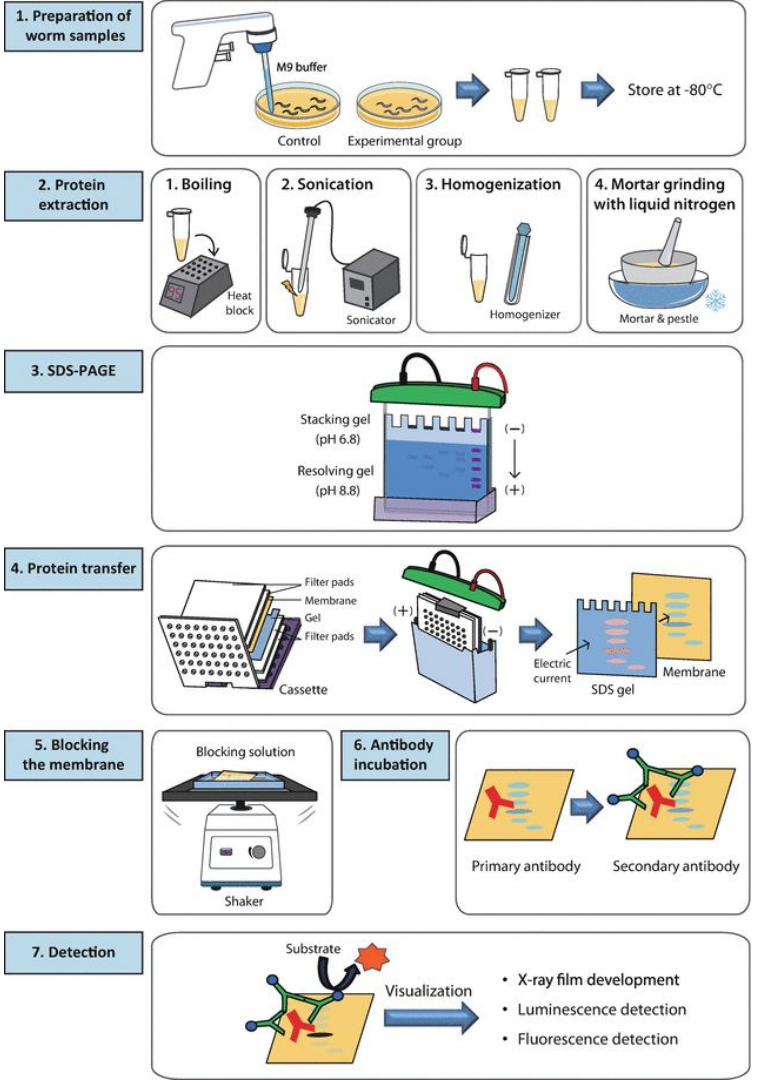
far eastern blot: glycolipids

southwestern blot: DNA binding proteins

Southern blot: DNA

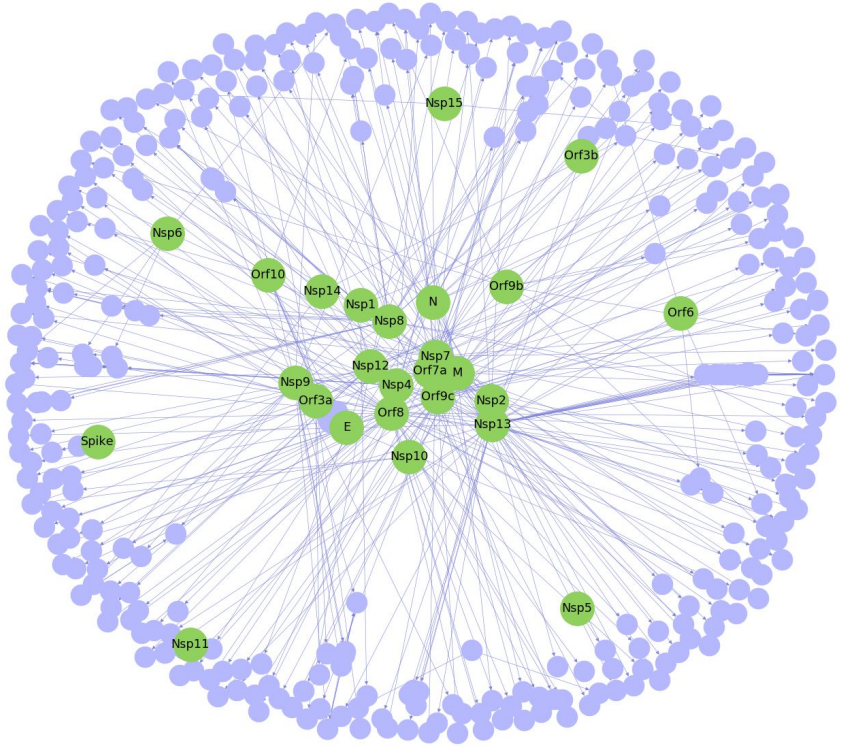
Western Blot

- Used to detect a specific protein of interest among a mixture of proteins
- Protein lysate runs on SDS-PAGE gel
  - Proteins separate based on molecular weight
- Protein transfer from gel to membrane for antibody staining and detection
- Results may vary



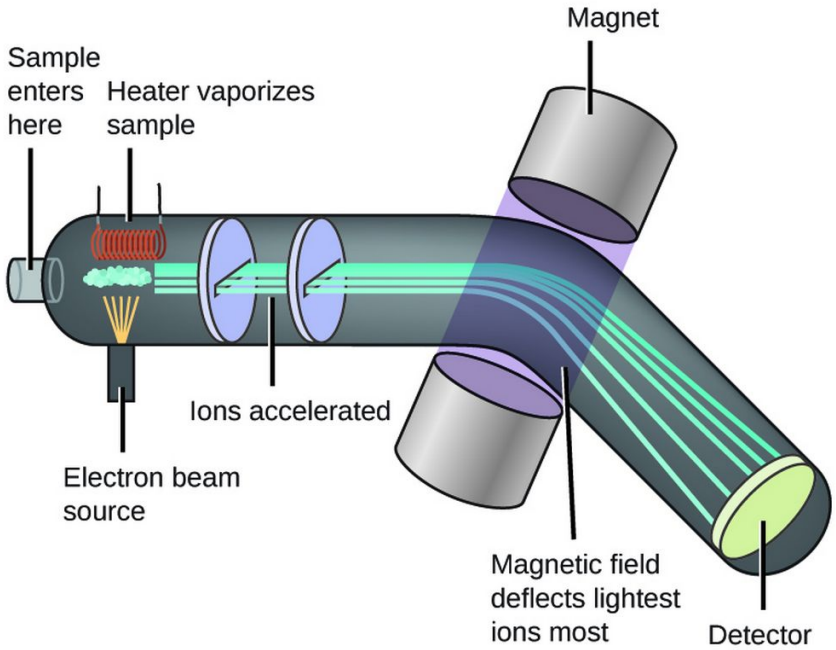
Mass Spectrometry-based proteomics

- Elucidate structure and chemical properties of different molecules
- Global measurement of protein levels
- ID post-translational modifications
- Protein complexes
- Localize proteins to organelles



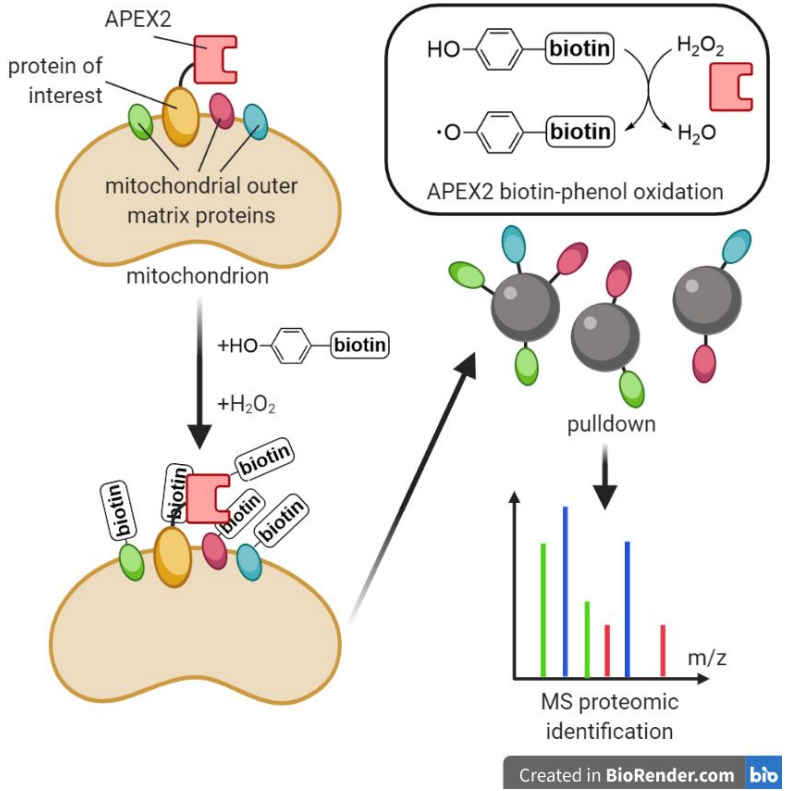
SARS-CoV-2 protein-protein interaction network
Gordon, Nature 2020

Mass Spectrometry



- Digest sample into peptides with unique charges
- Ionize samples to produce gas phase ions which accelerate over mag field
- Ions are
    1. separated according to their mass to charge ratio
    2. Detected in proportion to abundance
- Mass spectrum can ID & quantify abundance of unknown compound

Proximity labeling Mass Spec

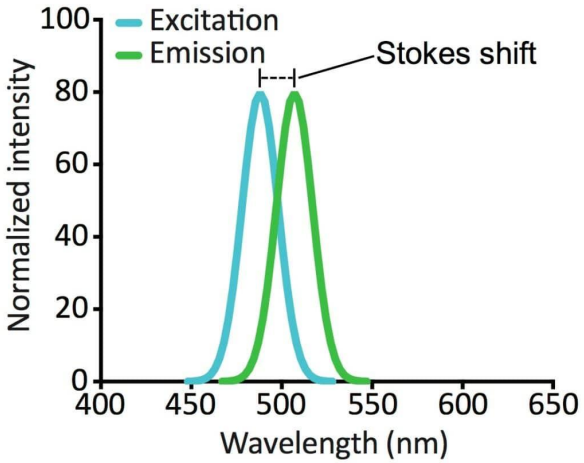


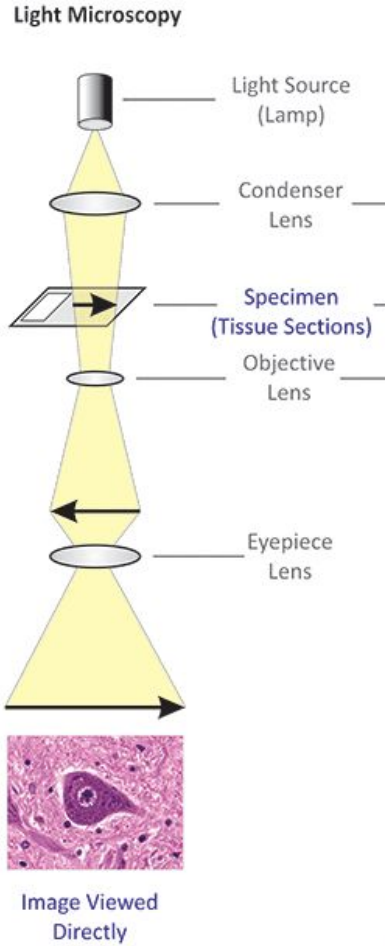
- Protein of interest is fused to a labeling enzyme that can biotinylate nearby molecules promiscuously
- Fuse APEX (Ascorbic acid PEroXidase) to protein of interest & biotinylate proximal proteins
- Capture organelle proteomes with temporal resolution

Light microscopy

Visible light passes through condenser lenses that focus a beam of light onto an object specimen & convex objective lenses enlarge the image formed. Can be performed on living cells.

- Brightfield
- Fluorescence microscopy
  - **Confocal microscopy** excite thin layer of sample to produce sharp image without interference from molecules in surrounding layers
- Two-photon
  - 2 low energy photons simultaneously strike a location causing 1 emission wavelength

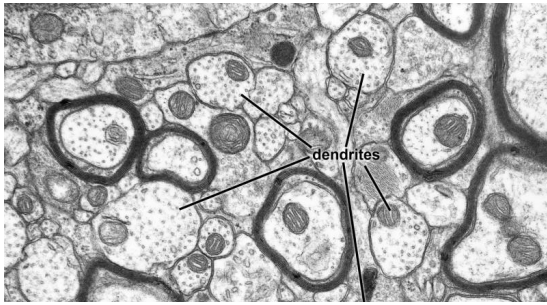


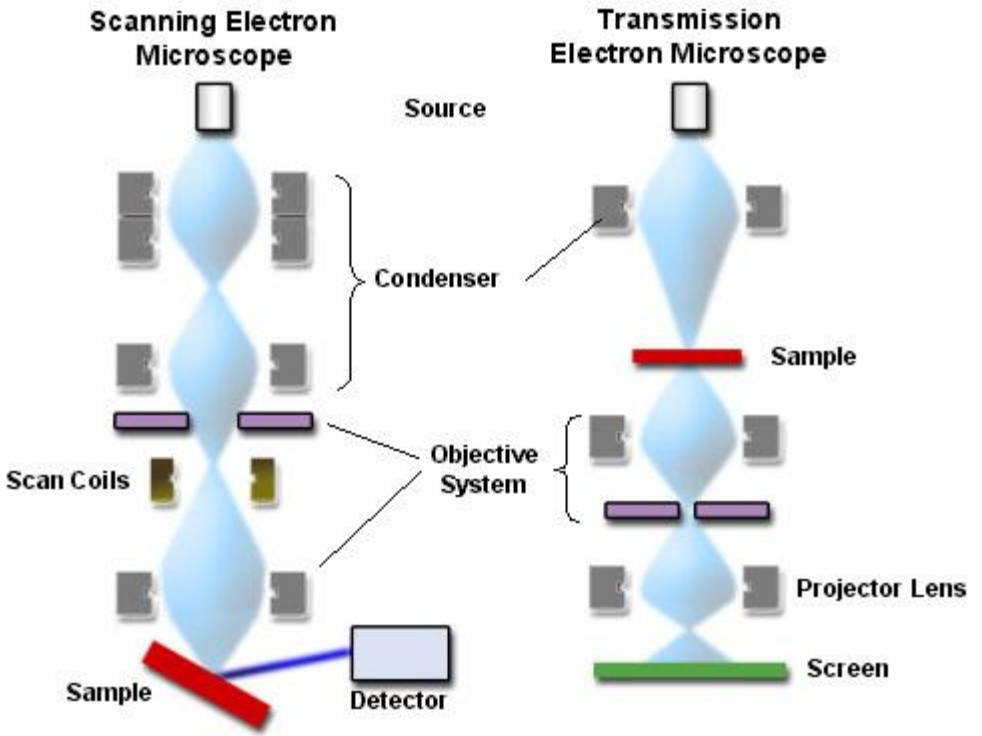


Electron Microscopy

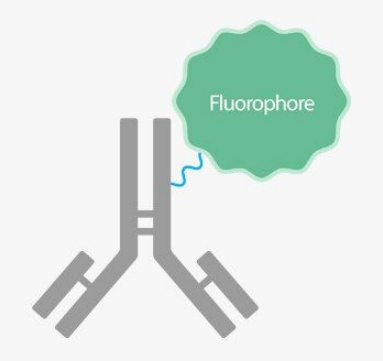
- Beam of electrons (shorter nm that visible light) produce higher-resolution image than light microscopy
    - Subcellular structures
- Samples must be placed under vacuum and cannot be live
- Scanning electron microscopy (SEM)
- Transmission electron microscopy (TEM)

transversely sectioned cluster of apical dendrites in visual cortex of a 27 year old monkey





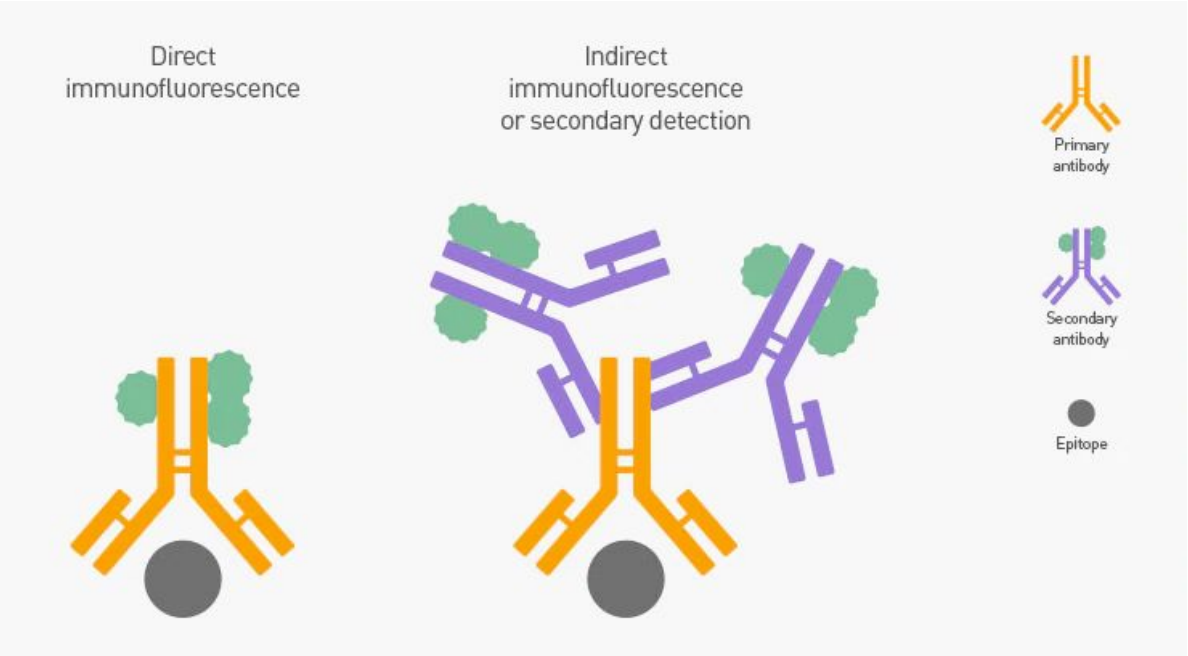
What is Immunofluorescence?



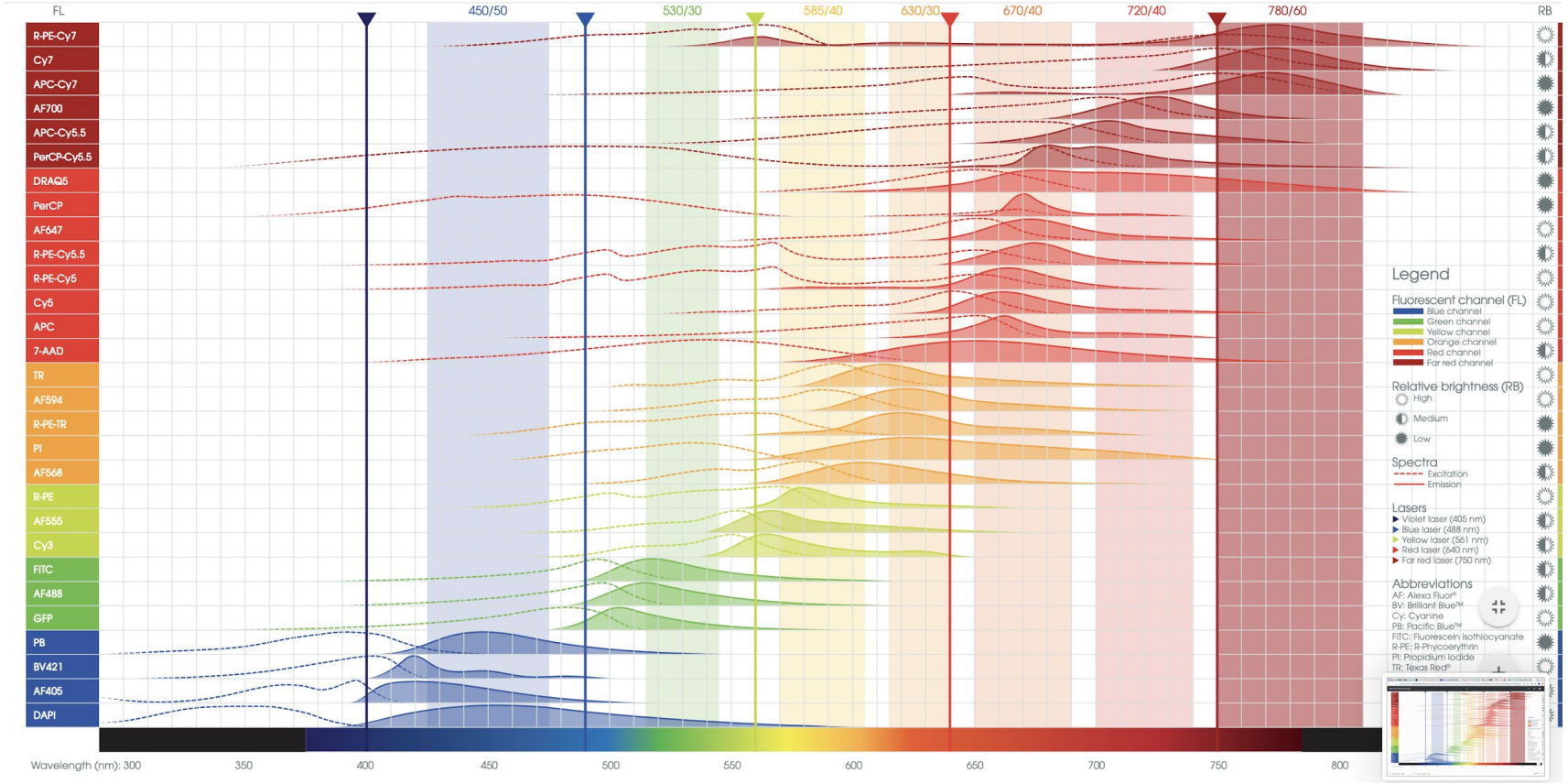
- Technique to fluorescently label a specific biological target within a sample using an **antibody**
- Fluorophore-conjugated antibodies allow for fluorescent imaging after immunolabeling

- Primary: binds specific target or epitope
- Secondary: binds primary

- Direct: fluorophore on primary
- Indirect: fluorophore on secondary

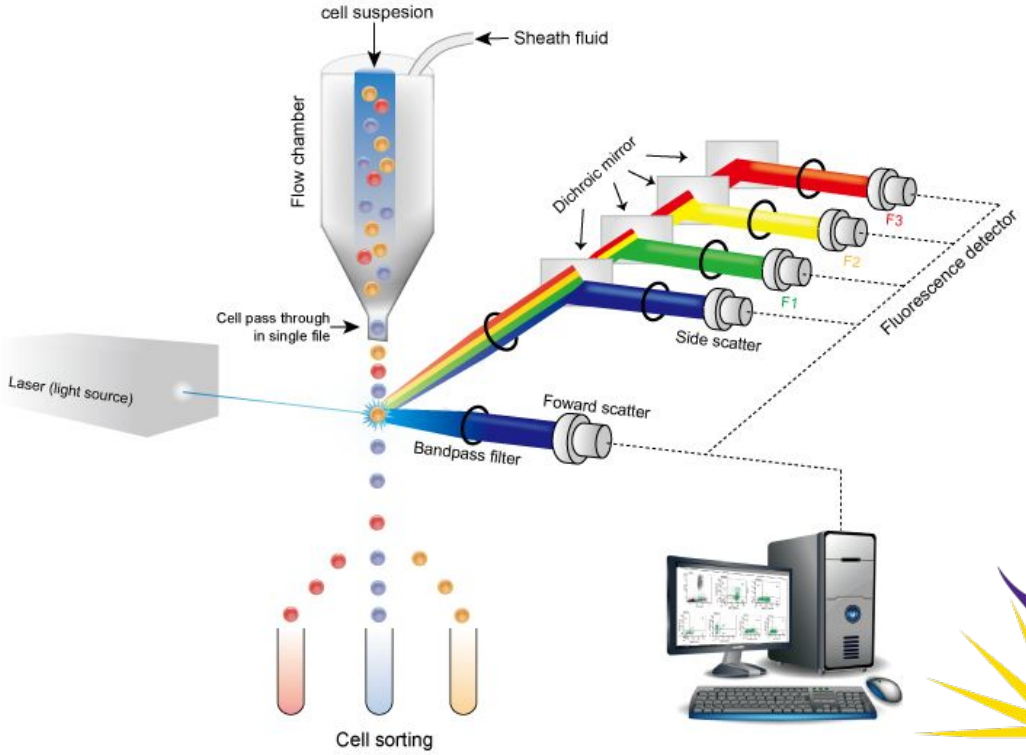


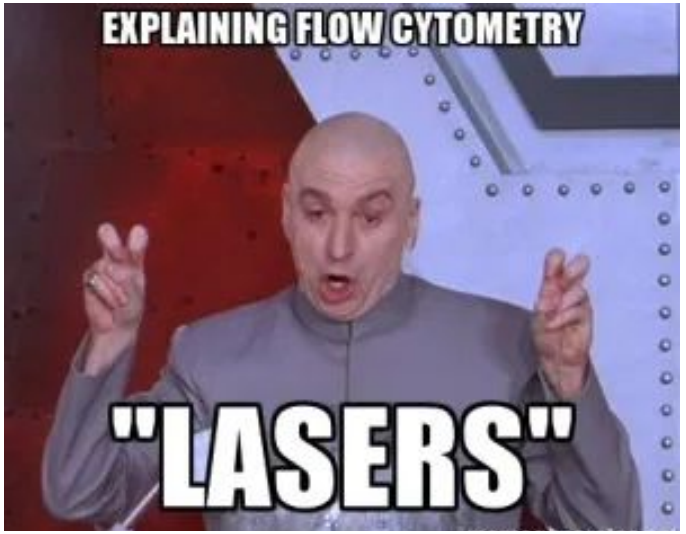
Guide to Fluorophores



abcam

Flow Cytometry





BioLegend®

Enabling Legendary Discovery™

Flow Cytometry vs FACS

Flow cytometry:

-Run on an analyzer such as LSR Fortessa

-Focuses on cell analysis such as protein levels or co-expression

-You do not get your cells back

FACS:

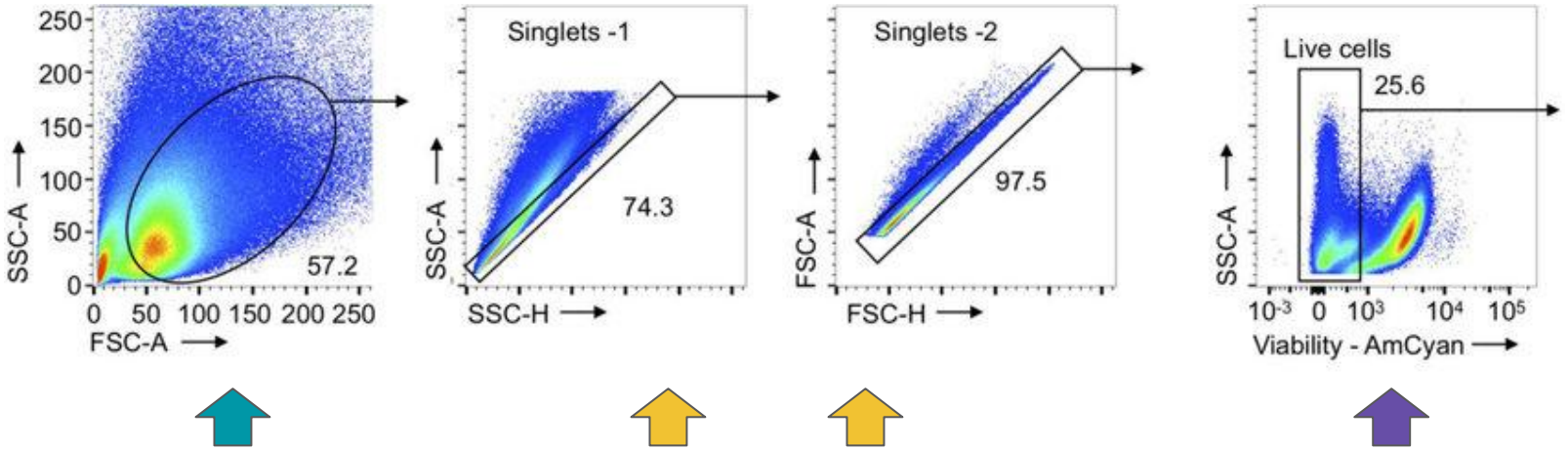
-Fluorescent-activated cell sorting

-Run on a sorter such as an Aria

-Focuses on sorting out cells for a specific and enriched population

-Cells can be used for downstream analysis such as RNA seq

Selecting Live and Single Cells



Selects cells over debris

Removes doublets

Removes dead cells

Herrisson et al 2018

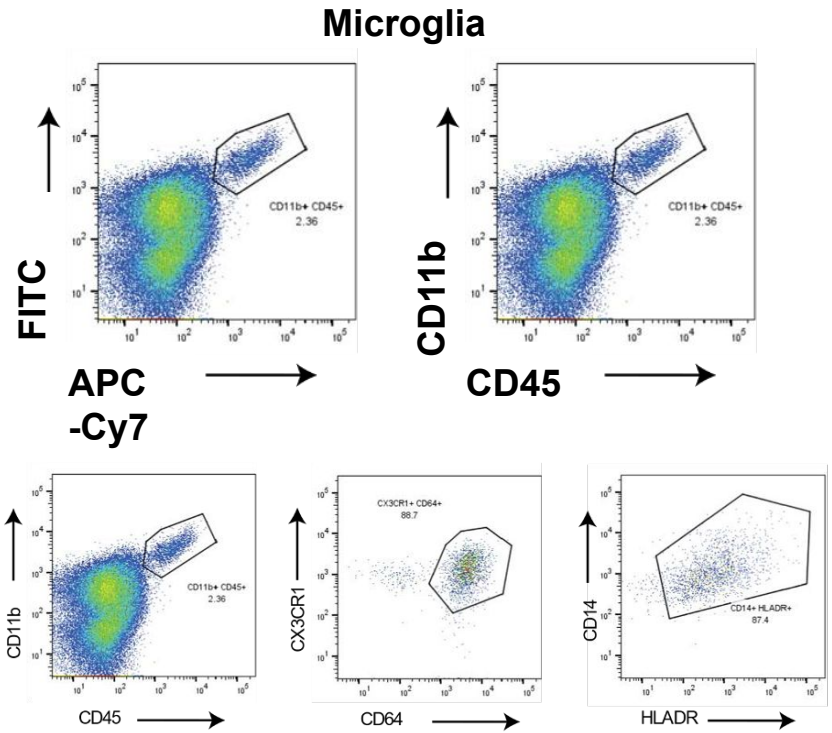
Selecting Your Population of Interest

Select markers using different fluorophores for genes of interest

Make sure you select for spectra that are far apart from each other

-If spectra overlap, you will need to also include compensation

For all experiments: Ensure you have unstained, isotype, and single color controls



Where to Get Your Wet Lab Done

Institute of Genomic Medicine (Leichtag)

Nikon Imaging Center (Leichtag)

LJI Microscopy Core (LJII)

Flow Cytometry Cores (SCRM, Salk, LJI)

Molecular Mass Spectrometry Core (Urey Hall)

Stem Cell Cores (SCRM and Salk)

Transgenic and Knockout Mouse Core (CMME)

Questions?


Kitchen vs Laboratory
Follow protocols
Analyze results
Clean up the mess
Sketching Science