

# Genetic Manipulations Part 2 and sequencing



Lecture 10  
Anita Autry, Ph.D.

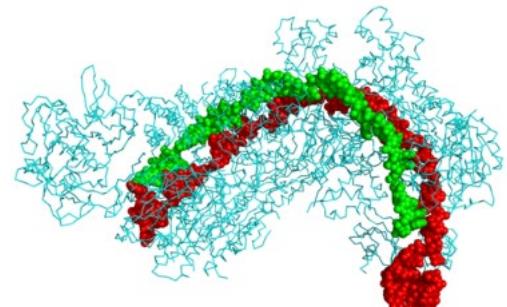
# Topics

- CRISPR-Cas9
  - Inducible systems
    - Tagging active cell populations
  - Sequencing and PCR
    - Sanger
    - PCR/QPCR
    - RNASeq
    - Single Cell RNASeq
  - In situ hybridization
    - MERFISH



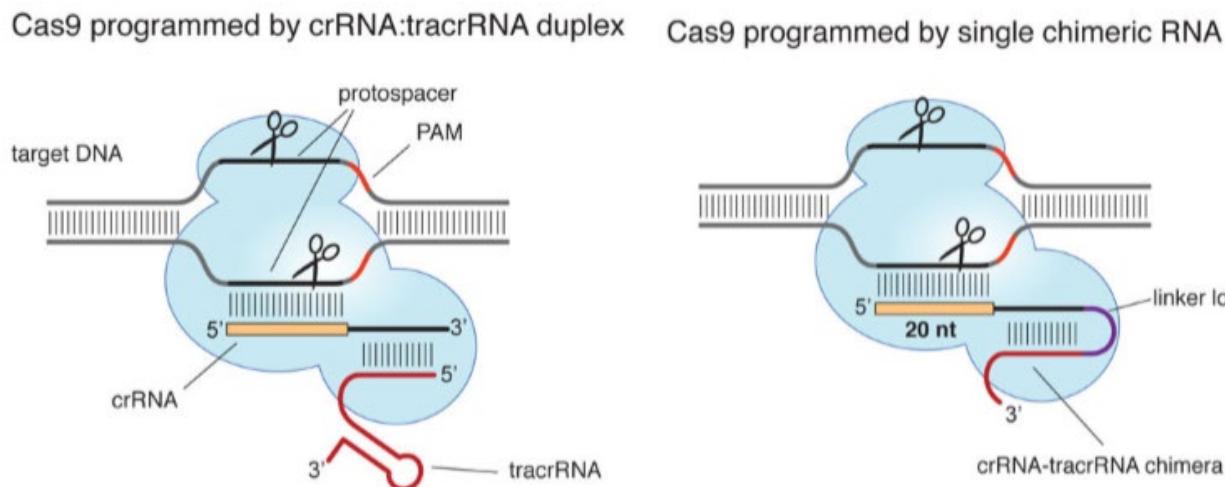
# CRISPR-Cas9 based expression systems

- Clustered regularly interspaced short palindromic repeats (CRISPR)
- Sites bind to **CRISPR associated protein 9** (Cas9)
- Site specific
- Can be multiplexed
- Next-gen CRISPR technology allowing knockout or knockin



# CRISPR-Cas9 development

- CRISPR sequences from bacteria have been studied since 1987
- Proposed to be an adaptive immune system 2005-2010
- Doudna and Charpentier proposed an engineered system based on the simplified CRISPR-Cas9 system in *Streptococcus pyogenes* to modify DNA in 2012



crRNA: CRISPR RNA

tracrRNA: trans-activating

Jinek, Science, 2012.

# CRISPR-Cas9 basics

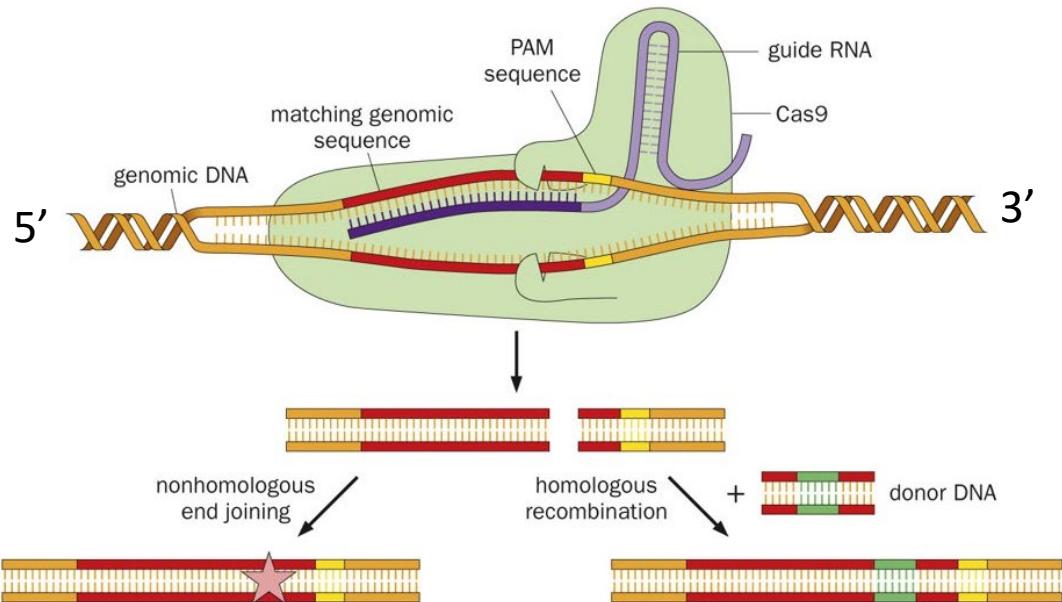


Figure 13-8 Principles of Neurobiology (© Garland Science 2016)

- Occurs over 95% of the time with DSB
- indel mutations

- Occurs 2-5% of the time after DSB, prefers an overhang
- With CRISPR/Cas9, even in cases when HDR occurred, indels also occurred

- Cas9 endonuclease is targeted to genomic location by sgRNA (single guide RNA)
- Cas9 protein and gRNA can be separately encoded/expressed
- PAM sequence (protospacer adjacent sequence): a 3 base pair sequence that occurs fairly regularly in the genome every 8-12 bp
- Guide RNA must contain the PAM (3 bp) and can be ~20nt in length but must be unique to prevent off-target binding

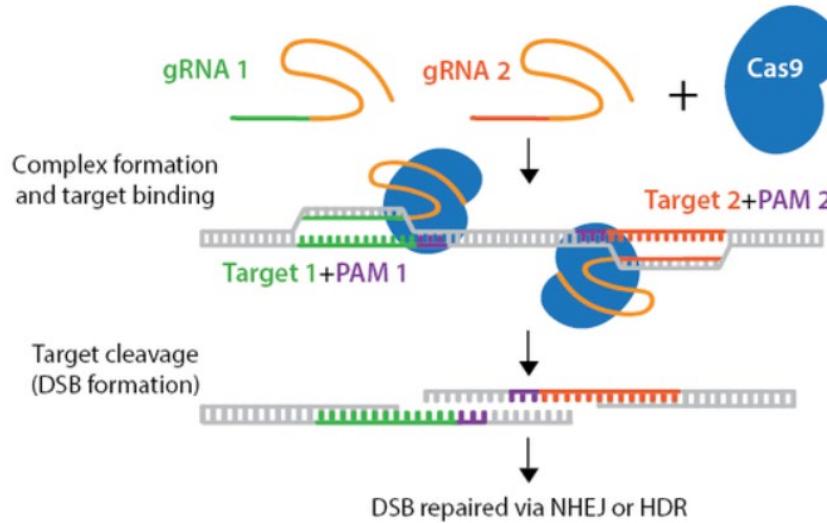
# Cas9 orthologs

Cas9 species	PAM sequence (5' to 3')	
<i>Streptococcus pyogenes</i> ( <i>Sp</i> )	NGG	4.1 kb
<i>Staphylococcus aureus</i> ( <i>Sa</i> )	NGRRT or NGRRN	3.3 kb
<i>Neisseria meningitidis</i> ( <i>Nm</i> or <i>Nme</i> )	NNNNGATT	
<i>Campylobacter jejuni</i> ( <i>Cj</i> )	NNNNRYAC	
<i>Streptococcus thermophilus</i> ( <i>St</i> )	NNAGAAW	
<i>Treponema denticola</i> ( <i>Td</i> )	NAAAAC	
~20 additional Cas9 species	PAM sequence may not be characterized	

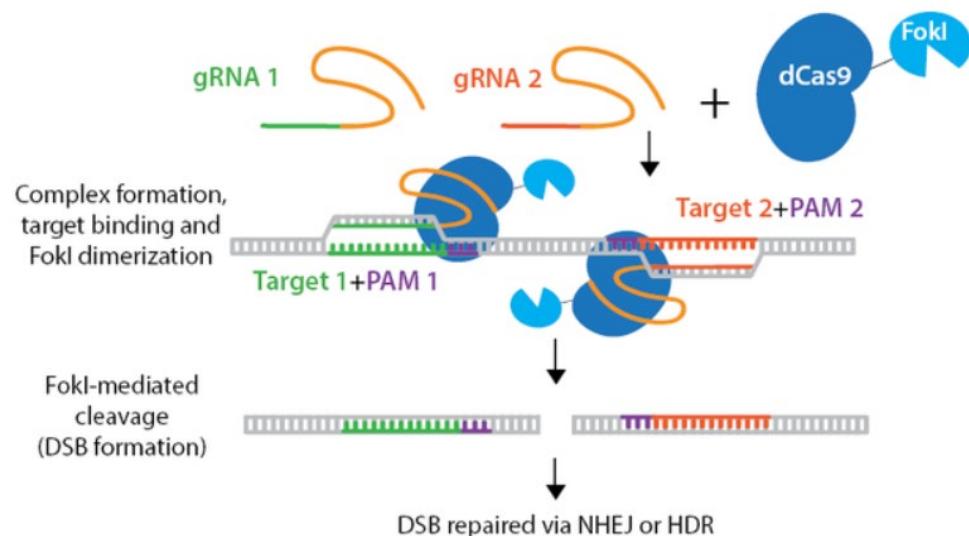
Friedland et al., Genome Biol., 2015.: paper describing saCas9  
[Addgene.org](http://Addgene.org)

# Cas9: targeting enhancement

Cas9-nickase (Cas9n)



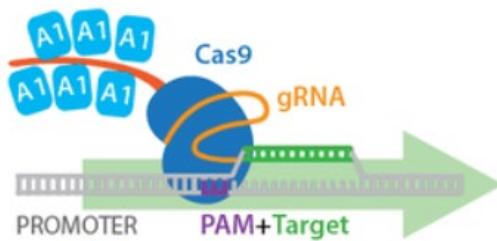
dCas9-FokI



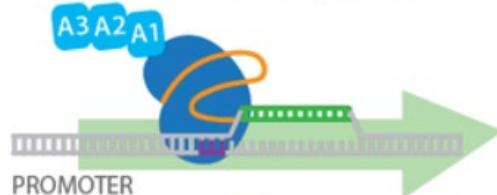
# Cas9: transcriptional regulation

## CRISPR Activation Systems

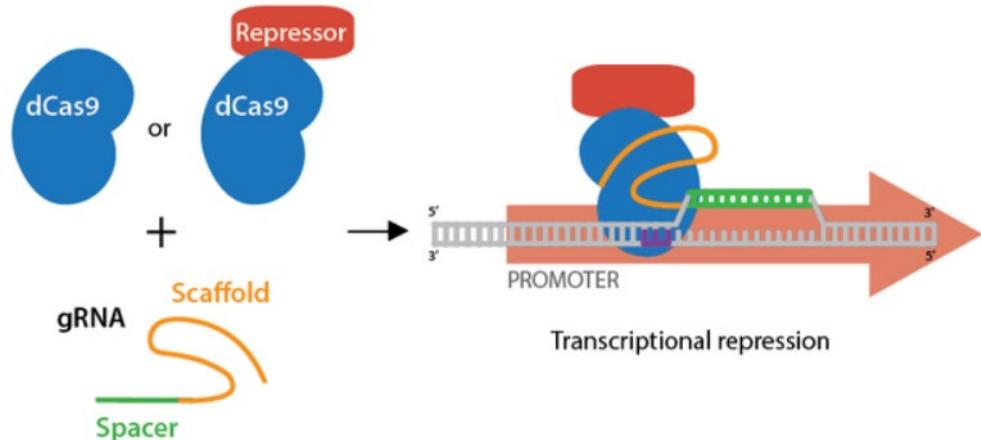
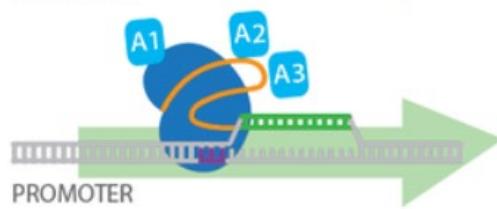
Strategy 1: dCas9 is fused to a scaffold that recruits activator peptides (e.g. SunTag)



Strategy 2: dCas9 is fused to a series of activation domains (e.g. dCas9-VPR)



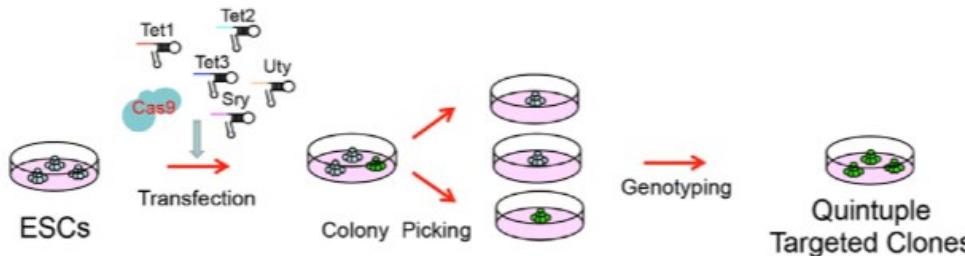
Strategy 3: dCas9 is fused to an activator and a tagged gRNA recruits other activators (e.g. SAM)



# CRISPR-Cas9: Making Mutants

A

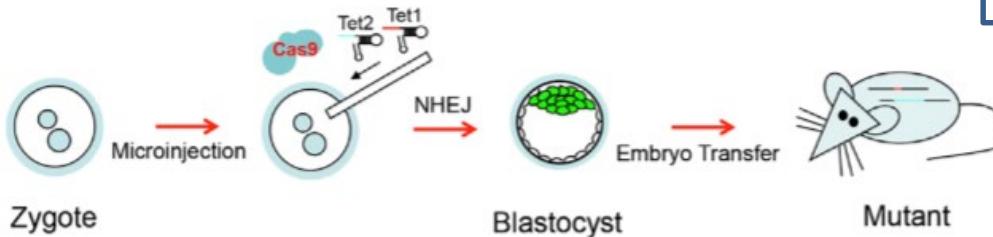
Multiple Gene targeting in ES cells



B

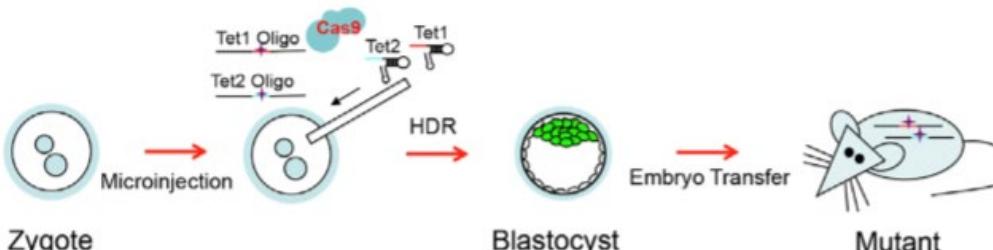
One Step Generation of Mice With Multiple Mutations

Targeted Mutations (Deletion / Insertion)



-Site-specific  
-Directly edit ES cell DNA

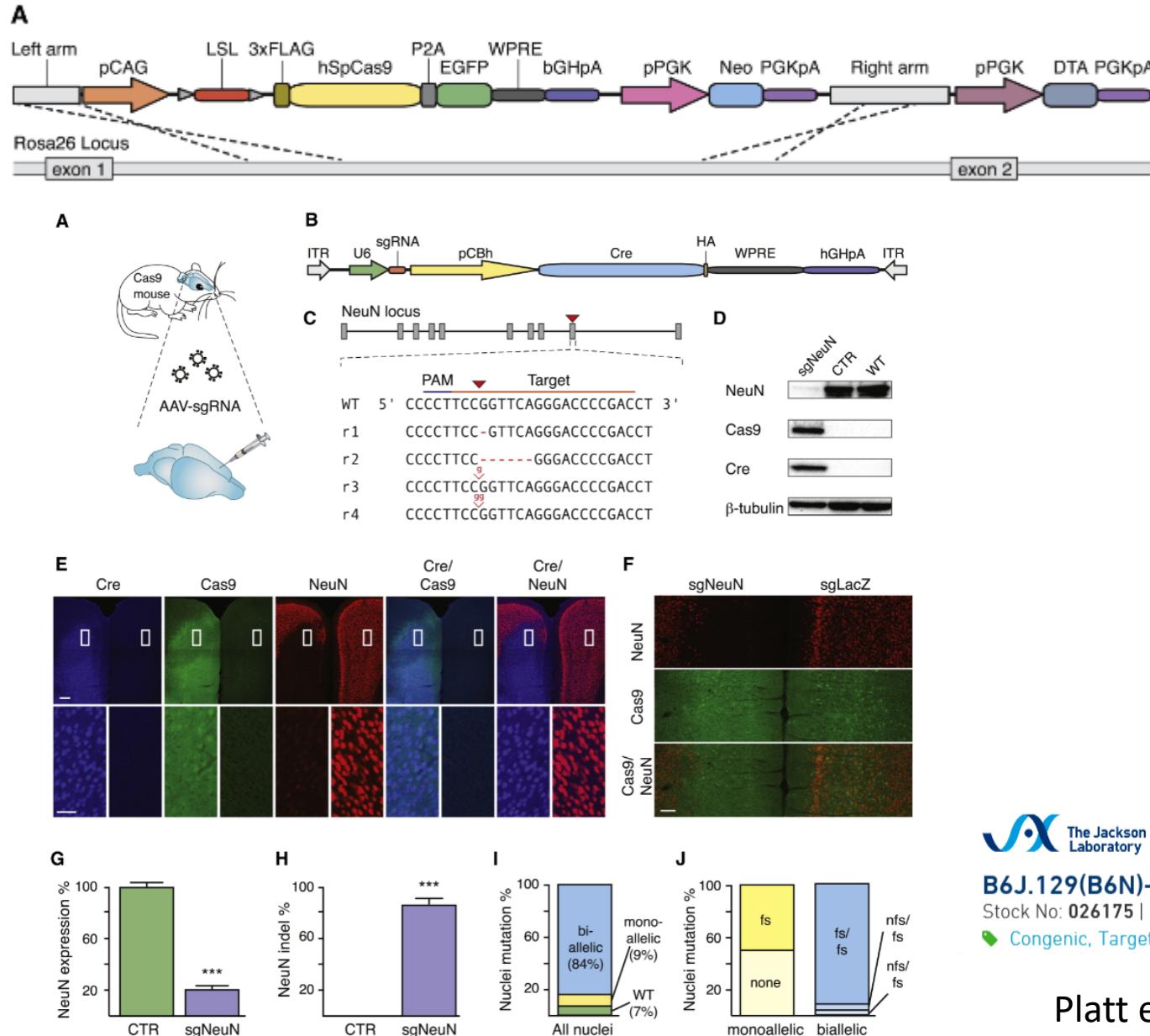
Predefined Precise Mutations



# CRISPR-Cas9 in the brain

Expression System	Components of System	Application
Lentiviral transduction	Cas9 and gRNA can be present in a single lentiviral transfer vector or separate transfer vectors. May contain reporter gene (e.g. GFP) to identify and enrich positive cells. Likely contains a selection marker to generate stable cell lines. <a href="#">Packaging and Envelope plasmids</a> provide the necessary components to make lentiviral particles.	Stable, tunable expression of Cas9 and/or gRNA in a wide variety of mammalian cell lines. Useful for difficult to transfect cell types and can be used <i>in vivo</i> . A common choice for conducting genome-wide screens using CRISPR.
AAV transduction	Only compatible with <a href="#">SaCas9</a> (packaging limit ~4.5kb). CRISPR elements are inserted into an AAV transfer vector and used to generate AAV particles.	Transient or stable expression of SaCas9 and/or gRNA. Infects dividing and non-dividing cells. AAV is least toxic method for <i>in vivo</i> viral delivery.
Cas9 mRNA and gRNA	Plasmids containing gRNA and Cas9 are used in <i>in vitro</i> transcription reactions to generate mature Cas9 mRNA and gRNA. RNA is delivered to target cells using microinjection or electroporation.	Transient expression of CRISPR components, expression decreases as RNA is degraded within the cell. Can be used for generating transgenic embryos.
Cas9-gRNA ribonucleoprotein complexes	Purified Cas9 protein and <i>in vitro</i> transcribed gRNA are combined to form a Cas9-gRNA complex and delivered to cells using cationic lipids.	Transient expression of CRISPR components, expression decreases as gRNA and Cas9 protein are degraded within the cell.

# Conditional Cas9 system



The Jackson Laboratory

**B6J.129(B6N)-Gt(ROSA)26Sor<sup>tm1(CAG-cas9\*, -EGFP)Fezh</sup>** / J

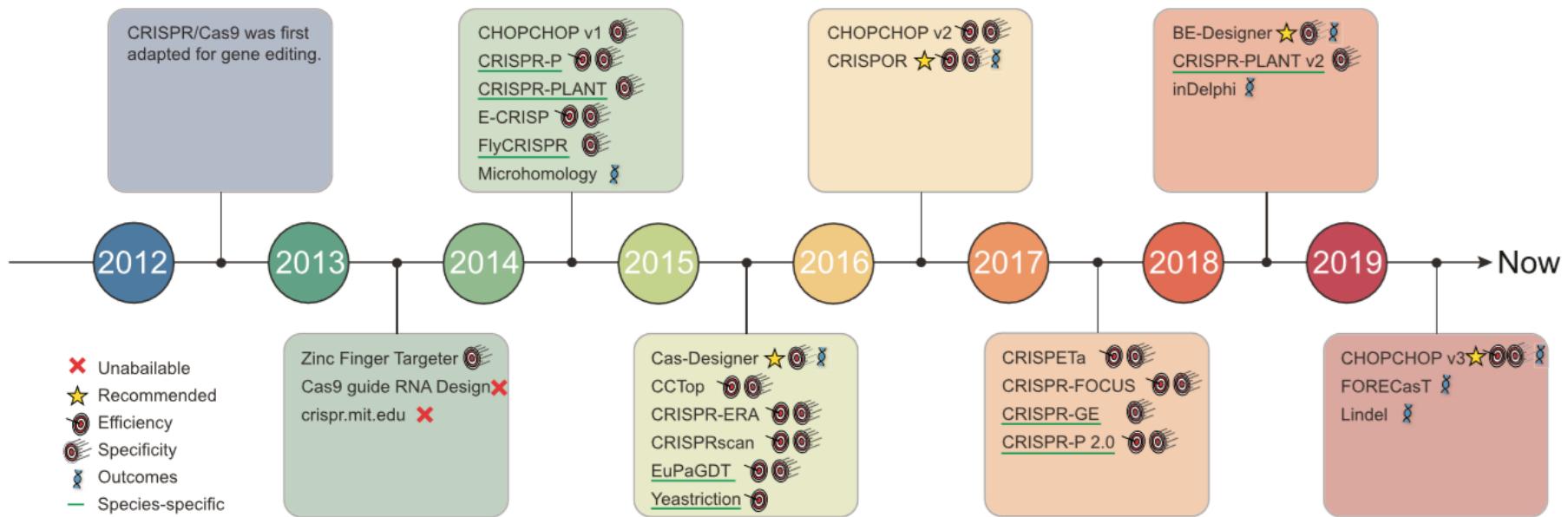
Stock No: 026175 | Rosa26-LSL-Cas9 knockin on B6J

Congenic, Targeted Mutation

Platt et al., Cell, 2014.

# Guide RNA

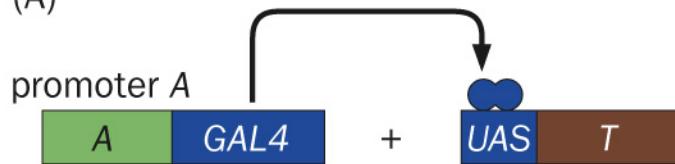
- Efficiency (do what it's supposed to)
- Specificity (not do anything else)



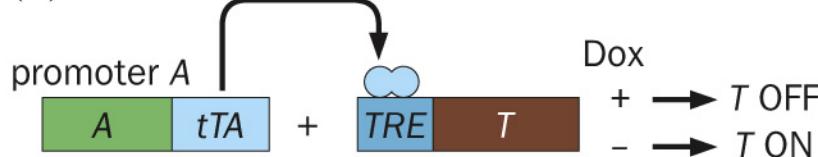
**Fig. 2.** Timeline of the development of web-based tools for CRISPR guide RNA design.

# Inducible systems

(A)



(B)



promoter A  
A      tTA

Dox  
+ → T OFF  
- → T ON

promoter A  
A      rtTA

Dox  
+ → T ON  
- → T OFF

(C)

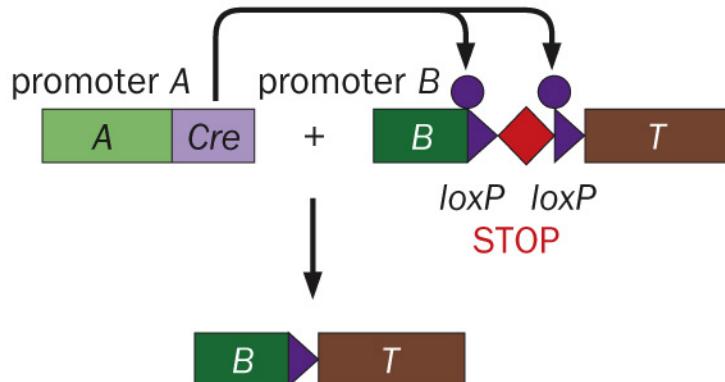
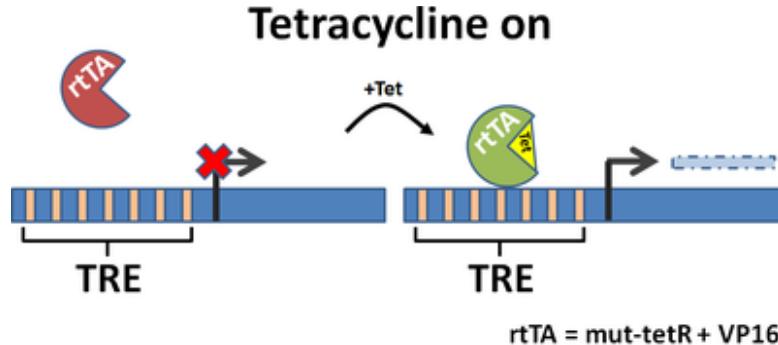


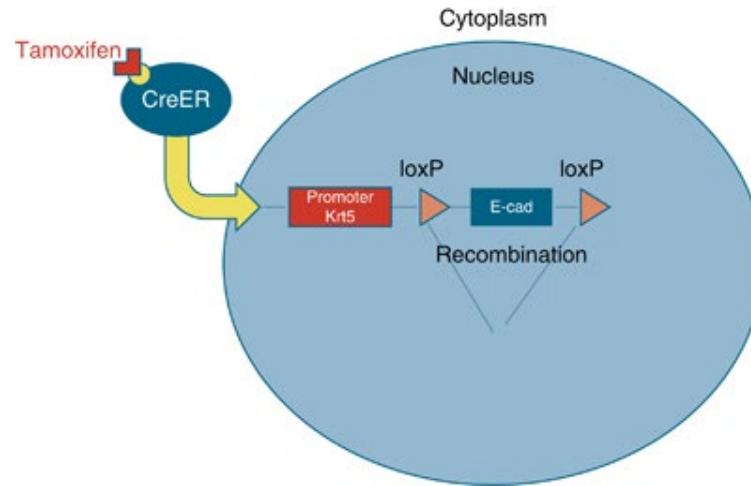
Figure 13-13 Principles of Neurobiology (© Garland Science 2016)

# Inducible Cre Expression *in vivo*

- Tetracycline

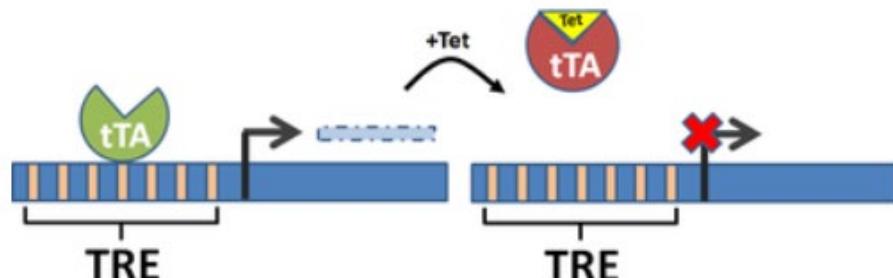


- Tamoxifen



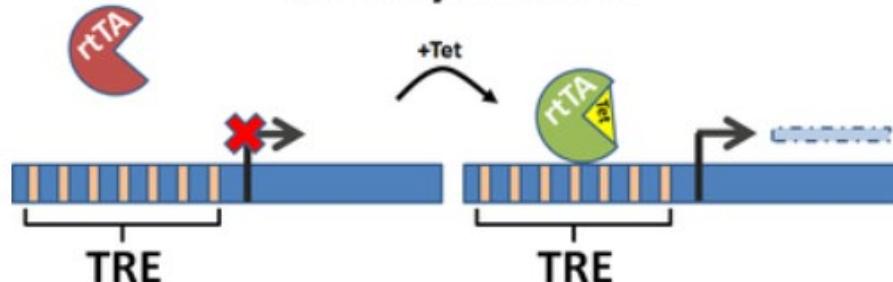
# Tet on/off

## Tetracycline off



tTA = tetR + VP16

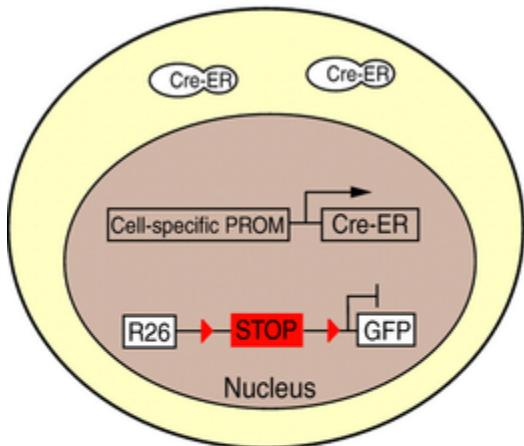
## Tetracycline on



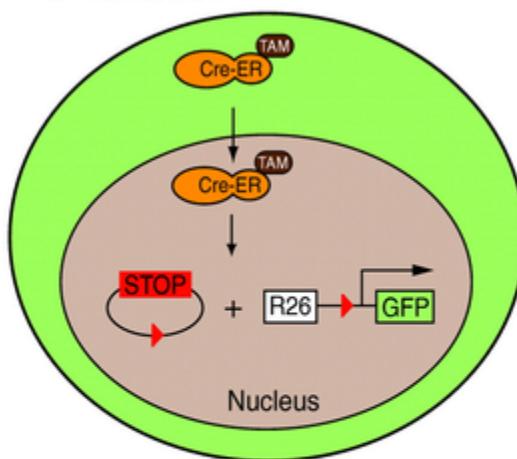
rtTA = mut-tetR + VP16

# Tamoxifen-inducible Cre

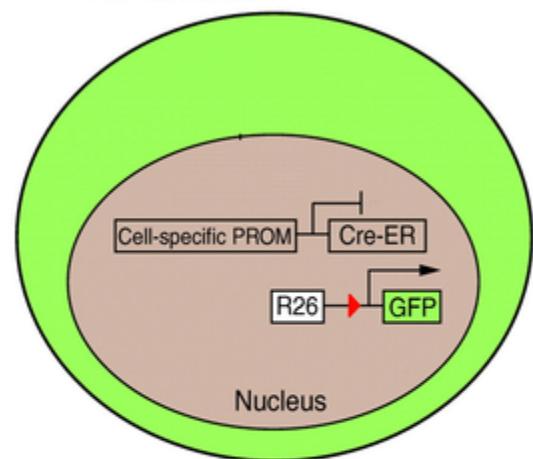
A No Tamoxifen



B Tamoxifen



No Tamoxifen



Key

R26 Rosa 26 promoter

► LoxP sites

Cre-ER Inactive Cre

Cre-ER Active Cre

TAM Tamoxifen

## Complications

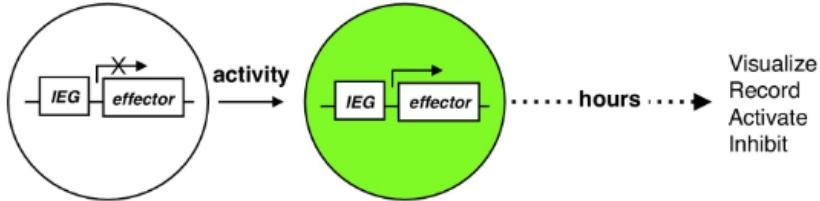
- Leaky systems
- Drug half-life
- Effects of chemical on cells
- Effects of chemical on desired readout (i.e. behavior)



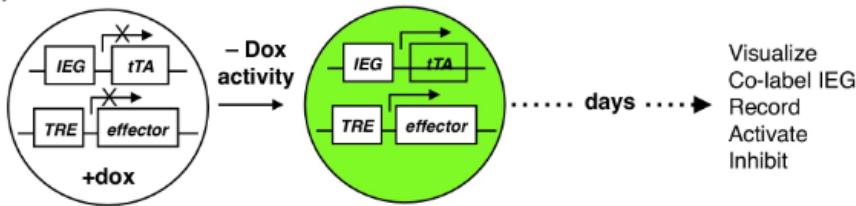
# Tagging Active Cell Populations

## IEG-based strategies

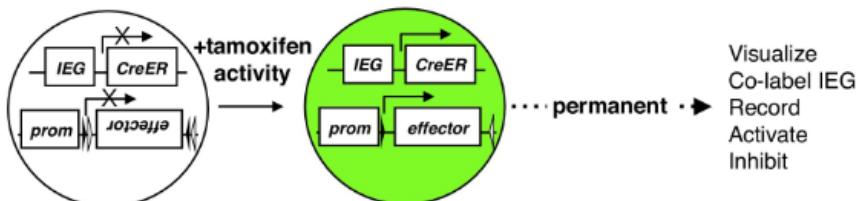
(a) *Fos-EGFP, Fos-LacZ, Arc-XFP, AAV-Fos-ChR2, LV-Fos-ChR2*



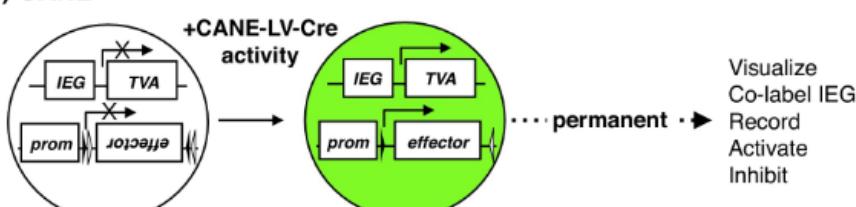
(b) *Fos-tTA*



(c) *FosTRAP, ArcTRAP, ArcCreERT<sup>2</sup>, AAV-Fos-CreERT<sup>2</sup>*

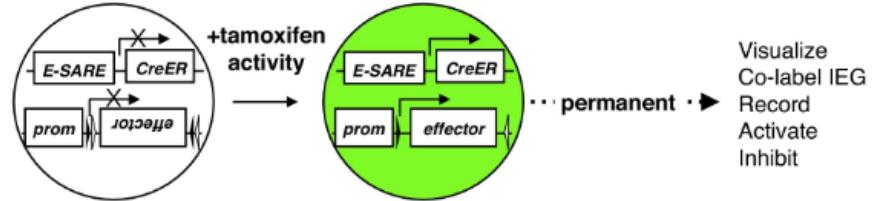


(d) *CANE*

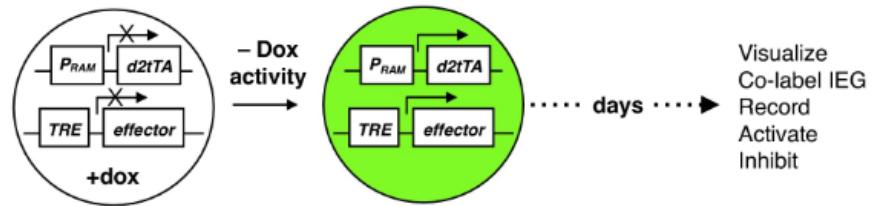


## Viral strategies using synthetic promoters

(e) *E-SARE*

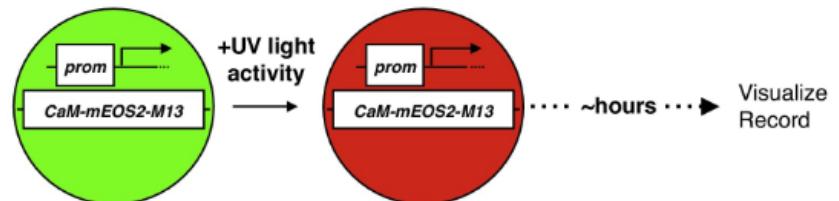


(f) *RAM*

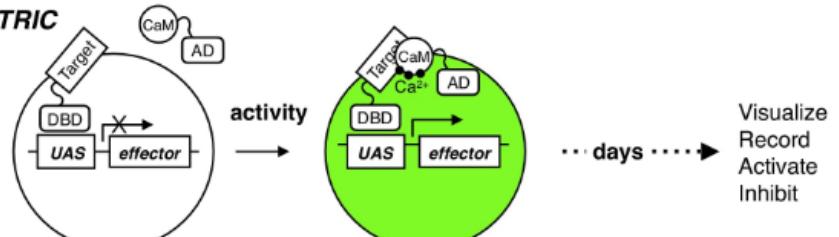


## Ca<sup>2+</sup>-based strategies

(g) *CaMPARI*



(h) *TRIC*

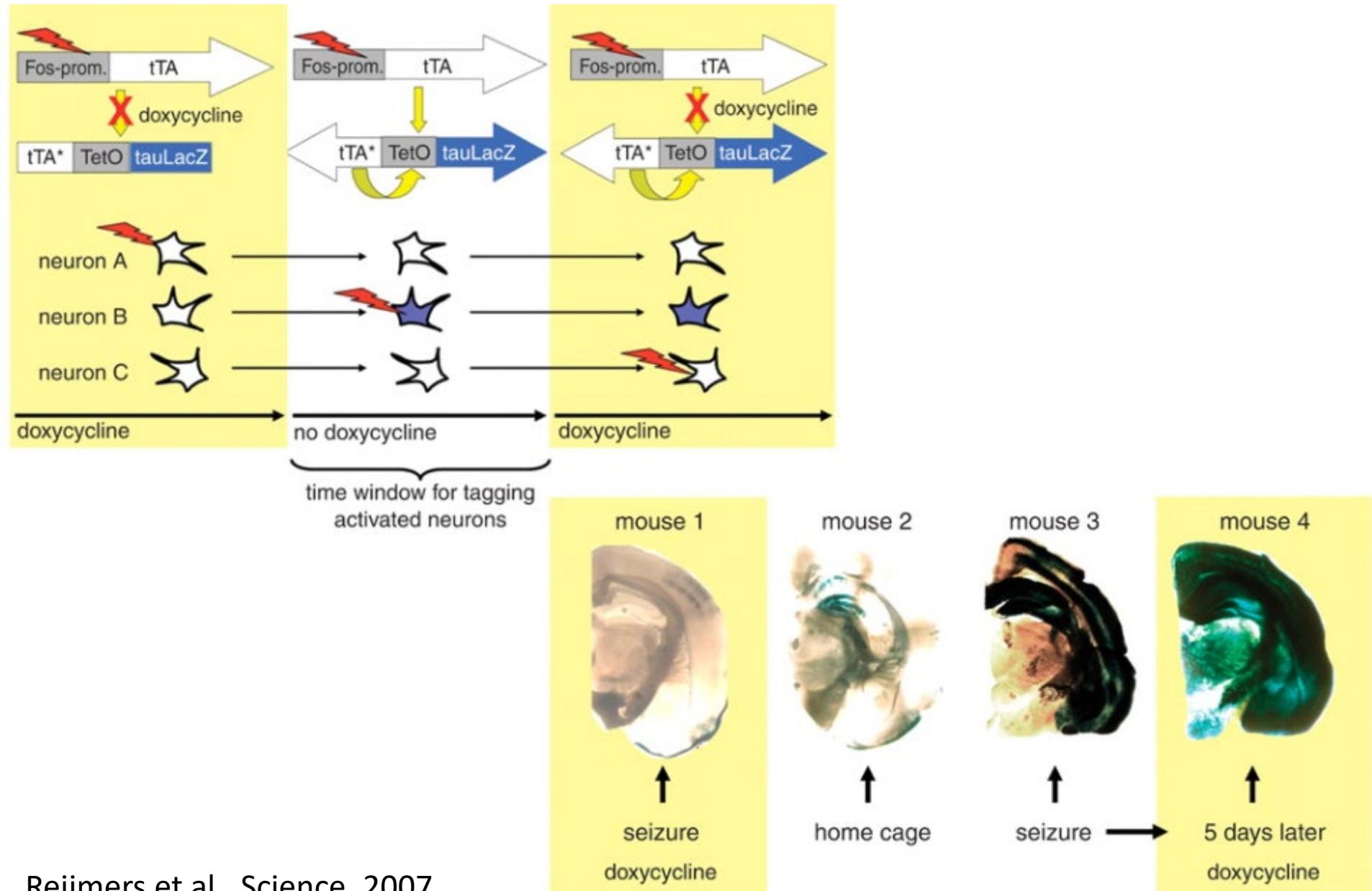


# Immediate early gene-based systems

- **TetTag:** tet inducible system (2007)
- **TRAP2:** targeted recombination in active populations (2013)
- **CANE:** capturing active neuronal ensembles (2016)
- **vGATE:** virus-delivered genetic activity-induced tagging of cell ensembles

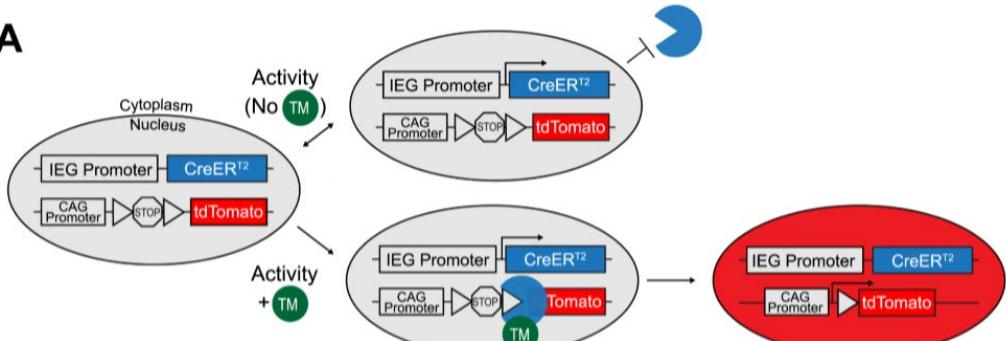


# TetTag

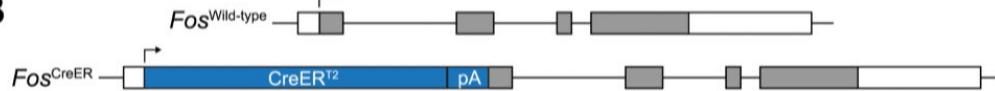


# TRAP2

A



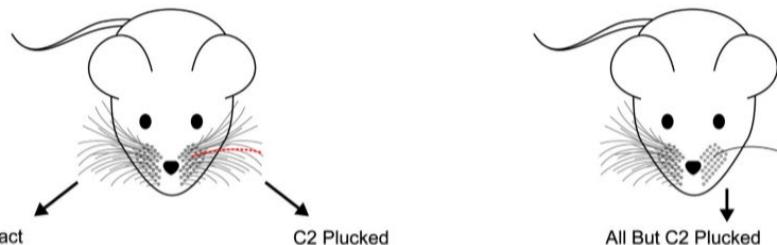
B



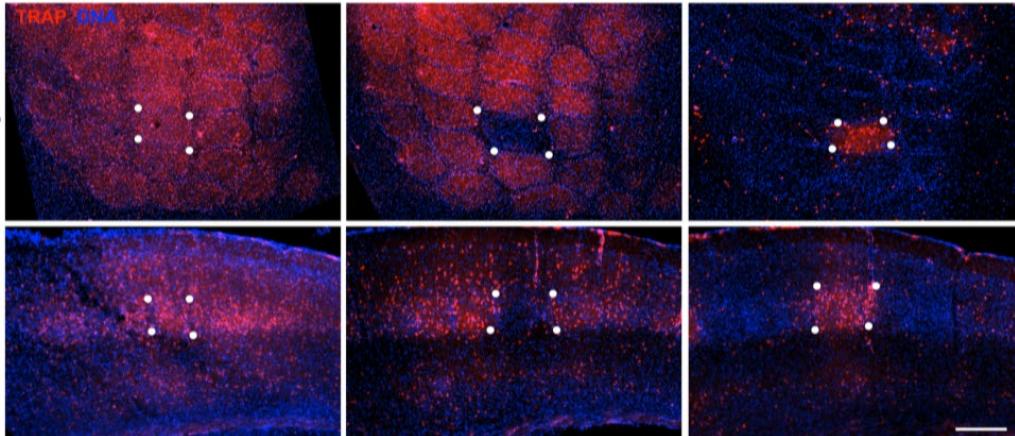
C

Pluck Whiskers → 2 days → Inject Tamoxifen → 7 days → Sacrifice

D

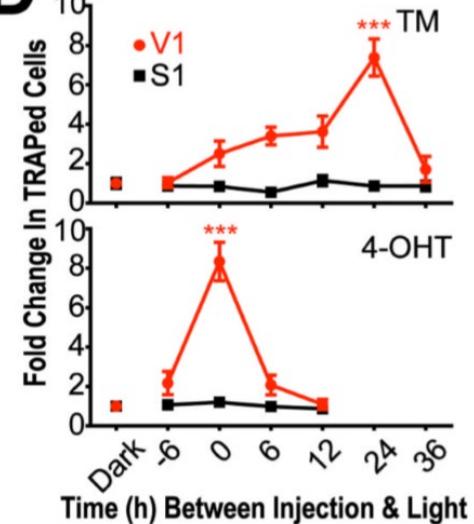


Tangential



Pro-tip: use OHT instead of tamoxifen

D



TRAP2: no disruption of endogenous c-fos expression



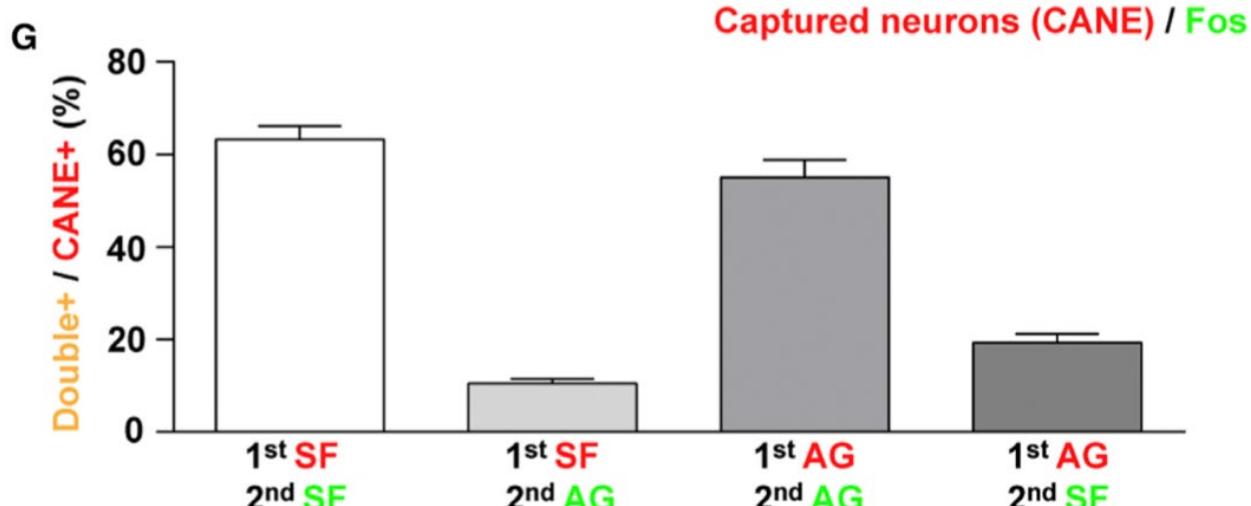
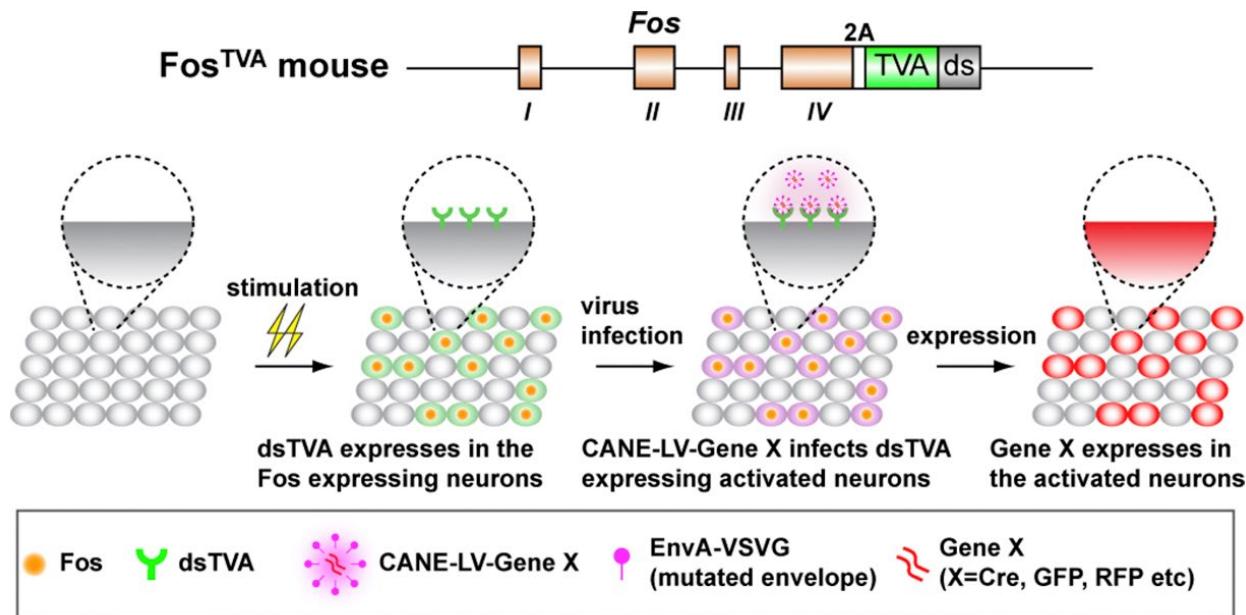
**STOCK Fos** *tm2.1(cre/ERT2)Luo* /J POPULAR

Stock No: 030323 | Fos <sup>2A-creER</sup> (TRAP2)

Targeted Mutation

Guenthner et al., Neuron, 2013.

# CANE



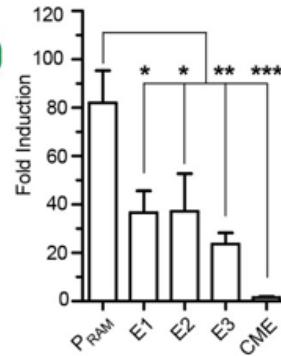
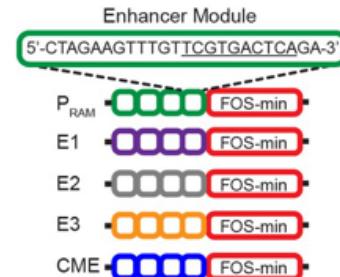
# Synthetic activity promoter systems

- eSARE: enhanced synaptic activity-responsive element (2015)
- **RAM**: robust activity marking (2016)

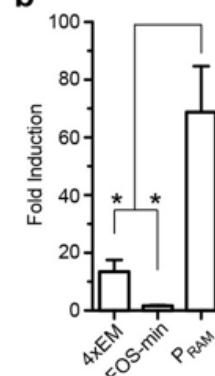


# RAM

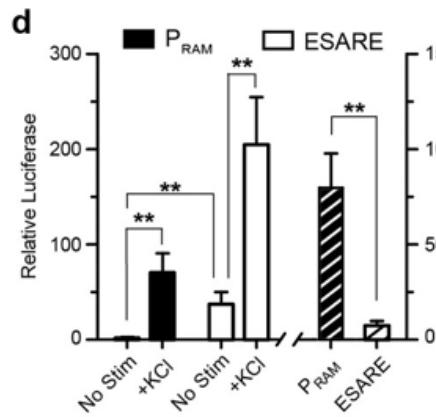
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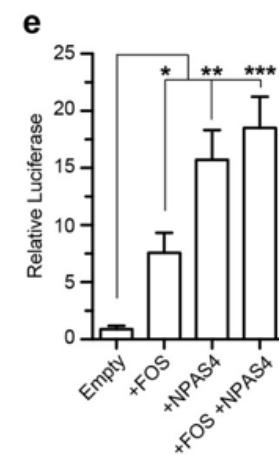
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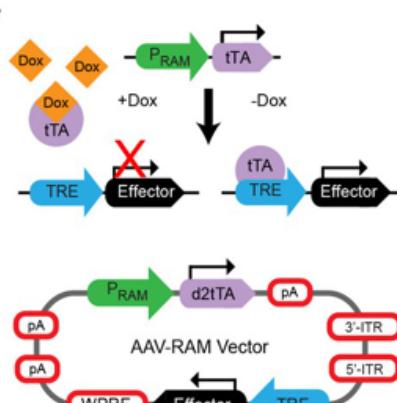
c



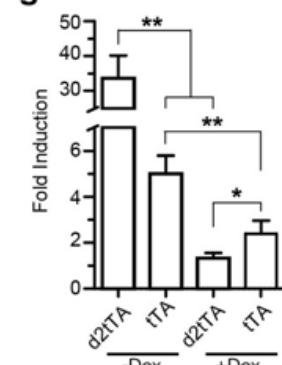
e



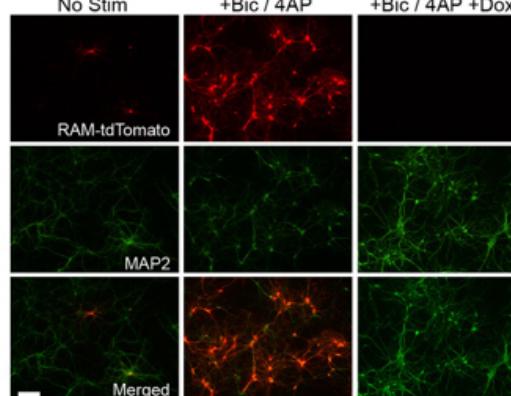
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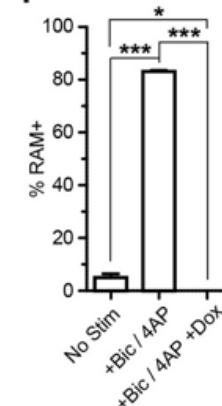
g



h



i

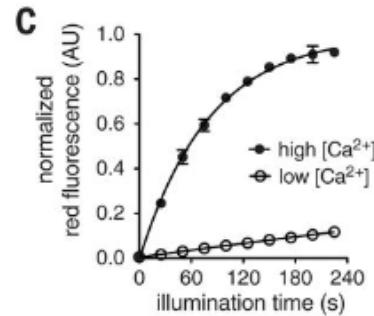
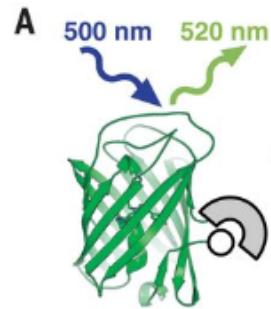


# Calcium and calcium/photoactivatable systems

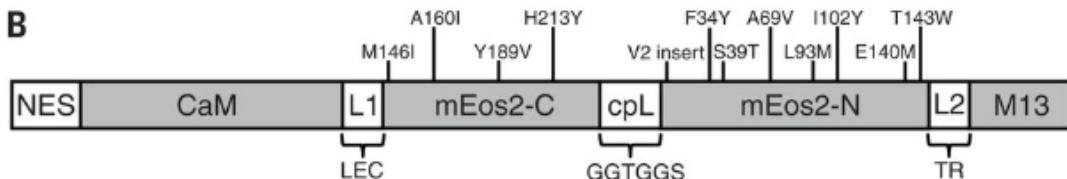
- **CaMPARI:** calcium-modulated photoactivatable ratiometric integrator
- **Cal-light:** calcium and light induced gene handling toolkit
- **FLARE:** Fast-light and activity regulated expression
- **PHOTSeq**



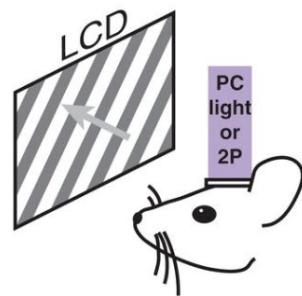
# CaMPARI



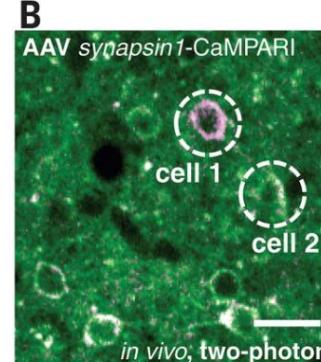
**B**



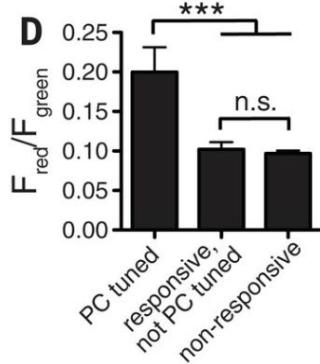
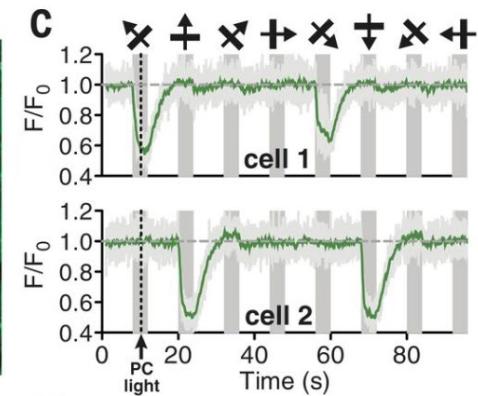
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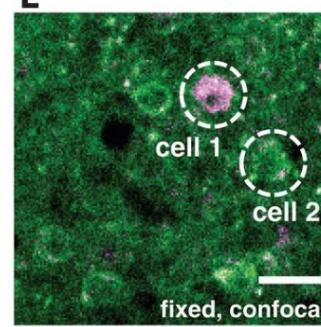
**B**



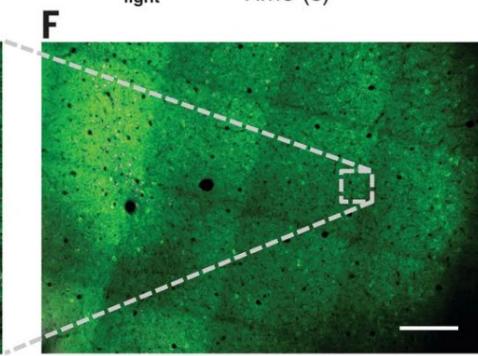
**C**



**E**

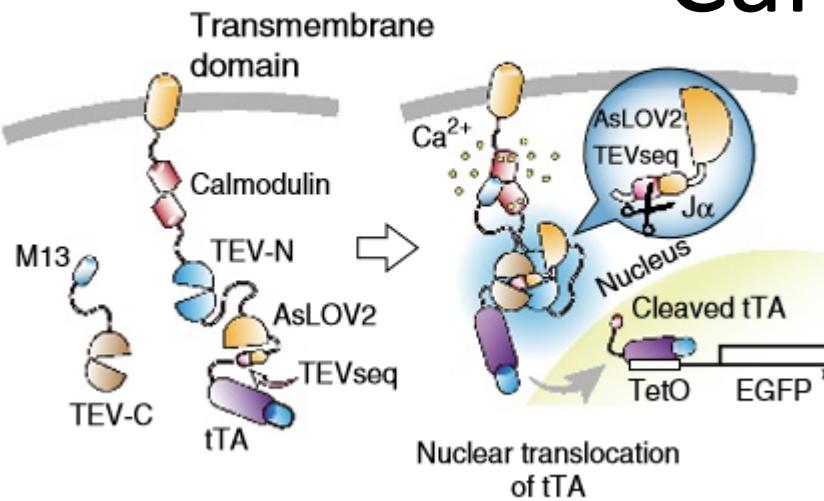


**F**



# Cal-Light

a

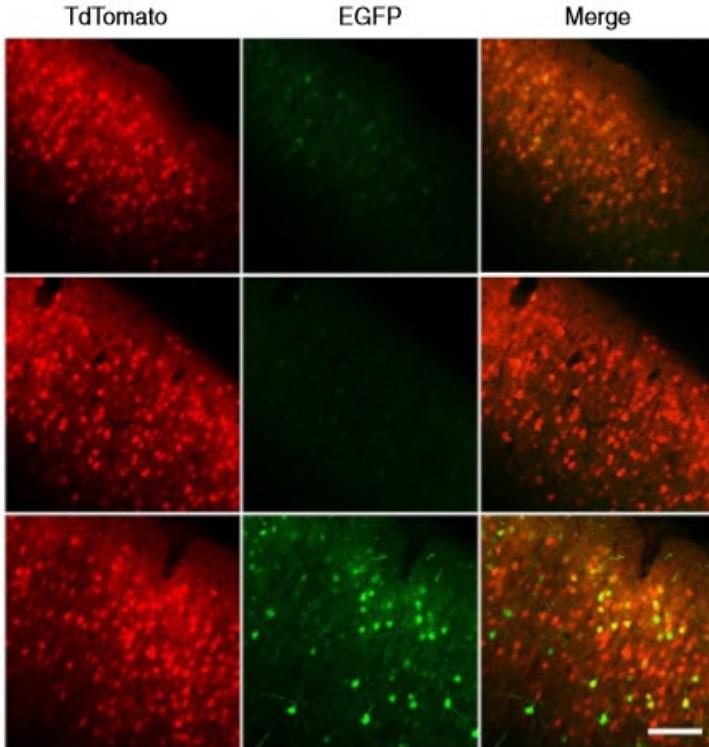


Tobacco etch virus protease

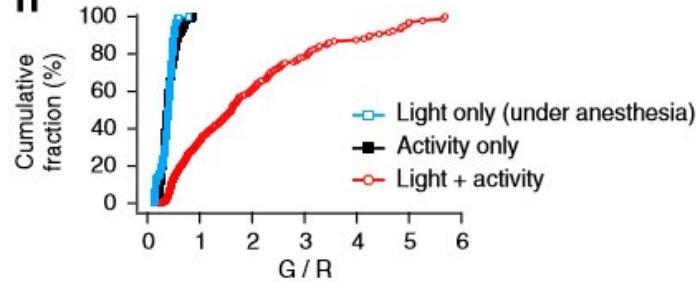
Lee et al., Nature Biotech., 2017.

g

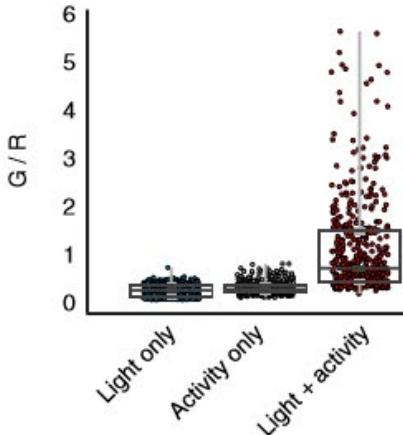
Activity only  
Light only  
Light + activity



h

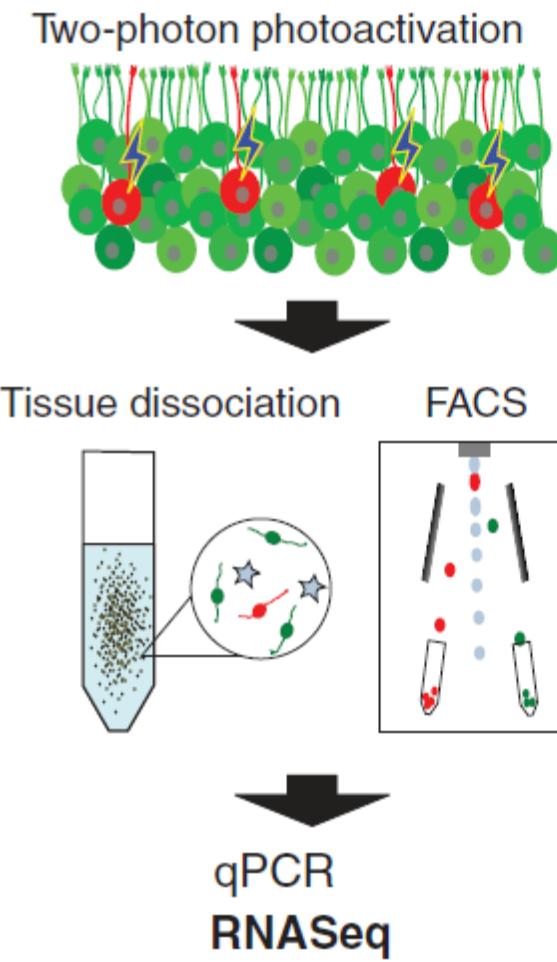
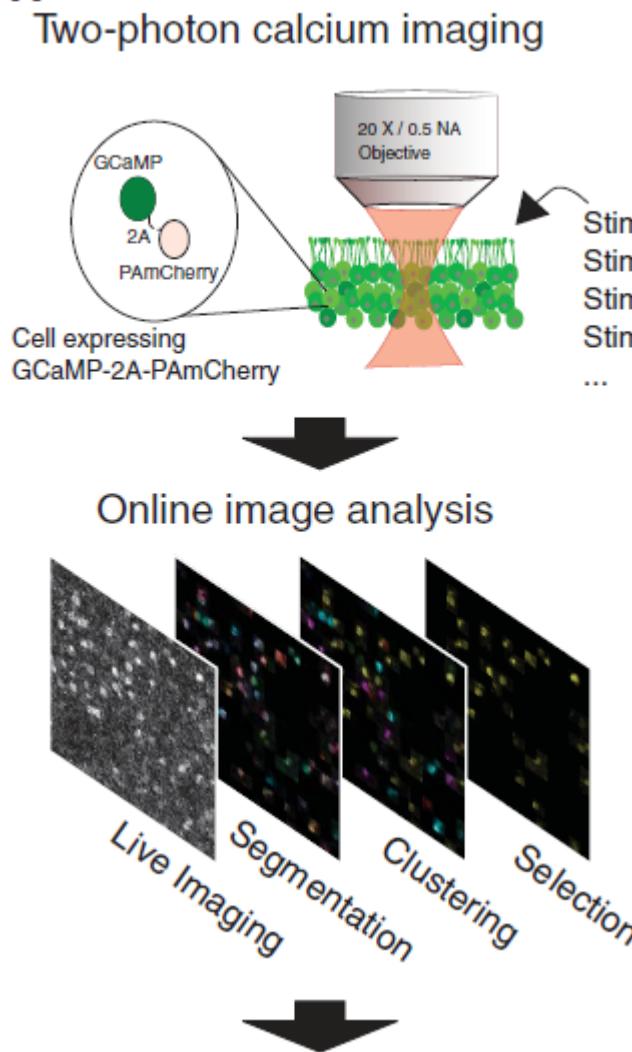


i



# PHOTSeq: physiological optical tagging sequencing

A



# Tagging resources

**Table 1**

Summary of tools for visualizing active neural ensembles and active synapses.

Tools	Activity-capturing components	Other regulatory components	Available resources	Refs <sup>a</sup>
[IEG promoter-based gene expression]				
TetTag	<i>Fos</i> promoter	tTA-TRE	JAX 018306, 008344; MMRRC 031756-MU	1
vGATE	<i>Fos</i> promoter	rtTA-TRE, Cre-LoxP	N/A	2
TRAP	<i>Fos</i> or <i>Arc</i> promoter	CreERT <sup>2</sup> -LoxP	JAX 021881, 021882, 022357, 030323	3-5
CANE	<i>Fos</i> promoter	dsTVA, EnvA-coated virus, Cre-LoxP	JAX 027831; Addgene 86641, 86666, 87221	6
E-SARE	SARE enhancers, mini- <i>Arc</i> promoter	Cre- or CreERT <sup>2</sup> -LoxP	N/A	7
RAM	AP-1 site, Npas4 motif, mini- <i>Fos</i> promoter	tTA-TRE	Addgene 63931	8
[Photoactivatable gene expression]				
Cal-Light	CaM, Ca <sup>2+</sup> /CaM-domain, light	TEVp-TEVseq, LOV, tTA-TRE	Addgene 92391, 92392	9
FLARE	CaM, Ca <sup>2+</sup> /CaM-domain, light	TEVp-TEVseq, LOV, tTA-TRE	Addgene 92213, 92214	10
PA-Cre	Split Cre, light	Photo-dimers CRY2-CIBN	Addgene 26888, 26889, 75367, 75368	11-13
PA-Flp	Split Flp, light	Photo-dimers Magnet	N/A	14
[Activated synapse visualization]				
SynaptoZIP	Synaptobrevin	ZIP-Synbond	N/A	15
SynTagMA	CaM, Ca <sup>2+</sup> /CaM-domain in CaMPARI2	Synaptophysin (pre), PSD-95 intrabody (post)	Addgene 119723, 119736, 119738	16
syb:GRASP	Synaptobrevin	GRASP (split GFP)	Addgene 65830, 73698-73700	17
eGRASP	<i>Fos</i> promoter	tTA-TRE, GRASP	Addgene 111579-111590, 111597, 111598, 120309	18
[Activated synapse manipulation]				
AS-PaRac1	PSDΔ1.2, DTE of <i>Arc</i> mRNA, light	Rac1, LOV	N/A	19
PA-BoNT	Split botulinum toxin, synaptophysin	Photodimer iLID-SspB	Addgene 122976, 122979, 122981	20

<sup>a</sup> 1, Reijmers et al., 2007; 2, Hasan et al., 2019; 3, Guenthner et al., 2013; 4, Allen et al., 2017; 5, DeNardo et al., 2019; 6, Sakurai et al., 2016; 7, Kawashima et al., 2013; 8, Sørensen et al., 2016; 9, Lee et al., 2017; 10, Wang et al., 2017; 11, Kennedy et al., 2010; 12, Schindler et al., 2015; 13, Taslimi et al., 2016; 14, Jung et al., 2019; 15, Ferro et al., 2017; 16, Perez-Alvarez et al., 2019; 17, Macpherson et al., 2015; 18, Choi et al., 2018; 19, Hayashi-Takagi et al., 2015; 20, Liu et al., 2019.

# Intermission

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## Cell-Specific Targeting of Genetically Encoded Tools for Neuroscience

Lucas Sjulson,<sup>1,2</sup> Daniela Cassataro,<sup>2</sup>  
Shamik DasGupta,<sup>3,4</sup> and Gero Miesenböck<sup>3</sup>

<sup>1</sup>Department of Psychiatry, New York University School of Medicine, New York, NY 10016;  
email: lukesjulson@gmail.com

<sup>2</sup>Department of Neuroscience and Physiology, Smilow Neuroscience Program, and New York  
University Neuroscience Institute, New York, NY 10016

<sup>3</sup>Centre for Neural Circuits and Behaviour, University of Oxford, Oxford, OX1 3SR,  
United Kingdom; email: gero.miesenboeck@cncb.ox.ac.uk

<sup>4</sup>Present address: Tata Institute of Fundamental Research, Mumbai, 400005, India

# Brief History of Sequencing Technology

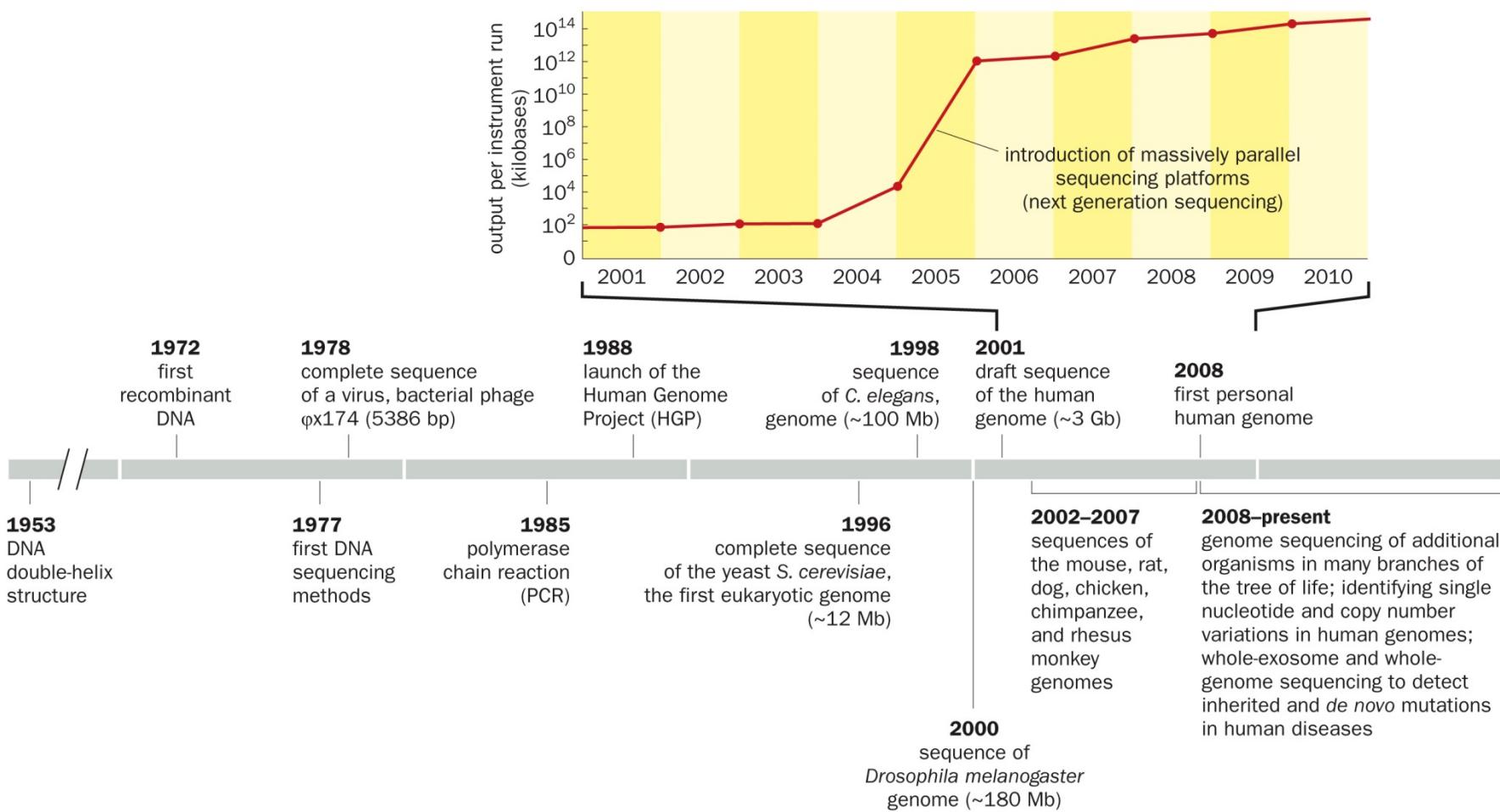


Figure 13-17 Principles of Neurobiology (© Garland Science 2016)

# Original Sanger sequencing

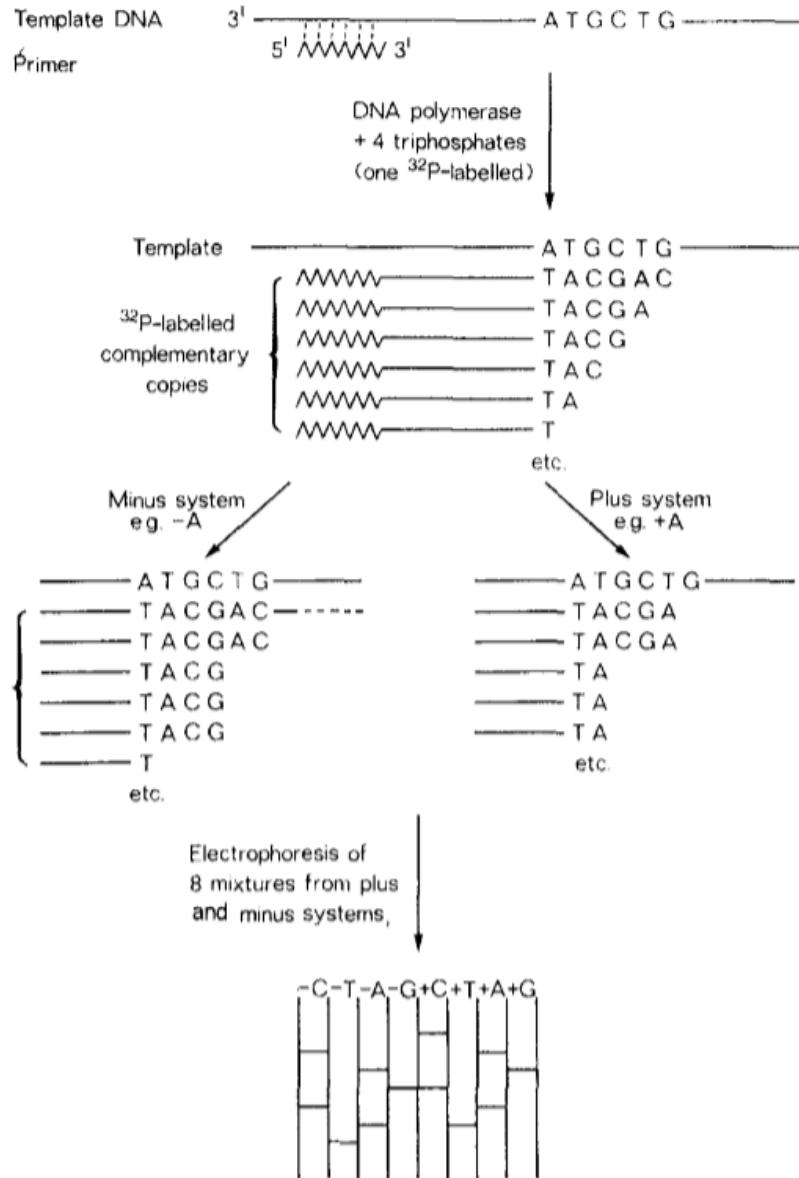
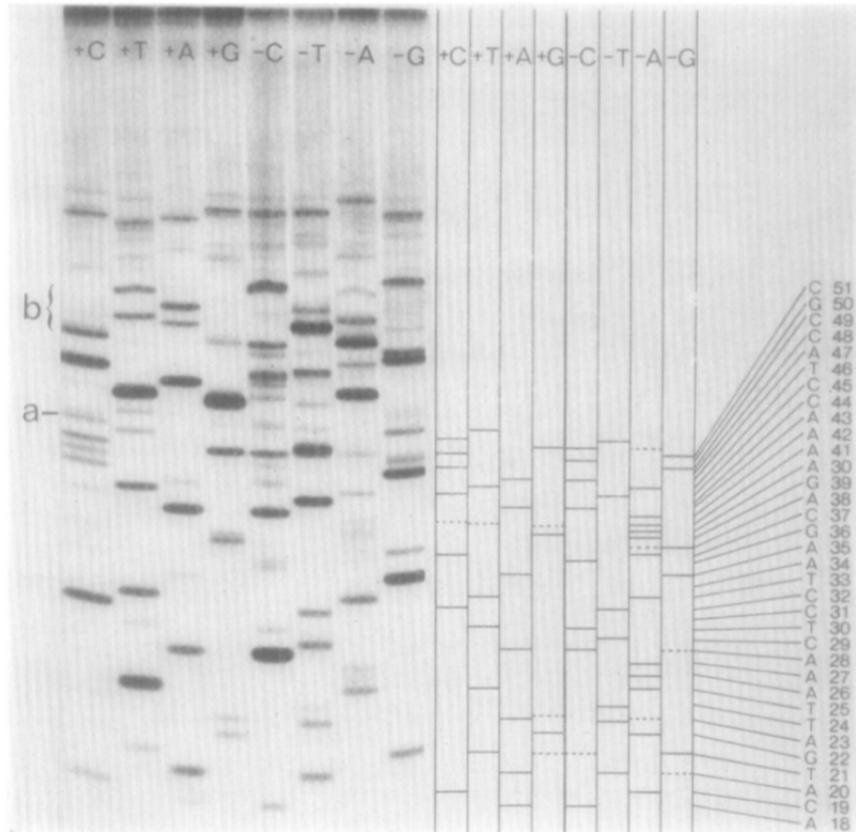


FIG. 1. The principle of the method.

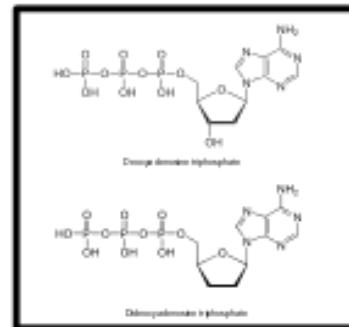
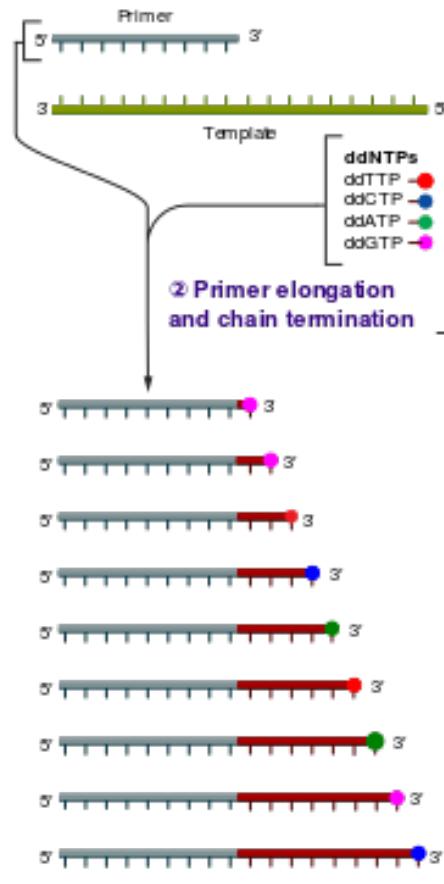


Sanger and Coulson, J. Mol. Bio., 1975.

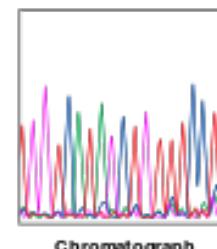
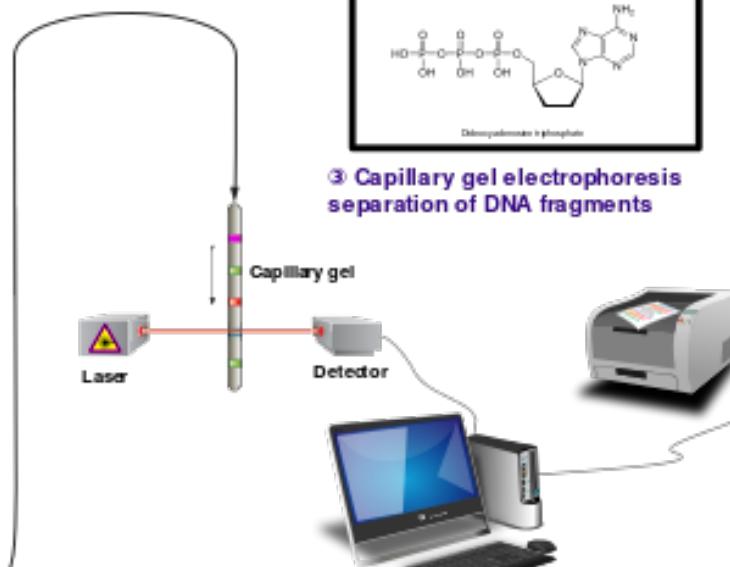
# Sanger sequencing today

## ① Reaction mixture

- Primer and DNA template → DNA polymerase
- ddNTPs with flourophoreses → dNTPs (dATP, dCTP, dGTP, and dTTP)



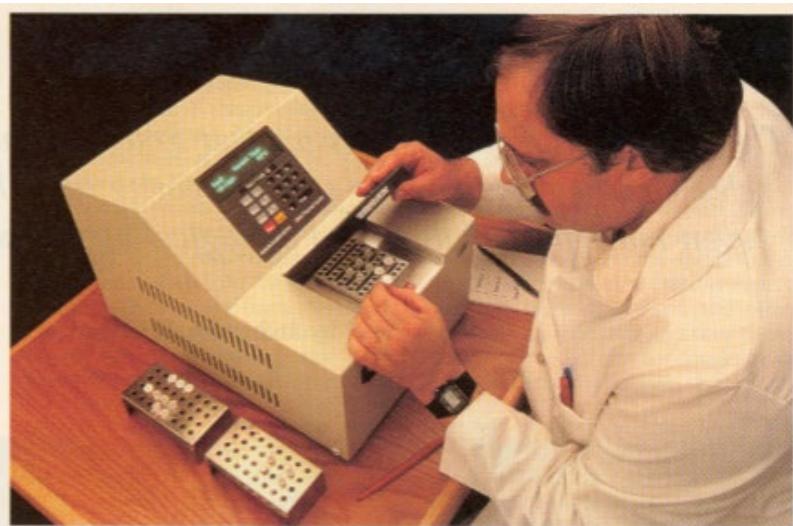
## ③ Capillary gel electrophoresis separation of DNA fragments



## ④ Laser detection of flourophoreses and computational sequence analysis

# Polymerase chain reaction

- Invented by Kary Mullis in 1983 while working at Cetus Corporation and won the Nobel Prize in Chemistry in 1993
- Amplify DNA in vitro (cell free)



MACHINE that performs the polymerase chain reaction is shown being loaded with samples of DNA. Such devices are rapidly becoming common fixtures in laboratories.

Sometimes a good idea comes to you when you are not looking for it. Through an improbable combination of coincidences, naiveté and lucky mistakes, such a revelation came to me one Friday night in April, 1983, as I gripped the steering wheel of my car and snaked along a moonlit mountain road into northern California's redwood country. That was how I stumbled across a process that could make unlimited numbers of copies of genes, a process now known as the polymerase chain reaction (PCR).

## The Unusual Origin of the Polymerase Chain Reaction

*A surprisingly simple method for making unlimited copies of DNA fragments was conceived under unlikely circumstances—during a moonlit drive through the mountains of California*

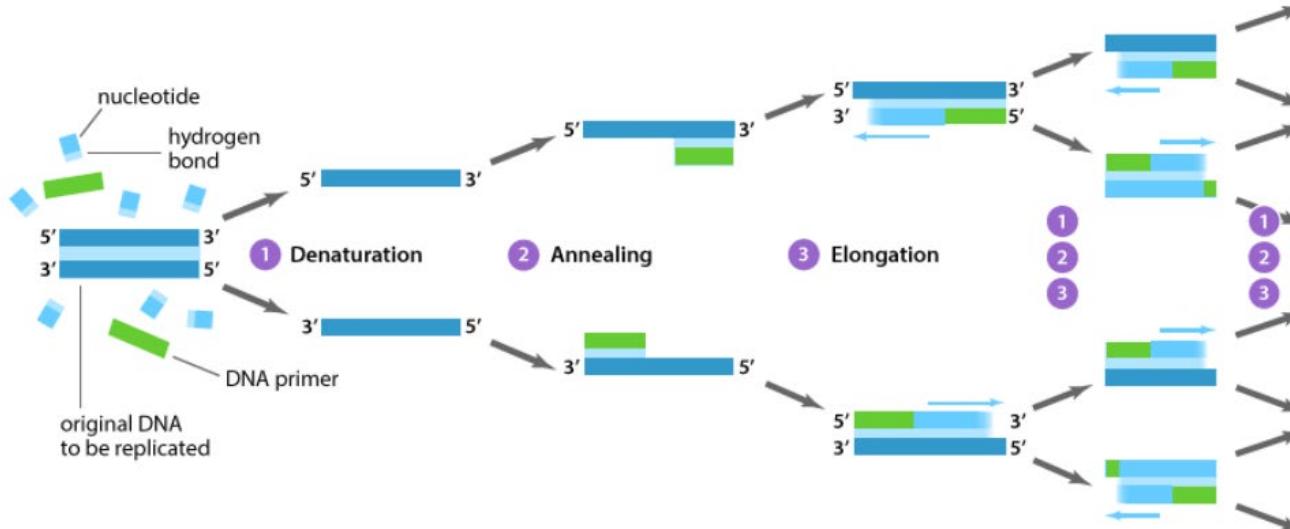
# PCR ingredients

- Taq: DNA polymerase originally isolated from thermus aquaticus that is very thermostable (range 72-95 degrees)
  - Discovered in hot springs of Yellowstone in 1969
  - Was “Molecule of the Year” in 1989
- Probes
- Buffer
- dNTPs



*Thermus aquaticus* was first discovered in the hot springs of Yellowstone National Park

# PCR

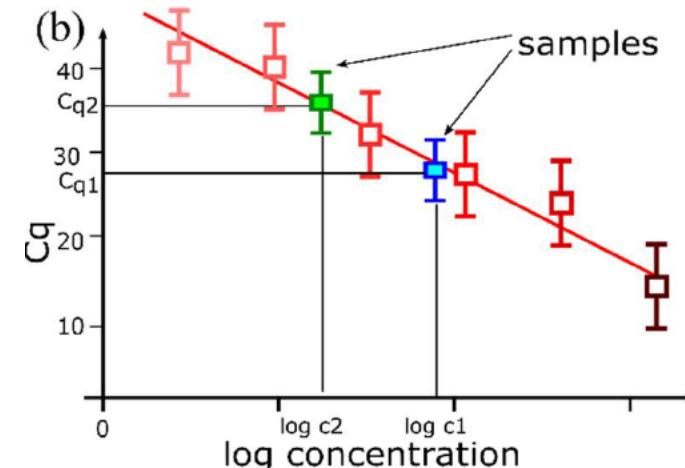
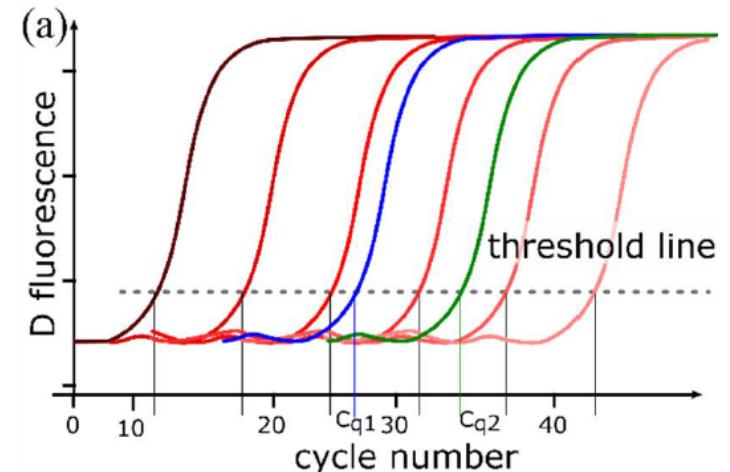
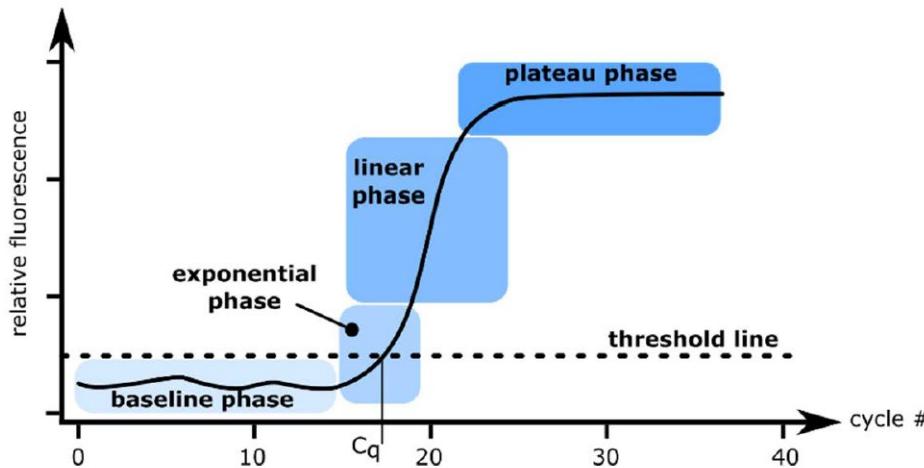
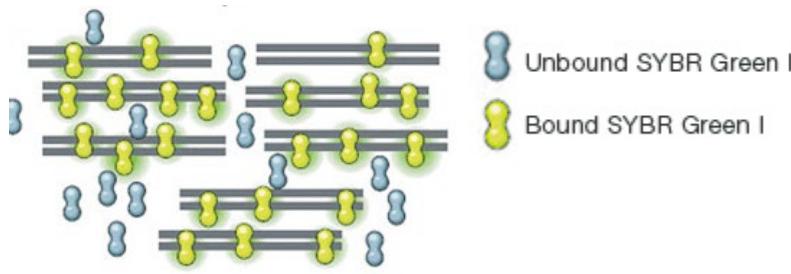


**Figure 1 – How Polymerase Chain Reaction works:** 1) Denaturation: the reaction is heated to 94–98 °C for 20–30 seconds to break the hydrogen bonds between the strands. 2) Annealing: the reaction temperature is lowered to 50–65 °C for 20–40 seconds to allow primers to anneal to the template strands. 3) Elongation: the temperature is increased (optimal temperature dependent on DNA Polymerase used e.g. 72–78 °C for Taq Polymerase) to allow for the addition of dNTPs. The amount of target sequence doubles with each thermal cycle which leads to an exponential amplification represented by  $2^{(\# \text{ of cycles})}$ .

- Template design considerations
  - GC (AT) content
  - Length (usually 16-21 bp)
- Temperatures
  - Melting temp of your primers
  - Annealing temp
- pH
  - higher pH can decrease truncations and increase yield)
- Length of steps
  - Shorten denaturation step
  - Lengthen elongation step
- DMSO/glycerol/Betaine to destabilize DNA and thus allow lower denaturation temps and less secondary structure formation

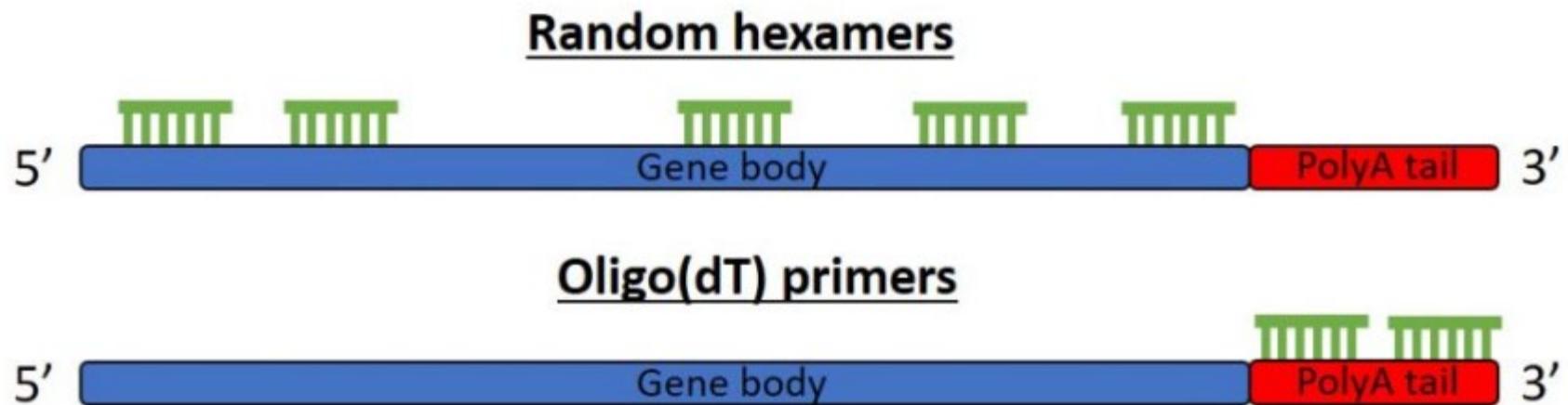
# QPCR (RT-PCR)

- Quantitative PCR/Real time PCR
- Uses a polymerase that fluoresces when bound to double stranded DNA



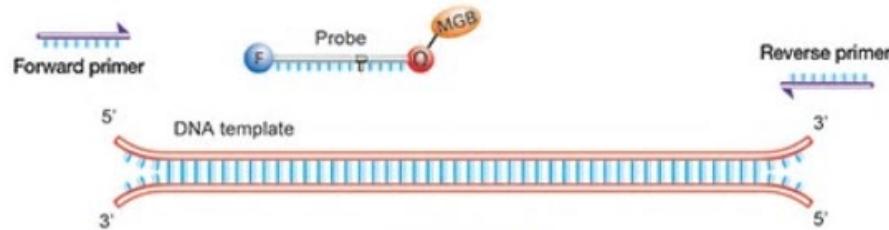
# Reverse transcription

- Step 1: Isolate and quantify RNA from sample
- Step 2: First strand synthesis-reverse transcriptase is used to create cDNA from RNA template
- Step 3: PCR (combination also confusingly called RT-PCR)

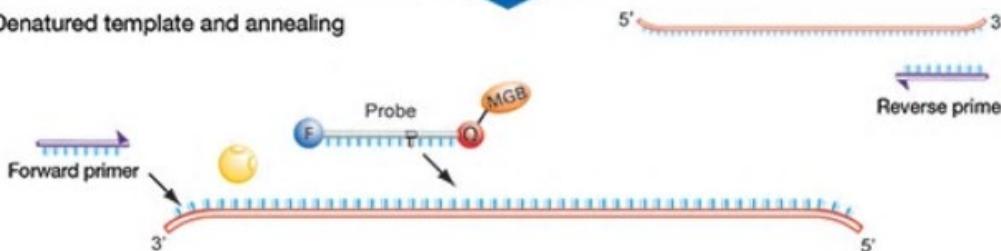


# Taqman assay

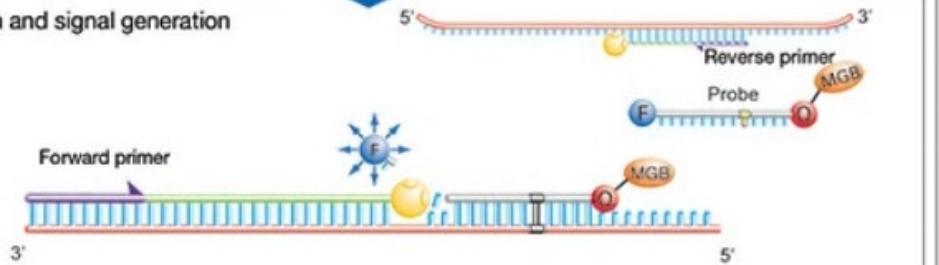
## 1. Assay components and DNA template



## 2. Denatured template and annealing



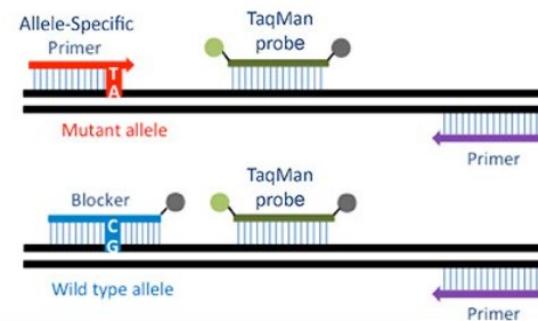
## 3. Polymerization and signal generation



## Legend

- F Applied Biosystems™ FAM™ or VIC™ dye
- Q Nonfluorescent quencher (NFQ)
- MGB Minor groove binder
- AmpliTaq Gold™ DNA Polymerase
- Probe
- Primer
- Template
- Newly synthesized DNA

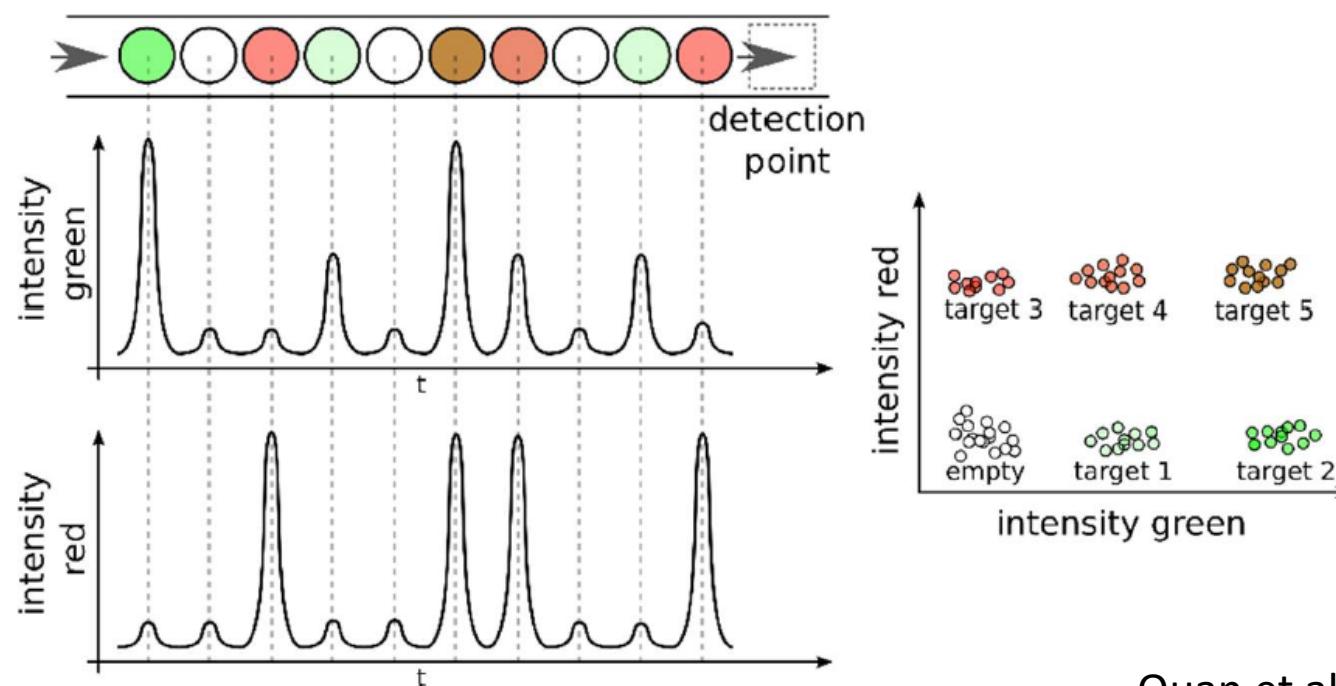
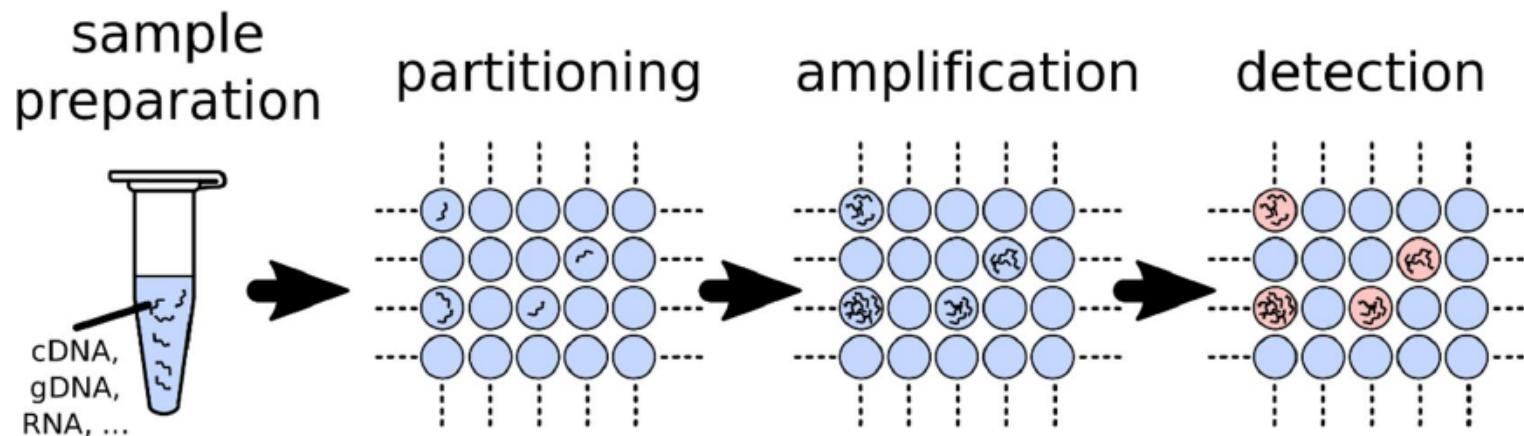
## Mutant allele assay



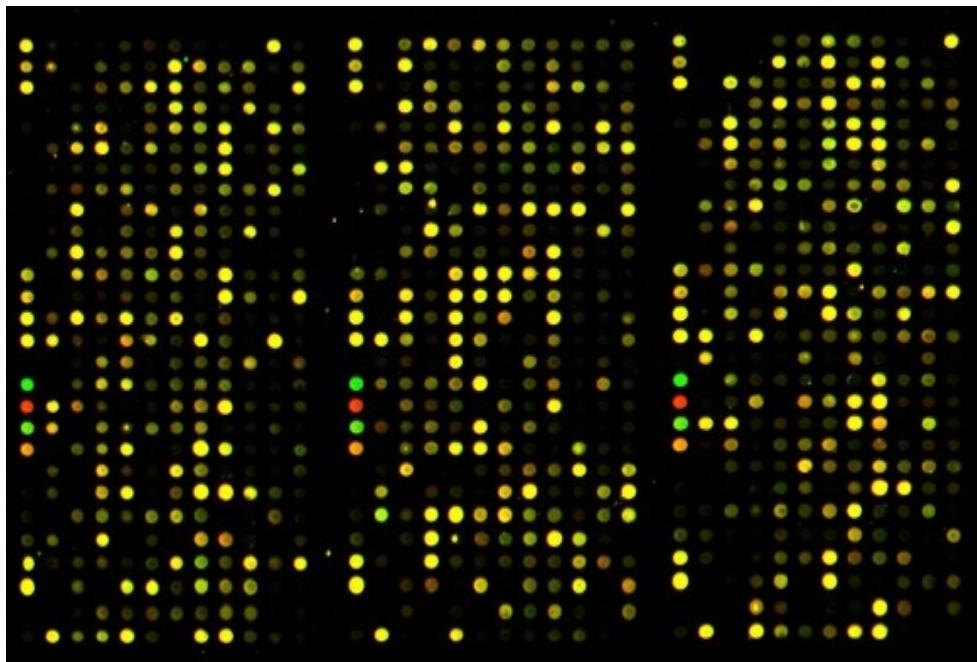
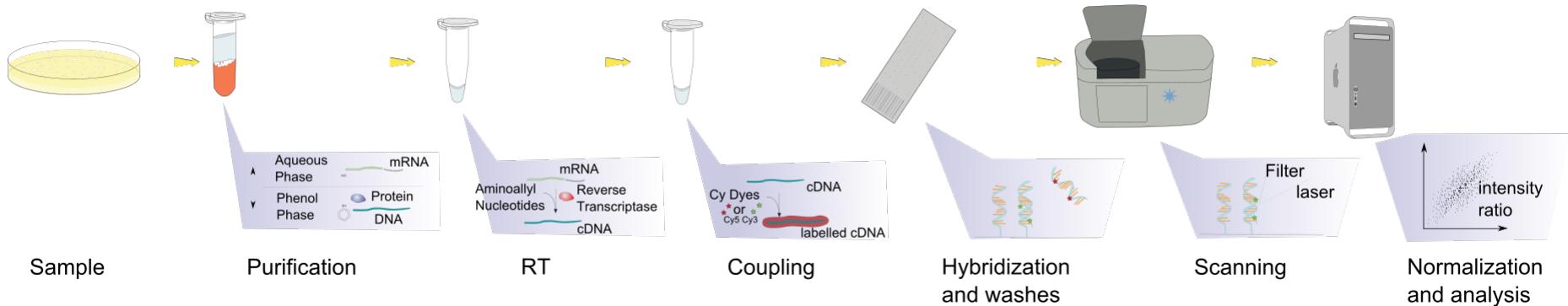
Allele-specific primer drives amplification of the mutant allele

MGB blocker oligonucleotide suppresses amplification of the wild type allele

# Digital PCR (droplet dPCR)



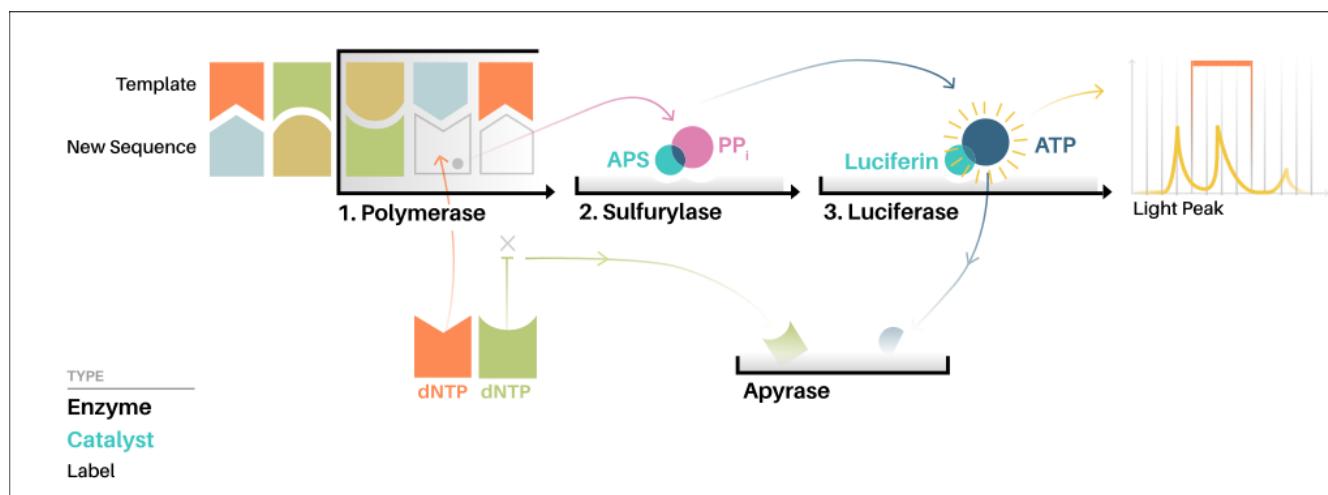
# Microarray (chip)



Invented by Pat Brown

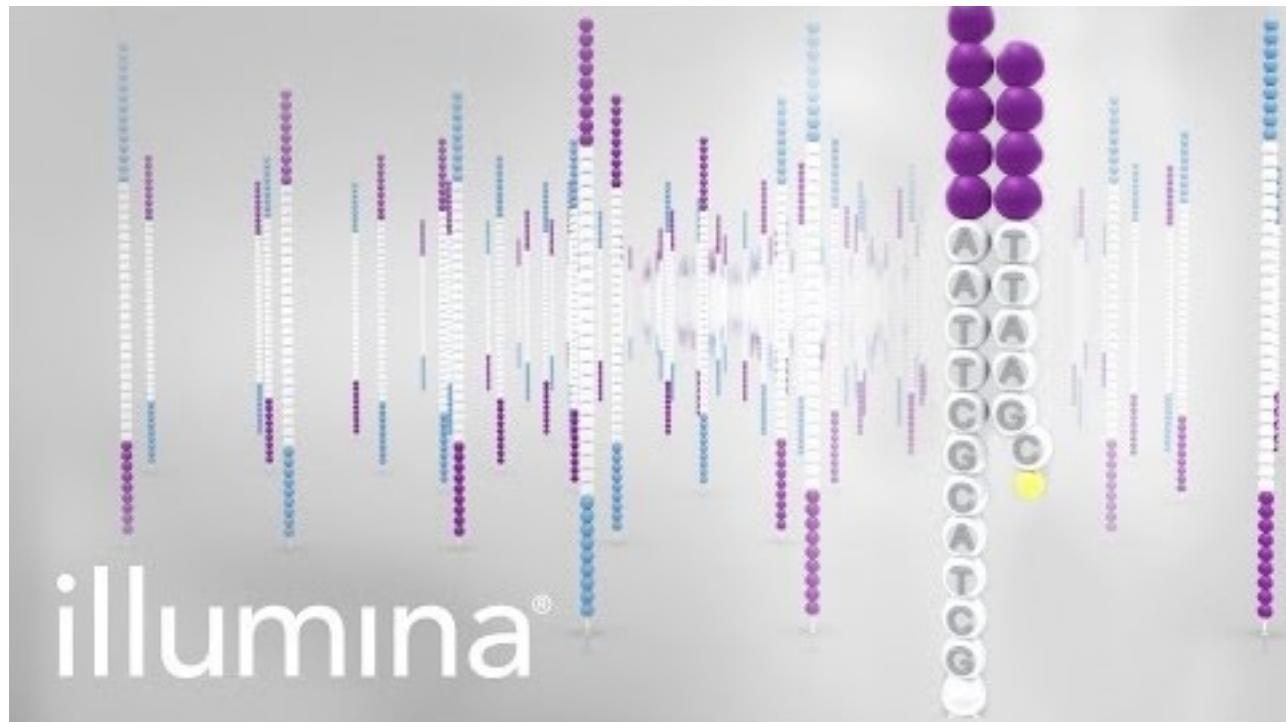
# Next Gen Sequencing

- Sequencing by synthesis
- Sequencing by ligation
- Pyrosequencing\*
- Ion semiconductor sequencing



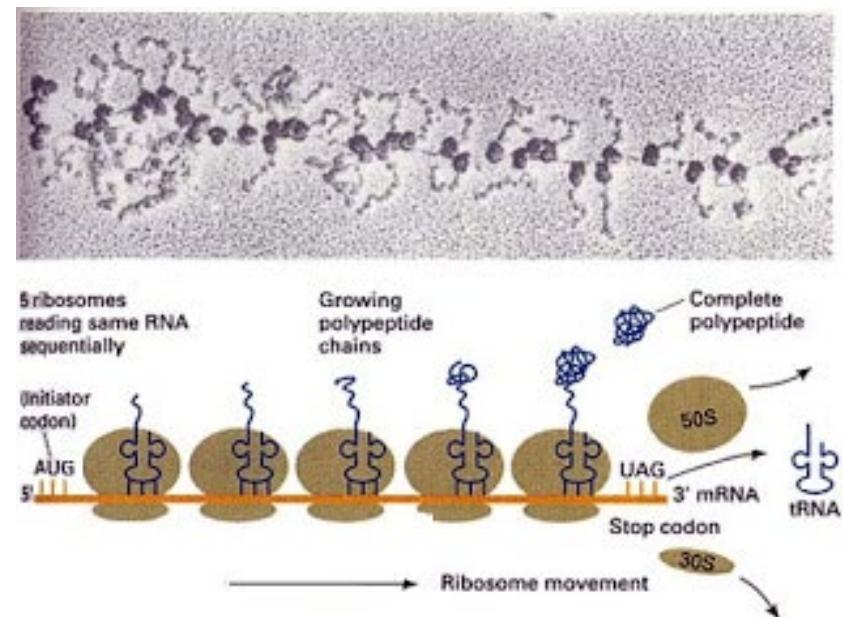
# Sequencing by

- Sample prep
- Cluster generation
- Sequencing
- Analysis



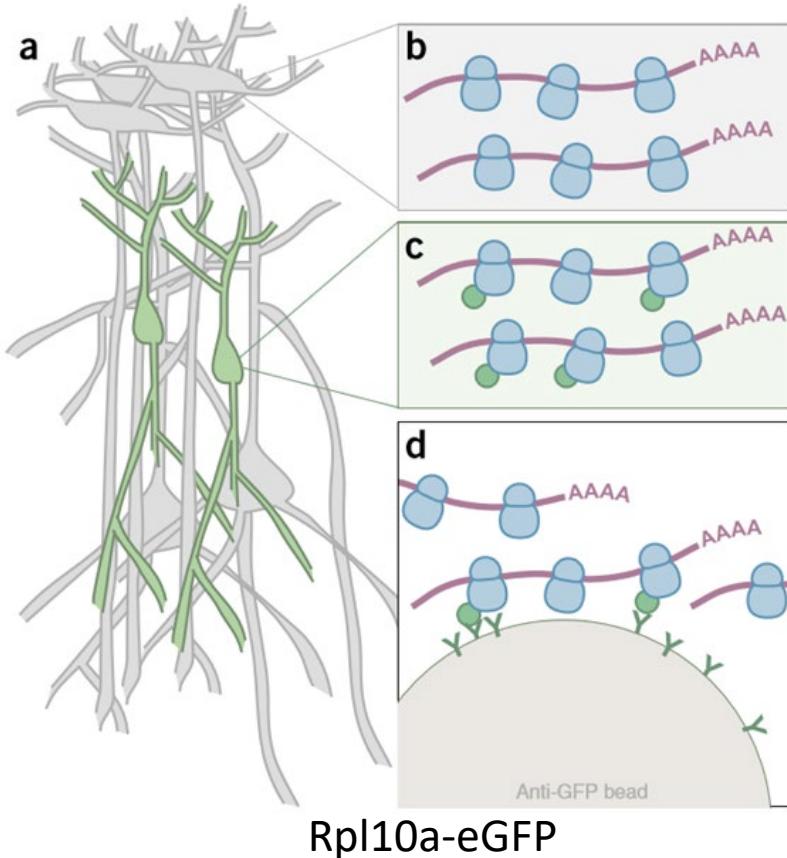
# Cell-type specific RNASeq via immunoprecipitation of ribosomes

- Based on gene expression: TRAP (translating ribosome affinity purification) and Ribotag
- Based on IEG: pS6



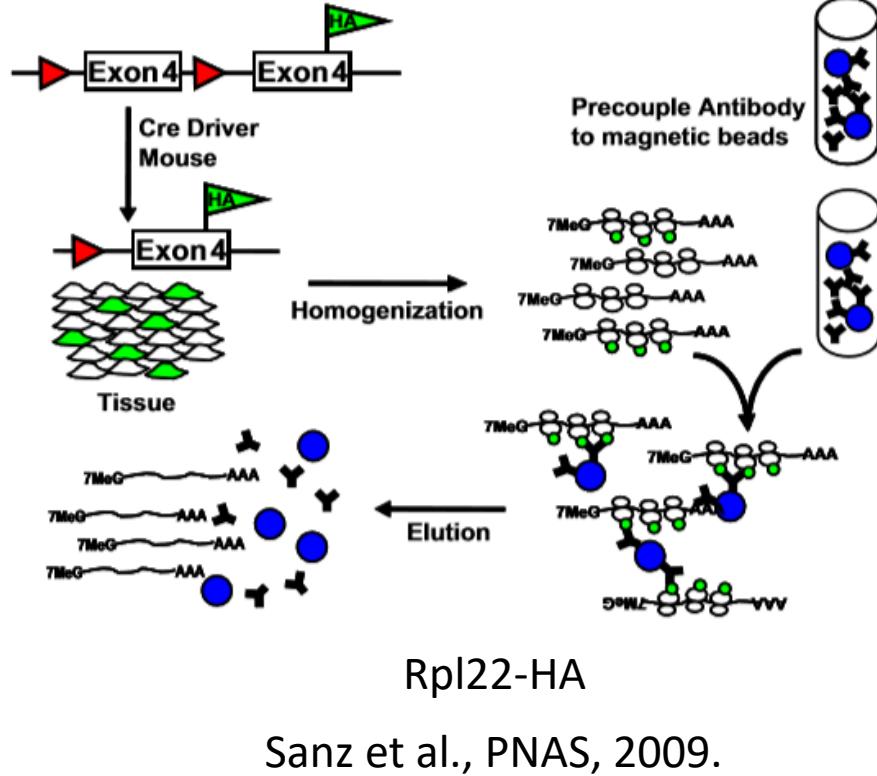
# TRAP/Ribotag

The “original” TRAP



Rpl10a-eGFP

Heiman et al., Cell, 2008.



Rpl22-HA

Sanz et al., PNAS, 2009.

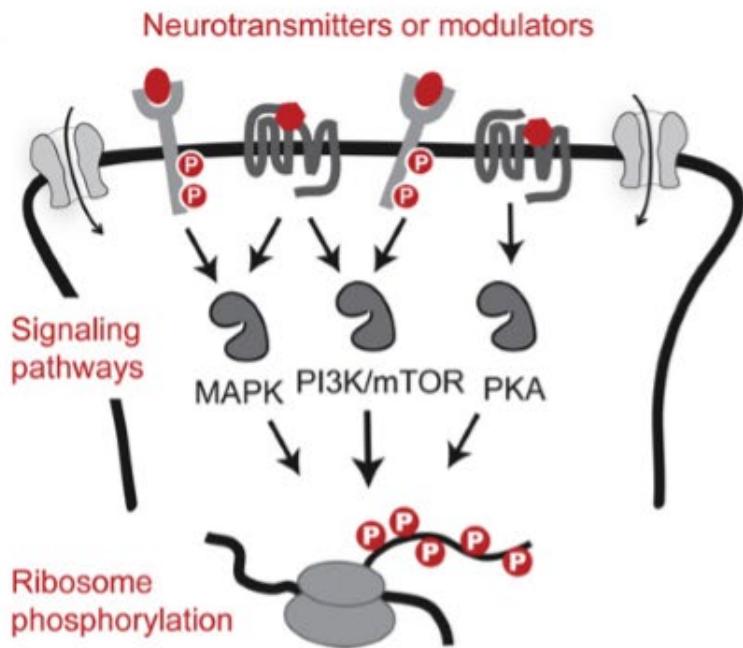


Protip: You can TRAP TRAP'ed cells

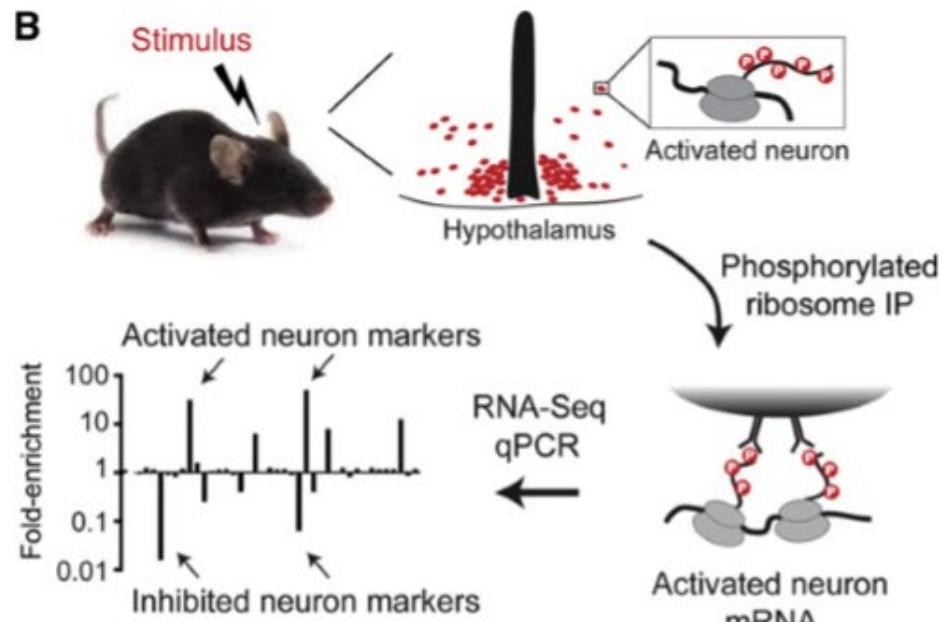
10/10 would recommend: Heiman et al., Nat. Protoc., 2014.

# pS6 pulldown

A

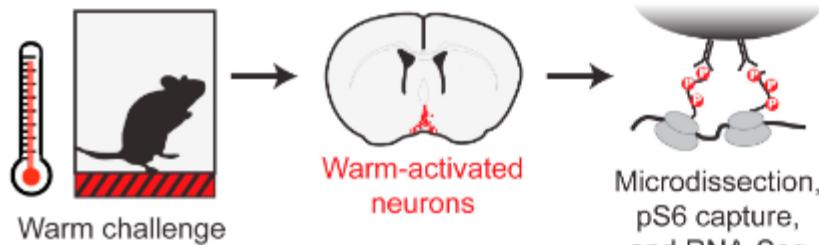


B

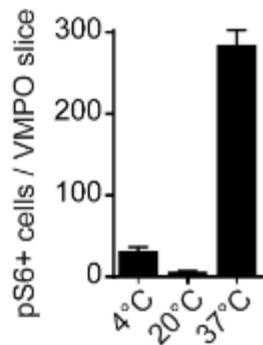


Knight et al., Cell, 2012.

A



B

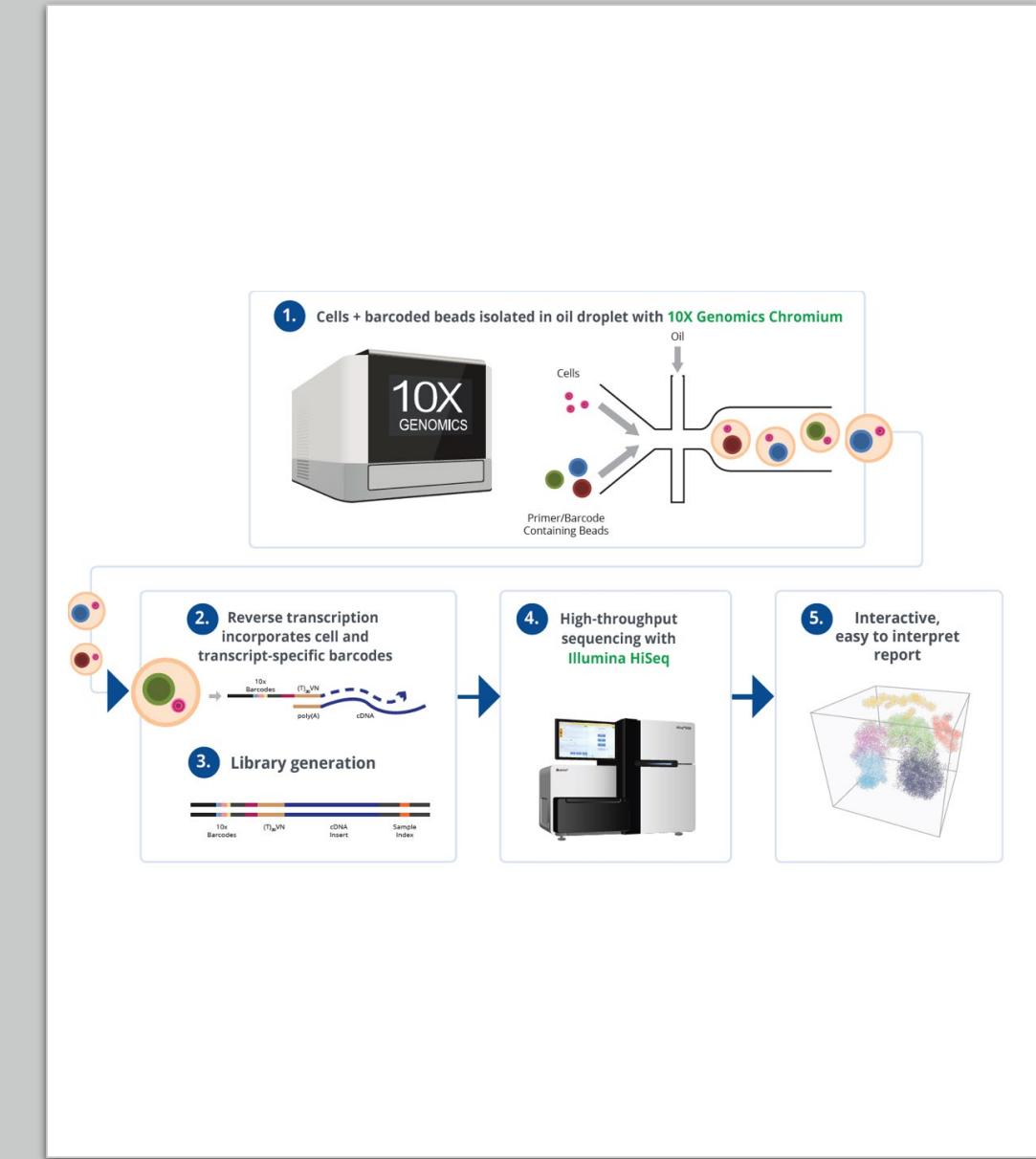


Protip: phosphoS6(244/247) (Invitrogen #44923G)

Tan et al., Cell, 2016.

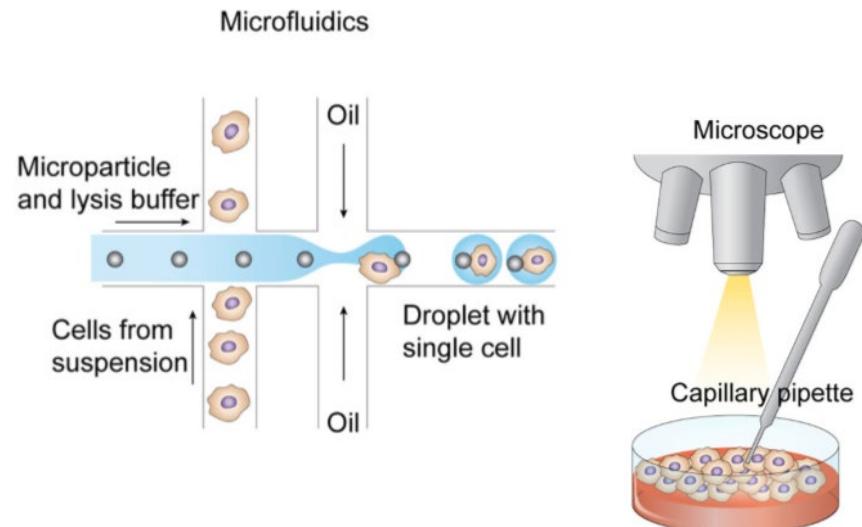
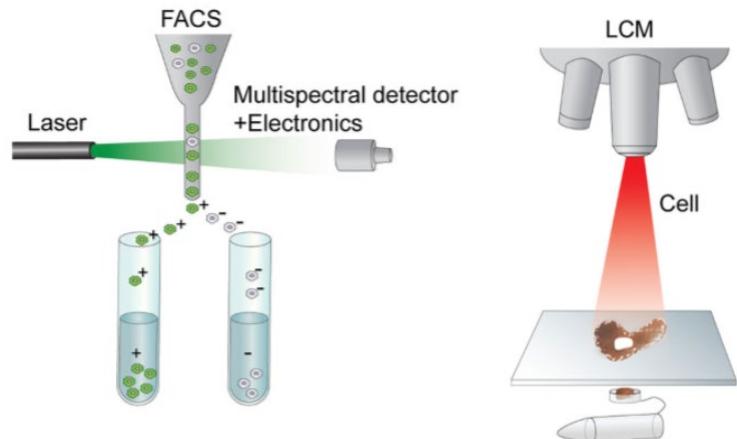
# Single cell RNASeq

- scSeq (single cell RNASeq)
- snSeq (single nucleus Seq)
- Steps:
  - Single cell dissociation
  - Single cell isolation
  - Library construction
  - Sequencing

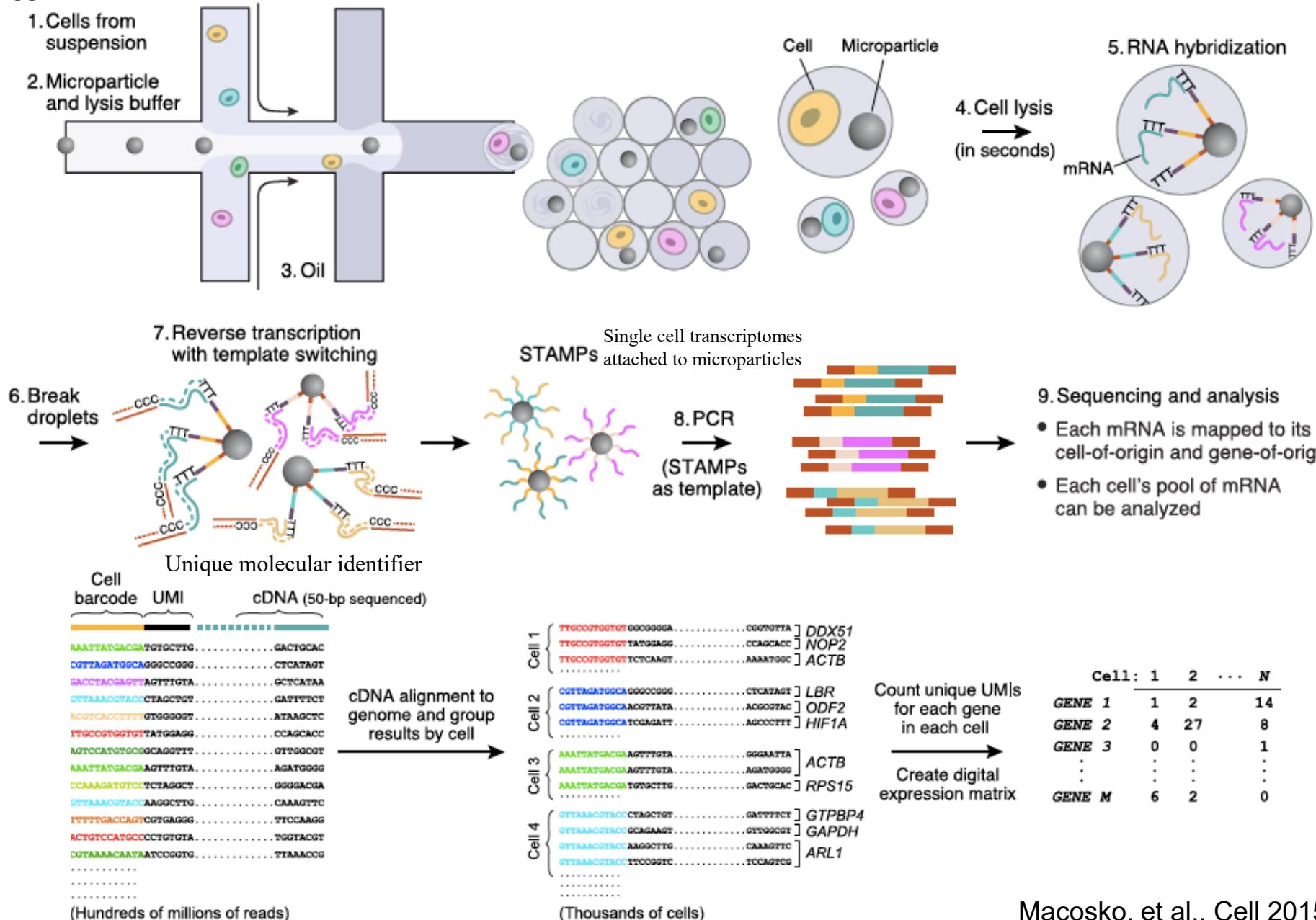


# Capturing single cells/nuclei

- Cells (neurons)
  - FACS sorting
  - Microfluidics (Drop-seq)
  - Laser capture microdissection
  - Pipette dissociated cells
- Nuclei
  - Lyse, pellet, FACS

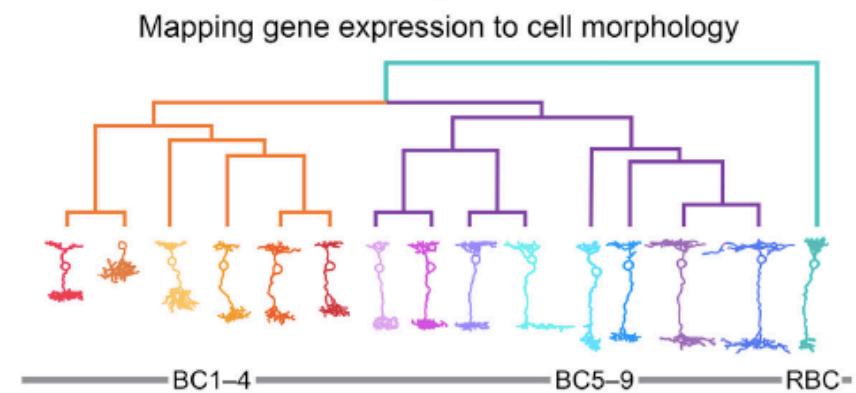
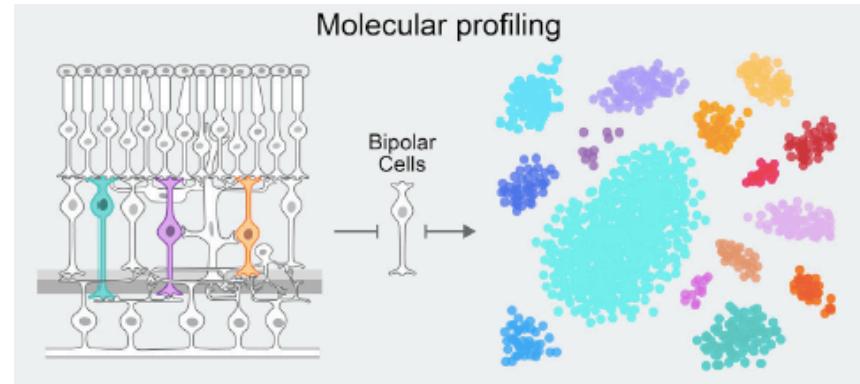
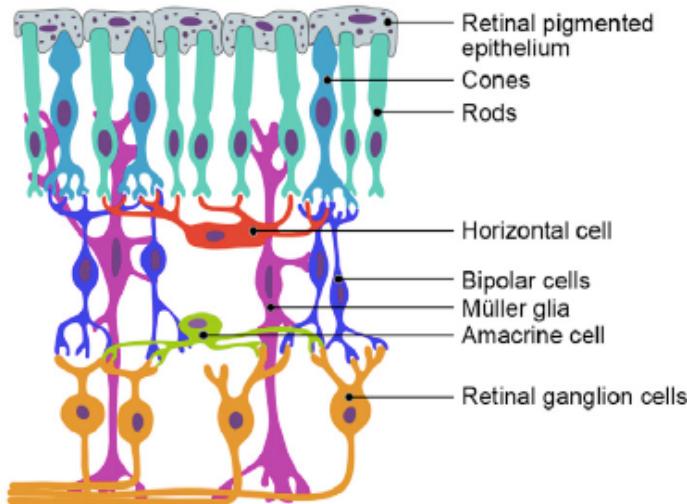


# Expression profiling of individual retinal cells using DropSeq



Macosko, et al., Cell 2015

# Expression profiling of individual retinal cells using DropSeq



- Previously, morphological data suggested 9-12 types of bipolar cells
- 25,000 bipolar cell transcriptomes reveals 15 types of bipolar cells

# Analysis at single cell level

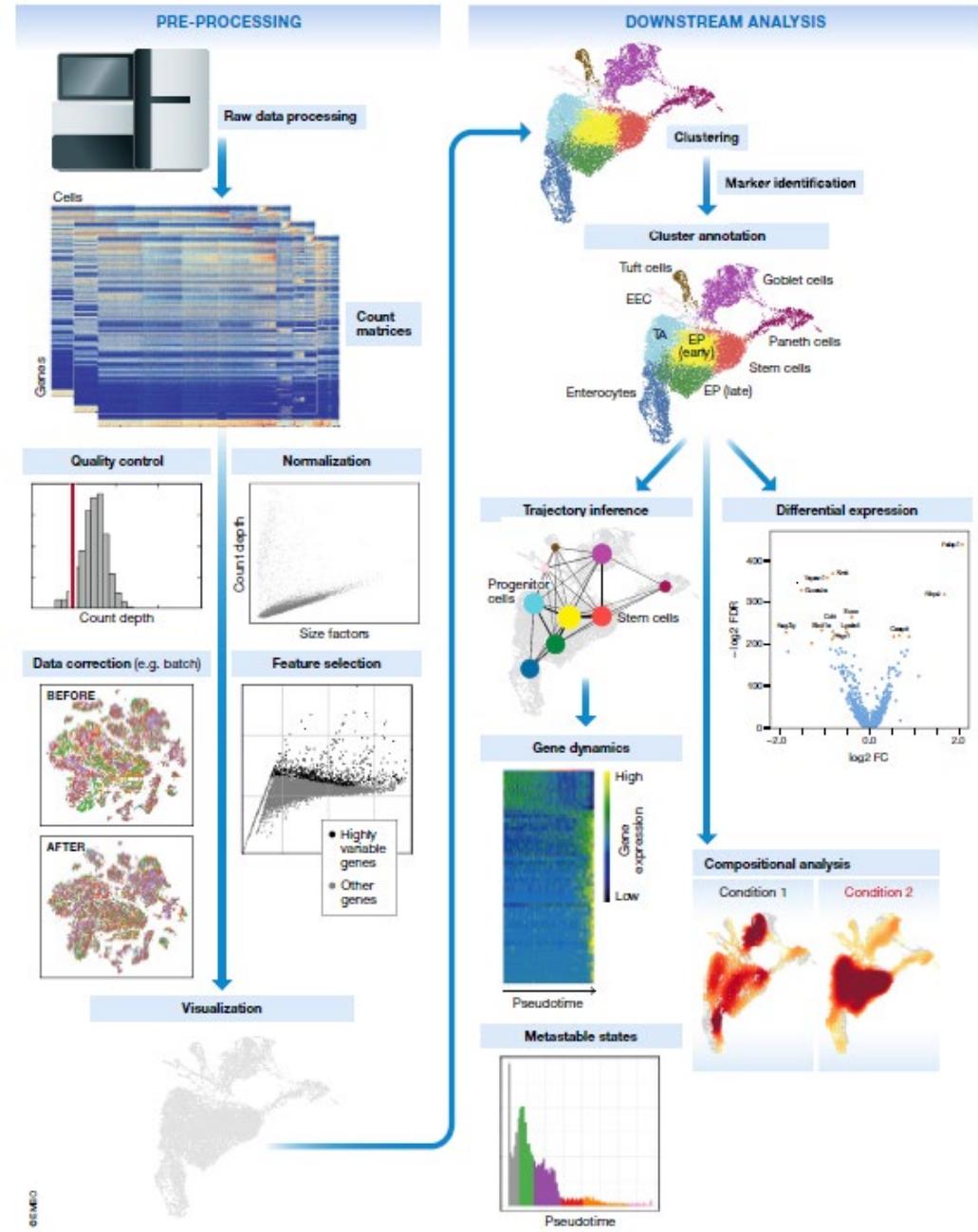


Figure 1. Schematic of a typical single-cell RNA-seq analysis workflow.

Raw sequencing data are processed and aligned to give count matrices, which represent the start of the workflow. The count data undergo pre-processing and downstream analysis. Subplots are generated using the best-practices workflow on intestinal epithelium data from Haber et al (2017).

# Python libraries for single cell analysis

kallisto | bustools

About Download Introduction Tutorials

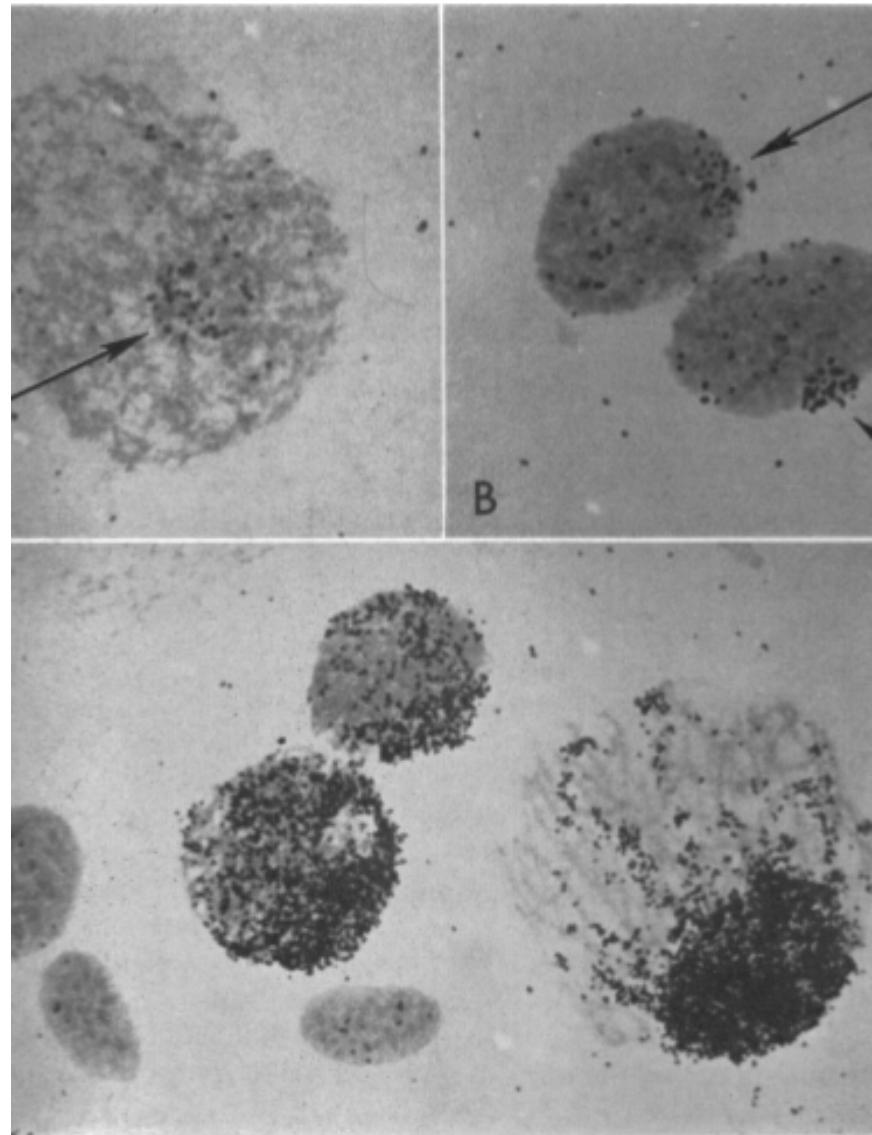
## Tutorials

Note: All Google Colab notebooks can be run by selecting `Runtime > Run all > Run anyway` within the notebook.

Tutorial	Description	Colab Link	Preview	Time (min)
Introduction 0	Introduction to single-cell RNA-seq	<a href="#">python</a>	<a href="#">python</a>	0.58
Introduction 1	Pre-processing and quality control	<a href="#">python</a> , <a href="#">R</a>	<a href="#">python</a> , <a href="#">R</a>	1.36
Introduction 2	Getting started with analysis	<a href="#">python</a> , <a href="#">R</a>	<a href="#">python</a> , <a href="#">R</a>	13.5
Analysis 0	Building and annotating an atlas	<a href="#">python</a> , <a href="#">R</a>	<a href="#">python</a> , <a href="#">R</a>	31.2
Analysis 1	Perform pseudotime analysis	<a href="#">R</a>		
Analysis 2	Process single-nuclei RNA-seq	<a href="#">python</a>		
Analysis 3	Perform RNA velocity analysis	<a href="#">python</a> , <a href="#">R</a>		
Analysis 4	Quantify multi-modal data	<a href="#">python</a>		
FAQ 0	Finding and downloading data	<a href="#">python</a>		
FAQ 1	Building consistent and custom indices	<a href="#">python</a>		
FAQ 2	Building RNA velocity indices	<a href="#">python</a>		
FAQ 3	Combining two count matrices	<a href="#">python</a>		
FAQ 4	Processing multiple sets of FASTQs	<a href="#">python</a>		
FAQ 5	Processing multi-species data	<a href="#">python</a> , <a href="#">R</a>		
FAQ 6	Pseudotime with Monocle2	<a href="#">R</a>		
FAQ 7	Parsing a BUS file in R	<a href="#">R</a>		

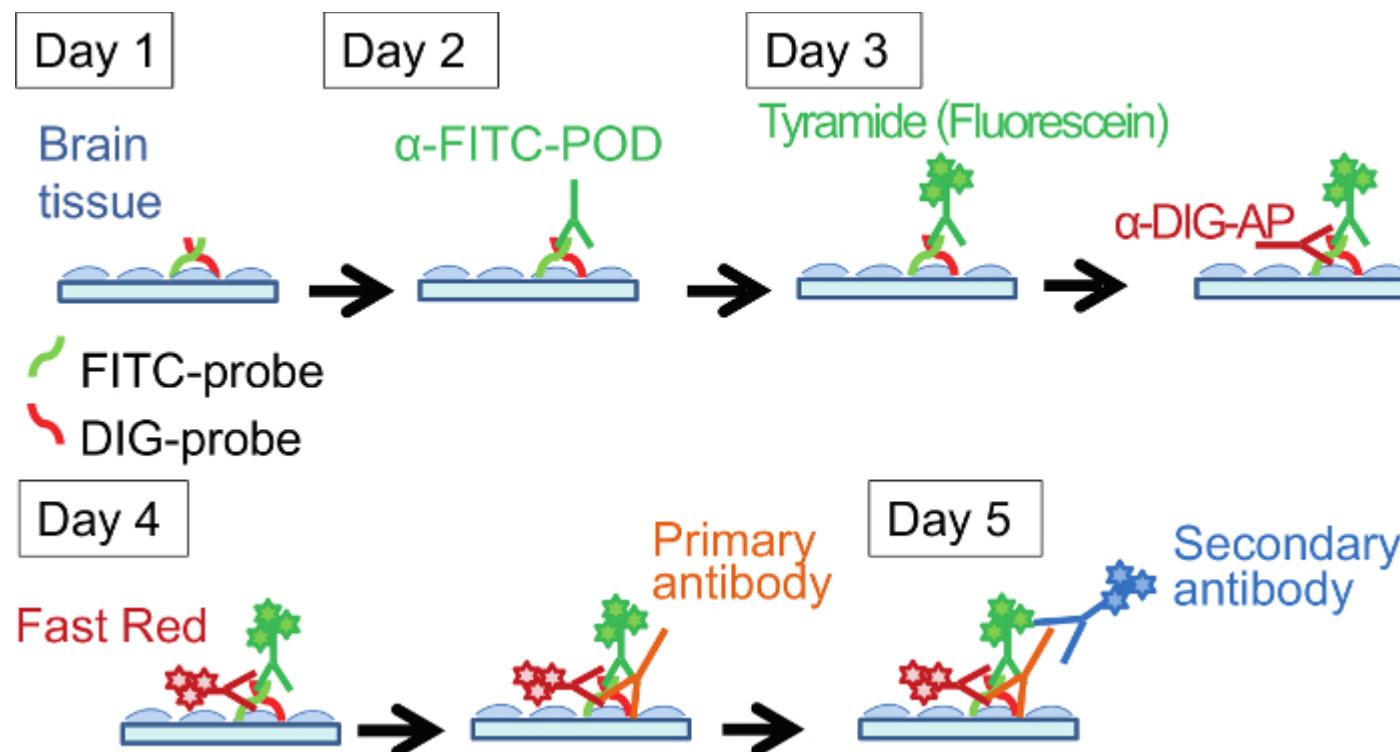
# In Situ Hybridization (ISH)

- Labeled complementary probe localizes a specific sequence in tissue
- Invented by Gall and Pardue in 1969
- Originally probes were labeled using radioactive UTPs, then other labels introduced that could be detected by light or fluorescence microscopy



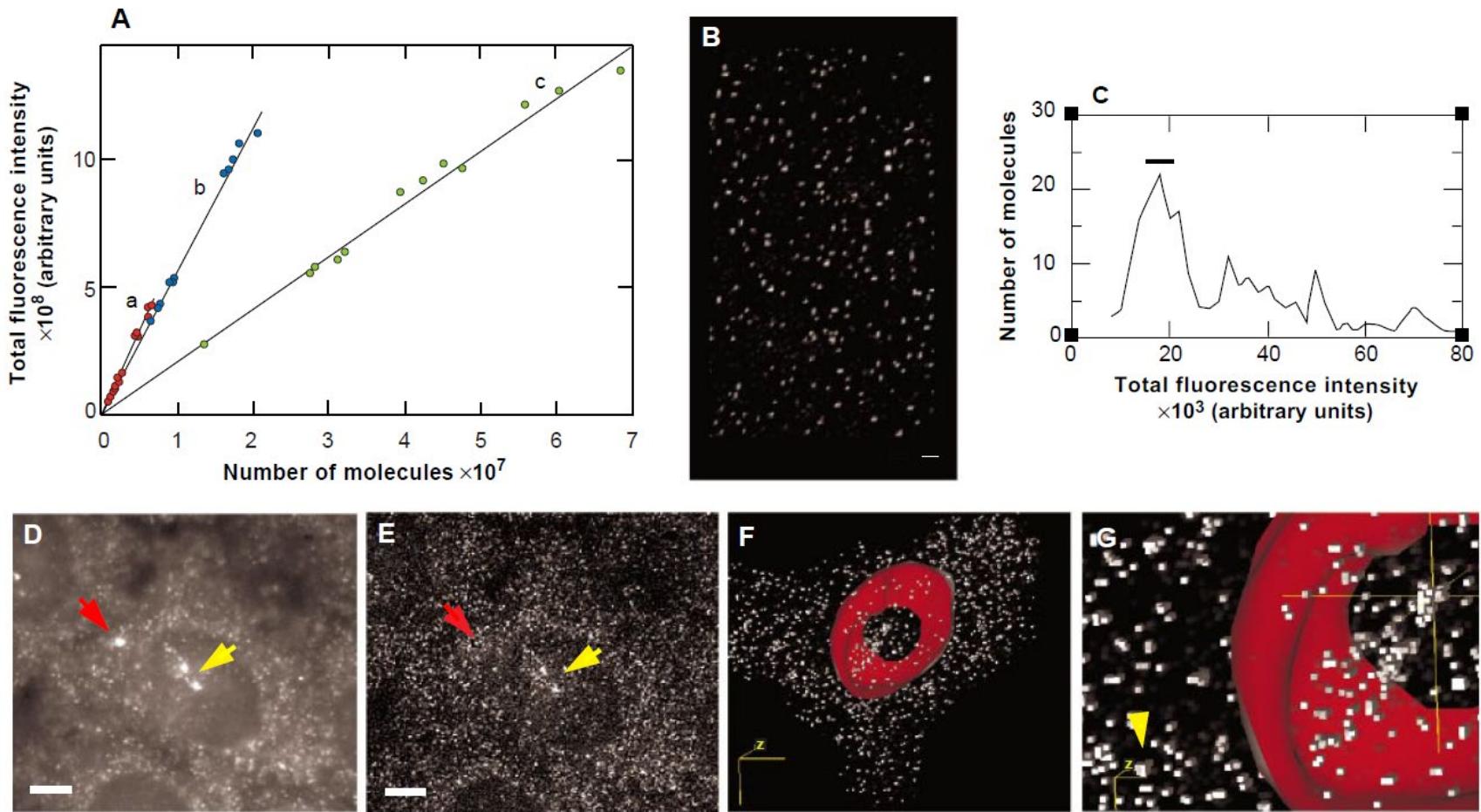
Gall and Pardue, Genetics, 1969.

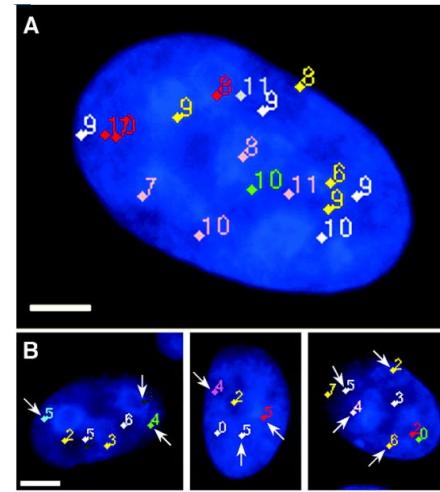
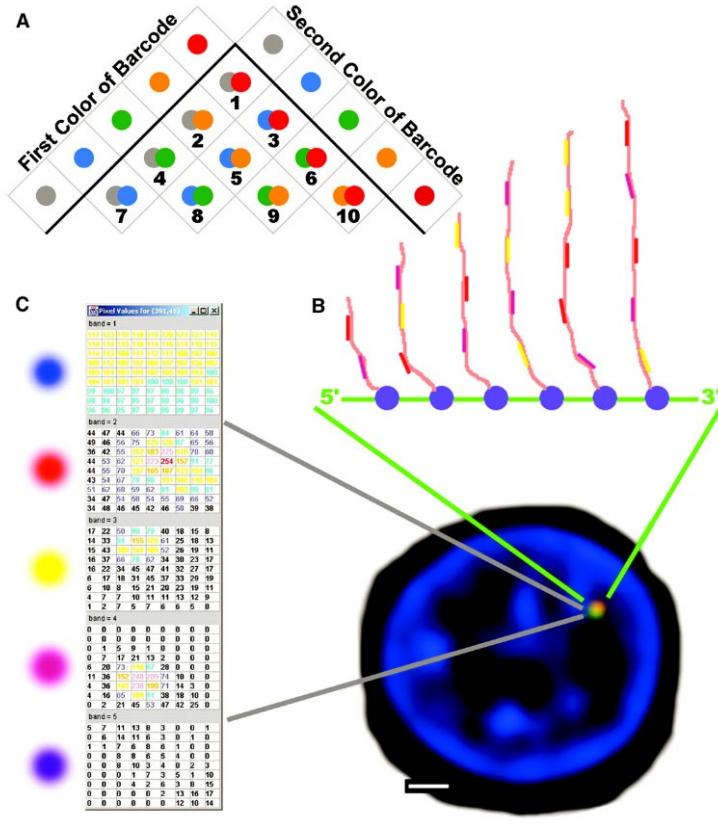
# Fluorescent ISH



# Single Molecule FISH (smFISH)

- Invented by Dr. Rob Singer in 1998





C	Pseudo FITC	Cy3	Cy3.5	Cy5
EGR-1	Red	Green	Black	Black
$\beta$ -actin	Pink	Green	Black	Orange
$\gamma$ -actin	White	Green	Yellow	Orange
c-myc	Yellow	Green	Black	Red
c-jun	Yellow	Green	Black	Black
Cyclin D1	Purple	Green	Black	Orange
IL-8	Cyan	Black	Yellow	Orange
MCL-1	Black	Black	Green	Red
TIEG-1	Light Green	Black	Black	Orange
DUSP-1	Green	Black	Yellow	Red

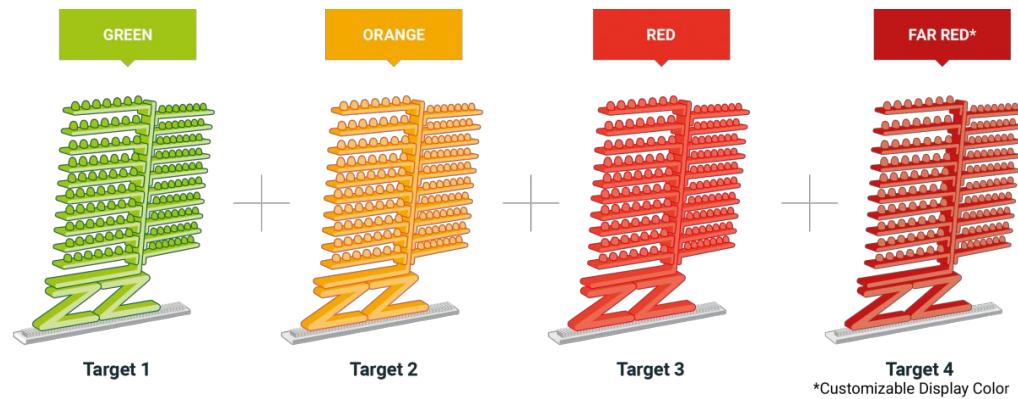
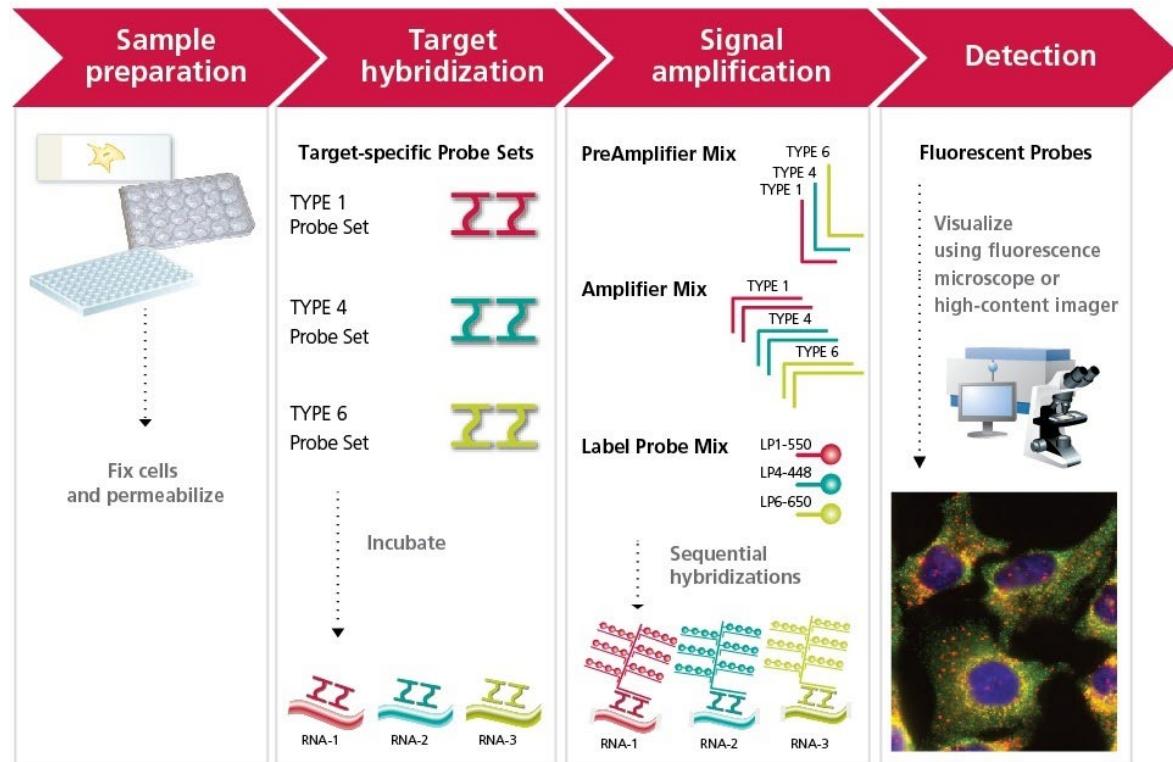
# smFISH with barcoding

Levsky et al., Science, 2002.

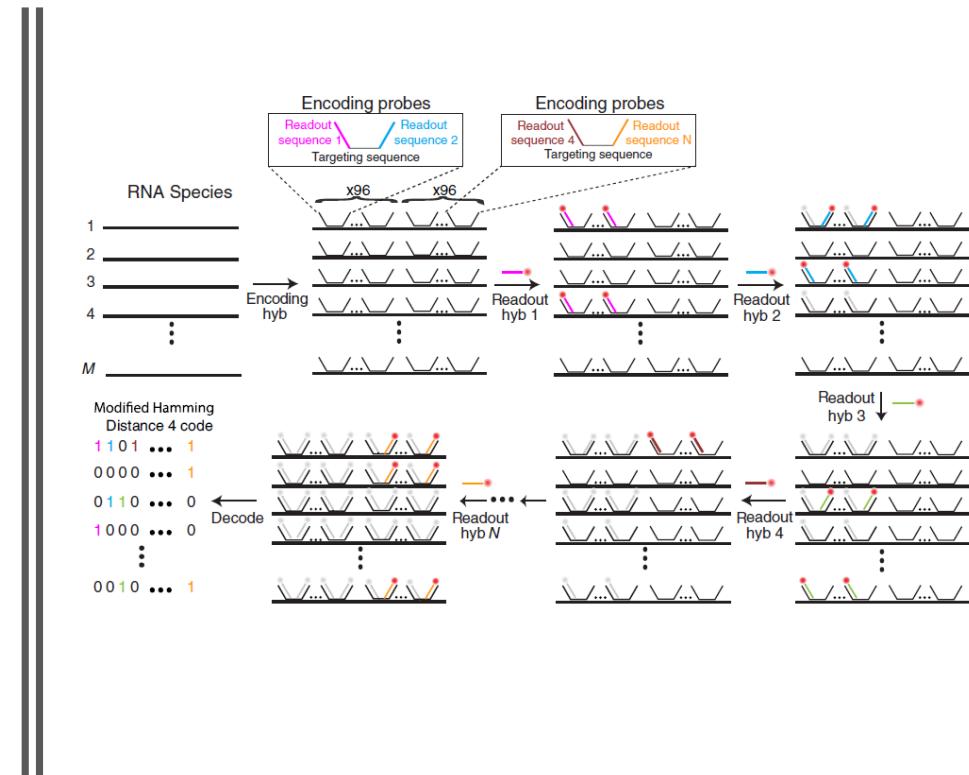
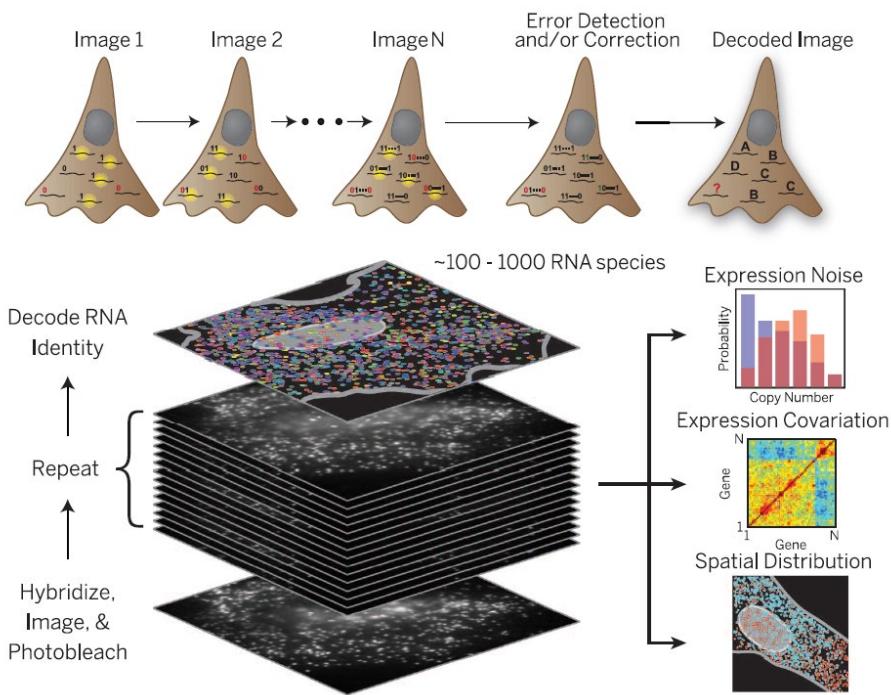


# Commercial FISH kits

- ViewRNA (Thermo)
- RNAScope (ACDBio)

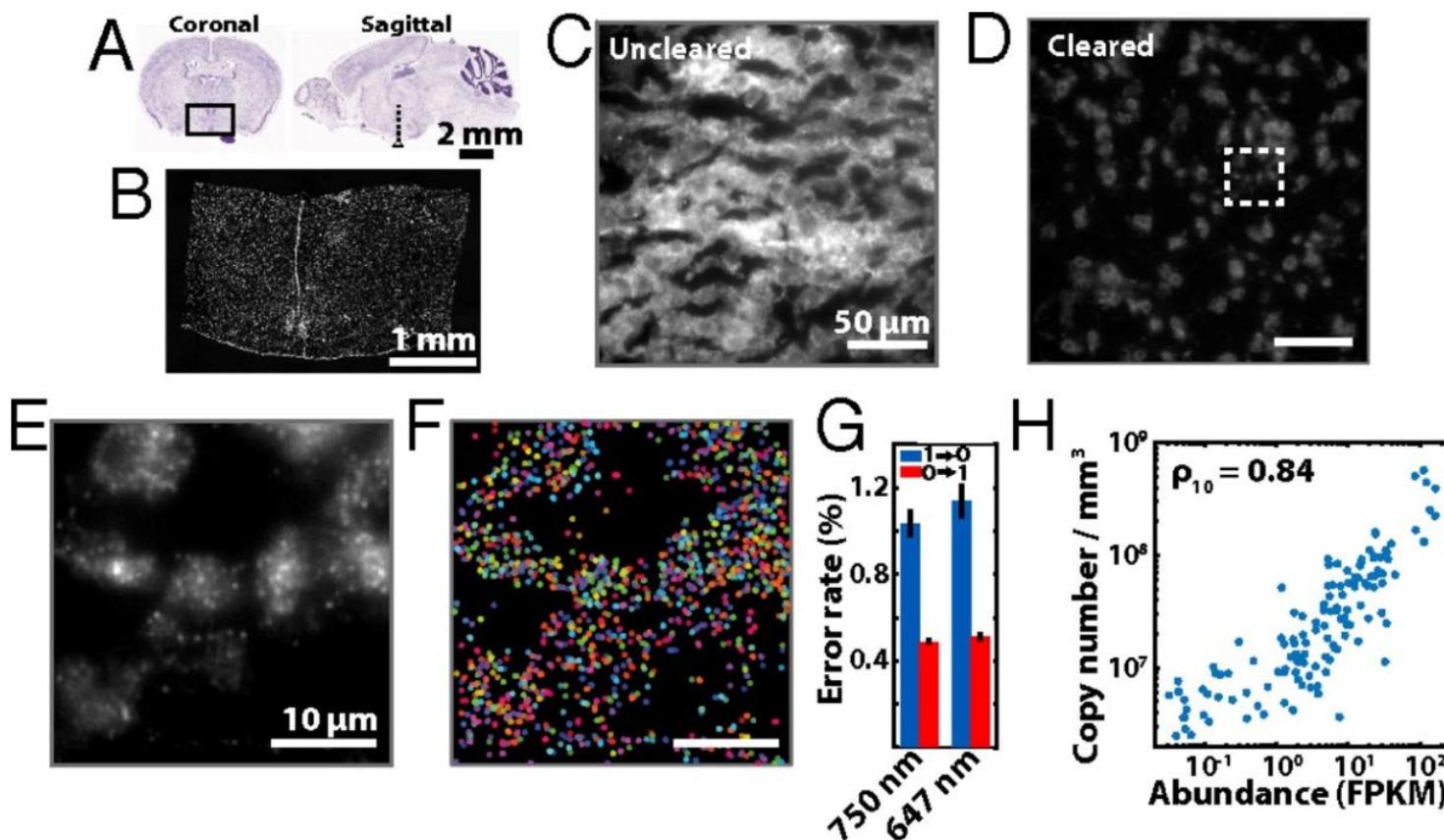


# Multiplexed error robust FISH

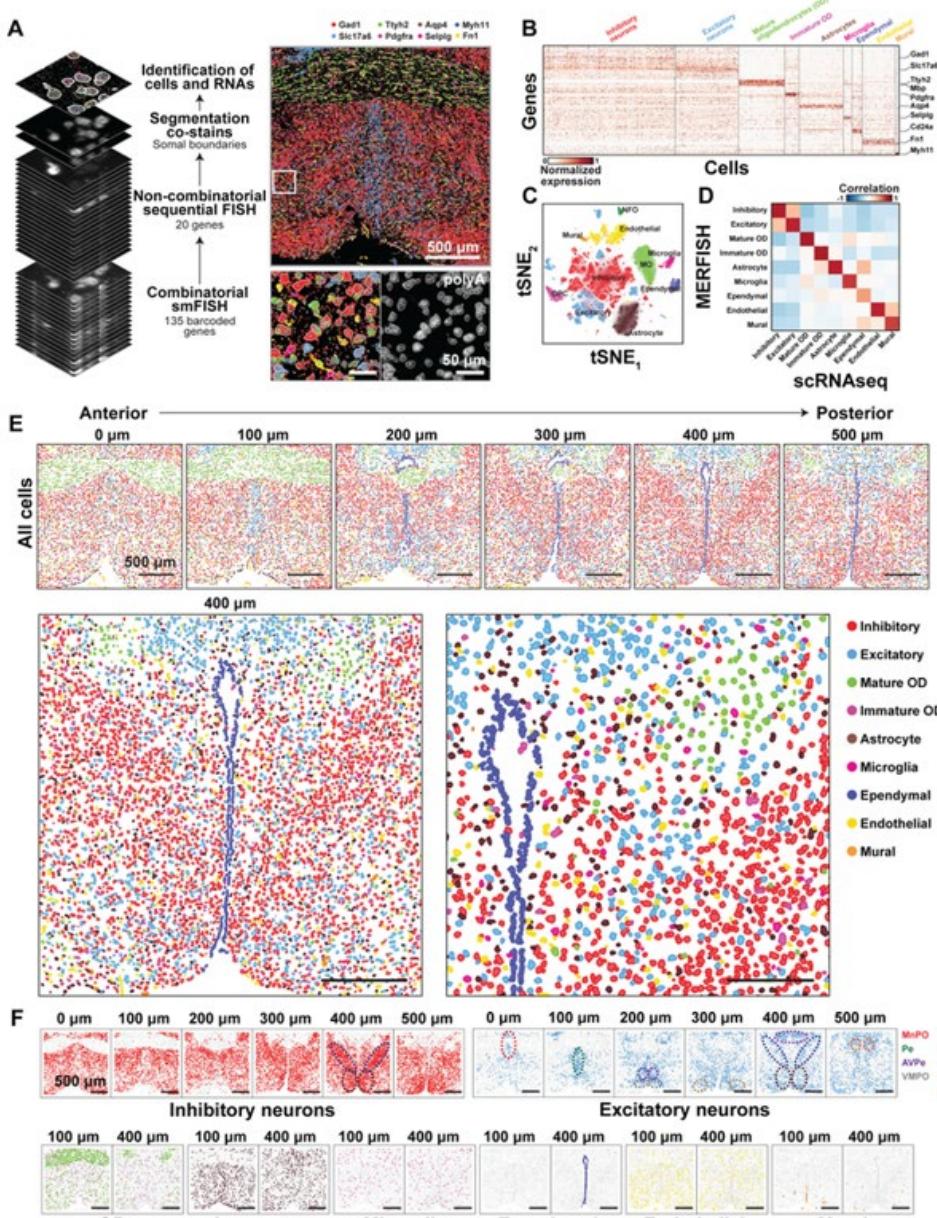
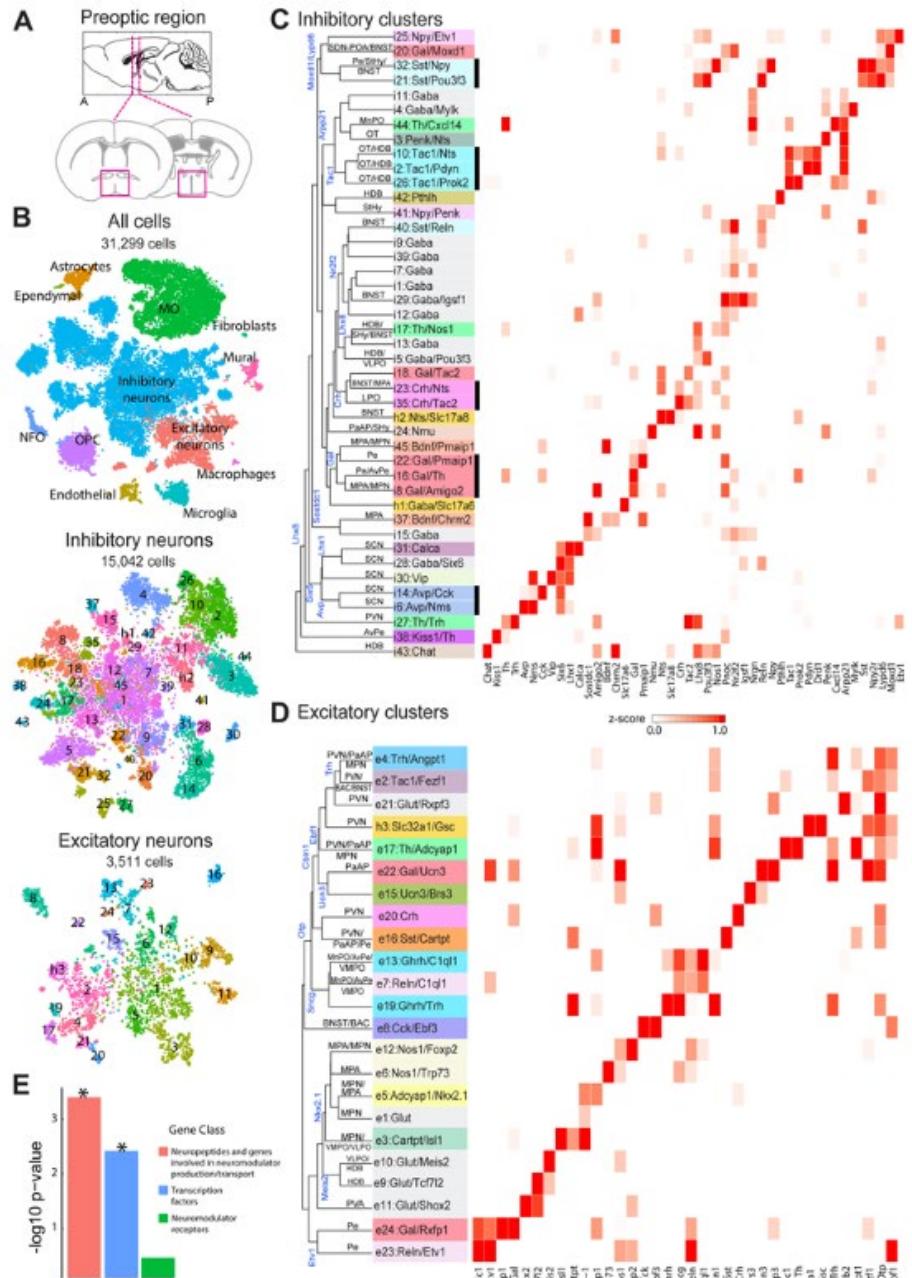


# MERFISH in brain tissue

- Tissue: high autofluorescence and non-specific probe binding to proteins and lipids
- Solution: gel embed and clear



# scRNASeq + MERFISH



Moffitt\*, Bambah-Mukku\*, Science, 2018.