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Techniques in cellular neurobiology

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Neuroscience read out

Neuroscience *in vivo* :

1) behavioral
2) metabolism
3) toxicology
4) electrophysiology

etc

NB (In vivo only on KNOWN TARGET)

Neuroscience *in vitro* :

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TARGETS

DNA

RNA

PROTEIN

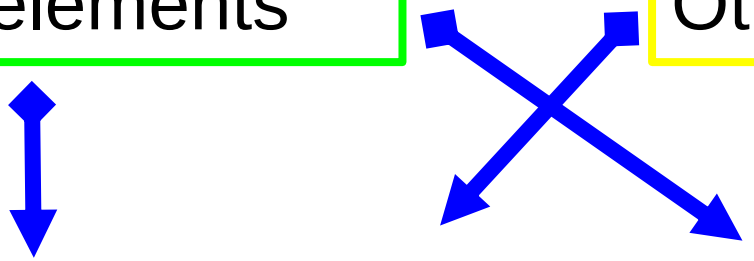
Other elements

DNA – Protein

RNA-Protein

PROTEIN-Protein

Other elements



UNKNOWN TARGET

KNOWN TARGET

Neuroscience *in vitro* : a NOT complete list

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DNA Microarray Methods

- DNA Microarray Maker — Unknown target
- cDNA production — Unknown target
- Random Priming
- *in situ* Hybridization — Known target
- Genome-wide response to Glucose Consumption — Unknown target
- Chuck Close and DNA Micorarrays — Unknown target

Genomic Circuits Methods

- Plasmids with inducible promoters — Known target
- CAT Assays — Known target
- GFP and reporter proteins/genes — Known target
- Growth Curves — Known target, Unknown target
- Homologous Recombination — Known target
- Brain Anatomy — Known target, Unknown target

Neuroscience *in vitro* : a NOT complete list

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Proteomics Methods

•Domain Functions	Known target
•	
•Yeast Two Hybrid	Unknown target
•Cre / *lox* P recombination	Known target
•Biotin and Avidin binding	Known target
•Affinity Chromotography	Known target Unknown target
•Kinase and enzyme assays	Known target
•RNAi (RNA interference)	Known target
•Mass Spectroscopy	Unknown target

Neuroscience *in vitro* : a NOT complete list

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Genomic Methods

- Pathology/Histology Slides — Unknown target
- Karyotypes — Known target, Unknown target
- Immunoprecipitation
- PCR — Unknown target
- SDS-PAGE — Known target, Unknown target
- Coomassie Staining — Unknown target
- Western Blot — Known target
- Southern Blot — Known target
- Northern blot — Known target
- Immunofluorescence — Known target
- Chromosomal Walking — Unknown target
- RFLP
- Knockout Mouse and Homologous Recombination — Known target
- Liposomes

Neuroscience *in vitro* : a NOT complete list

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- Capillary Electrophoresis — Known target — Unknown target
- ELISA (see animated version) — Known target
- FACS (Fluorescence Activated Cell Sorting) — Known target
- Knockout Mouse and Homologous Recombination — Known target
- PCR — Known target
- Real-time PCR — Known target
- RT-PCR (reverse transcriptase-PCR) — Known target
- Whole-Genome Sequencing — Unknown target

Neuroscience *in vitro* : a NOT complete list

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UNKNOWN TARGET

BIOINFORMATICS

http://www.ebi.ac.uk/

MicroARRAYS

mRNA

miRNA

ncRNA

SNP

Protein

Biochemical read out

ALL TARGETS

Biochemical read out

2 Gel-Electrophoresys

2 Hybrid Screen

Biochemical read out

High-throughput screening

screening

Biochemical read out

etc

DNA

cDNA Microarray

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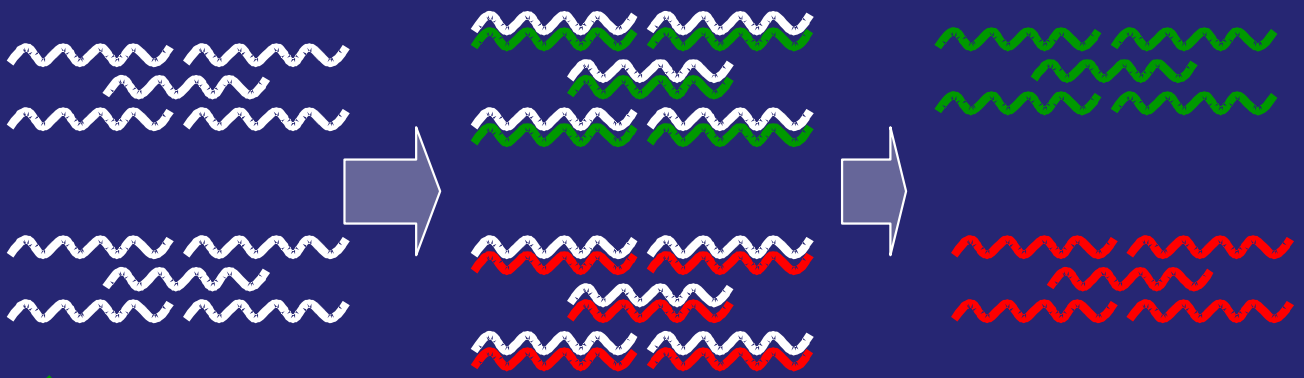
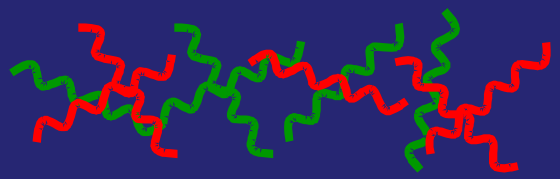
mRNA

Reverse transcription

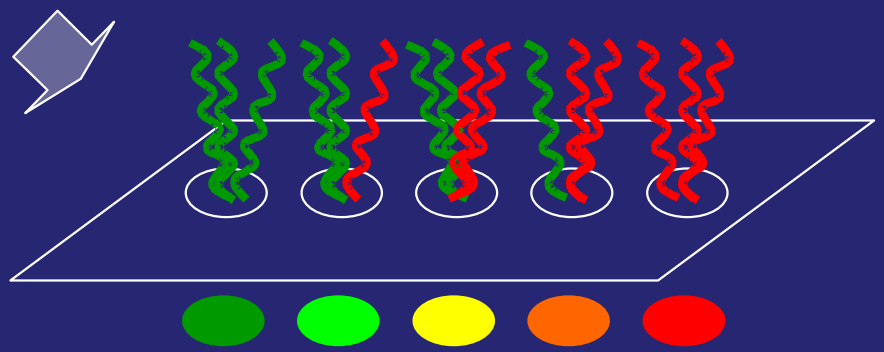
Labeled 1st strandcDNA

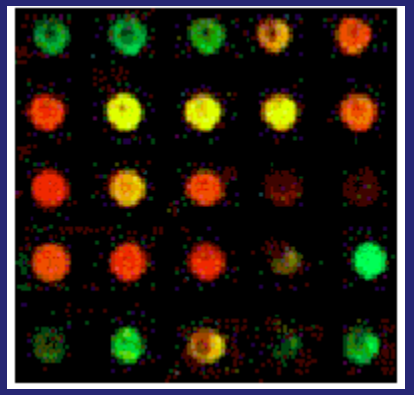
Sample 1 (test)





Hybridization on thecDNA microarray





cDNA Microarray

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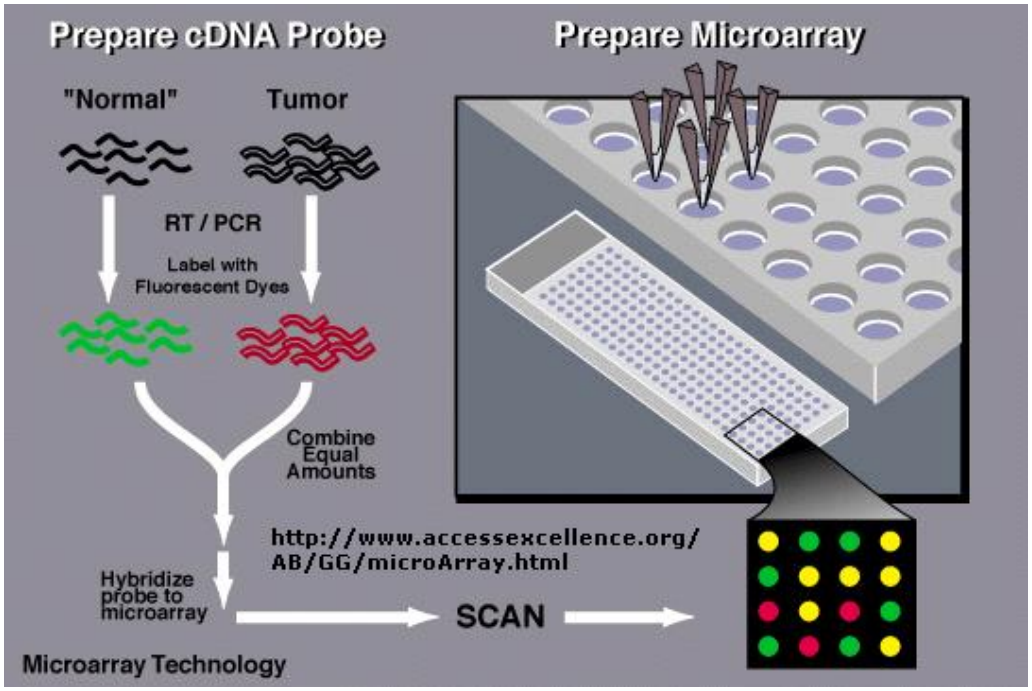
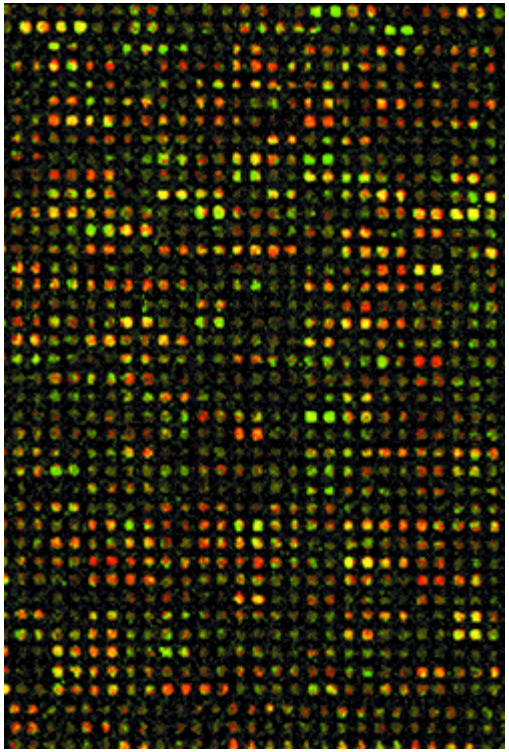
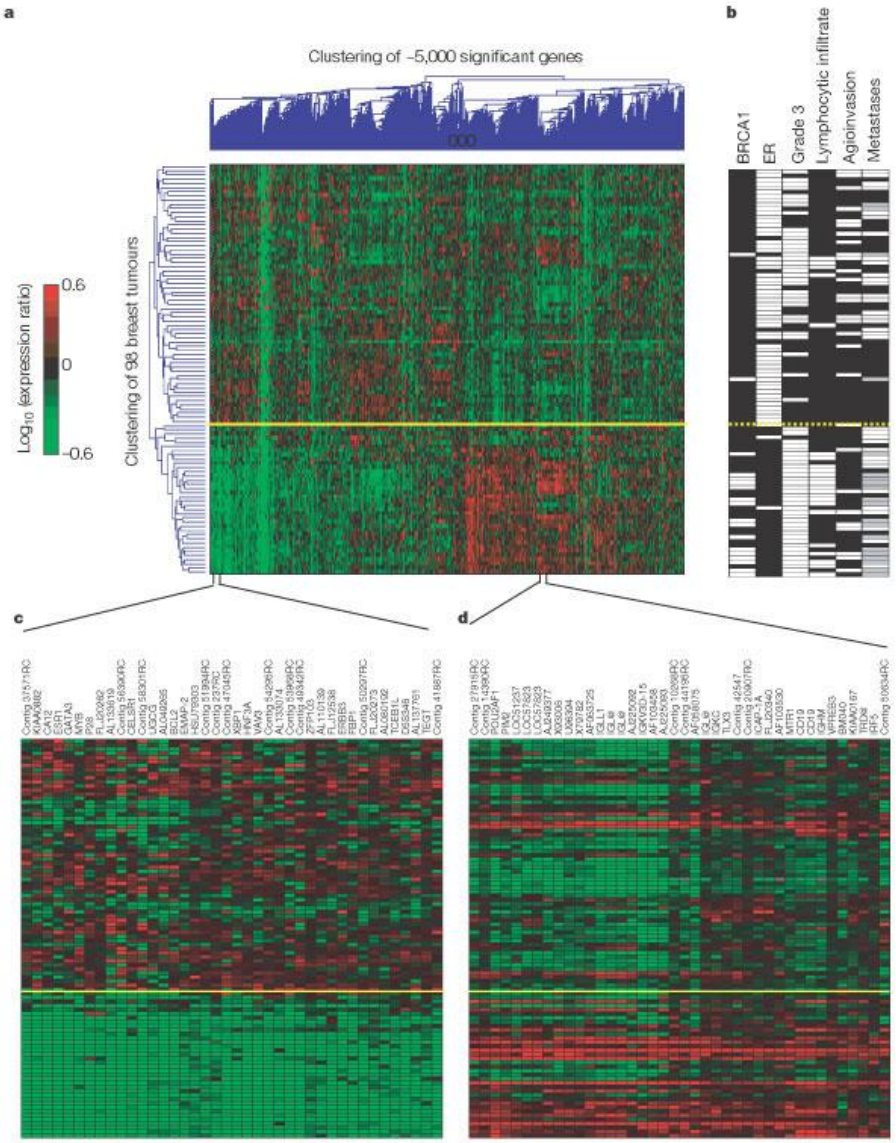




cDNA Microarray read out



Available microarrays
also
for RNAs and Proteins

Protein

2D-Gel electrophoresis

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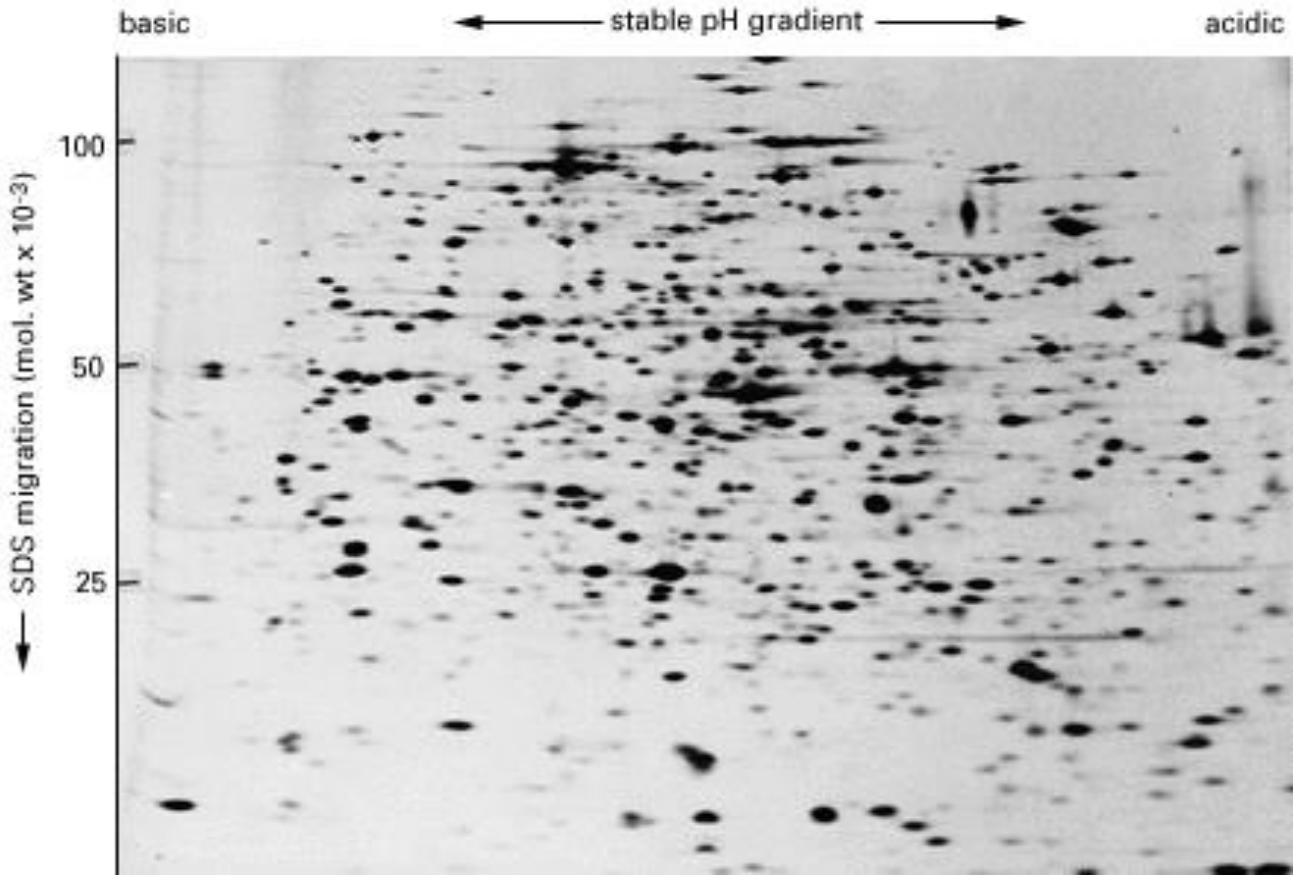

Neuroscience *in vitro* : a NOT complete list

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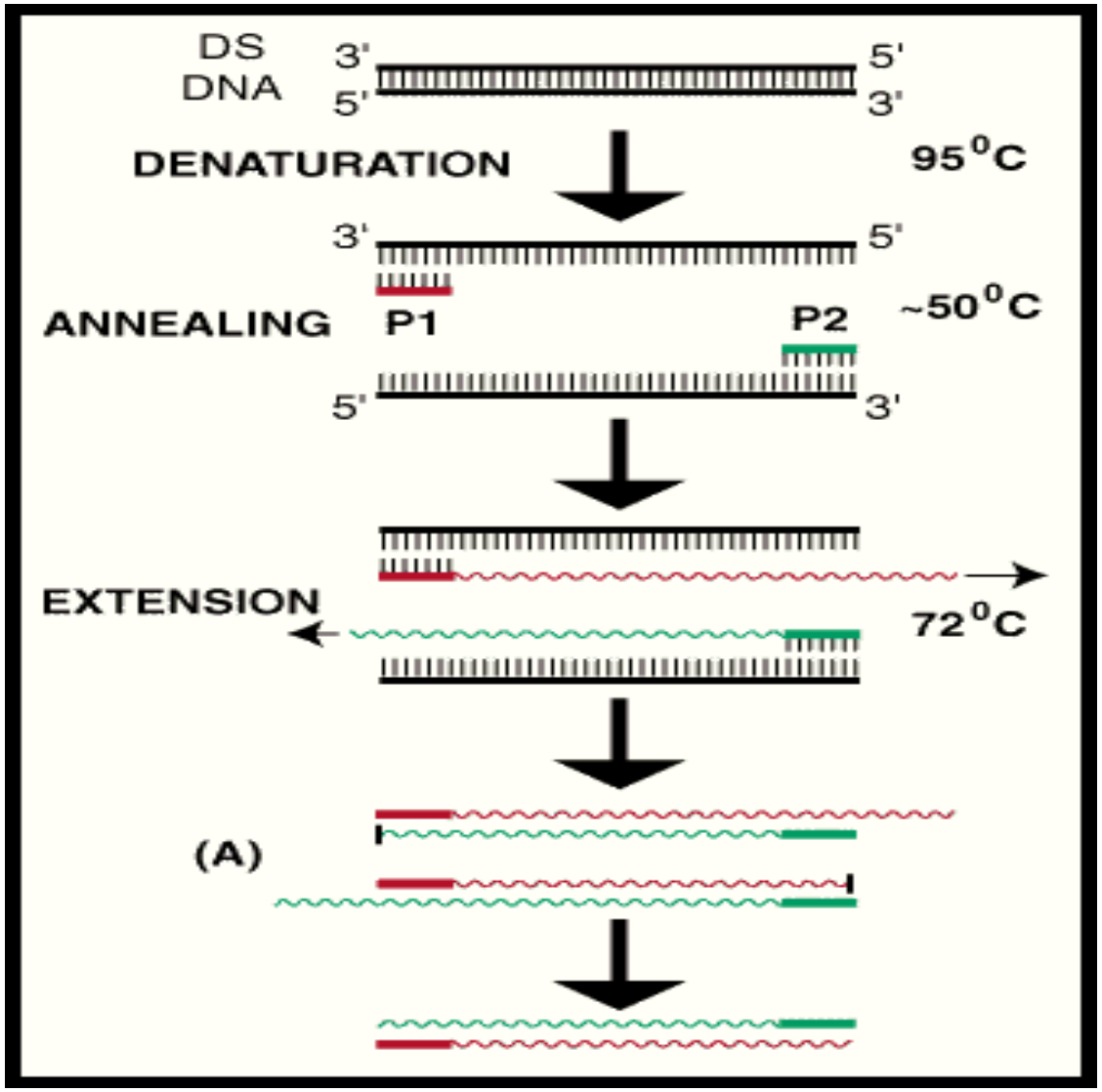
KNOWN TARGET

BIOINFORMATICS

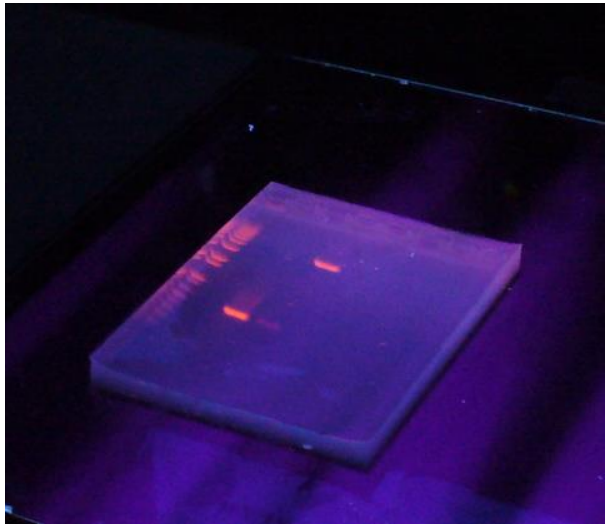
ALL TARGETS

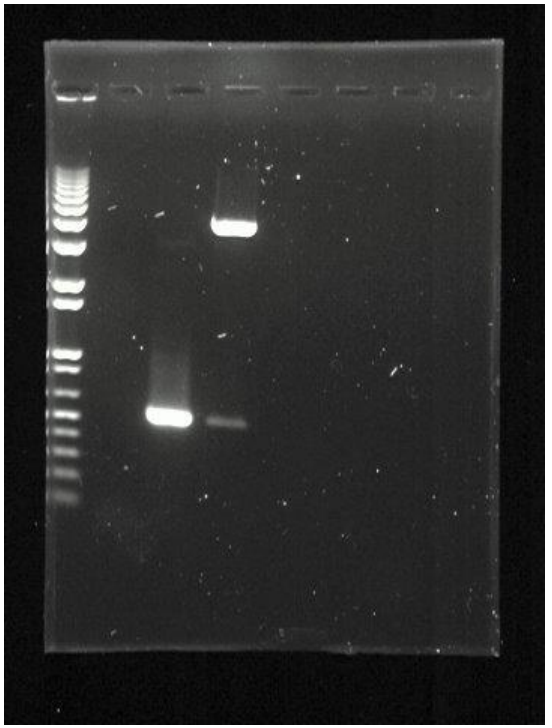
DNA	PCR	Biochemical read out
	Southern Blot	Biochemical read out
	Sequencing	Biochemical read out

PCR


DS
DNA
3'
5'
5'
3'
DENATURATION
95°C
3'
5'
ANNEALING
P1
P2
~50°C
5'
3'
EXTENSION
72°C
(A)

PCR





Western Blot

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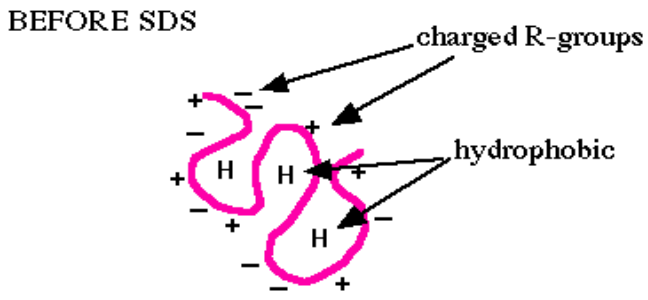
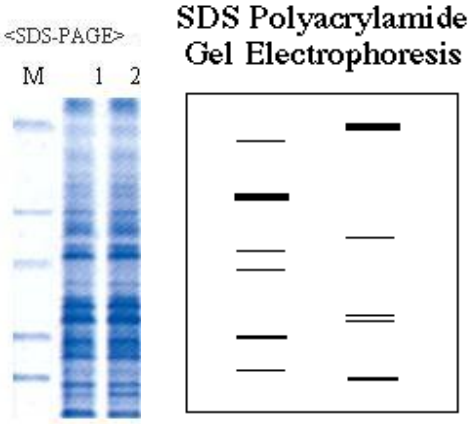
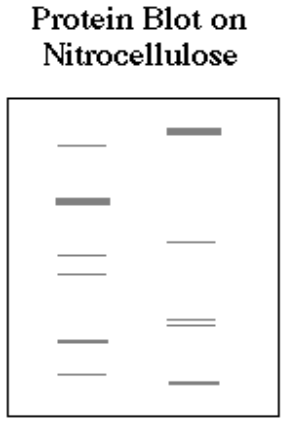
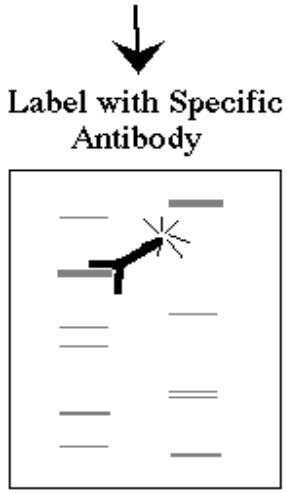
- Western blots allow investigators to determine the molecular weight of a protein and to measure relative amounts of the protein present in different samples.
- Proteins are separated by gel electrophoresis, usually SDS-PAGE.
- The proteins are transferred to a sheet of special blotting paper called nitrocellulose or PVDF.
- The proteins retain the same pattern of separation they had on the gel.

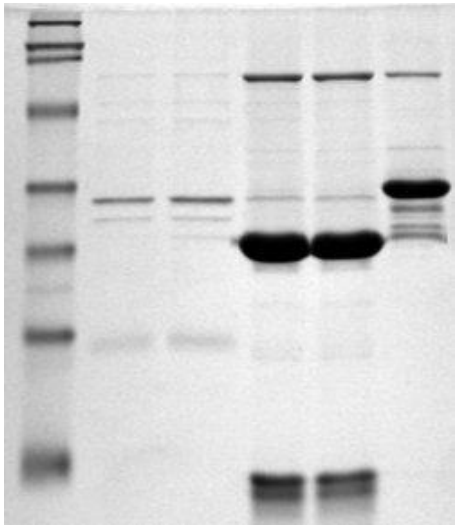
Western Blot

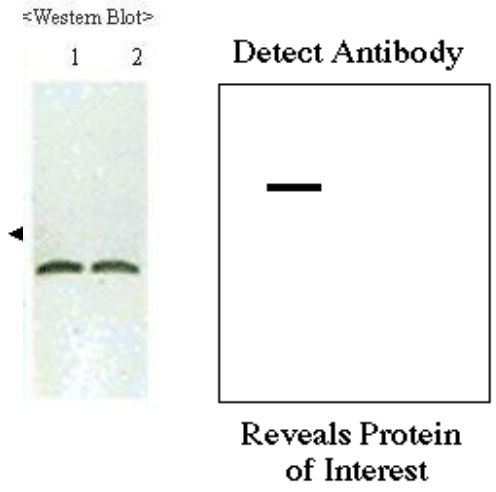




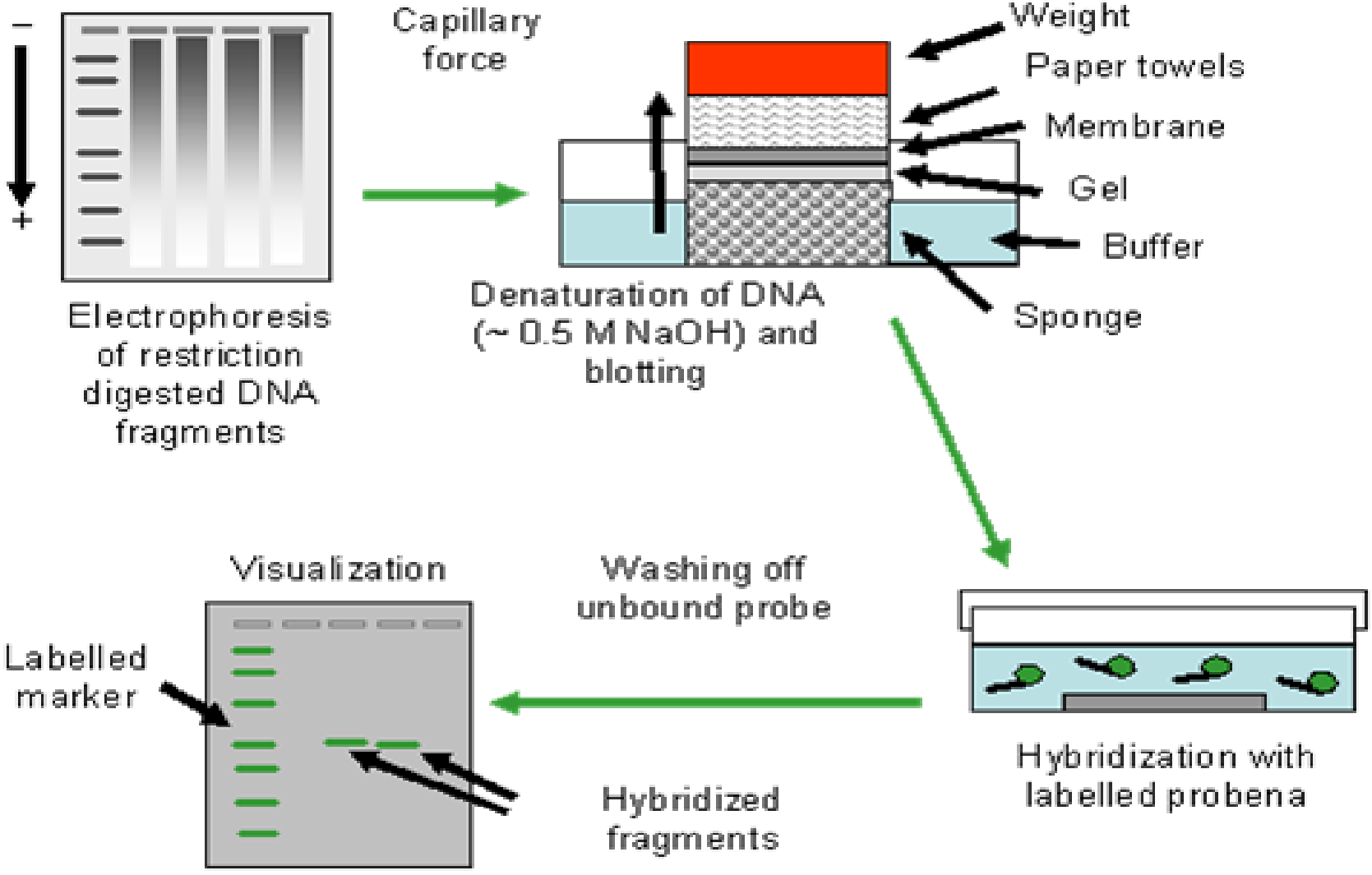








Southern blot



Southern blot

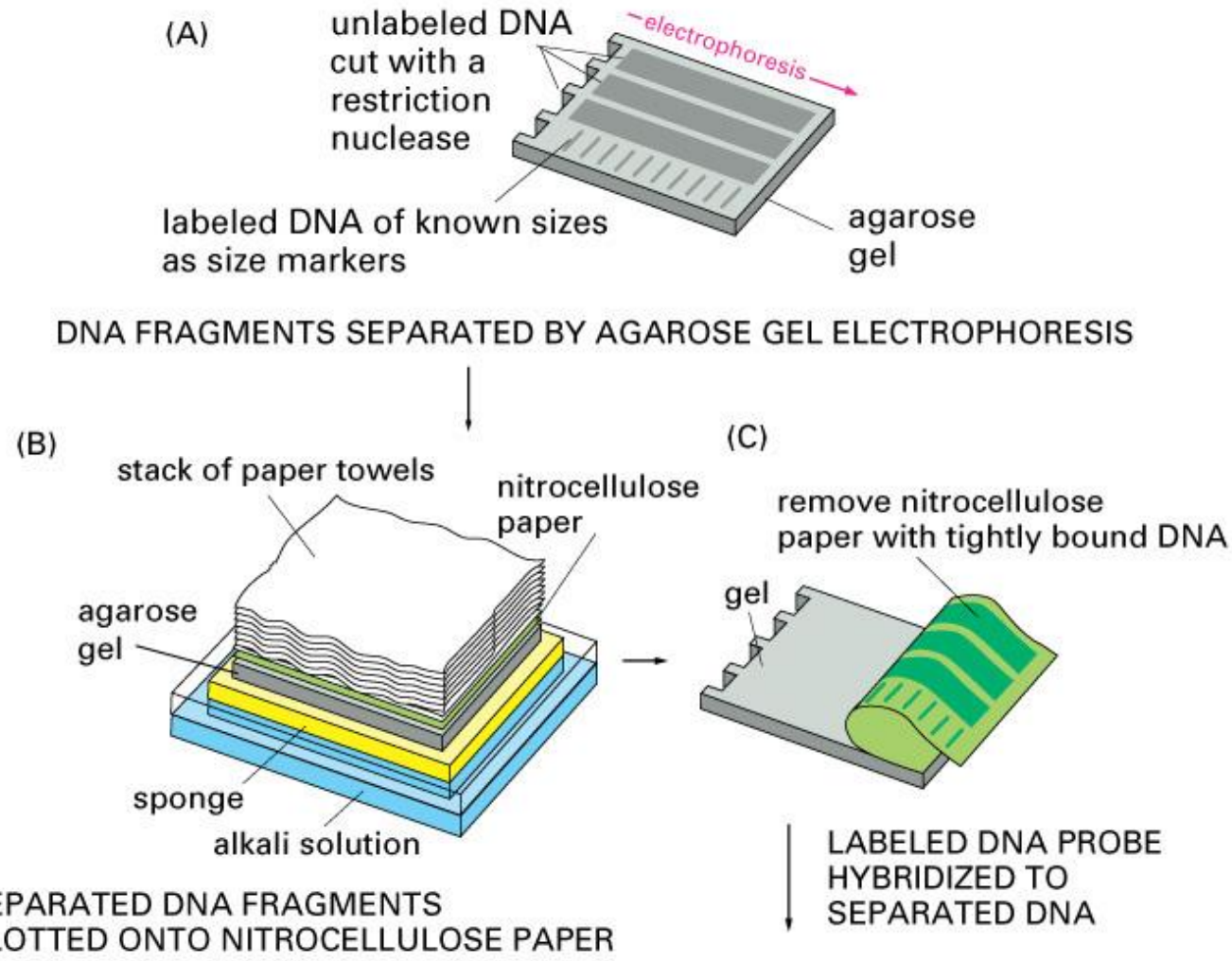


Figure 10-14 part 1 of 2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Neuroscience *in vitro* : a NOT complete list

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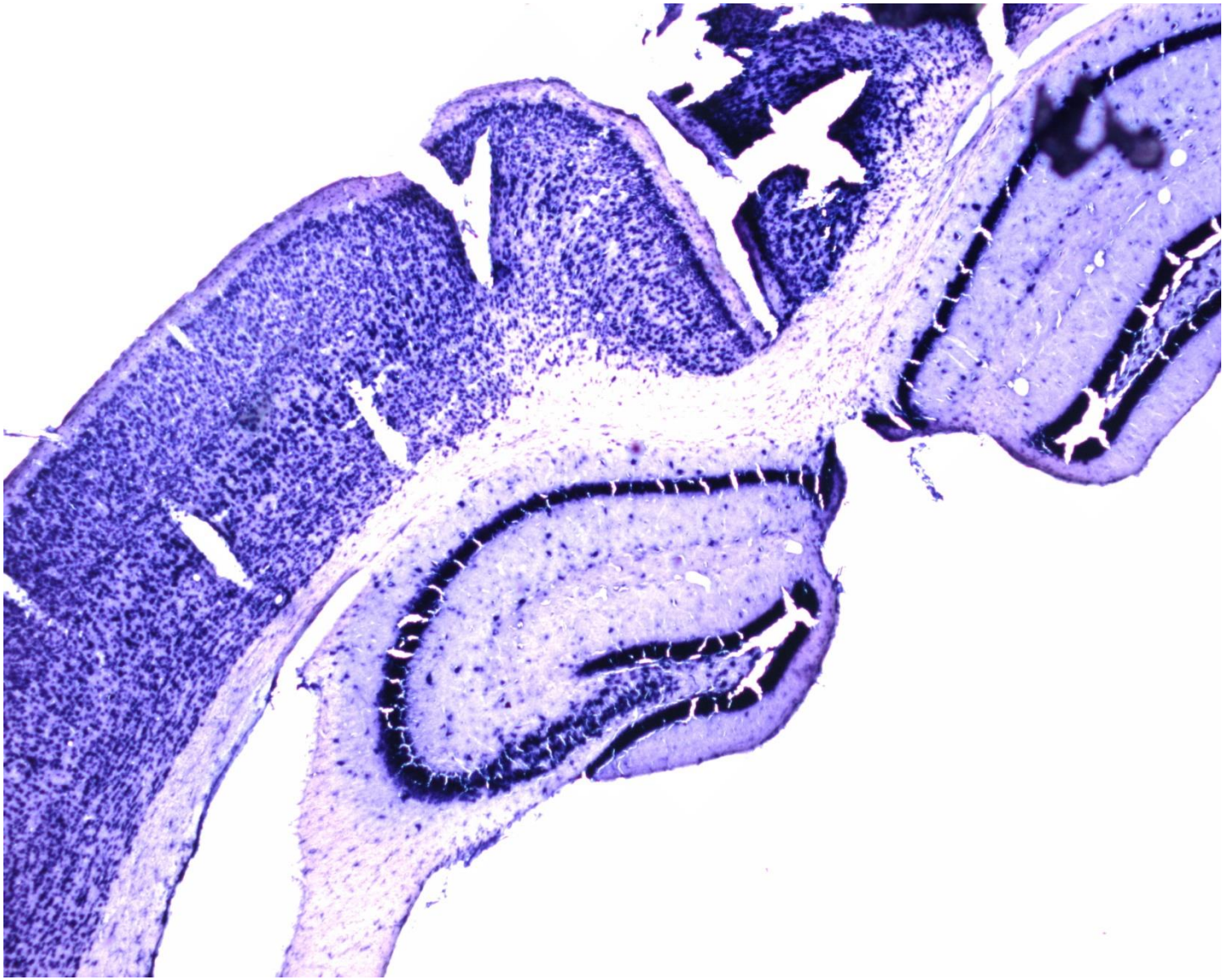
KNOWN TARGET

BIOINFORMATICS

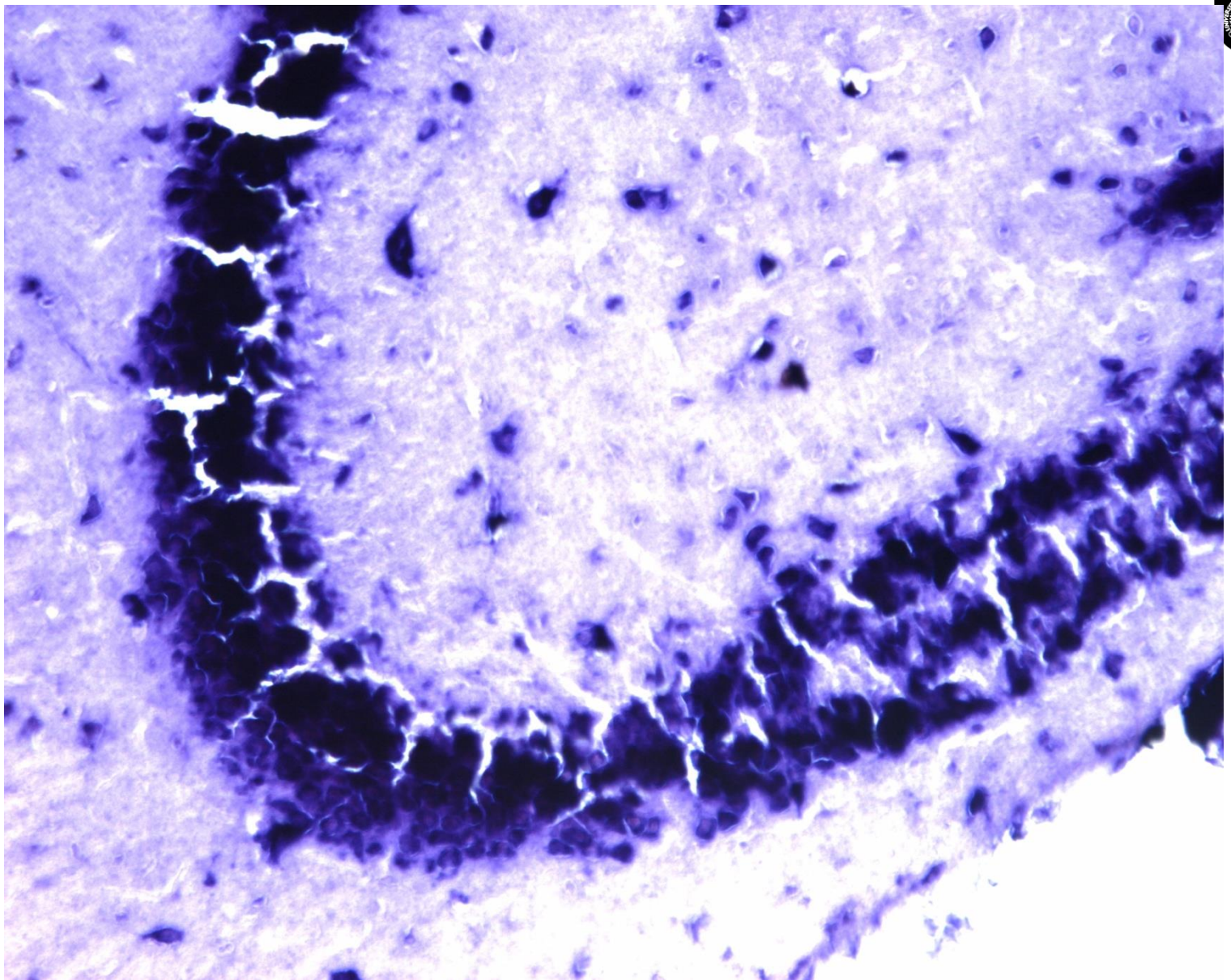
ALL TARGETS

RNA	PCR	Biochemical read out
	Northern Blot	Biochemical read out
	Sequencing	Biochemical read out
	In situ	subcellular read out

In situ



In situ



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Southern/Northern blot

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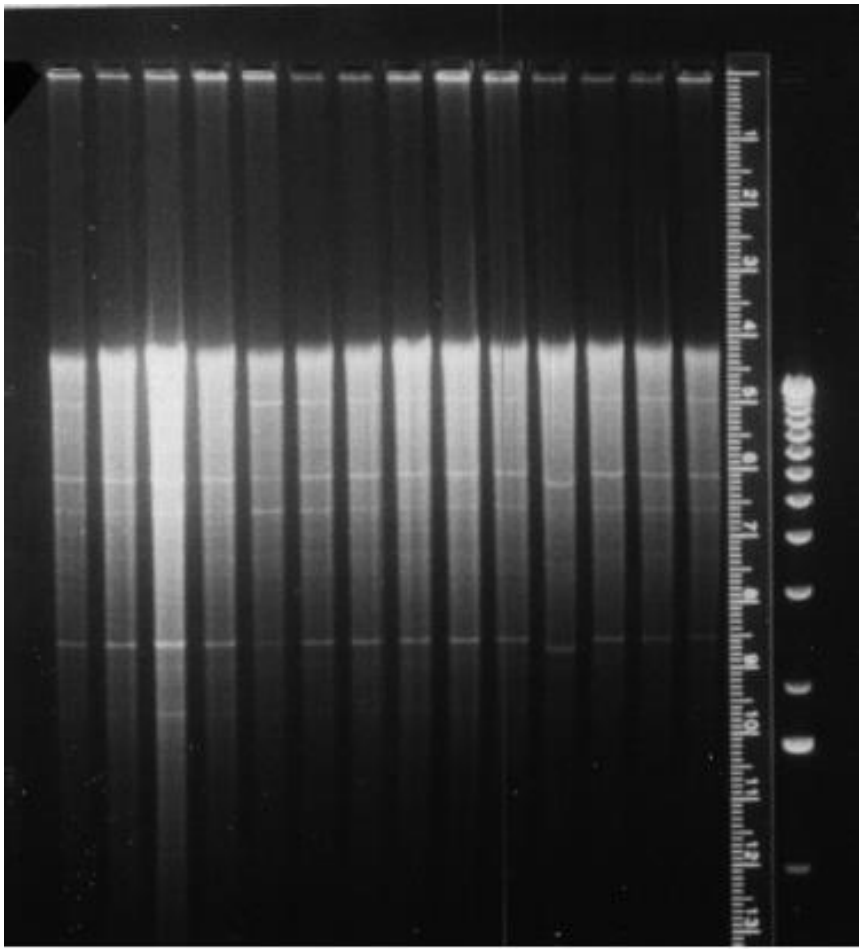

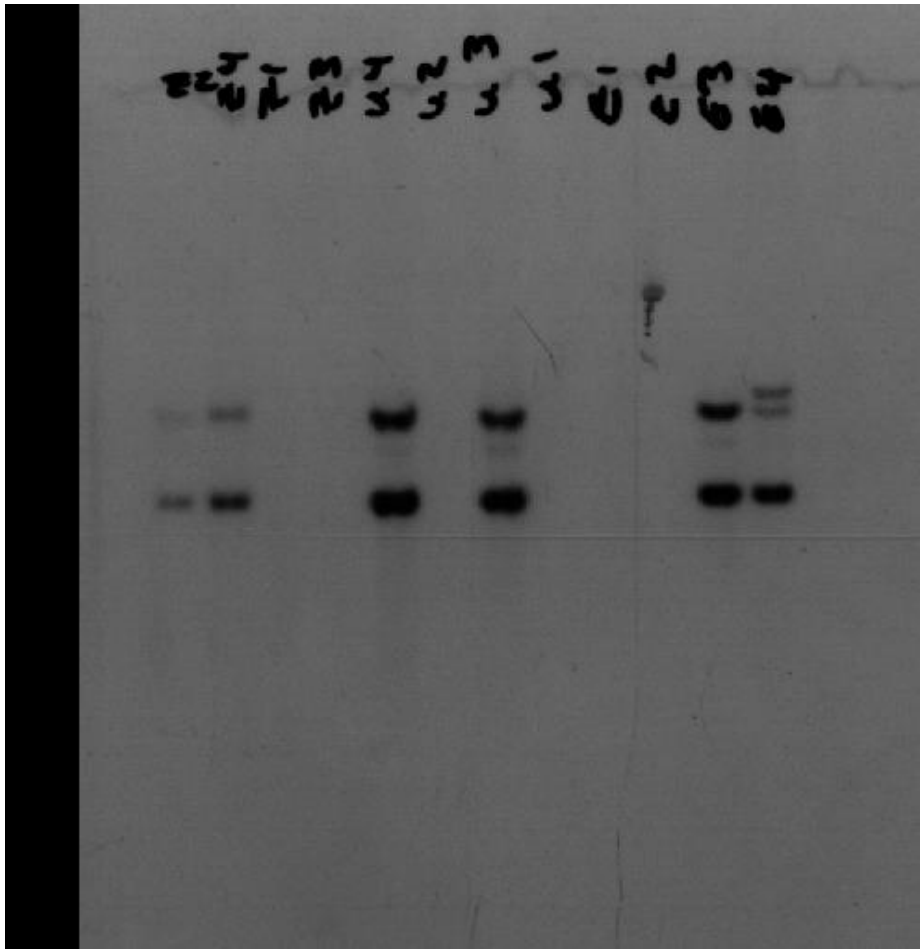

Comparison of Southern, Northern and Western blot hybridization

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Blot type	Target	Probe	Applications
Southern	DNA	DNA or RNA (Agarose Gel)	mapping genomic clones estimating gene numbers, etc
Northern	RNA	DNA or RNA (Formaldehyde agarose gel)	RNA sizes and abundance (gene expression level)
Western	Protein	Antibodies (Polyacrylamide gel)	protein size and abundance (gene expression level)

Neuroscience *in vitro* : a NOT complete list

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KNOWN TARGET

BIOINFORMATICS

ALL TARGETS

Protein

Western Blot	Biochemical read out
Elisa	Biochemical read out
Sequencing	Biochemical read out
Immuno Istochemistry	subcellular read out
Immuno Citochemistry	subcellular read out

Immunohistochestry
Immunocytochemistry

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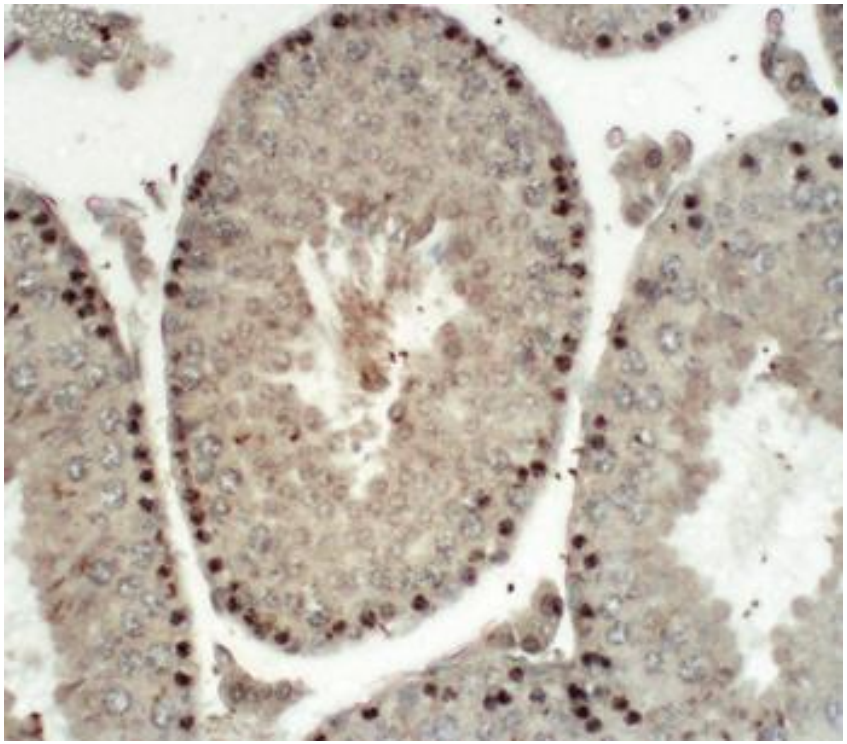

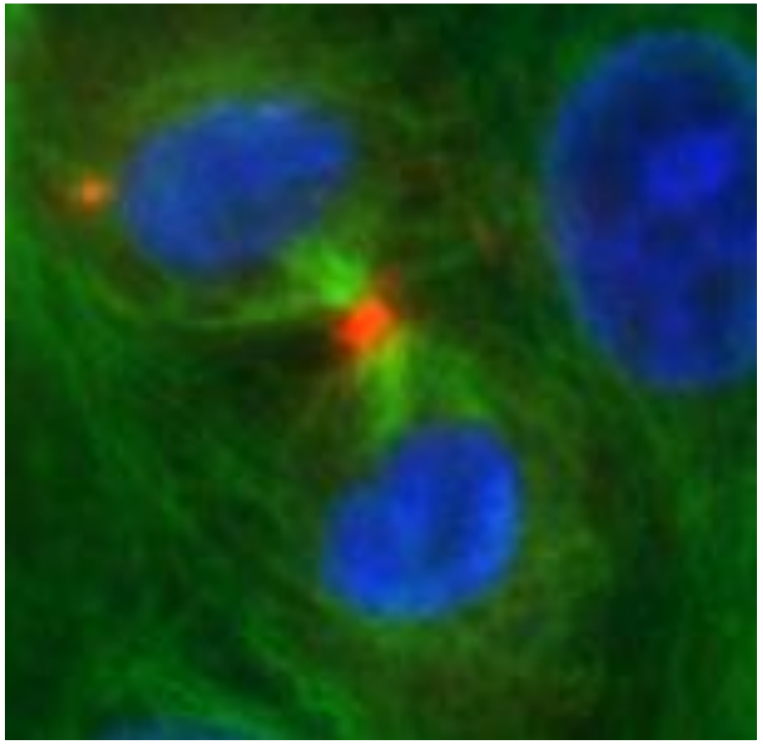

Neuroscience *in vitro* : a NOT complete list

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KNOWN TARGET

BIOINFORMATICS

ALL TARGETS

BASAL CONDITION

"MODIFIED CONDITIONS"

DNA-Protein

Chromatin immunoP

Biochemical read out

RNA-Protein

Co immunoP + RT PCR

EMSA

SuperShift

Gel Shift

Biochemical read out

Protein-Protein

FRET

subcellular read out

Neuroscience *in vitro* :The choice of a Model

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	BASAL CONDITION	"MODIFIED CONDITIONS"
• Cell Lines	Biochemical read out subcellular read out	Biochemical read out subcellular read out
• Primary culture	Biochemical read out *limited* by the *availability* MORFOLOGICAL READ OUT subcellular read out	MORFOLOGICAL READ OUT subcellular read out
• Primary culture from Transgenic/ KO/KIN Animal	Biochemical read out MORFOLOGICAL READ OUT subcellular read out	Biochemical read out MORFOLOGICAL READ OUT subcellular read out

Neuroscience *in vitro* :The choice of a Model

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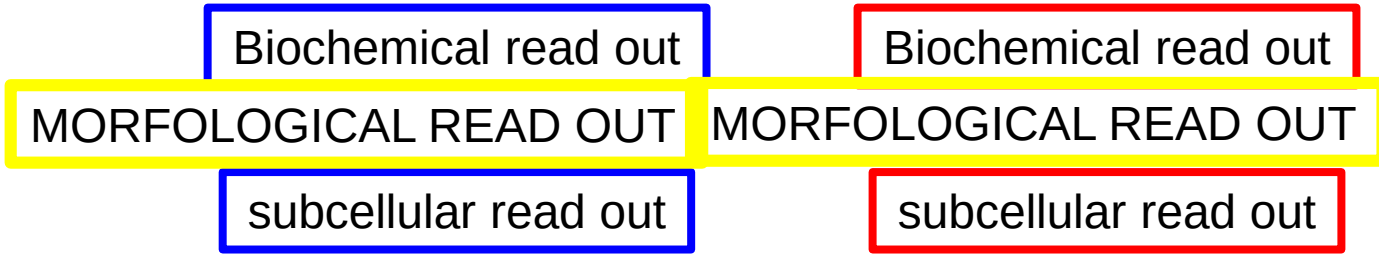
BASAL CONDITION **"MODIFIED CONDITIONS"**

- Organotypic cultures

- Organotypic cultures

from Transgenic KO/KIN Animal



Neuroscience *in vitro* :The choice of a Model

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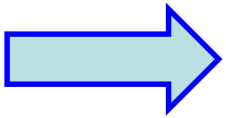
- Cell lines = NO REAL NEURONS

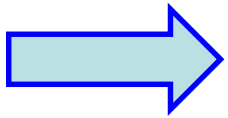
NO REAL MORPHOLOGICAL READ OUT IN NEUROSCIENCE

STRUCTURE  FUNCTION

Neuroscience *in vitro* :The choice of a Model

- Primary culture = NO REAL TISSUE

MORPHOLOGICAL READ OUT
IN NEUROSCIENCE

STRUCTURE  FUNCTION

LIMITED TO SINGLE FAMILY OF CELLS

Neuroscience *in vitro* :The choice of a Model

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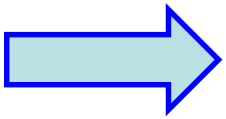
- Organotypic slice= NO REAL BRAIN

MORPHOLOGICAL READ OUT
IN NEUROSCIENCE

STRUCTURE  FUNCTION

LIMITED TO SINGLE TISSUE

Consider Technical LIMITATIONS

Neuroscience *in vitro* :The choice of a Model

- Animal = NO HUMAN BRAIN

MORPHOLOGICAL READ OUT
IN NEUROSCIENCE

STRUCTURE → FUNCTION

LIMITED TO SINGLE ANIMAL SPECIEs

Consider many Technical LIMITATIONS

Neurons

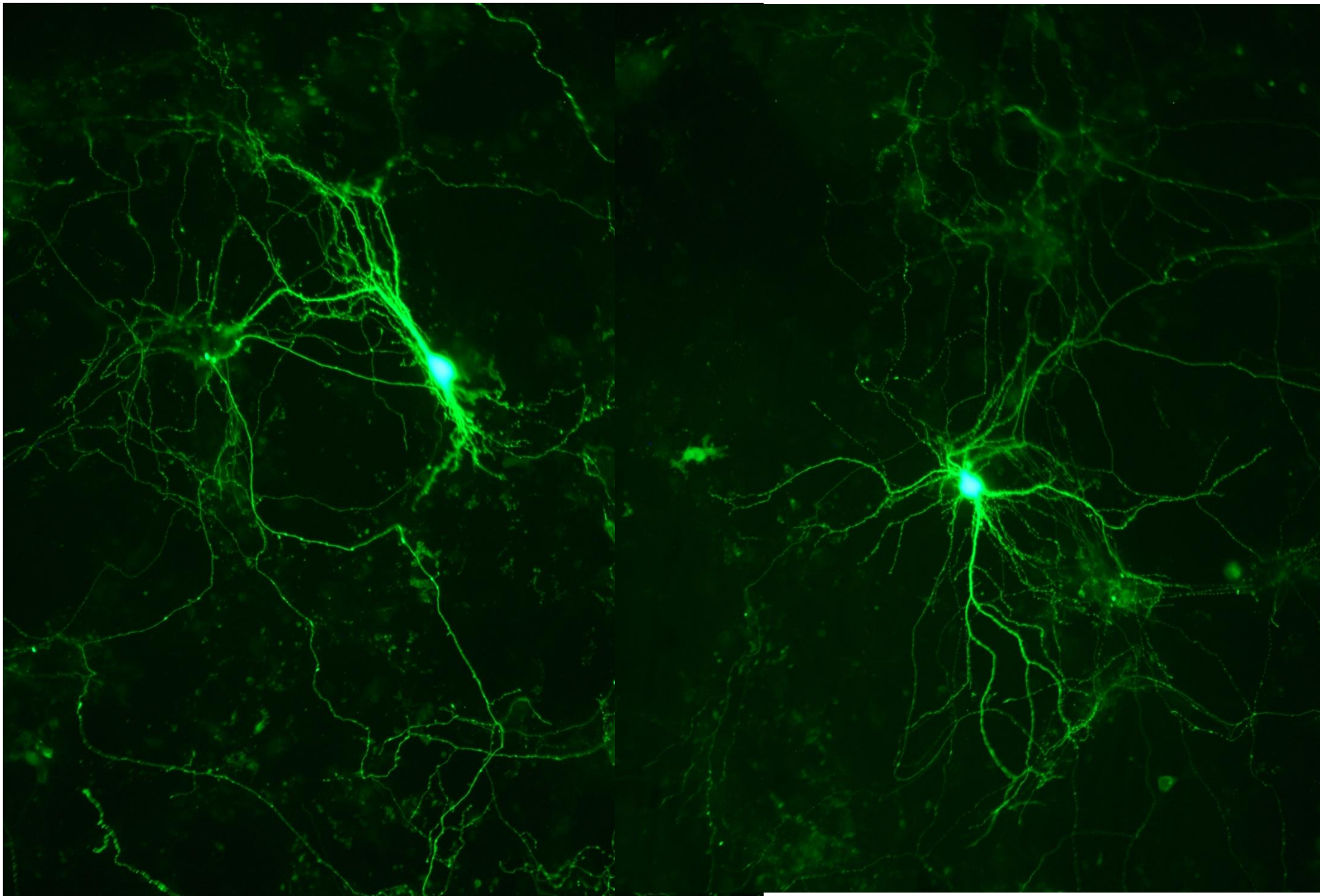


Image J

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http://rsbweb.nih.gov/ij/

or simply type : imagej on