Cell-type specific mRNA analysis by translating ribosome affinity purification (TRAP)

Mario Nuvolone

Technical Journal Club

16th September 2014

University Hospital Zurich
Institute of Neuropathology

Bulk vs cell-specific mRNA analyses

Limitations in studies on bulk mRNA from whole tissue/organ:

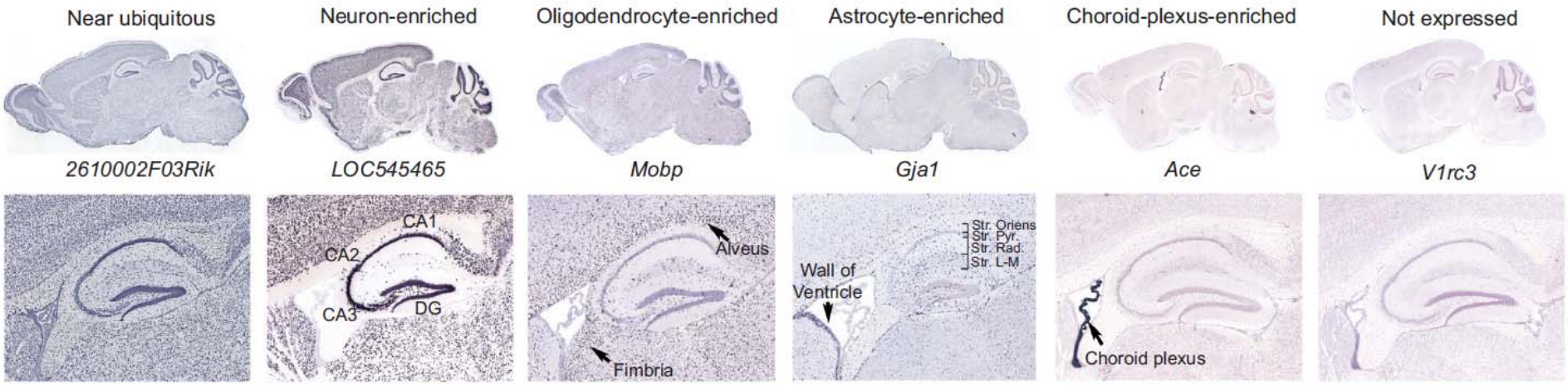
- Upregulation of a transcript in a cell type can be masked by downregulation in a different cell type

- Changes in rare cell types are difficult to detect

→ Changes in specific cell types are not analysed

Cell-specific mRNA analysis: current approaches

In situ hybridization:



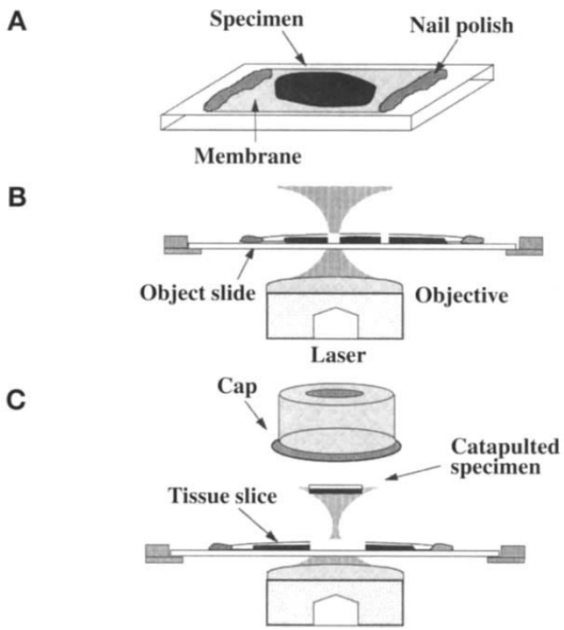
Limitations:

- Not quantitative
- Identity of cells expressing the transcript not always evident
- Genome-wide studies are difficult and restricted to reference samples (Allen Brain Atlas, Eurexpress)

Lein ES Nature 2007
The Allen Brain Atlas

Cell-specific mRNA analysis: current approaches

Laser capture microdissection

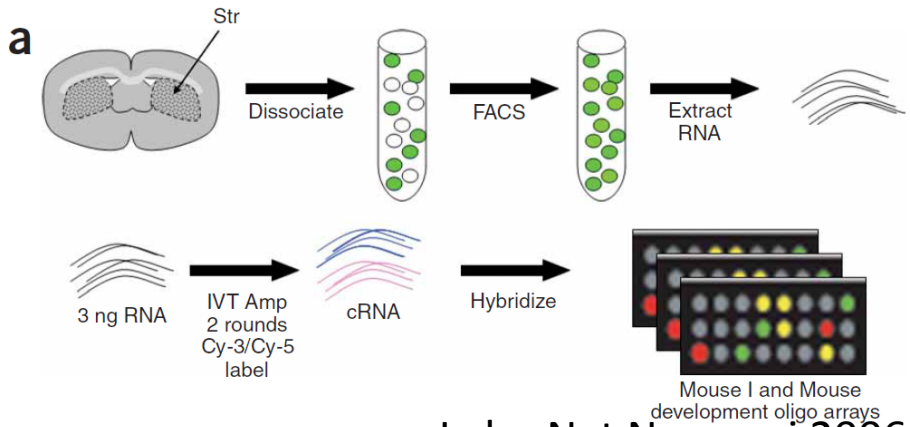


Schütze Nat Biotech 1998

Limitations:

- Fixation required
- Laborious
- Special instruments required
- Cell projections not captured

Microdissection & FACS

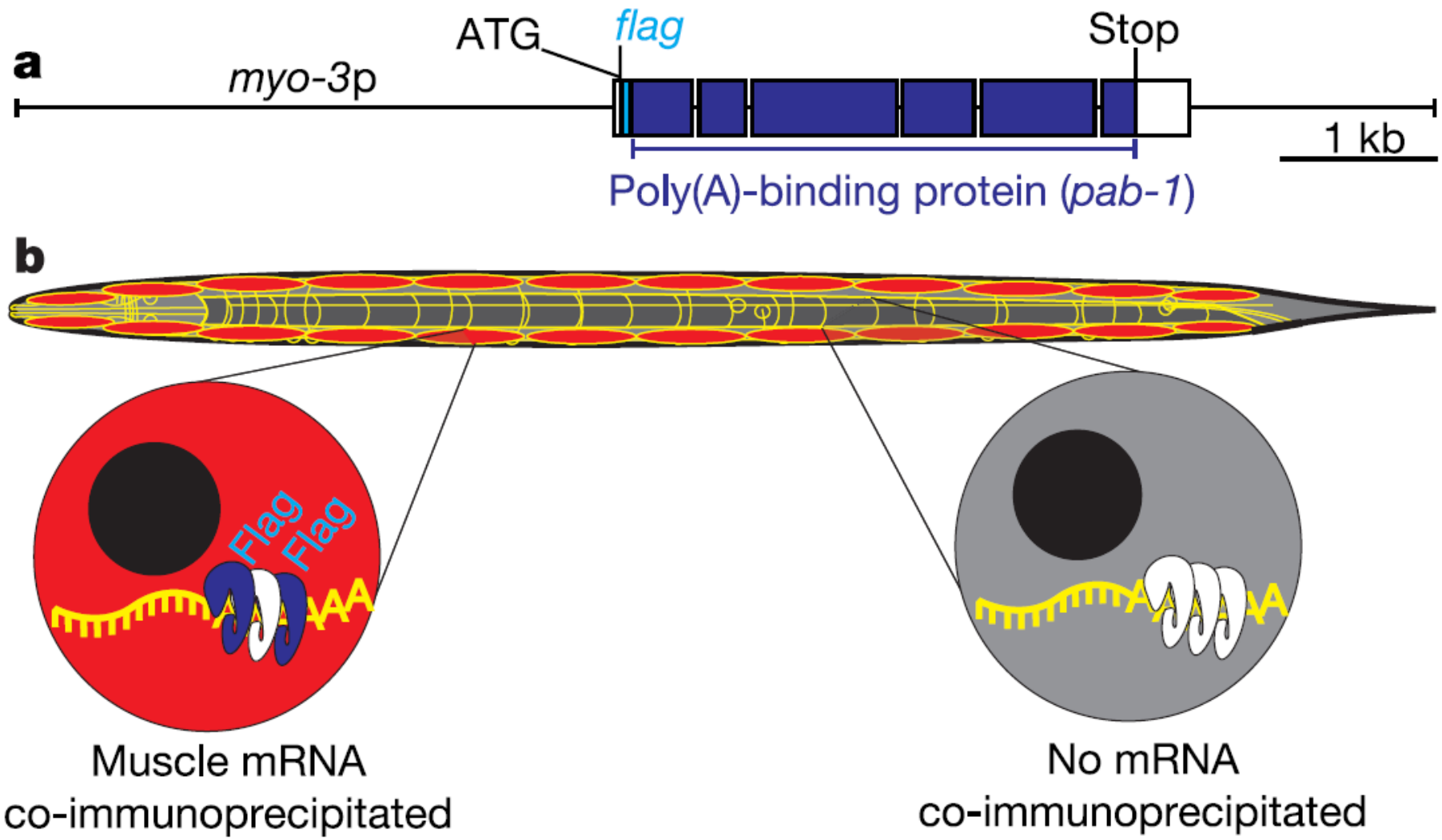


Lobo Nat Neurosci 2006

Limitations:

- Artefacts from sample processing

mRNA tagging in *C. elegans*



Roy Nature 2002

Cell

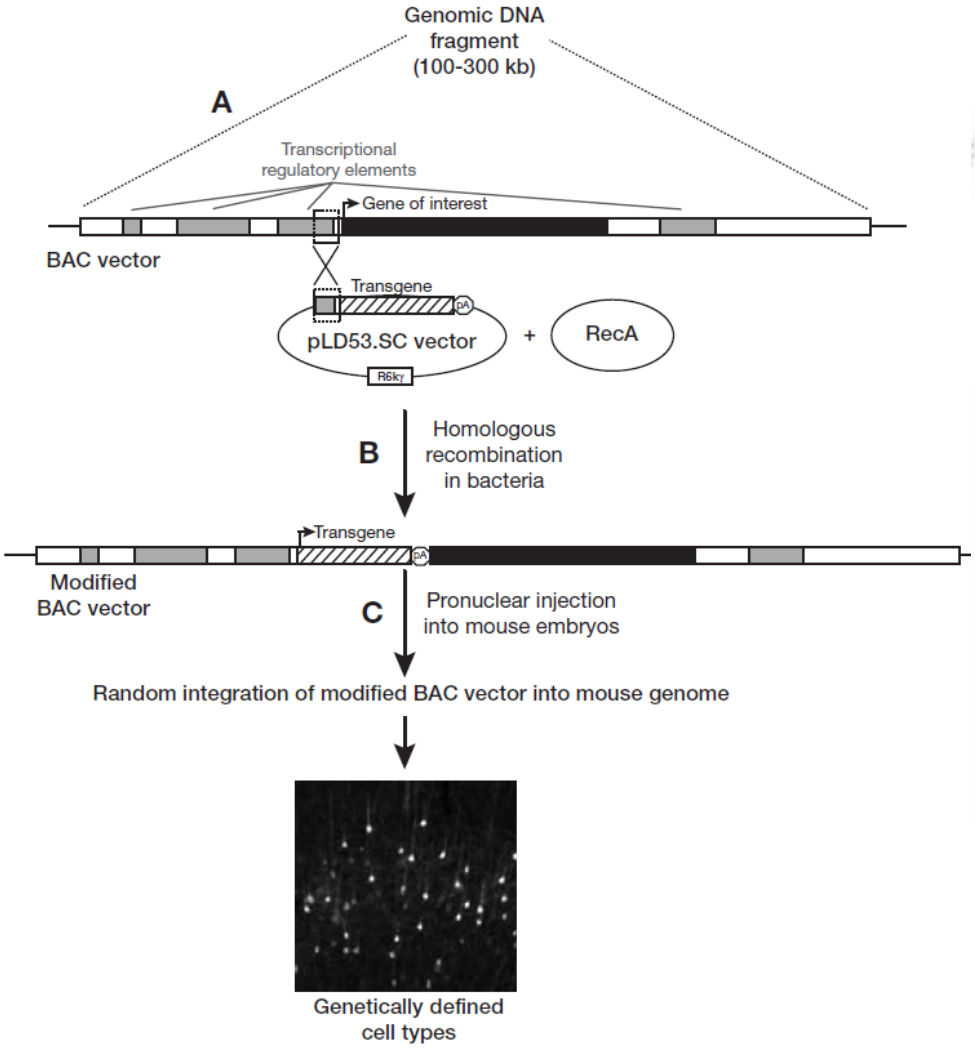
Resource

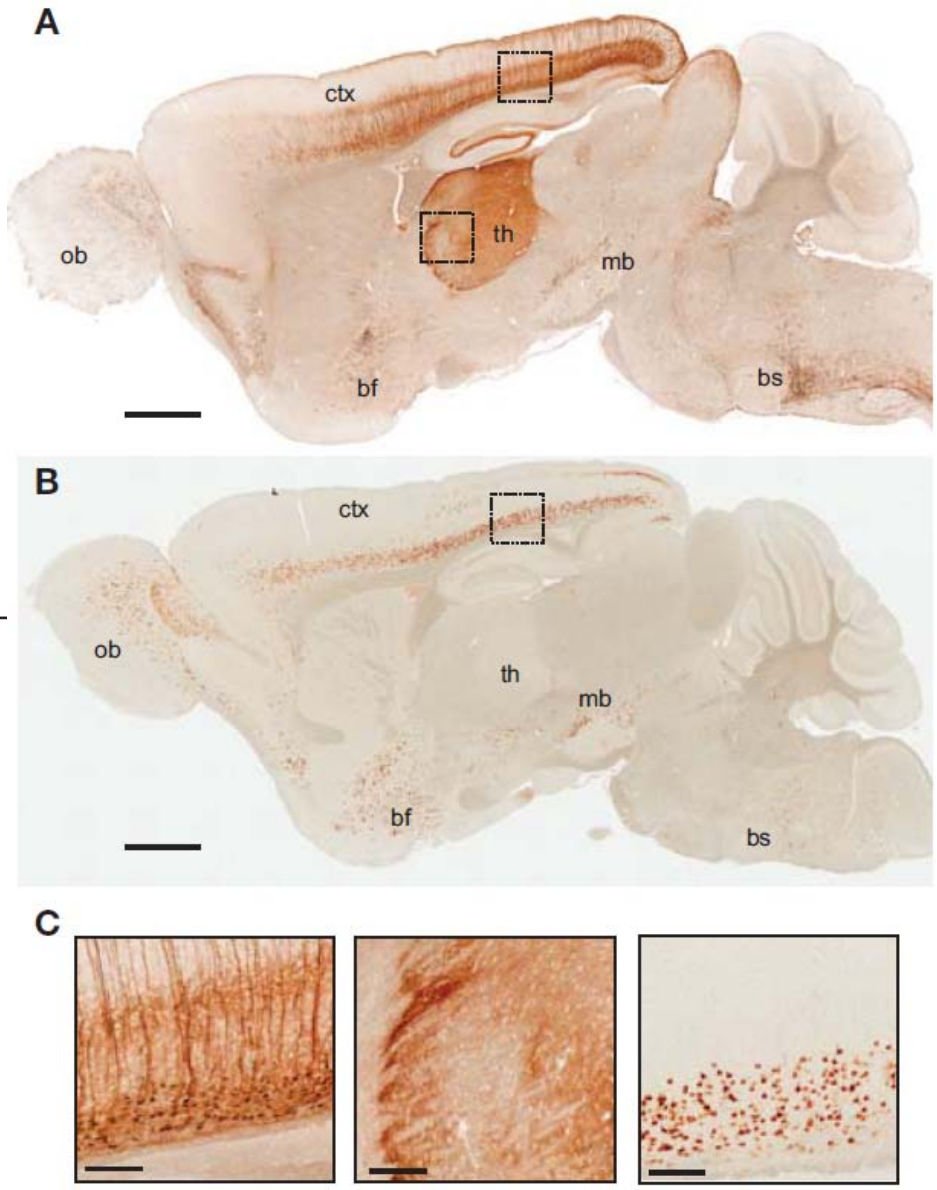
A Translational Profiling Approach for the Molecular Characterization of CNS Cell Types

Myriam Heiman,[1] Anne Schaefer,[1] Shiaoching Gong,[2] Jayms D. Peterson,[5] Michelle Day,[5] Keri E. Ramsey,[6] Mayte Suárez-Fariñas,[4] Cordelia Schwarz,[3] Dietrich A. Stephan,[6] D. James Surmeier,[5] Paul Greengard,[1] and Nathaniel Heintz[2,3,*]

[1]Laboratory of Molecular and Cellular Neuroscience
[2]GENSAT Project
[3]Laboratory of Molecular Biology, Howard Hughes Medical Institute
[4]The Rockefeller University Hospital
The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA
[5]Department of Physiology, Feinberg School of Medicine, Northwestern University, 303 East Chicago Avenue, Chicago, IL 60611, USA
[6]Neurogenomics Division, Translational Genomics Research Institute, 445 North 5th Street, Phoenix, AZ 85004, USA
*Correspondence: heintz@mail.rockefeller.edu
DOI 10.1016/j.cell.2008.10.028

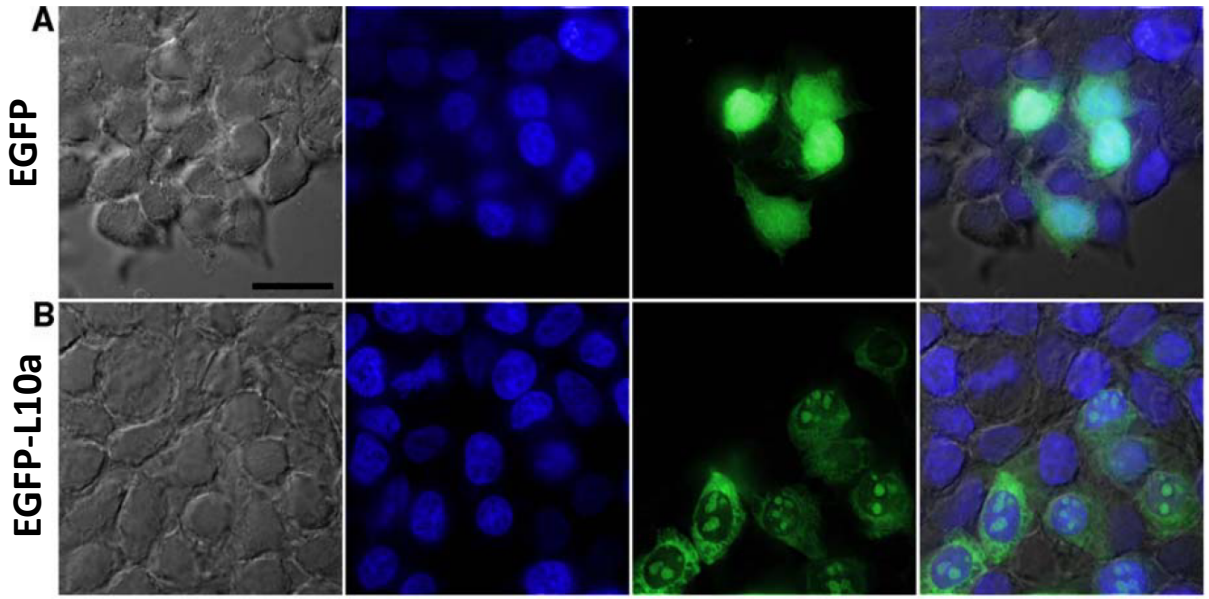
BAC Transgenic mice and the GENSAT Database





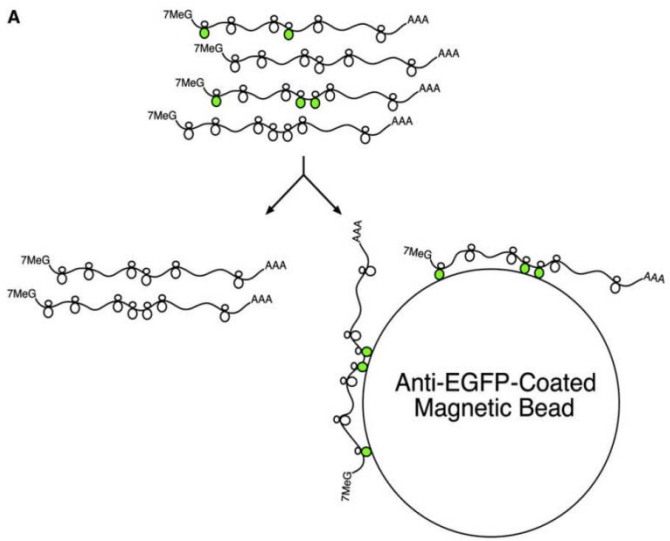
Gong Nature 2003
Schmidt Cold Spring Harb Protoc 2013

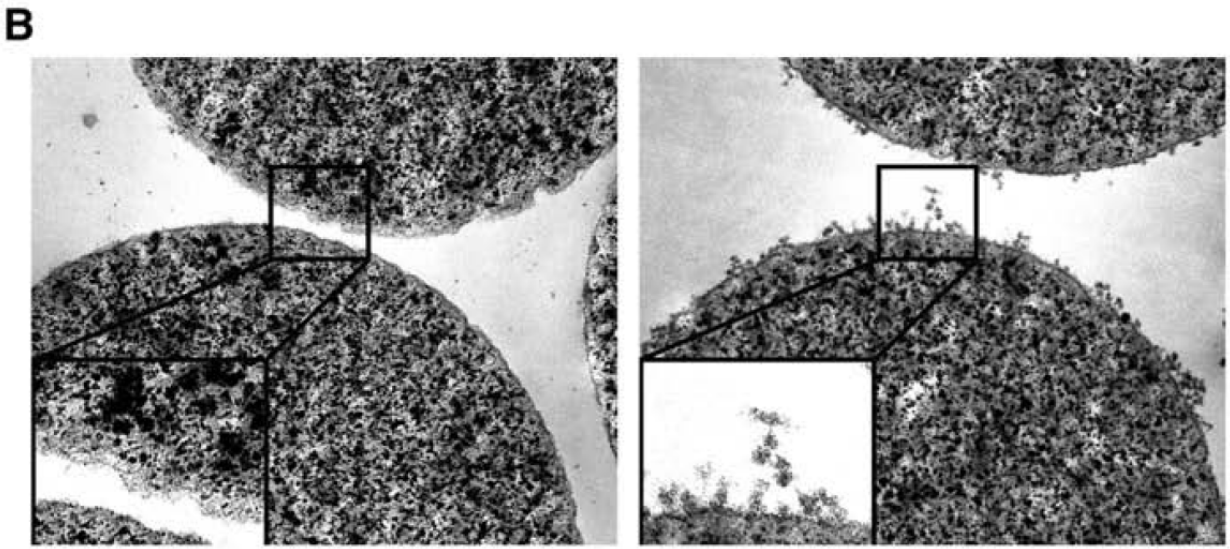
TRAP strategy: screens for ribosome tagging



Screening of candidate ribosomal proteins:
→L10a

Screening of affinity tags
→ EGFP

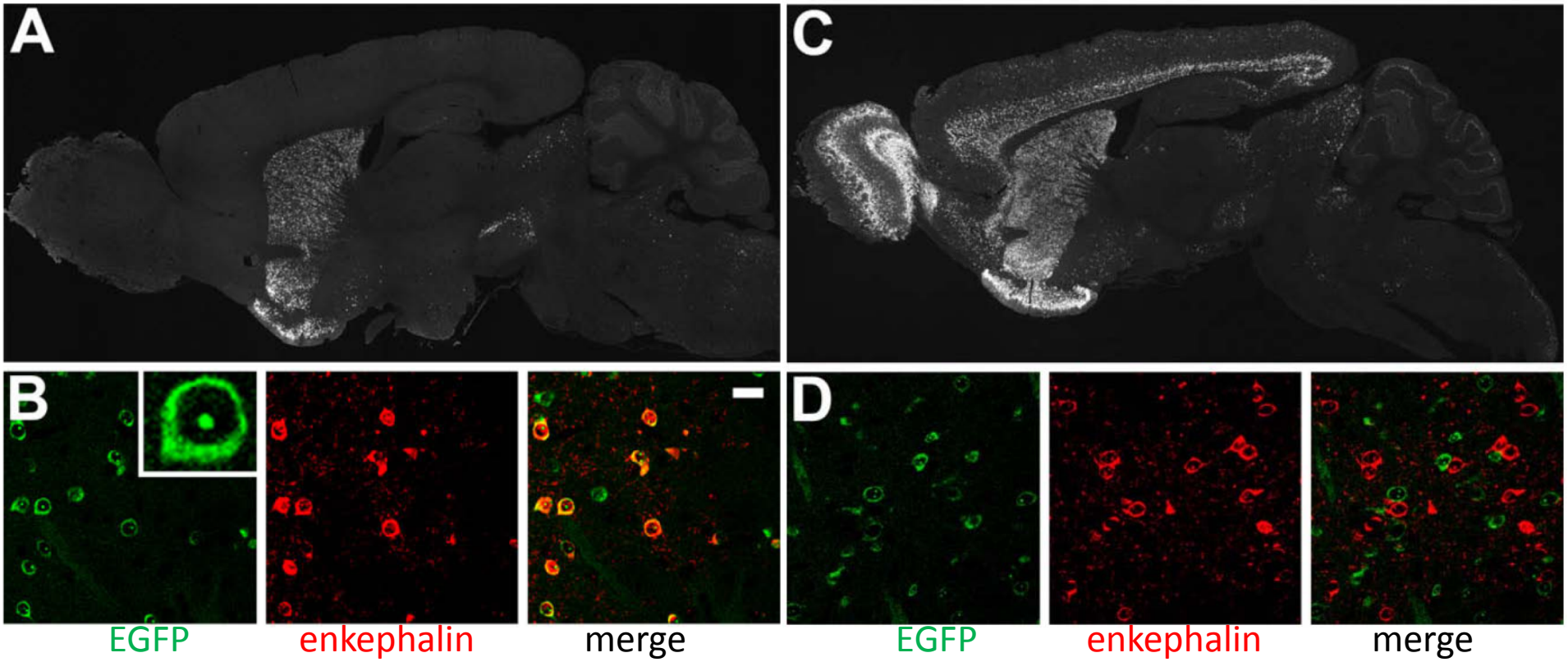




Generation of BAC-TRAP transgenic mouse lines

Drd2 bac-TRAP line

Drd1a bac-TRAP line



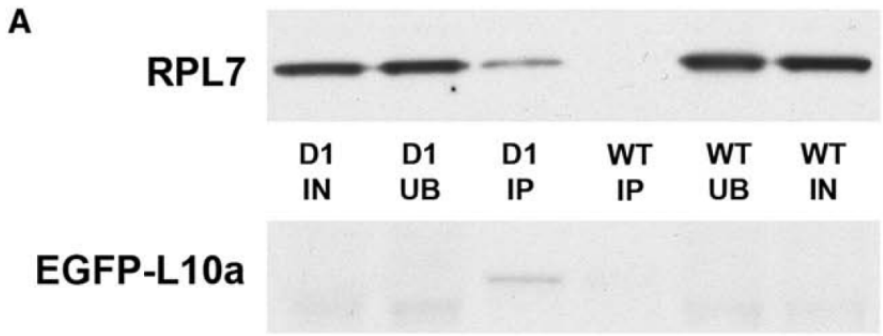
Striatopallidal neurons:

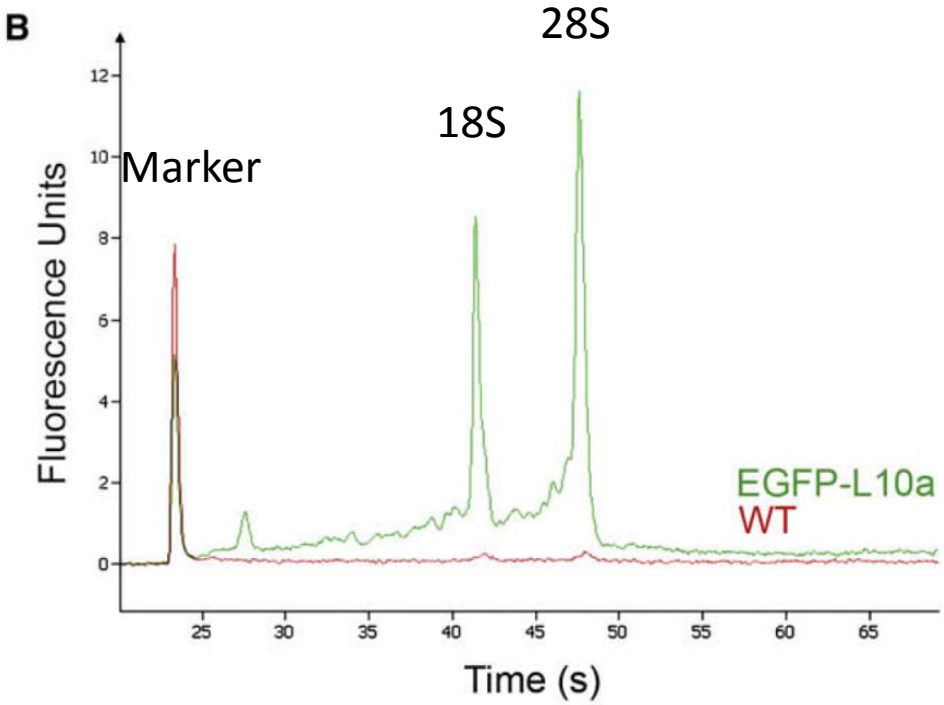
- Dorsal and ventral striatum
- Olfactory tubercle
- Hippocampus
- Substantia nigra pars compacta
- Ventral tegmental area

Striatonigral neurons:

- Dorsal and ventral striatum
- Olfactory tubercle
- Olfactory bulbe
- Cortical layers 5 and 6

Protein and mRNA purification from bac-TRAP lines

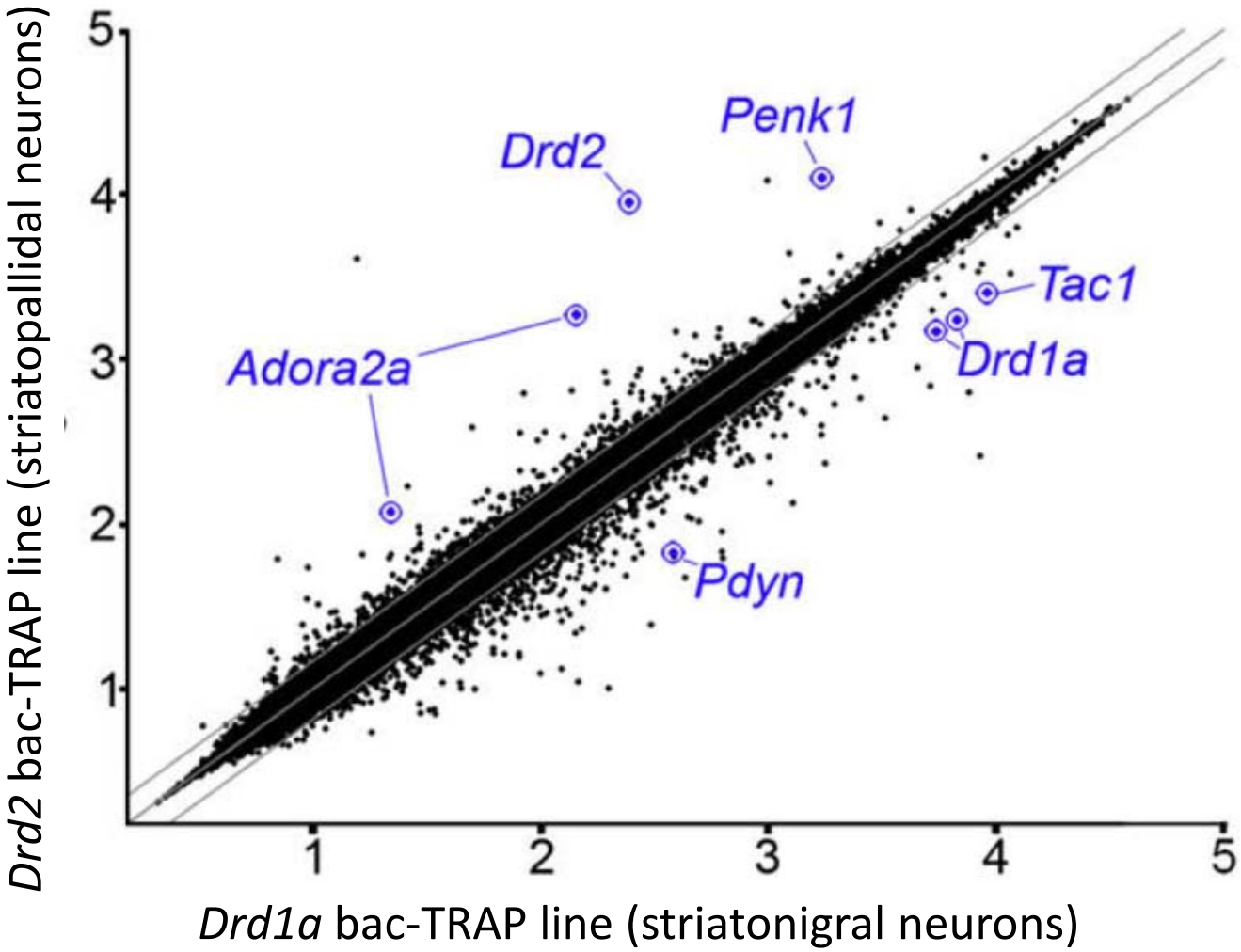




Optimization of purification protocol:

- Rapid homogenization of fresh tissue
- Mg^{2+} and cycloheximide in lysis buffer
- Inhibition of endogenous Rnase
- Solubilization of RER-bound polysomes
- High-affinity anti-EGFP antibodies
- High-salt washes after

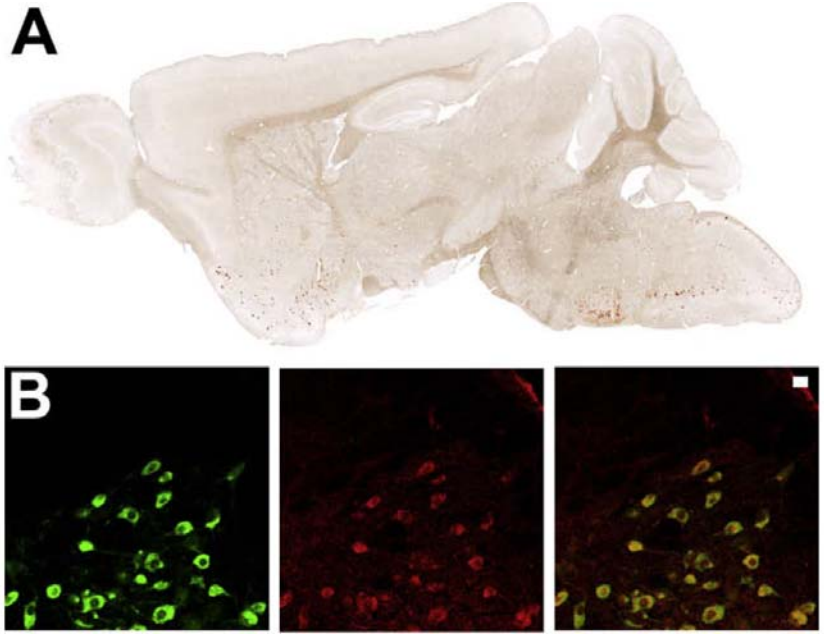
Translational profiling with TRAP



Replication of known enriched transcript (in blue)
Identification of ≈70 striatopallidal and ≈150 striatonigral-enriched transcripts

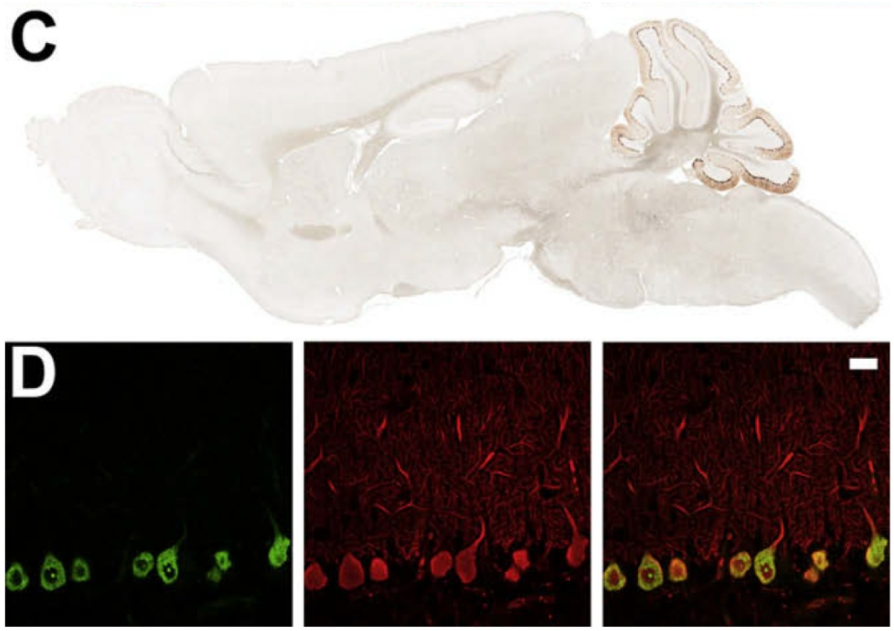
Generalization of TRAP methodology

Chat bac-TRAP line



EGFP chat merge

Pcp2 bac-TRAP line



EGFP calbindin merge

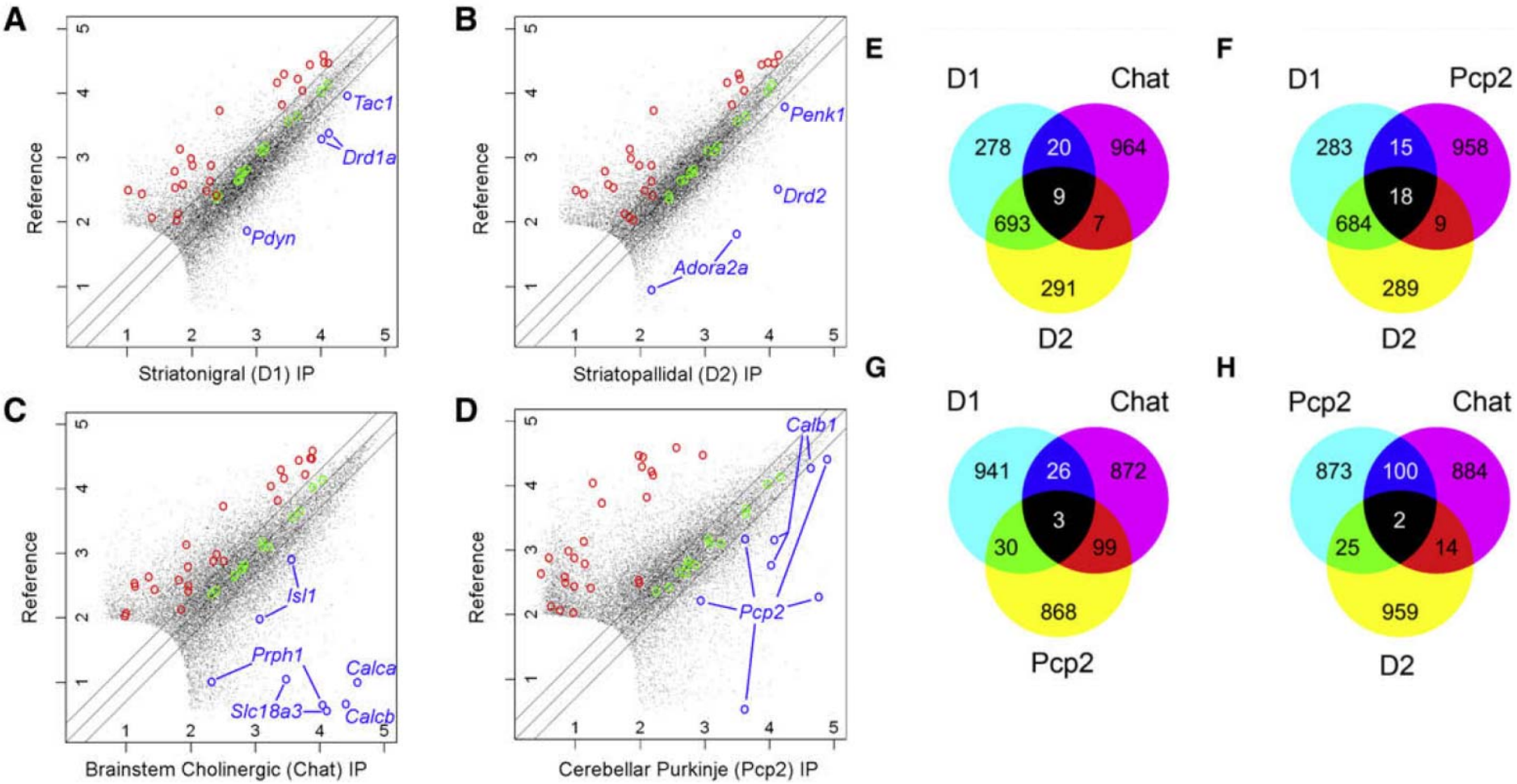
Cholinergic motor neurons:

- Dorsal and ventral striatum
- Basal forebrain
- Brain stem
- Spinal chord
- Medial habenula

Purkinje neurons:

- Cerebellum

Generalization of TRAP methodology



Summary

- Establishment and proof-of-principle of TRAP technology
- Generation of 4 bac-TRAP lines
- Identification of novel physiological differences between striatopallidal and striatonigral neurons, at steady state and after cocaine treatment (not shown)

Resource

Cell

Application of a Translational Profiling Approach for the Comparative Analysis of CNS Cell Types

Joseph P. Doyle,[1,4] Joseph D. Dougherty,[1,4] Myriam Heiman,[2] Eric F. Schmidt,[1] Tanya R. Stevens,[1] Guojun Ma,[1] Sujata Bupp,[1] Prerana Shrestha,[1] Rajiv D. Shah,[1] Martin L. Doughty,[3] Shiaoching Gong,[1,3] Paul Greengard,[2] and Nathaniel Heintz[1,3,*]

[1]Laboratory of Molecular Biology, Howard Hughes Medical Institute

[2]Laboratory of Molecular and Cellular Neuroscience

[3]GENSAT Project

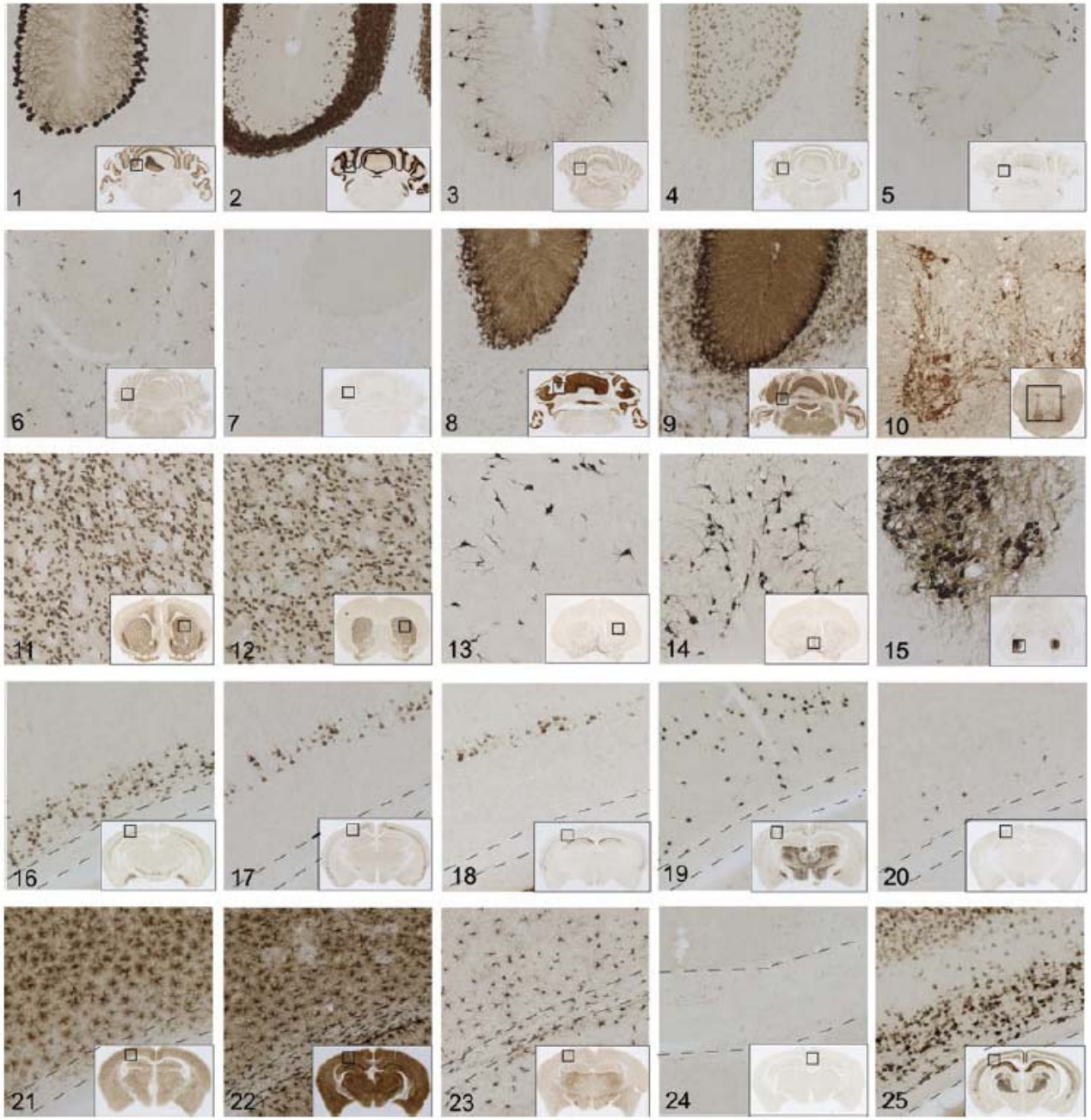
The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA

[4]These authors contributed equally to this work

*Correspondence: heintz@mail.rockefeller.edu

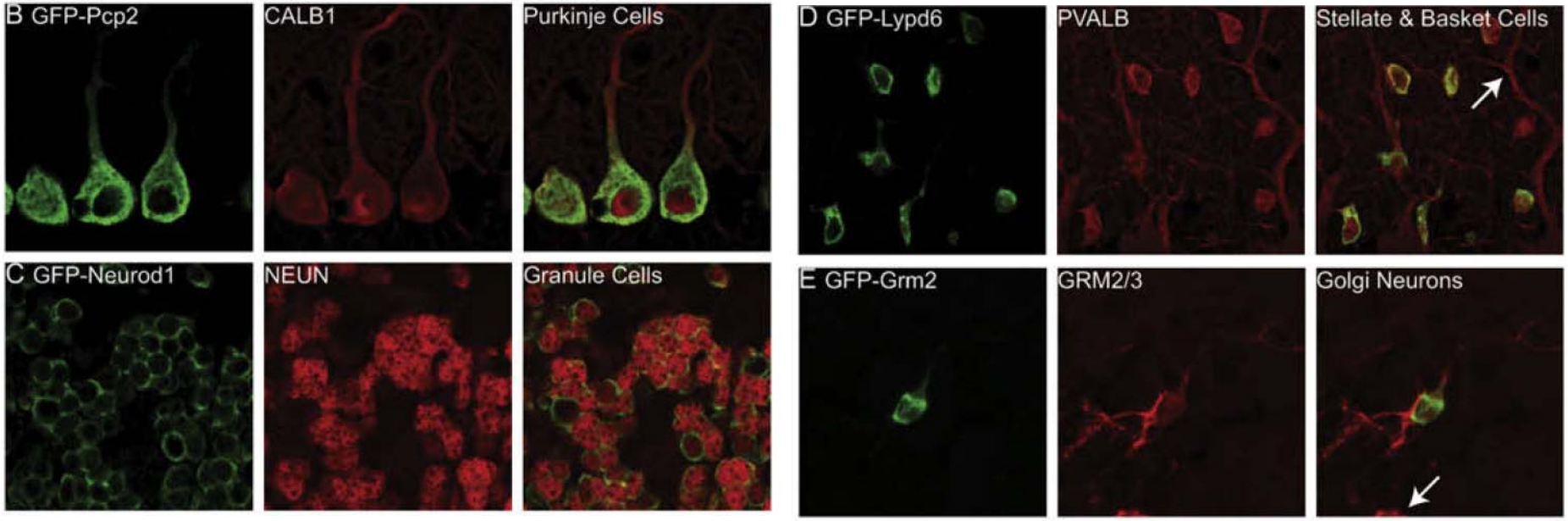
DOI 10.1016/j.cell.2008.10.029

Generation of novel bac-TRAP lines

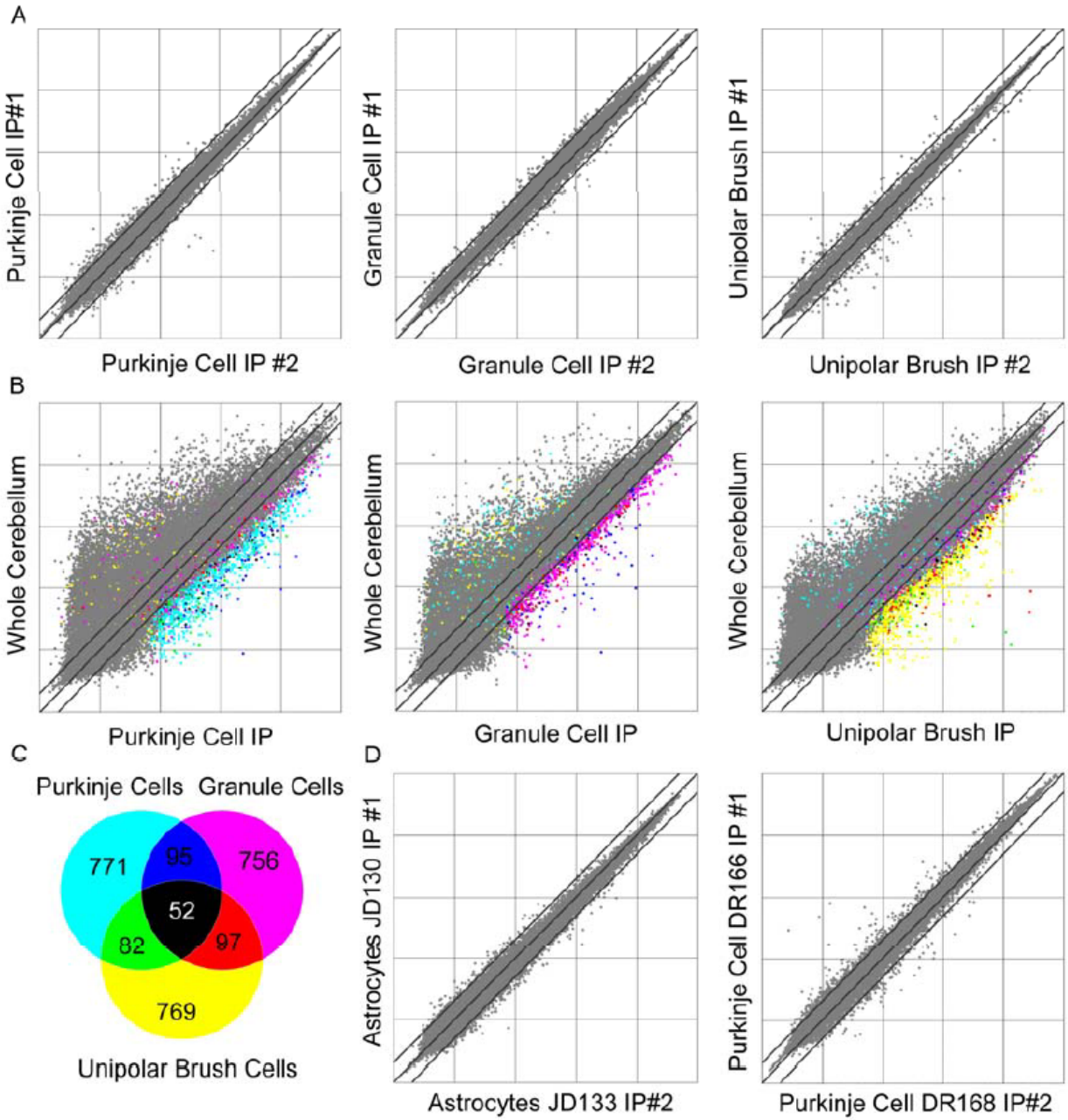


Panel	Region	BAC Driver
1	Cerebellum	Pcp2
	Cerebellum	Pcp2
2	Cerebellum	NeuroD1
3	Cerebellum	Grm2
4	Cerebellum	Lypd6
5	Cerebellum	Grp
6	Cerebellum	Olig2
7	Cerebellum	Cmtm5
8	Cerebellum	Sept4
9	Cerebellum	Aldh1L1
10	Spinal Cord	Chat
11	Striatum	Drd1
12	Striatum	Drd2
13	Corpus Striatum	Chat
14	Basal Forebrain	Chat
15	Brain Stem	Chat
16	Cortex	Ntsr1
17	Cortex	Glt25d2
18	Cortex	Etv1
19	Cortex	Pnoc
20	Cortex	Cort
21	Cortex	Aldh1L1
22	Cortex	Aldh1L1
23	Cortex	Olig2
24	Cortex	Cmtm5
25	Cortex	Cck

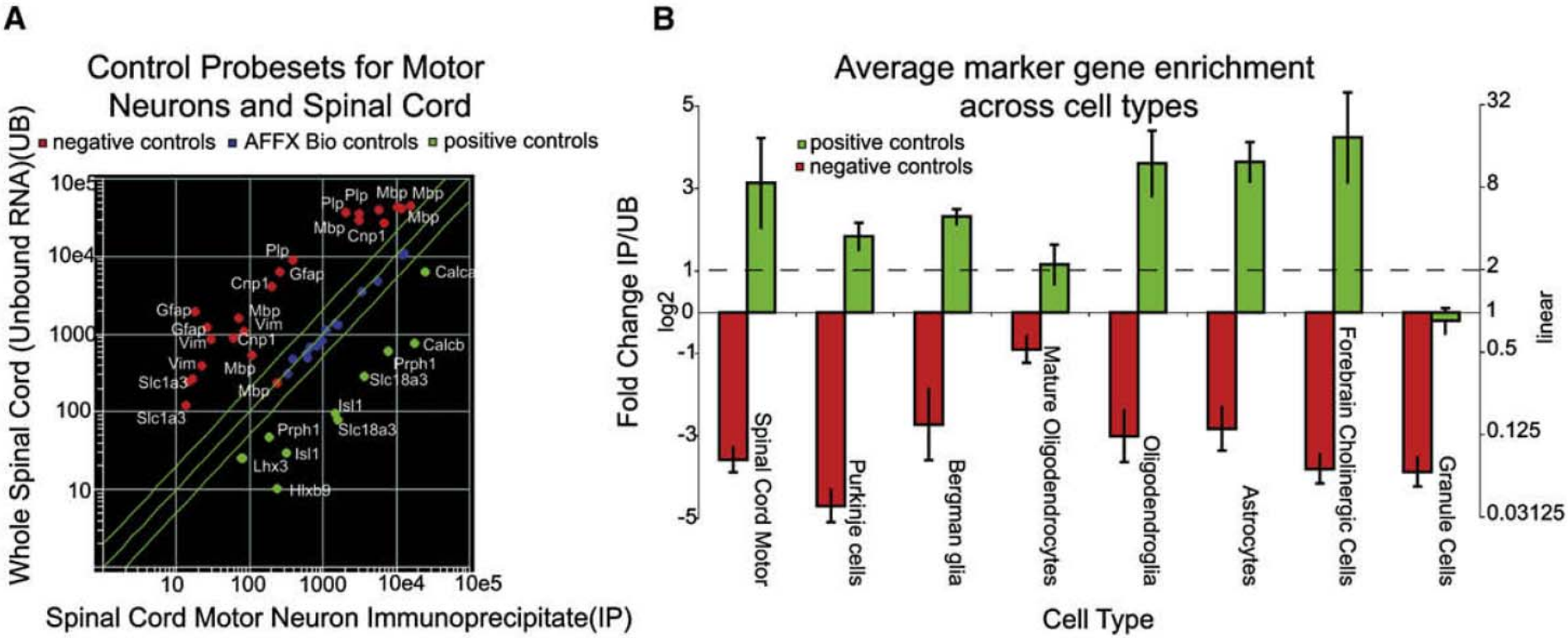
In depth-characterization of bac-TRAP lines



Measurement of mRNA enrichment by TRAP

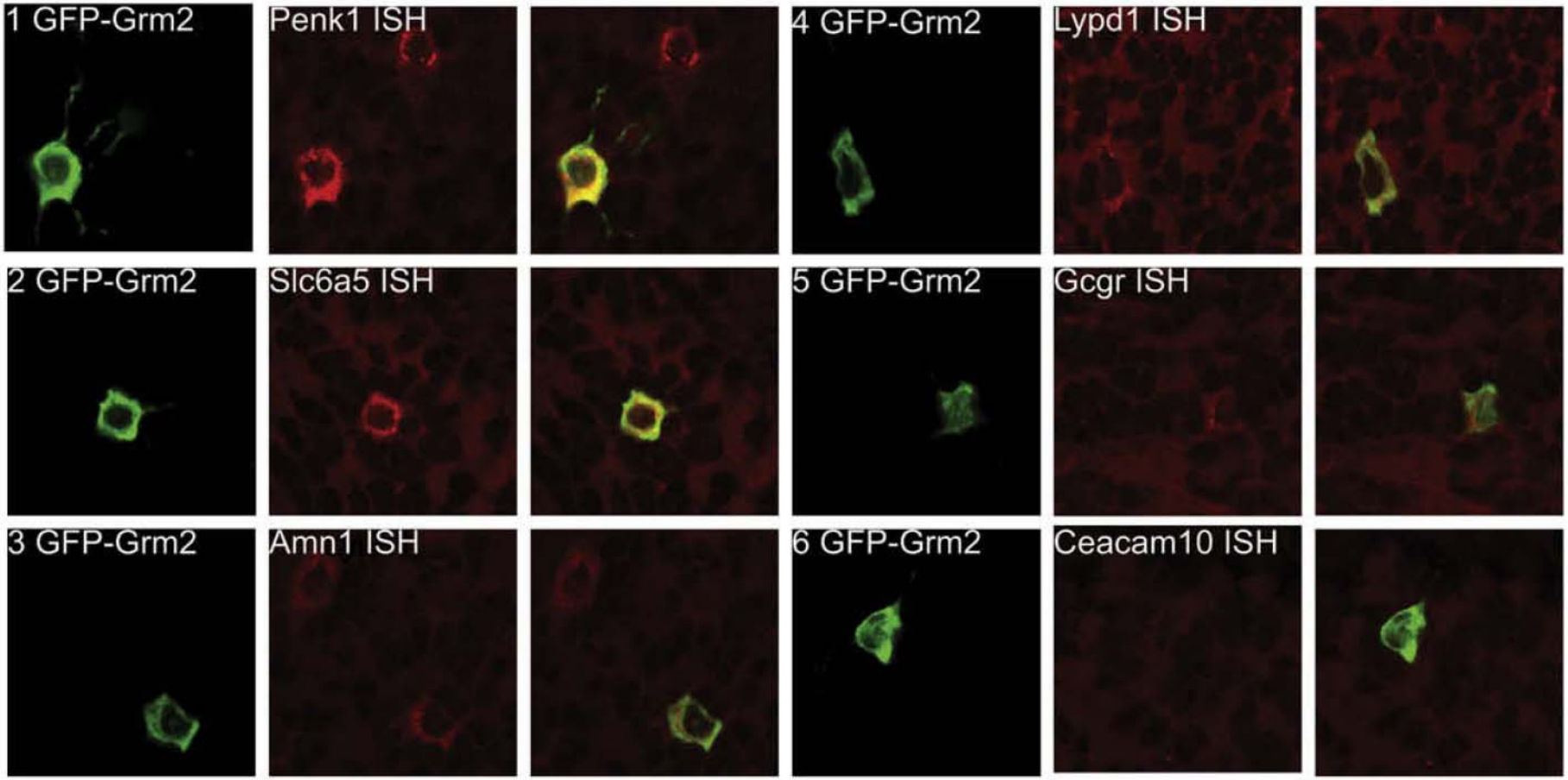


Identification of known cell-specific markers

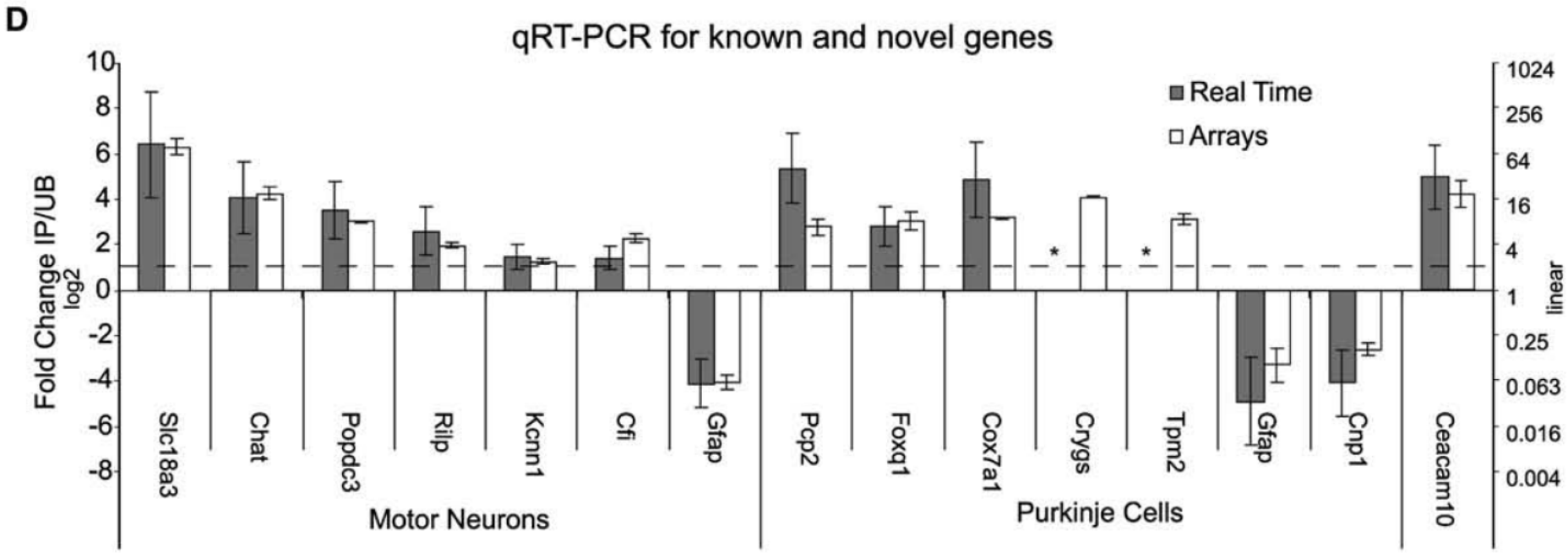


Identification of novel cell-specific markers by TRAP

Cerebellar Golgi cells



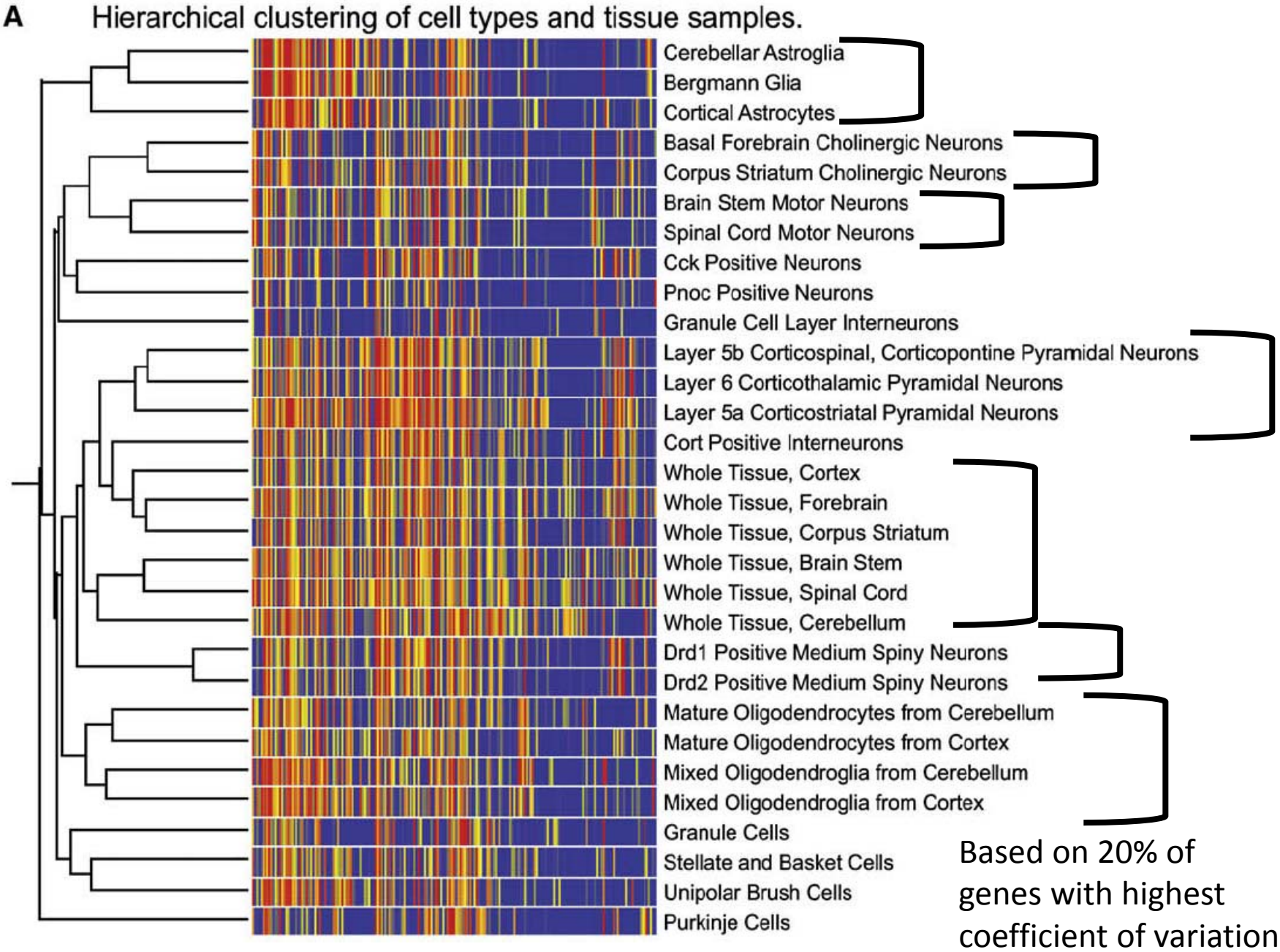
Validation of quantitative aspects of TRAP



Chat bac-TRAP

Pcp2 bac-TRAP

Comparative analysis of TRAP microarray data



Based on 20% of genes with highest coefficient of variation

Summary

- Confirmation and extension of TRAP technology
- Generation of 12 additional bac-TRAP lines (16 in total)
- Comparative microarray analysis of different CNS cell types (incl. data not shown)

→ All plasmids and mouse lines are available upon request

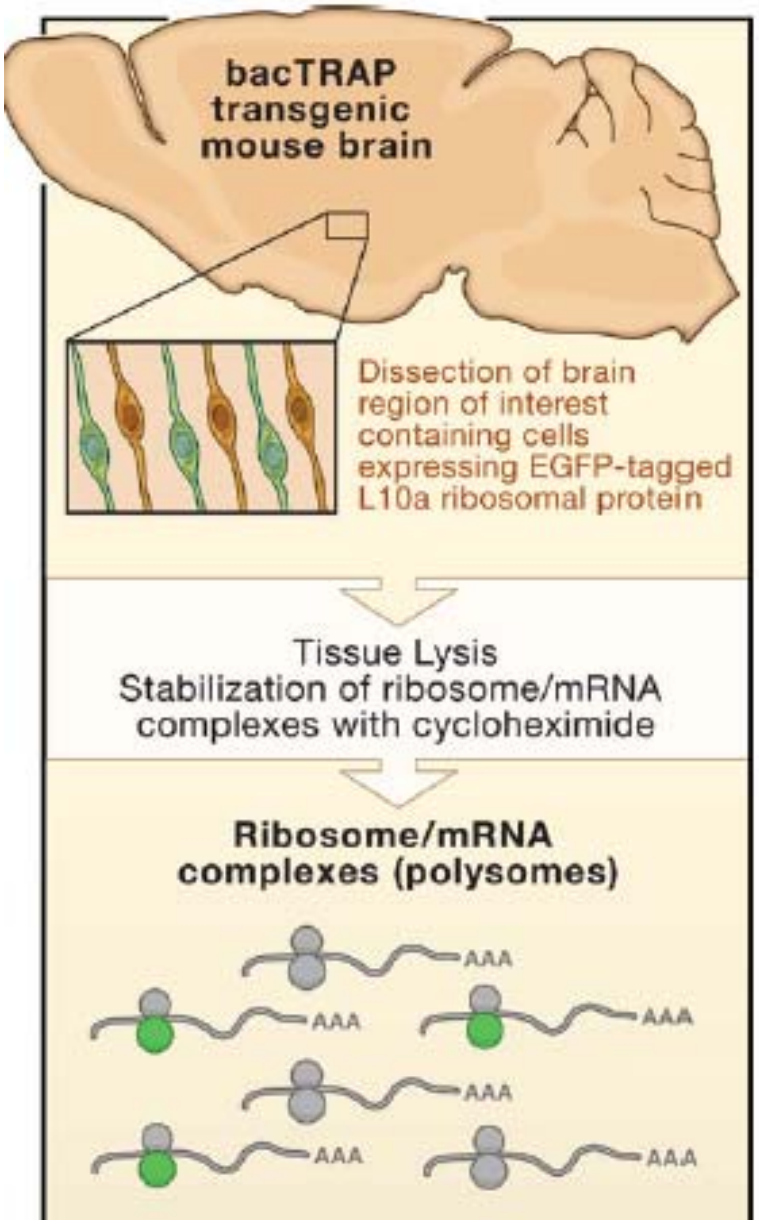
Advantages:

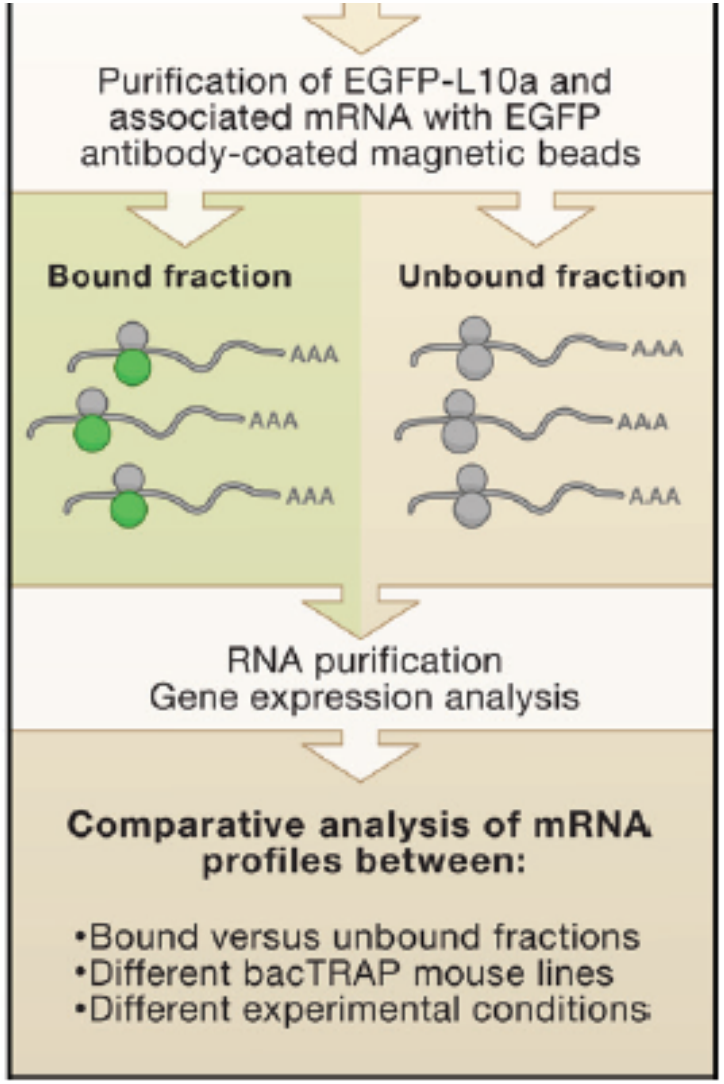
- *In situ*, avoiding artefacts from dissociation or tissue fixation
- EGFP tagging allows visualization of cells under study (e.g. electrophysiology)
- Translatome, rather than transcriptome
- Relatively rapid
- Does not require special instruments or reagents (besides bac-TRAP lines)

Limitations:

- A genetic element to drive cell-type specific expression of TRAP transgene is needed
- Specific bac-TRAP lines for the cell of interest have to be generated
- Low mRNA yield
- Translatome, rather than transcriptome

TRAP strategy





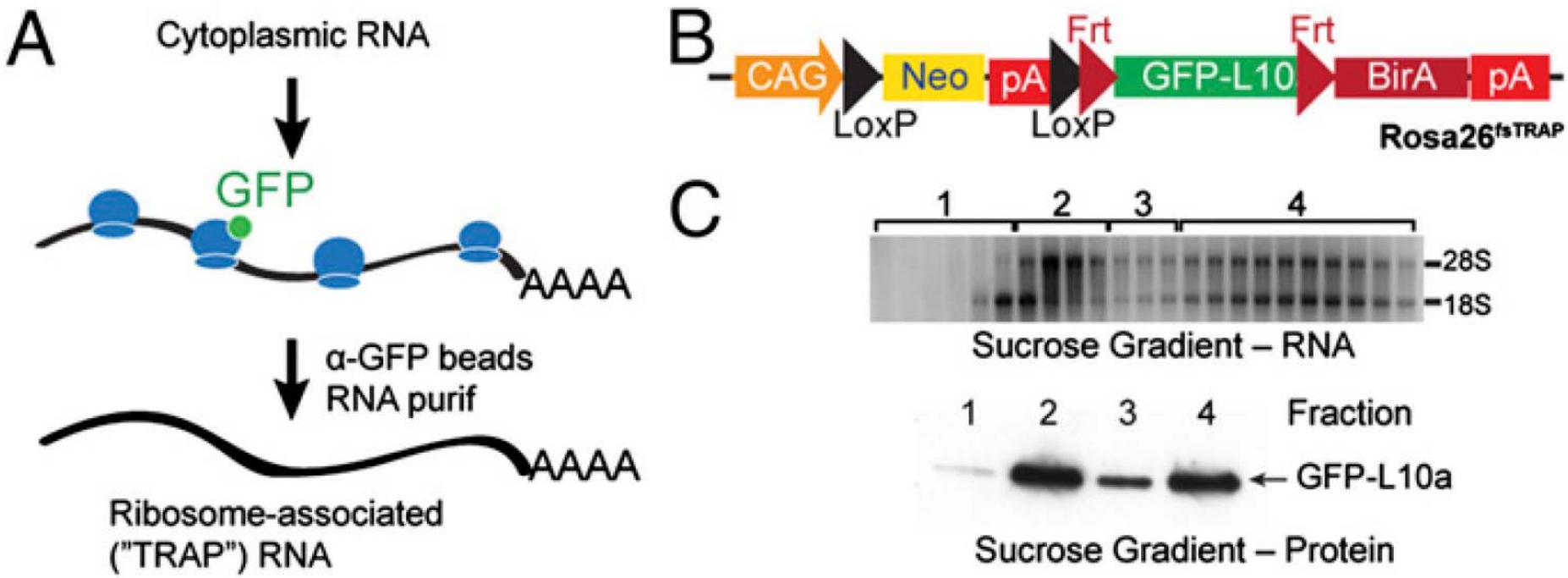
Emery & Barres Cell 2008

Interrogating translational efficiency and lineage-specific transcriptomes using ribosome affinity purification

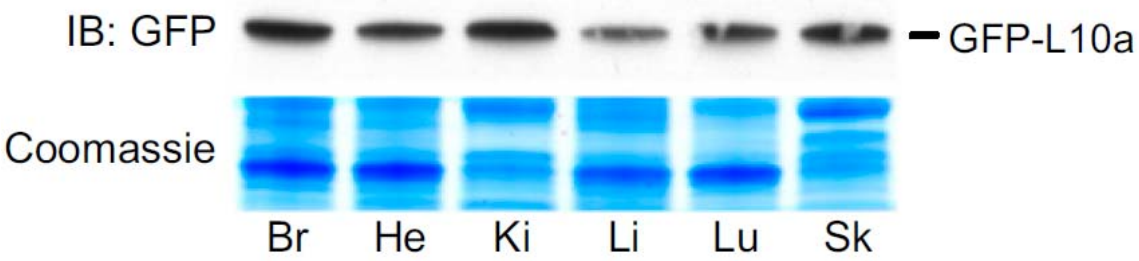
Pingzhu Zhou[a], Yijing Zhang[a], Qing Ma[a], Fei Gu[a], Daniel S. Day[b,c], Aibin He[a], Bin Zhou[a,1], Jing Li[d], Sean M. Stevens[a], Daniel Romo[d], and William T. Pu[a,e,2]

[a]Department of Cardiology, Boston Children's Hospital, Boston, MA 02115; [b]Center for Biomedical Informatics, Harvard Medical School, Boston, MA 02115; [c]Harvard/Massachusetts Institute of Technology Division of Health Sciences and Technology, Cambridge, MA 02139; [d]Laboratory for Innovative, Chemistry, and Natural Products-Based Interdisciplinary Drug Discovery, Texas A&M University, College Station, TX 77842; and [e]Harvard Stem Cell Institute, Harvard University, Cambridge, MA 02138

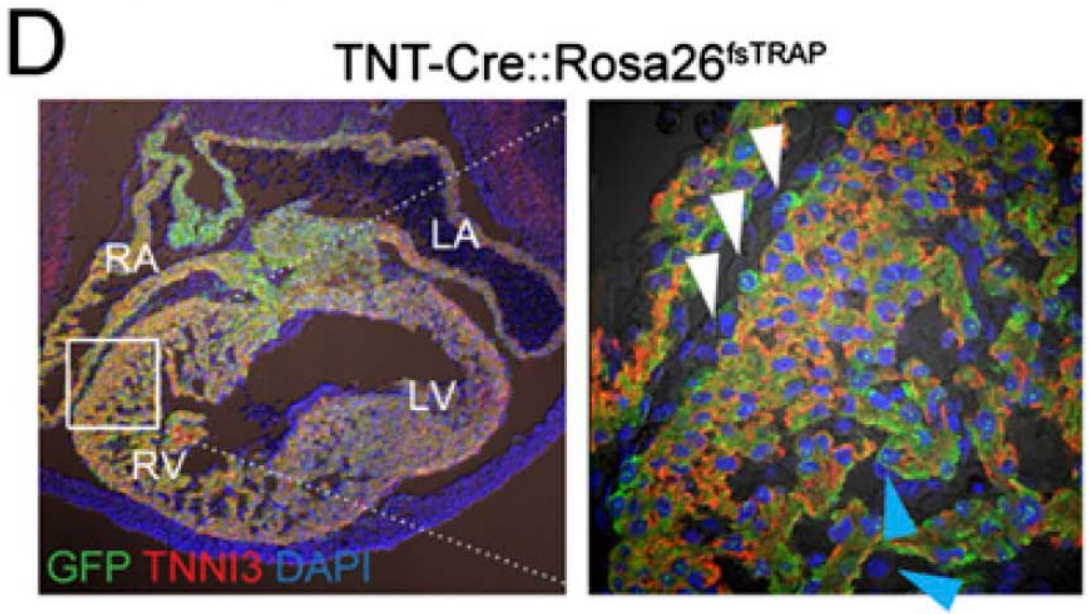
Generation of a Cre-activated TRAP mouse line

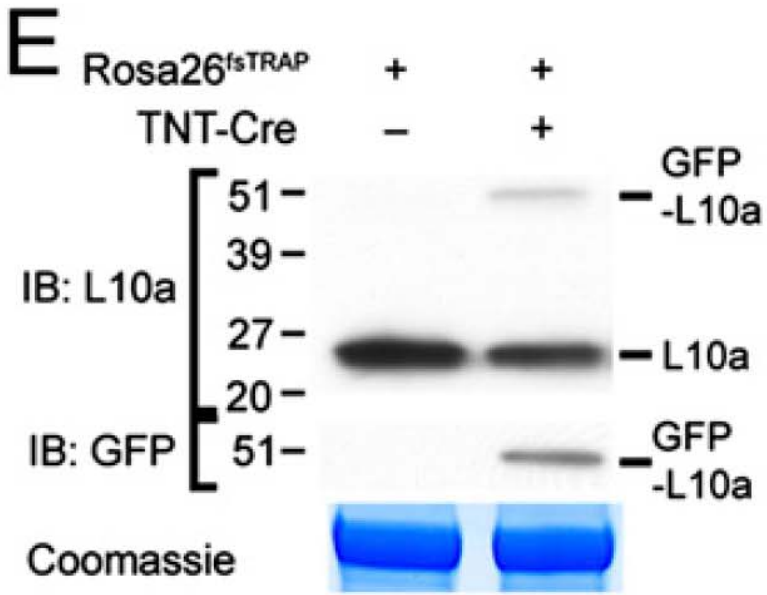


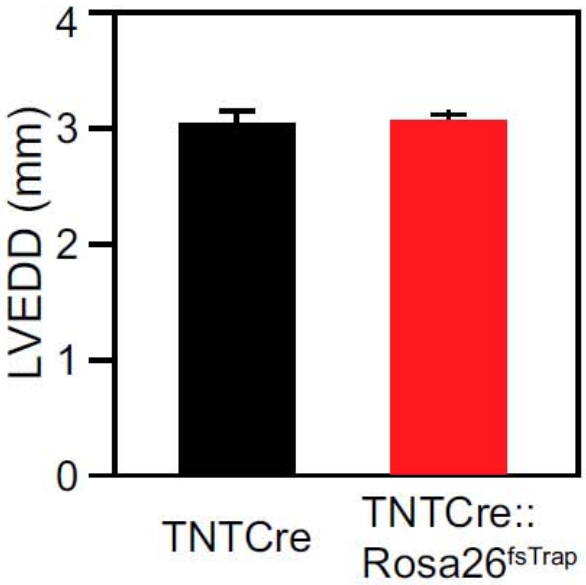
Ubiquitous Cre-mediated activation of TRAP transgene

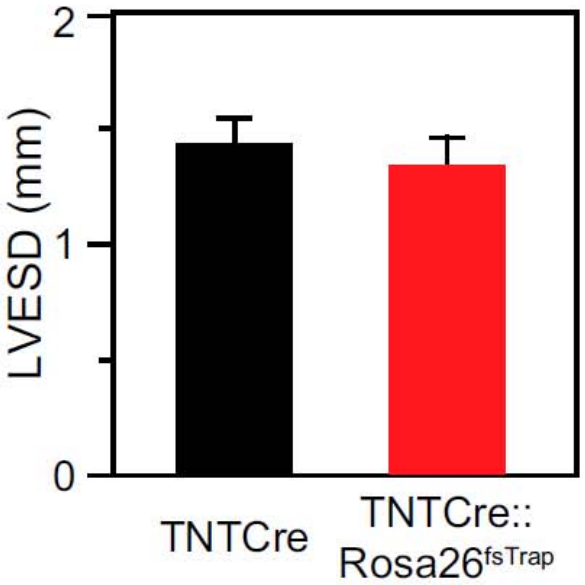


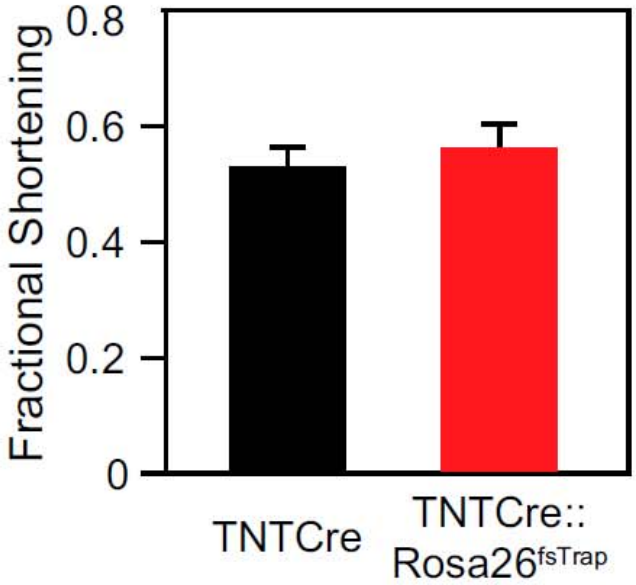
Cardiomyocyte-restricted activation of TRAP transgene



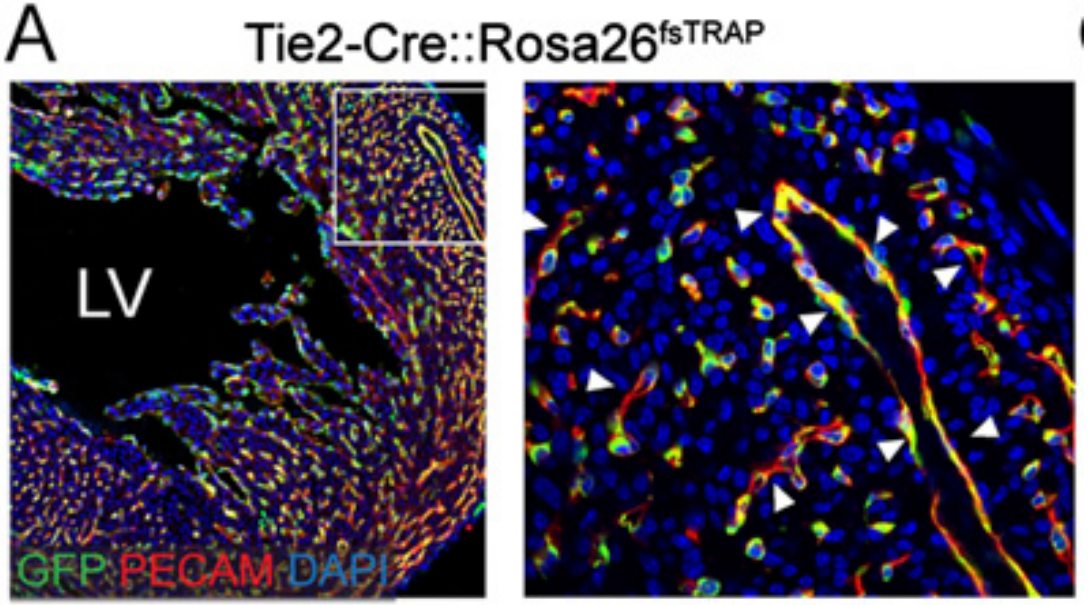


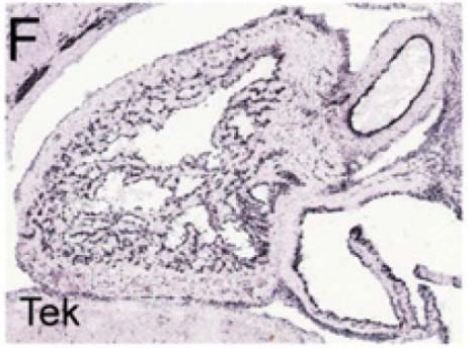


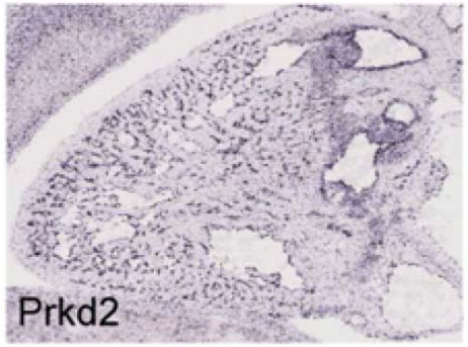


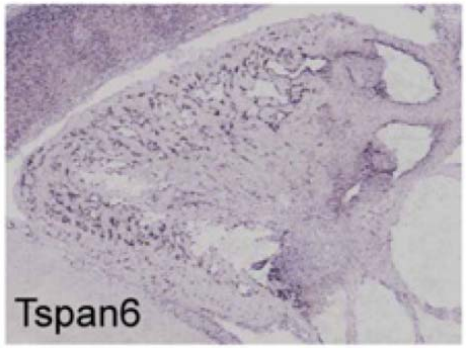


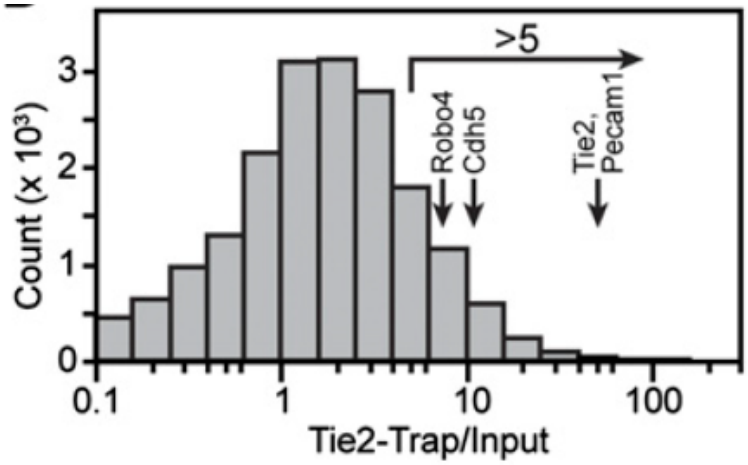
Endothelial cell-restricted activation of TRAP transgene

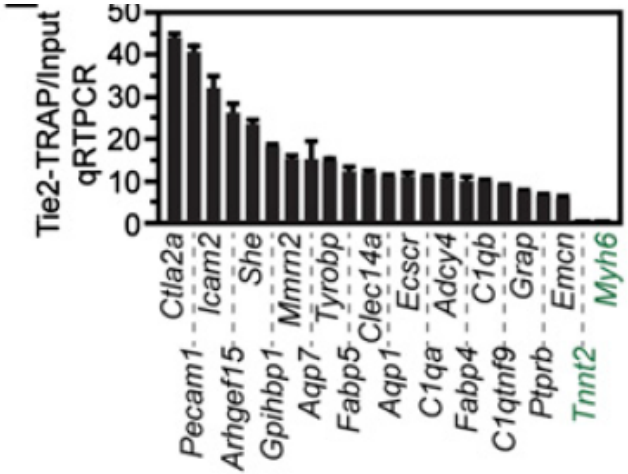












Summary

- Establishment and proof-of-principle of Cre-conditional TRAP transgene

- Study of endothelial-cell enriched/specific genes in heart

- Study of translational modifications in cardiomyocytes after aortic banding (not shown)

→ Rosa26^{fsTRAP} and Rosa26$^{CAG\text{-}TRAP}$ lines are available at JAX laboratories

Published online 27 October 2013

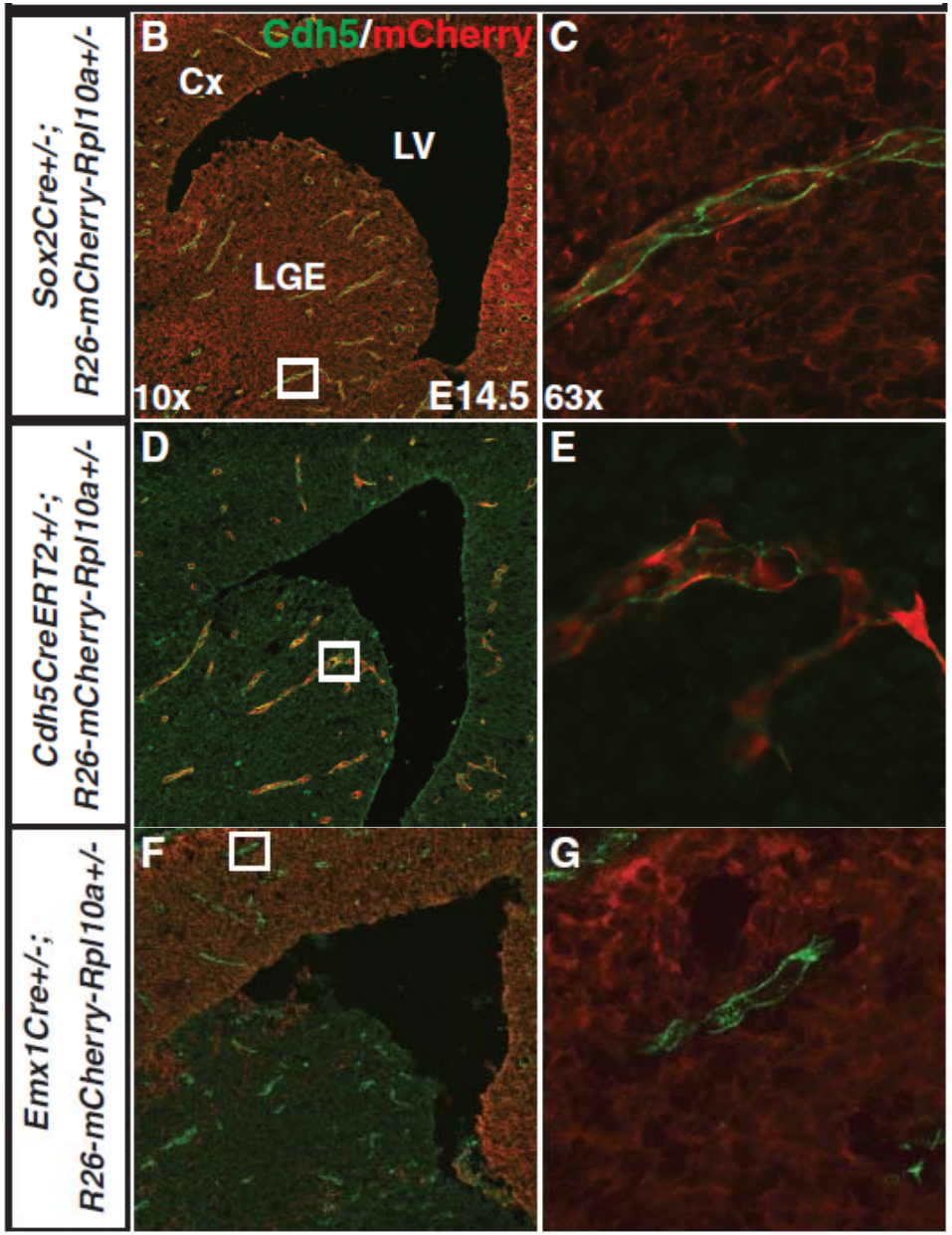
Nucleic Acids Research, 2014, Vol. 42, No. 2 ***e14***
doi:10.1093/nar/gkt995

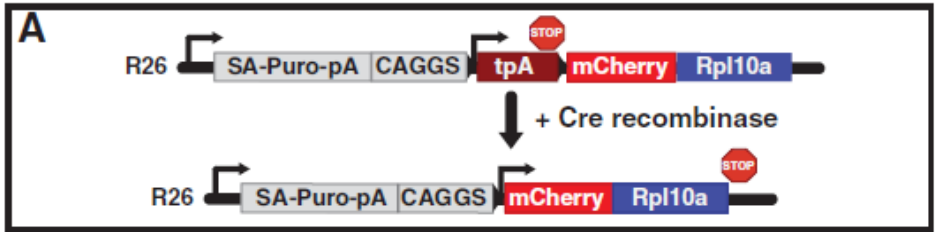
Evaluation of TRAP-sequencing technology with a versatile conditional mouse model

Mike Hupe[1], Minerva Xueting Li[1,2], Karin Gertow Gillner[1], Ralf H. Adams[3] and Jan M. Stenman[1,*]

[1]Ludwig Institute for Cancer Research Ltd, Box 240, Stockholm SE-171 77, Sweden, [2]Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm SE-171 77, Sweden and [3]Max-Planck-Institute for Molecular Biomedicine, Department of Tissue Morphogenesis, and University of Münster, Faculty of Medicine, D-48149 Muenster, Germany

Generation of a Cre-activated TRAP mouse line





All epiblast derivatives

Endothelial cells

Dorsal telencephalon

Summary

- Establishment and proof-of-principle of an additional Cre-conditional TRAP transgene

- Study of endothelial-cell enriched/specific genes in brain and kidney (data not shown)

- Study of differences in transcriptome and translatome in different organs (not shown)

Dmitry A. Belostotsky (ed.), *Plant Systems Biology, vol. 553*

DOI 10.1007/978-1-60327-563-7_6 Springerprotocols.com

Isolation of Plant Polysomal mRNA by Differential Centrifugation and Ribosome Immunopurification Methods

Angelika Mustroph, Piyada Juntawong, and Julia Bailey-Serres

PLoS ONE | www.plosone.org 1 July 2012 | Volume 7 | Issue 7 | e40276

A Versatile Method for Cell-Specific Profiling of Translated mRNAs in *Drosophila*

Amanda Thomas[1], Pei-Jung Lee[1], Justin E. Dalton[2], Krystle J. Nomie[1], Loredana Stoica[3,4], Mauro Costa-Mattioli[3,4,5], Peter Chang[6], Sergey Nuzhdin[6], Michelle N. Arbeitman[2]*, Herman A. Dierick[1,3,5,7]*

Cell Type–Specific Translational Profiling in the *Xenopus laevis* Retina

DEVELOPMENTAL DYNAMICS 241:1960–1972, 2012

F. L. Watson,[1]* E. A. Mills,[2] X. Wang,[1] C. Guo,[3] D. F. Chen,[3] and N. Marsh-Armstrong[2]

Development of Translating Ribosome Affinity Purification for Zebrafish

genesis 51:187–192 (2013)

Robert C. Tryon,* Nilambari Pisat, Stephen L. Johnson, and Joseph D. Dougherty

PROTOCOL

1282 | VOL.9 NO.6 | 2014 | NATURE PROTOCOLS

Cell type–specific mRNA purification by translating ribosome affinity purification (TRAP)

Myriam Heiman[1,3], Ruth Kulicke[1,3], Robert J Fenster[1,3], Paul Greengard[1] & Nathaniel Heintz[2]

- Improved protocol, yielding higher RNA amount upon TRAP purification
- Two days from sample preparation to RNA purification
- Freshly isolated samples give higher RNA yields
- Compatible with Northern blotting, qPCR, microarray and RNA-Seq

Summary

- Allows cell-specific studies of translatome

Advantages:
- *In situ*, avoiding artefacts from dissociation or tissue fixation
- EGFP or mCherry tagging allows visualization of cells under study (e.g. electrophysiology)
- Translatome, rather than transcriptome
- Relatively rapid
- Does not require special instruments or reagents (besides bac-TRAP or conditional TRAP lines)

Limitations:
- A genetic element to drive cell-type specific expression of TRAP transgene is needed
- Limited temporal control of TRAP transgene expression
- Translatome, rather than transcriptome