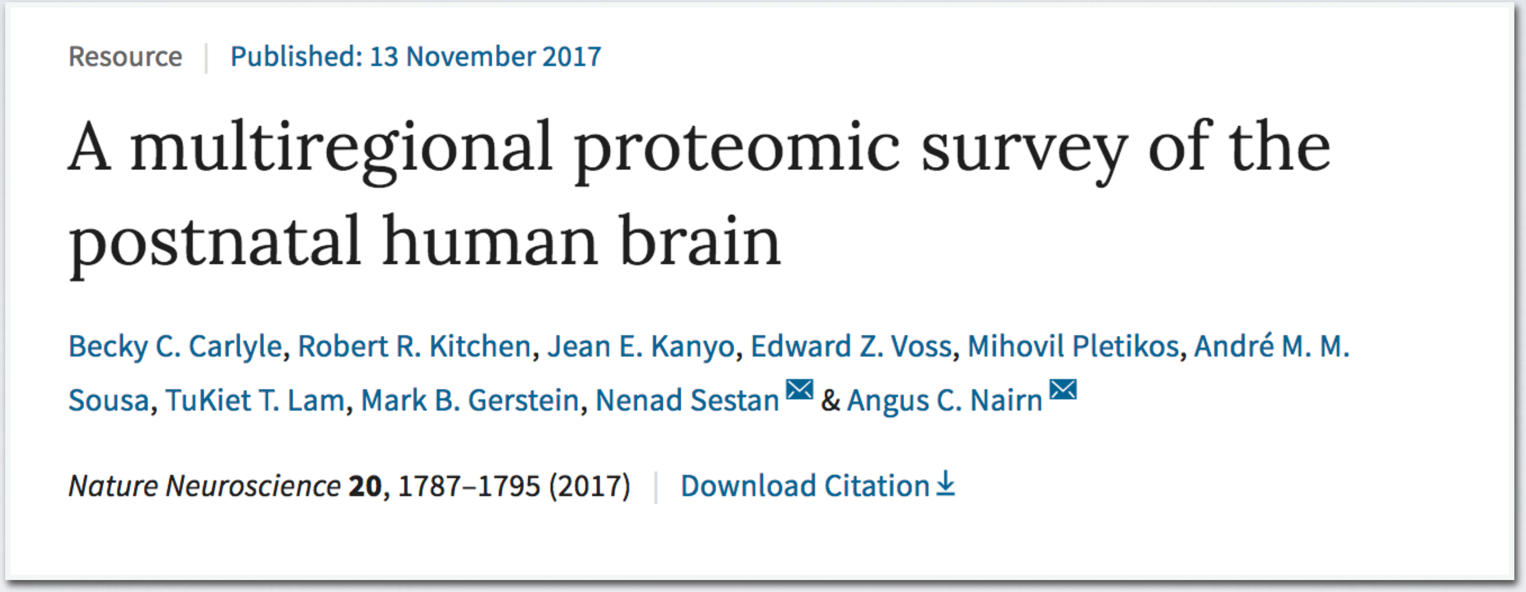
A Multiregional Proteomic Survey of the Postnatal Human Brain

Becky Carlyle, PhD
currently: Alzheimer’s Clinical and Translational Research Unit
Massachusetts General Hospital
previously: Nairn Lab, Yale Department of Psychiatry

Talk outline

- Key take homes from the Brainspan neuroproteomic survey



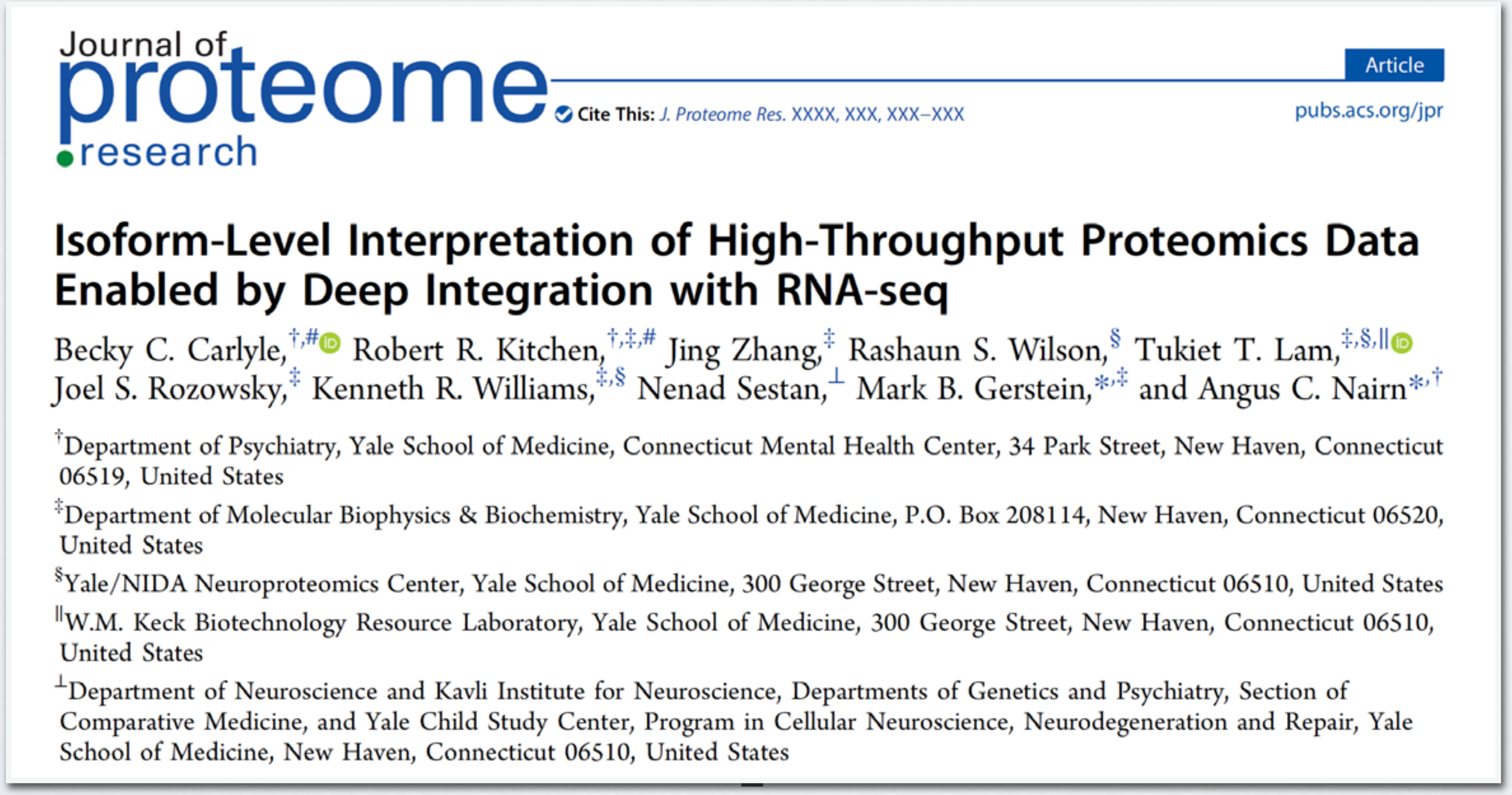
Resource | Published: 13 November 2017

A multiregional proteomic survey of the postnatal human brain

Becky C. Carlyle, Robert R. Kitchen, Jean E. Kanyo, Edward Z. Voss, Mihovil Pletikos, André M. M. Sousa, TuKiet T. Lam, Mark B. Gerstein, Nenad Sestan ✉ & Angus C. Nairn ✉

Nature Neuroscience **20**, 1787–1795 (2017) | Download Citation

- Utility of the EMPIRE algorithm for signaling research



Journal of proteome research

Article

Cite This: *J. Proteome Res.* XXXX, XXX, XXX−XXX

pubs.acs.org/jpr

Isoform-Level Interpretation of High-Throughput Proteomics Data Enabled by Deep Integration with RNA-seq

Becky C. Carlyle,[†,#] Robert R. Kitchen,[†,‡,#] Jing Zhang,[‡] Rashaun S. Wilson,[§] Tukiet T. Lam,[‡,§,∥] Joel S. Rozowsky,[‡] Kenneth R. Williams,[‡,§] Nenad Sestan,[⊥] Mark B. Gerstein,[*,‡] and Angus C. Nairn[*,†]

[†] Department of Psychiatry, Yale School of Medicine, Connecticut Mental Health Center, 34 Park Street, New Haven, Connecticut 06519, United States

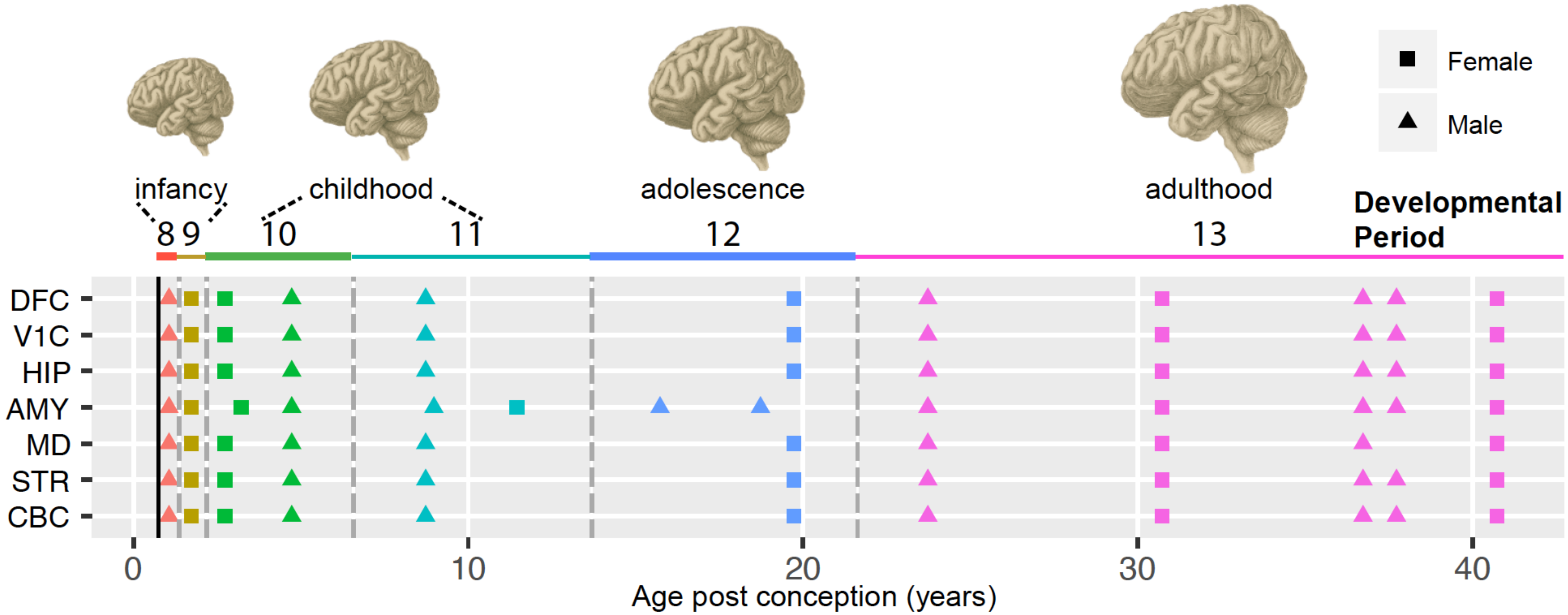
[‡] Department of Molecular Biophysics & Biochemistry, Yale School of Medicine, P.O. Box 208114, New Haven, Connecticut 06520, United States

[§] Yale/NIDA Neuroproteomics Center, Yale School of Medicine, 300 George Street, New Haven, Connecticut 06510, United States

[∥] W.M. Keck Biotechnology Resource Laboratory, Yale School of Medicine, 300 George Street, New Haven, Connecticut 06510, United States

[⊥] Department of Neuroscience and Kavli Institute for Neuroscience, Departments of Genetics and Psychiatry, Section of Comparative Medicine, and Yale Child Study Center, Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale School of Medicine, New Haven, Connecticut 06510, United States

BrainSpan/psychENCODE project samples

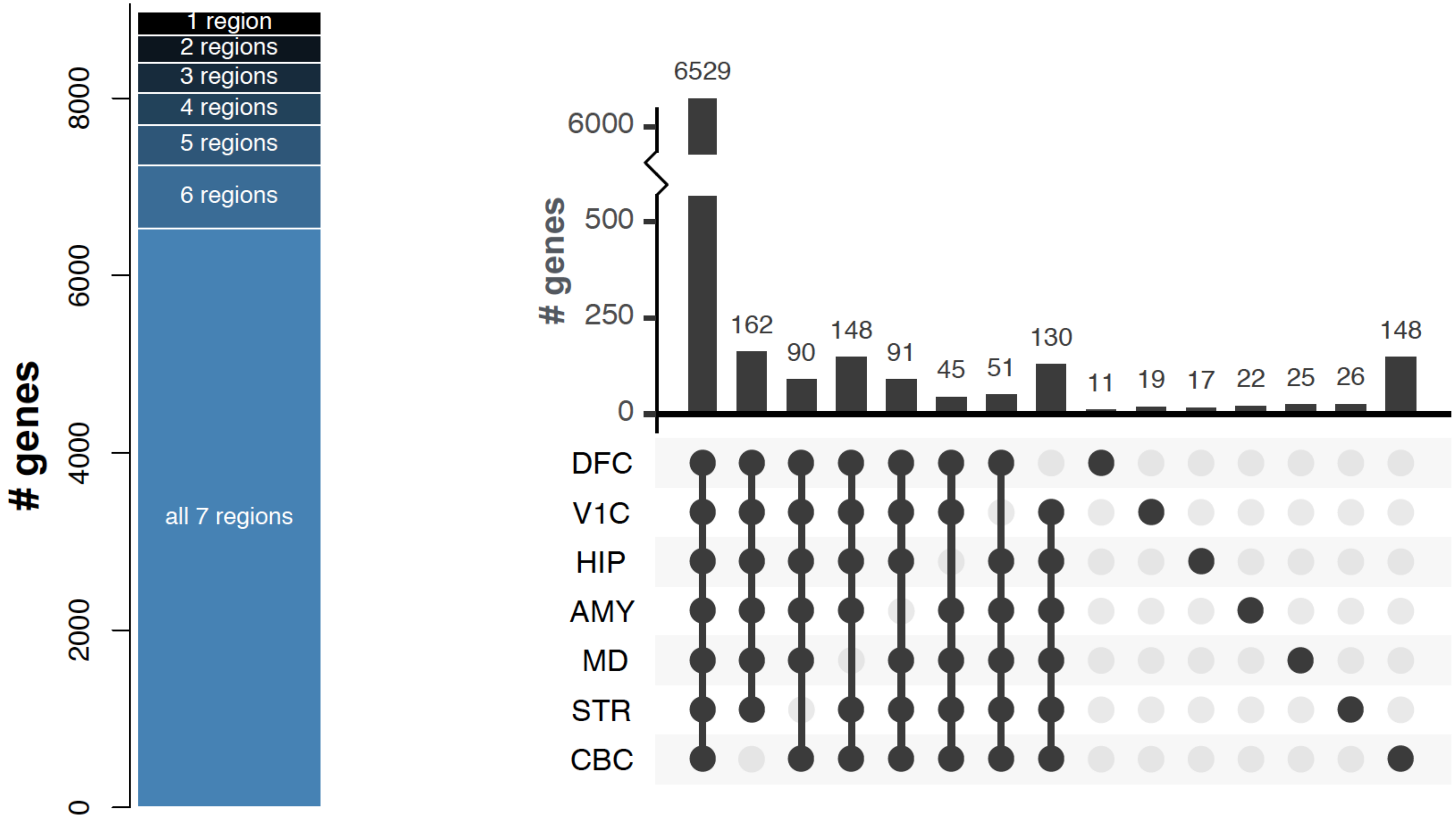


Same pulverized tissue samples as used in BrainSpan for RNA-seq

~6 subjects spanning postnatal development

5 adult subjects, 7 brain regions

Fractionated regions

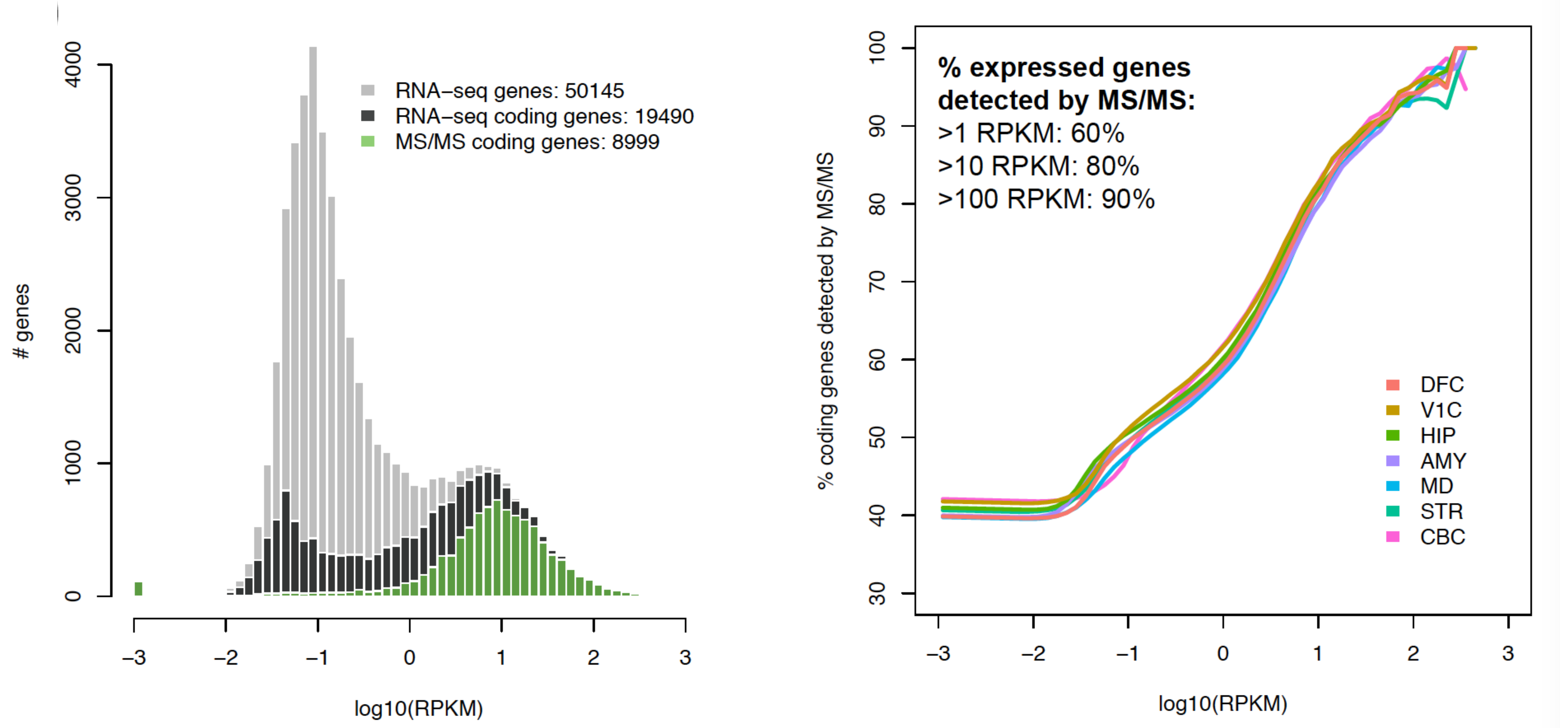


Total protein numbers are comparable to other studies showing ~11,000 proteins (in relatively simpler mixtures)

Most proteins are common to all regions

The cerebellum is a clear outlier

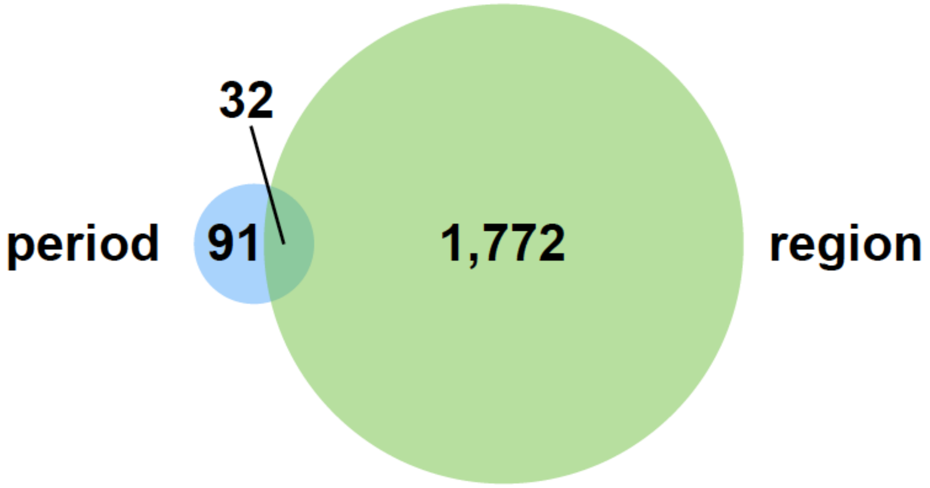
Fractionated regions - Comparison with RNA-seq



% expressed genes detected by MS/MS:
>1 RPKM: 60%
>10 RPKM: 80%
>100 RPKM: 90%

Unsurprisingly, coverage improves the more abundant a gene is

Single shot data: DEX genes - regions

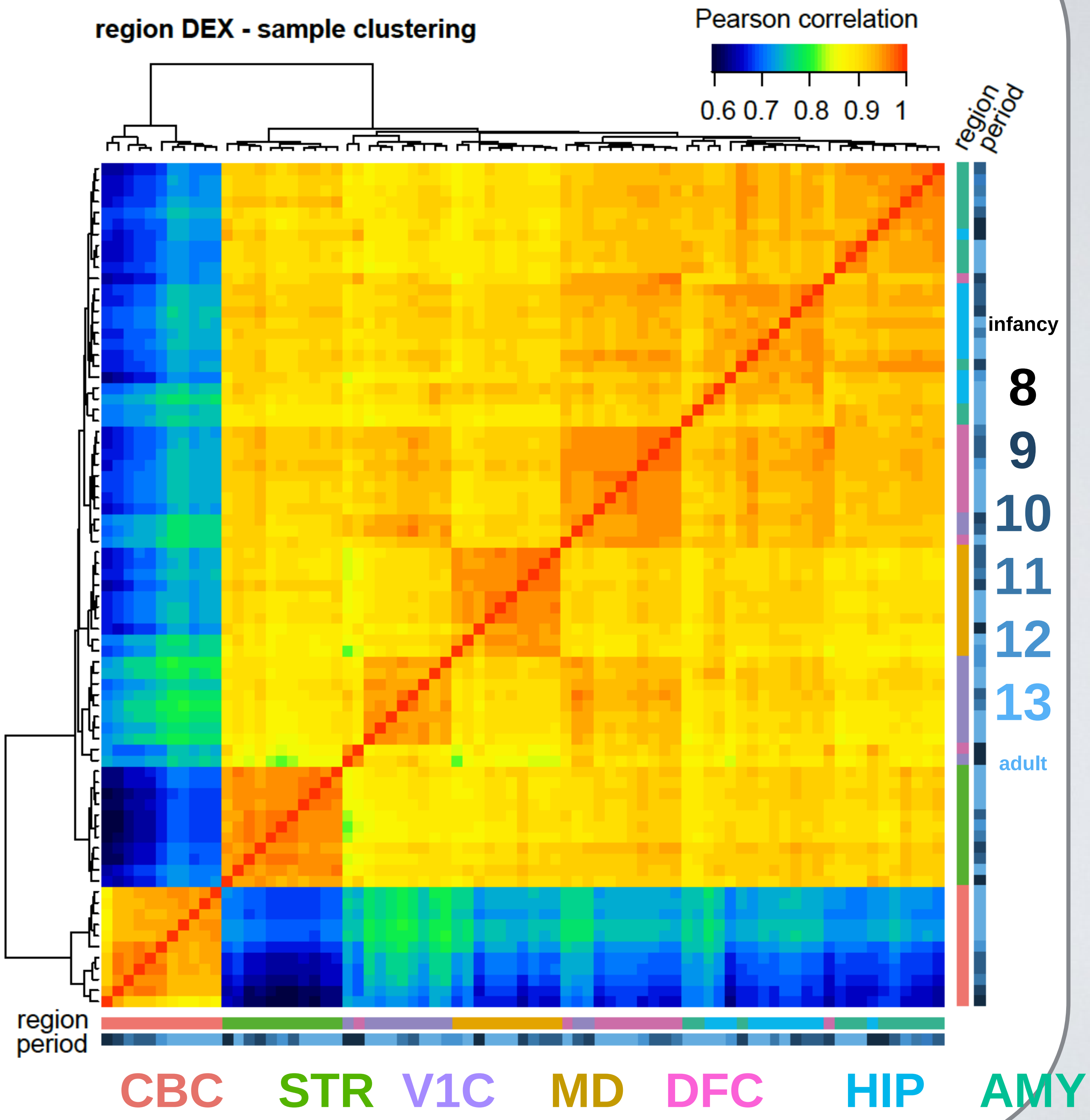


5151 proteins were reliably quantified

Samples were clustered on the basis of DEX genes

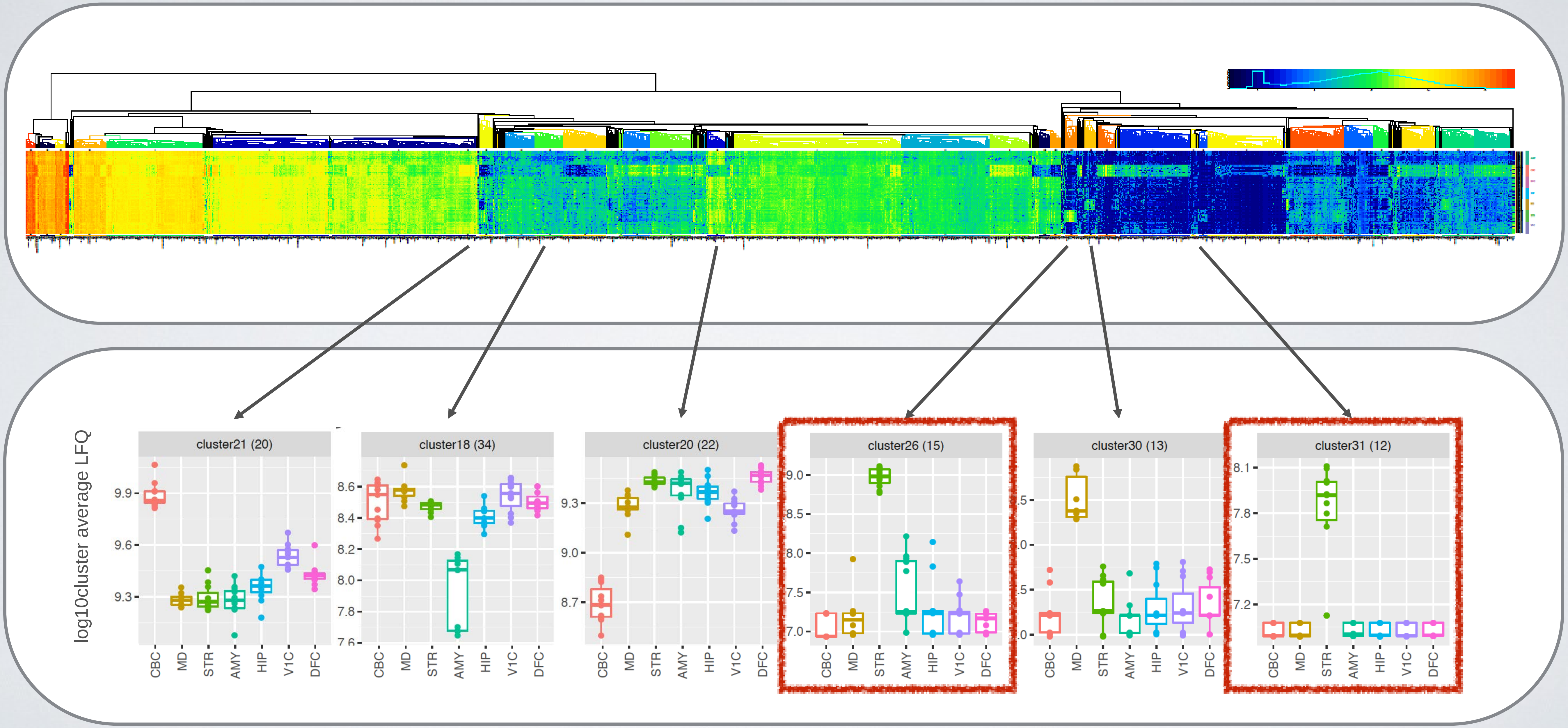
Cerebellum and striatum are clearly defined by their proteome in both development and adulthood

These two regions are markedly more homogenous with regards to cell type than the other 5 regions



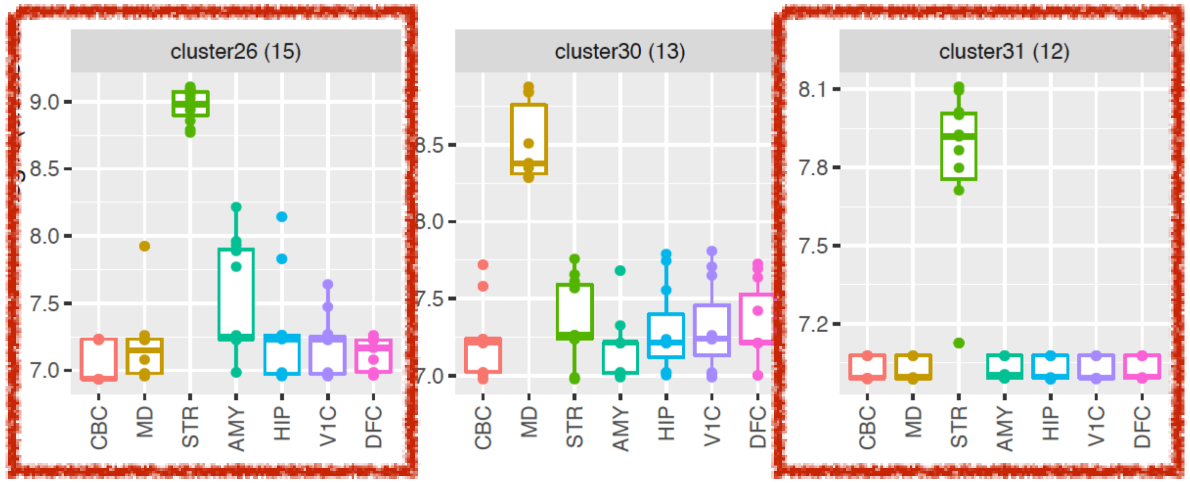
Single shot data: DEX - genes

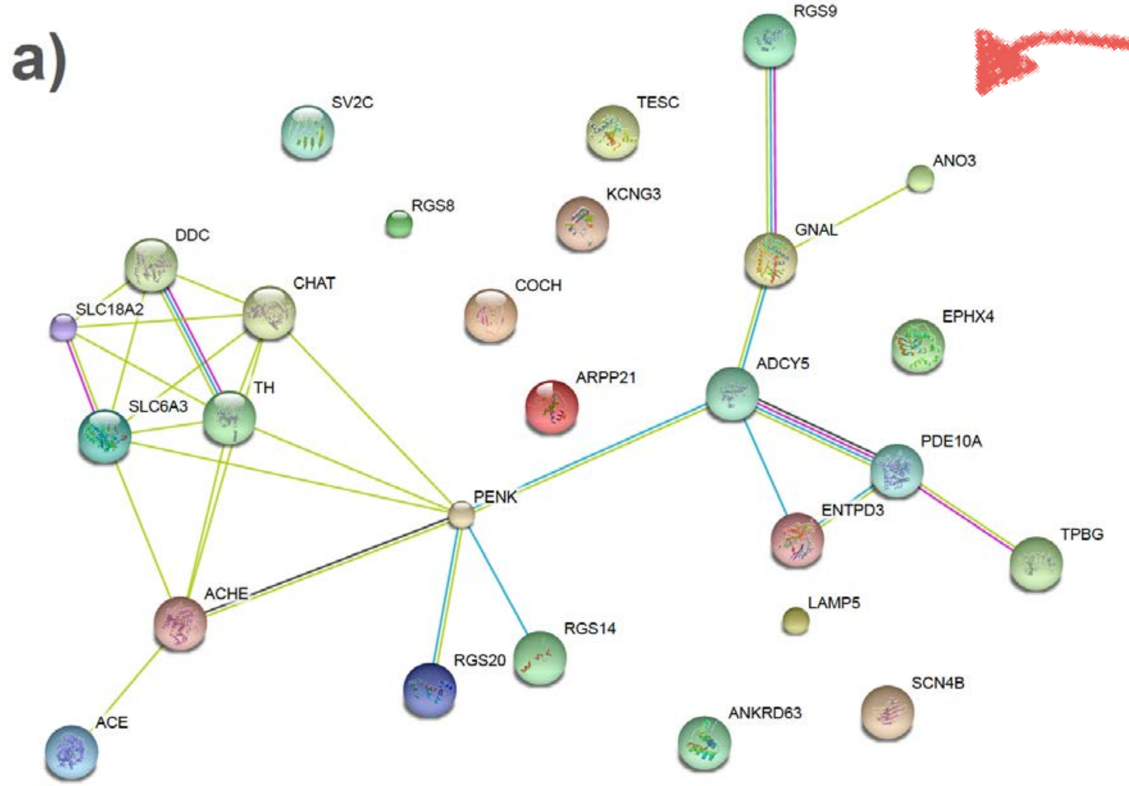
32 region DEX clusters



Striatally enriched clusters

DEX genes - clustering

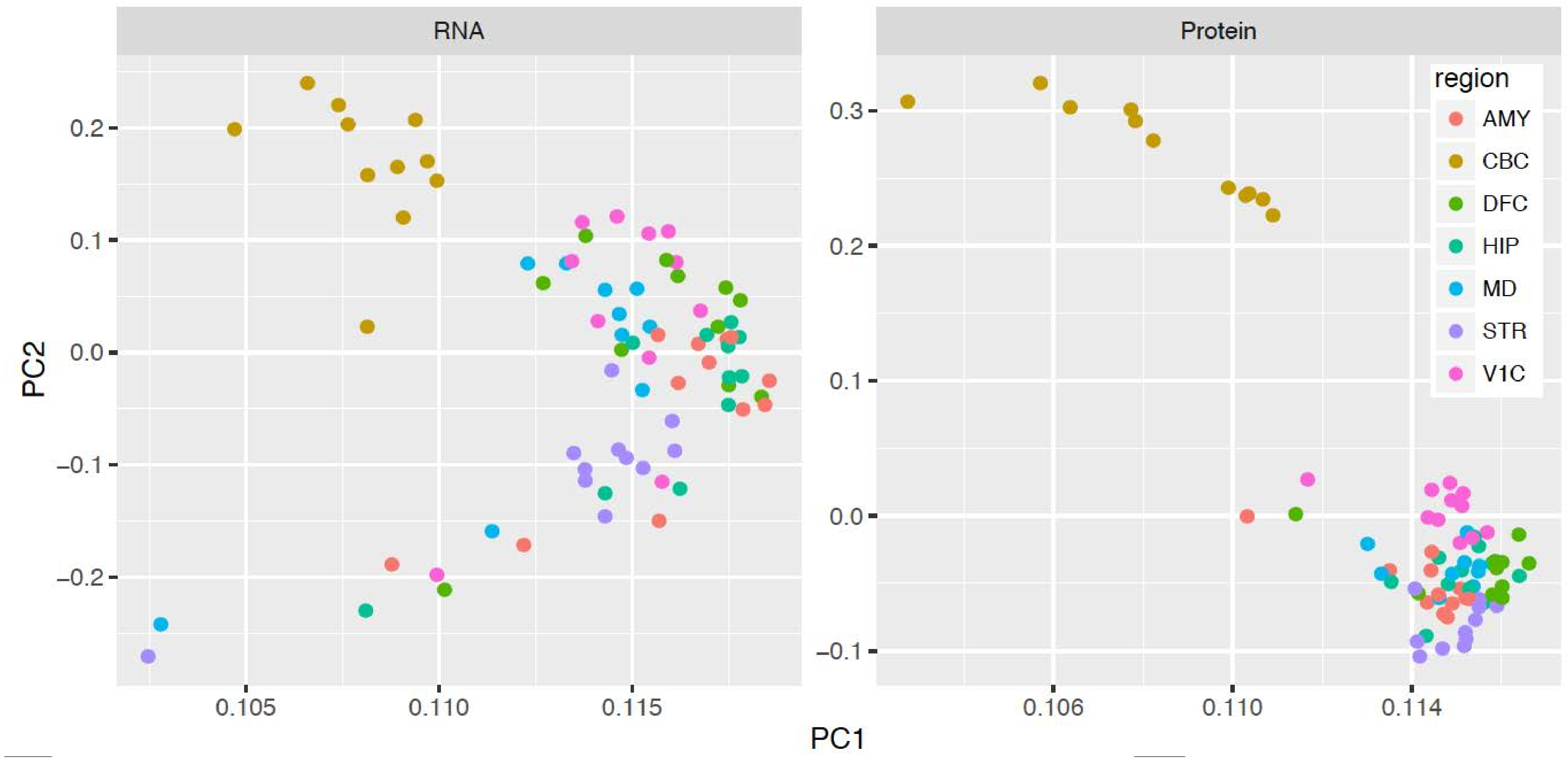




KEGG pathway	pAdj	proteins
Cocaine addiction	2.55E-06	ADCY5, DDC, RGS9, SLC18A2, SLC6A3, TH
Amphetamine addiction	0.0006	ADCY5, DDC, SLC18A2, SLC6A3, TH
Dopaminergic synapse	0.001	ADCY5, DDC, GNAL, SLC18A2, SLC6A3, TH
Parkinson’s disease	0.0147	ADCY5, GNAL, SLC18A2, SLC6A3, TH

Proteins from striatal enriched clusters are functionally related, and enriched for appropriate KEGG pathways

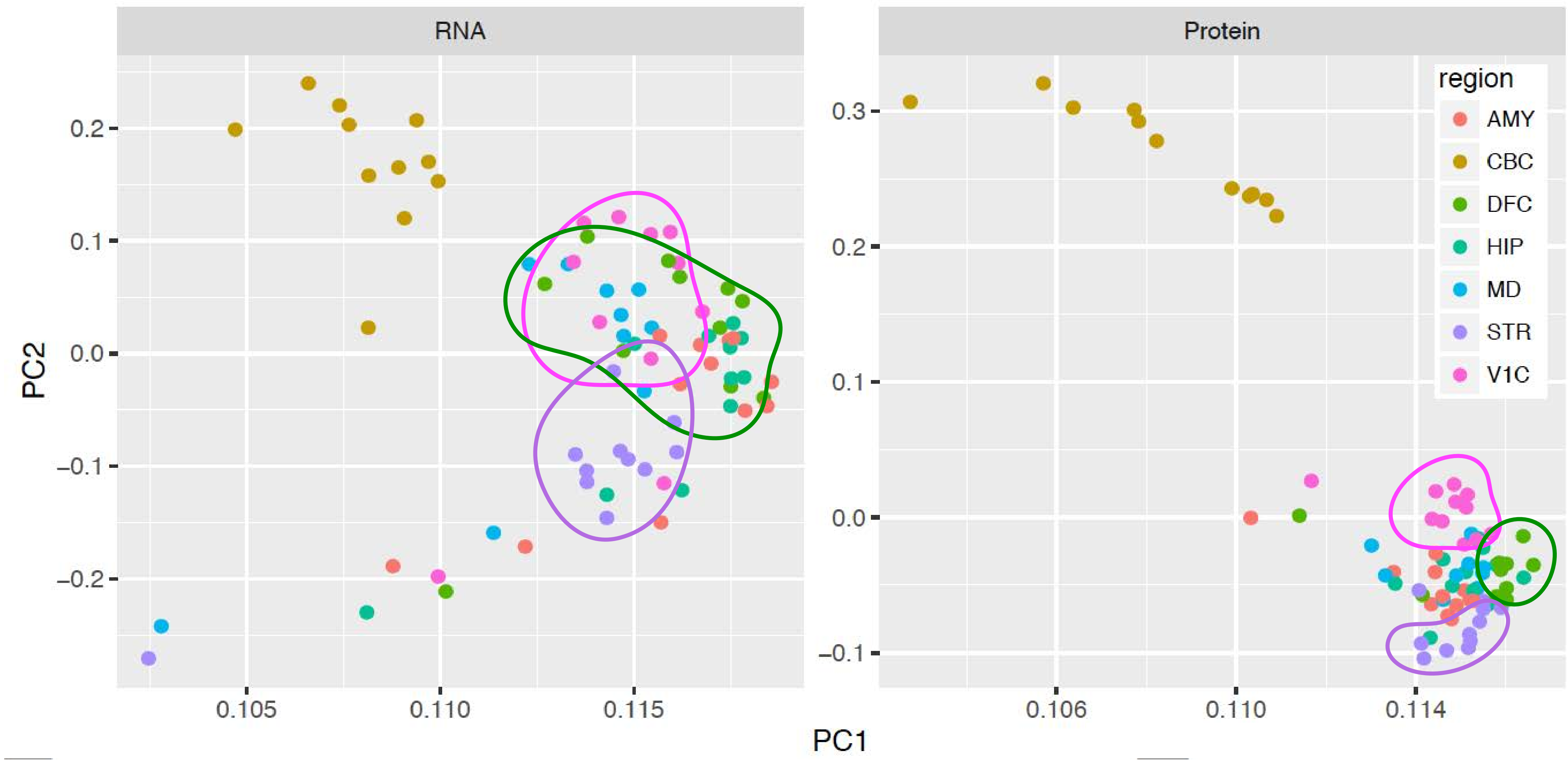
Comparison to RNA-seq



Cerebellum is more clearly separated from the other regions by protein

The other regions are easier to define by protein

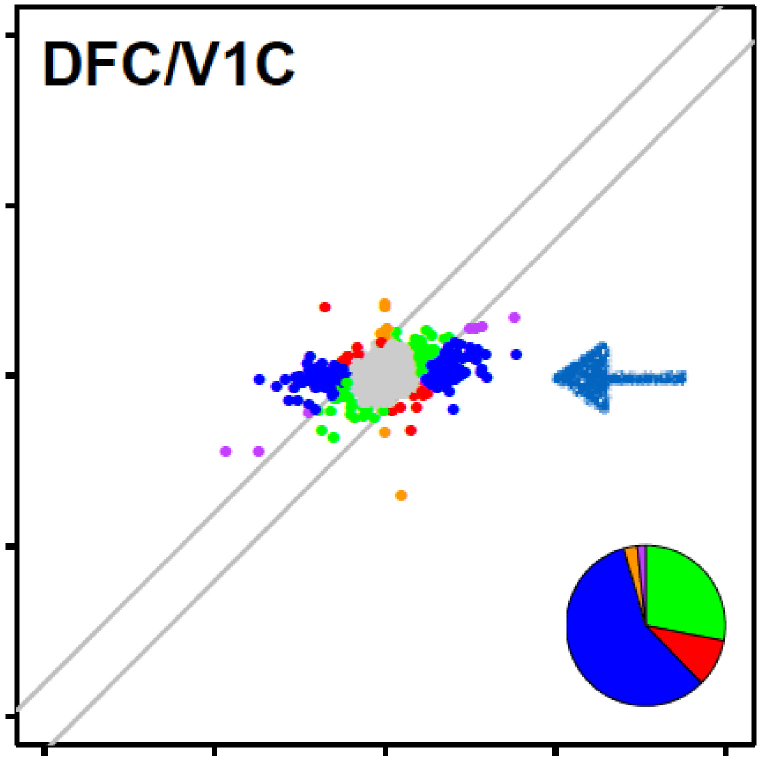
Comparison to RNA-seq



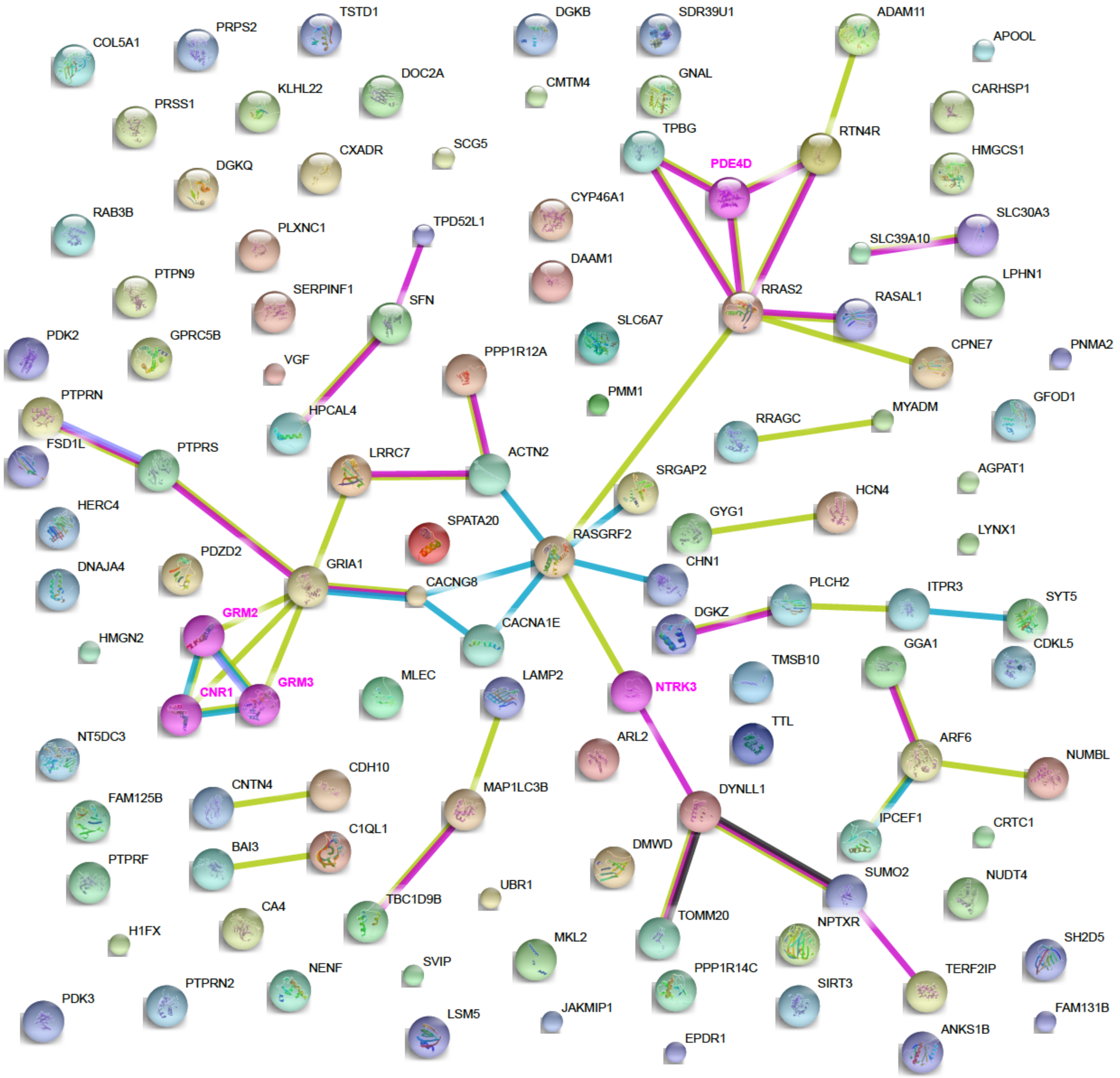
Cerebellum is more clearly separated from the other regions by protein

The other regions are easier to define by protein

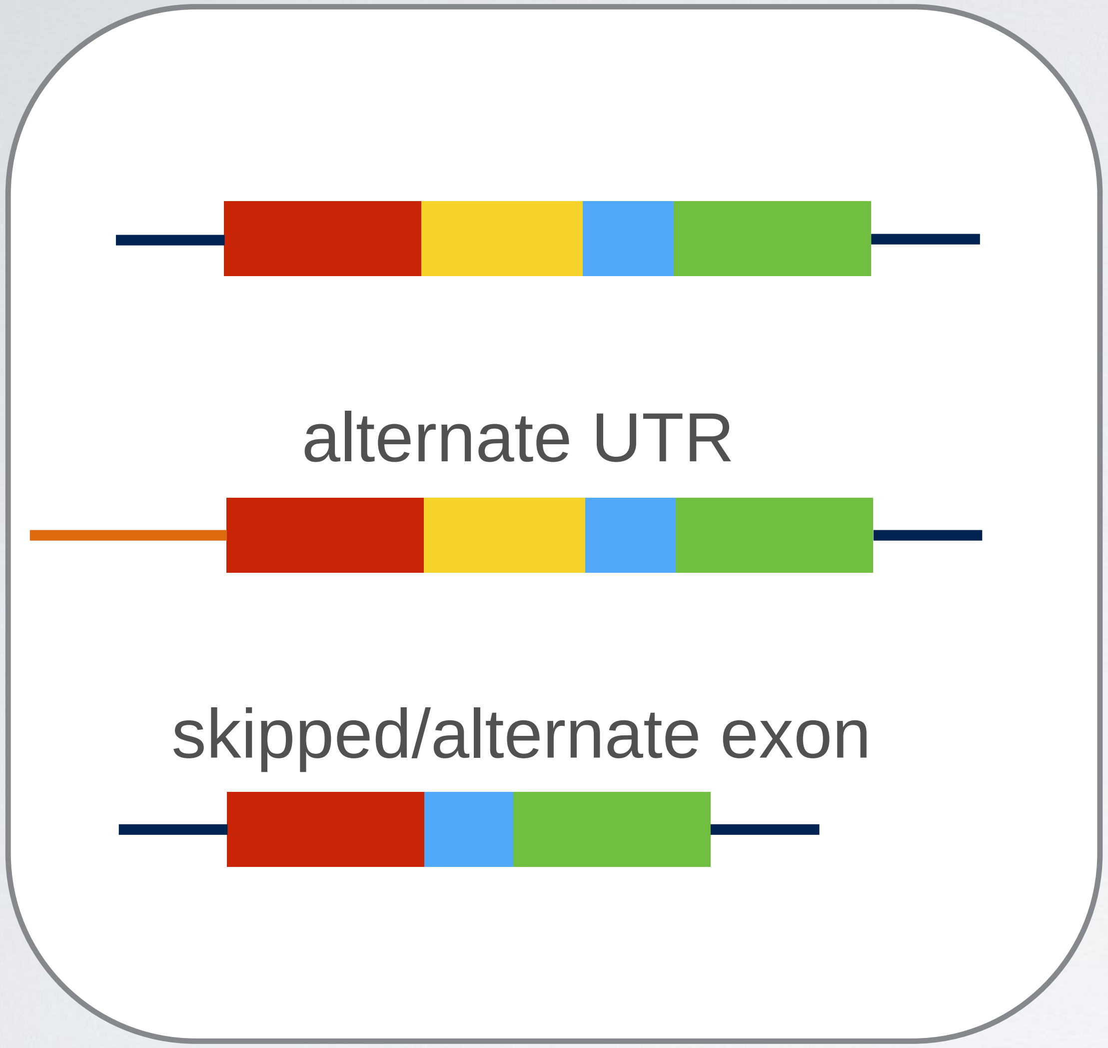
Proteins enriched in dlPFC compared to V1C

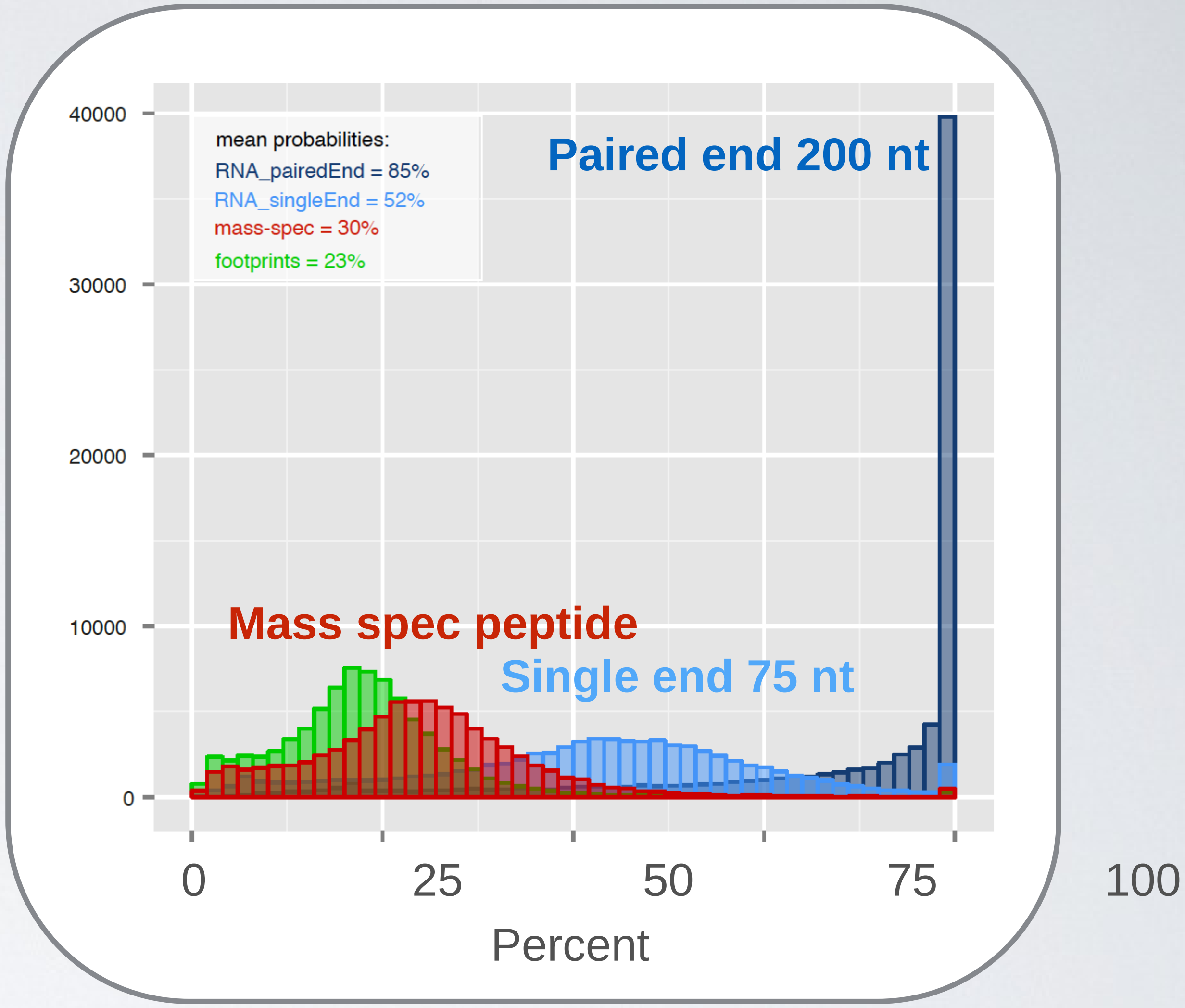


Proteins enriched in DFC vs V1C are functionally related



Deeper integration with mRNA - why?

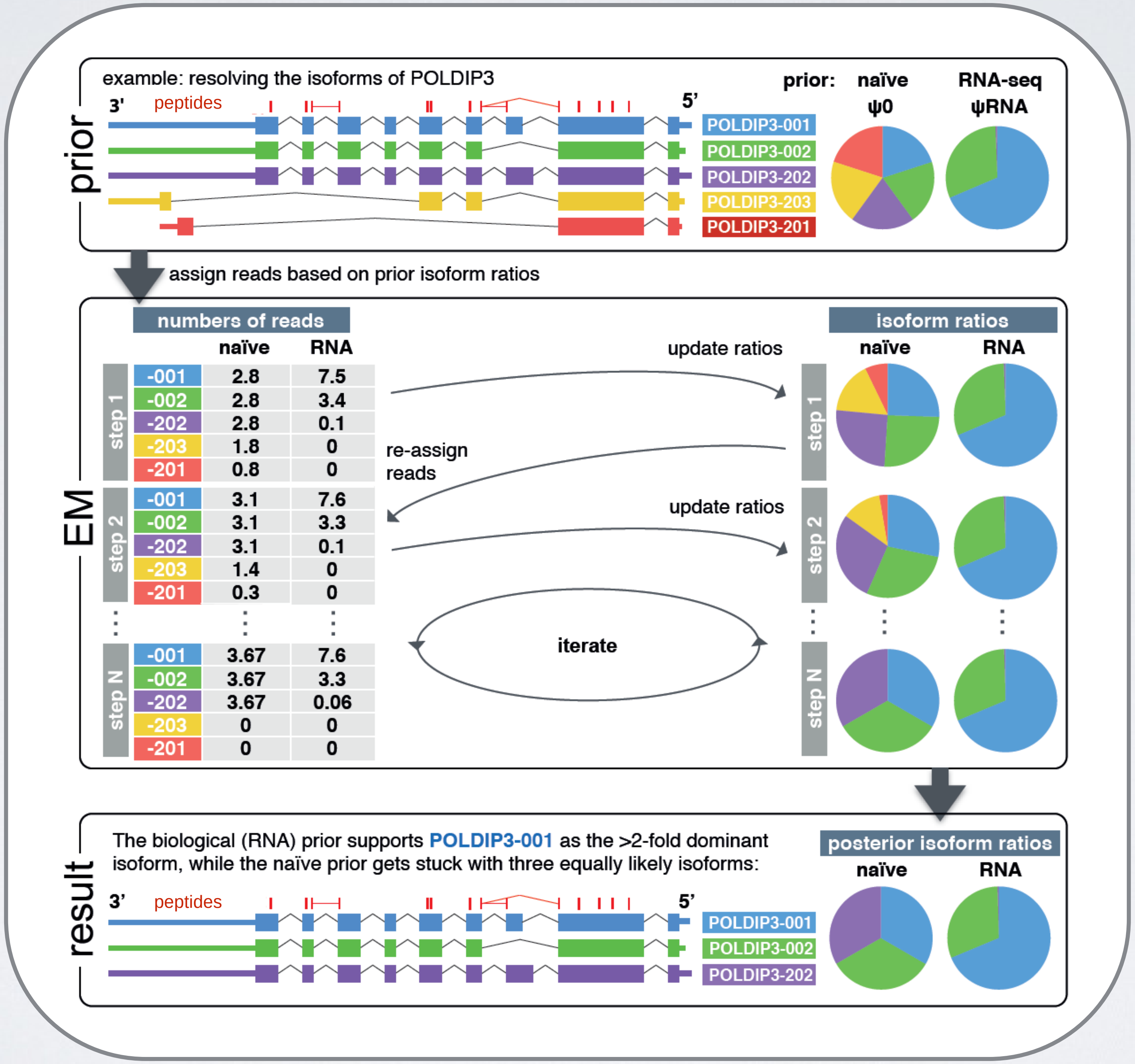




- Main benefits:
  - Libraries for searching peptides can be decreased in size - lose fewer proteins through FDR correction (fewer tests)
  - Peptides can be assigned to isoforms rather than genes - increased functional information
  - Proteins with redundant sequences can be defined more clearly

EMpire software

- Uses biologically relevant mRNA-seq priors to bias assignment of peptides to isoforms, through a process of expectation maximisation



assign reads based on prior isoform ratios

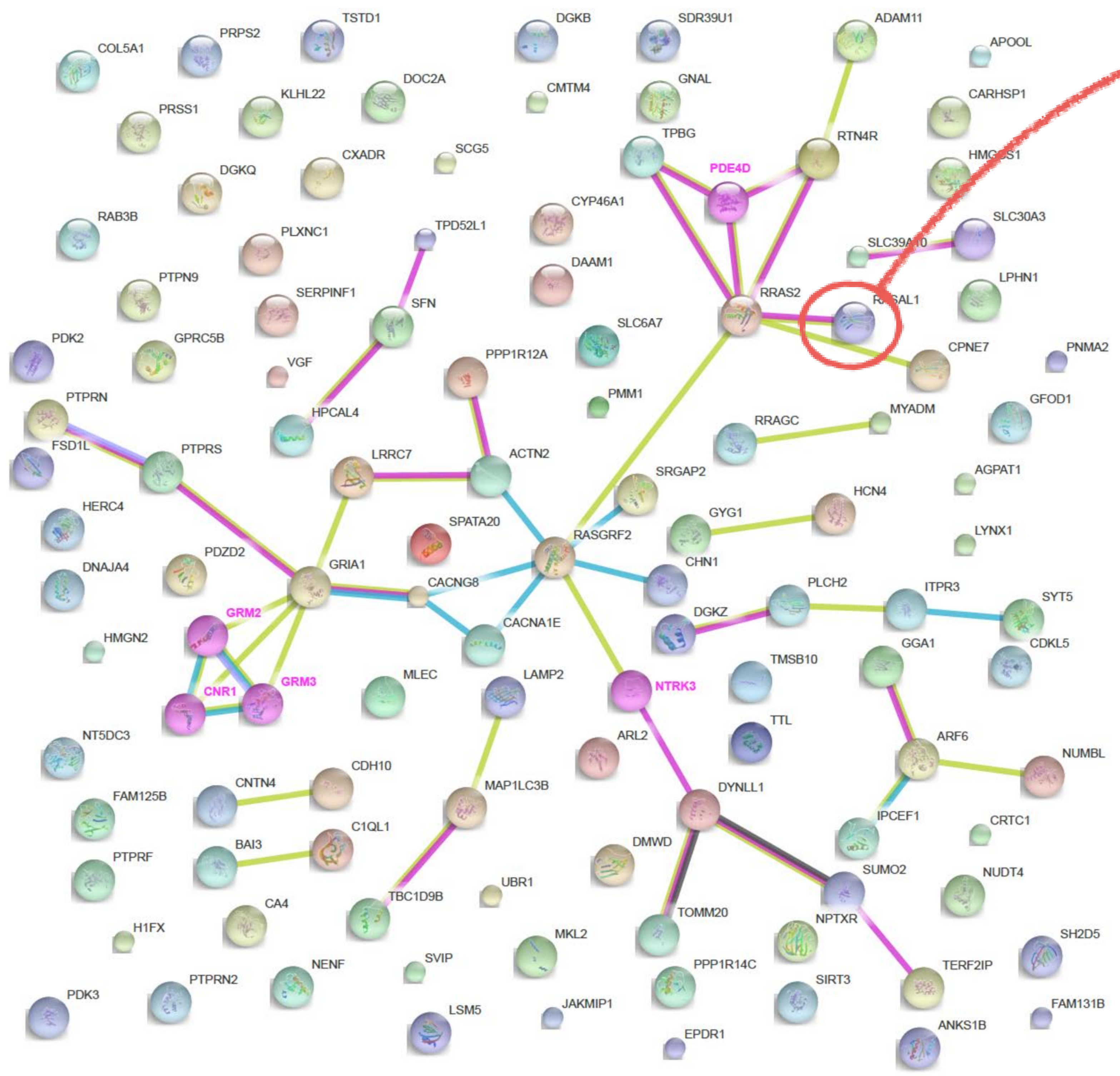
numbers of reads

		naïve	RNA
step 1	-001	2.8	7.5
	-002	2.8	3.4
	-202	2.8	0.1
	-203	1.8	0
	-201	0.8	0
step 2	-001	3.1	7.6
	-002	3.1	3.3
	-202	3.1	0.1
	-203	1.4	0
	-201	0.3	0
⋮		⋮	⋮
step N	-001	3.67	7.6
	-002	3.67	3.3
	-202	3.67	0.06
	-203	0	0
	-201	0	0

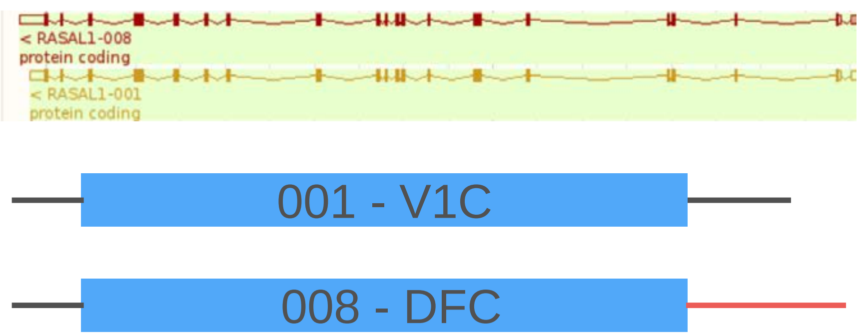
The biological (RNA) prior supports **POLDIP3-001** as the >2-fold dominant isoform, while the naïve prior gets stuck with three equally likely isoforms:

Rob Kitchen

Isoform differences in dlPFC vs V1C



RASAL1



008 DFC principal transcript has additional predicted **enhancers** and **miRNA binding sites** in the 3’ UTR

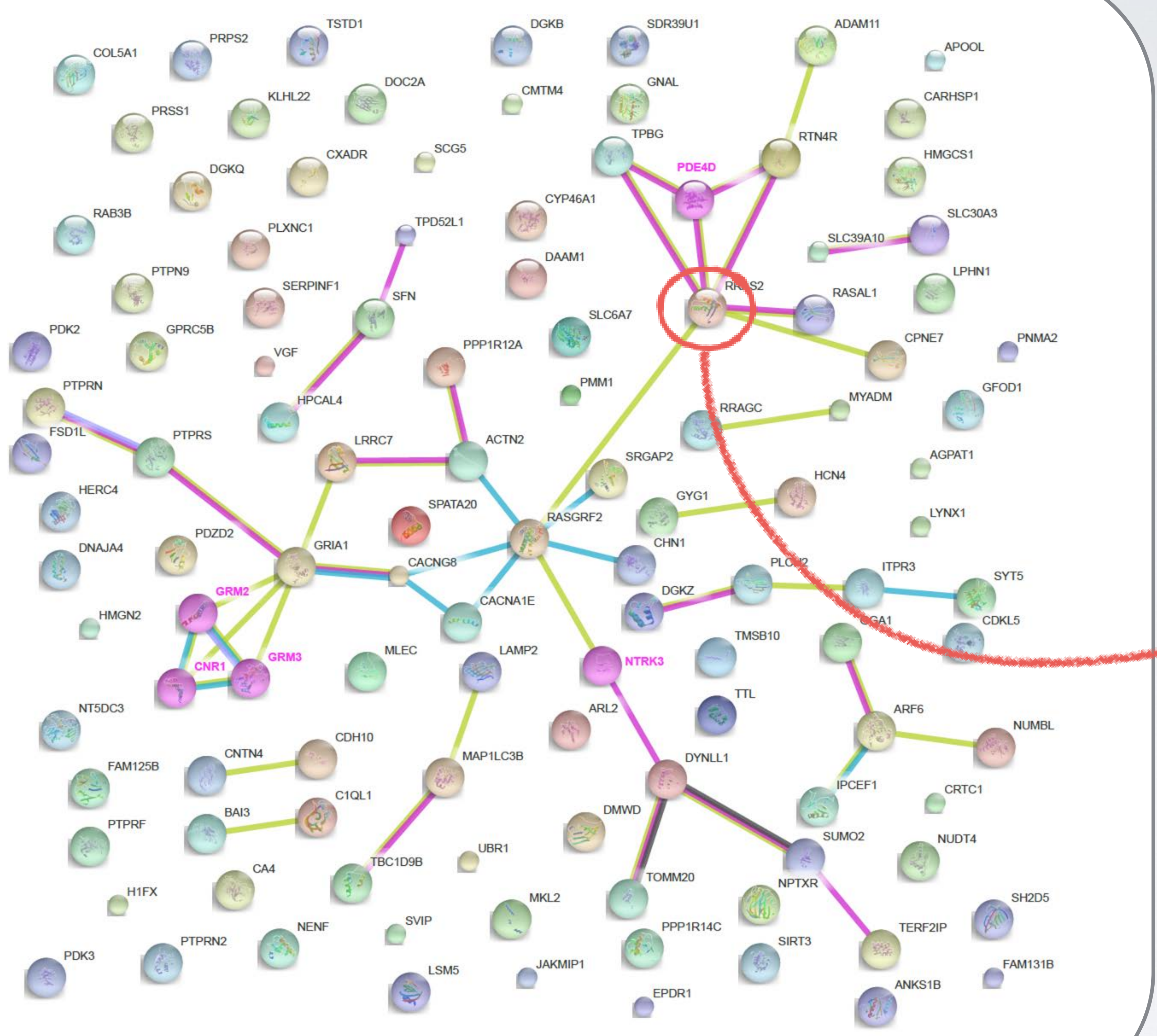
RegRNA 2.0

RASAL1 is involved in ERK/PKA signaling regulation

Expression levels have been linked to the regulation of GABA receptor subunit expression

Mulligan et al., 2012, PLoS One

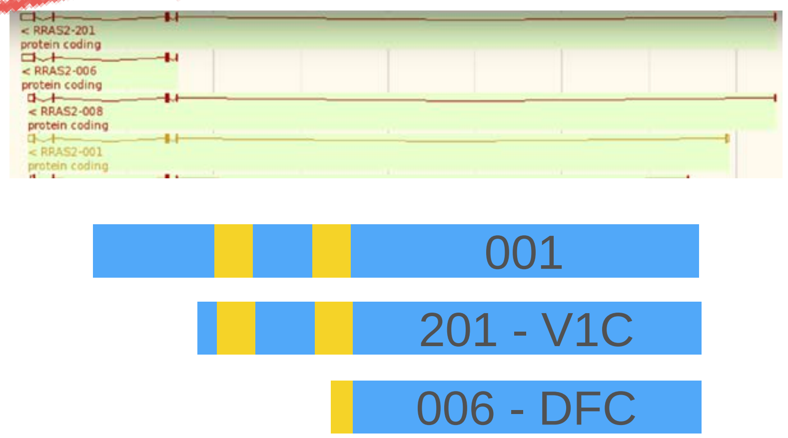
Isoform differences in dlPFC vs V1C



RRAS2 is a membrane bound small GTPase

Switch domains mediate the interaction of RRAS2 with downstream effectors, changing resultant activity of the protein

RRAS2



006 DFC principal transcript is lacking intact switch domains

Interpro Scan 5

Summary

- Proteomic measurements can detect between-region changes in expression not detected by RNA-level measurements

- Integration of RNA-seq level data with proteomics may lead to increased functional insight into protein-protein interactions and regulation

- There is a caveat here - the sensitivity of proteomics may change the isoform outcome (for example, if a key isoform defining peptide is suppressed by a more abundant protein), so individual targets should be followed up carefully

Thanks!

The psychENCODE Neuroproteomics team: Angus Nairn, Rob Kitchen, Nenad Sestan, Tukiet Lam

Funding:
Yale/NIDA Neuroproteomics Centre: AD018343
NIDA R21: DA040454
NIA: AG047270
NIMH: MH110926, MH106934, MH103339
NIH SIGs: 1S10OD019967, 1S10ODOD018034
State of Connecticut Department of Mental Health & Addiction Services
NARSAD Young Investigator