

GPMTG

RK

7th September 2012



overview

brains!

- 1** intro / motivation
- 2** human, chimp, macaque miRNA analysis strategy
- 3** rebuilding the chimp & macaque miRNA reference
- 4** inter-species & inter-tissue expression differences
- 5** miRNA-mRNA

human brain

cortex

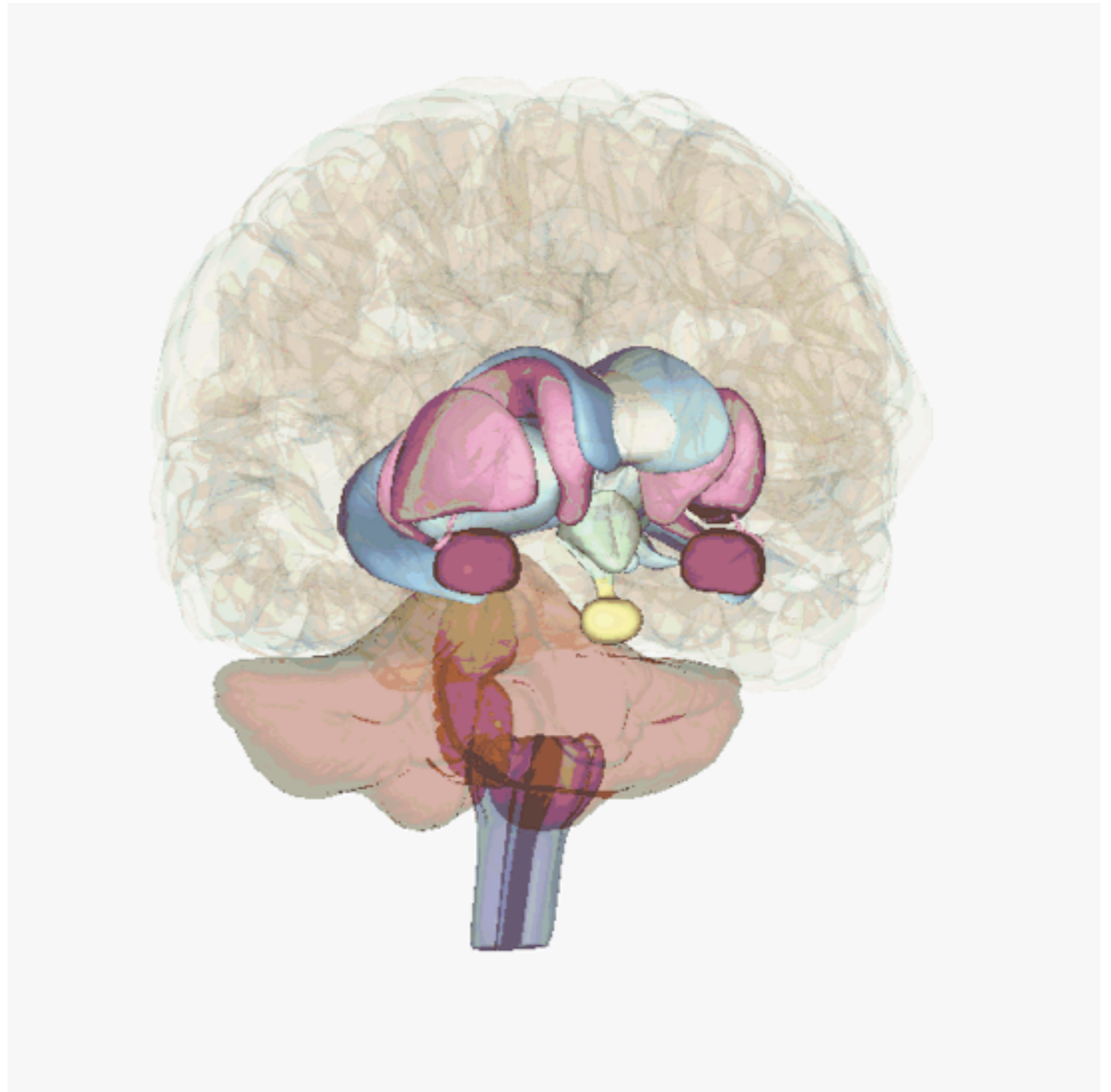
amygdala

cerebellum

hyppocampus

striatum

thalamus



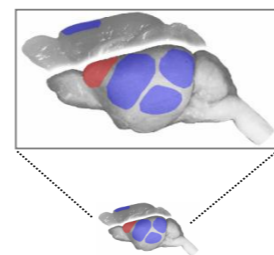
brain comparison

human brain is composed of **functionally distinct regions** and **cell types**

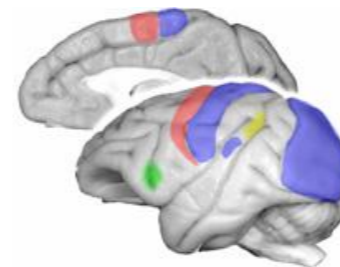
'wiring' of the cortex is what makes us human

process of wiring depends on precise **spatiotemporal** regulation of **gene expression**

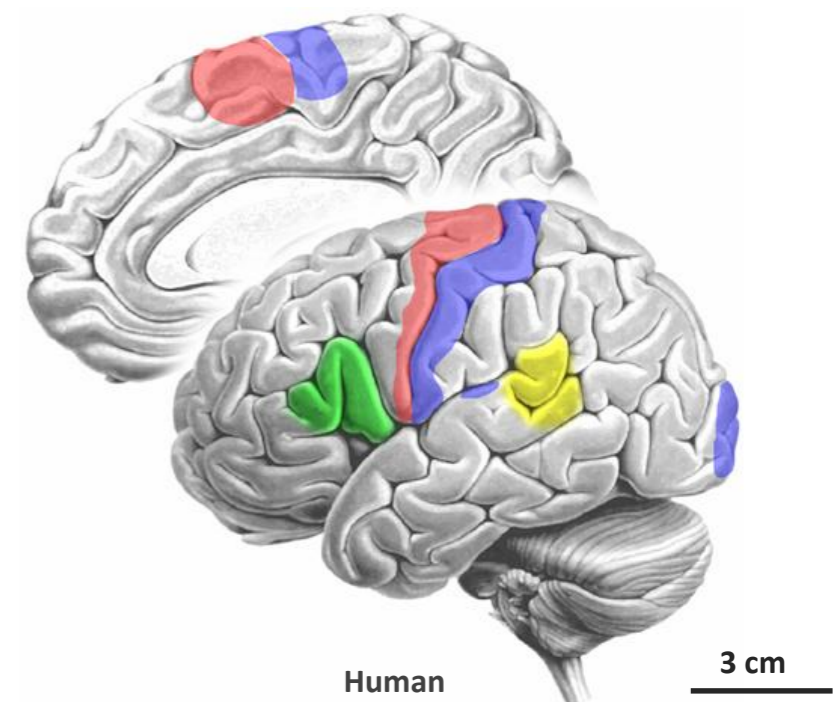
- Primary motor areas
- Primary sensory areas
- Perisylvian language areas



Mouse
0.416 g
71 M neurons



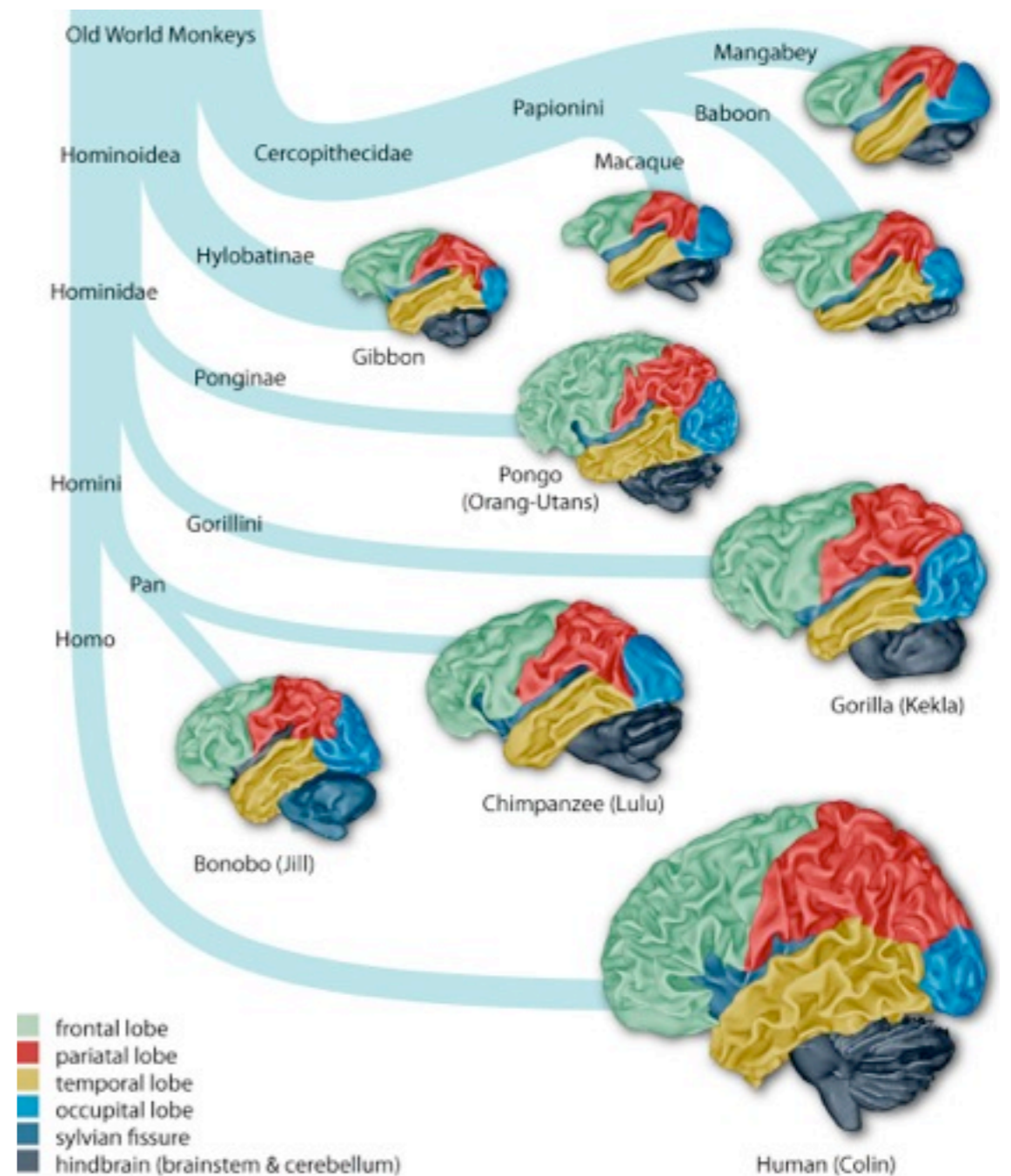
Macaque monkey
87.35 g
6376 M neurons



Human
1508 g
86000 M neurons

brain comparison

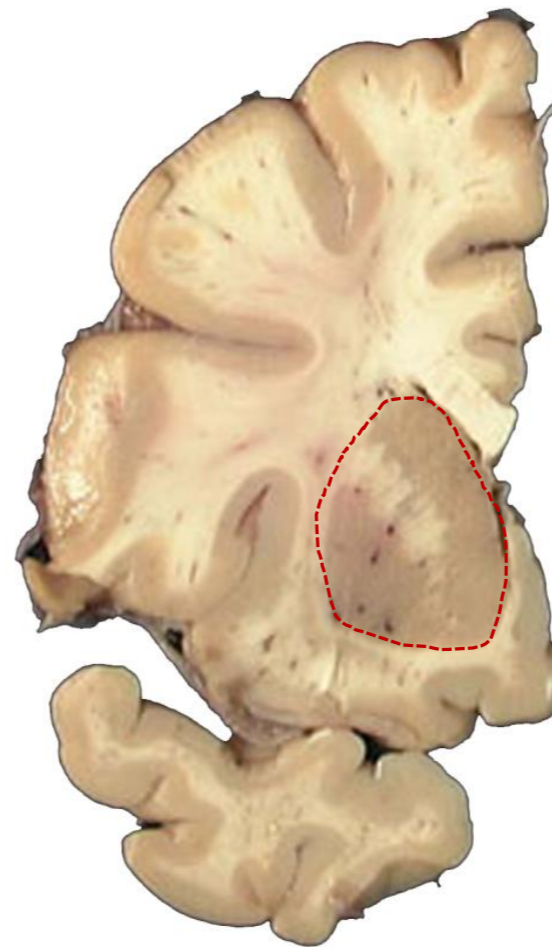
organism	N neurons	N synapses	synapses per neuron
worm	300	10^4	33
fly	100 thousand	10^7	100
mouse	75 million	10^{11}	1,000
chimp	7 billion	$\sim 10^{13}$	$\sim 1,000$
human	85 billion	10^{15}	10,000



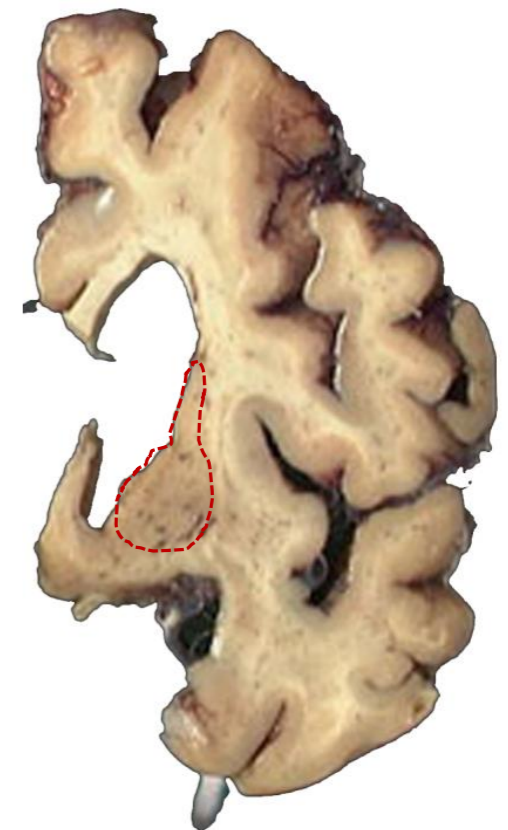
brain disease

Huntington's disease affects the whole brain, but the **striatum** is more vulnerable

Control brain



Huntington's disease brain

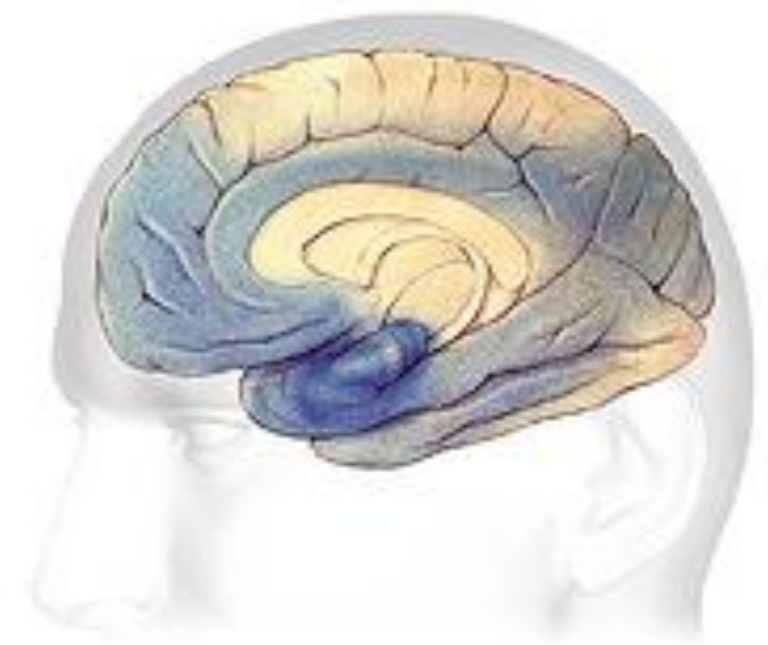


brain disease

Earliest stage

Mild to moderate stage

Severe stage



plaques resulting from **Alzheimer's disease** progresses through the cortex in a predictable pattern

brain disorder

specific brain areas implicated in **Autism Spectrum Disorders**

blue regions

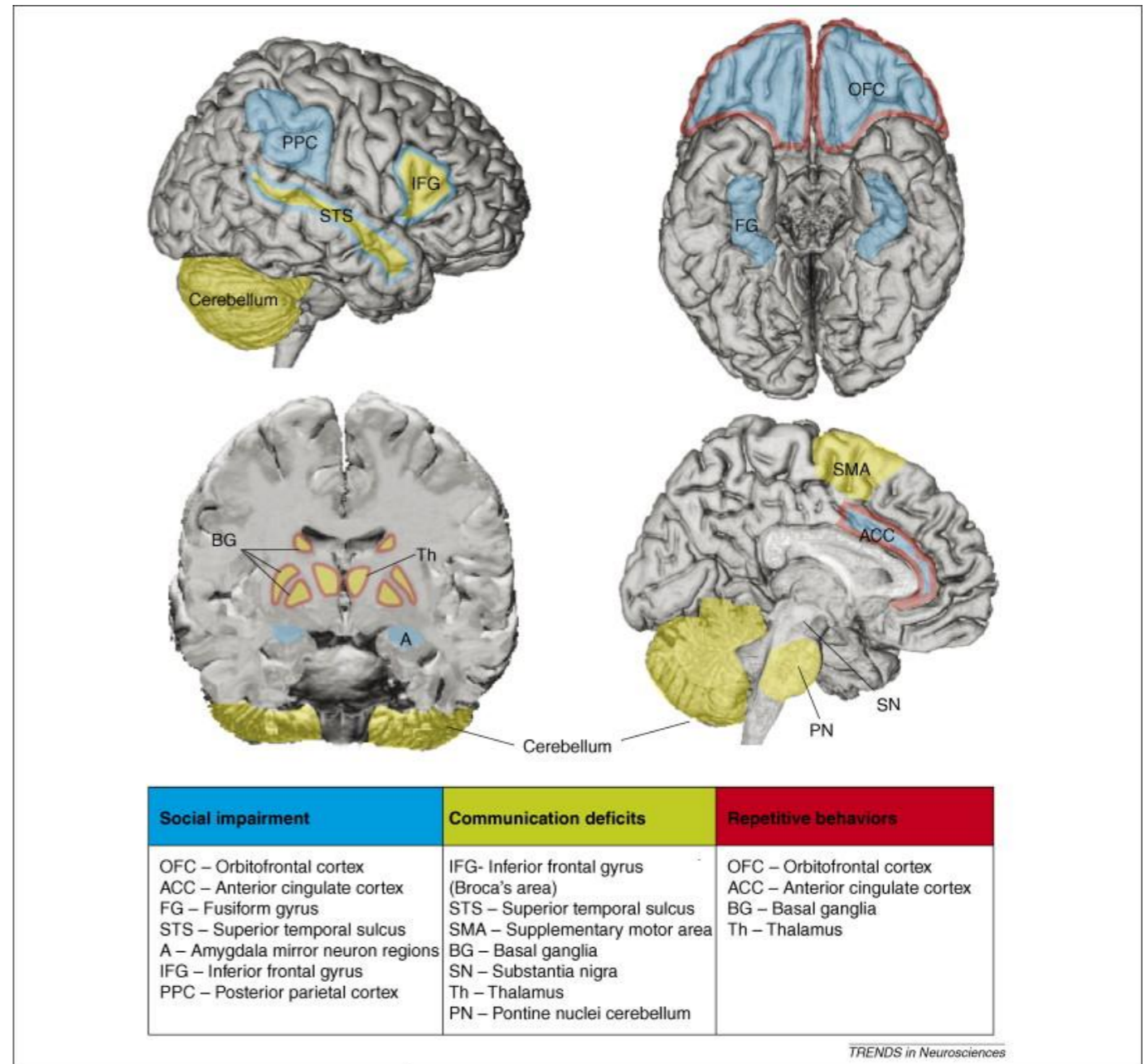
social impairment

yellow regions

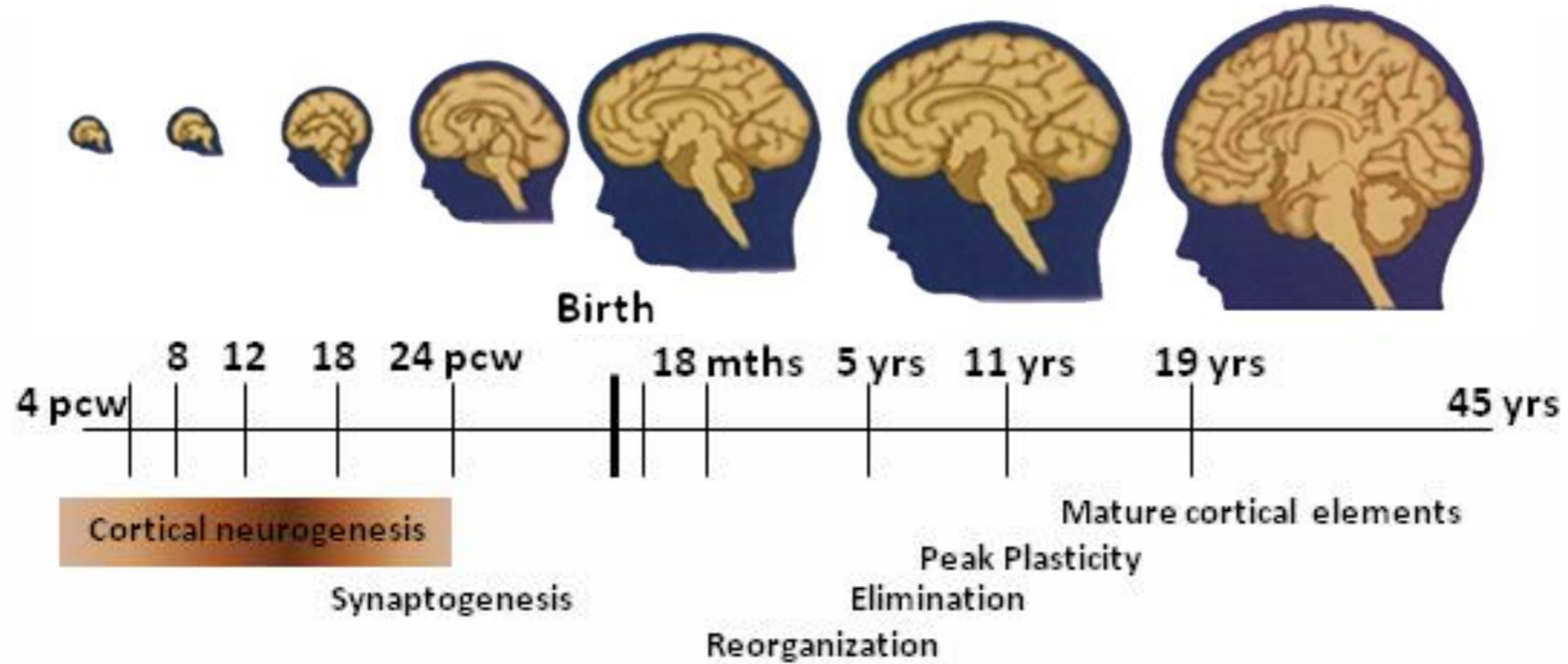
communication deficits

red regions

repetitive behaviour



brain development



development of the human brain is a complex and prolonged process

brain development

can lose an **entire hemisphere** in early development and still function [fairly] normally

fairly common that to remove a **brain tumour**, a surgeon will remove an **entire lobe**



brainspan xSpecies

human (hsa)



chimp (ptr)

macaque (mml)

brainspan xSpecies

human (hsa)



6 brains

chimp (ptr)



5 brains

macaque (mml)



5 brains

sequenced mRNA and miRNA from 16 regions of each brain:

Frontal lobe

DFC Dorsolateral prefrontal cortex
M1C Primary motor cortex
MFC Medial prefrontal cortex
OFC Orbital prefrontal cortex
VFC Ventrolateral prefrontal cortex

Occipital lobe

V1C Primary visual cortex

Parietal lobe

IPC Posterior inferior parietal cortex
S1C Primary sensory cortex

Temporal lobe

A1C Primary auditory cortex
ITC Inferior temporal cortex
STC Posterior superior temporal cortex

Sub-cortex

AMY Amygdala
CBC Cerebellum
HIP Hippocampus
MD Thalamus
STR Striatum

human brain

cortex

amygdala

cerebellum

hyppocampus

striatum

thalamus



brainspan xSpecies

Number	Species	Sex	Age (years)	Stage	Hemisphere
HSB 123	Homo sapiens	Male	37	Adulthood	Right
HSB 126	Homo sapiens	Female	30	Adulthood	Right
HSB 130	Homo sapiens	Female	21	Adulthood	Left
HSB 145	Homo sapiens	Male	36	Adulthood	Right
HSB 135	Homo sapiens	Female	40	Adulthood	Right
HSB 136	Homo sapiens	Male	23	Adulthood	Right
PTB 162	Pan troglodytes	Female	22.5	Adulthood	Left
PTB 164	Pan troglodytes	Female	30.8	Adulthood	Right
PTB 165	Pan troglodytes	Male	31.2	Adulthood	Right
PTB 166	Pan troglodytes	Male	26.4	Adulthood	Right
PTB 167	Pan troglodytes	Male	29.8	Adulthood	Right
RMB 160	Macaca mulatta	Female	10.7	Adulthood	Left
RMB 161	Macaca mulatta	Male	11	Adulthood	Left
RMB 196	Macaca mulatta	Female	11	Adulthood	Left/Right
RMB 218	Macaca mulatta	Male	7	Adulthood	Left
RMB 219	Macaca mulatta	Male	7	Adulthood	Left

previously...

Hu et al. 2011 -->

431 miRNAs expressed in HSA
CBC and/or DFC

of these:

385 map to PTR

390 map to MML

375 PTR and 366 MML miRNAs
expressed

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PLOS GENETICS

MicroRNA Expression and Regulation in Human, Chimpanzee, and Macaque Brains

Hai Yang Hu^{1,3}, Song Guo^{1,3}, Jiang Xi¹, Zheng Yan¹, Ning Fu², Xiaoyu Zhang³, Corinna Menzel⁴, Hongyu Liang³, Hongyi Yang³, Min Zhao³, Rong Zeng^{2*}, Wei Chen^{4,5}, Svante Pääbo⁶, Philipp Khaitovich^{1,6*}

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Abstract

Among other factors, changes in gene expression on the human evolutionary lineage have been suggested to play an important role in the establishment of human-specific phenotypes. However, the molecular mechanisms underlying these expression changes are largely unknown. Here, we have explored the role of microRNA (miRNA) in the regulation of gene expression divergence among adult humans, chimpanzees, and rhesus macaques, in two brain regions: prefrontal cortex and cerebellum. Using a combination of high-throughput sequencing, miRNA microarrays, and Q-PCR, we have shown that up to 11% of the 325 expressed miRNA diverged significantly between humans and chimpanzees and up to 31% between humans and macaques. Measuring mRNA and protein expression in human and chimpanzee brains, we found a significant inverse relationship between the miRNA and the target genes expression divergence, explaining 2%–4% of mRNA and 4%–6% of protein expression differences. Notably, miRNA showing human-specific expression localize in neurons and target genes that are involved in neural functions. Enrichment in neural functions, as well as miRNA-driven regulation on the human evolutionary lineage, was further confirmed by experimental validation of predicted miRNA targets in two neuroblastoma cell lines. Finally, we identified a signature of positive selection in the upstream region of one of the five miRNA with human-specific expression, miR-34c-5p. This suggests that miR-34c-5p expression change took place after the split of the human and the Neanderthal lineages and had adaptive significance. Taken together these results indicate that changes in miRNA expression might have contributed to evolution of human cognitive functions.

Citation: Hu HY, Guo S, Xi J, Yan Z, Fu N, et al. (2011) MicroRNA Expression and Regulation in Human, Chimpanzee, and Macaque Brains. *PLoS Genet* 7(10): e1002327. doi:10.1371/journal.pgen.1002327

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Competing Interests: The authors have declared that no competing interests exist.

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† These authors contributed equally to this work.

Introduction

Phenotypic differences between species, including human-specific features such as language and tool-making, are thought to have arisen, to a large extent, through changes in gene expression [1]. Indeed, humans and the closest living primate relatives, chimpanzees, display substantial gene expression diver-

endogenous, single-stranded RNA involved in post-transcriptional gene expression silencing. Mature miRNA function as part of the RNA-induced silencing complex (RISC), mediating post-transcriptional gene expression inhibition [6–8]. In animals, the predominant mechanism of miRNA-mediated gene silencing employs complementary base-pairing between the miRNA seed region and the mRNA 3' UTR region [9,10]. This interaction

previously...

reported miRNAs with significant divergence in abundance:
35 between hsa/ptr & 106 between hsa/mml
totalRNA from several individuals sequenced in 1 or 2 pool(s) per species

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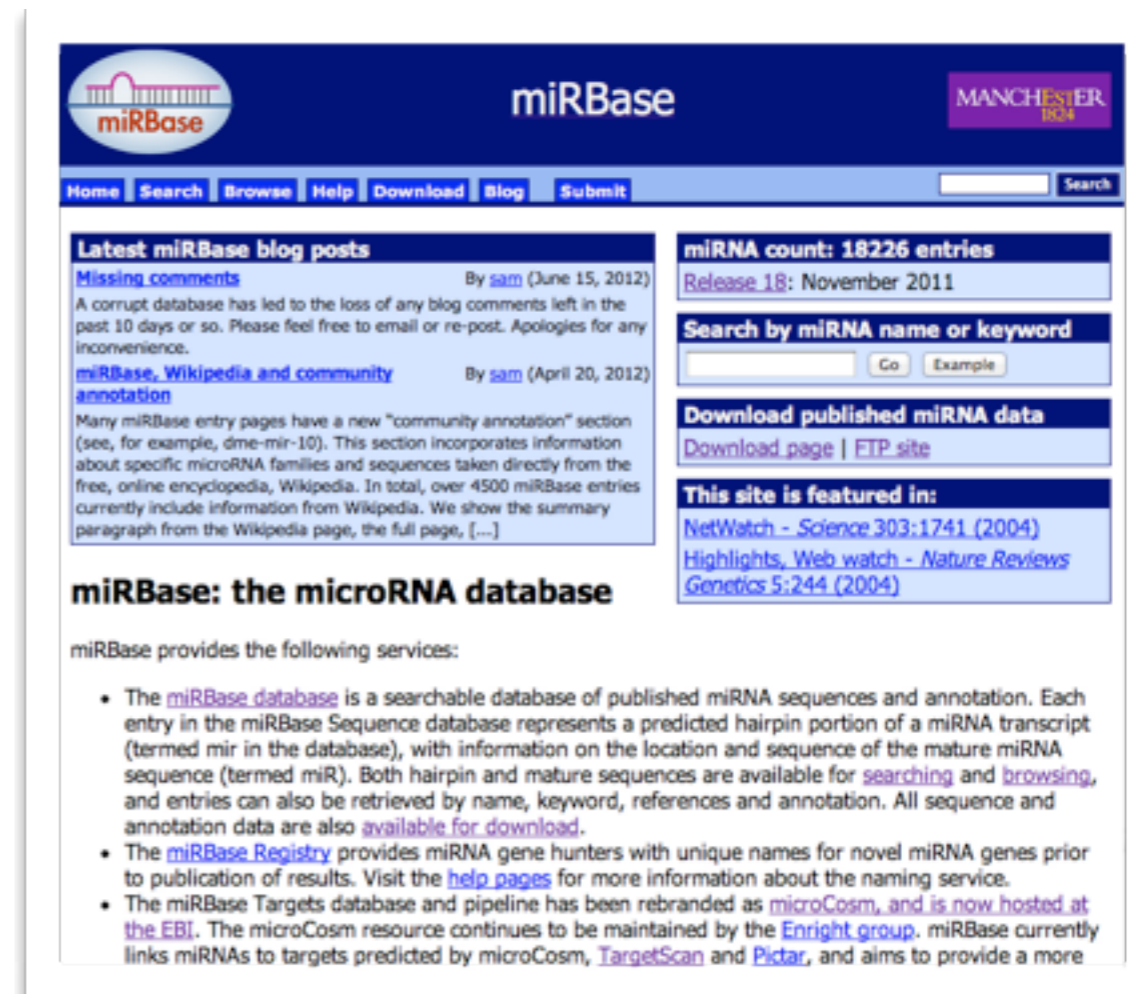
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mapping reads to known miRNAs

mapping to the genome is potentially error-prone due to small size of mature miRNAs

hope to gain sensitivity by mapping to known sequences of hairpin/mature miRNAs

miRBase is a well-maintained collection of miRNAs for a variety of species



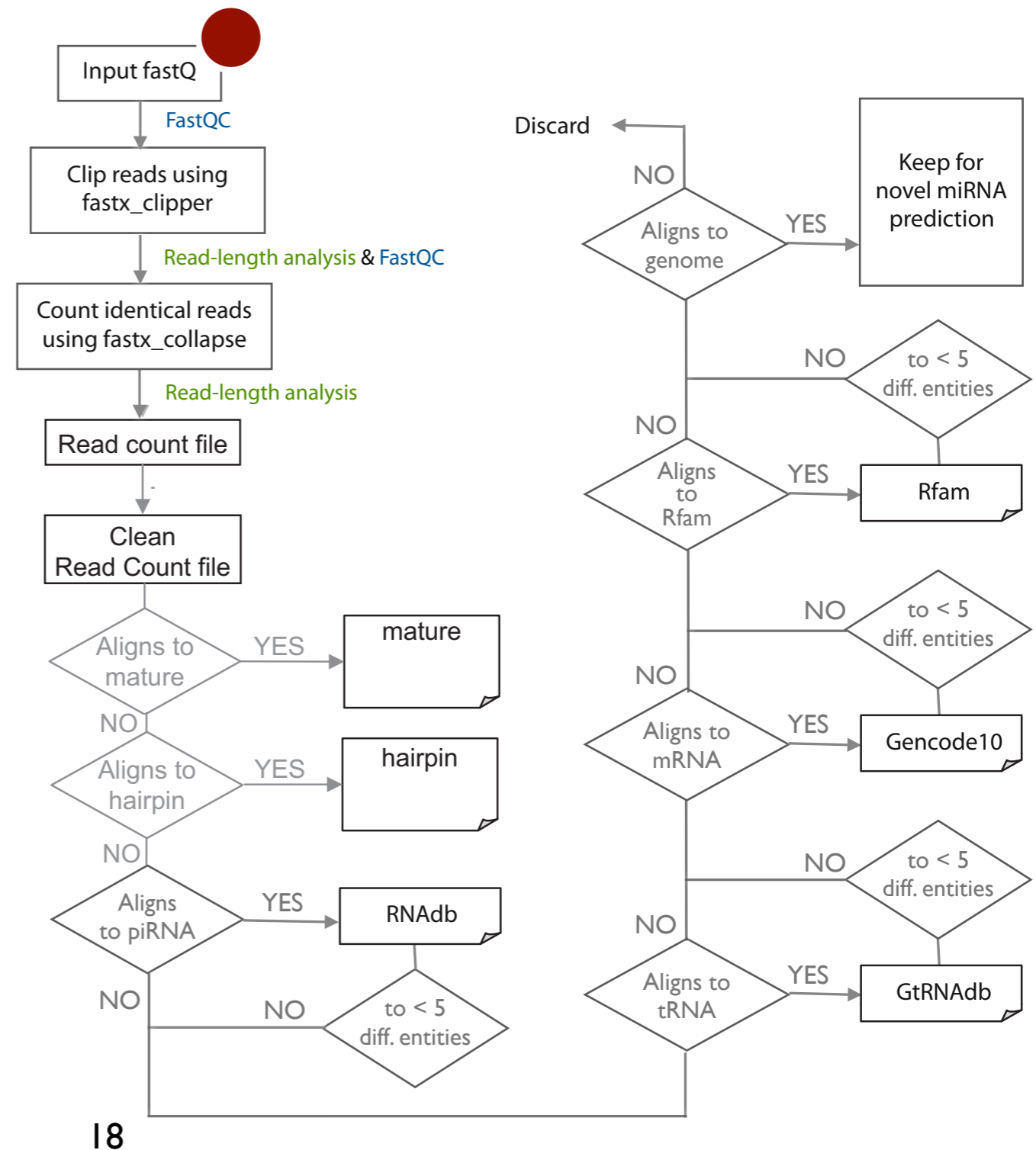
The screenshot shows the miRBase website homepage. At the top, there is a navigation bar with links for Home, Search, Browse, Help, Download, Blog, and Submit. The main content area is divided into several sections:

- Latest miRBase blog posts:** Two posts are listed, including "Missing comments" and "miRBase, Wikipedia and community annotation".
- miRNA count: 18226 entries:** A box indicating the total number of entries and the release date (Release 18: November 2011).
- Search by miRNA name or keyword:** A search box with a "Go" button and an "Example" button.
- Download published miRNA data:** A box with links for "Download page" and "FTP site".
- This site is featured in:** A box listing several publications that have featured the site.
- miRBase: the microRNA database:** A section describing the database and its services, including a searchable database of published miRNA sequences and annotation, a registry for novel miRNA genes, and a targets database.

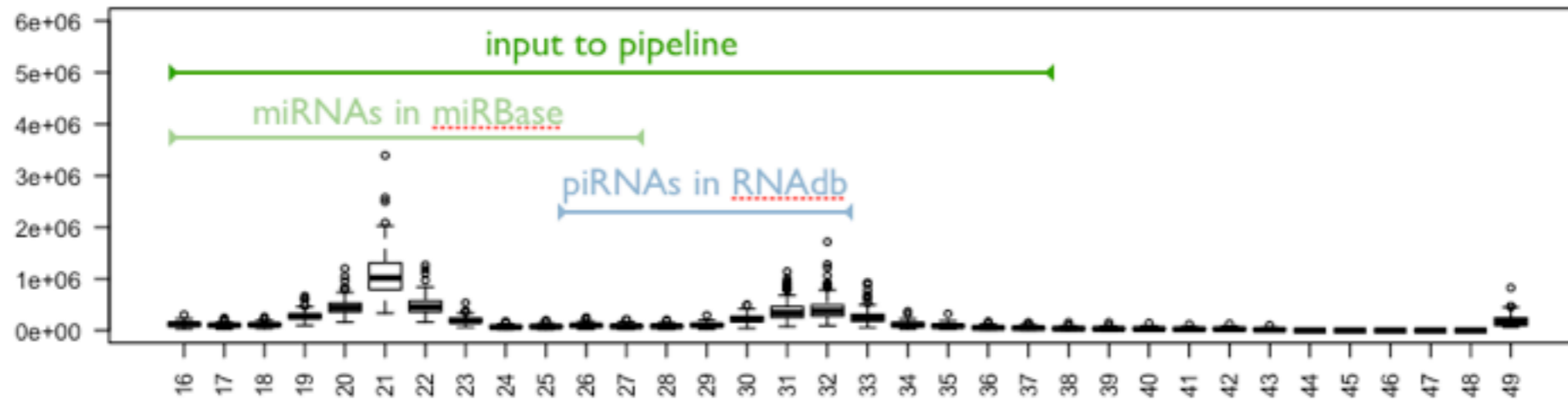
mapping strategy

for each species, reads were mapped to the following references:

- 1 - miRNA
- 2 - miRNA homologs
- 3 - piRNA
- 4 - tRNA
- 5 - mRNA
- 6 - misc/contaminants
- 7 - novel miRNA

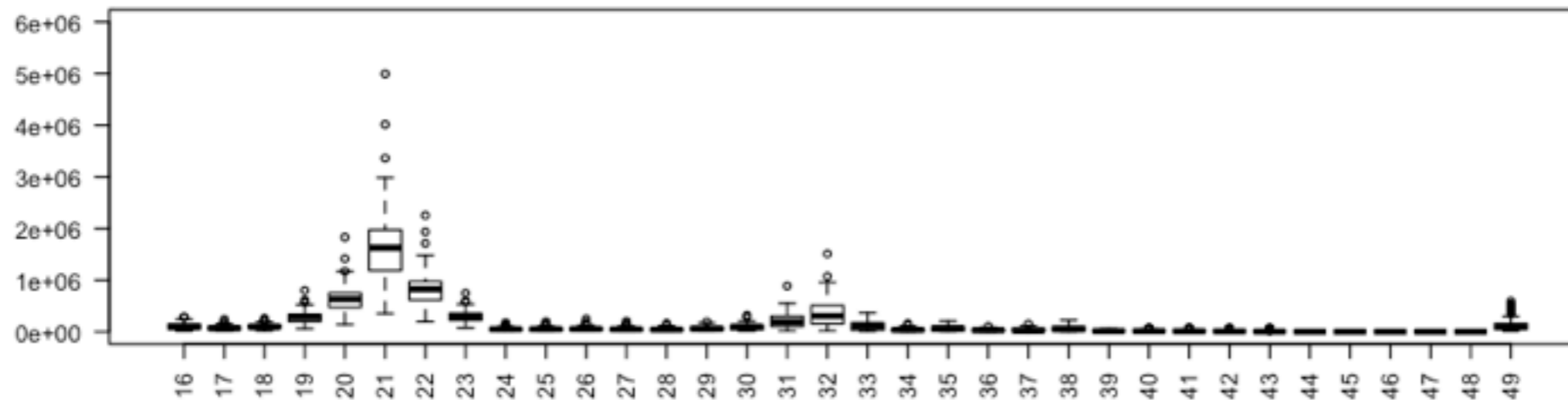


read-length distributions

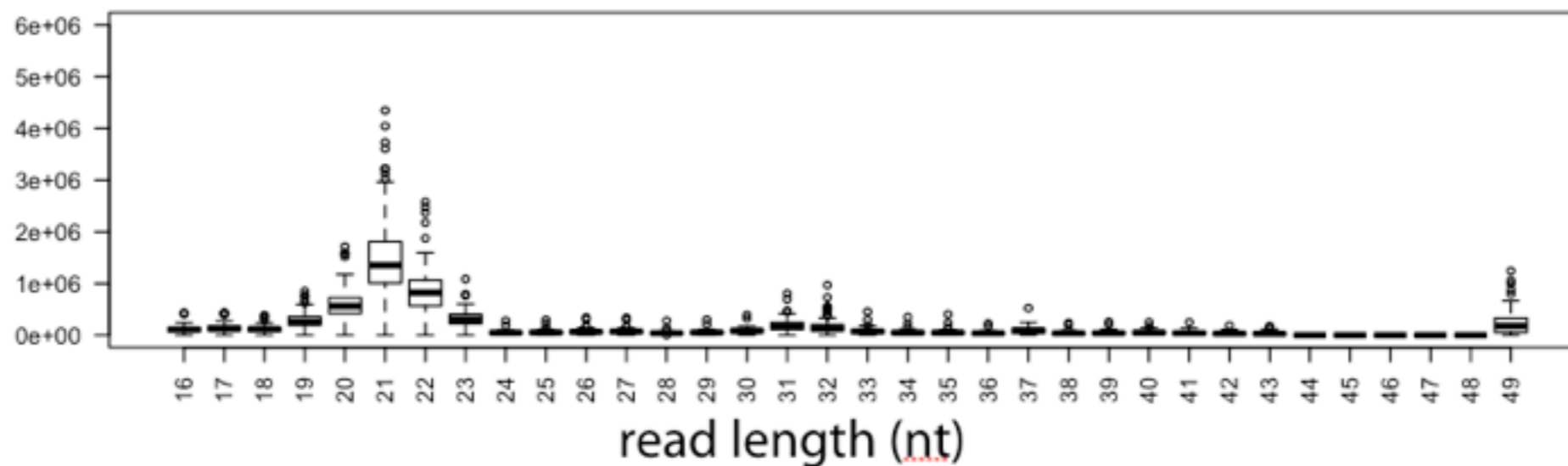


human

read count



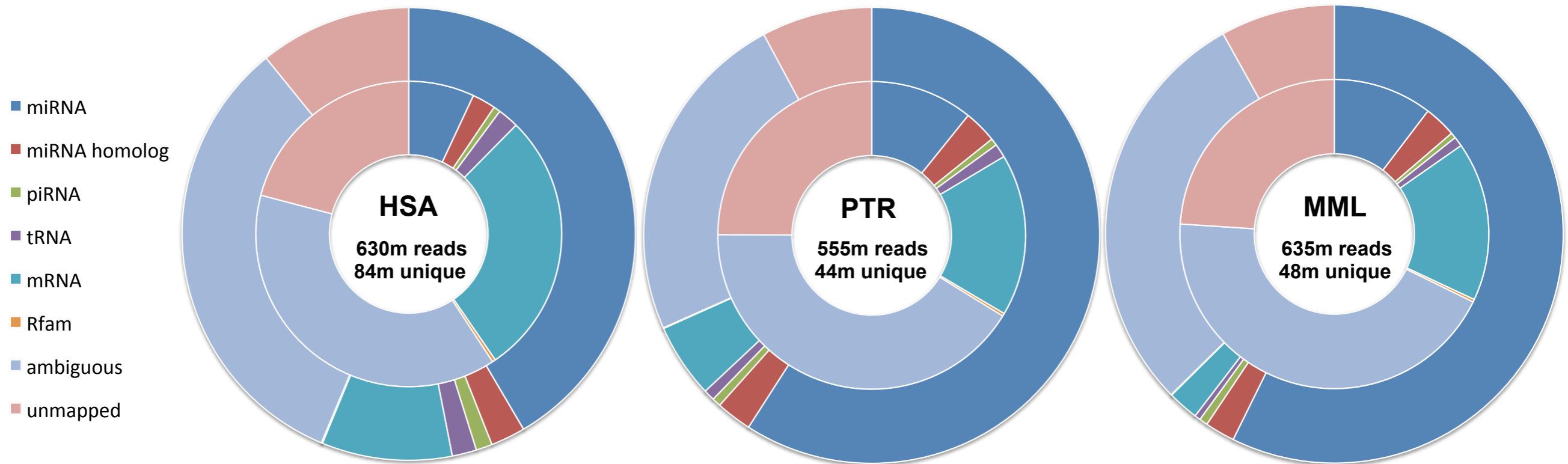
chimp



macaque

read length (nt)

mapping stats



inner ring total number of unique read-sequences

outer ring total number of sequenced reads

miRBase (release 18)

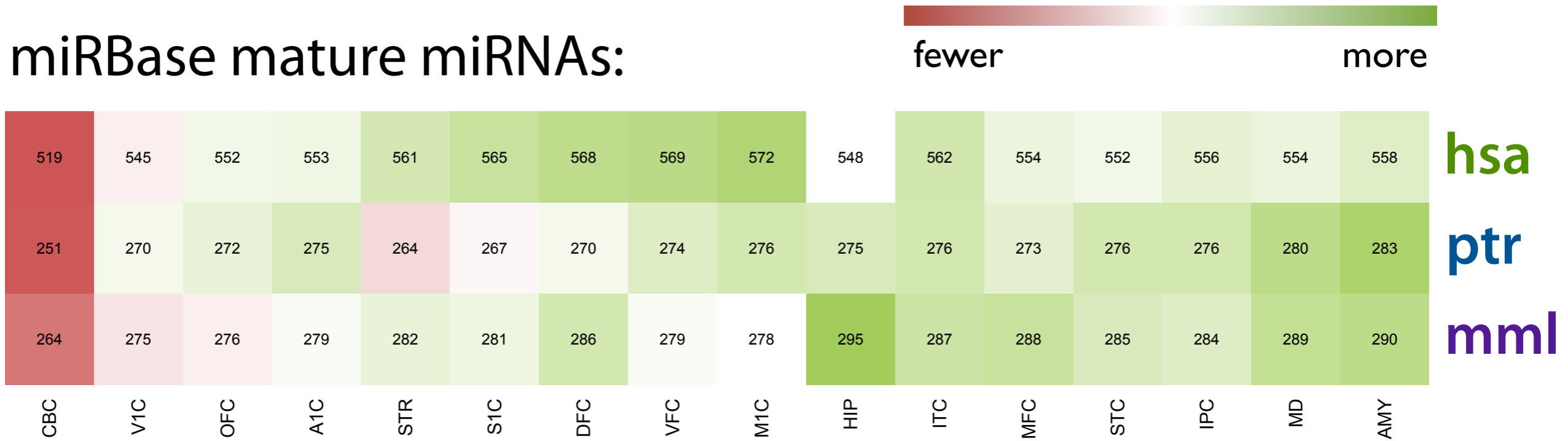
source	human	chimp	macaque
miRBase mature miRNAs	1,921	525	488
miRBase detected	746	312	308
Hu et al. detected	413	375	366

only 678 human miRNAs were known when the chimp reference was updated in 2009

& only 533 when the macaque reference was updated in 2008

per-tissue detection

miRBase mature miRNAs:



detected in human: ~550

detected in chimp: ~270

detected in macaque: ~280

reannotating the ptr/mml miRNAs

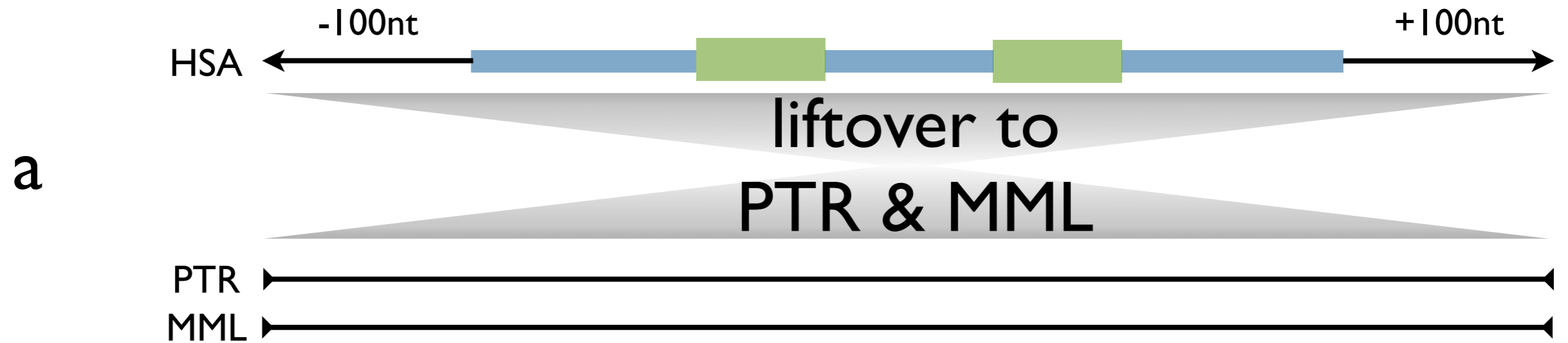
human (hsa)



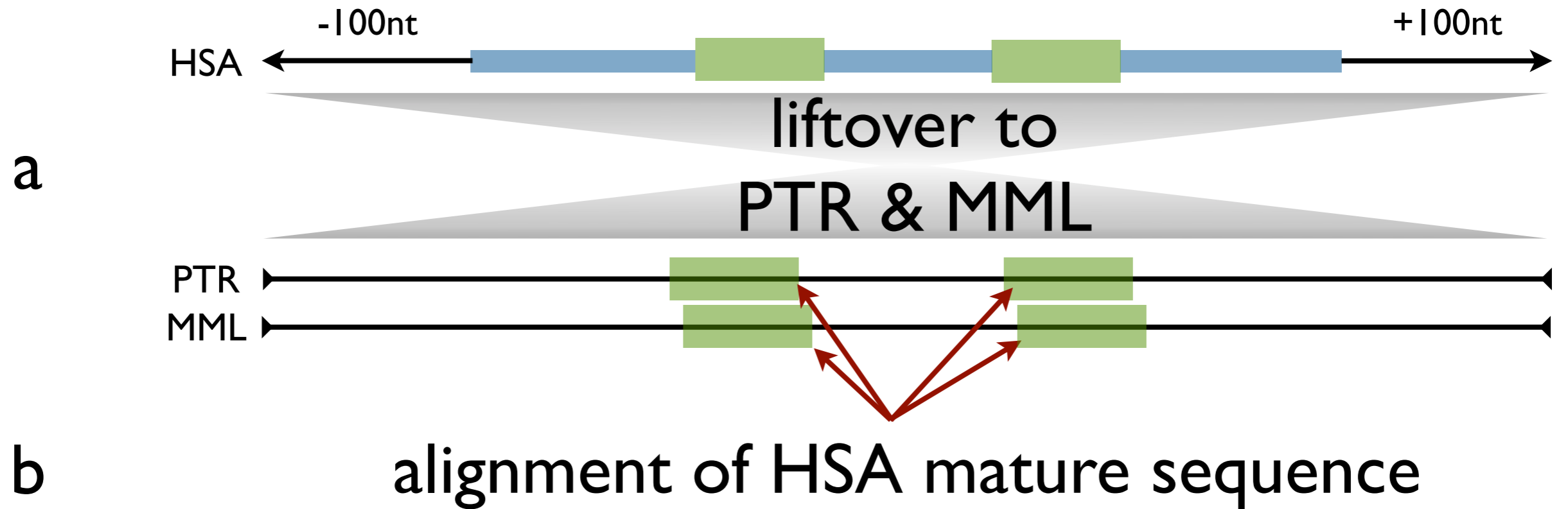
chimp (ptr)

macaque (mml)

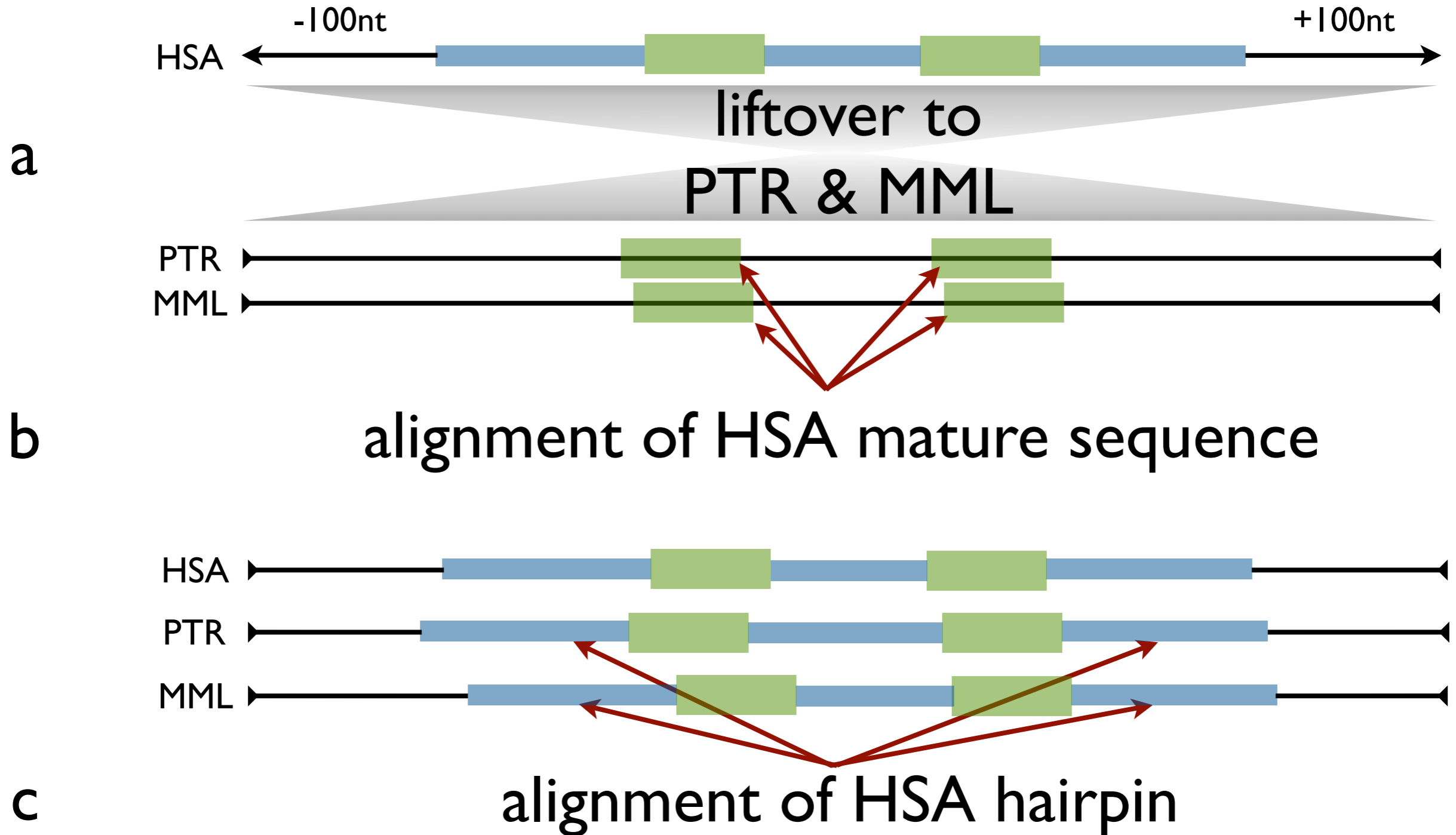
re-annotation



re-annotation



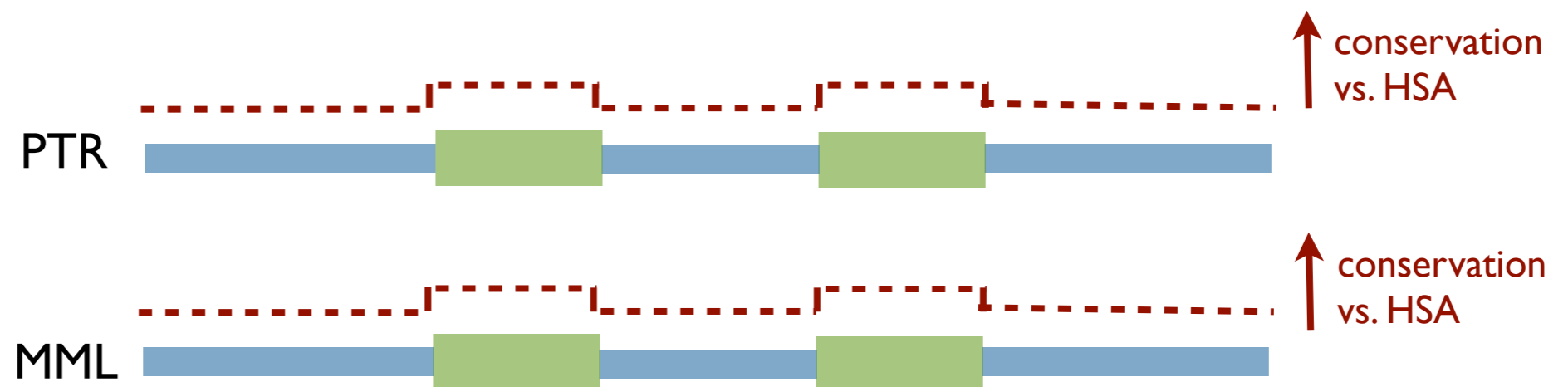
re-annotation



re-annotation (benefits)

d

patterns in identity between
PTR/HSA and MML/HSA?



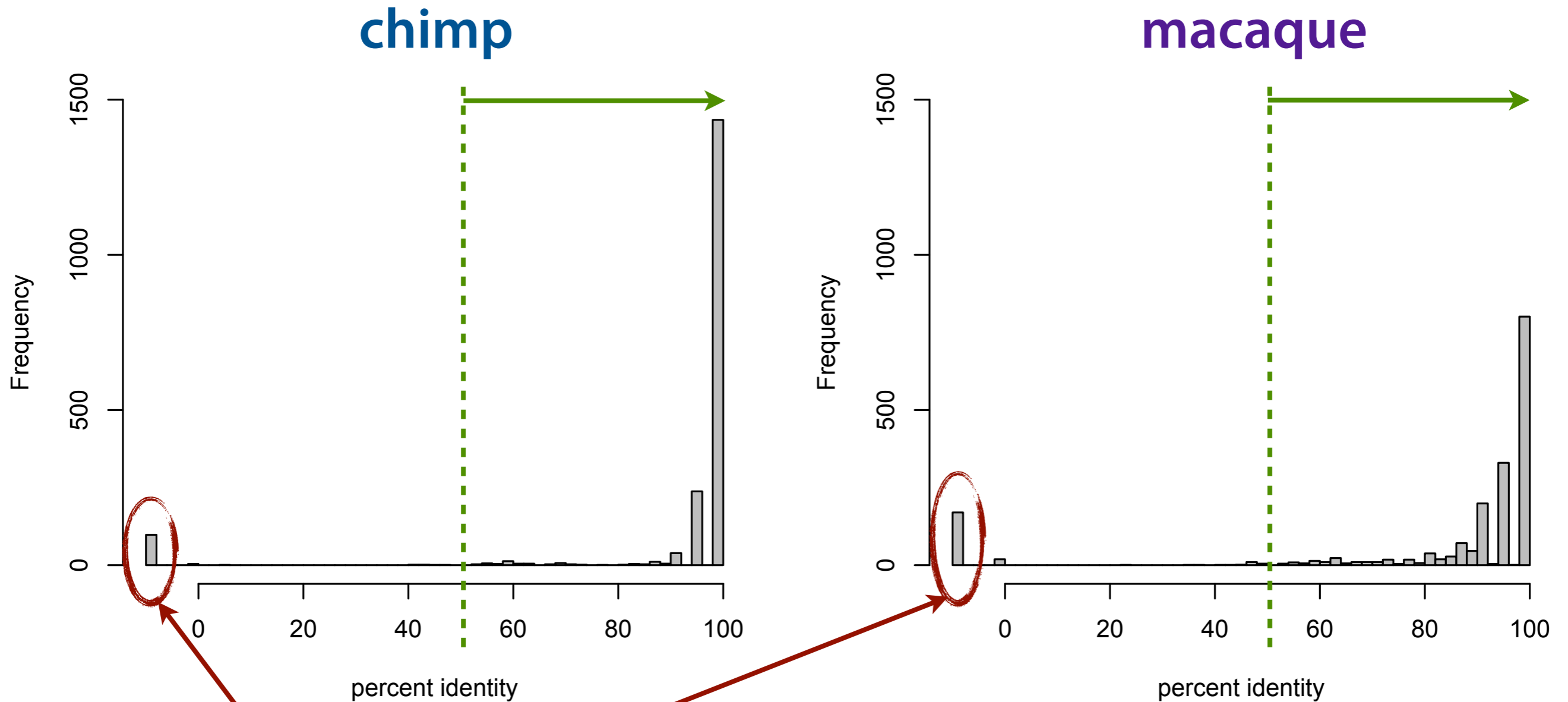
e

3' or 5' conservation bias?

f

identification of ~8nt
Argonaute-binding seed region?

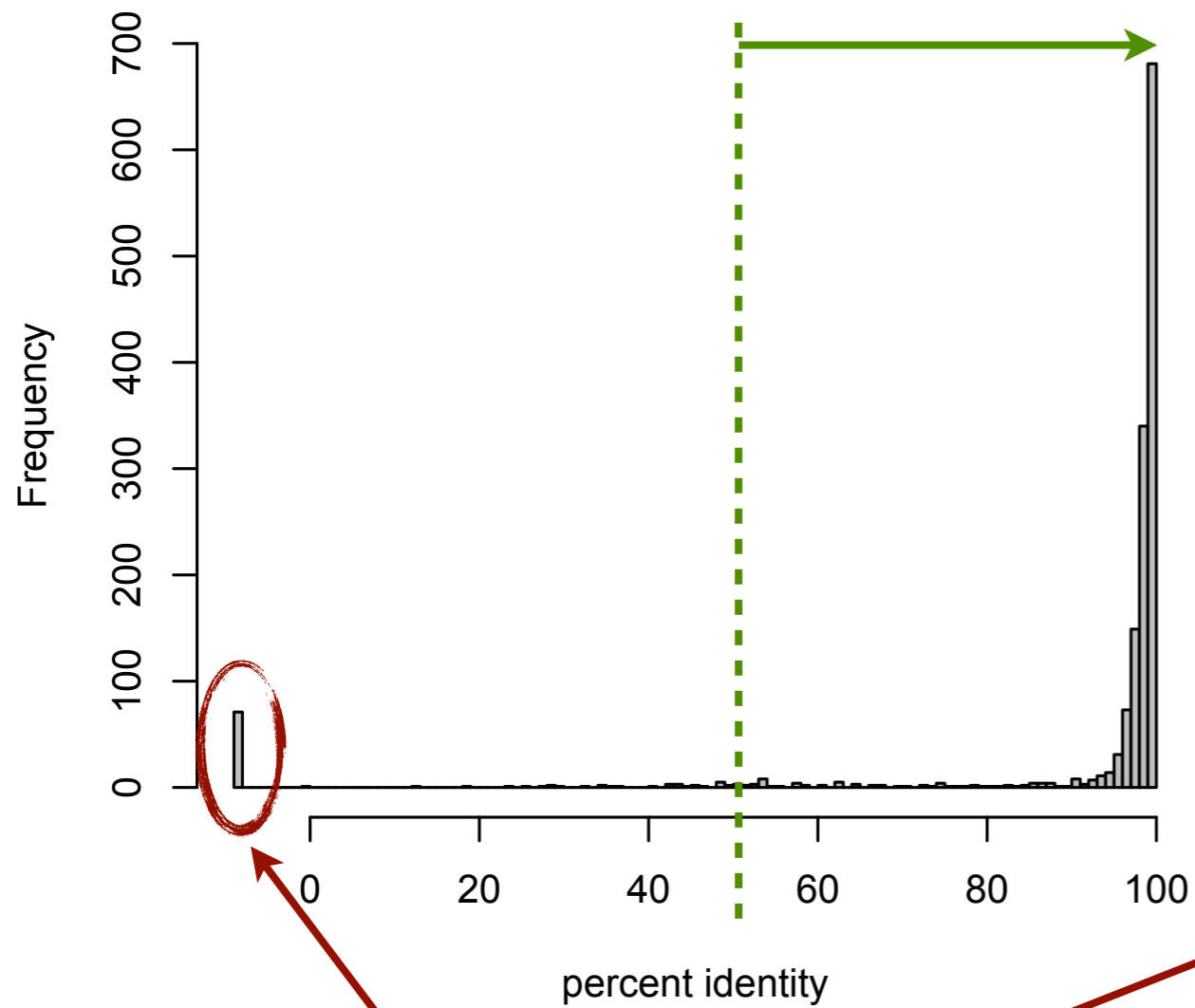
mature miRNA identity



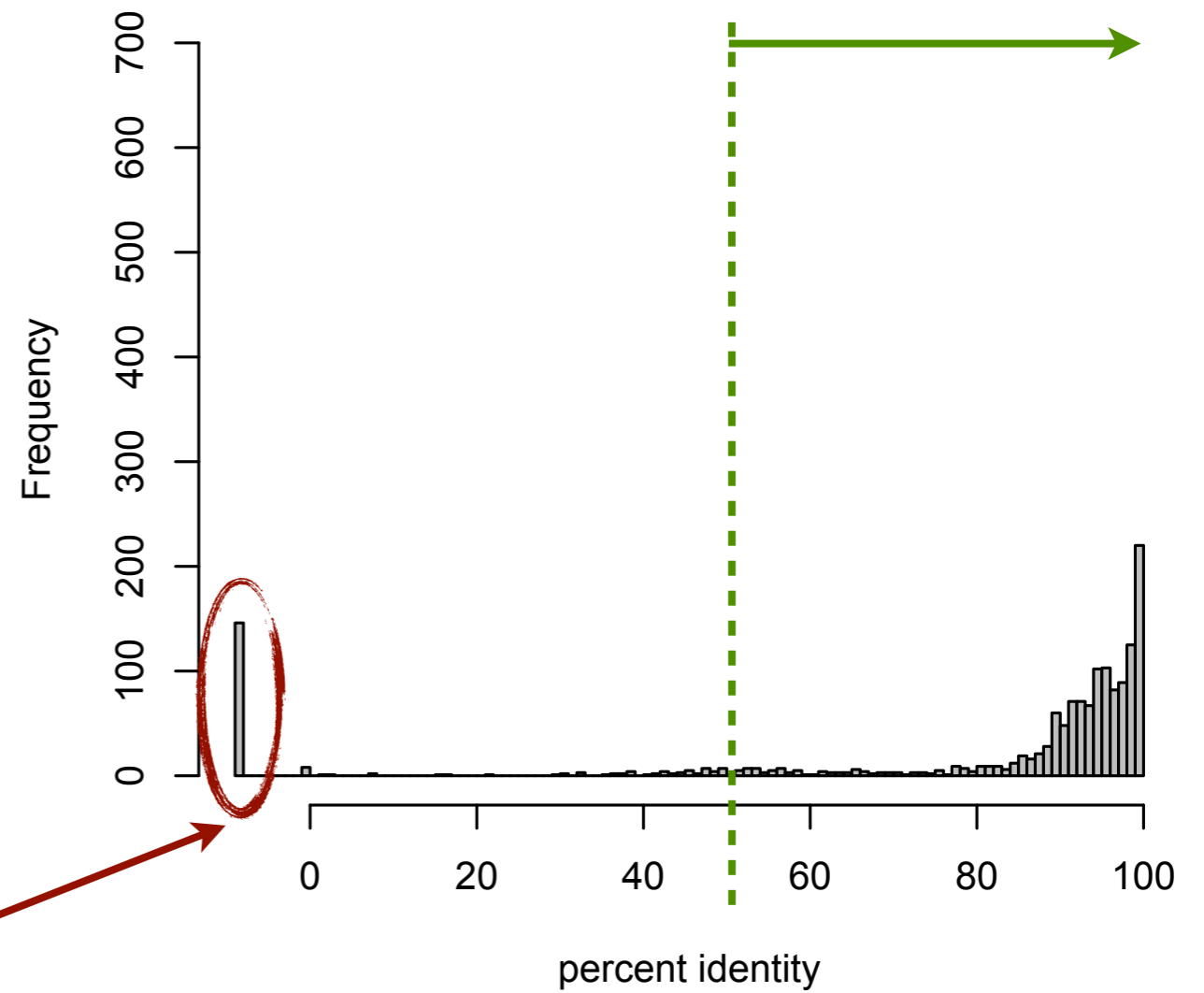
human pre-miRNA
did not lift-over

pre-miRNA identity

chimp



macaque



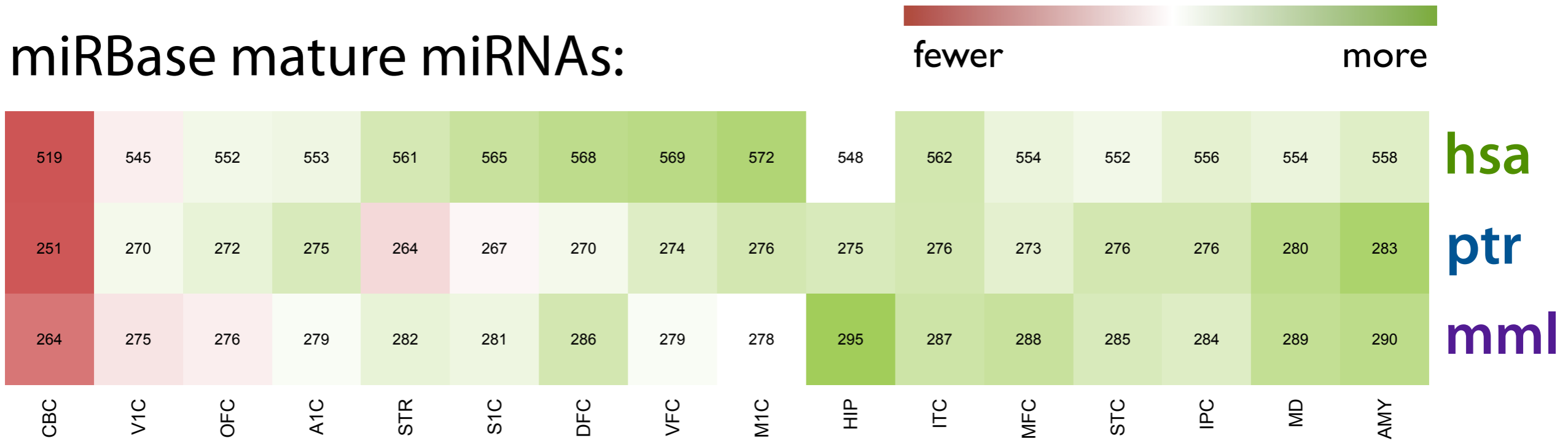
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mature miRNA detection- updated

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liftover mature miRNAs	1,898	1,789	1,692
liftover detected	746	606	502

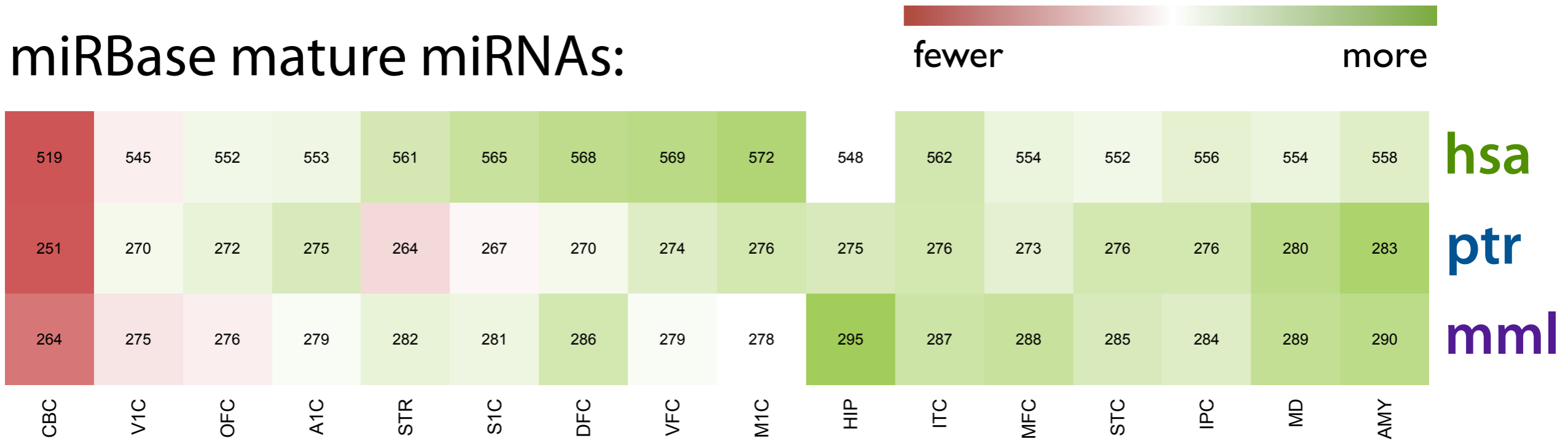
per-tissue detection (again)

miRBase mature miRNAs:

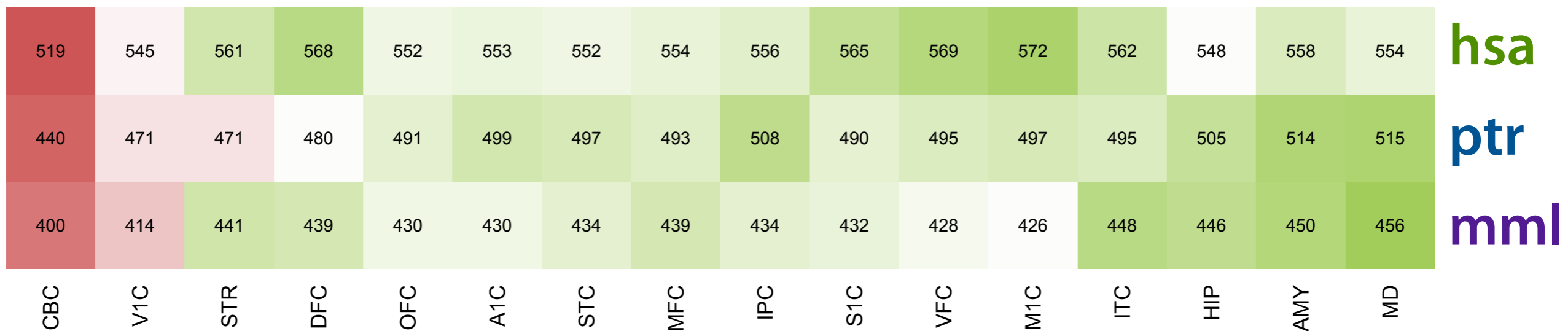


per-tissue detection (again)

miRBase mature miRNAs:



ptr/mml re-annotated mature miRNAs:



mRNA expression comparison

tissue comparison



species comparison



154	273	151	196	54	125	208	92	316	265	234	68	87	394	262	99	h>p>r
397	527	439	339	276	390	396	310	486	481	482	337	383	766	566	277	h>p=r
170	185	183	153	125	135	160	143	149	195	214	157	159	301	239	112	h>r>p
1216	1330	1187	1453	768	1146	1396	935	1402	1133	1114	776	814	1398	1212	910	h=p>r
14112	12186	13953	12901	15672	13614	12659	15311	12604	13352	13459	15282	15235	11551	12350	15075	h=p=r
240	507	244	322	215	356	441	219	330	252	331	177	147	271	377	260	h=r<p
47	60	59	38	27	64	74	35	54	66	71	41	32	44	74	35	h<r<p
215	400	288	242	237	361	281	207	382	396	463	256	266	848	570	278	h<p=r
73	93	74	72	26	73	91	57	91	92	90	43	57	95	86	55	h<p<r
OFC	DFC	VFC	MFC	M1C	S1C	IPC	A1C	STC	ITC	V1C	HIP	AMY	STR	MD	CBC	

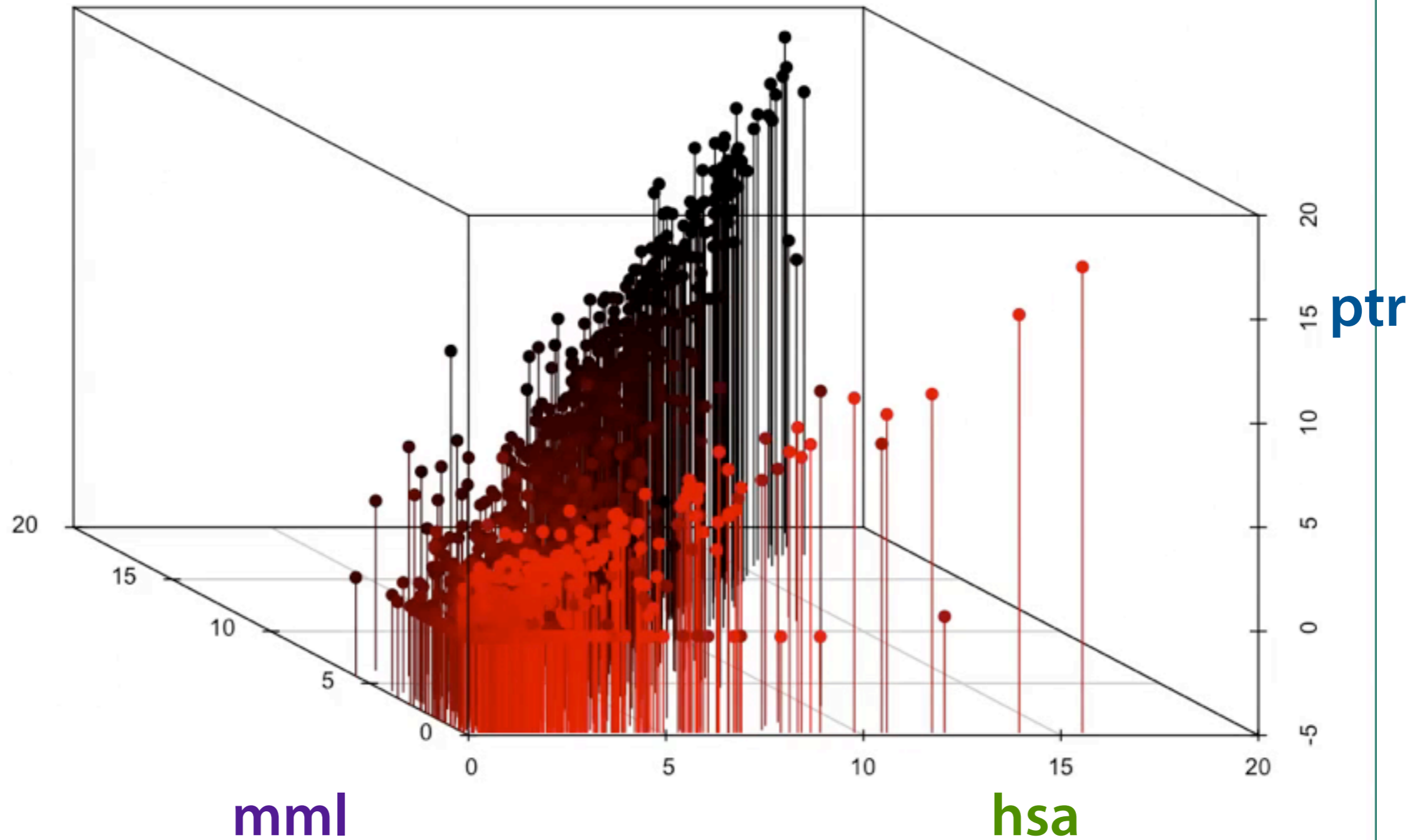
miRNA

91	77	123	121	70	105	89	104	107	102	115	99	116	70	105	103	h<p<r
24	24	15	15	23	16	28	28	11	20	16	22	19	24	32	20	h<p=r
24	24	10	13	26	11	21	23	21	18	8	27	10	39	25	9	p>r>h
28	44	64	45	39	51	41	31	63	35	41	36	51	45	41	43	h=p<r
828	821	803	809	834	824	813	822	805	815	827	828	809	816	812	812	h=p=r
5	6	2	5	9	5	6	2	2	5	4	4	5	5	5	6	h=r>p
7	7	3	4	6	4	4	3	3	2	3	3	4	4	2	8	h>r>p
18	24	20	20	21	20	20	16	19	22	17	13	12	18	14	27	h>p=r
15	13	13	20	13	13	10	14	15	14	15	14	13	12	8	16	h>p>r
16	22	21	22	20	21	30	19	25	22	26	12	24	15	15	26	r>h>p
16	10	9	12	16	13	14	17	10	20	13	17	15	12	9	11	h=p>r
18	15	13	14	13	11	14	13	16	13	12	18	16	22	19	13	p>h>r
16	19	10	6	16	12	16	14	9	18	9	13	12	24	19	12	h=r<p
OFC	DFC	VFC	MFC	M1C	S1C	IPC	A1C	STC	ITC	V1C	HIP	AMY	STR	MD	CBC	

mRNA

154	273	151	196	54	125	208	92	316	265	234	68	87	394	262	99	h>p>r
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170	185	183	153	125	135	160	143	149	195	214	157	159	301	239	112	h>r>p
1216	1330	1187	1453	768	1146	1396	935	1402	1133	1114	776	814	1398	1212	910	h=p>r
14112	12186	13953	12901	15672	13614	12659	15311	12604	13352	13459	15282	15235	11551	12350	15075	h=p=r
240	507	244	322	215	356	441	219	330	252	331	177	147	271	377	260	h=r<p
47	60	59	38	27	64	74	35	54	66	71	41	32	44	74	35	h<r<p
215	400	288	242	237	361	281	207	382	396	463	256	266	848	570	278	h<p=r
73	93	74	72	26	73	91	57	91	92	90	43	57	95	86	55	h<p<r
OFC	DFC	VFC	MFC	M1C	S1C	IPC	A1C	STC	ITC	V1C	HIP	AMY	STR	MD	CBC	

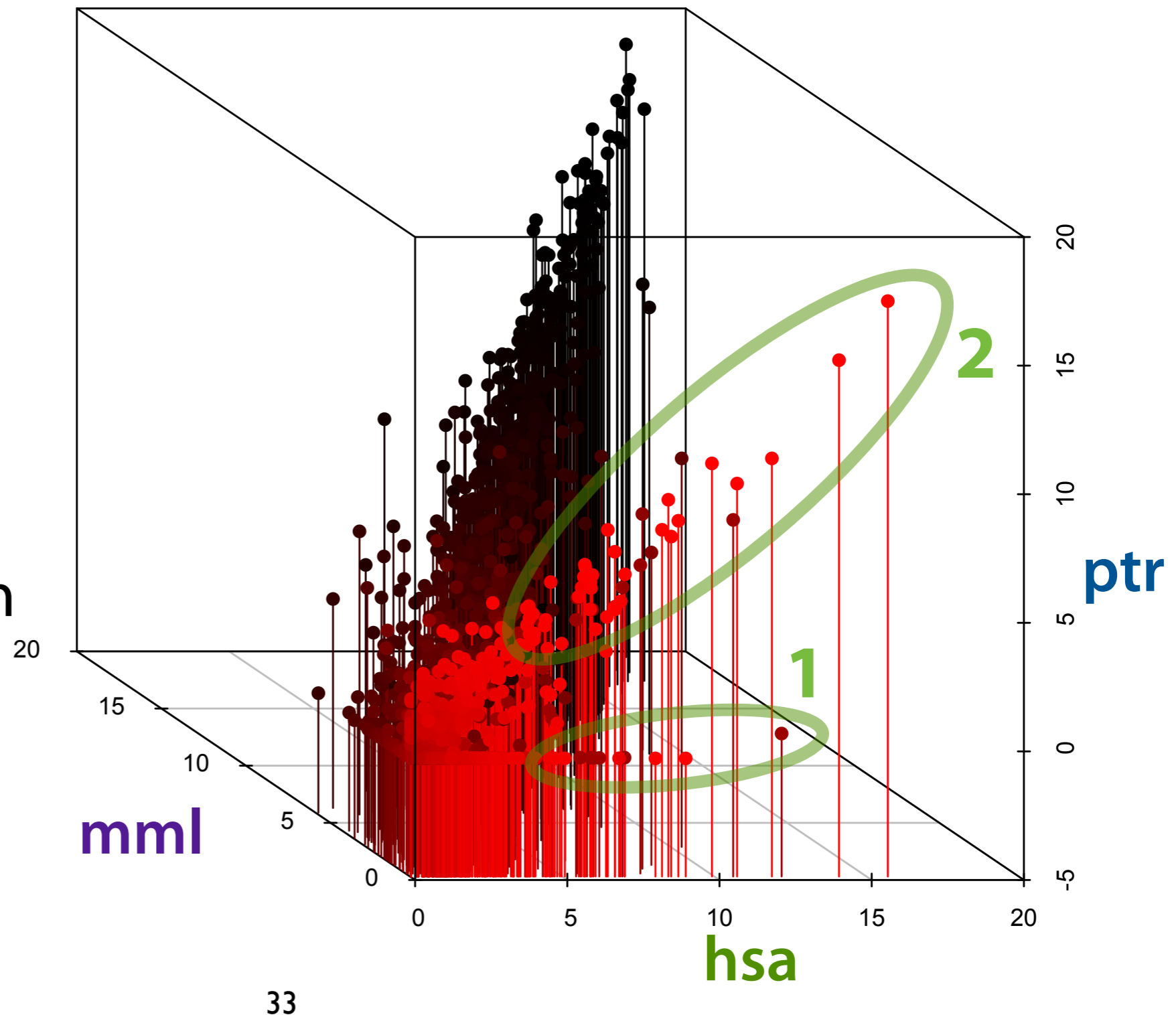
differential expression by species



differential expression by species

small number of miRNAs seem to be human-specific (#1)

larger number only expressed in human and chimp (#2)



pairwise differential expression

ptr

mml

	0	0	0	8	0	0	0	0	0	0	5	6	5	25	19	190	OFC
0		0	0	0	0	0	0	0	0	0	2	13	9	26	20	184	DFC
0	0		0	0	0	0	0	0	0	0	0	9	6	25	21	189	VFC
0	0	0		9	0	0	0	0	0	17	2	0	14	19	161	MFC	
0	0	0	3		0	0	0	4	27	5	40	35	21	17	215	M1C	
0	0	0	0	0		0	0	0	10	2	22	23	22	15	197	S1C	
0	0	0	0	0	0		0	0	0	2	18	16	17	17	183	IPC	
0	0	0	2	0	0	0		0	0	0	15	17	24	21	195	A1C	
0	0	0	0	0	0	0	0		0	3	9	8	27	21	163	STC	
0	0	0	0	0	0	0	0	0		21	2	0	21	29	162	ITC	
0	2	0	8	0	0	0	0	0	8		39	37	40	30	159	V1C	
5	7	10	3	9	8	10	7	5	4	15		0	14	29	218	HIP	
0	5	5	0	6	8	5	5	0	0	17	0		7	18	172	AMY	
20	20	17	20	16	13	23	19	20	19	26	2	11		21	188	STR	
12	14	12	19	10	10	11	8	14	19	13	11	13	21		203	MD	
200	212	205	214	187	184	185	192	190	215	166	212	223	207	198		CBC	
OFC	DFC	VFC	MFC	M1C	S1C	IPC	A1C	STC	ITC	V1C	HIP	AMY	STR	MD	CBC		

	0	0	0	3	2	0	0	0	0	0	6	15	15	28	3	143	OFC
0		0	0	3	0	0	0	0	0	0	7	25	27	30	4	155	DFC
0	0		2	0	0	0	0	0	0	0	3	21	22	31	7	143	VFC
0	0	0		5	4	0	3	0	0	12	3	0	10	6	125	MFC	
0	0	0	3		0	0	0	0	2	0	31	34	30	3	143	M1C	
0	0	0	0	0		0	0	0	2	0	18	33	24	4	103	S1C	
0	0	0	0	0	0		0	0	0	4	16	22	27	4	143	IPC	
0	0	0	2	0	0	0		0	2	2	29	51	37	3	144	A1C	
0	0	0	0	0	0	0	0		0	5	21	38	33	18	165	STC	
0	0	0	0	0	0	0	0	0		11	12	6	20	6	161	ITC	
0	2	0	8	0	0	0	0	0	8		59	88	49	23	103	V1C	
5	7	10	3	9	8	10	7	5	4	15		3	19	16	172	HIP	
0	5	5	0	6	8	5	5	0	0	17	0		11	19	195	AMY	
20	20	17	20	16	13	23	19	20	19	26	2	11		24	171	STR	
12	14	12	19	10	10	11	8	14	19	13	11	13	21		143	MD	
200	212	205	214	187	184	185	192	190	215	166	212	223	207	198		CBC	
OFC	DFC	VFC	MFC	M1C	S1C	IPC	A1C	STC	ITC	V1C	HIP	AMY	STR	MD	CBC		

hsa

hsa

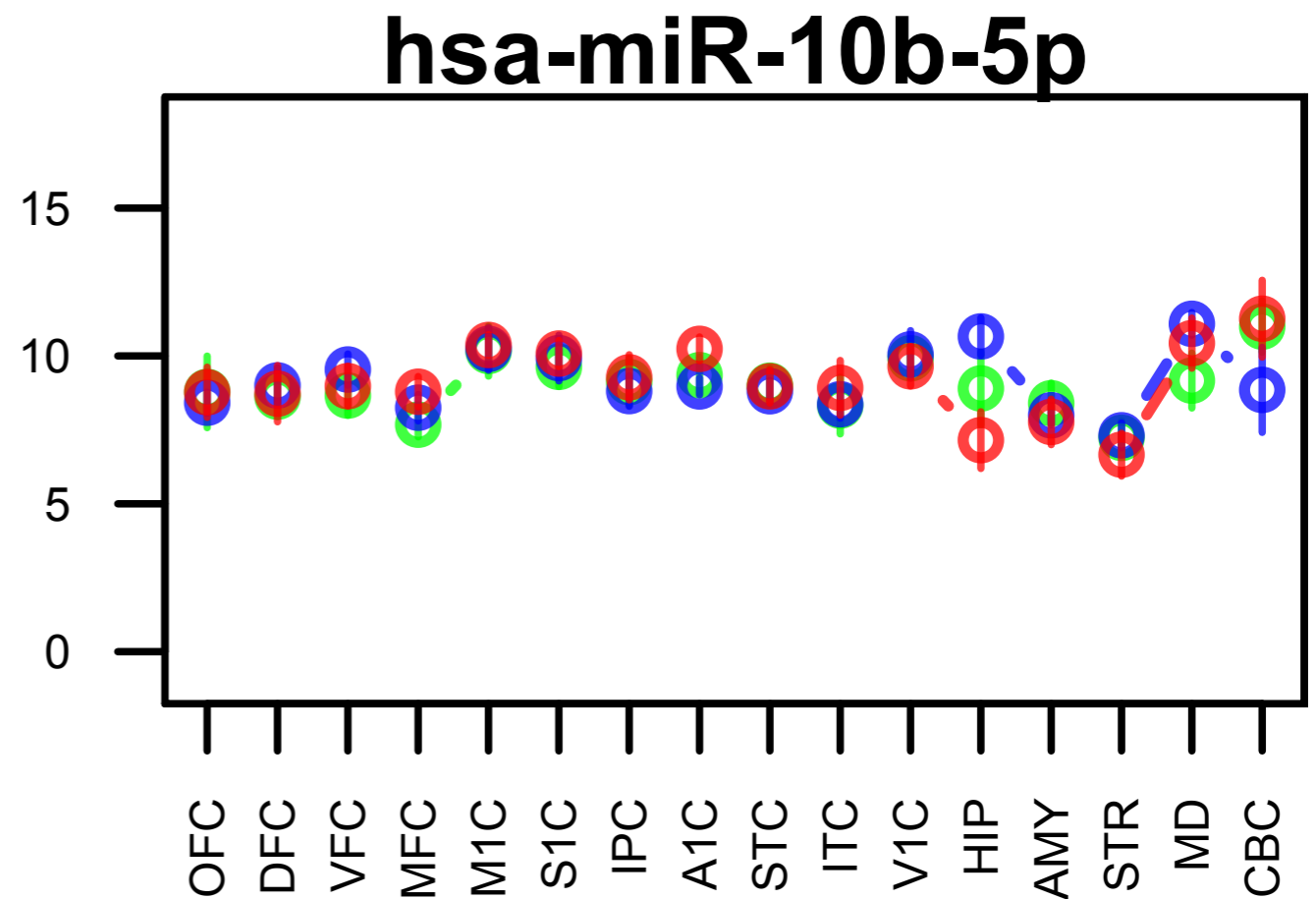
individual miRNA representation

plot normalised \log_2 expression values for a single miRNA over all 16 tissues in all 3 species:

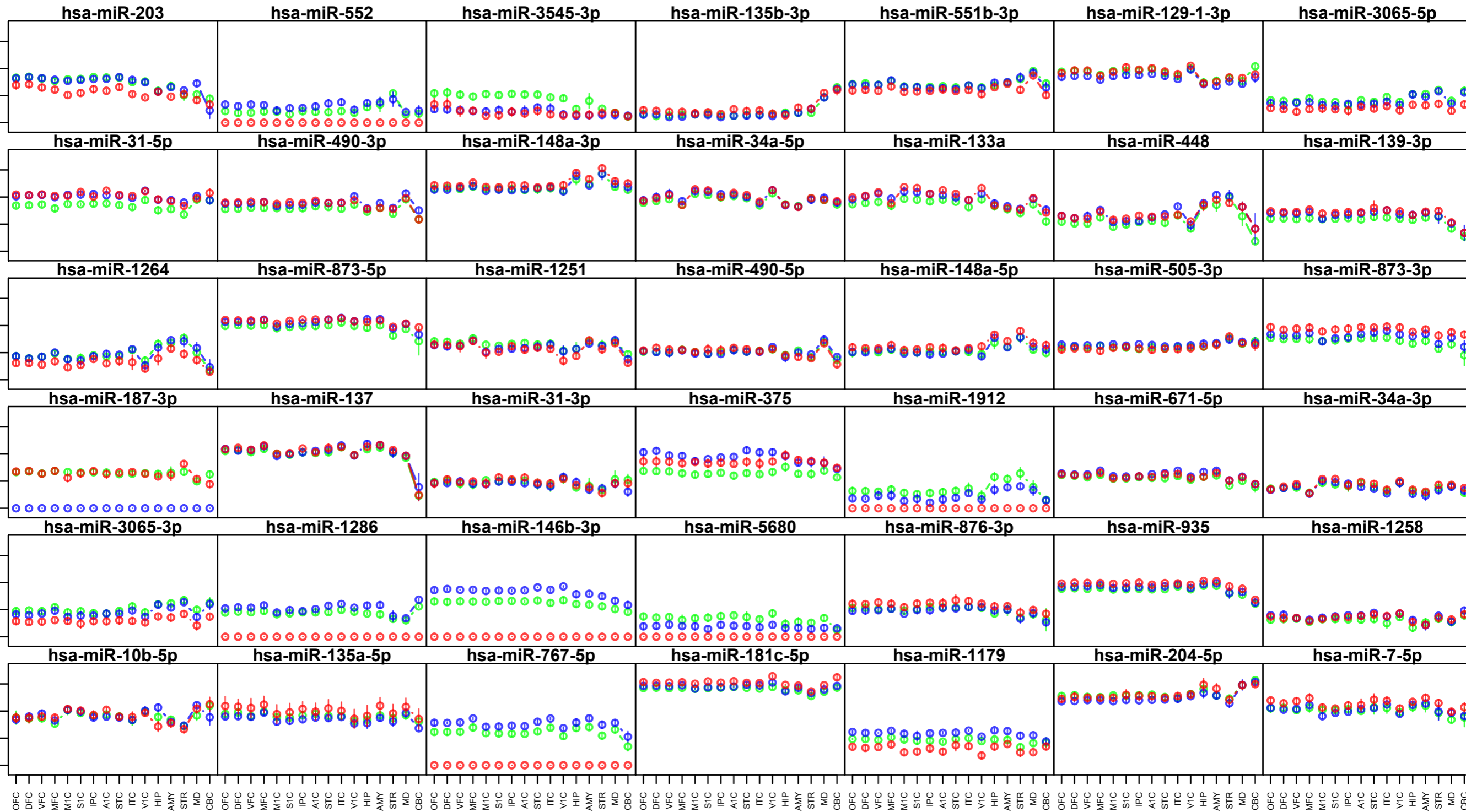
human

chimp

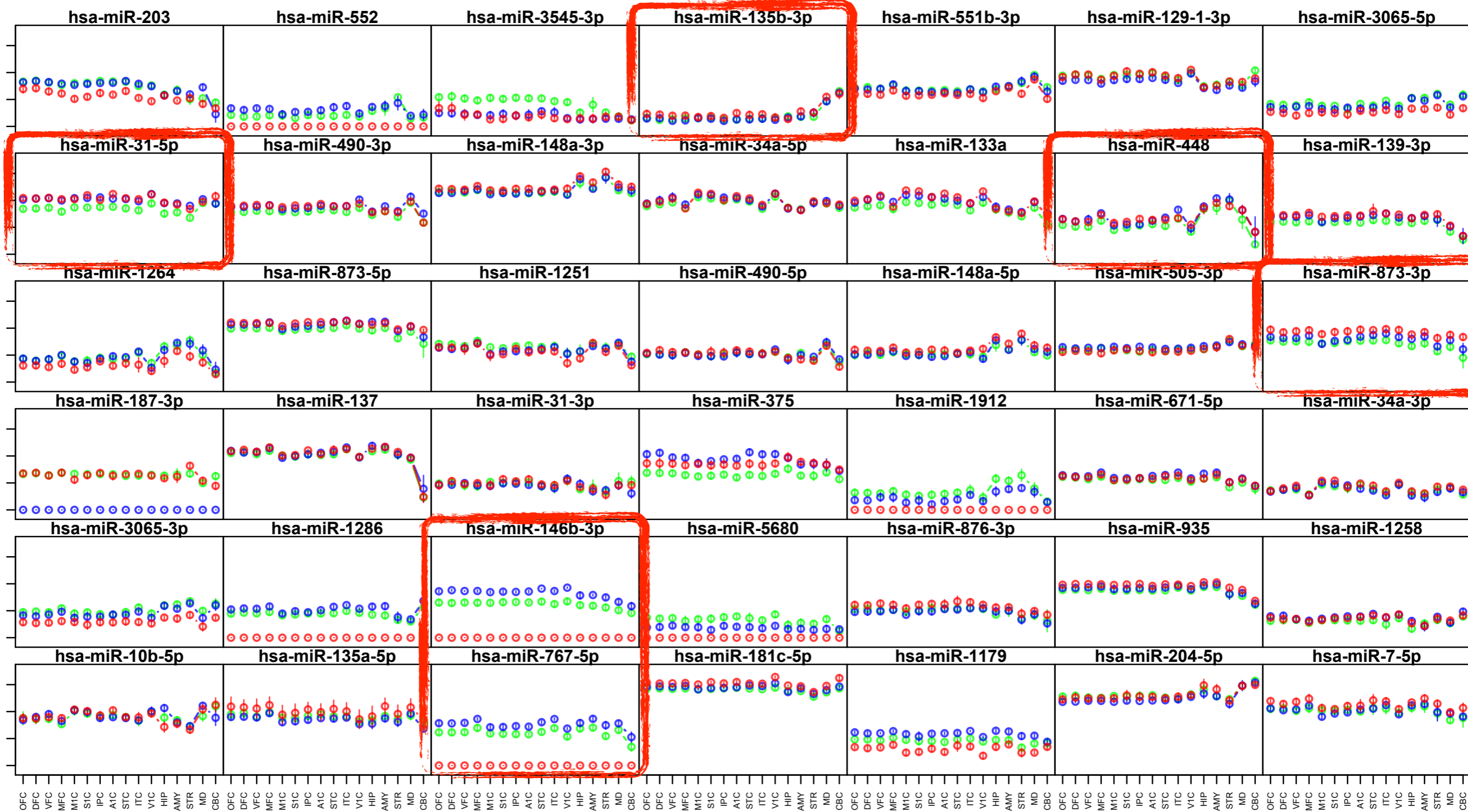
macaque



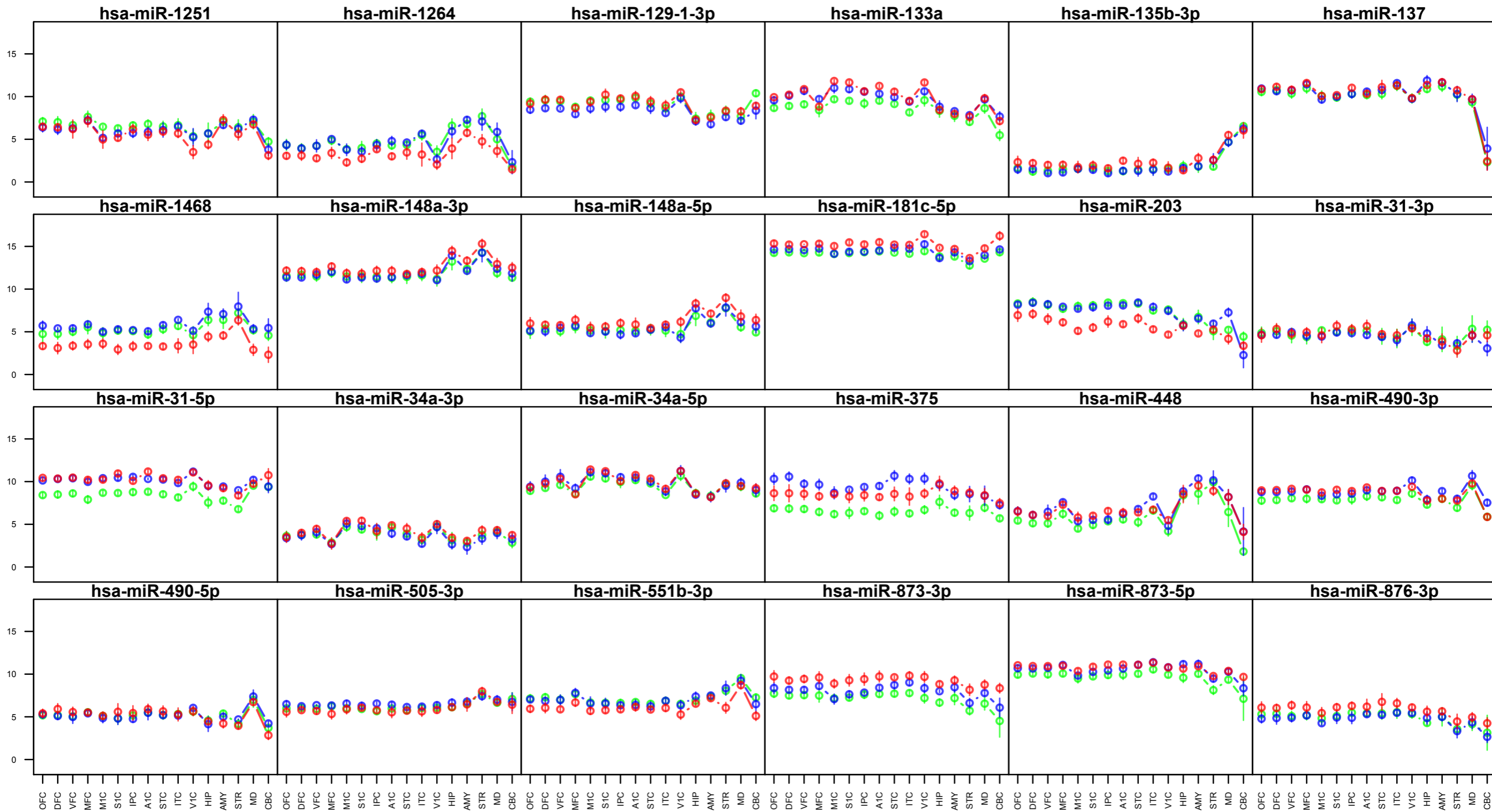
human DEX miRNAs



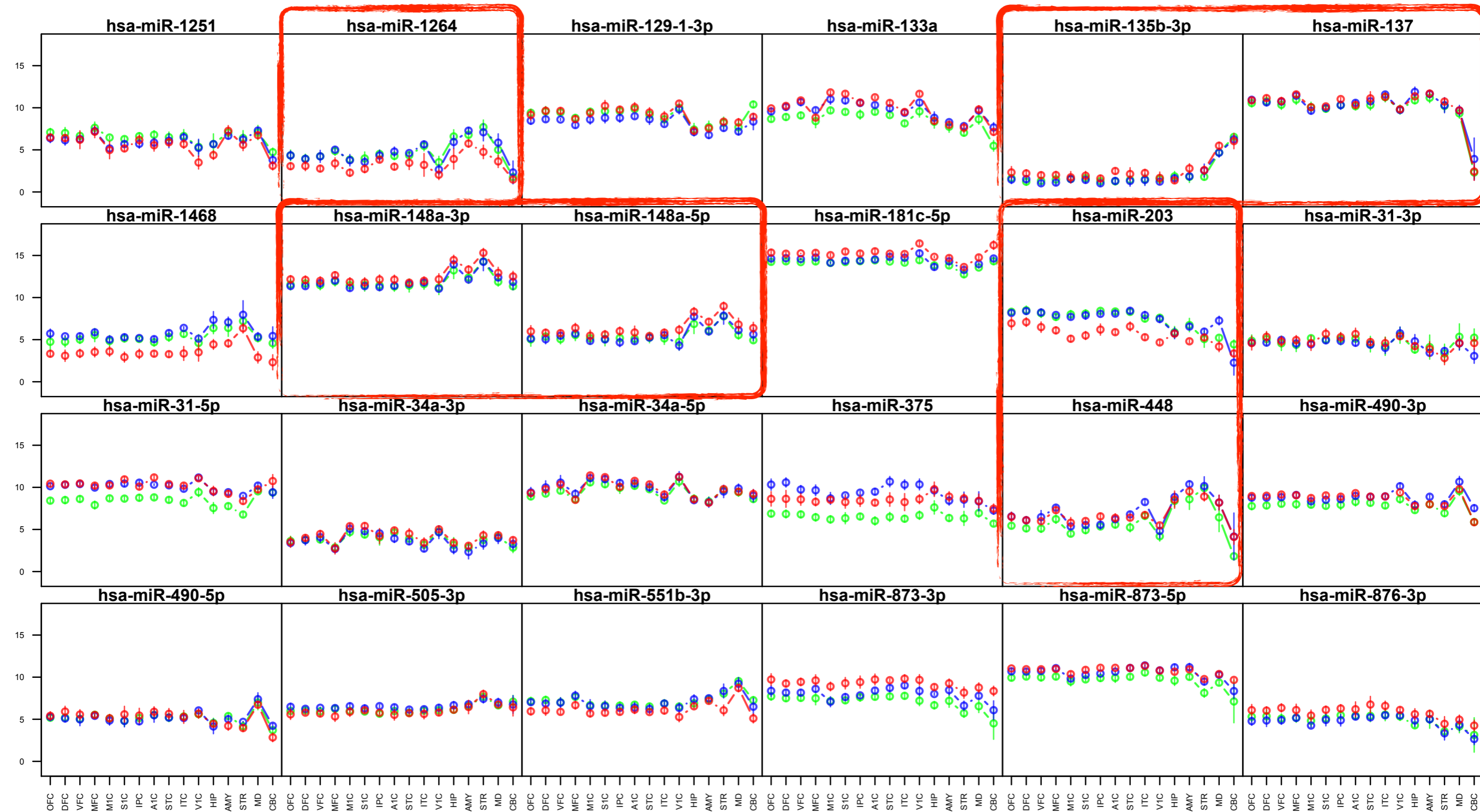
human DEX miRNAs



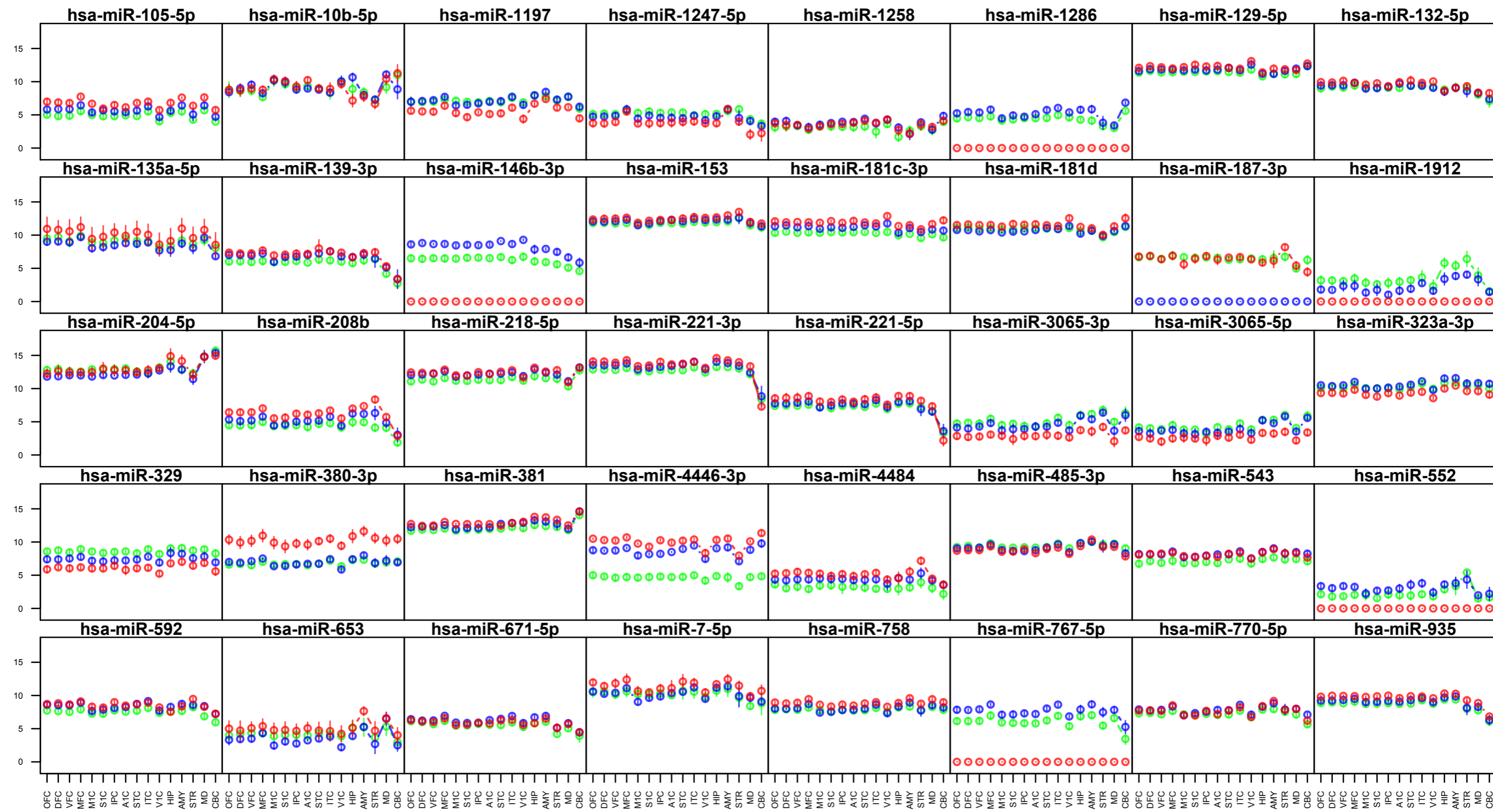
miRNAs DEX in all species



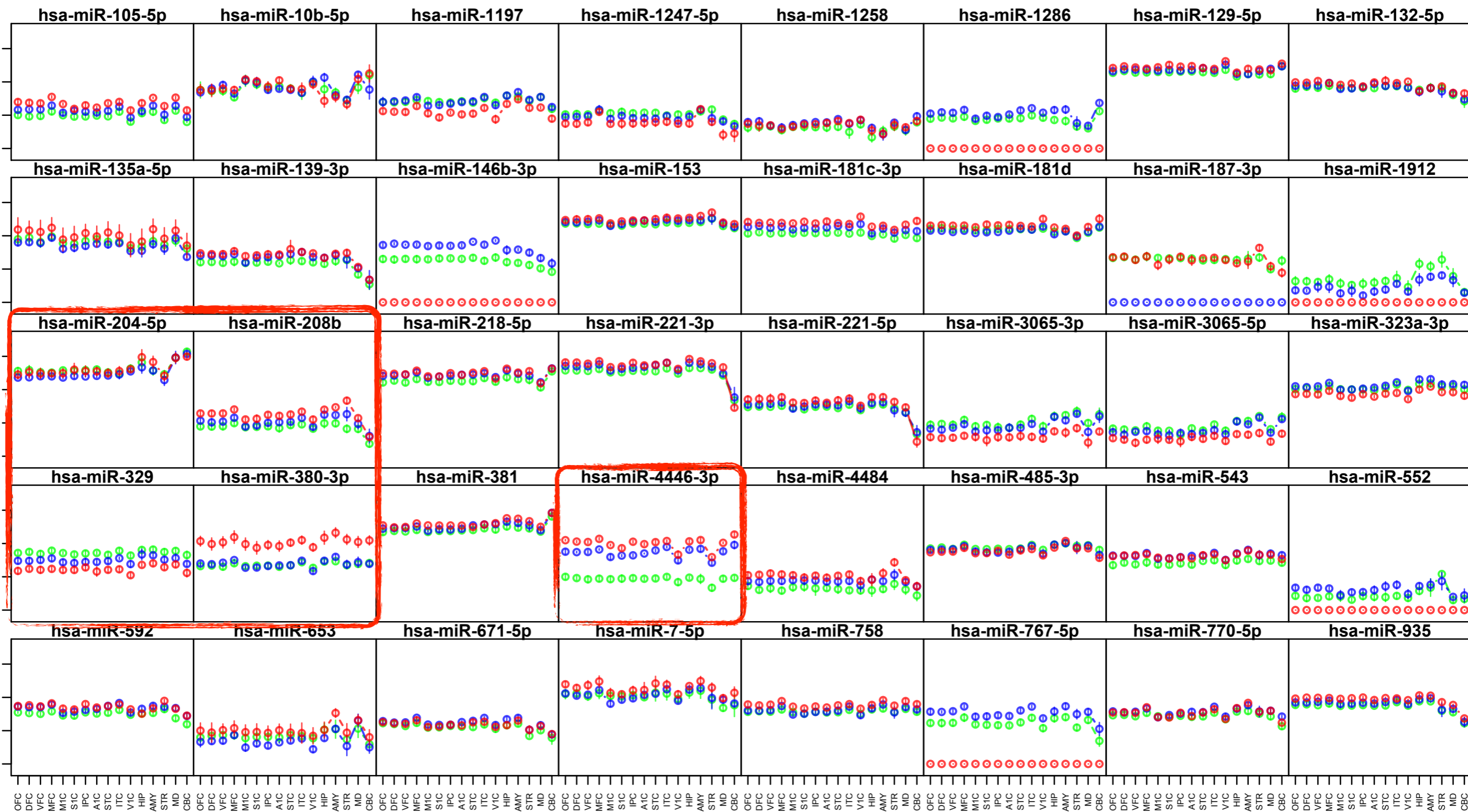
miRNAs DEX in all species



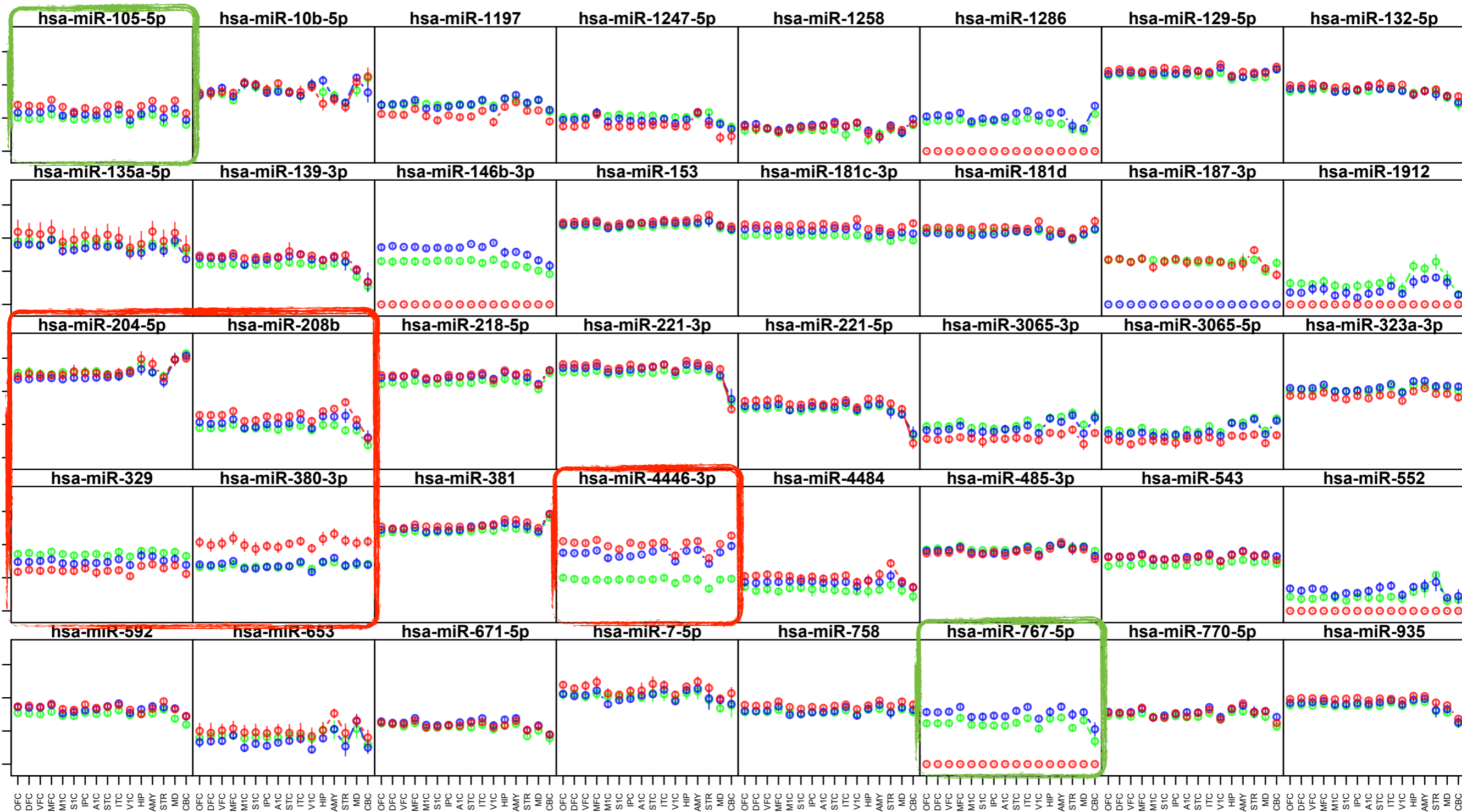
miRNAs DEX in 2 species



miRNAs DEX in 2 species



miRNAs DEX in 2 species



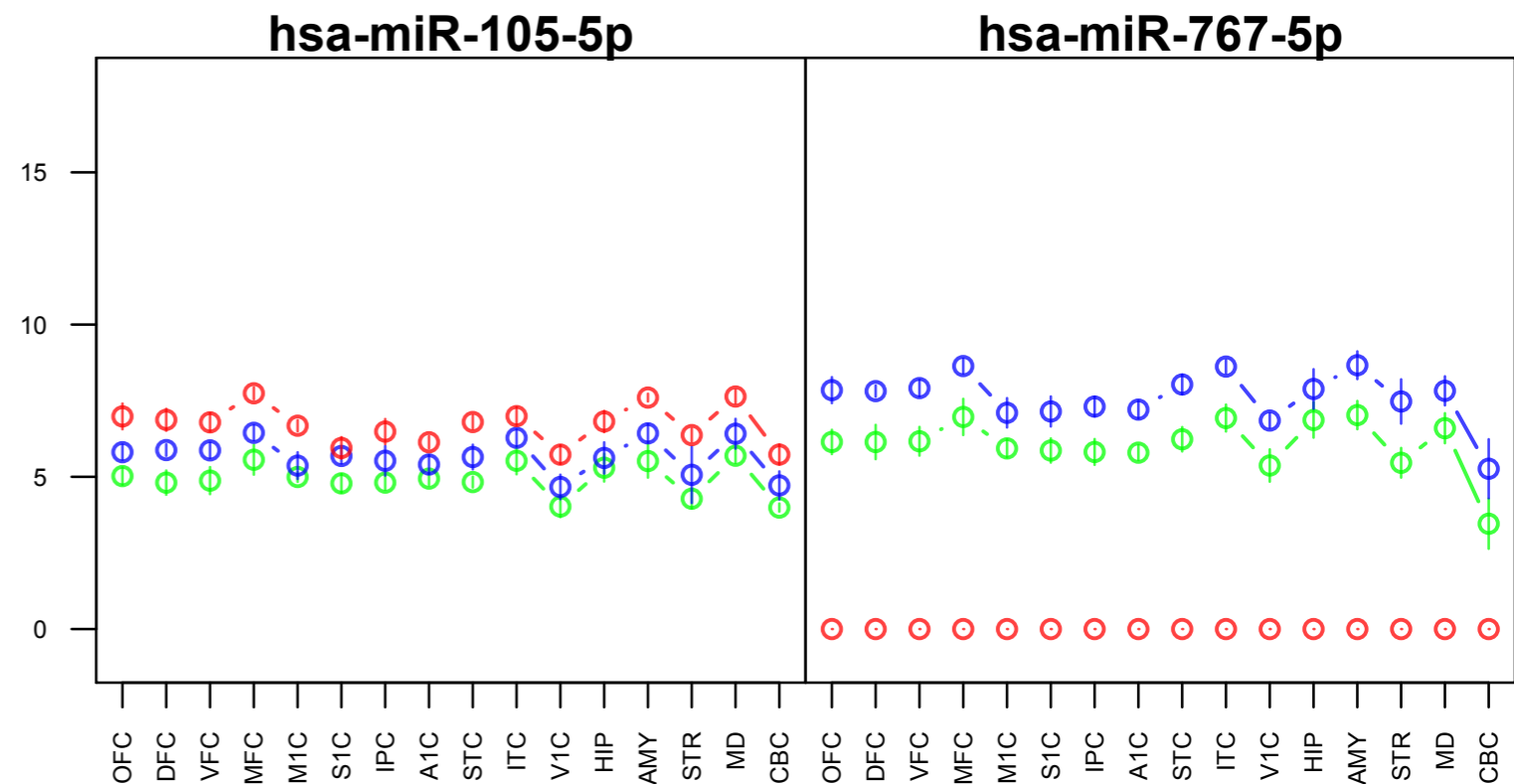
interesting miRNAs

miR-105, miR-767 < 1kb apart in an intron of **GABRA3**
(subunit of the GABA receptor)

interesting that **miR-767 is not expressed in mml**

miR-105 targets:
IL2RB, LIN7A, HOXA3

miR-767 targets:
SMAD6, BASP1, ATXN1

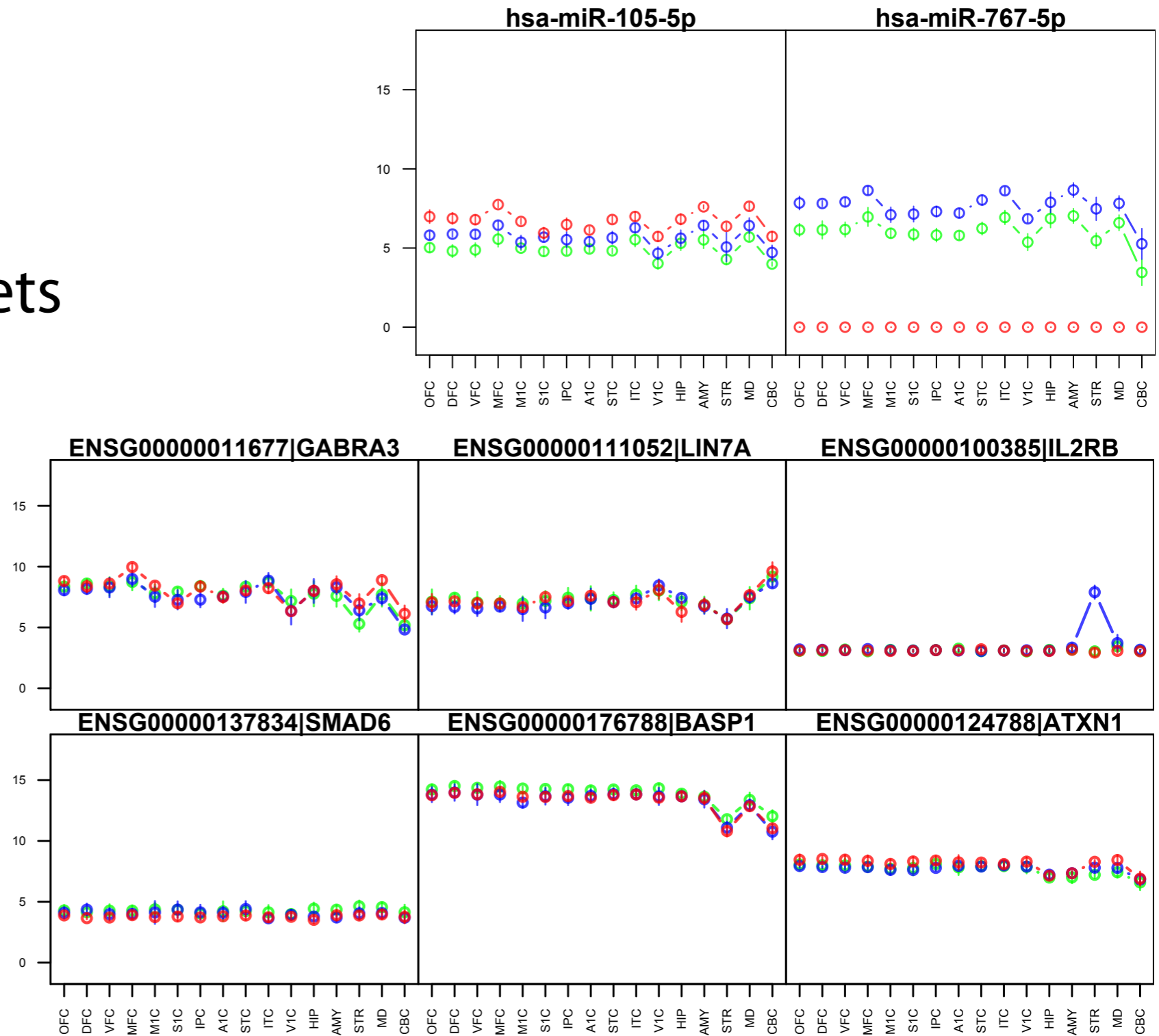


interesting miRNAs

however no obvious anticorrelation with these supposed targets

strong positive correlation with GABRA3 'parent' gene

search continues...



to do...

carefully integrate homolog & novel miRNA expressions into this analysis

examine hsa/ptr/mml sequence alignments for **conservation bias**

examine cases where sequences align well, but have a total loss of expression in 1+ species --> **pseudo-miRNAs?**

continue to examine individual DEX miRNAs to pick strong **validation candidates** and **concise exemplar network**

brainspan samples overview

adult samples

human



6 brains

chimp



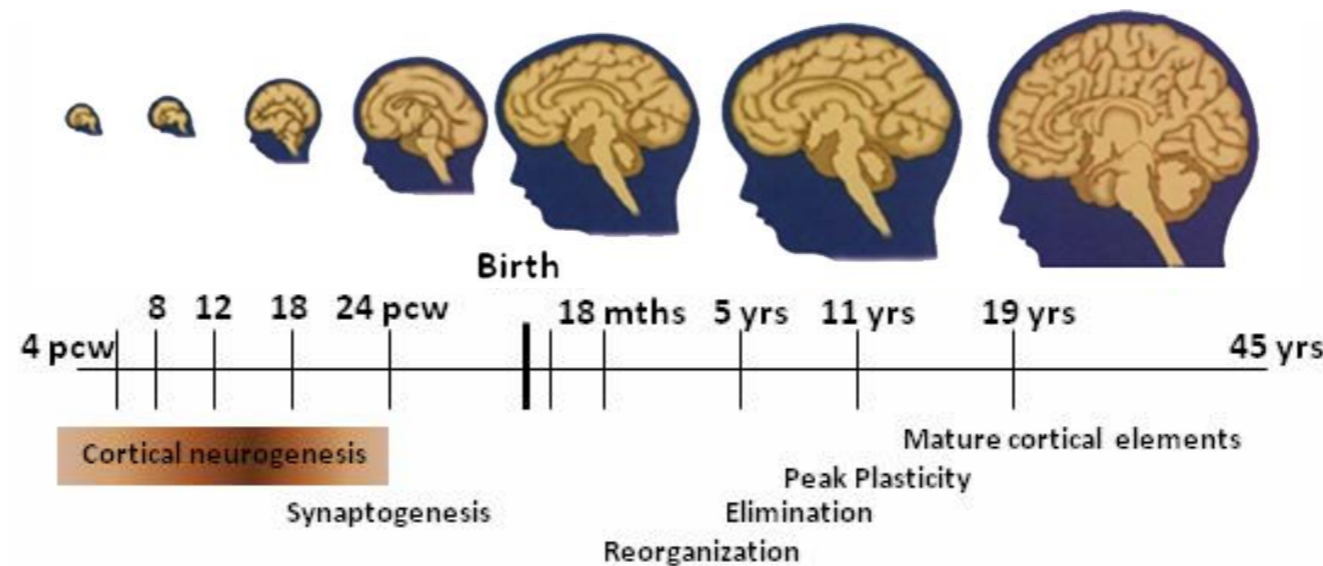
5 brains

macaque



5 brains

human-development samples



18 brains

brainspan data overview

	human adult	chimp adult	macaque adult	developing human	developing macaque
mRNA-seq	6 brains x 16 regions	5 brains x 16 regions	5 brains x 16 regions	18 brains x 16 regions	planned
miRNA-seq	6 brains x 16 regions	5 brains x 16 regions	5 brains x 16 regions	18 brains x 16 regions	planned
DNA	6 brains (chip) 5 brains (seq)	5 brains (seq, in progress)	...	18 brains (planned)	...
methylation	6 brains x 16 regions (chip)
ChIP-seq	3 IPs x 3 brains x 2 regions	?	?

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