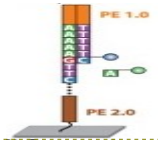
Sequencing resume

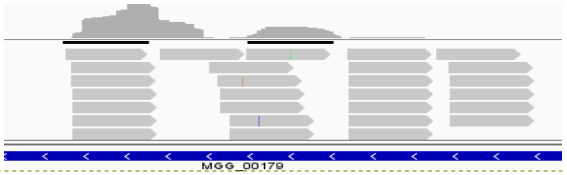
- 2 strains (*WT, Δrbp35*) x 4 conditions (*CM, MM, -N, -C*) x 3 replicates
- 4751592 – 11517077 total reads database
- ~62% - ~82% successfully mapped reads
- 43 bp mean read length
- ~92% - ~98% replicates correlation
- ~100bp mean pair ended distance
- ~400x coverage per poly(A) site*

** assuming an amount of 22000 mRNA molecules per cell*

Workflow

Program and details	Procedure	% of data left
	Illumina sequencing	100%
fasta-mcf, *adapter trimming*	Data prepping	96%
gsnap, *default parameters*	Alignment	96%
• *Low quality mapping (<30))* • *High A/T content (>90%)* • *Internal priming*	Filtering	75%
Assign reads to known features, Including all intergenic space up to 400 bp from gene end	Assignment	65%
Clustering of most significant poly(A) sites (at least 5 supporting reads and p-value < 0.05 over global gene expression) at 33 bp	poly(A) sites detection	27%

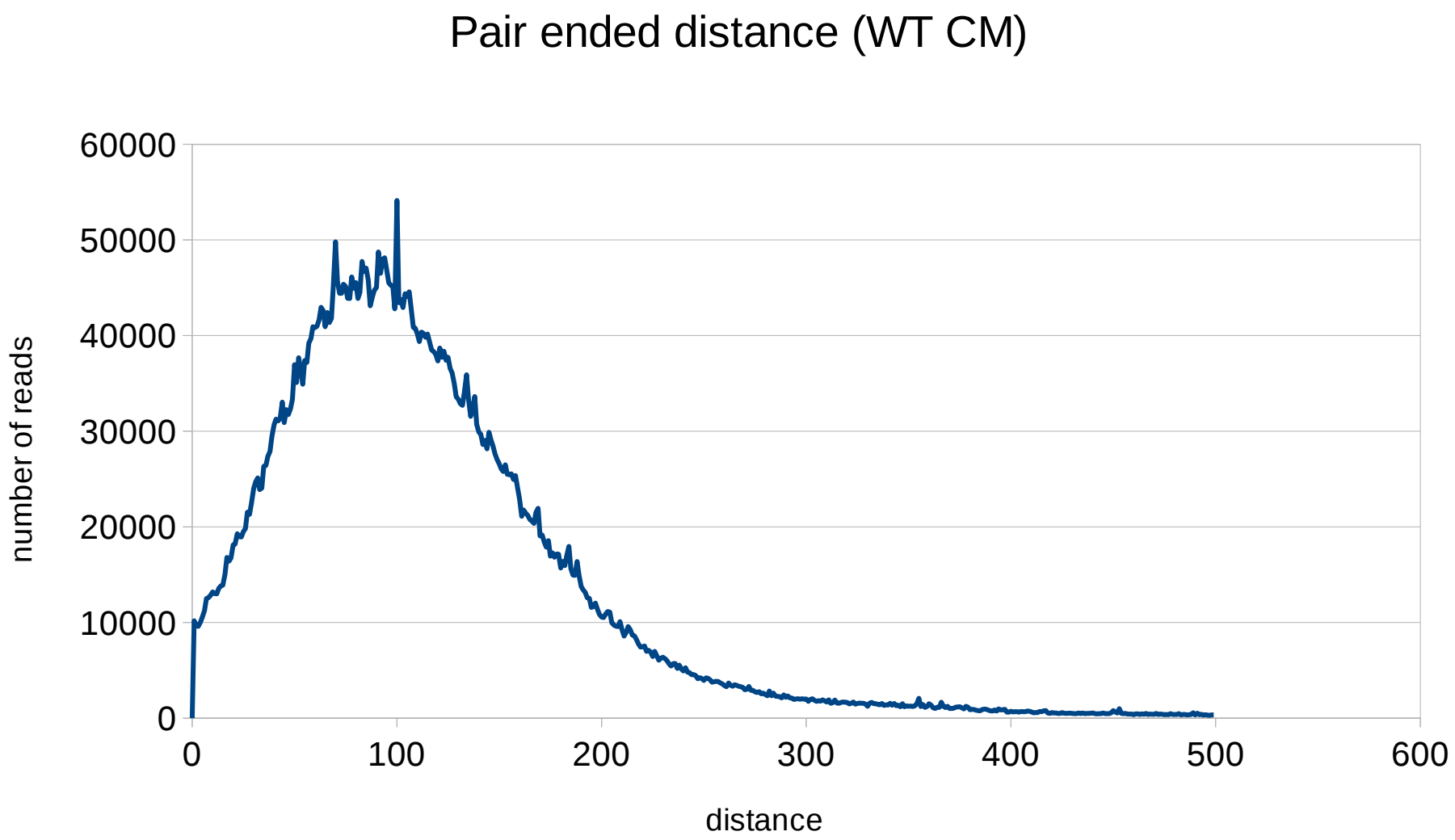




*Example: **Actin,** single cut poly(A):*

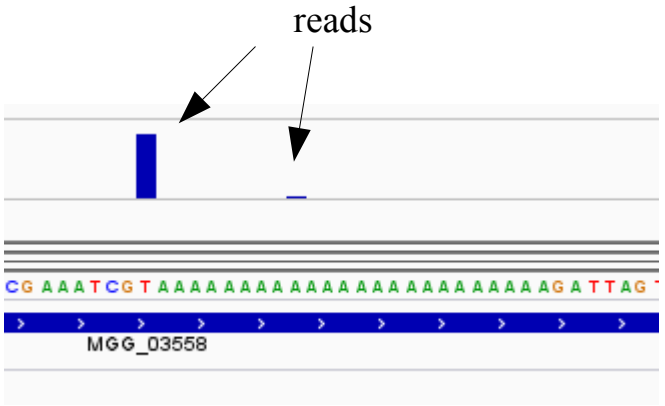
- whole gene expression ~7000 reads
- poly(A) site expression ~3000 reads

Pair ended reads distance

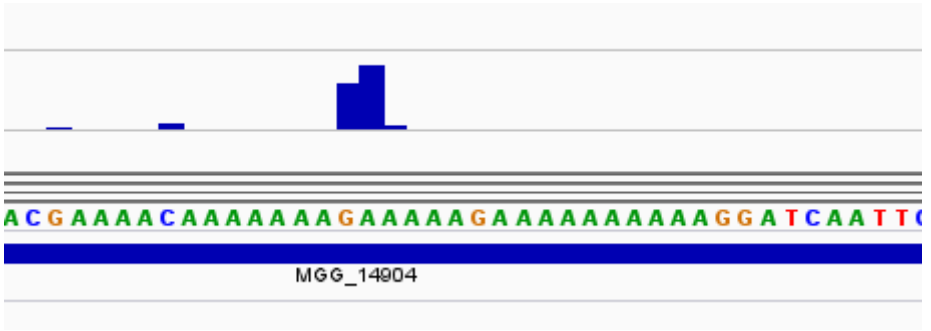


~2.5% of poly(A) sites are internal priming

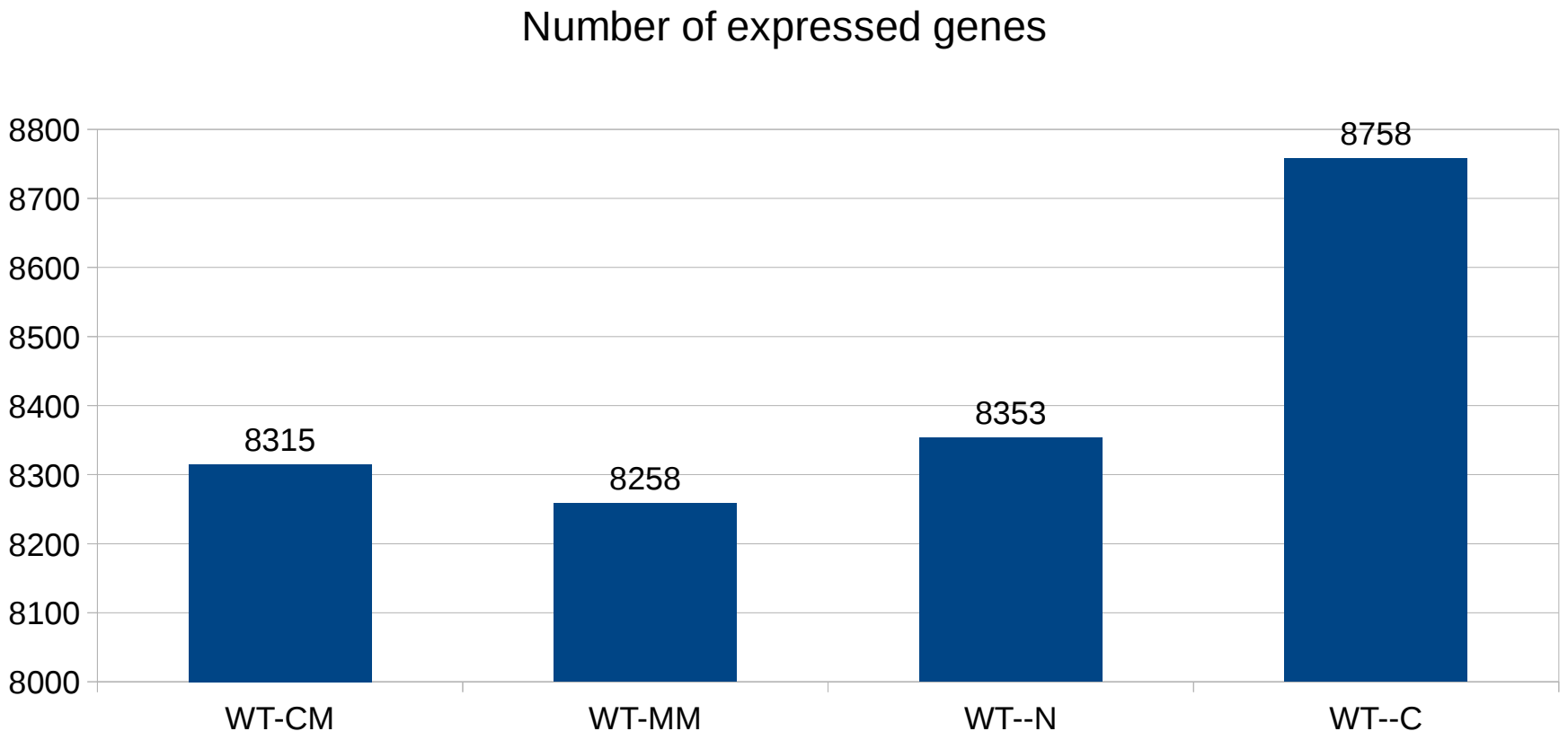
- Some poly(A) sites are just a side effect of poly(A) ***genomic*** regions



stretches of As



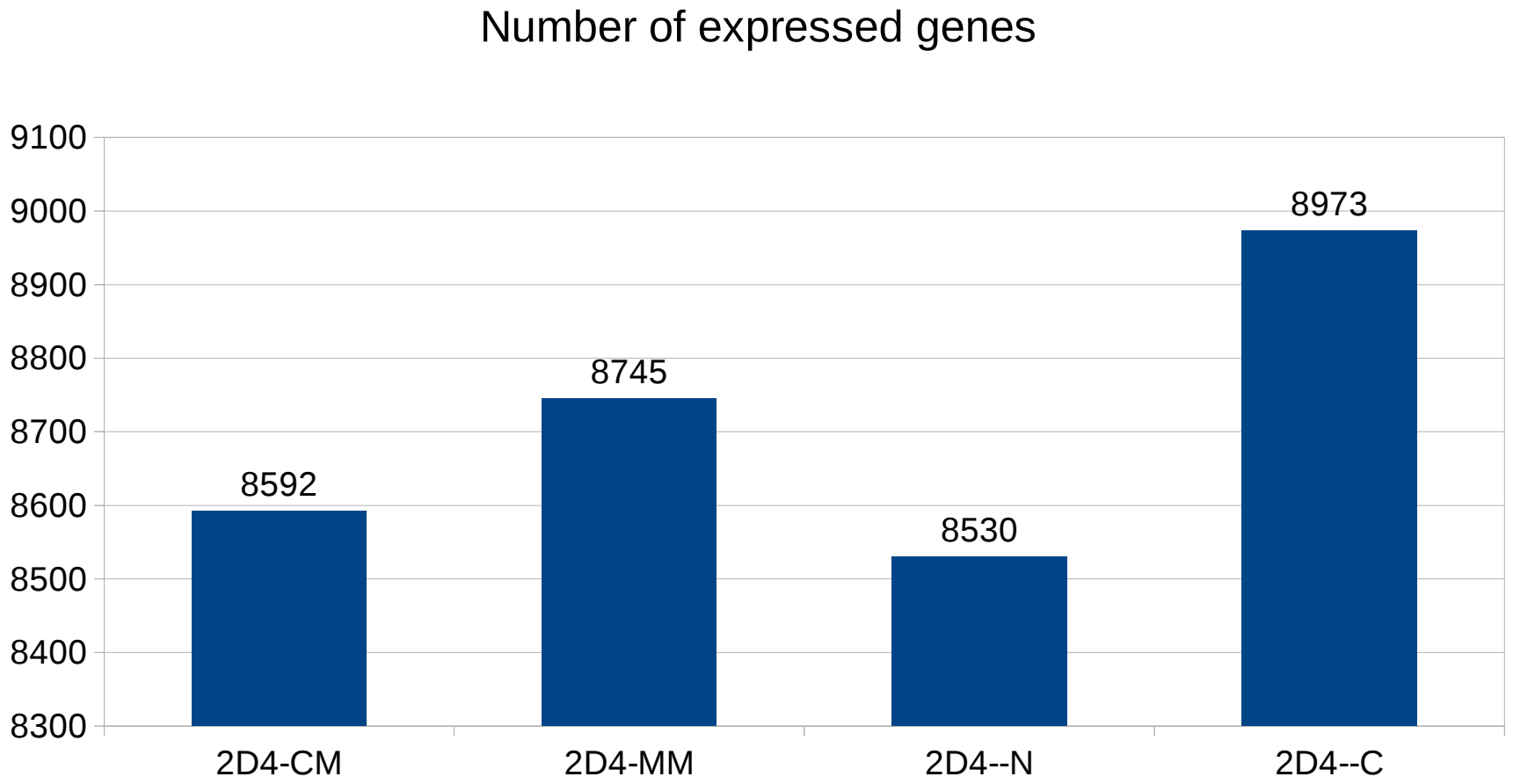
~8500 genes are expressed, out of a total of 13218 annotated genes (WT)



- 7662 genes are expressed in every condition (WT only)
- 3979 genes are never expressed (WT only)

A gene is considered as expressed when has at least 10 supporting reads in a least 2 replicates

~8500 genes are expressed, out of a total of 13218 annotated genes (Δrbp35)

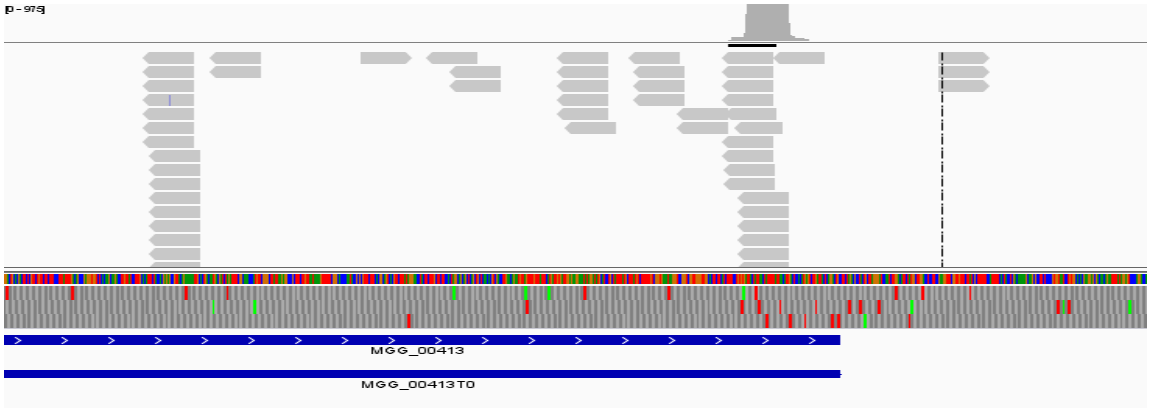


- 7993 genes are expressed in every condition (Δrbp35 only)
- 3757 genes are never expressed (Δrbp35 only)

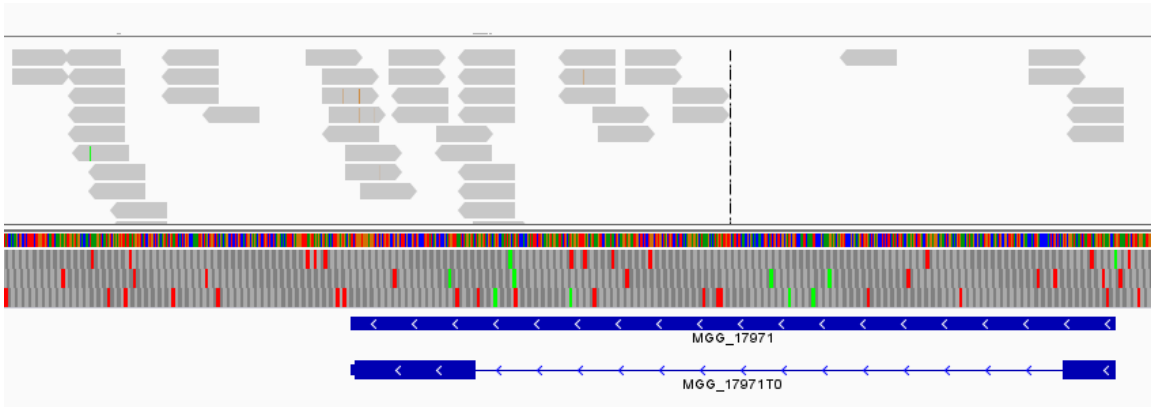
A gene is considered as expressed when has at least 10 supporting reads in a least 2 replicates

Not every expressed gene has a recognizable poly(A) site

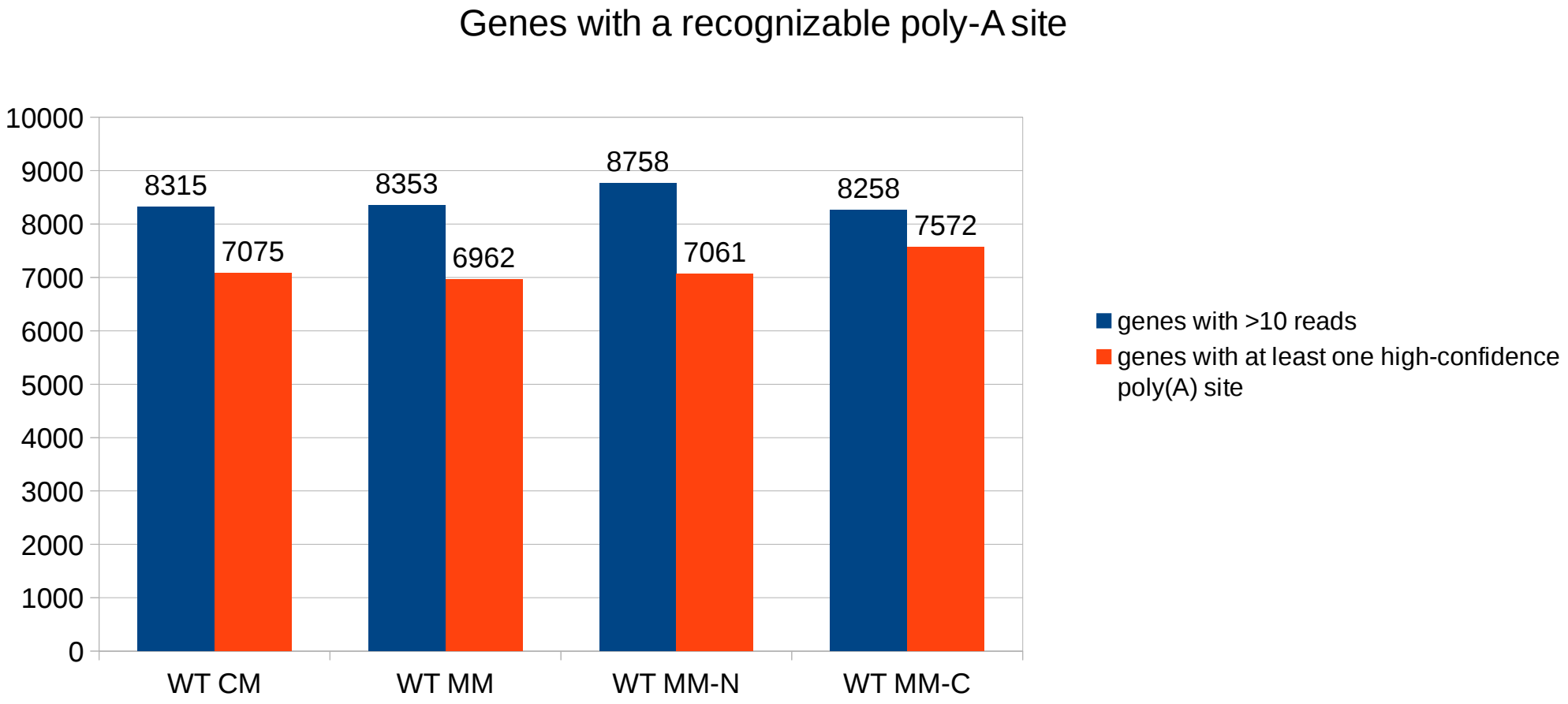
Expressed gene <u>with</u> a recognizable poly(A) site:



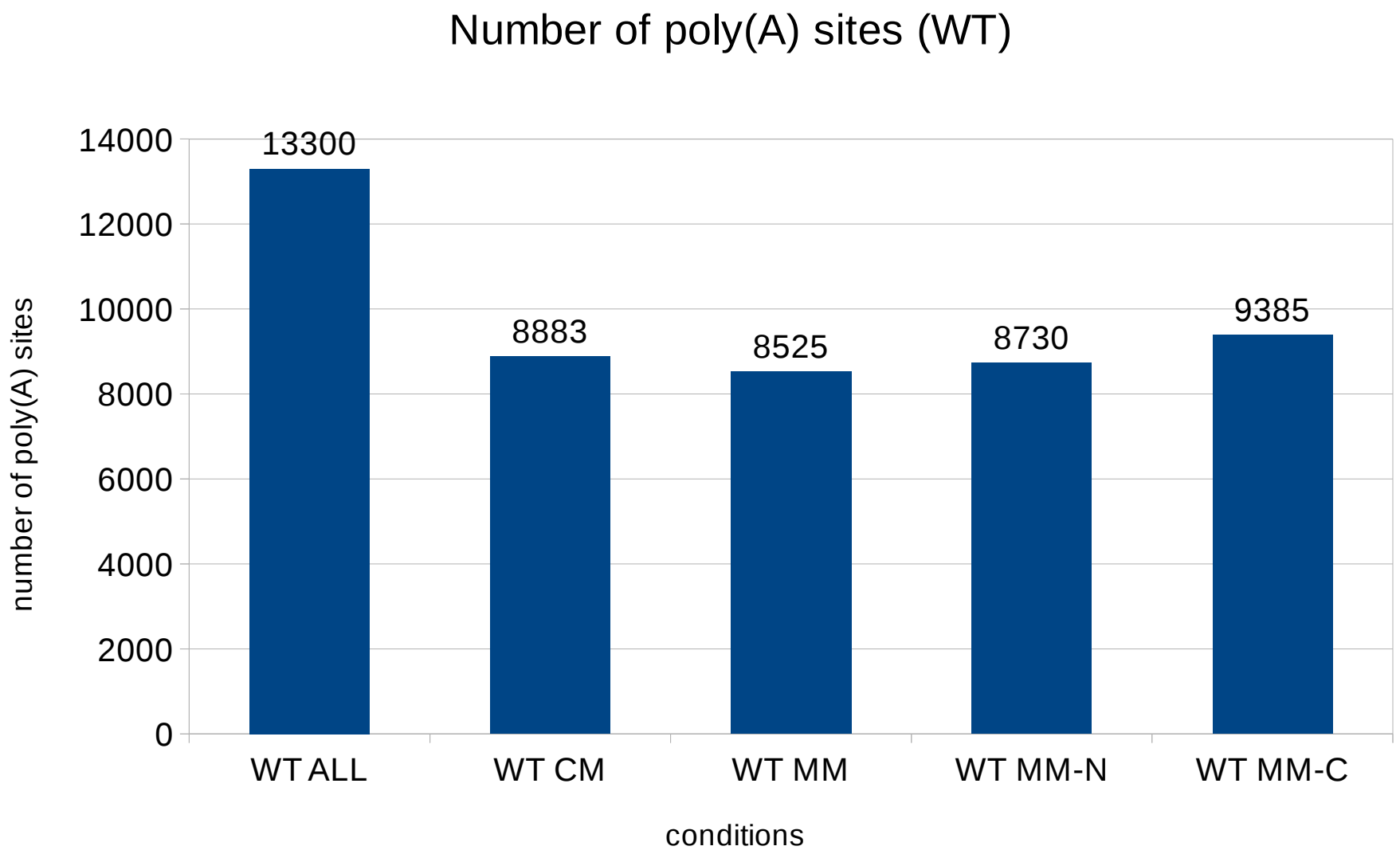
Expressed gene <u>without</u> a recognizable poly(A) site:



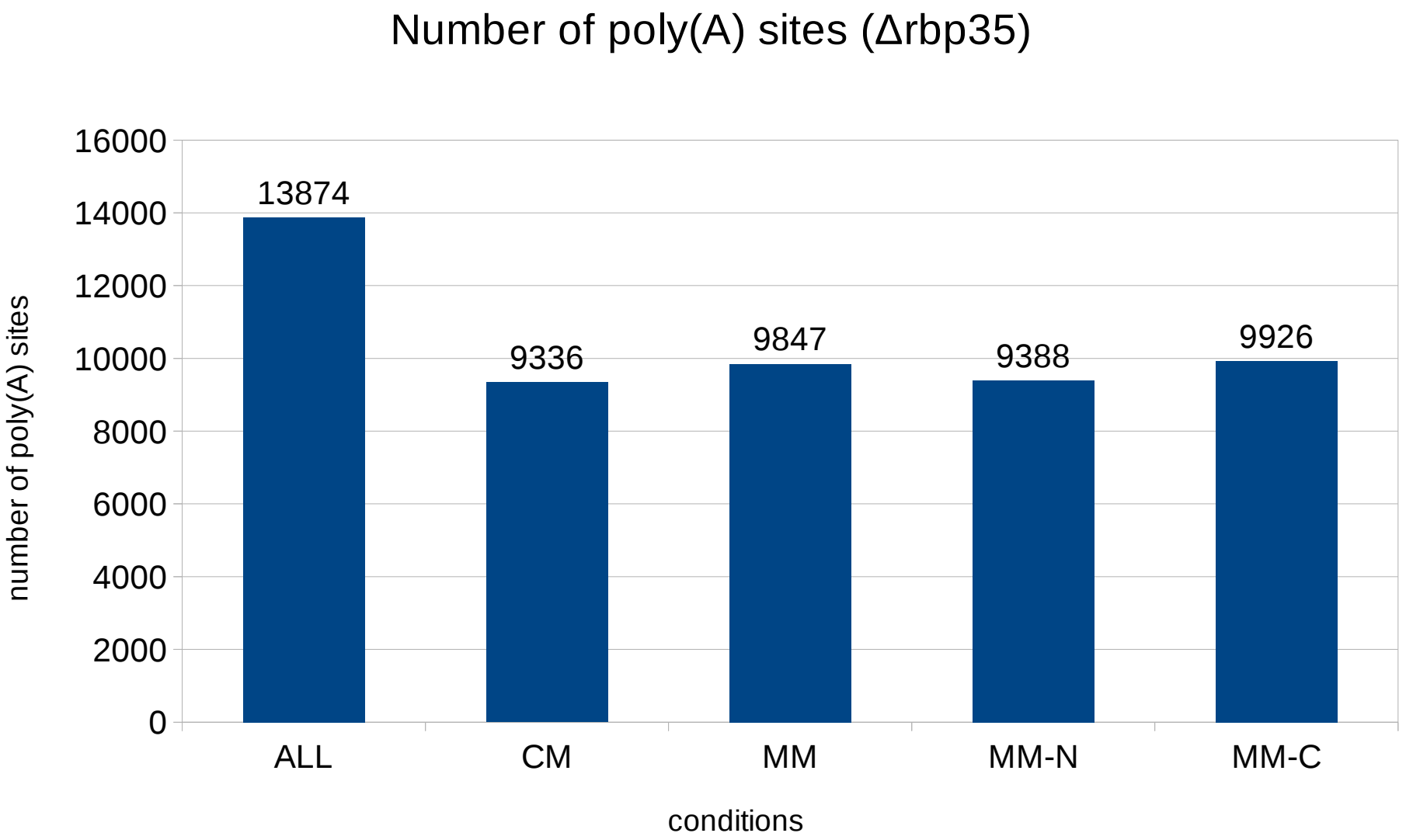
Only ~85% of genes expressed have a recognizable poly(A) site



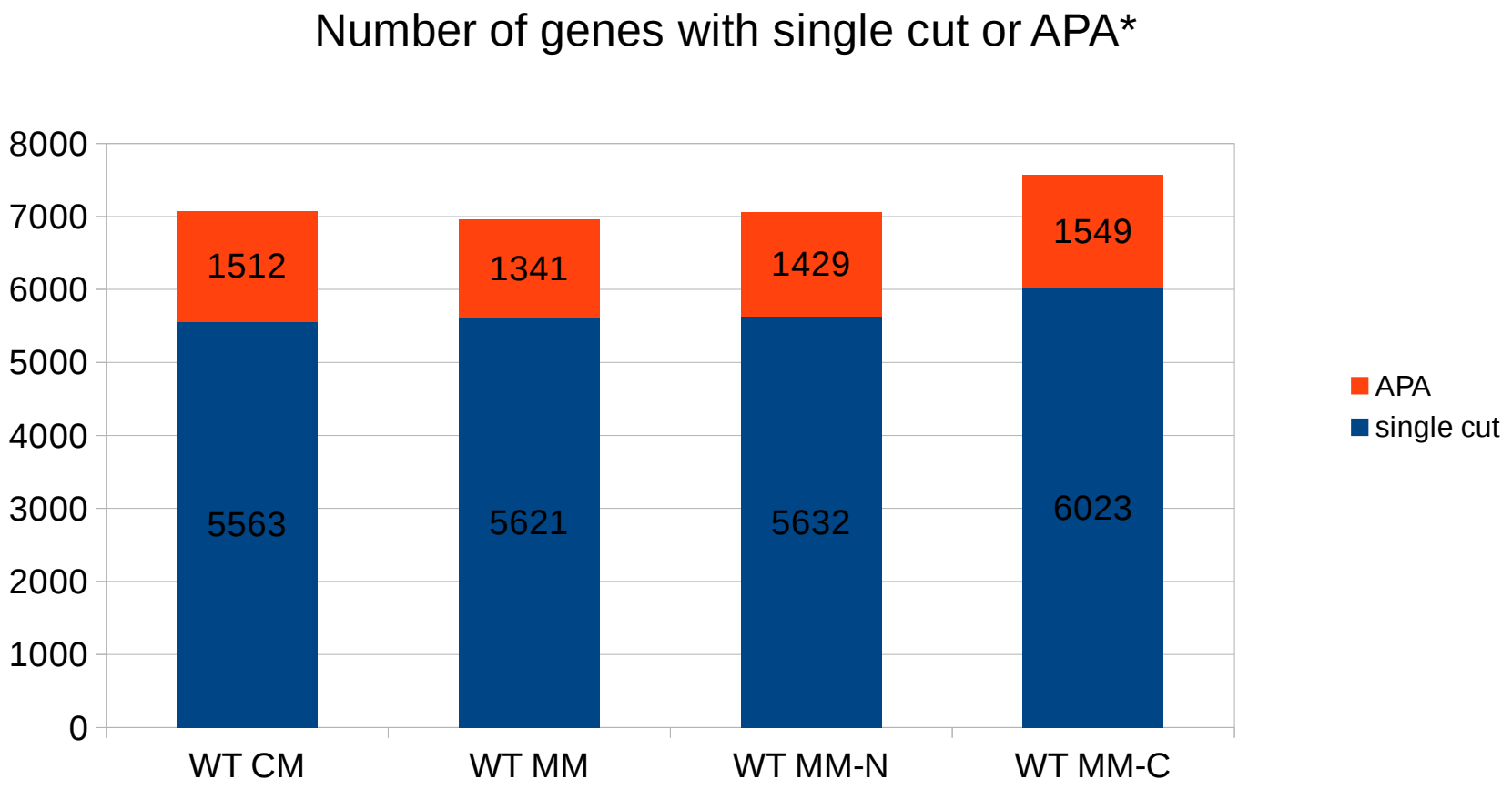
~13000 poly(A) site could be assigned to annotated genes



~13000 poly(A) site could be assigned to annotated genes (*Δrbp35*)

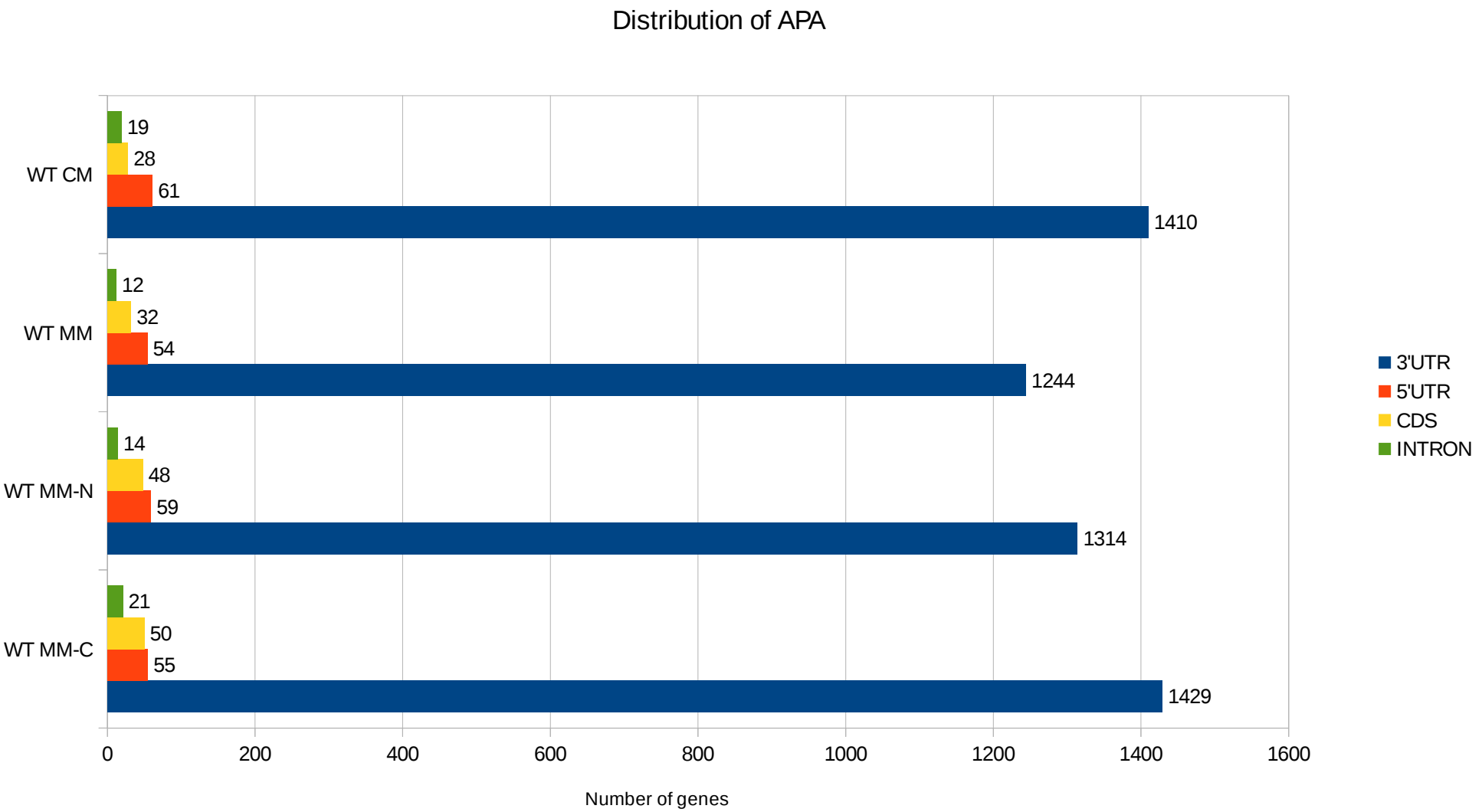


~20% of genes are alternatively polyadenilated

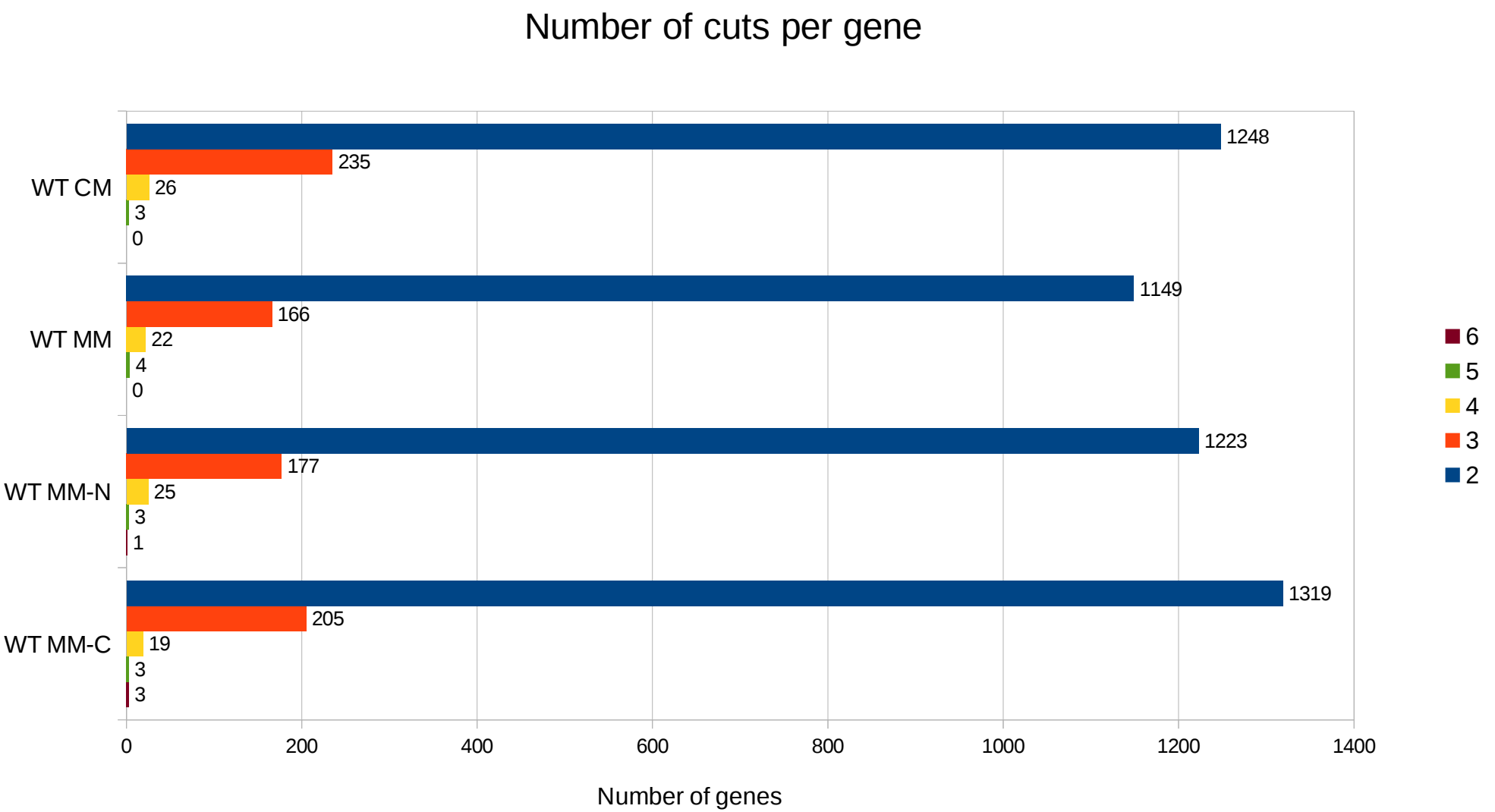


** calculated over the global number of expressed genes with a recognizable poly(A) site*

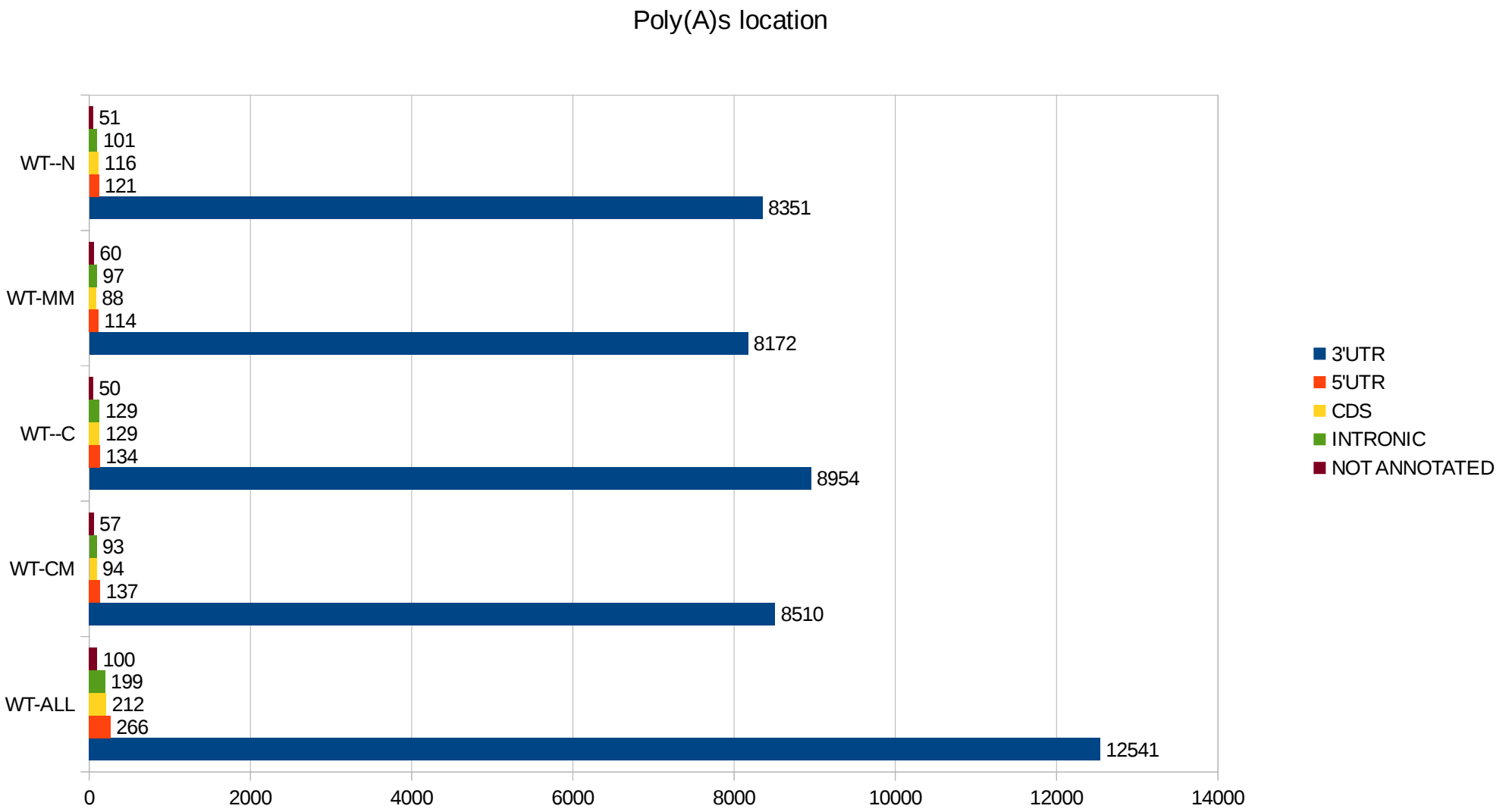
>90% of APA is located in the 3'UTR



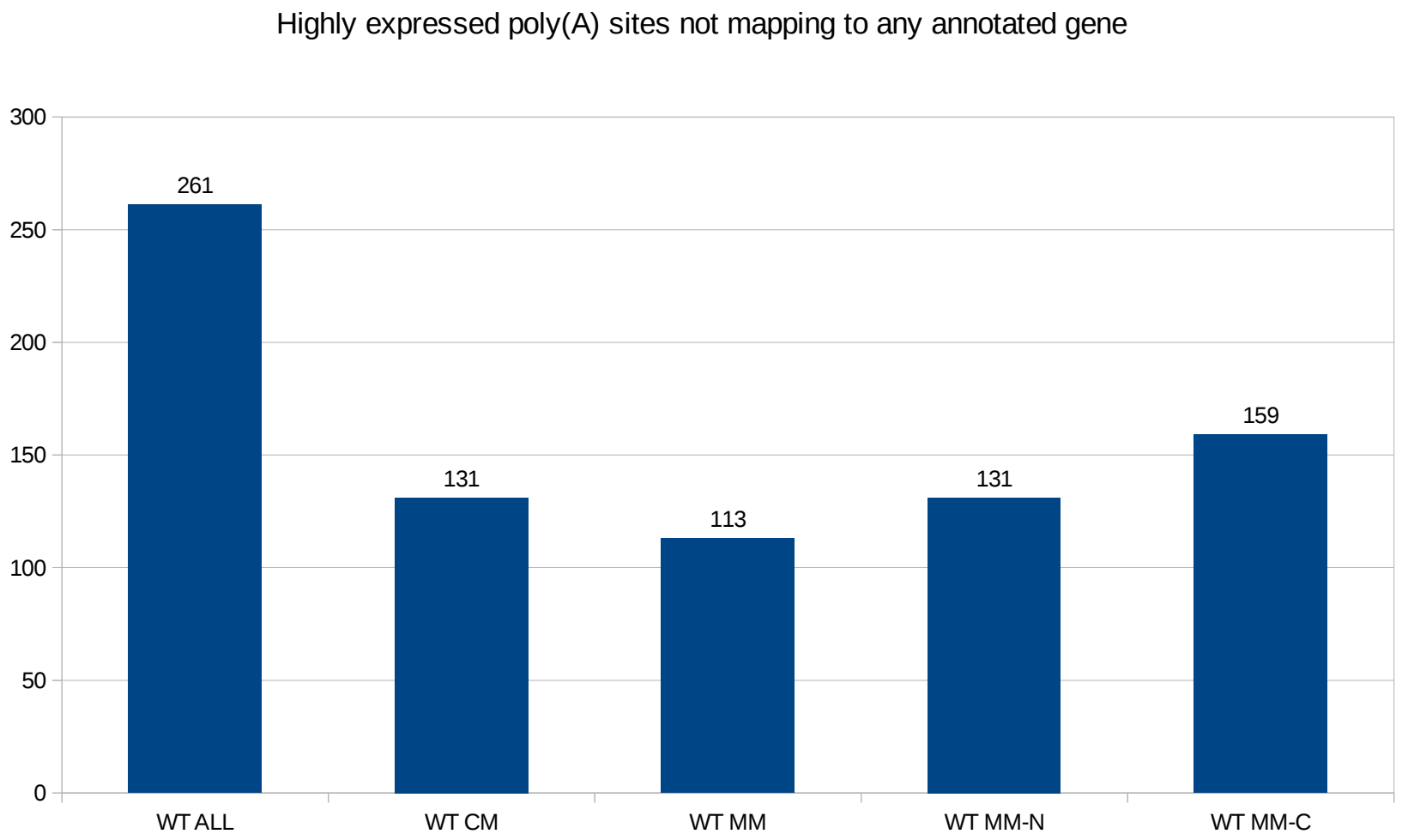
>80% of APA is composed of two cleveage sites



>90% of poly(A) sites are located in the 3'UTR



261 highly expressed (>100 reads) poly(A) sites could not be assigned to any annotated gene



261 orphan poly(A) sites highly expressed in WT (>100 reads)

- 14 hits against other gene copies in *M.oryzae*
- 44 hits against Uniprot nt/nr database
- 4 hits against Rfam(ncRNA) database
- 81 overlapping annotated genes antisense
- 63 matching CPA-sRNA sequences
- 16 matching retrotransposons
- 7 located in telemeric avirulence regions

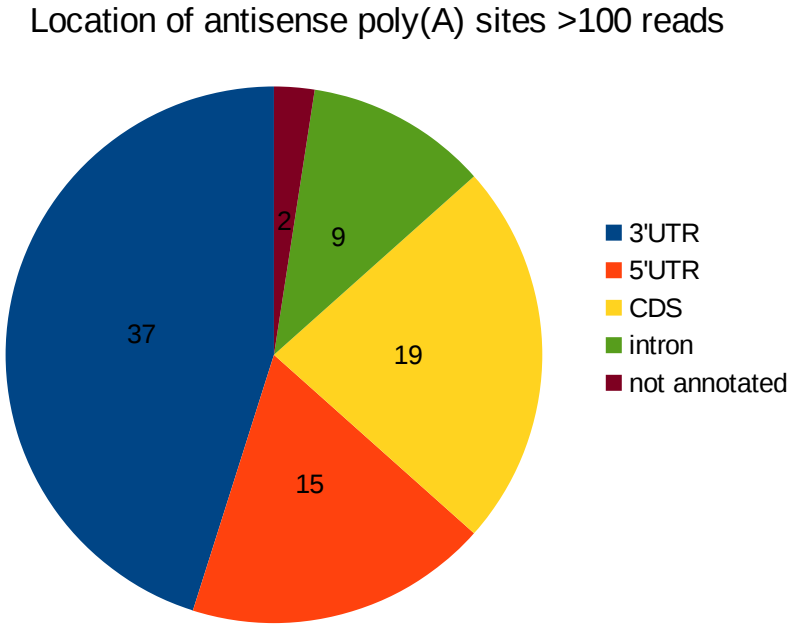
3165 orphan poly(A) sites expressed in WT (>10 reads)

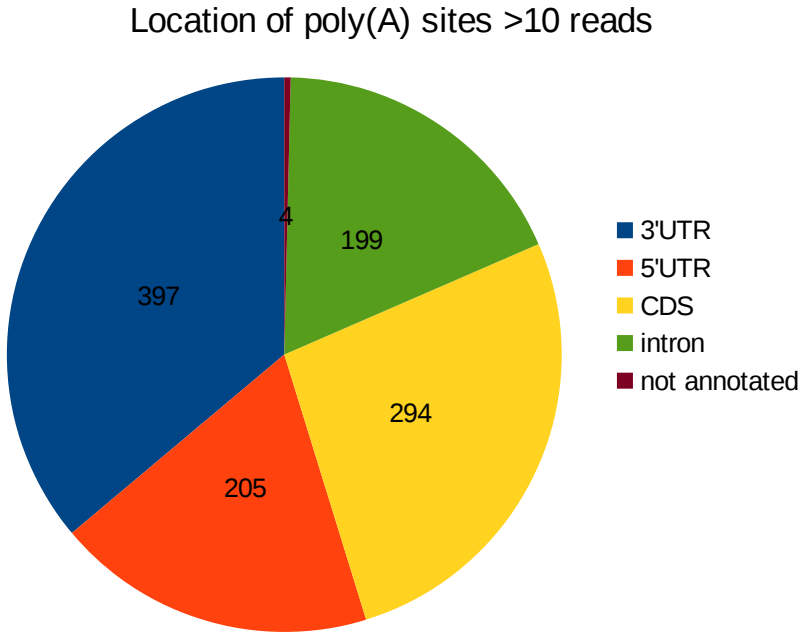
- 102 hits against other gene copies in *M.oryzae*
- 438 hits against Uniprot nt/nr database
- 10 hits against Rfam(ncRNA) database
- 1098 overlapping annotated genes antisense
- 253 matching CPA-sRNA sequences
- 129 matching retrotransposons
- 57 located in telemeric avirulence regions

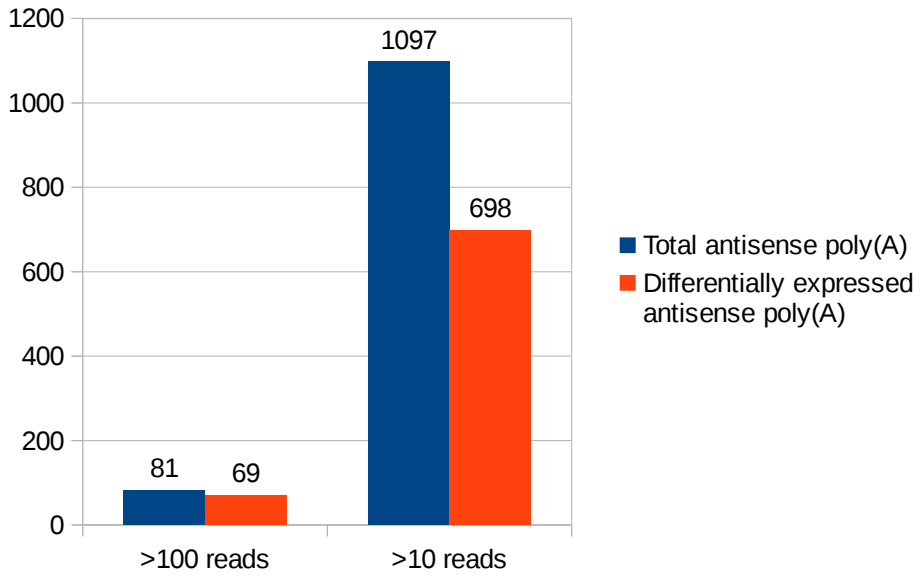
Orphans differentially expressed in WT

(>100 reads)	
CM → MM-C	167
CM → MM	36
CM → MM-N	51
MM → MM-C	129
MM → MM-N	0
(>10 reads)	
CM → MM-C	1499
CM → MM	177
CM → MM-N	285
MM → MM-C	1110
MM → MM-N	0

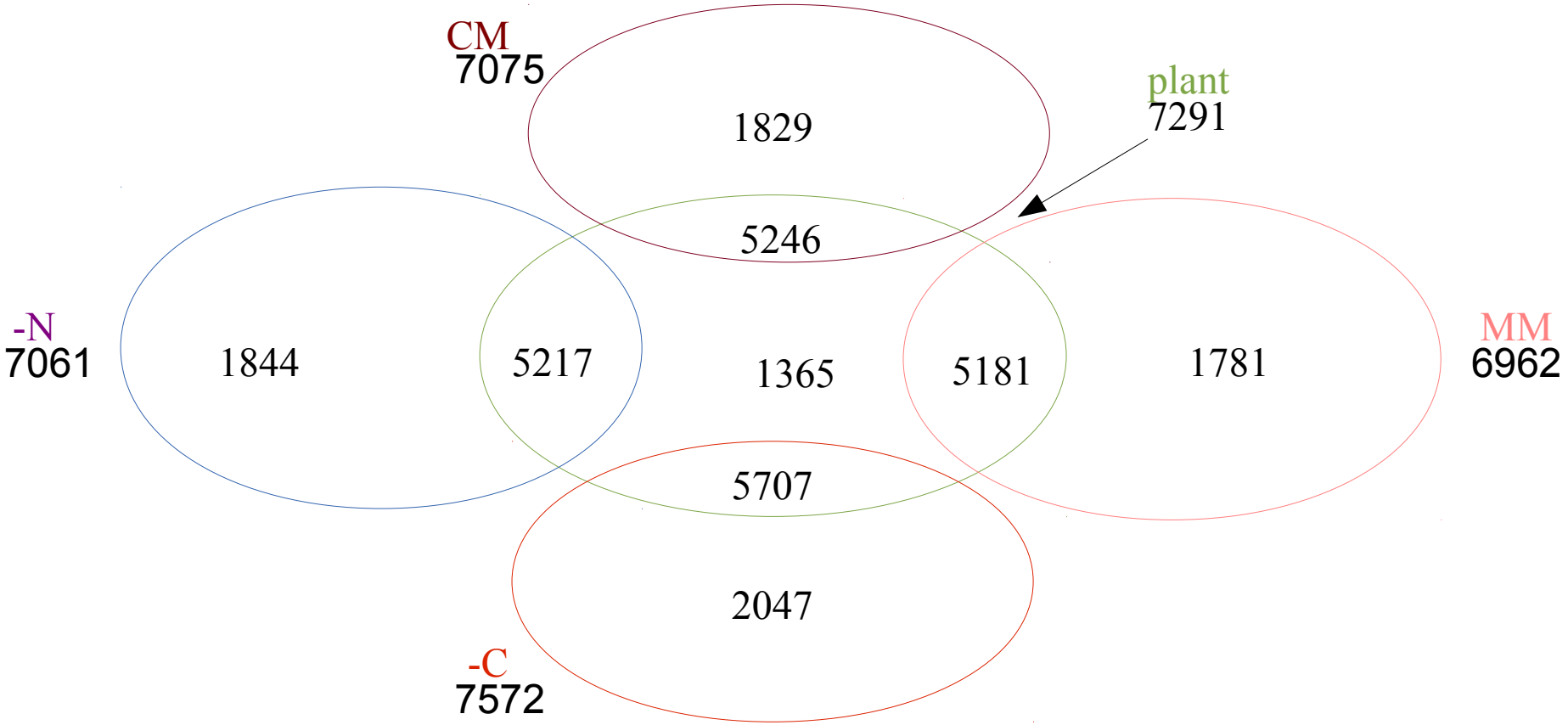
Antisense poly(A) are usually located in the 3'UTR, most of antisense poly(A) are differentially expressed in any condition







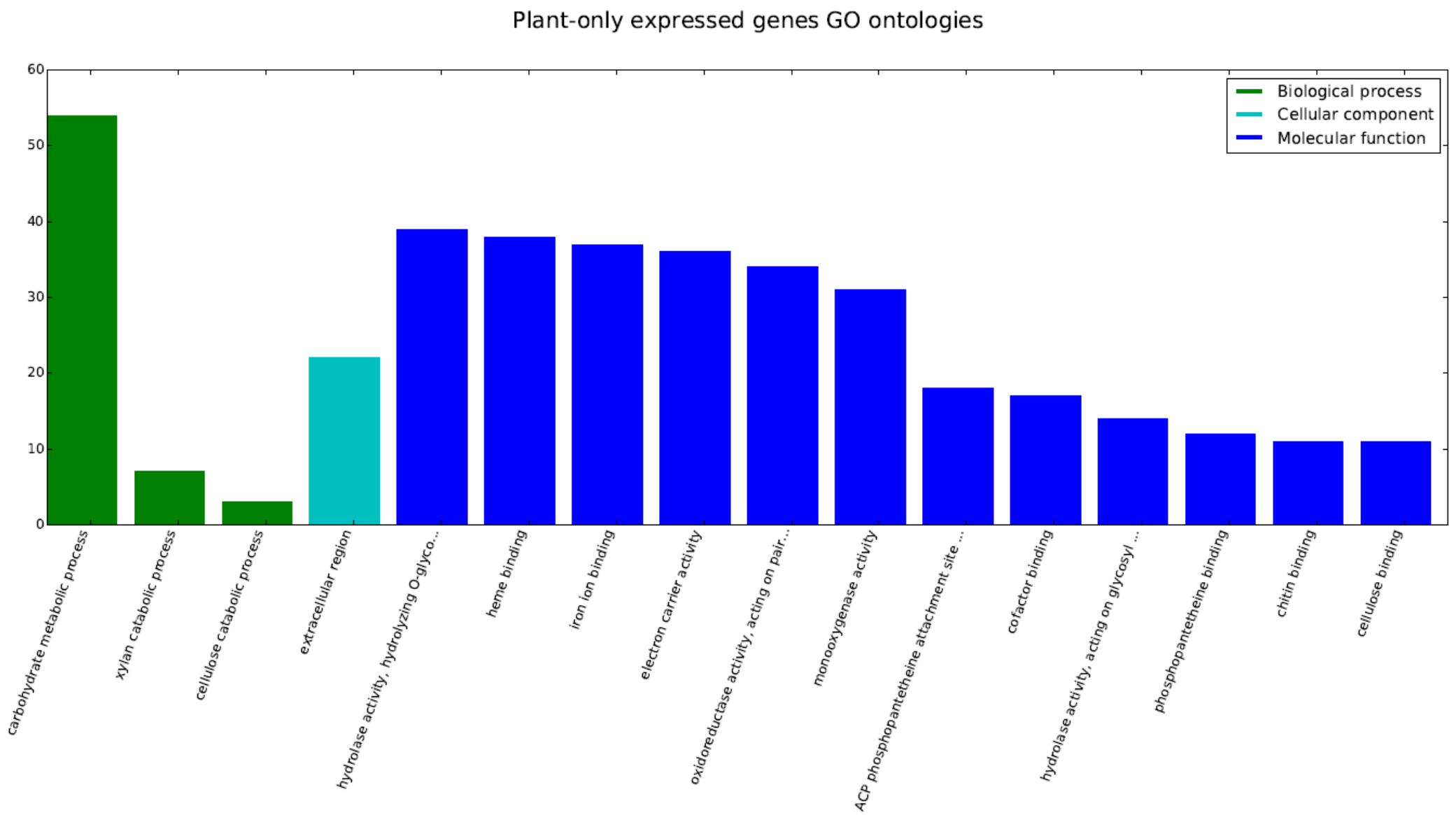
1365 genes expressed in plant are never expressed in vitro



Sage + mosquera = 8171 genes, 7291 still found in current annotation

1365 of these last ones. never found in our experiment

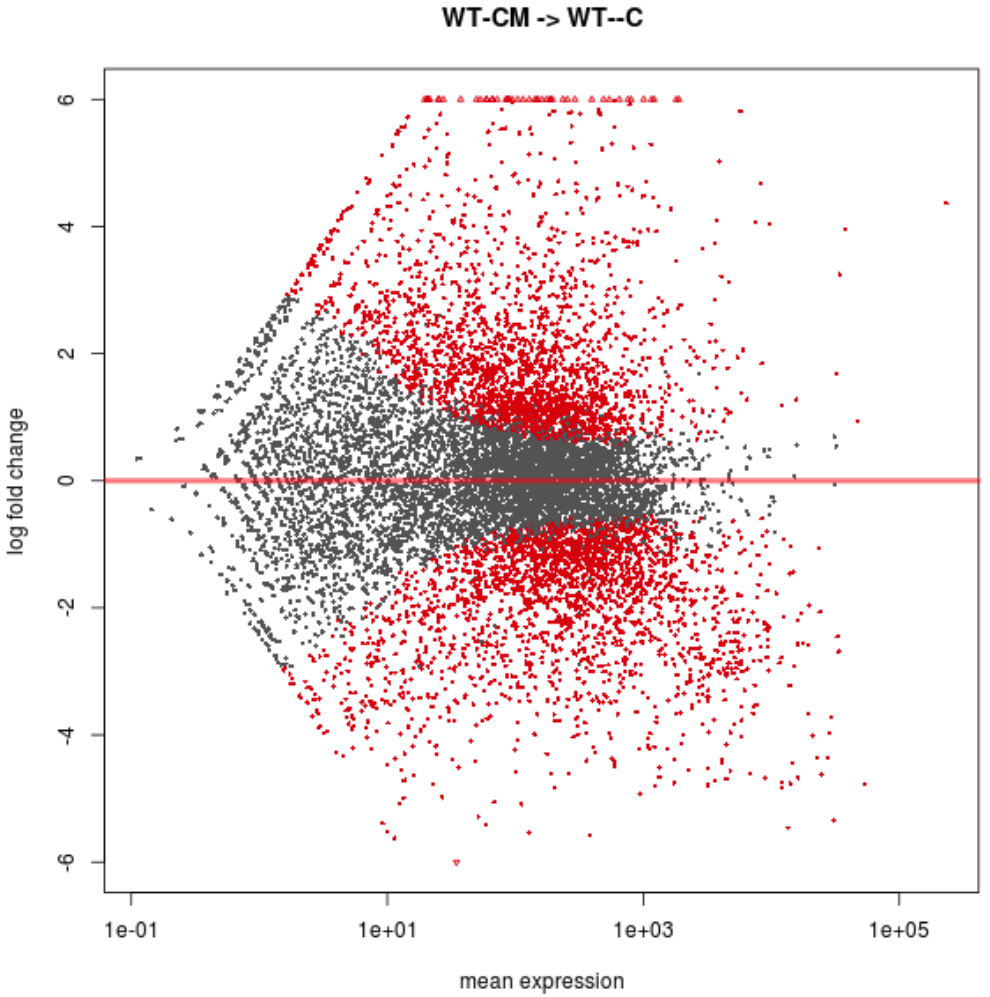
1365 genes expressed in plant are never expressed in vitro

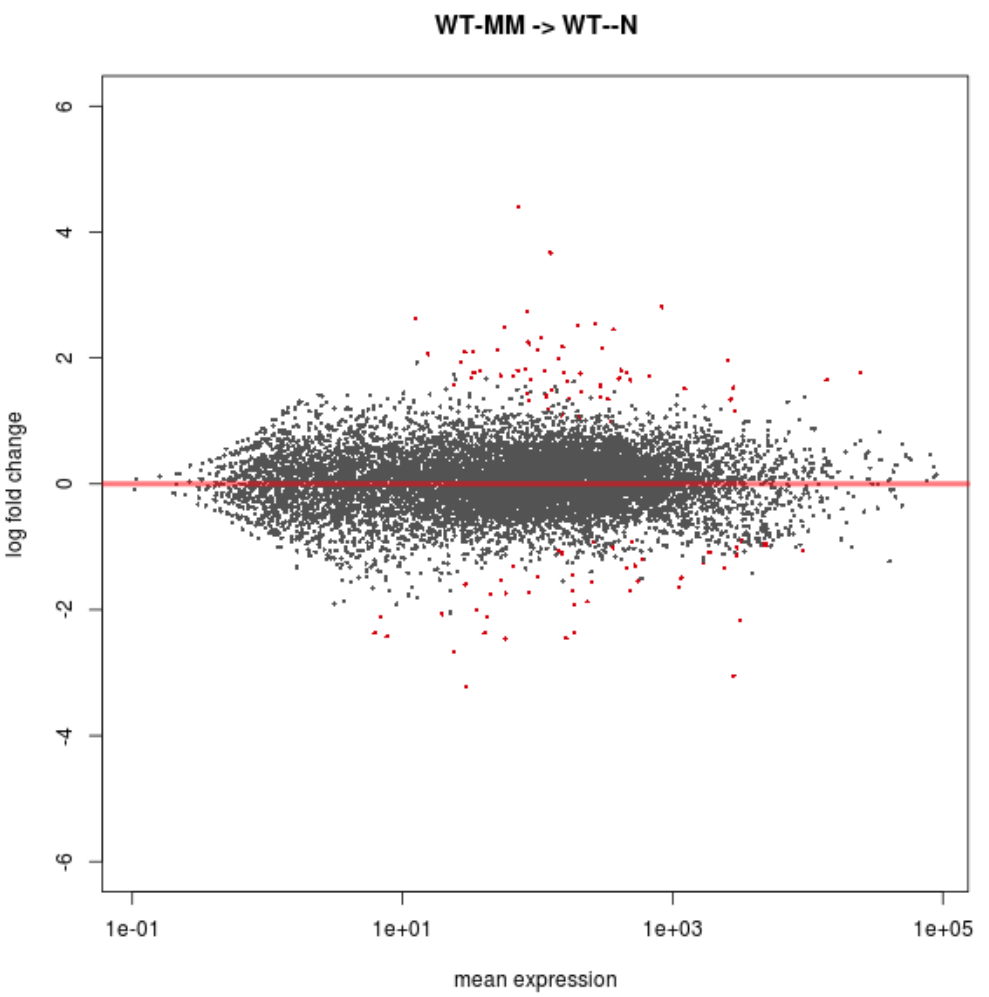


The CM → -C condition presents the highest number of differentially expressed genes, while MM → -N the lowest

DIFFERENTIALLY EXPRESSED GENES IN THE WT			
	DOWN	UP	TOTAL
CM → MM	314	559	*873*
CM → MM-N	630	874	*1504*
CM → MM-C	2307	2342	*4649*
MM → MM-N	48	59	*107*
MM → MM-C	1882	1589	*3471*

The CM → -C condition presents the highest number of differentially expressed genes, while MM → -N the lowest





The CM → -C condition presents the highest number of differentially expressed genes, while MM → -N the lowest

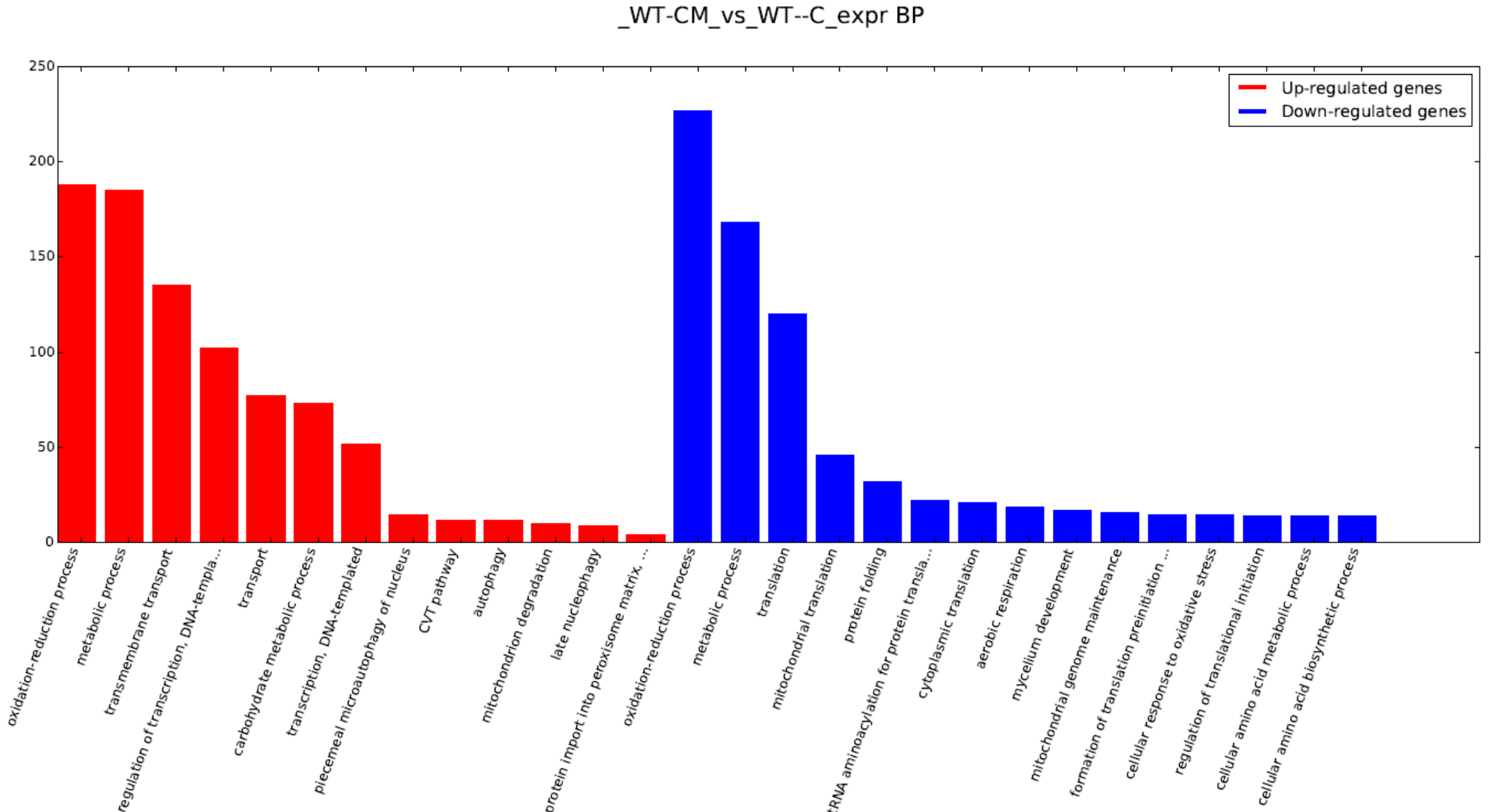
TOP 20 HIGHEST DIFFERENTIALLY EXPRESSED GENES CM → -C

gene	log2foldChange	description
MGG_09072	8.1538523515	Alcohol oxidase
MGG_00244	8.0070871451	15-hydroxyprostaglandin dehydrogenase
MGG_07210	7.9942521054	Putative uncharacterized protein
MGG_09607	7.4595914704	Maltose permease MAL31
MGG_01367	7.2171221376	Putative uncharacterized protein
MGG_07253	7.1375757829	Putative uncharacterized protein
MGG_11289	7.0894568362	Putative uncharacterized protein
MGG_08937	7.0375631939	Quinate permease
MGG_15267	6.9971932885	Putative uncharacterized protein
MGG_03793	6.8988221238	2,3-dihydroxybenzoic acid decarboxylase
MGG_06828	6.872238681	Putative uncharacterized protein
MGG_05941	6.8217314329	Maltose permease MAL31
MGG_02245	6.7008914884	Endoglucanase type F
MGG_00659	6.6866120353	Glucan 1,3-beta-glucosidase
MGG_10663	6.4805171445	cAMP-regulated D2 protein

gene	log2foldChange	description
MGG_17996	-5.6066608795	no_description
MGG_07973	-5.3026451979	Surface protein 1
MGG_08019	-5.156698481	F-box domain-containing protein
MGG_06234	-5.0149833139	Putative uncharacterized protein
MGG_04258	-4.9786465581	Putative uncharacterized protein
MGG_01952	-4.8319947186	Putative uncharacterized protein
MGG_17706	-4.7704849827	Putative uncharacterized protein
MGG_10456	-4.7234757359	Putative uncharacterized protein
MGG_09015	-4.700976561	Putative uncharacterized protein
MGG_17103	-4.6464513062	Putative uncharacterized protein
MGG_08360	-4.6275010826	Putative uncharacterized protein
MGG_17677	-4.5723814478	Putative uncharacterized protein
MGG_11608	-4.5509909646	Laccase-2
MGG_05344	-4.5356873581	SnodProt1
MGG_07966	-4.5297712533	Phosphate transporter

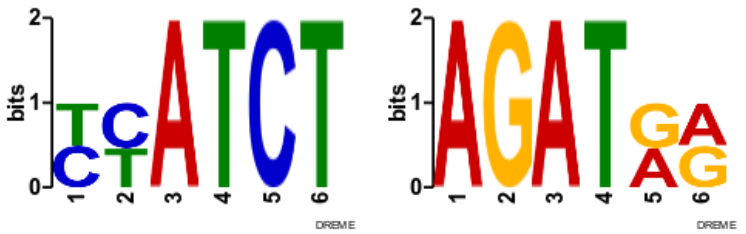
The CM → -C condition presents the highest number of differentially expressed genes, while MM → -N the lowest

AFFECTED GO ONTOLOGIES CM → -C



Up-regulated genes in nitrogen starvation show the typical GATA motif in the promoter region

1. YYATCT



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Details

Positives	Negatives	P-value	E-value	Unerased E-value
463/955	234/700	5.1e-10	0.0000028	0.0000028

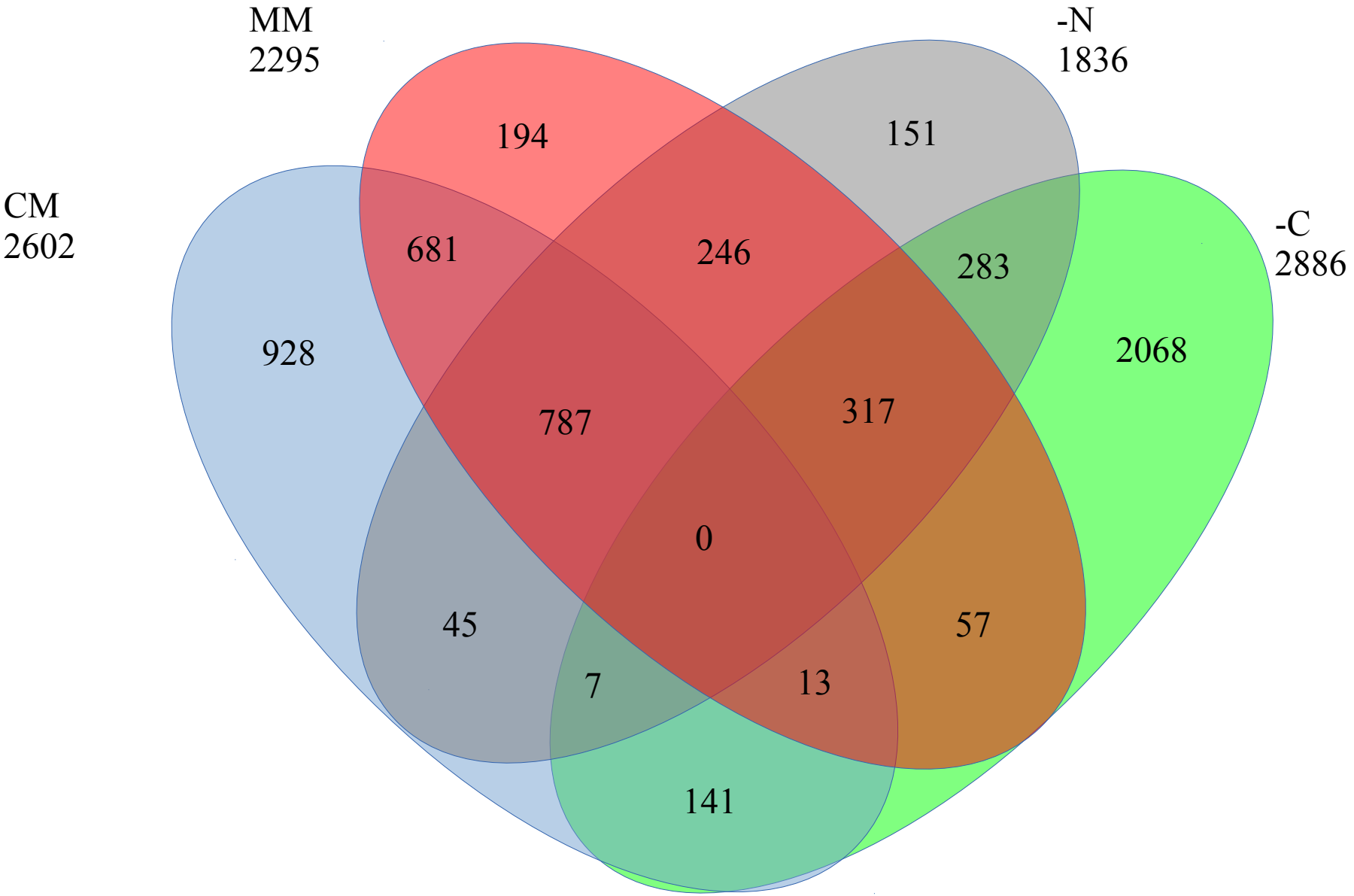
Enriched Matching Words

Word	Positives	Negatives	P-value	▼E-value
CTATCT	122/955	49/700	7.3e-5	4.1e-1
TTATCT	150/955	67/700	1.4e-4	8.0e-1
TCATCT	146/955	74/700	3.1e-3	1.7e+1
CCATCT	163/955	88/700	6.8e-3	3.8e+1

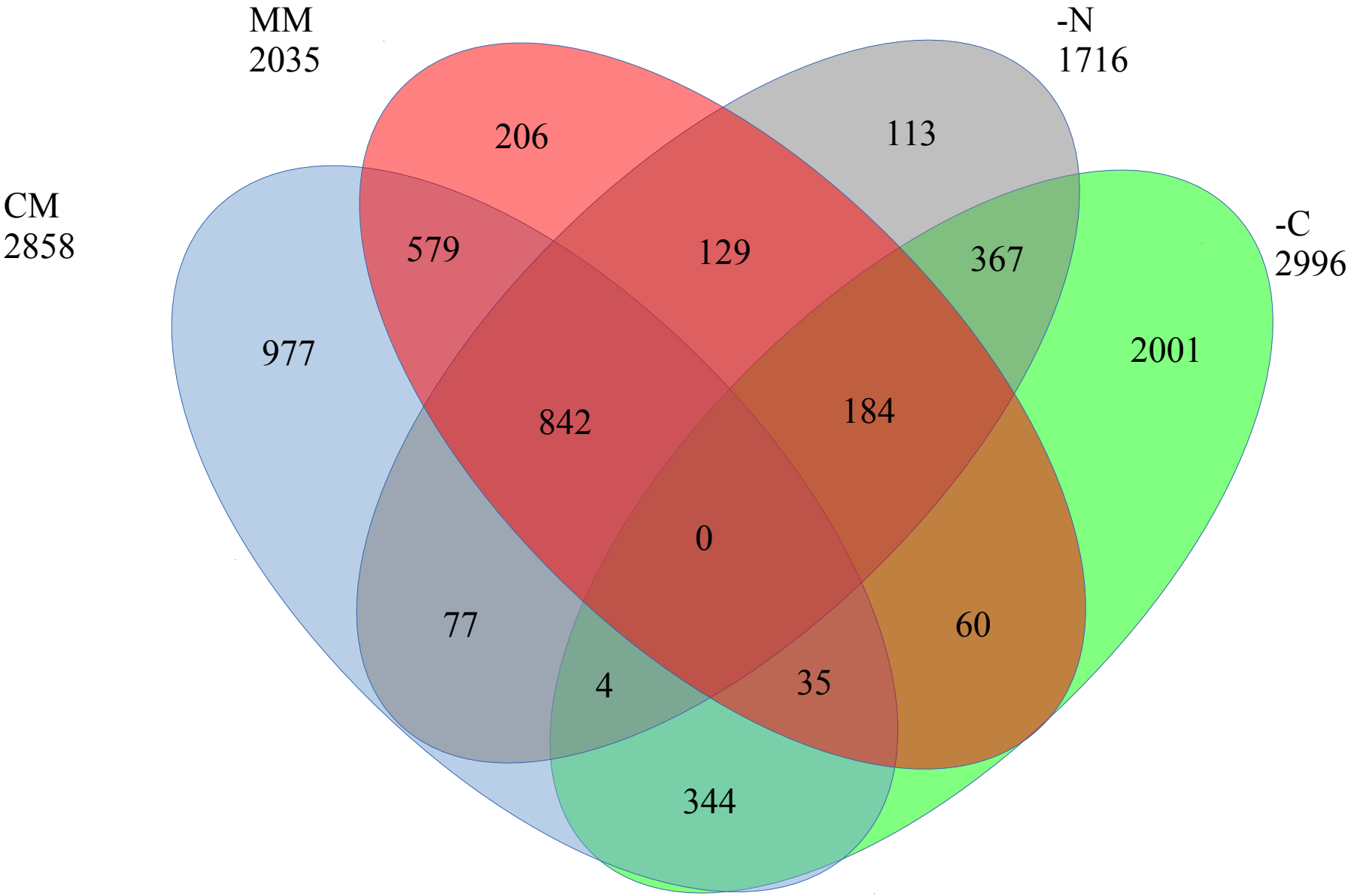
- *None of the known GATA-binding transcription factors is found to be significantly up-regulated in our 12h NS experiment*
- *NUT1, the important nitrogen-related TF is found to be generally down-regulated in Carbon-starvation*
- *30 of the 51 top up-regulated genes listed in www.ncbi.nlm.nih.gov/pubmed/16731015 are confirmed in our experiment*

- *Only two Transcription factors, MGG_05829 and MGG_01486, probably related with purine Regulation, are found to be up-regulated in MM → -N, MGG_05829 is down-regulated in the mutant*

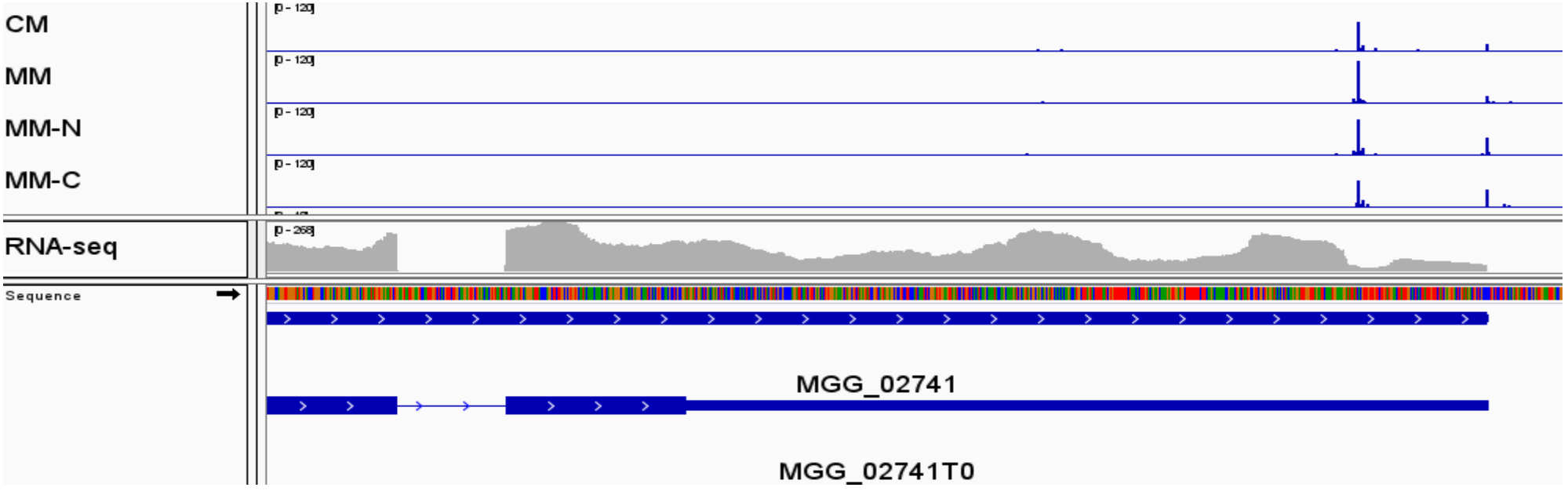
Up-regulated genes between conditions

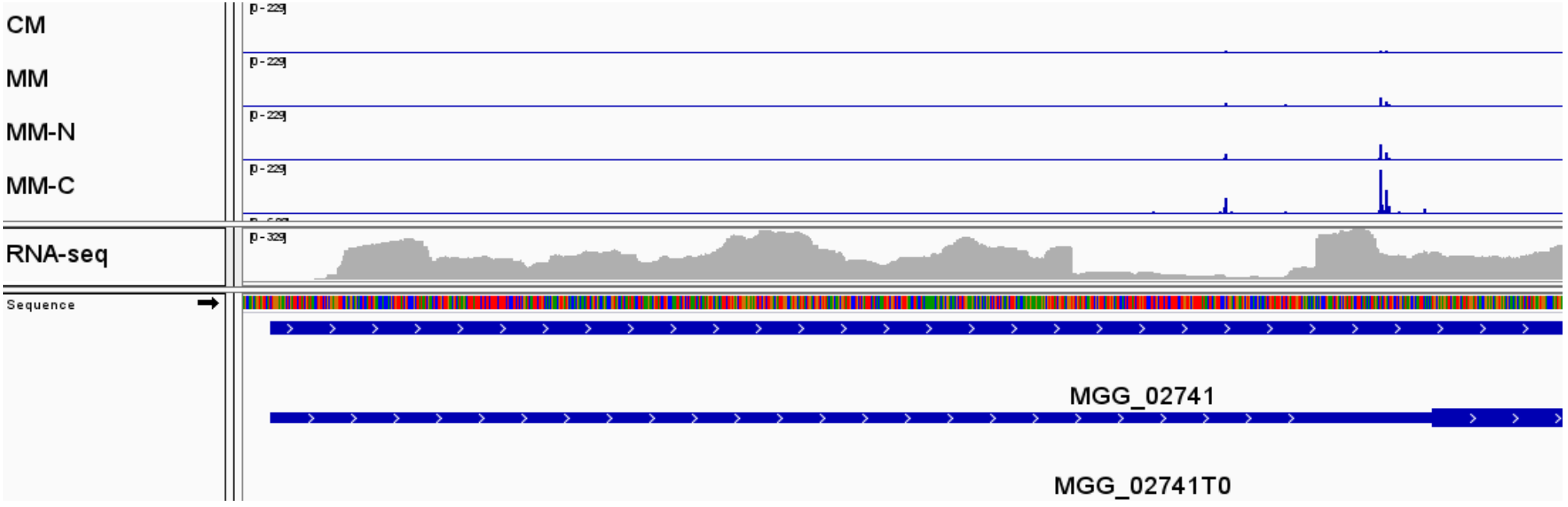


Down-regulated genes between conditions

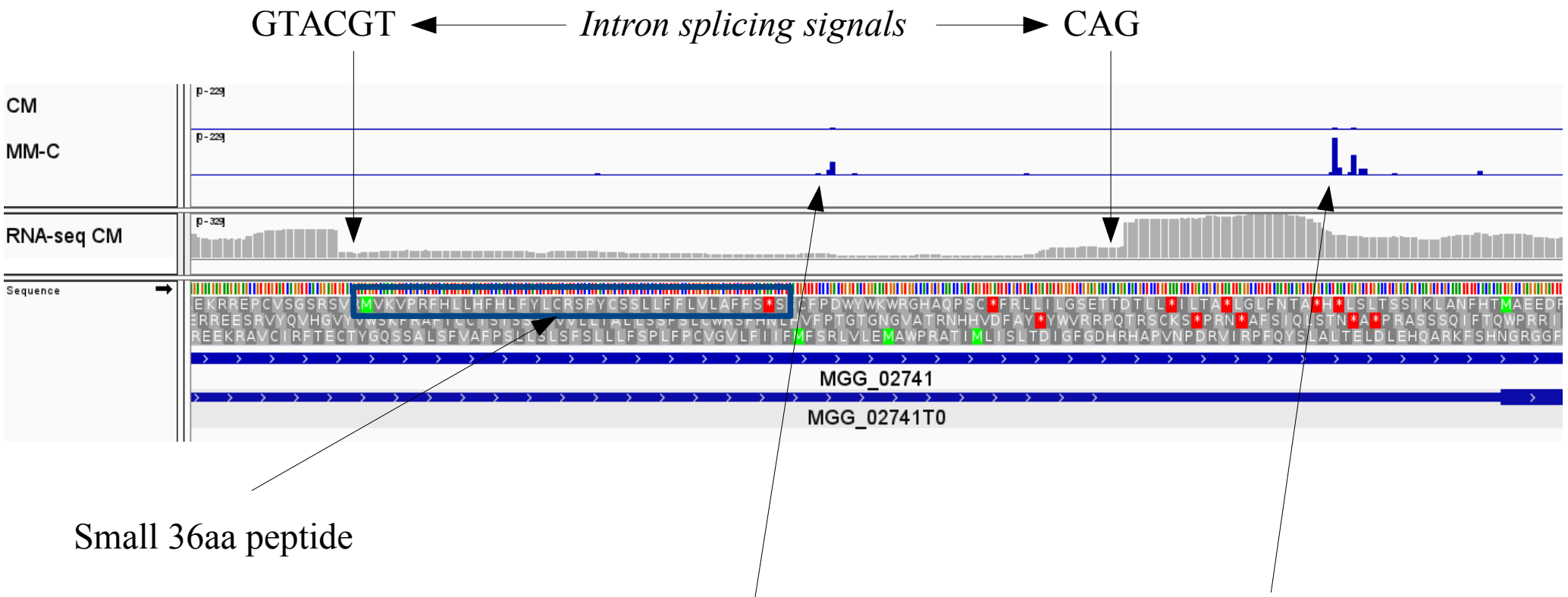


RBP35 shows different polyadenilation in each medium, with strong differencies in MM-C





RBP35 shows an alternative polyadenylated 5'UTR, putatively enconding a small peptide

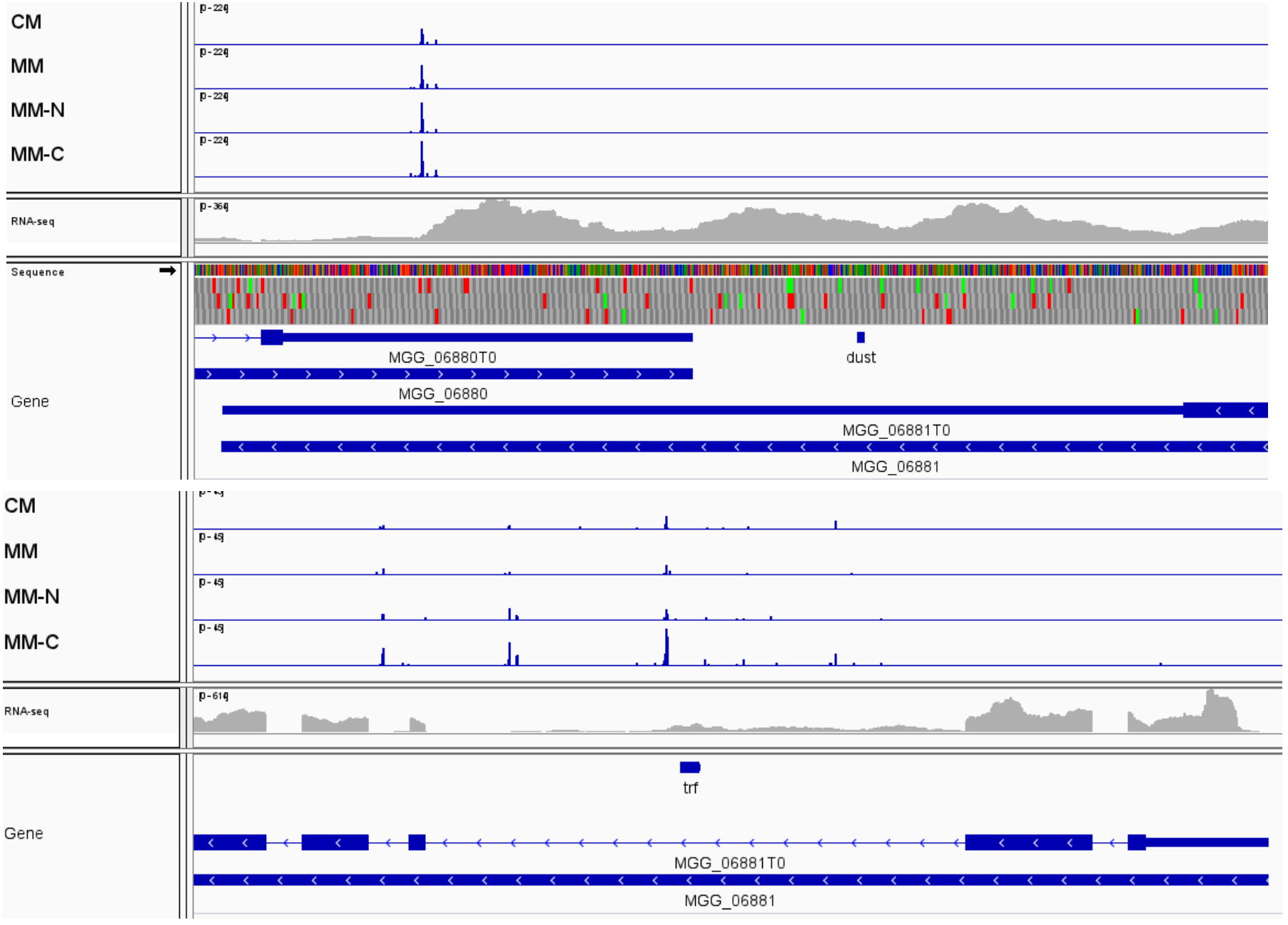


Small 36aa peptide

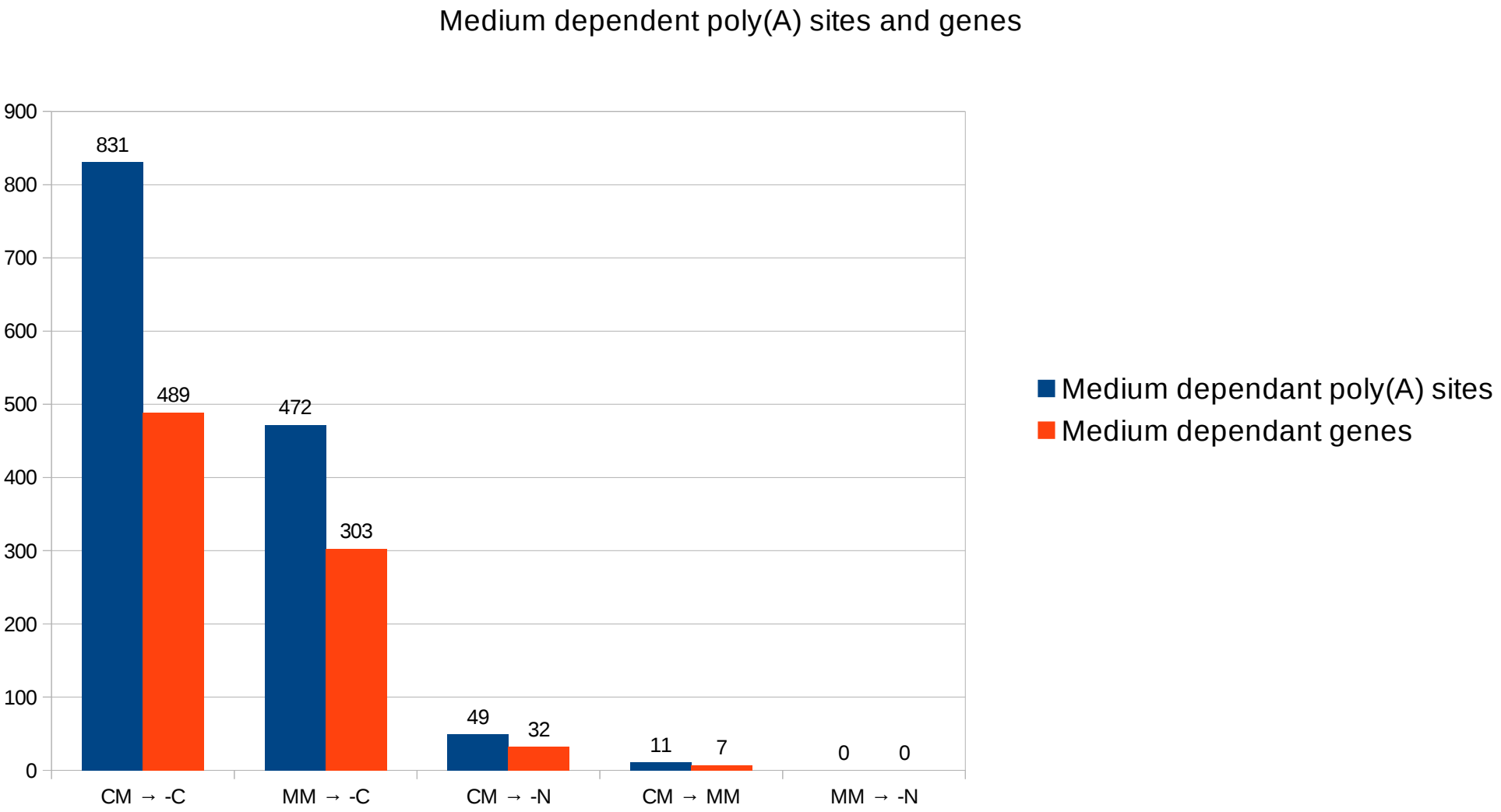
Poly(A) site used in absense of splicing, when the small petide **is** transcribed

Poly(A) site used after splicing, when The small petide **is not** transcribed
Up-regulated poly(A) site in MM-C

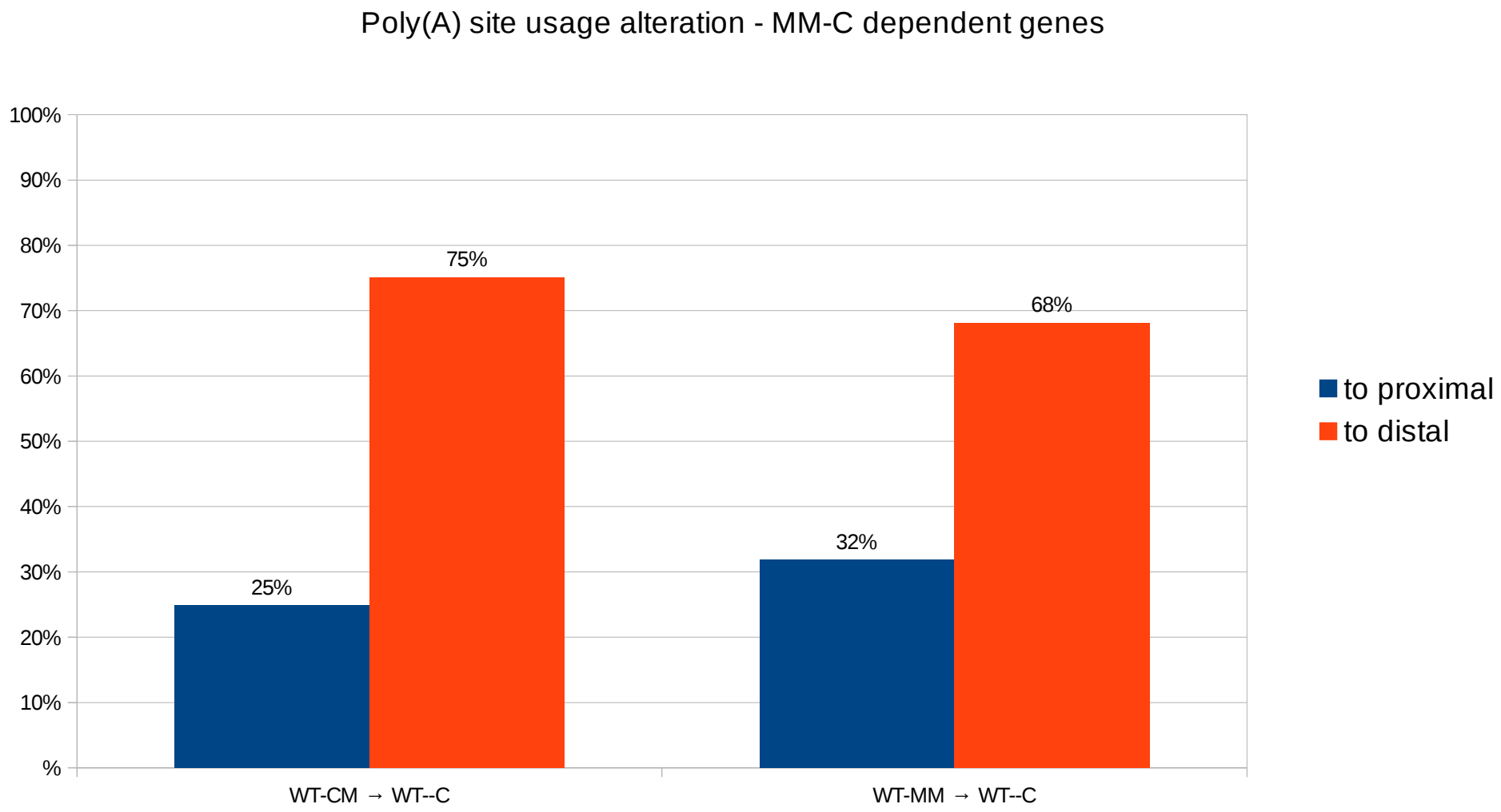
HRP1 shows an up-regulated intronic poly(A) site in MM-C



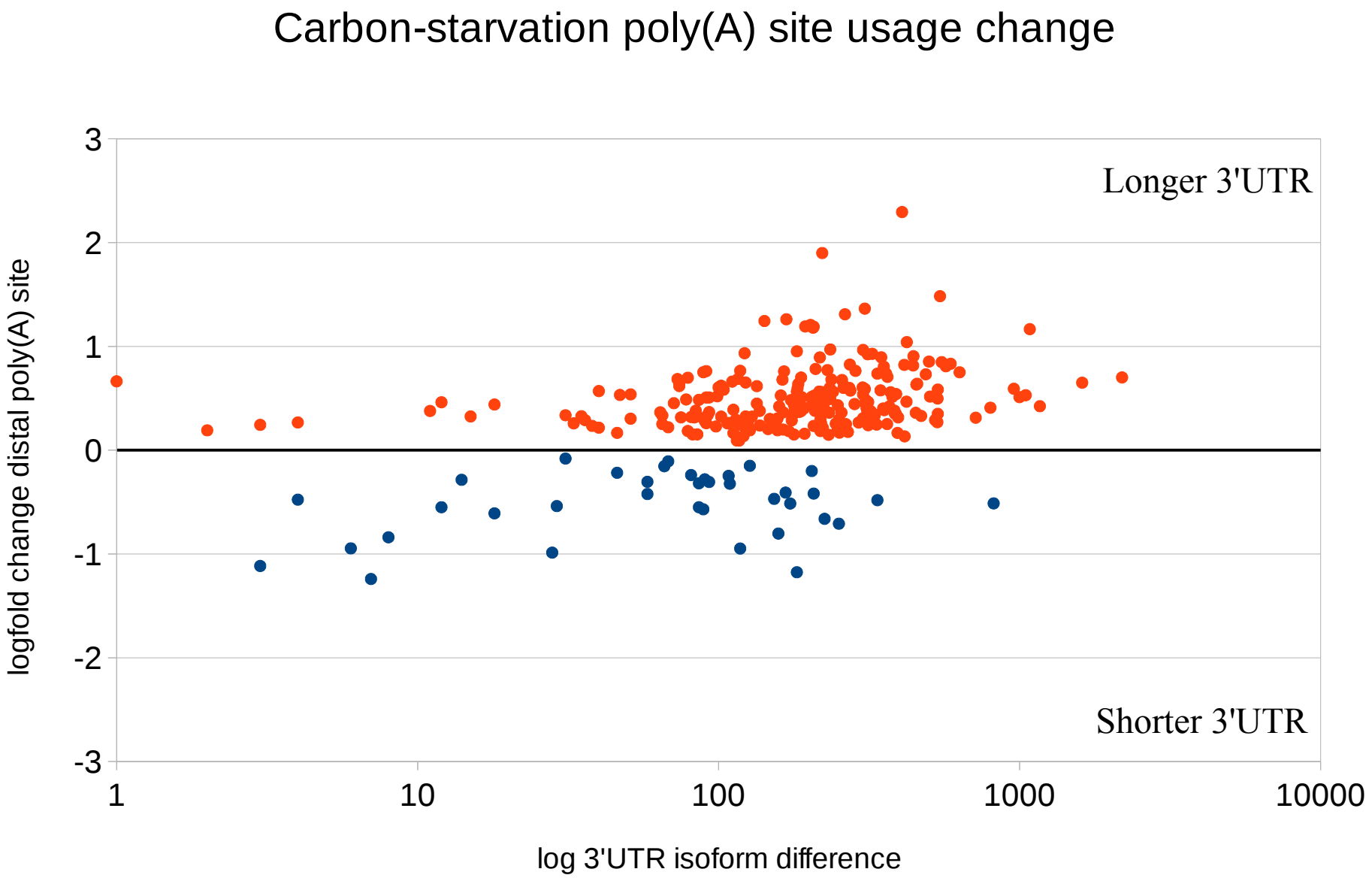
MM-C affects a great number of poly(A) sites



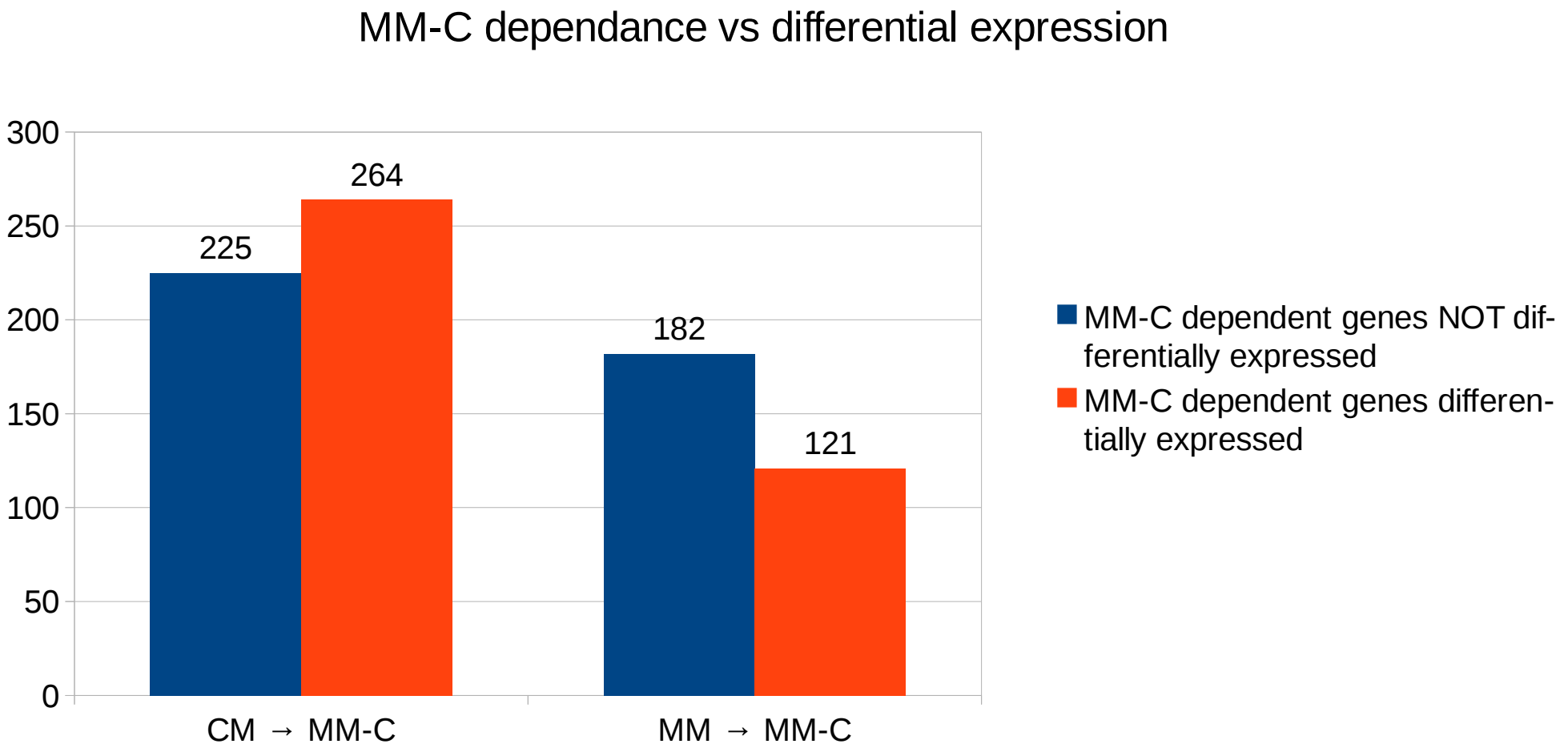
MM-C affects poly(A) sites usage, preferring distal cuts - percentages



MM-C affects poly(A) sites usage, preferring distal cuts - foldChange

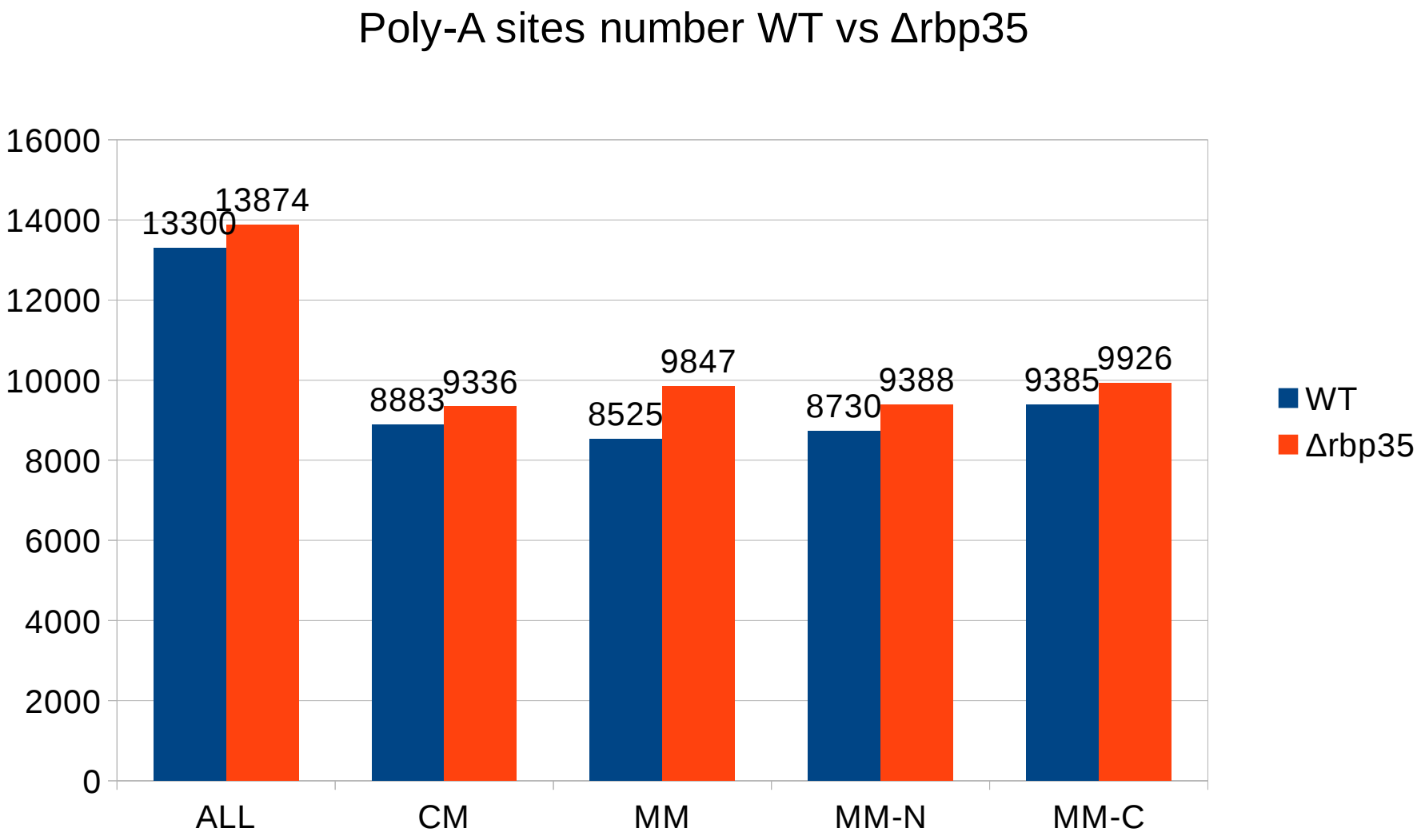


MM-C dependent genes are usually differentially expressed

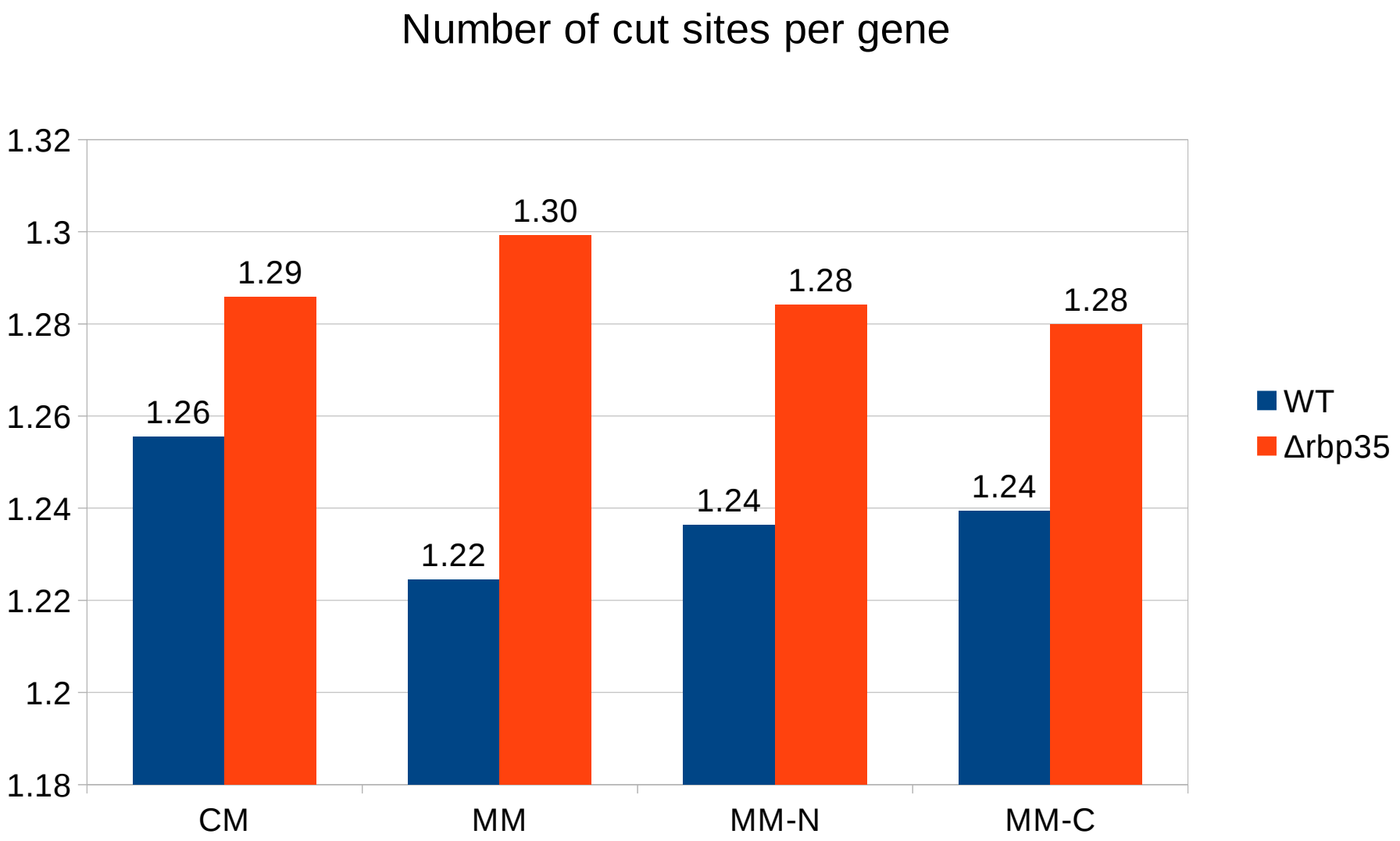


Differentially expressed gene are equally distributed between up & down regulated (data not shown)

Δrbp35 affects poly(A) sites number



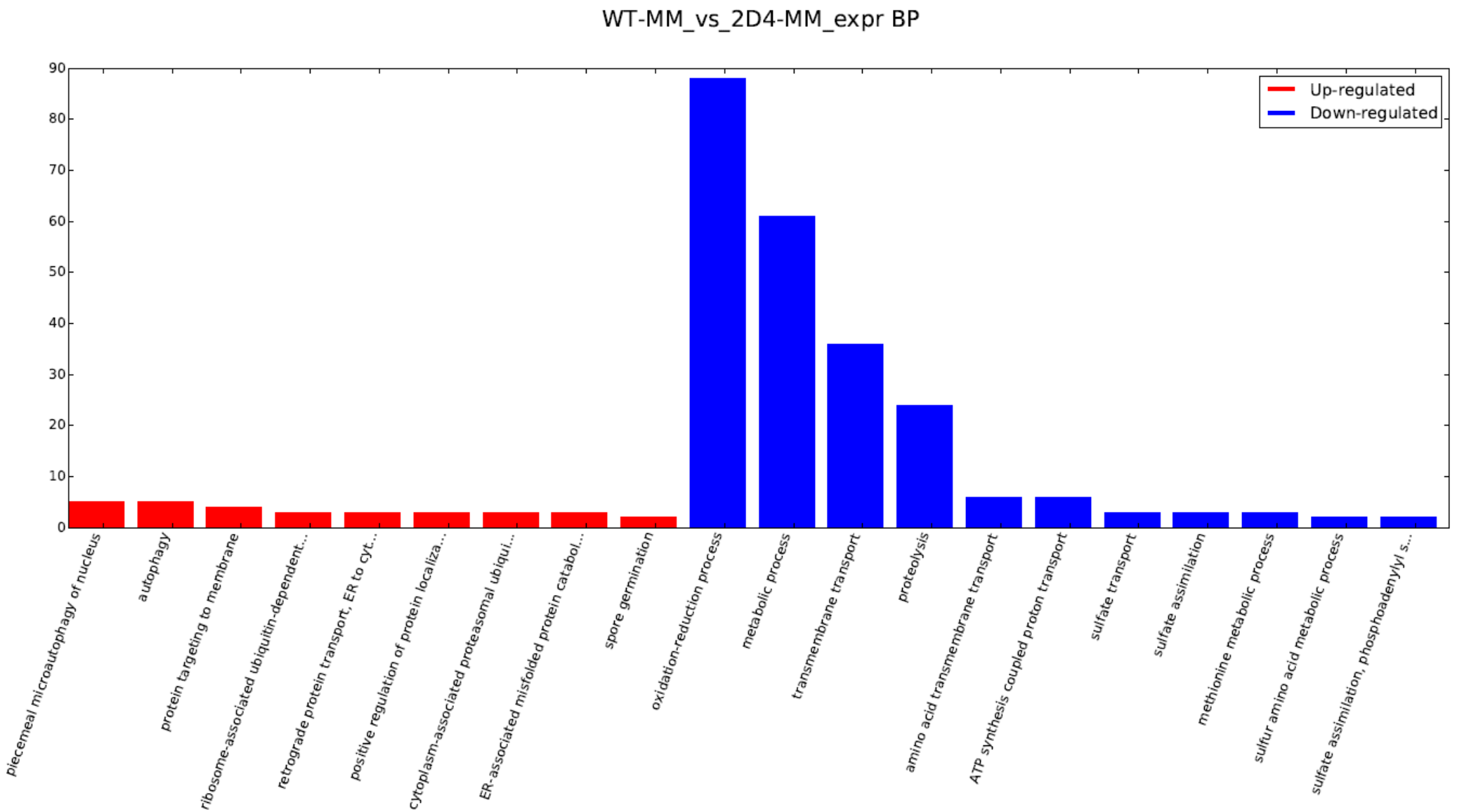
Δrbp35 affects number of cut sites per gene



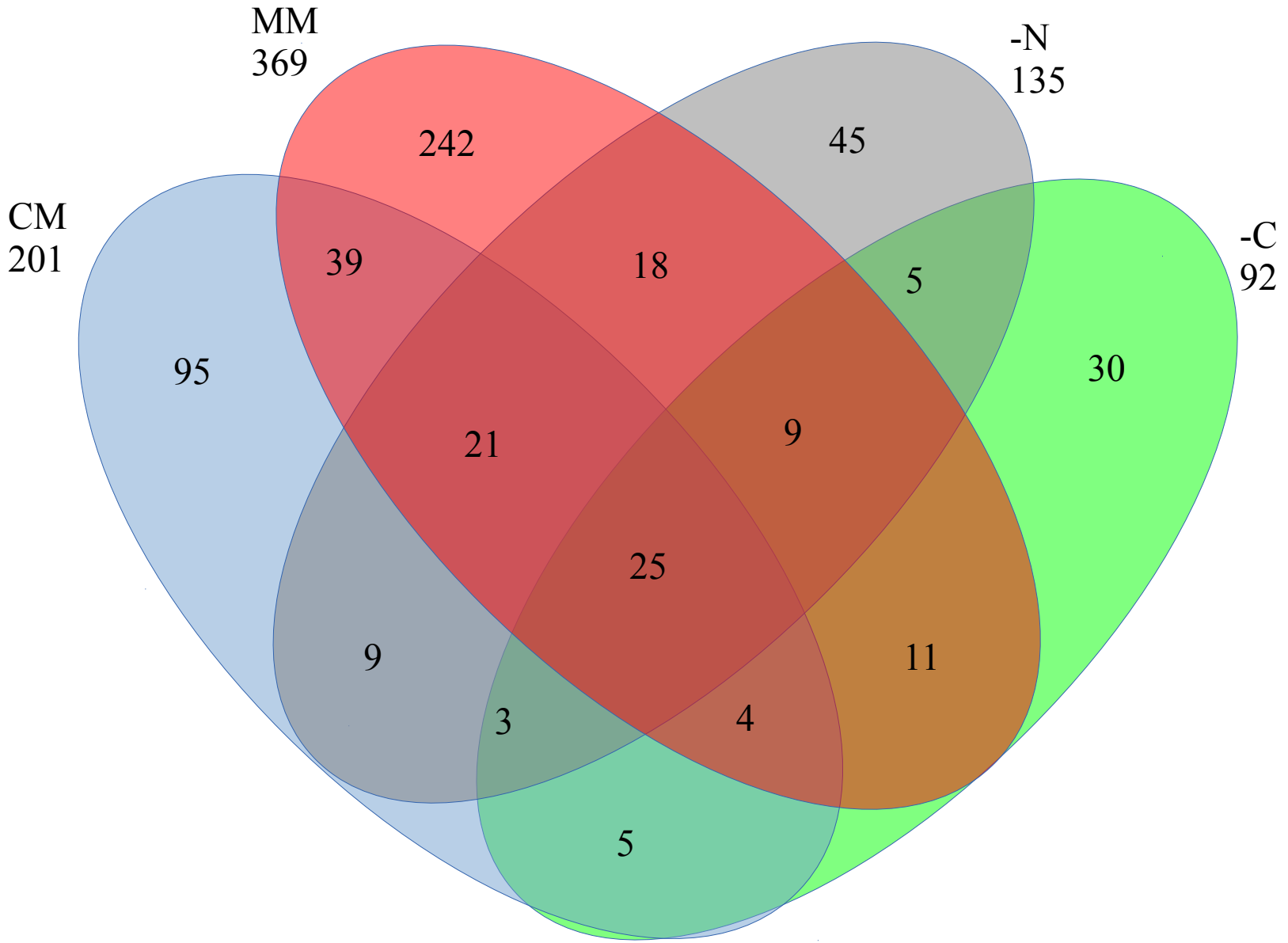
MM is the most affected condition in *Δrbp35,* MM-C the least affected

	down regulated genes	*up regulated gene*	*total*
WT → *Δrbp35* CM	224	201	425
WT → *Δrbp35* MM	529	369	898
WT→ *Δrbp35* MM-N	272	135	407
WT→ *Δrbp35* MM-C	136	92	228

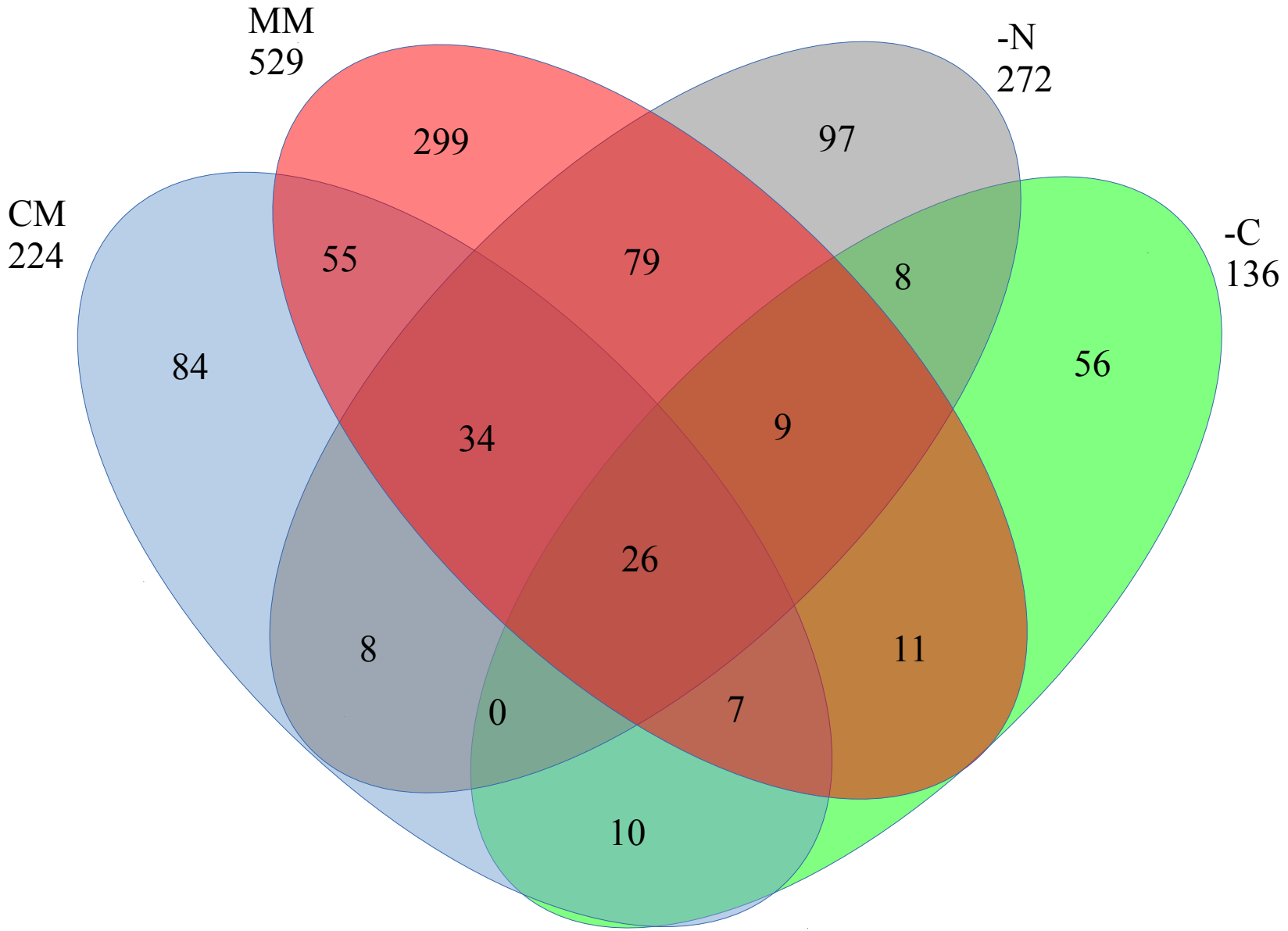
MM is the most affected condition in Δ*rbp35*, MM-C the least affected



Up-regulated genes WT → Δ*rbp35*



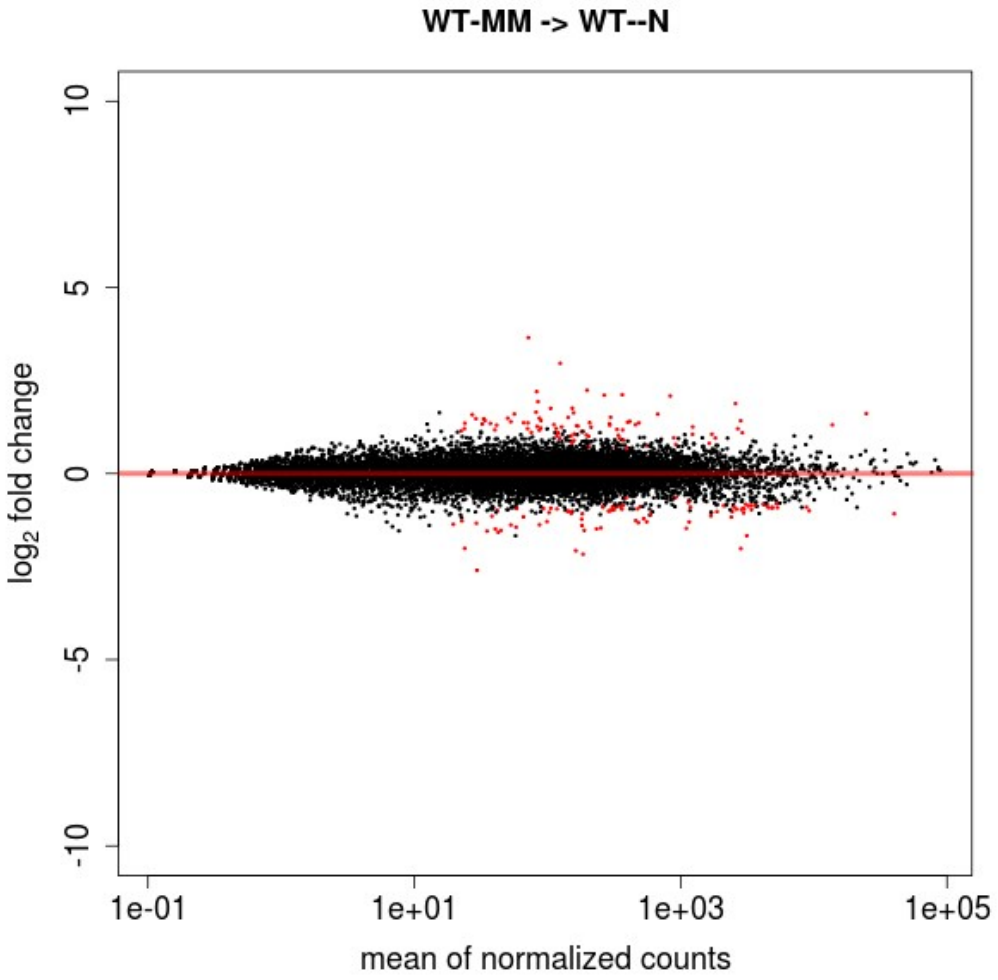
Down-regulated genes WT → Δ*rbp35*

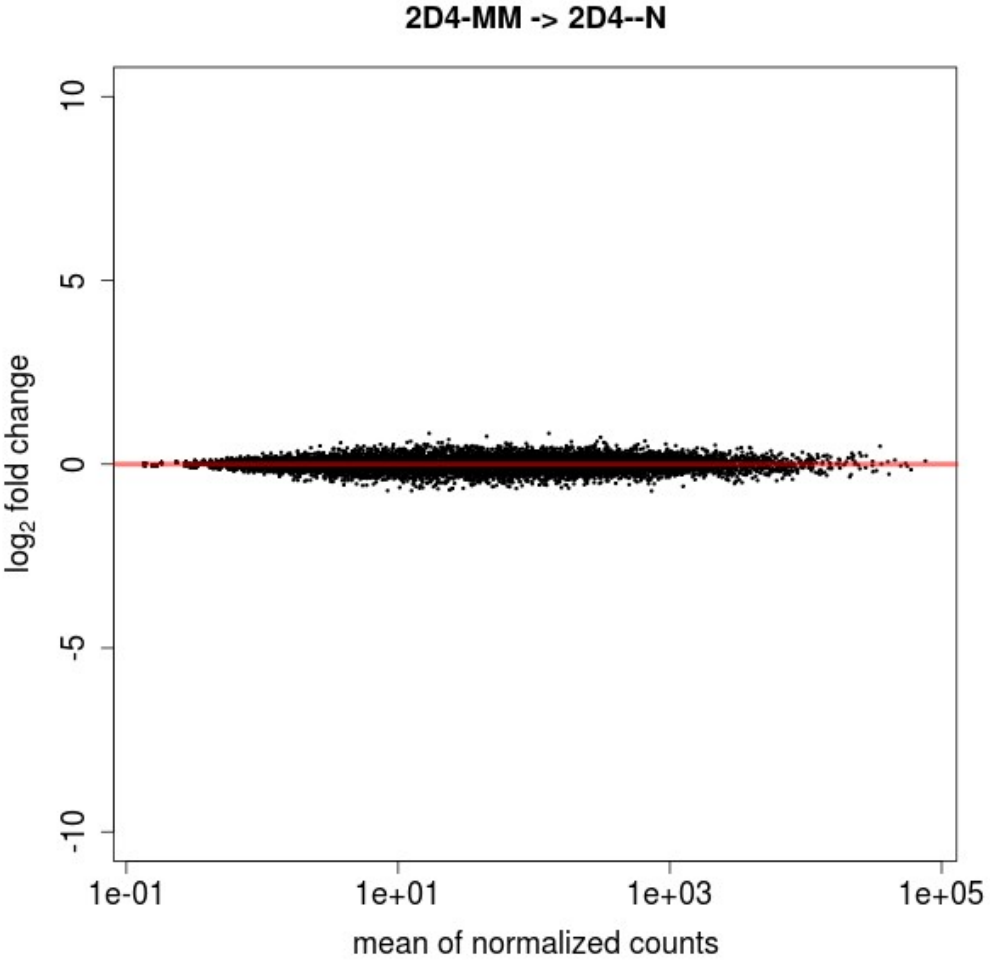


Δrbp35 appears to inhibit medium recognition in MM-N

DIFFERENTIALLY EXPRESSED GENES IN Δrbp35			
	DOWN	UP	TOTAL
CM → MM	508	405	913
CM → MM-N	461	404	865
CM → MM-C	1241	1136	2377
MM → MM-N	0	0	0
MM → MM-C	475	493	968

Δrbp35 appears to inhibit medium recognition in MM-N





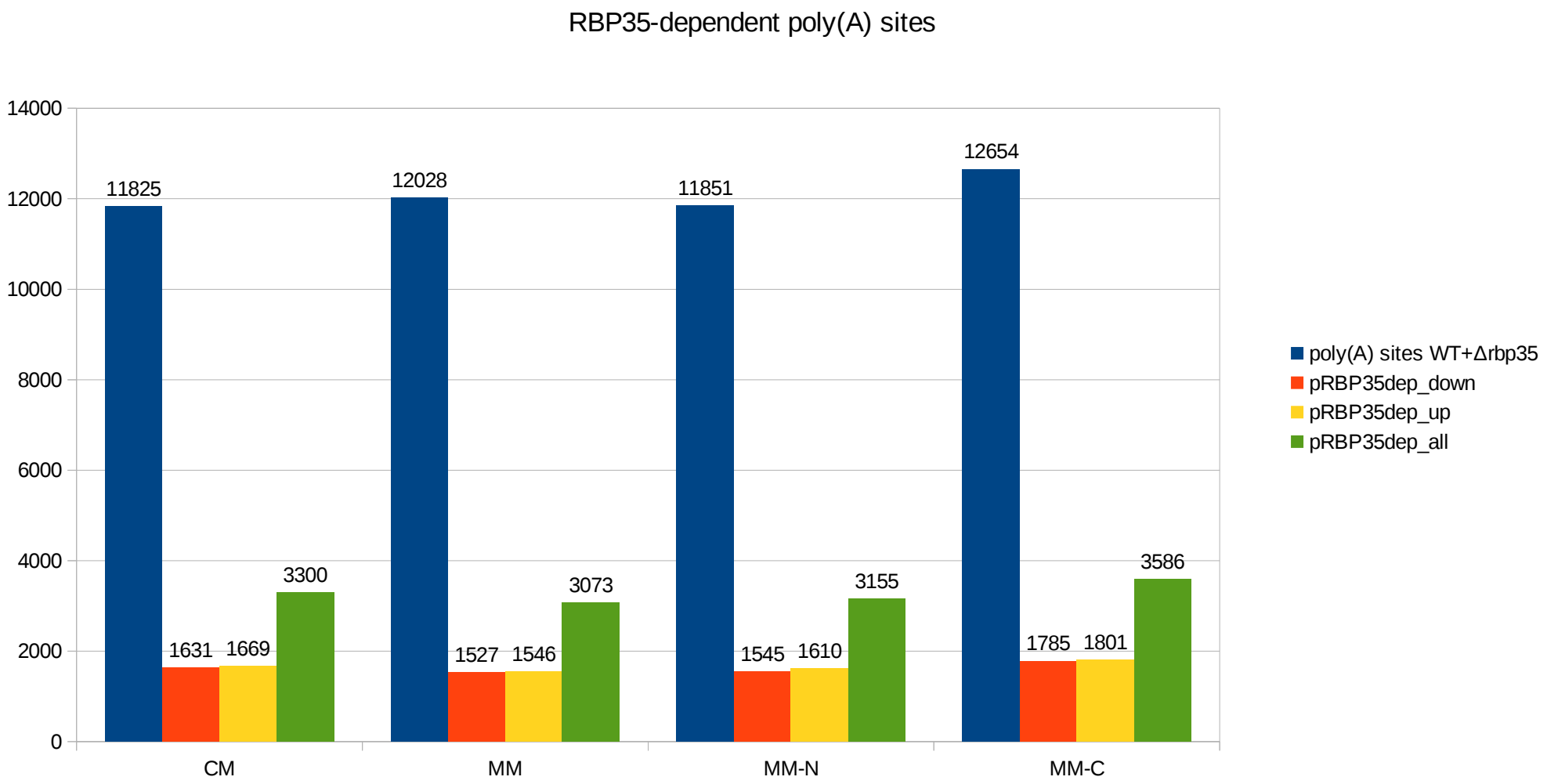
Terminology

- **pRBP35dep:** poly(A) sites that show a differential expression between wild-type and Δrbp35. We call it "RBP35 dependent poly(A) sites"
- **pRBP35dep_down**: a down-regulated RBP35 dependent poly(A):

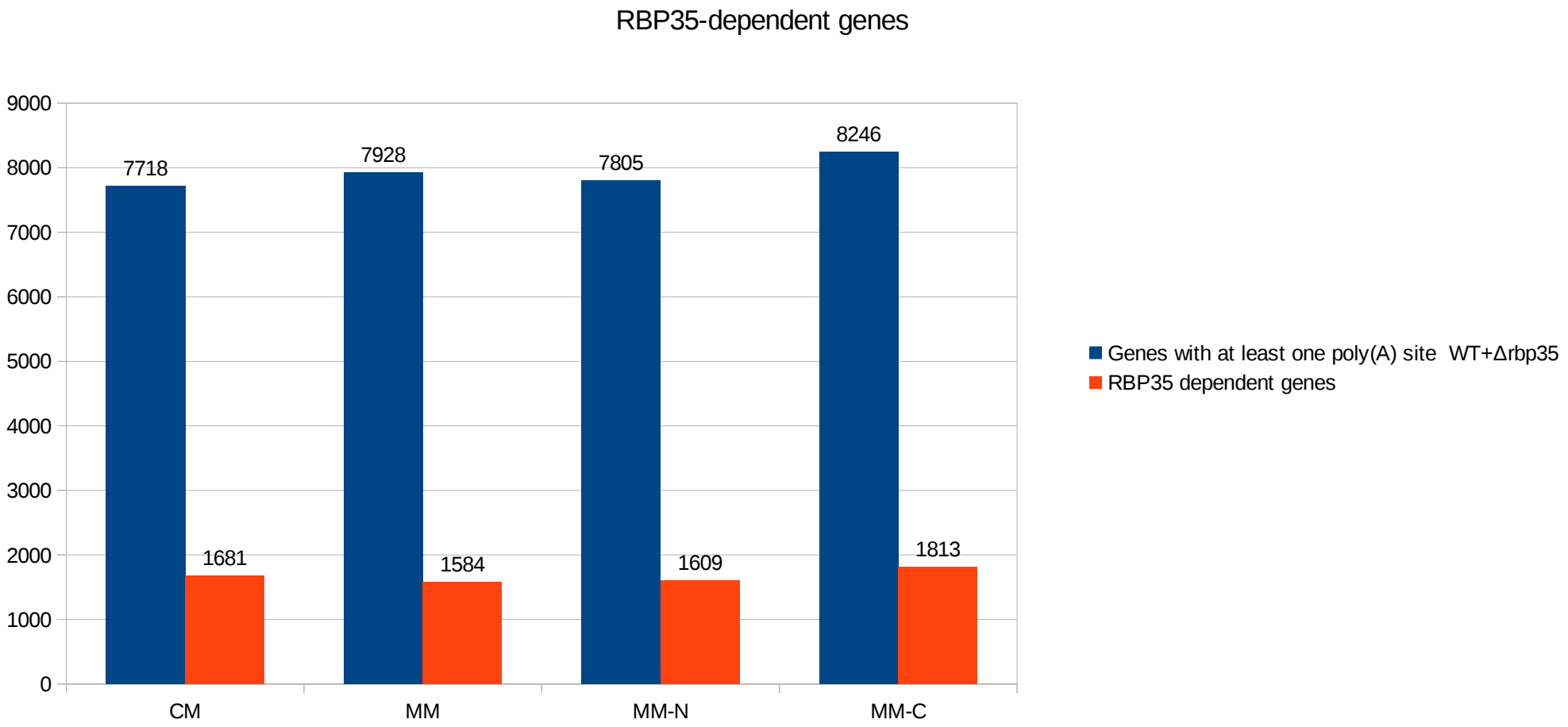
- **pRBP35dep_up**: an up-regulated RBP35 dependent poly(A):

- A gene is defined "RBP35 dependent gene" (or simply **RBP35dep**) when one or more of its poly(A) belong to the previous groups

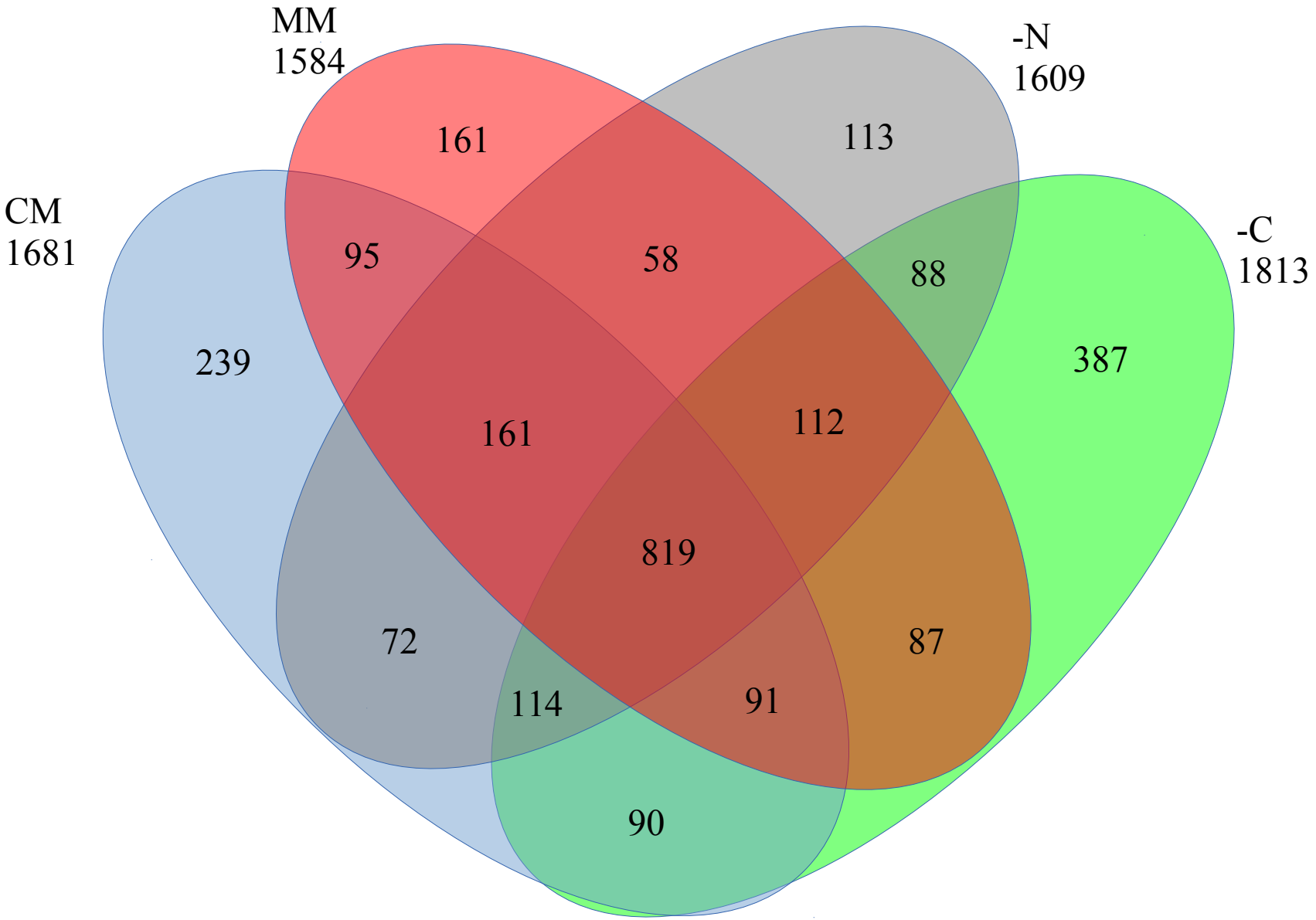
25%-28% of poly(A) sites are dependent from RBP35 in all media



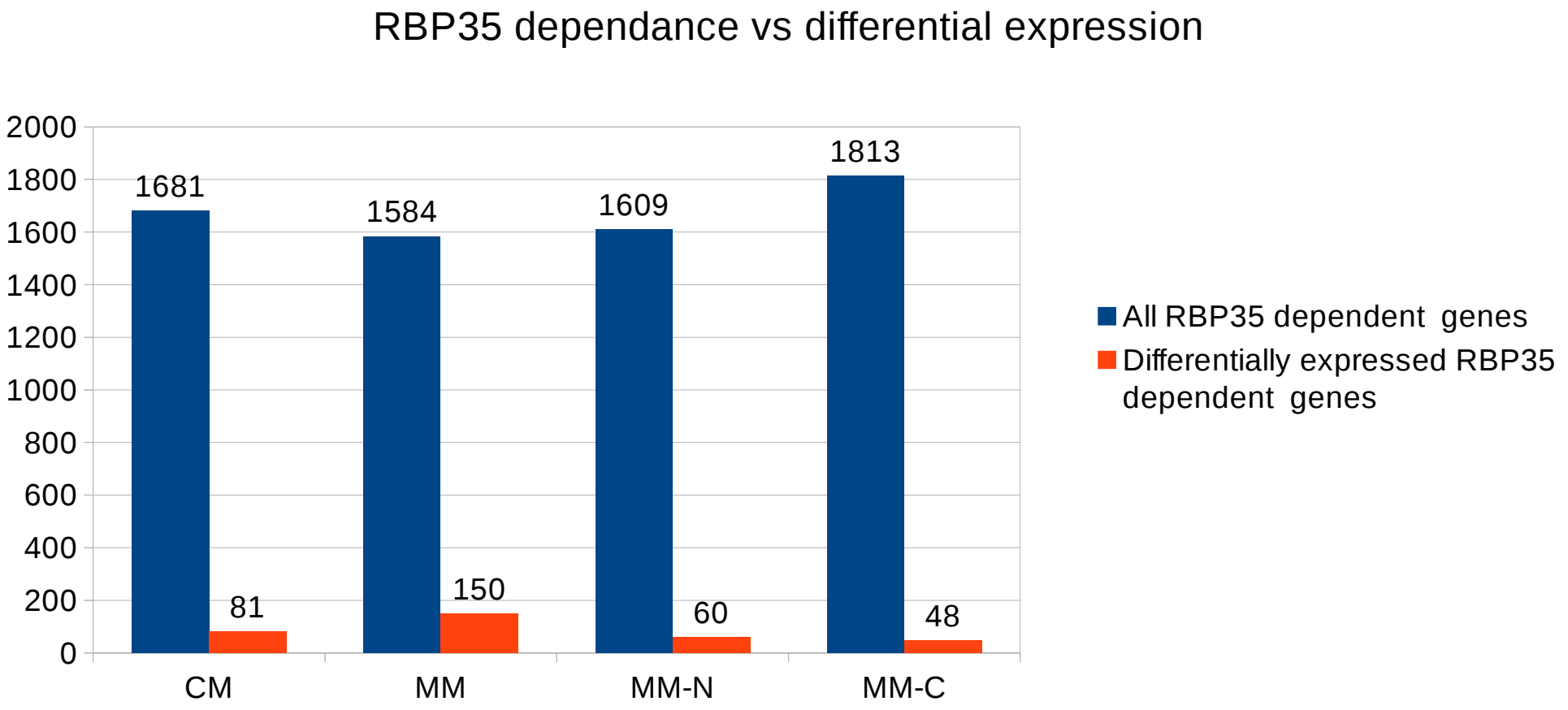
~20% of genes are dependent from RBP35 in all media



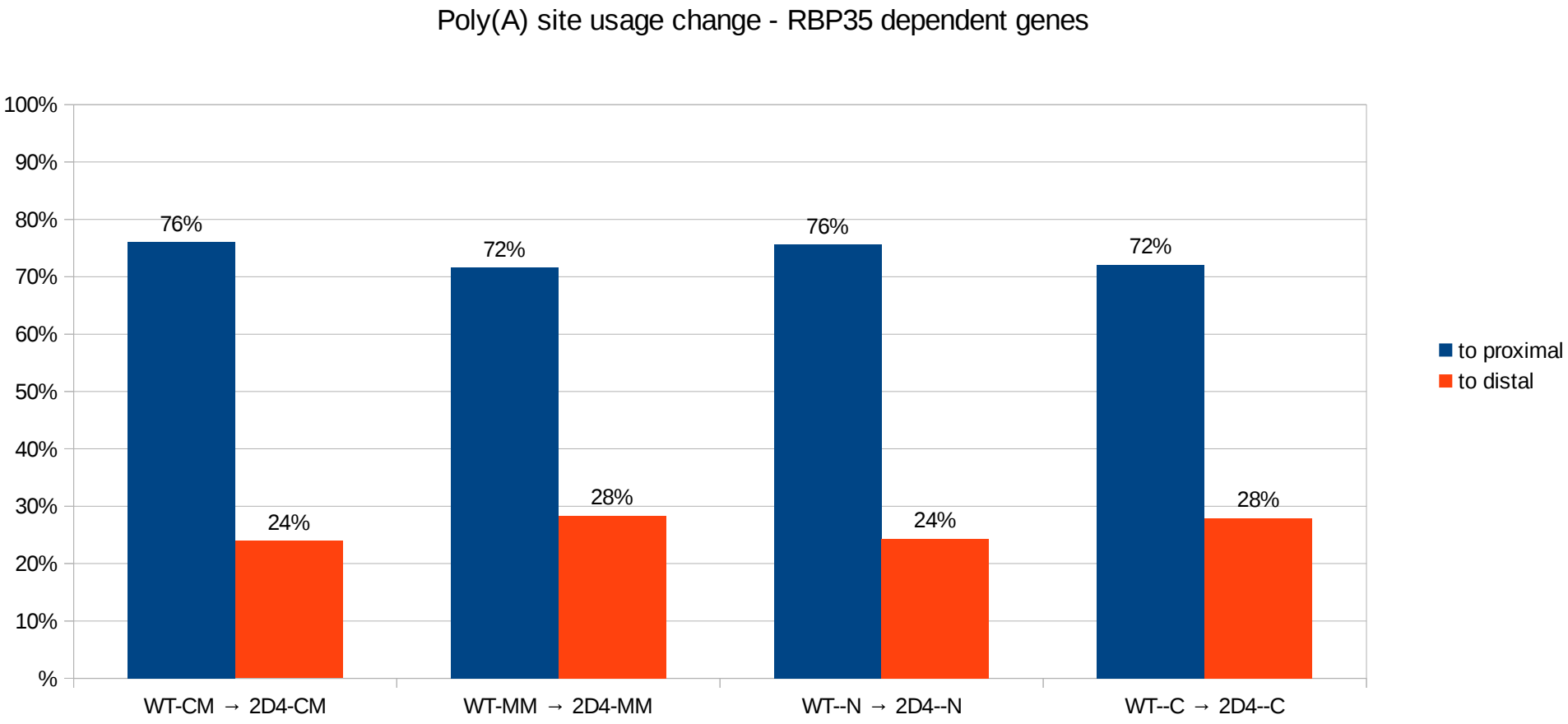
RBP35 dependant genes



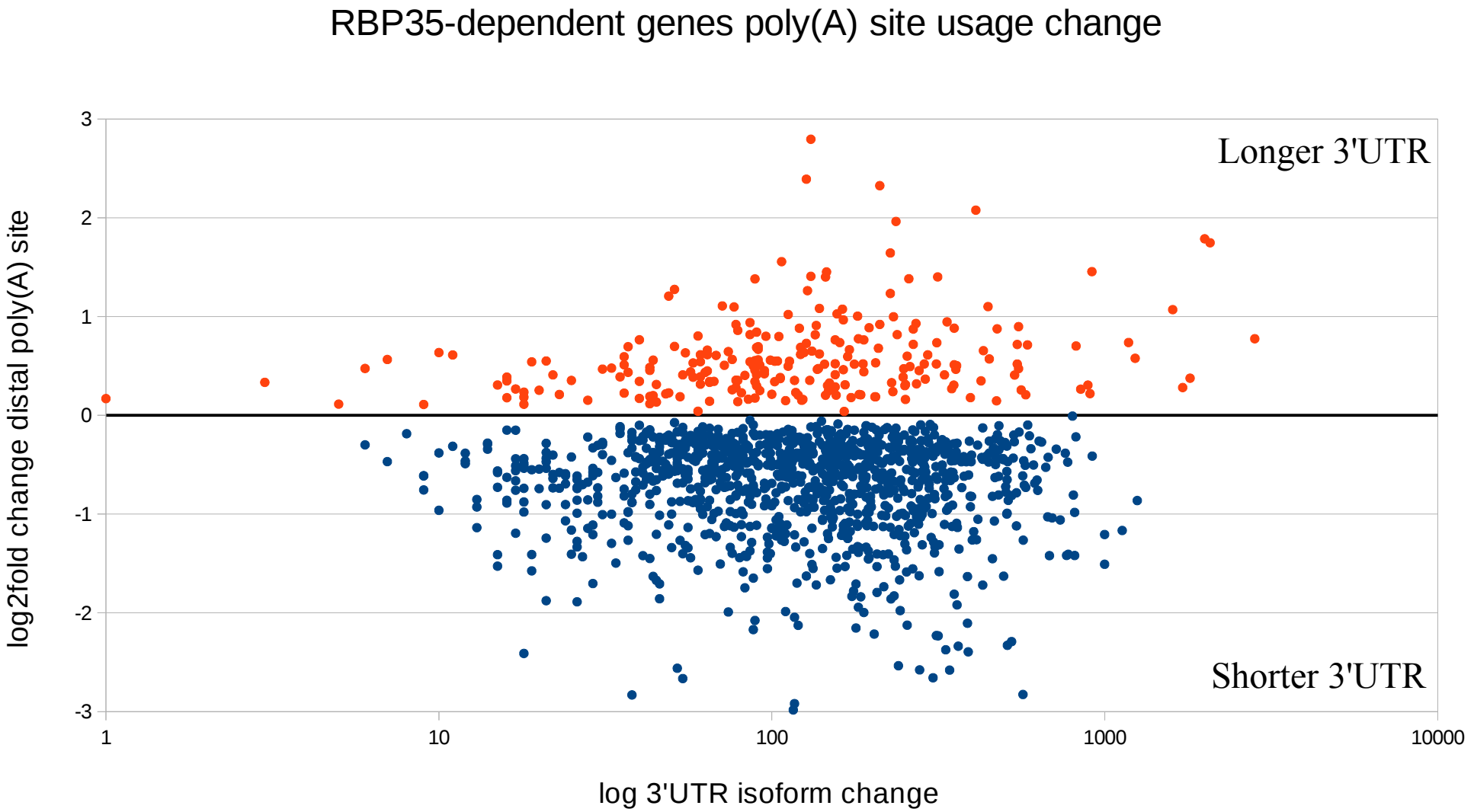
There is no correlation between RBP35 dependance and differential expression



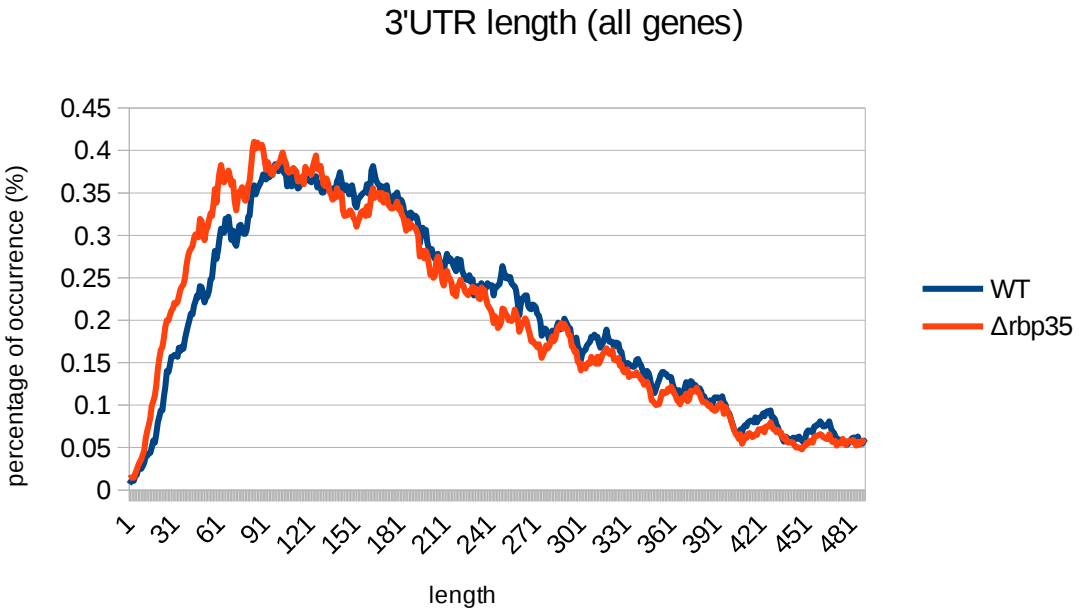
Δrbp35 affects poly(A) sites usage, preferring proximal cuts - percentages

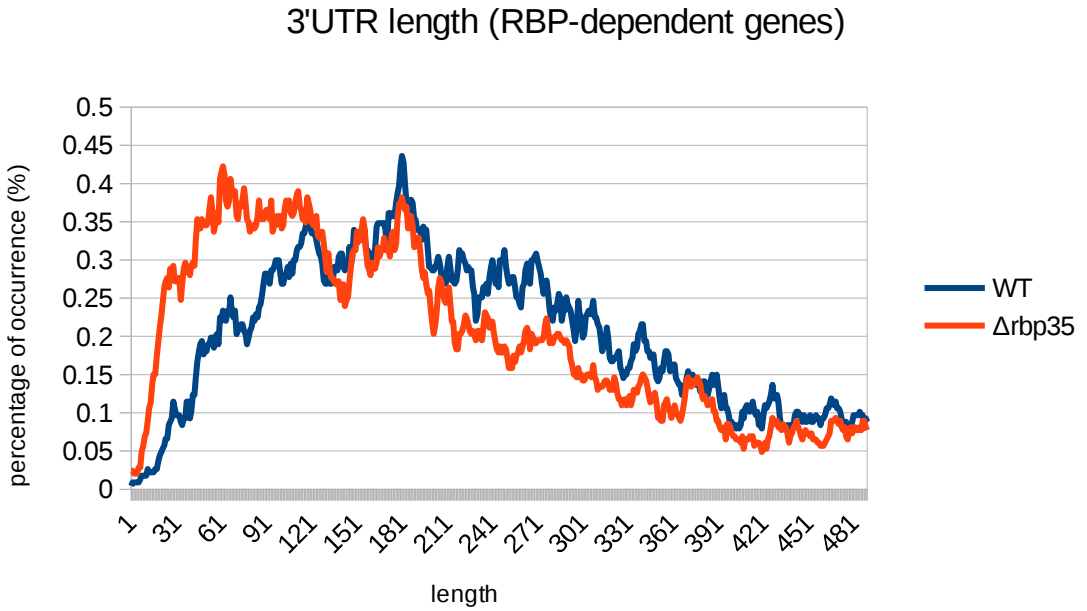


Δrbp35 affects poly(A) sites usage, preferring proximal cuts

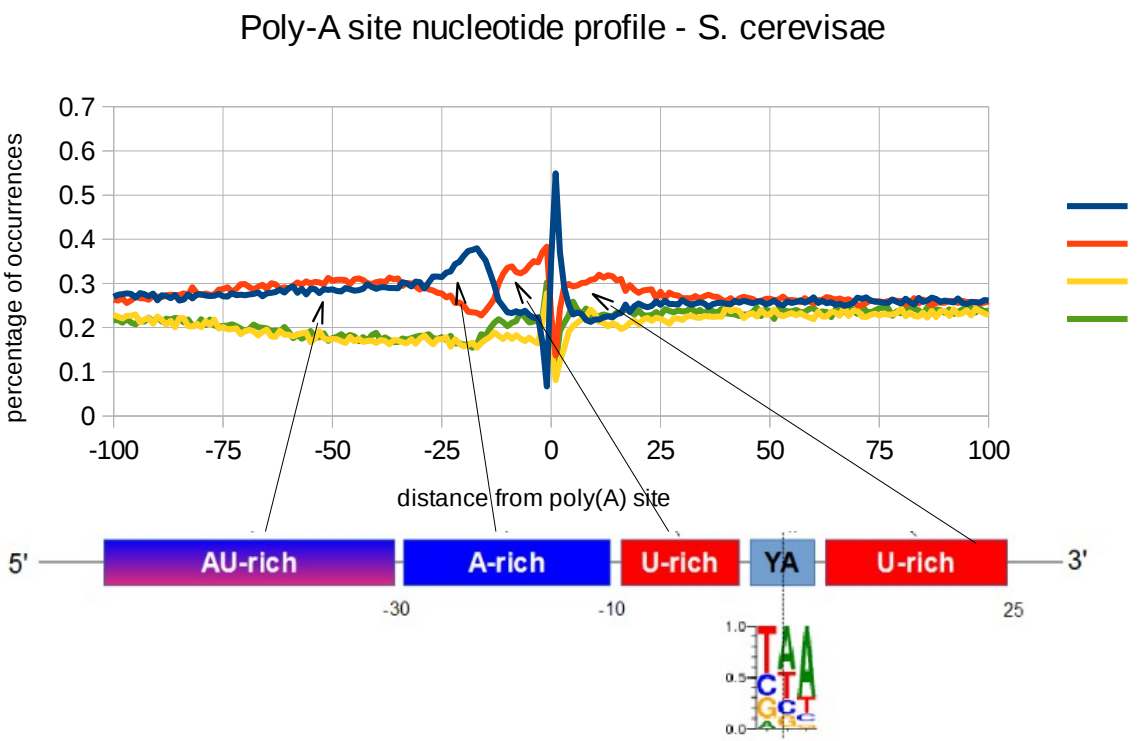


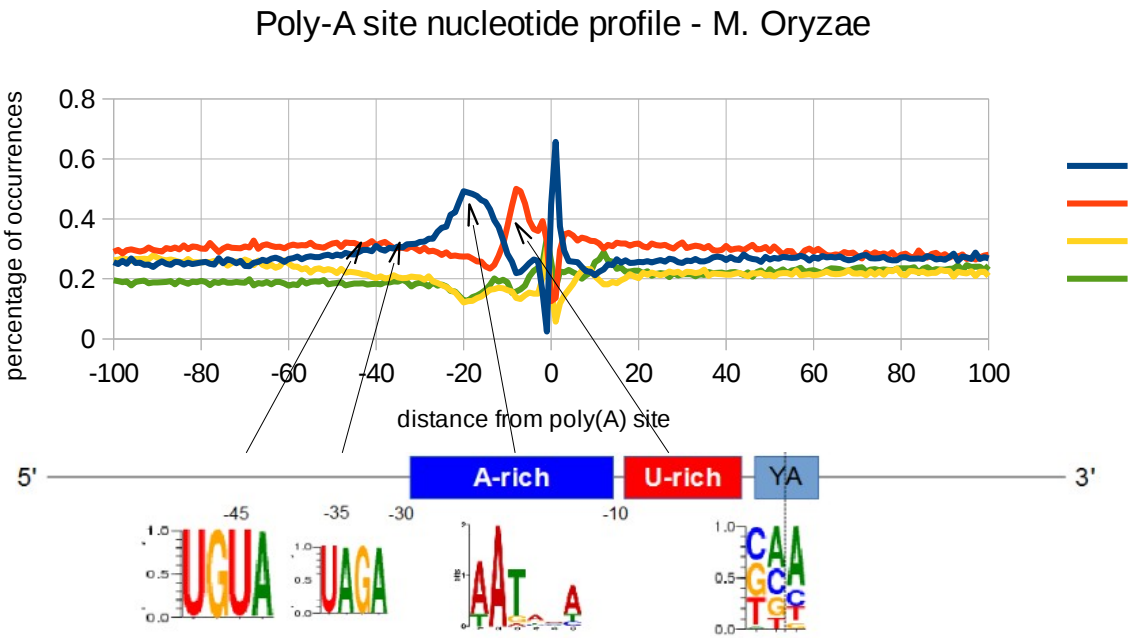
Δrbp35 affects 3'UTR length



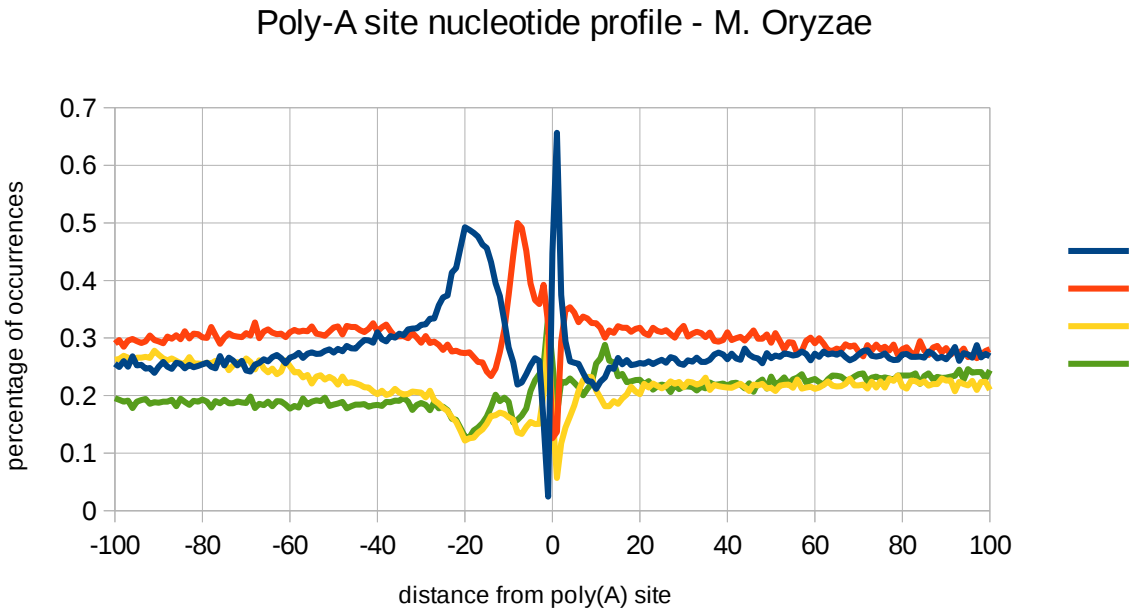


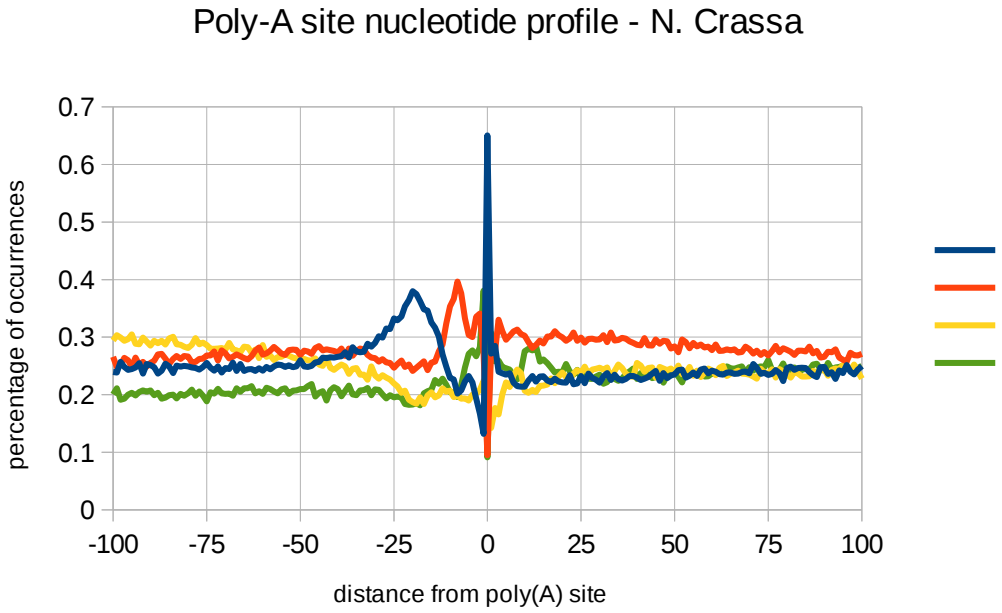
Nucleotides profile of poly(A) sites slighty differs from *S.cerevisae*

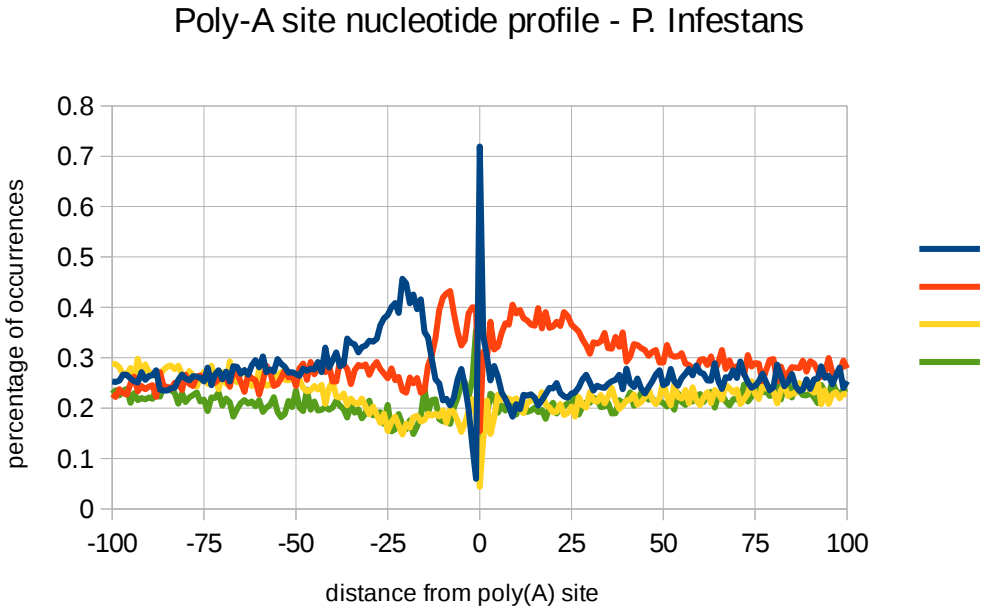




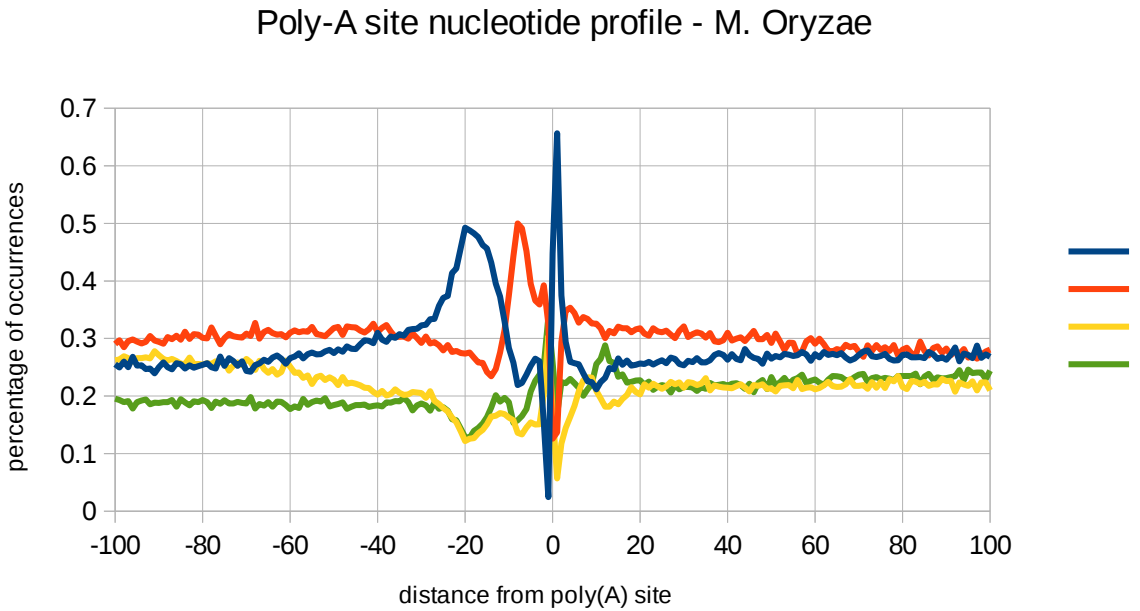
Nucleotides profile of poly(A) sites resembles *N. crassa*

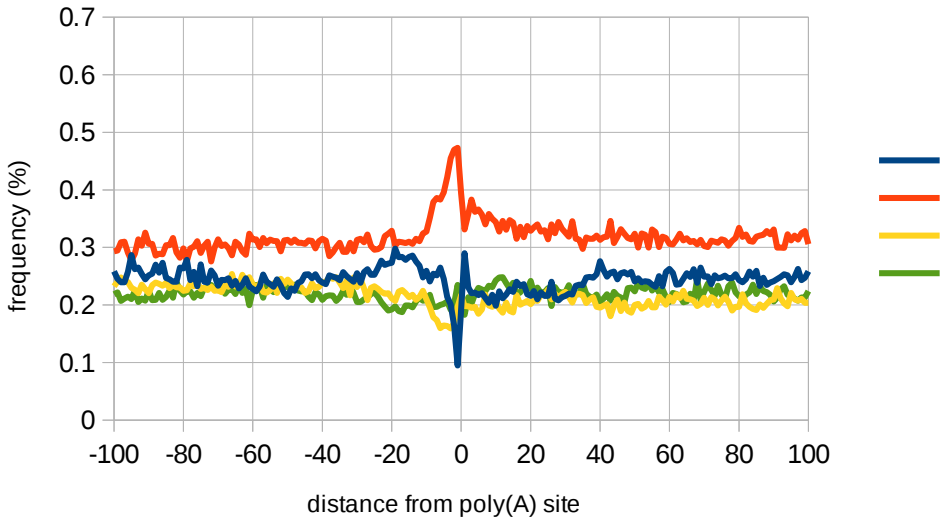


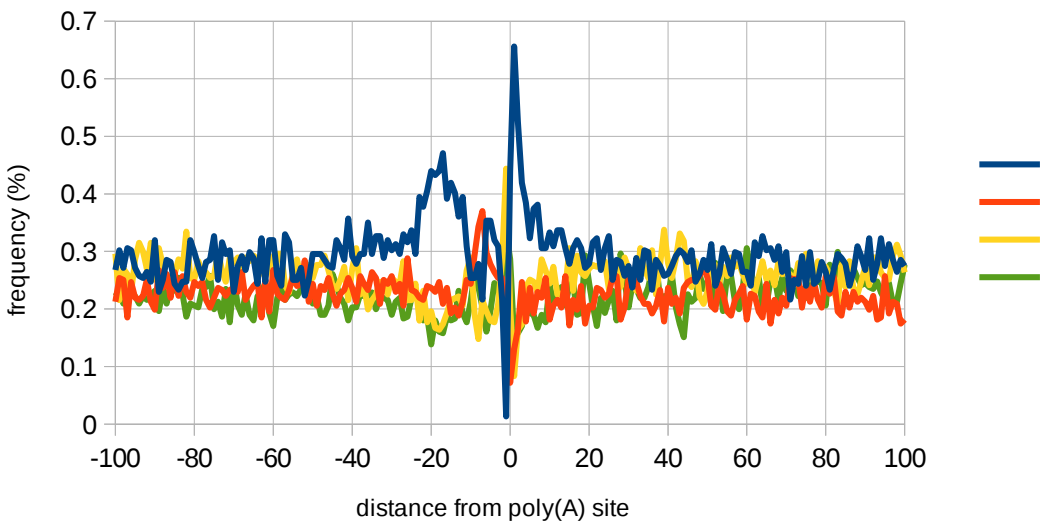




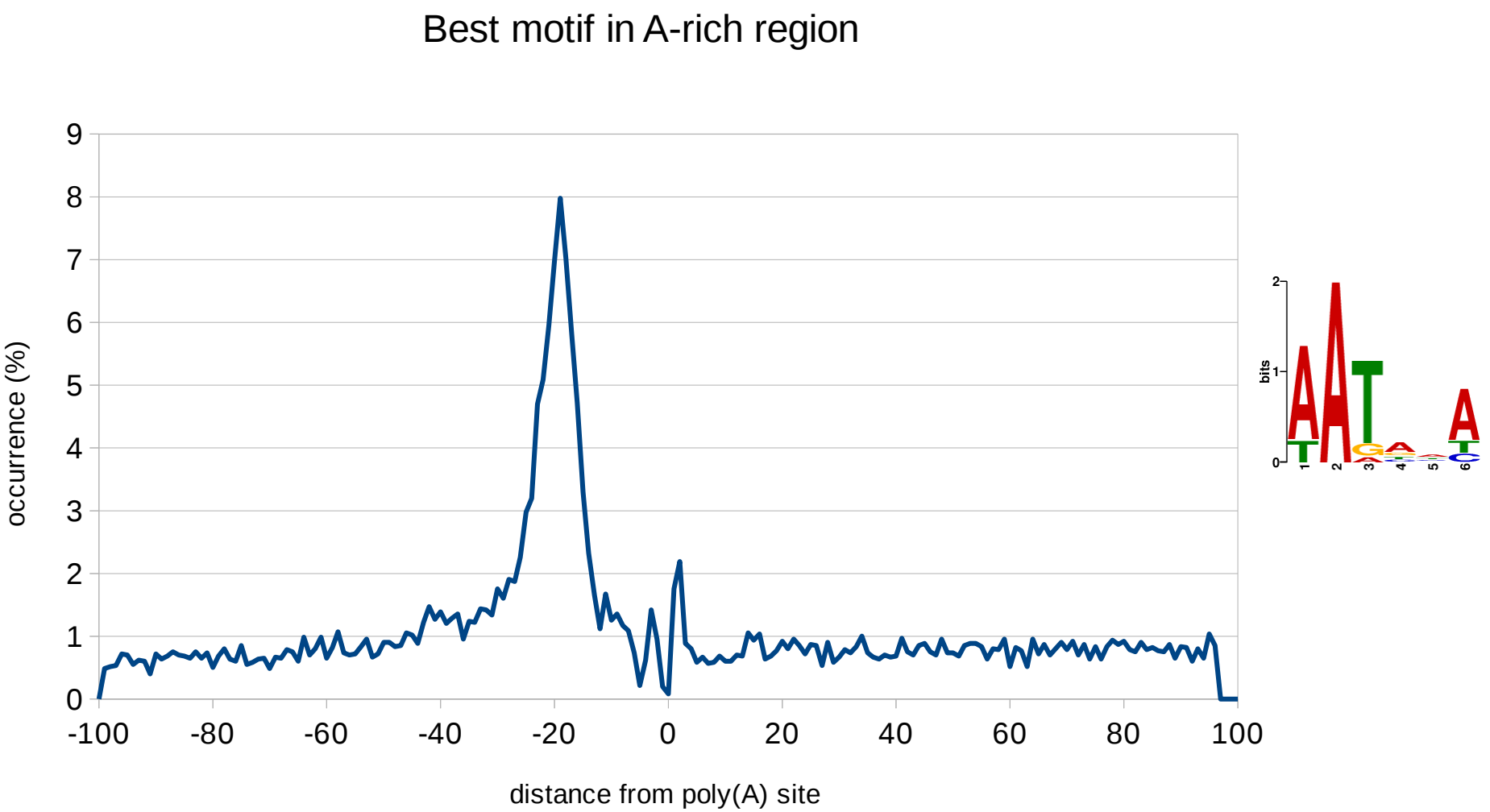
Nucleotides profile of poly(A) of ncRNA and CDS poly(A) is different



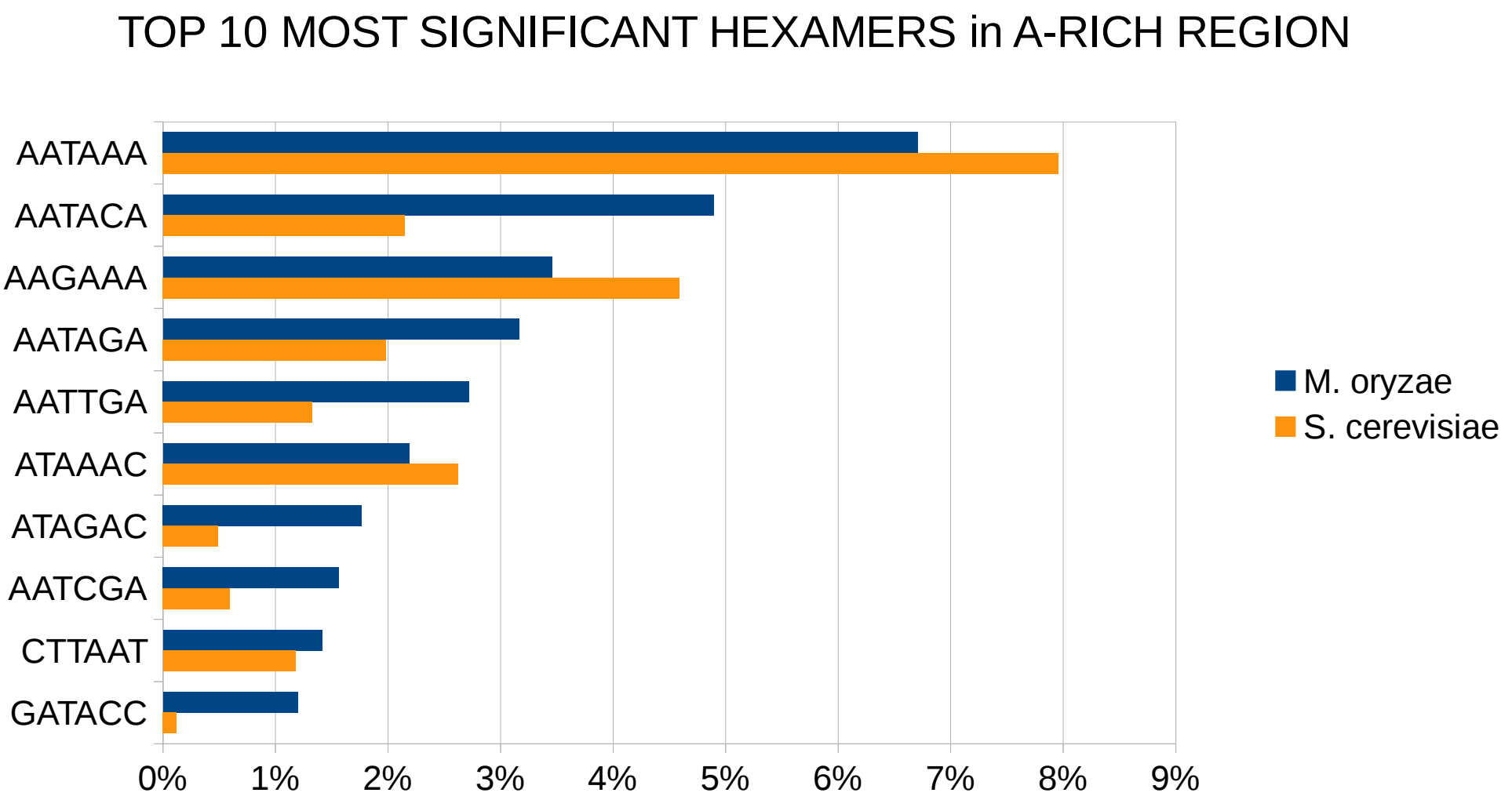




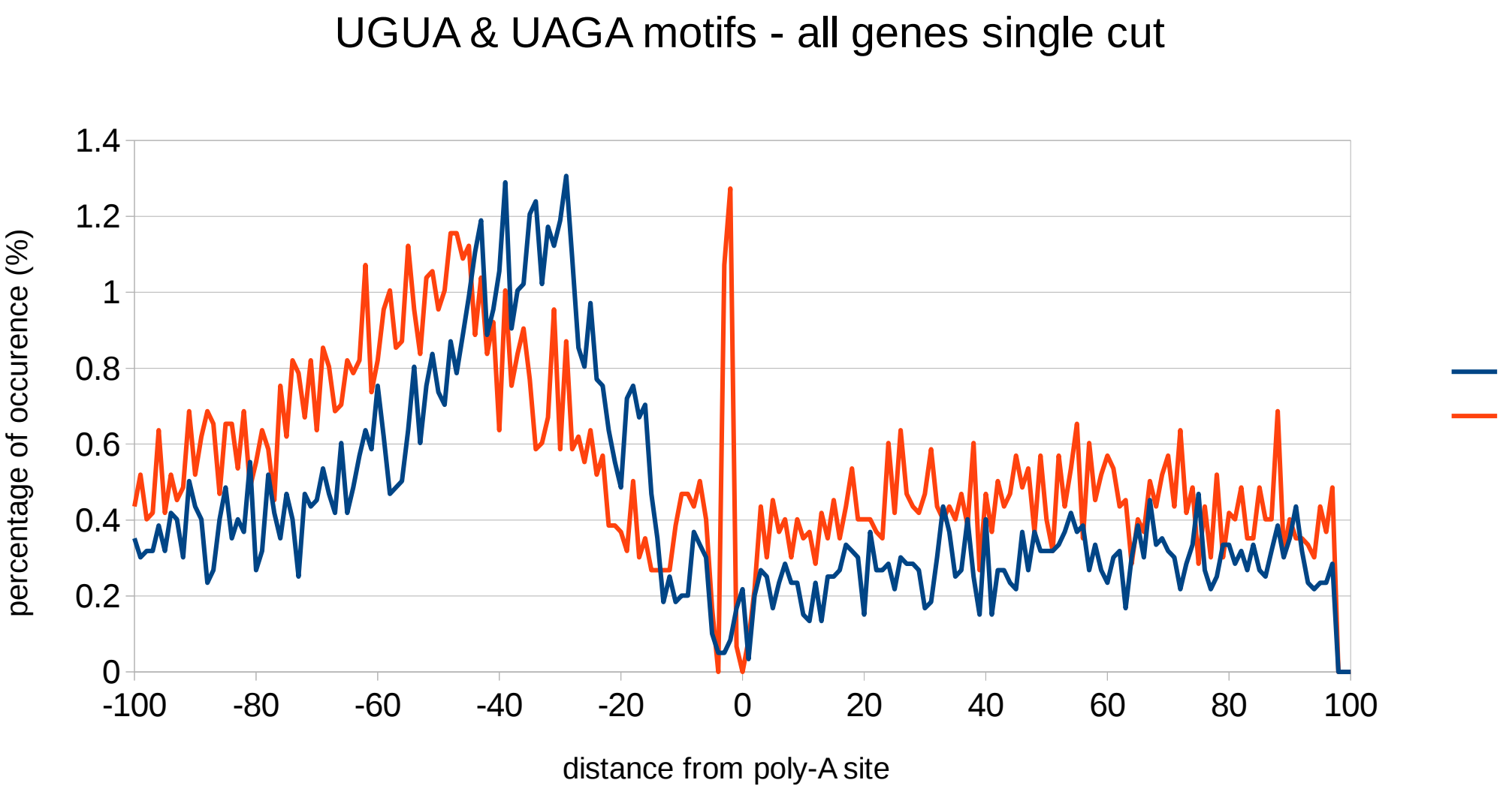
The A-RICH region is located -30 -10 bp upstream



The A-RICH region is located -30 -10 bp upstream



UAGA & UGUA motifs



Polyadenilation signals in common genes

MPG1

...GG**UAGA**GAAGUCUCUUCUCGUUCCACUCAUUU**AAUAAA**ACCCCUUCCAGACC**UA**...

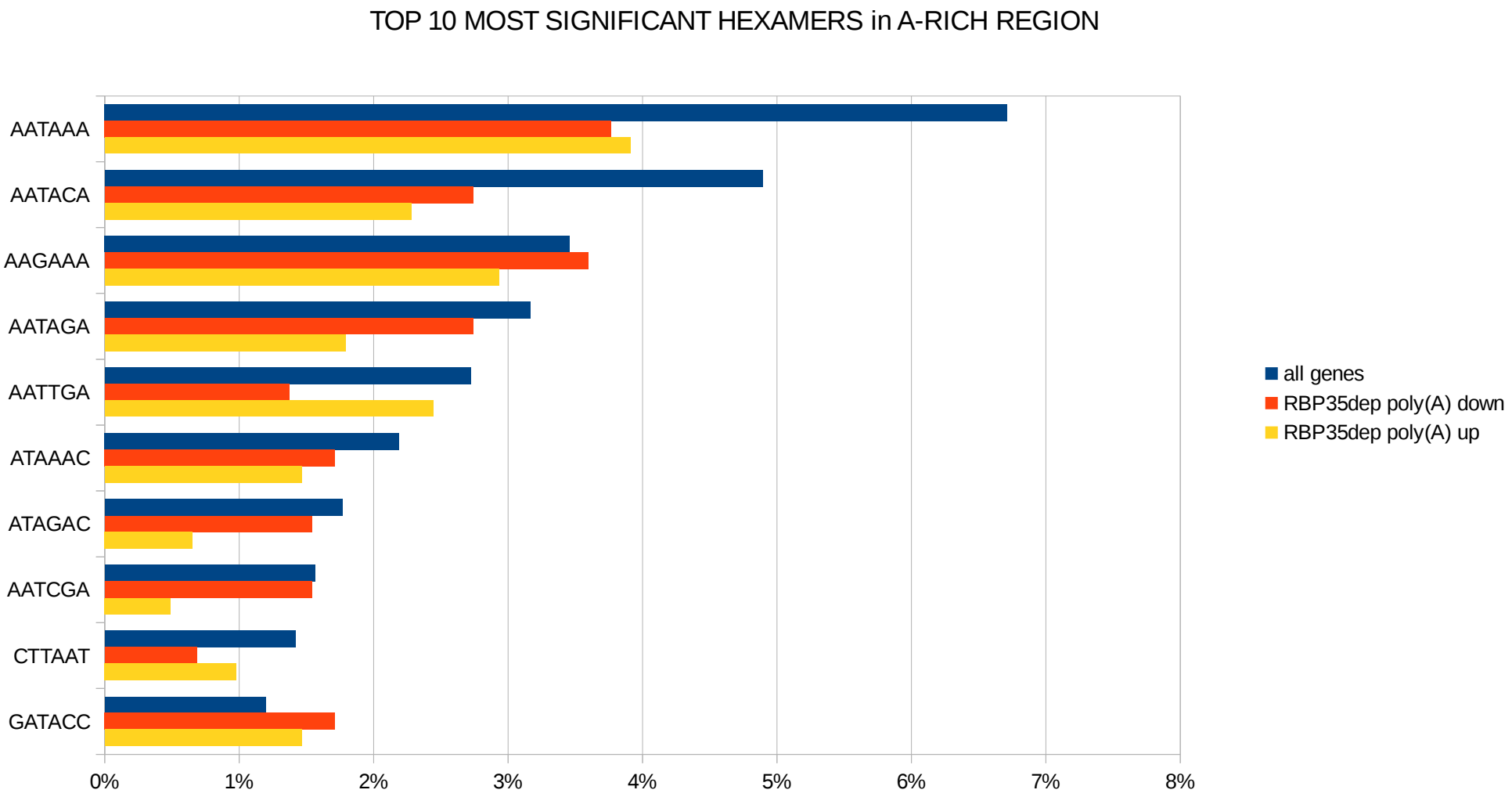
PMK1

...CGUU**UAGA**AUGUGCAGGAGACACGAGUGGGAAAAUG**AAUACA**UGGAUGCCAG**CA**...

MST12

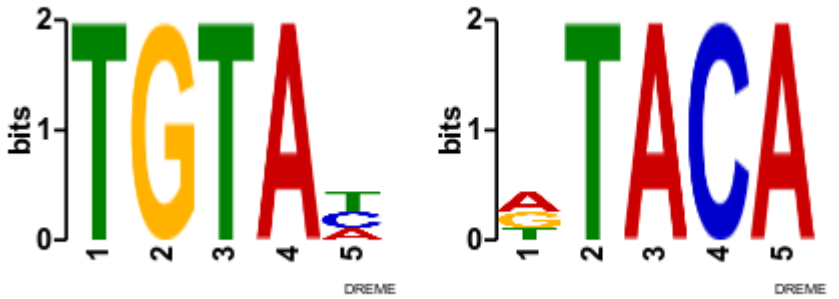
...CAGUGGCAUAAAAUCACAAAAUCUU**UAGA**AAGAUCAC**AGAAAA**CCUUUUGUC**CA**...

poly(A) sites dependent from *RBP35* are low in the canonical AATAAA signal



UGUAH motif is enriched in poly(A) sites dependent from *RBP35* down-regulated in Δrbp35, in the region -100 -30

1. TGTAH



Details

Positives	Negatives	P-value	E-value	Unerased E-value
1603/3193	836/3193	4.8e-88	3.8e-83	3.8e-83

Enriched Matching Words

Word	Positives	Negatives	P-value	▼E-value
TGTAT	797/3193	384/3193	3.2e-41	2.6e-36
TGTAC	624/3193	282/3193	2.1e-35	1.7e-30
TGTAA	470/3193	266/3193	6.2e-16	4.9e-11
TGTAC	413/3193	260/3193	2.5e-10	2.0e-5

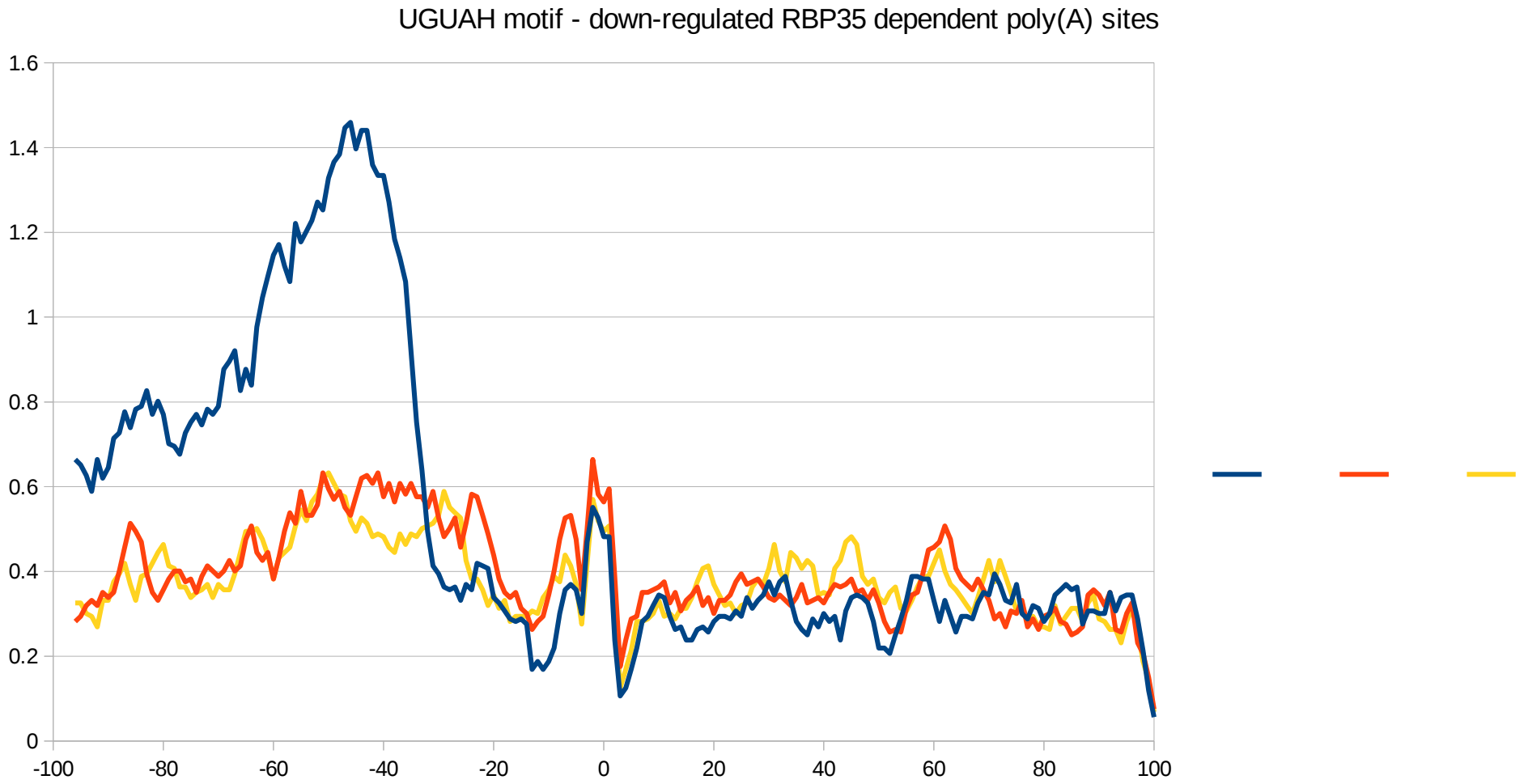
- Output of DREME, pRBP35dep as positive sequences list versus not-pRBP35dep negative list

UGUAH motif – pRBP35dep vs not pRBP35dep

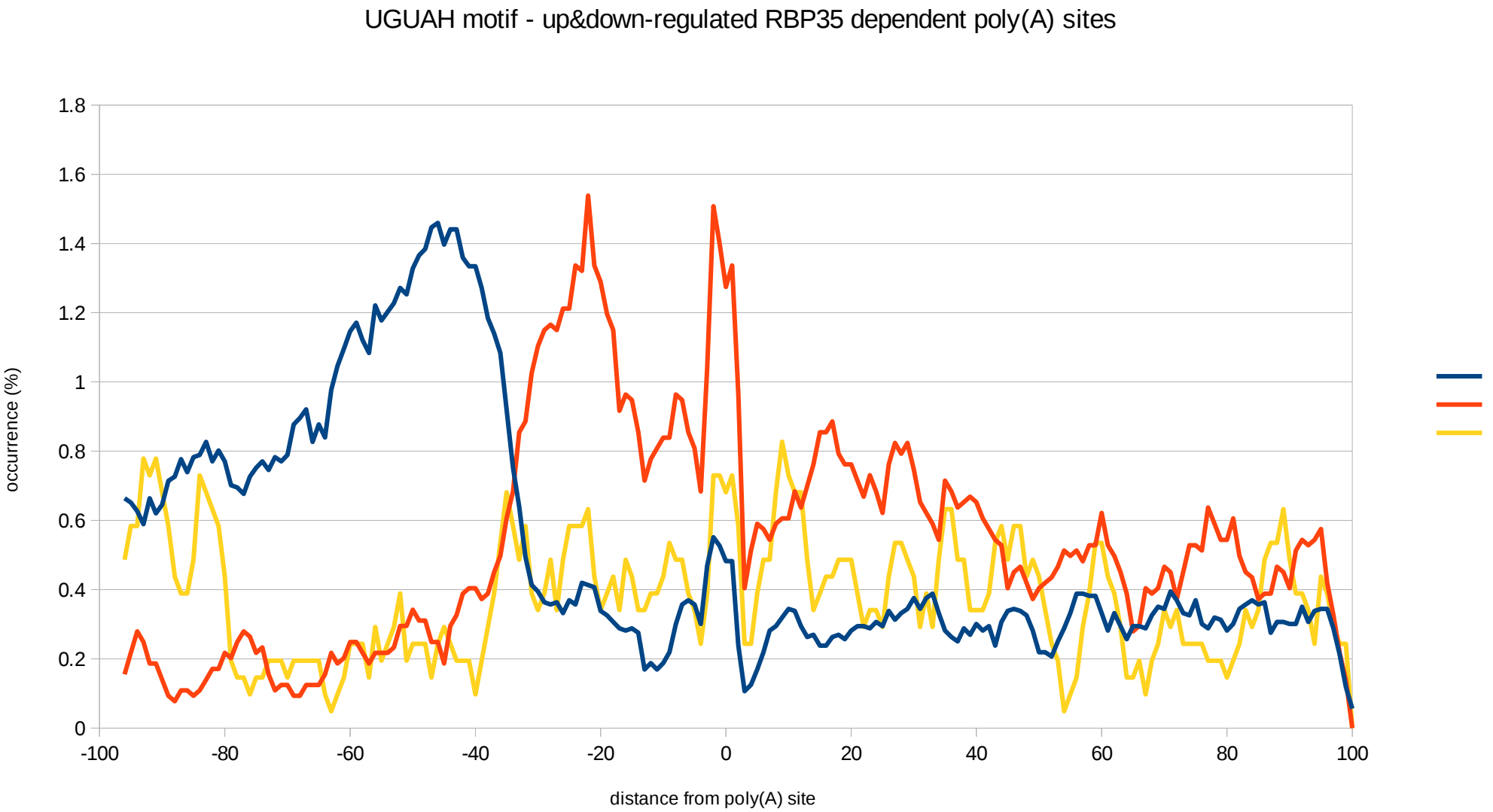
- In the first graph, we want to show how poly(A) sites dependent from *RBP35* display a different profile for the UGUAH motif in the respect to "regular" poly(A) sites

- We therefore plot down-regulated RBP35 dependent poly(A) sites against two groups of poly(A) not dependent from RBP35 of the same size, one group of poly(A) sites belonging to the same genes and one group of poly(A) sites belonging to other genes

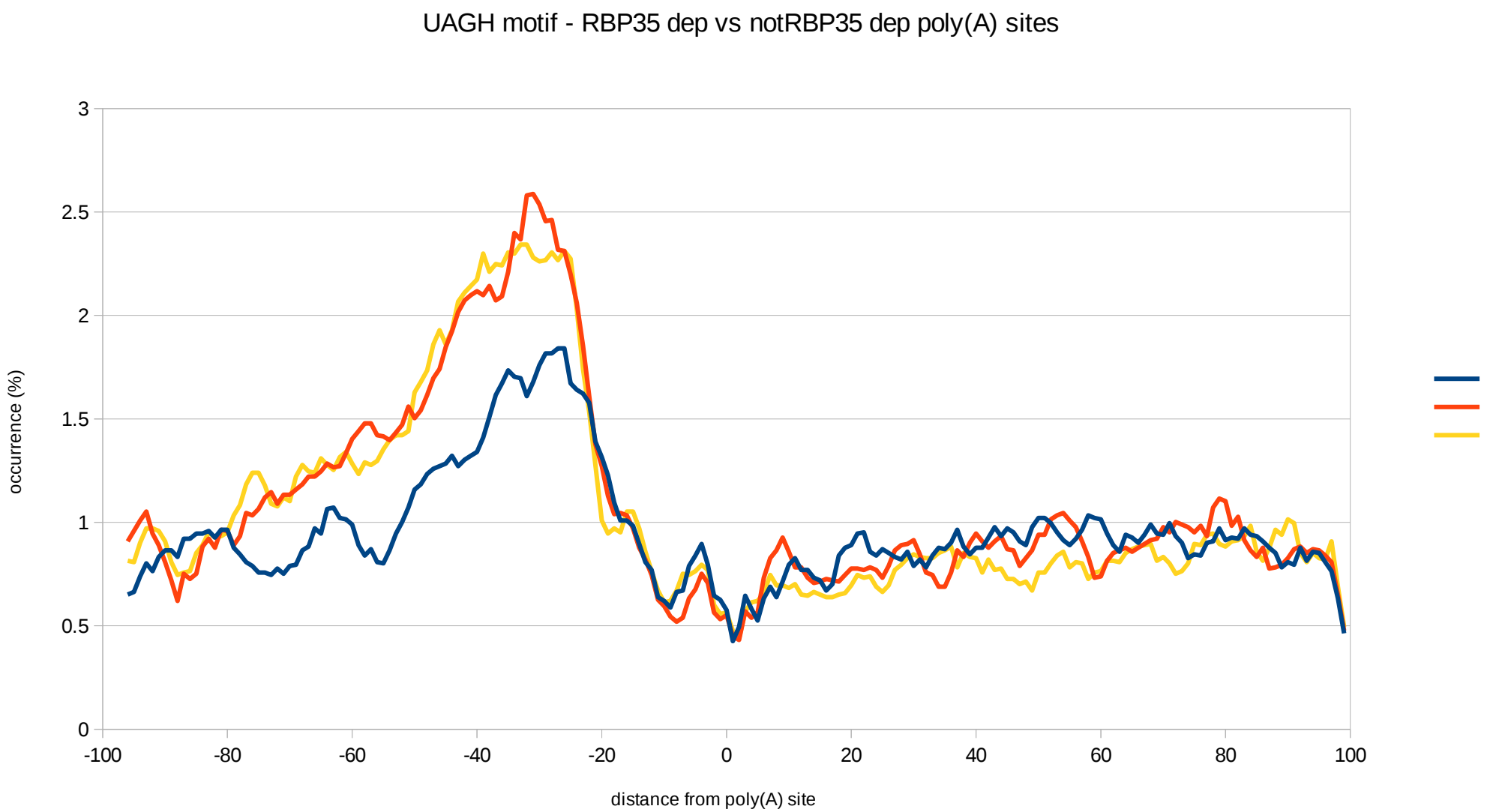
UGUAH is enriched at -45 in poly(A) sites dependent from *RBP35*



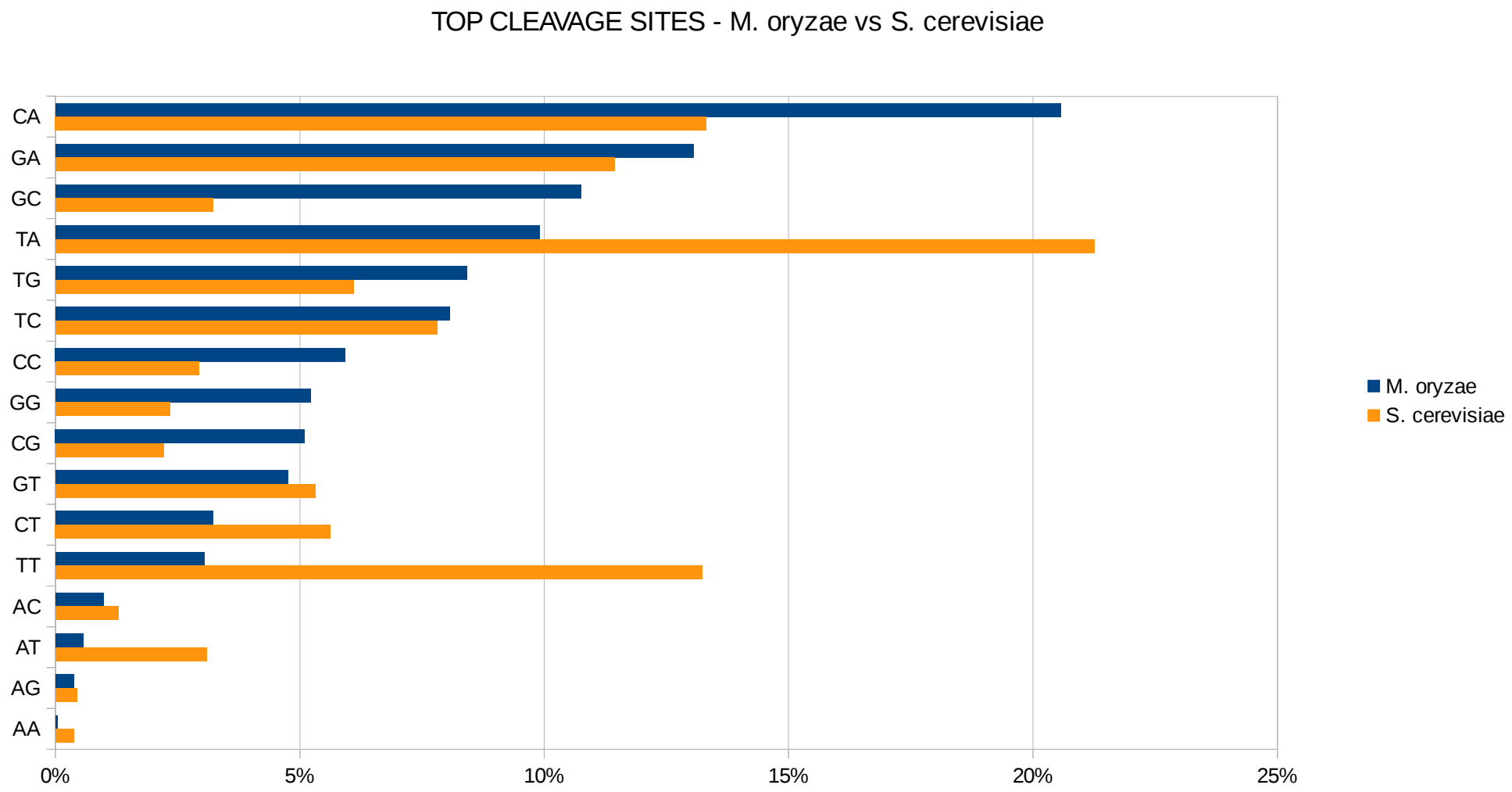
UGUAH motif – RBP35 dependent poly(A) sites (up vs down regulated)



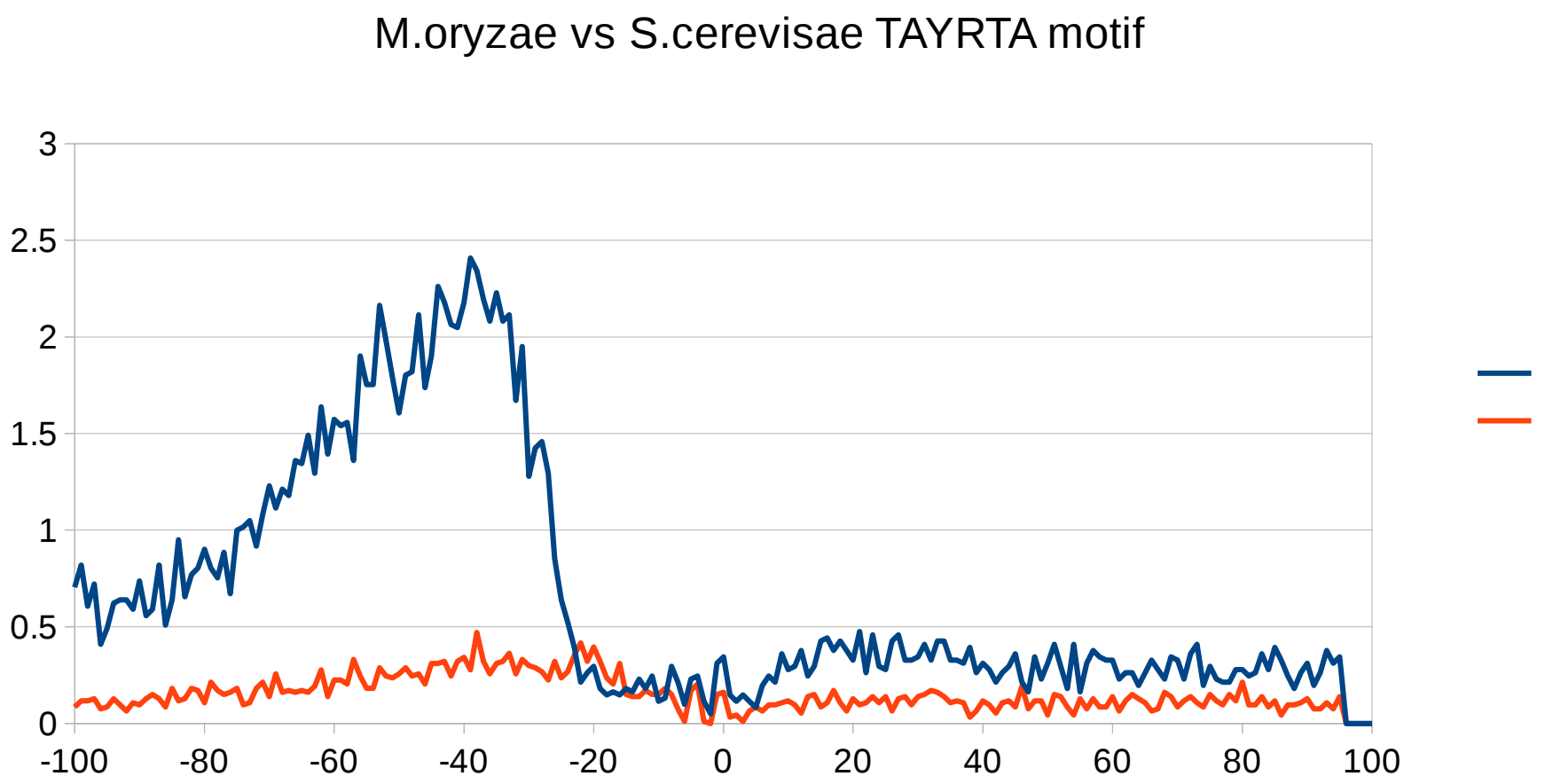
UAGH is impoverished at -35 in poly(A) sites dependent from *RBP35*



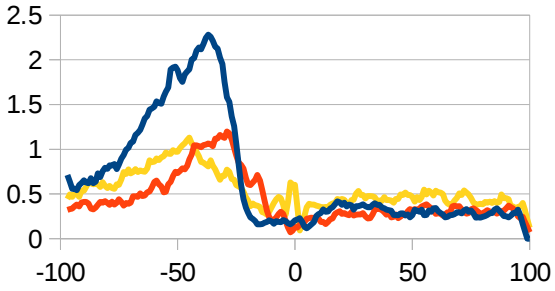
M. oryzae prefers SA as cutting-site instead of YA



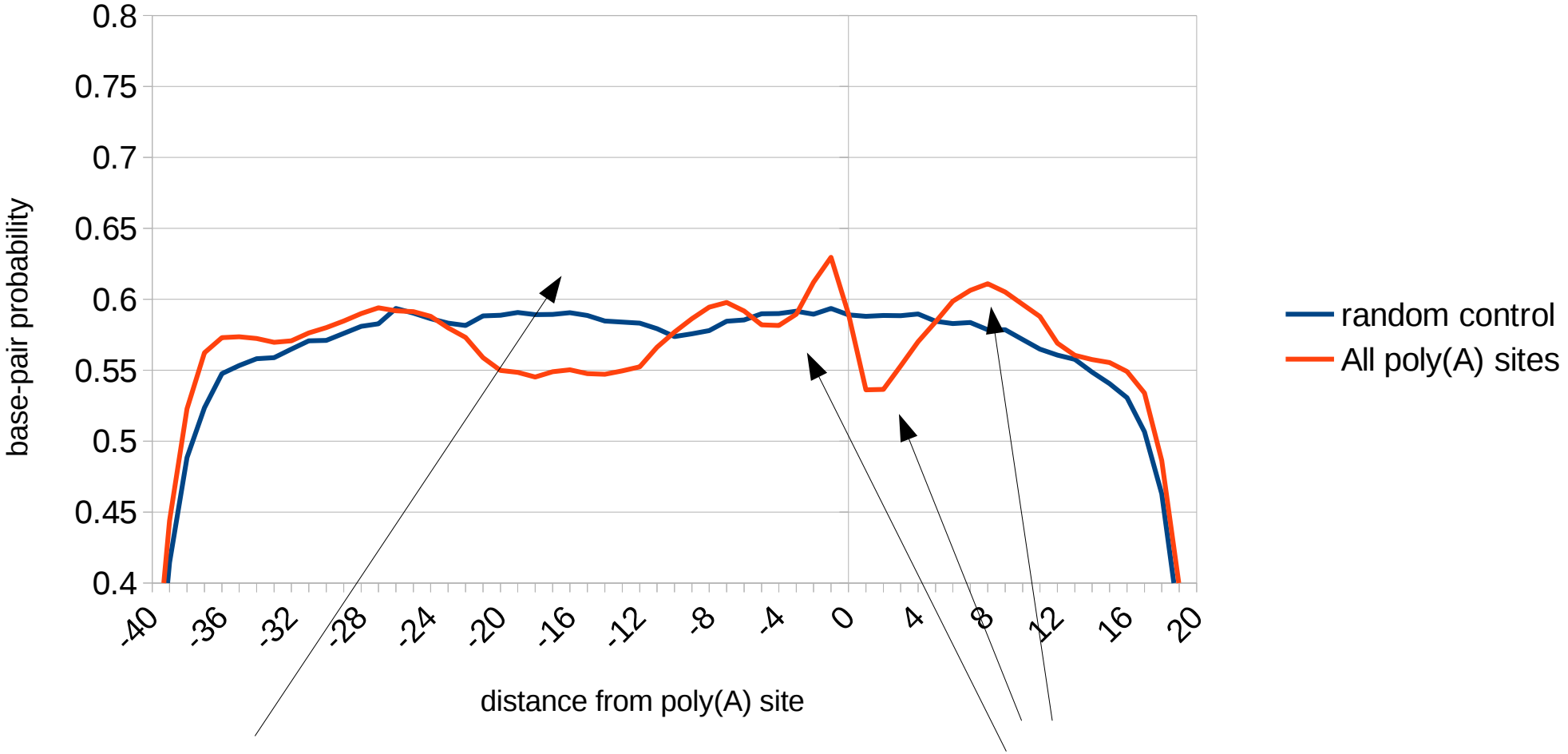
The HRP1 binding motif TAYRTA from *S.cerevisae* in not found in *M.oryzae*



Maybe in M.oryzae CFIm binds to UGUA and Hrp1 binds to UAGA?



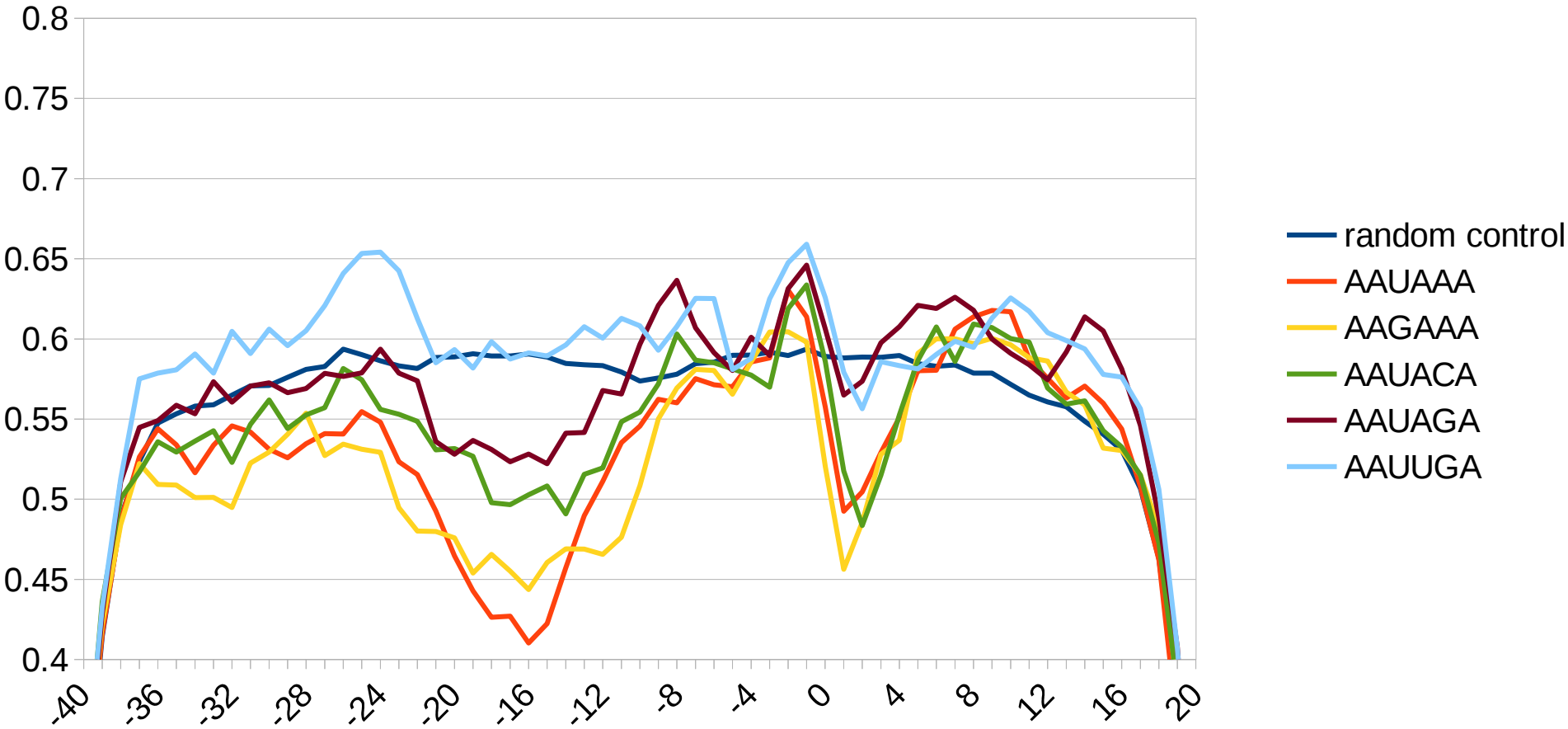
The polyadenylation site region has a defined structure - 1



The A-rich region is usually not structured

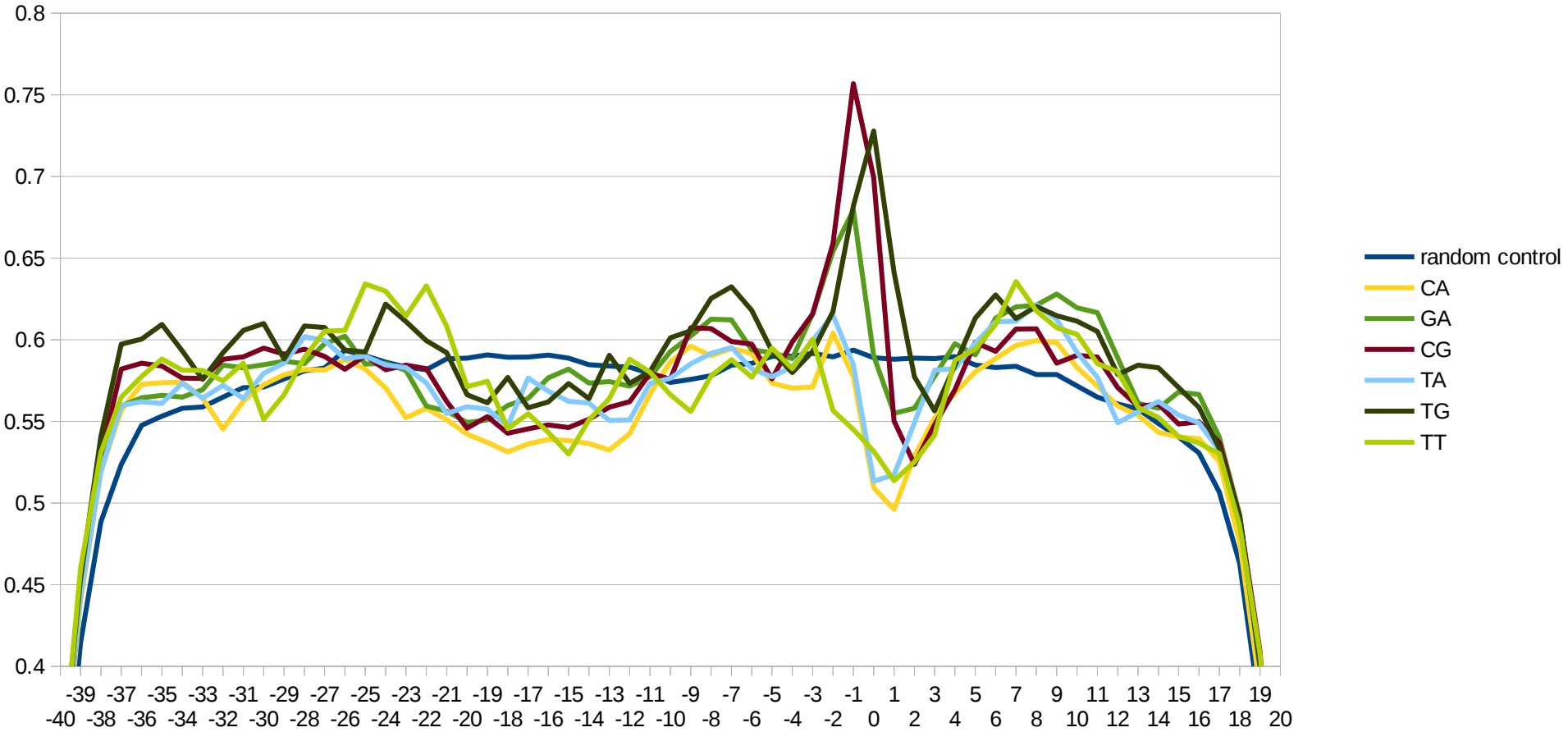
The polyadenilation site is usually structured and located in a hairpin-loop

The polyadenylation site region has a defined structure - 2



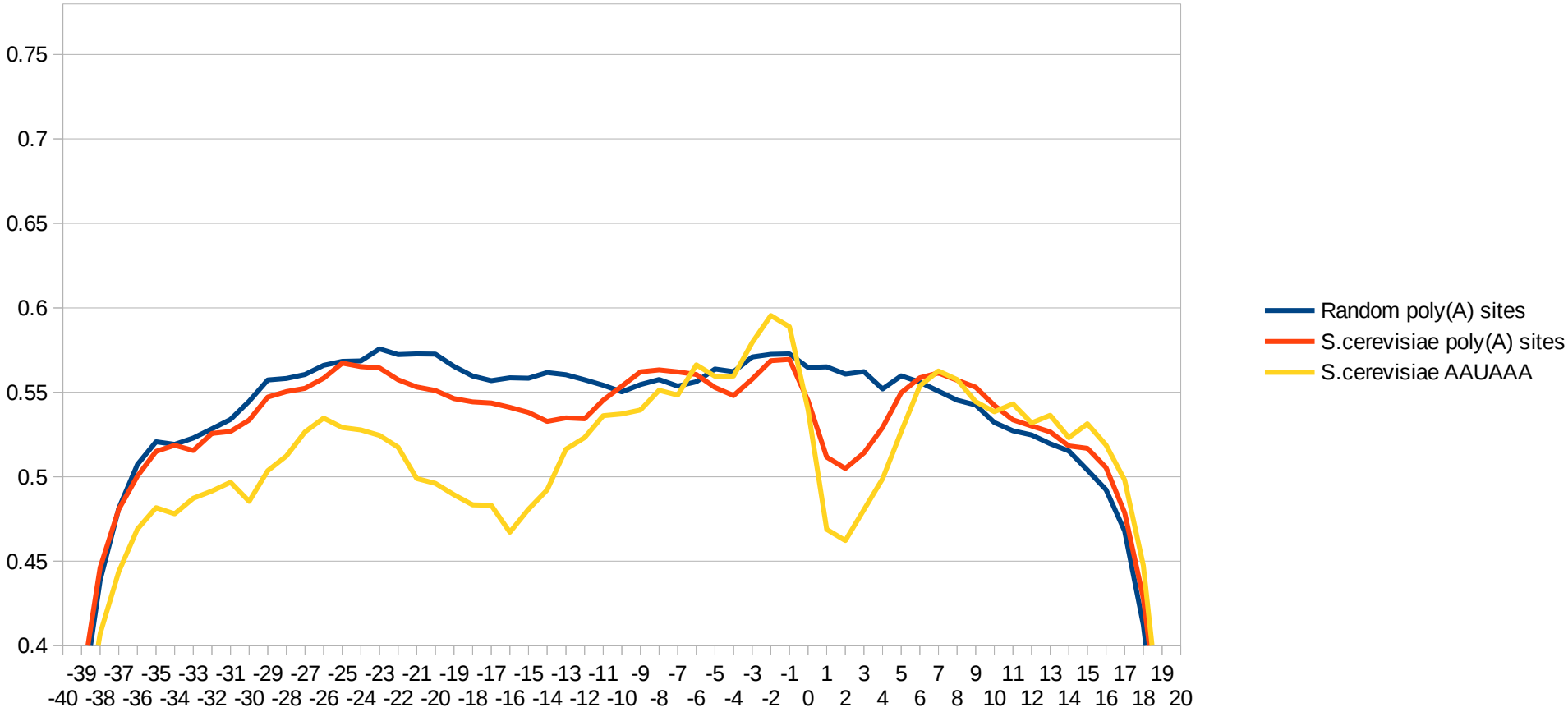
Different A-rich motifs results in different degrees of conformation, with AAUAAA the most unstructured

The polyadenylation site region has a defined structure – 3



Different cutsites have different base pais probabilites, with TG and CG the most structured. The most common poly(A) site CA has a average conformation

The polyadenylation site region has a defined structure – 4



In S.cerevisiae, the poly(A) site is not clearly structured