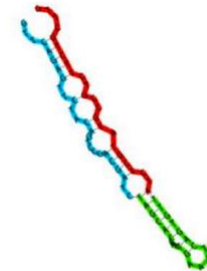


Magnaporthe oryzae small-RNA sequencing



CBGP
CENTRO DE BIOTECNOLOGÍA
Y GENÓMICA DE PLANTAS
UPM-INIA

Fundación **BBVA**



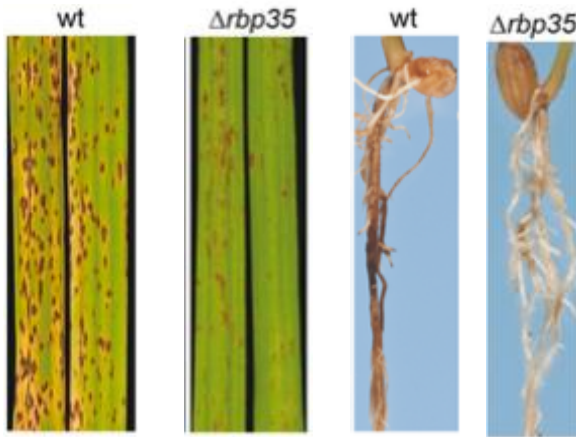
POLITÉCNICA

"Ingeniamos el futuro"

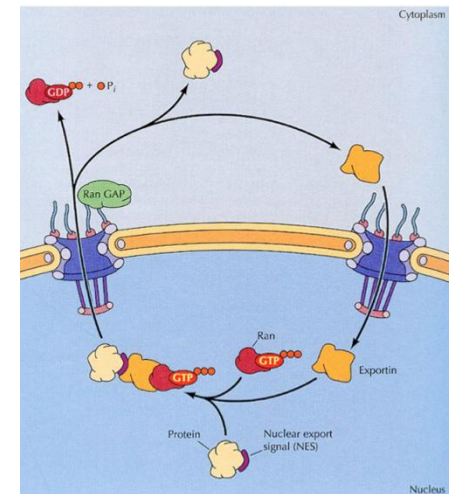
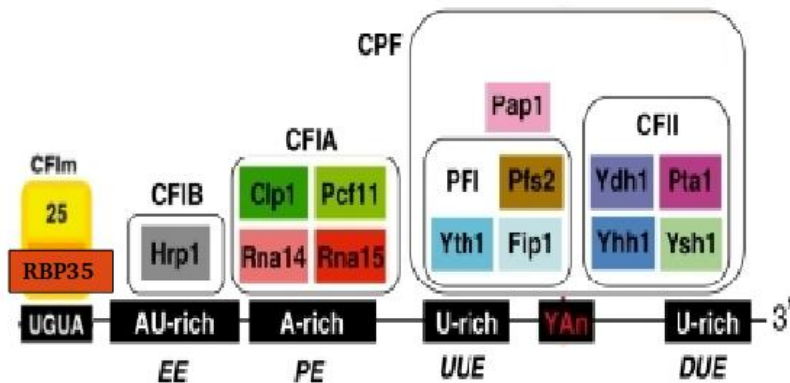
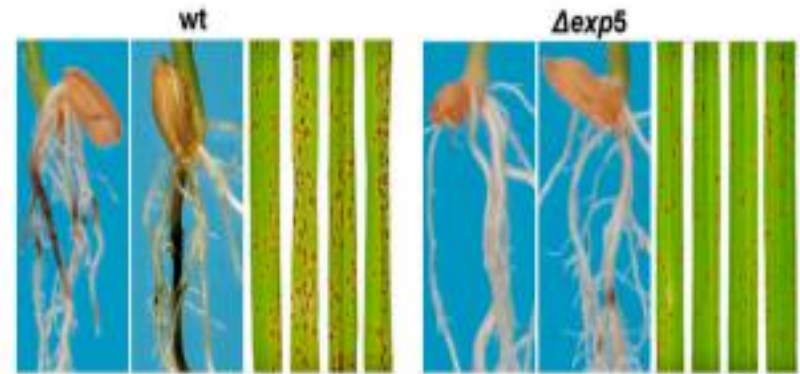
CAMPUS
DE EXCELENCIA
INTERNACIONAL

Introduction

RBP35



EXP5



Introduction – RBP35

- We performed a poly(A) specific RNA-seq
- Alternative polyadenylation is altered in *Δrbp35* in 25% of genes, belonging to specific functional groups
- UTR are usually shortened in *Δrbp35*
- *Δrbp35* probably binds to UGUA motif
-(previous work)

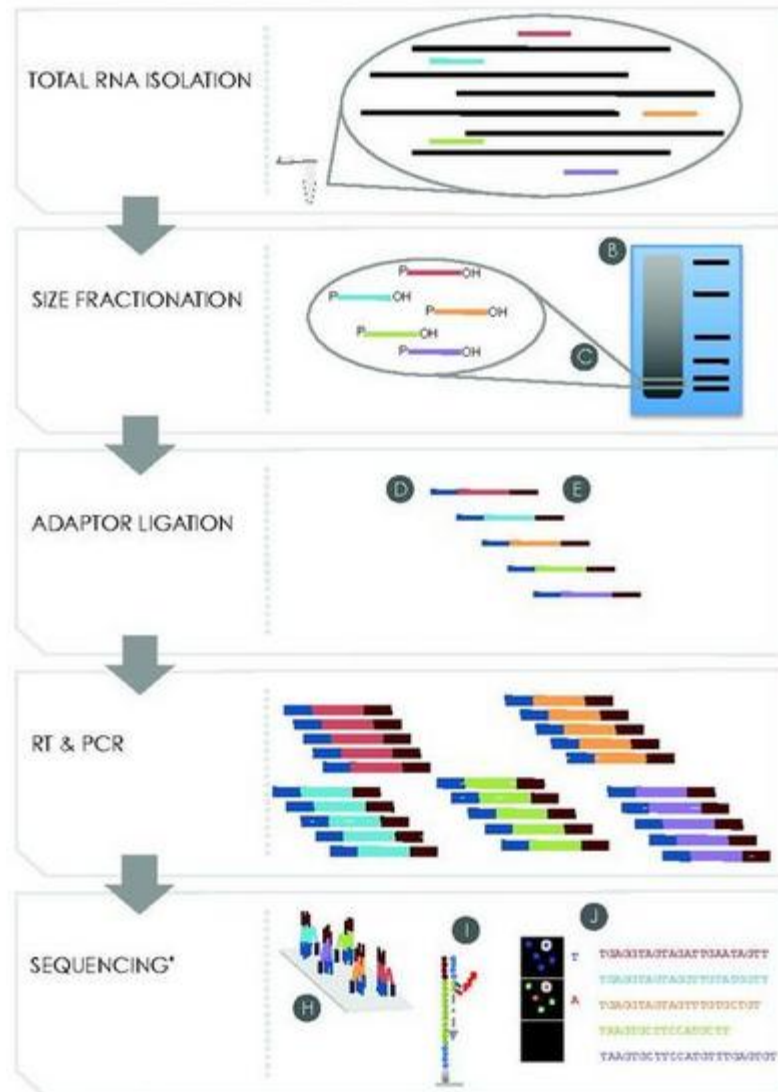
Introduction – EXP5

- In yeast, involved in nuclear import and export of several proteins, and required for re-export of mature tRNAs after their retrograde import from the cytoplasm
- In mammals also mediates the nuclear export of micro-RNA precursors, and double-strand RNA bearing proteins

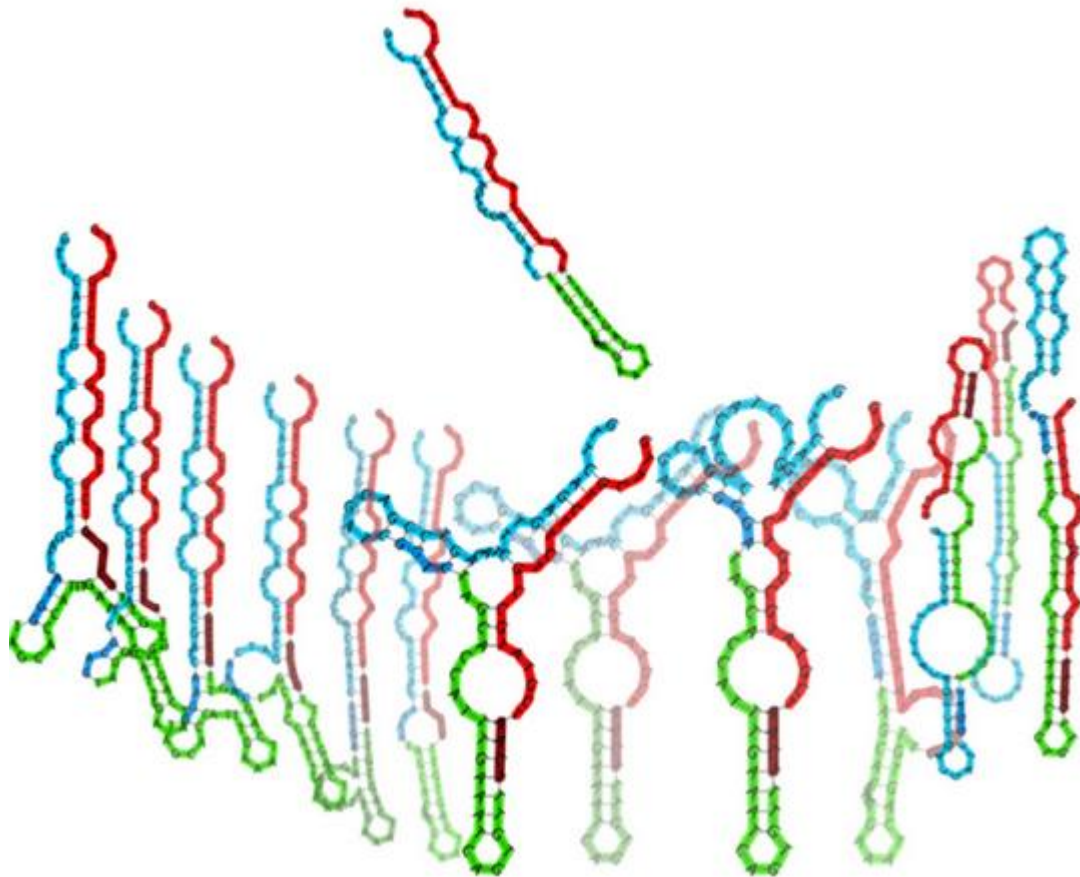
Introduction – purposes

- In order to elucidate a role for EXP5 in *M.oryzae*, and considering its role in smallRNA export in mammals, we performed a smallRNA sequencing.
- We also included the $\Delta rbp35$ mutant in the pool

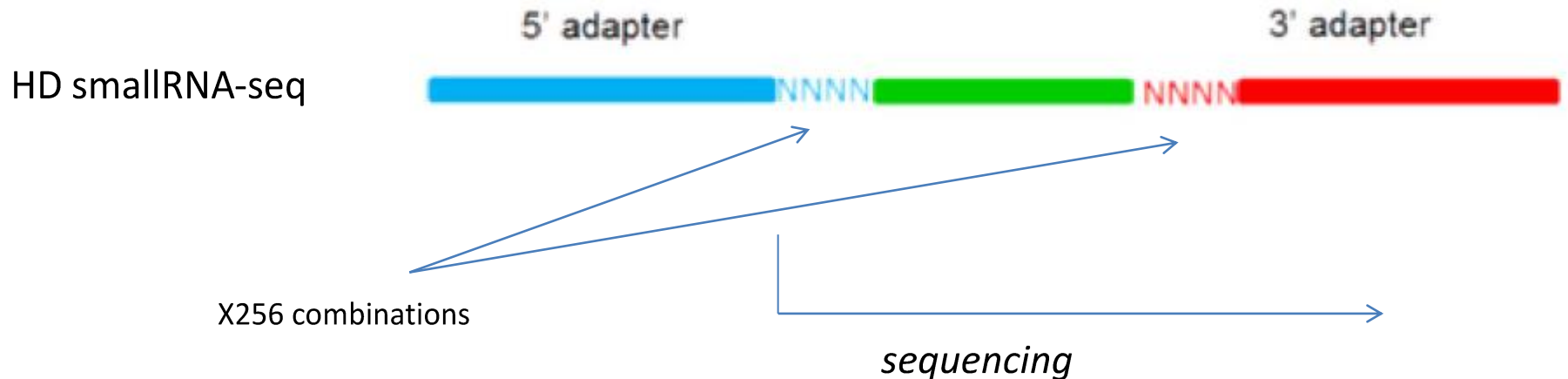
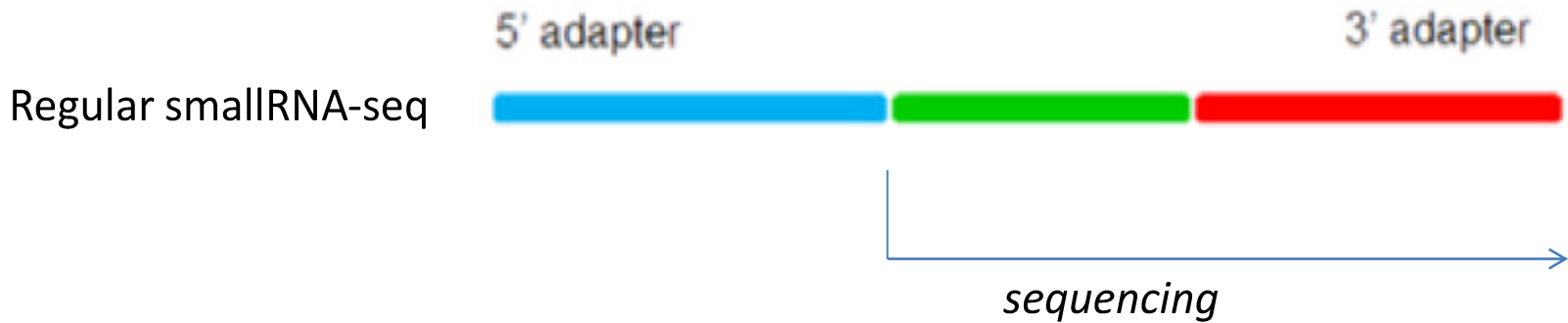
Small RNA library preparation and sequencing



Standard smallRNA-seq adapters have been shown to be biased for certain RNA structures



Our library was prepared with HD adapters (adriana in england)

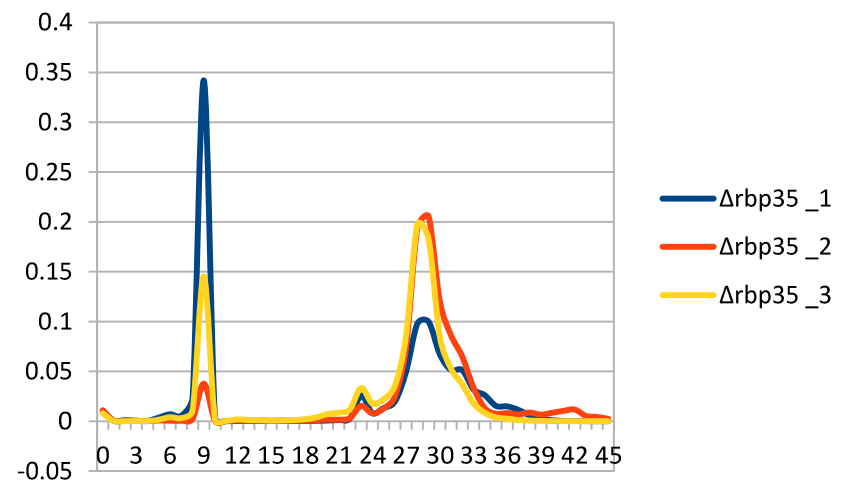
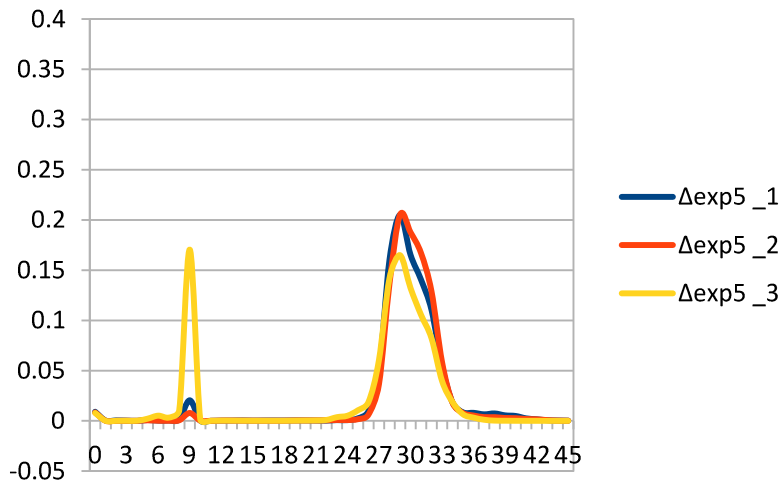
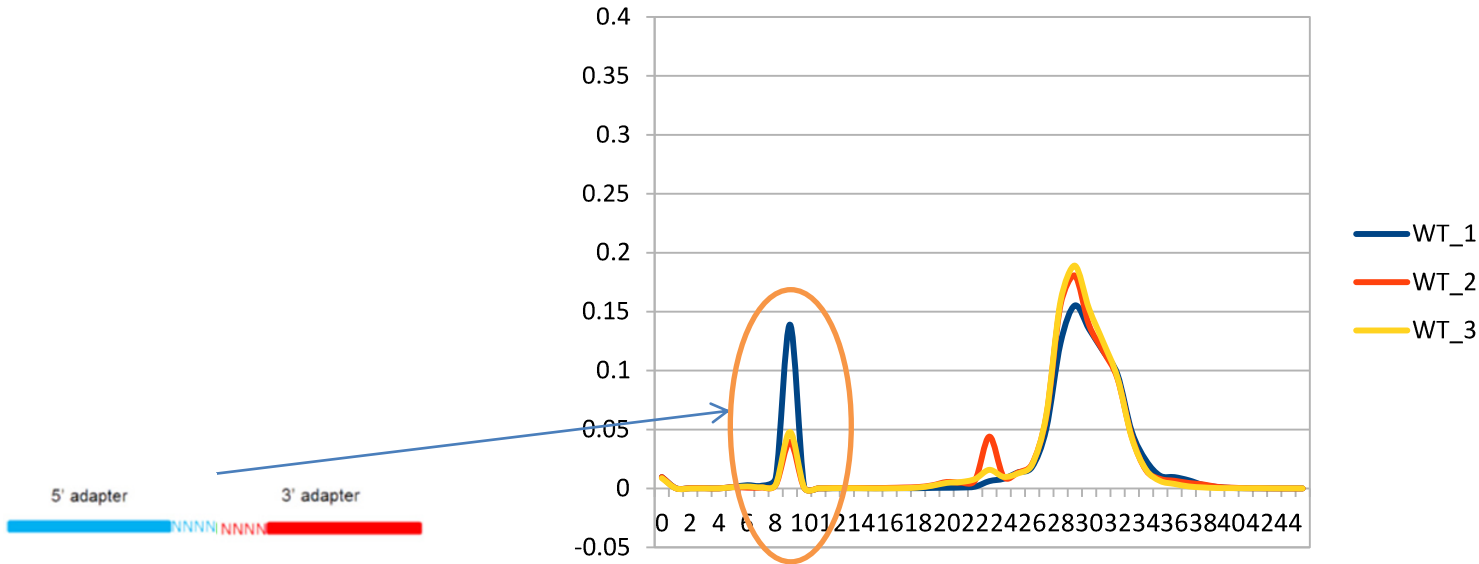


Sequencing resume

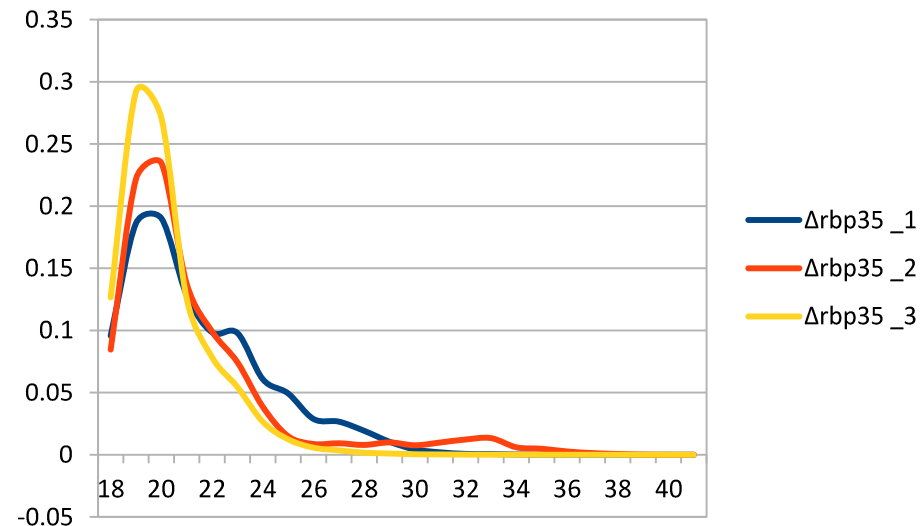
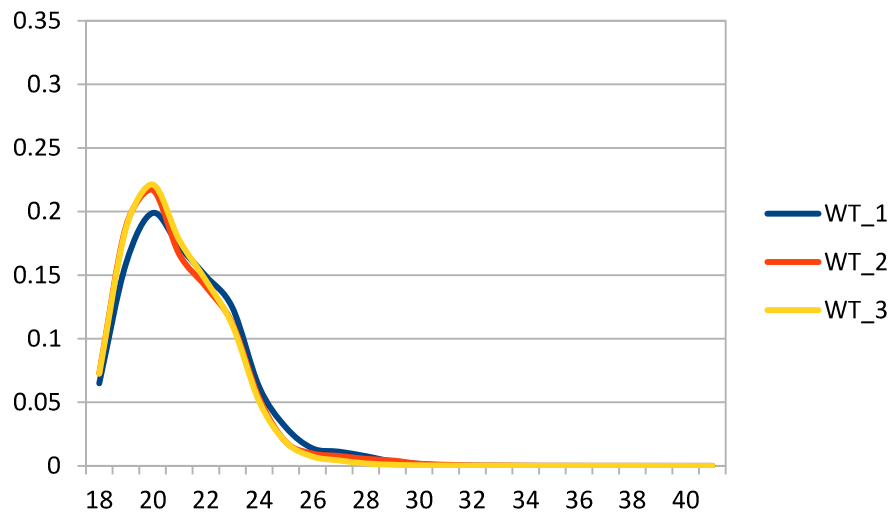
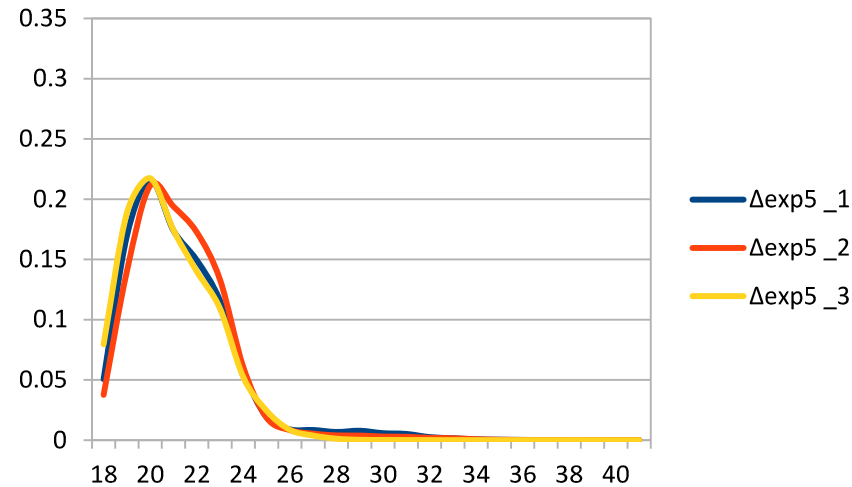
sample	total reads	After filtering*	% filtered reads	Unique reads
WT_3	14168509	11826616	16.53%	10.71%
WT_2	21412622	17398315	18.75%	8.97%
WT_1	13826696	10577152	23.50%	10.09%
Δrbp35 _3	32233131	21309230	33.89%	10.86%
Δrbp35 _2	25965551	22122147	14.80%	10.61%
Δrbp35 _1	14107800	7261472	48.53%	11.50%
Δexp5 _3	16606918	12293728	25.97%	10.11%
Δexp5 _2	39423030	37346923	5.27%	7.03%
Δexp5 _1	25580902	23662841	7.50%	8.29%

** No 3' adapter found, too short after trimming (<18) or low quality*

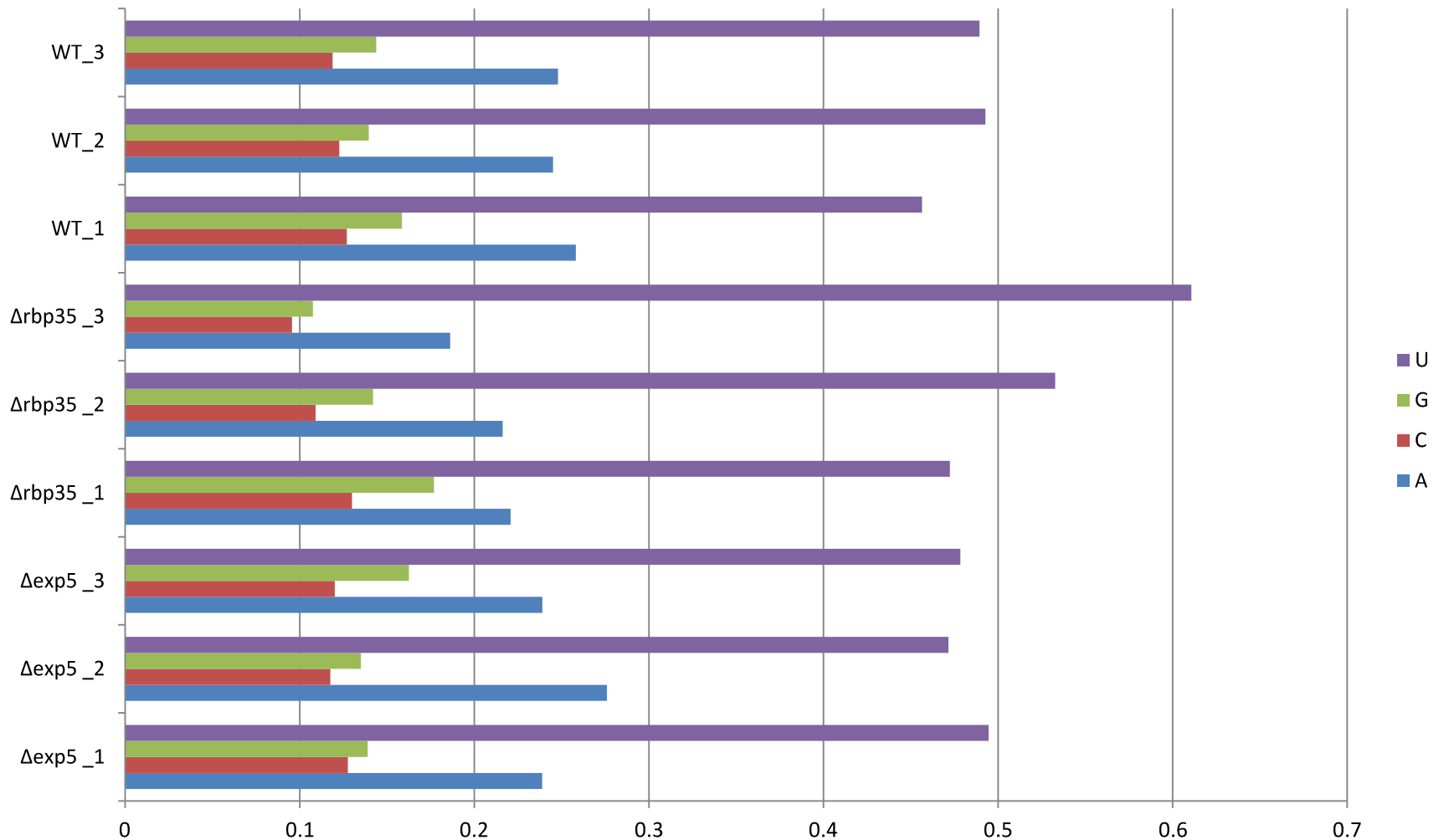
Adapters contamination is variable



Reads length is between 18-25 bp

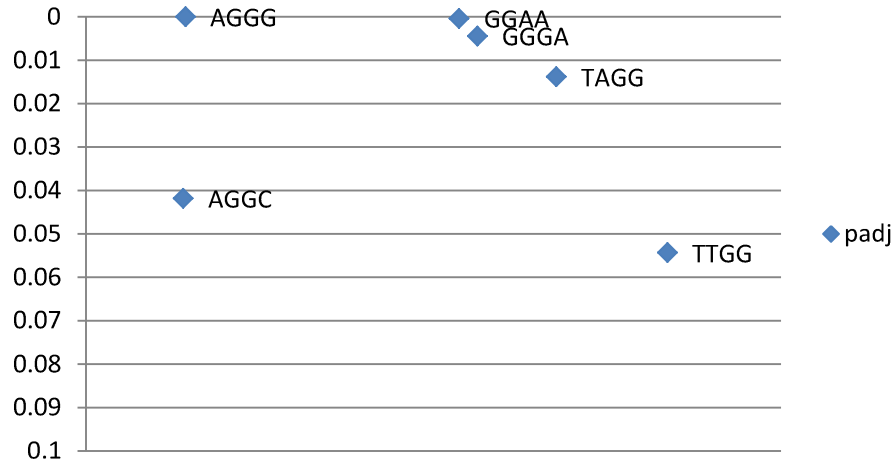


There is an overall U preference as 1st nucleotide

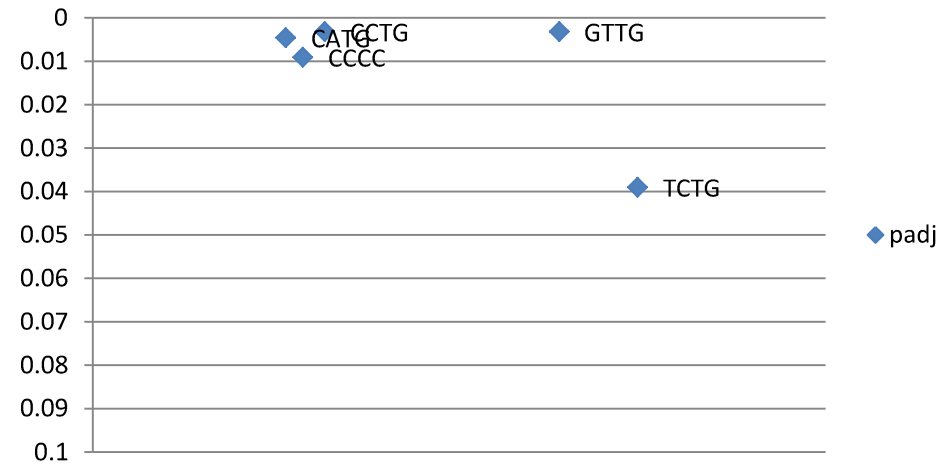


Some HD adapter are significantly changed

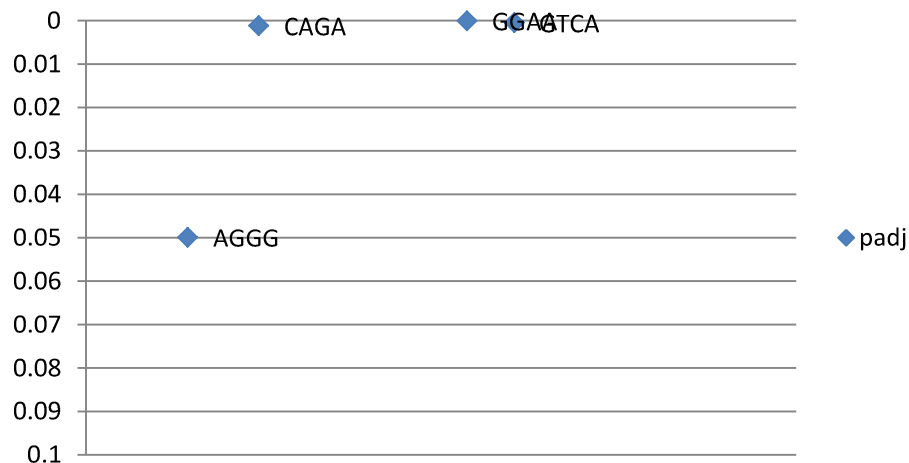
WT -> Δ exp5 5'adapters



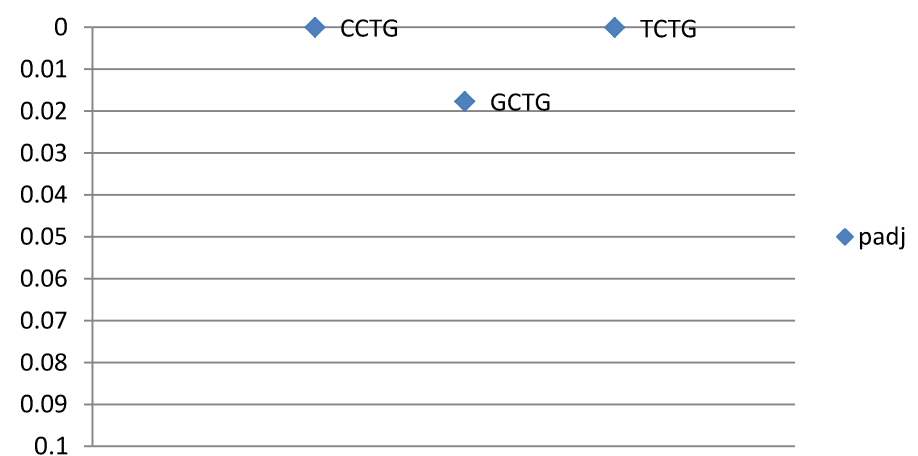
WT -> Δ exp5 3'adapters



WT -> Δ rbp35 5'adapters

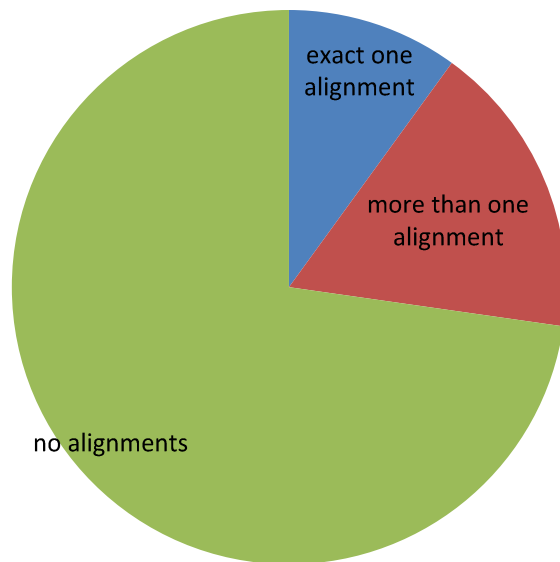


WT -> Δ rbp35 3'adapters

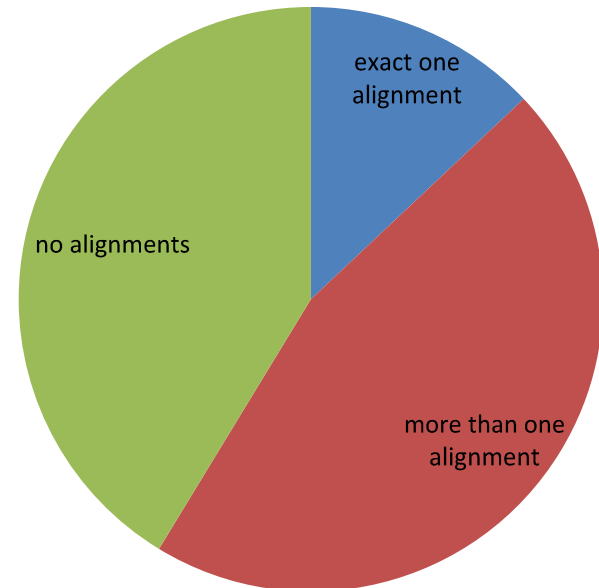


More than 70% of reads do not aligned perfectly on the genome

WT no mismatches

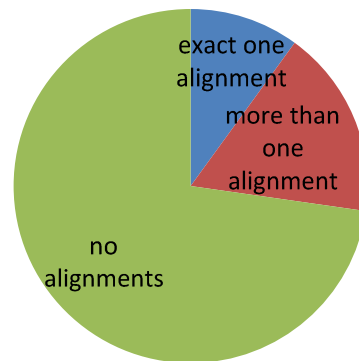


WT mismatches allowed

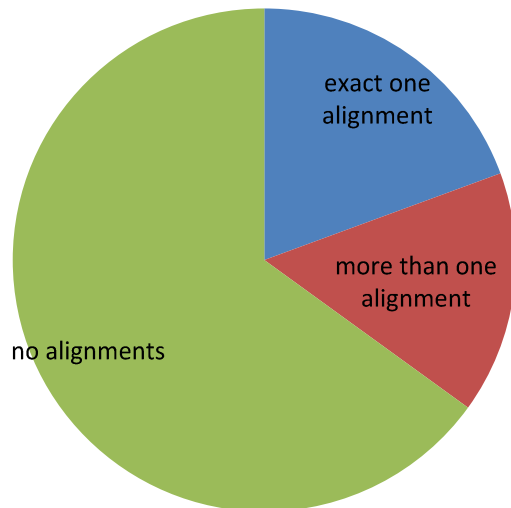


Δ rbp35 aligns a little more, Δ exp5 slightly less

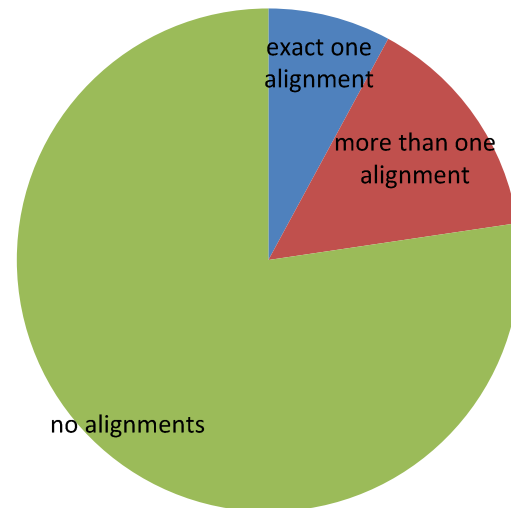
WT no mismatches



Δ rbp35 no mismatches

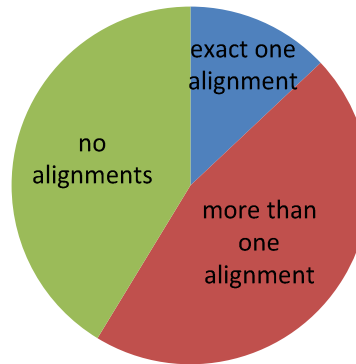


Δ exp5 no mismatches

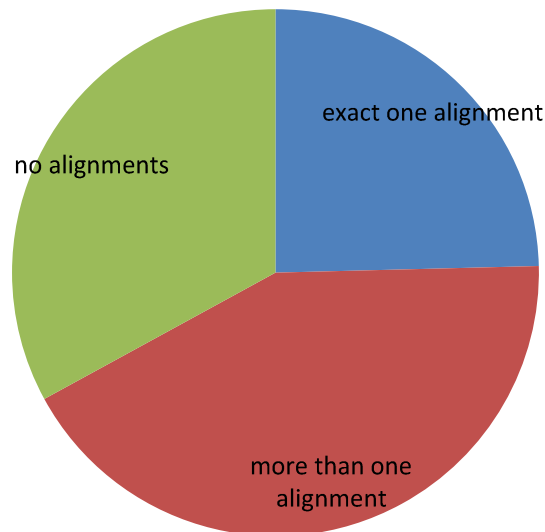


Δ rbp35 aligns a little more, Δ exp5 slightly less

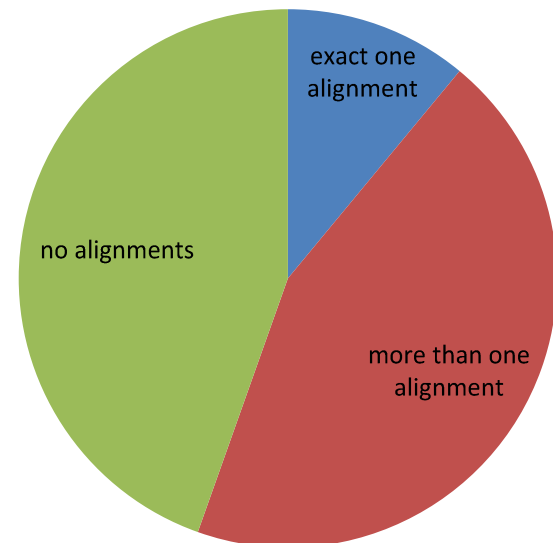
WT mismatches allowed



Δ rbp35 with mismatches

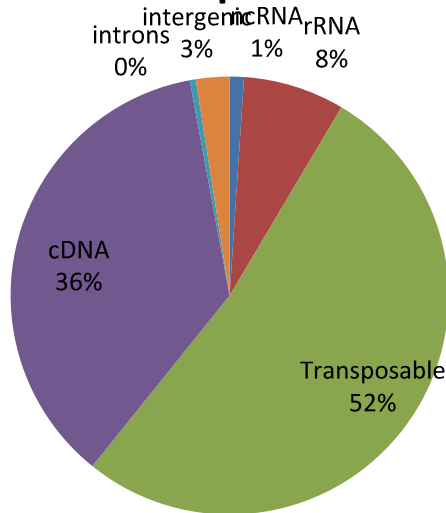


Δ exp5 with mismatches

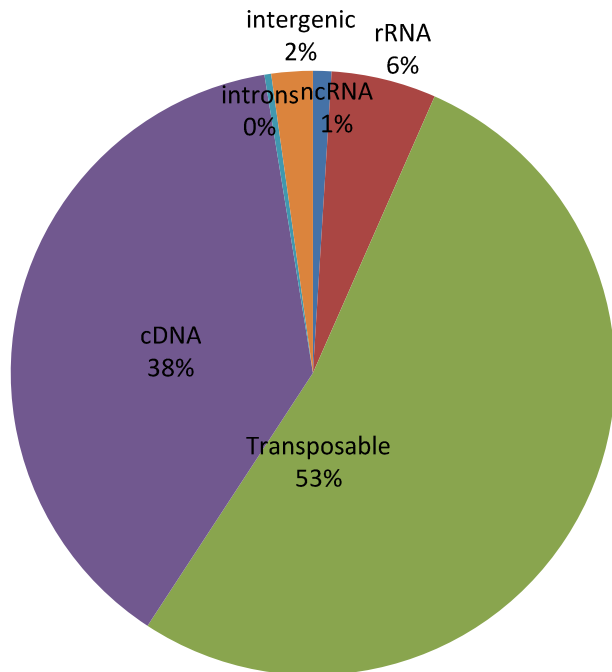


Δ rbp35 produces more reads from cDNA

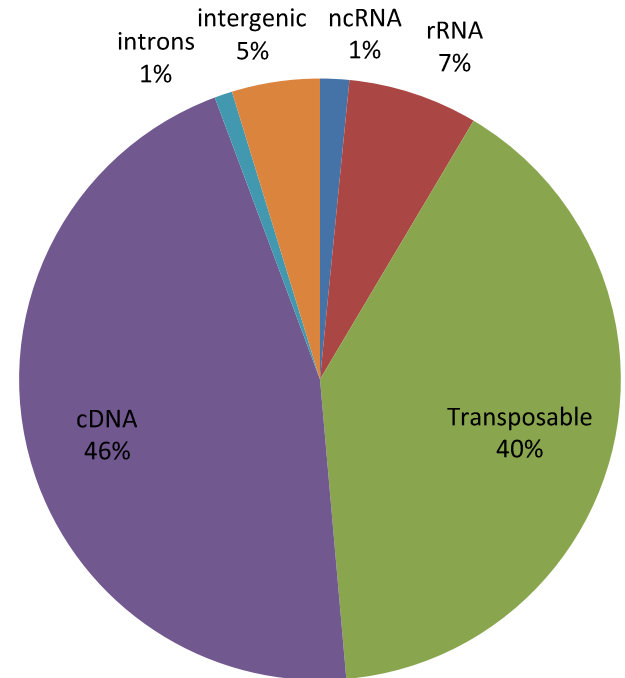
WT unique reads



Δ exp5 unique reads



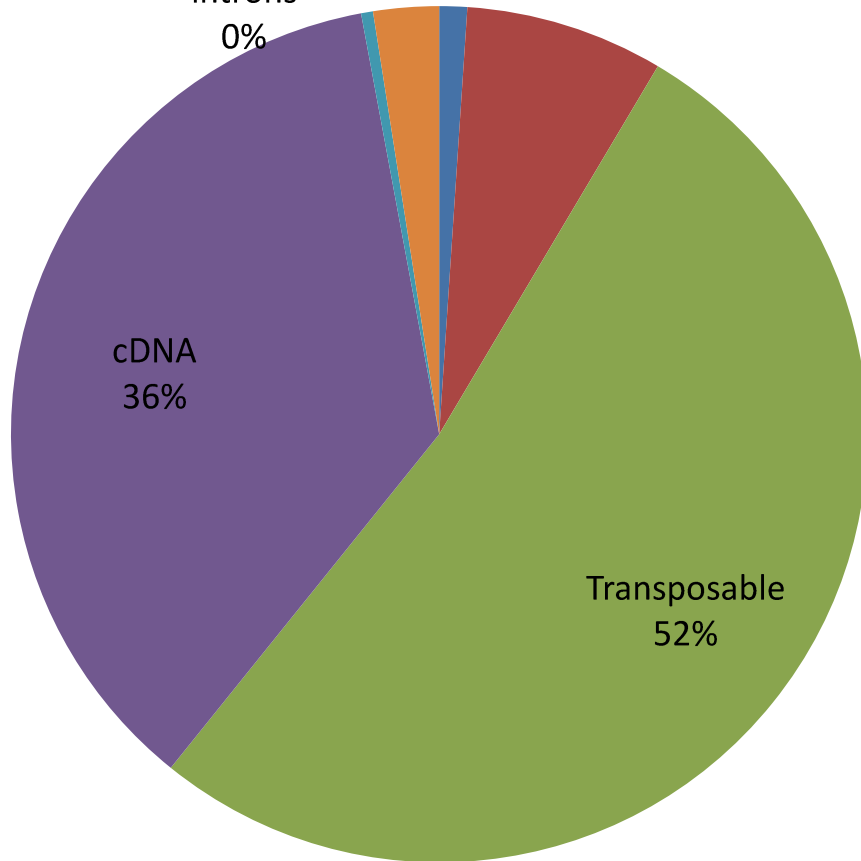
Δ rbp35 unique reads



Transposable elements represent most of the expression

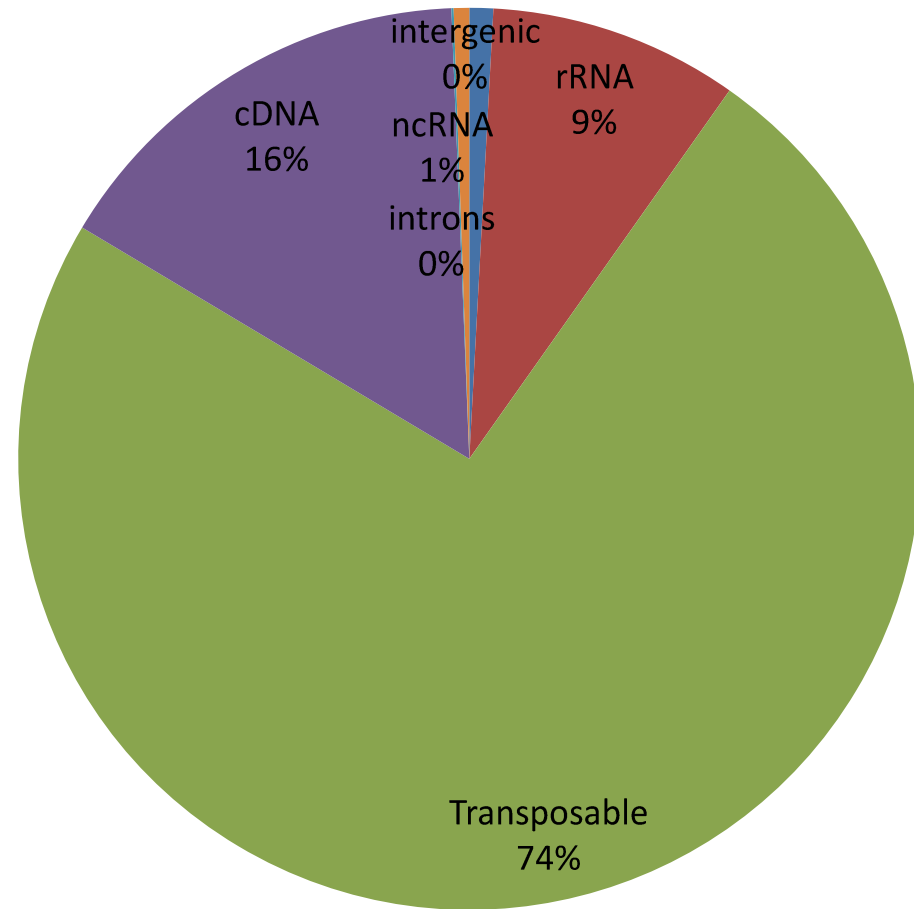
WT unique reads

intergenic 3%
ncRNA 1%
rRNA 8%
introns 0%



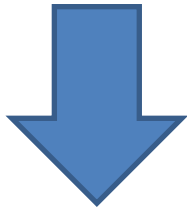
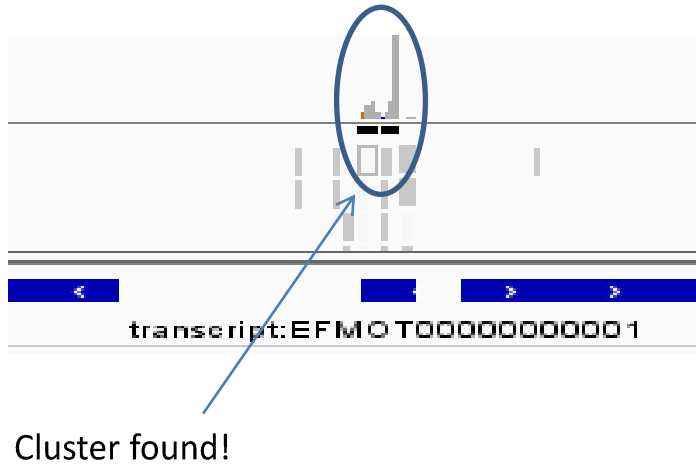
WT expression

intergenic 0%
ncRNA 1%
rRNA 9%
introns 0%

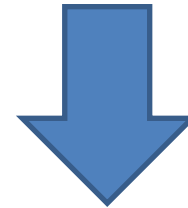
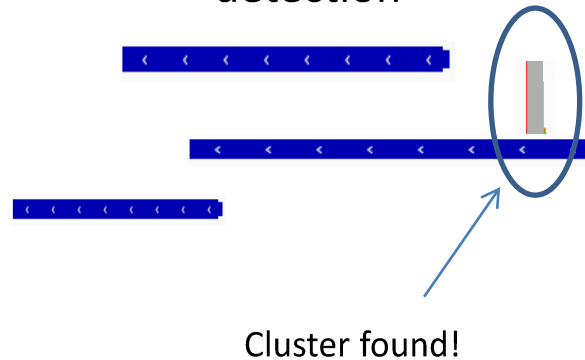


Three approaches to identify changes

Genome clusters detection

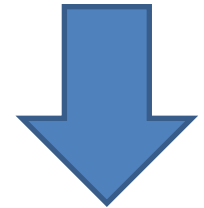


Transcriptome clusters detection



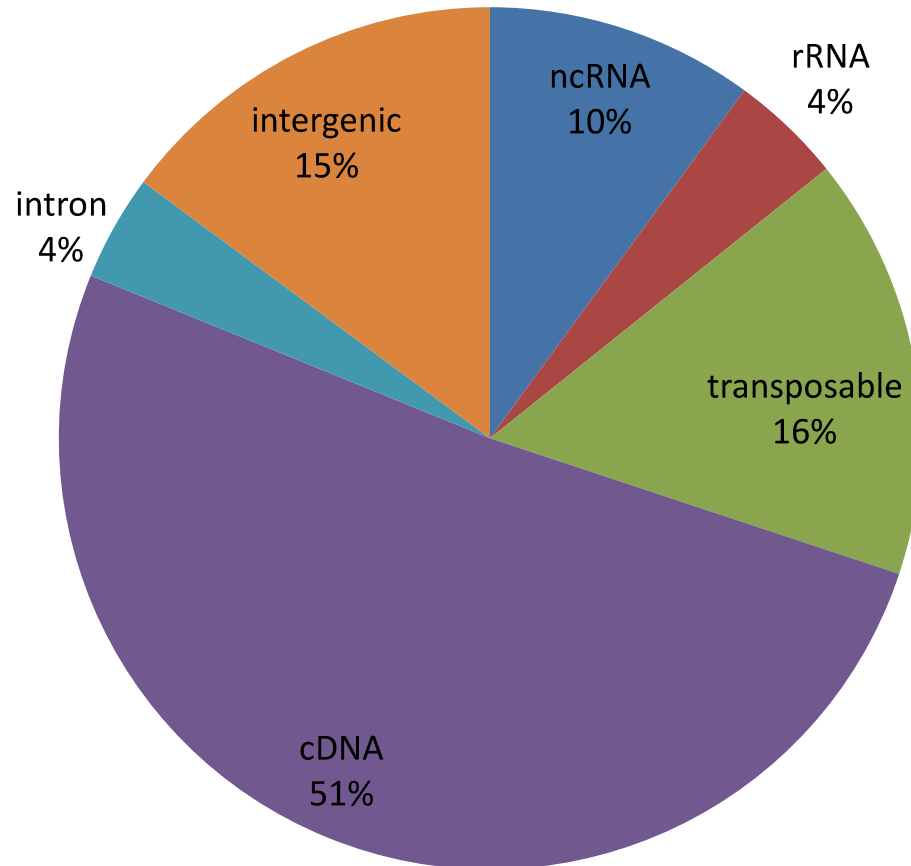
Single read analysis

GGCAACGTGCCTGAGTGGTTAAA
ACGACCATACCCACTG
TCCGGTATGGTGTAGGGGTTAGCATAC
...



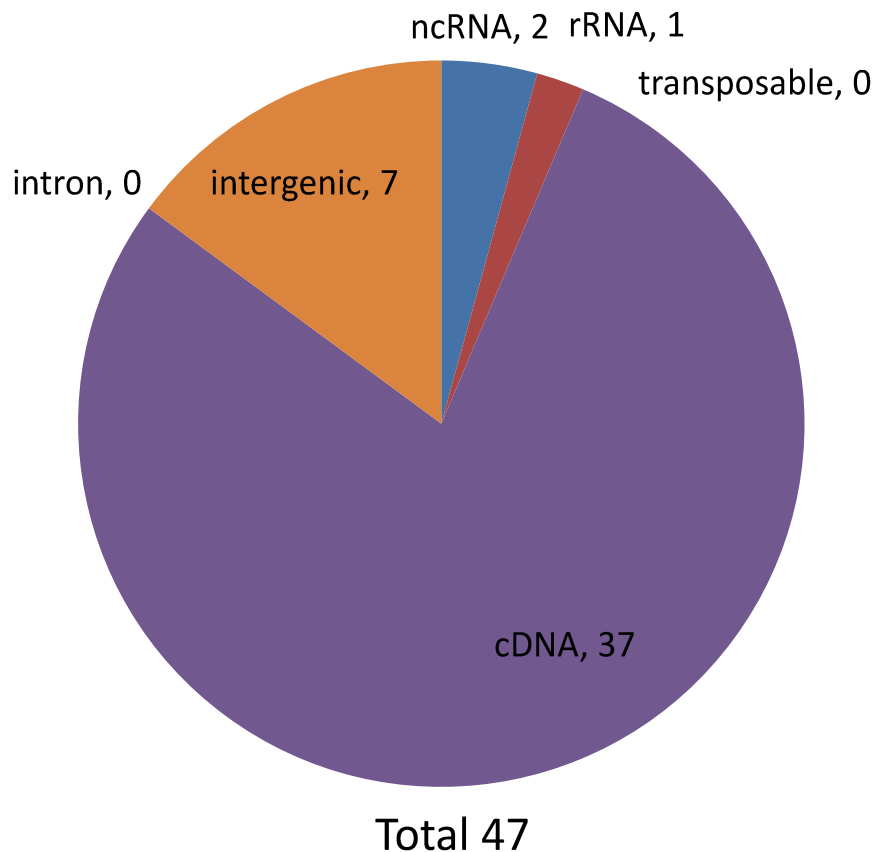
DIFFERENTIAL EXPRESSION

Most of genome cluster loci (3572) come from protein transcripts

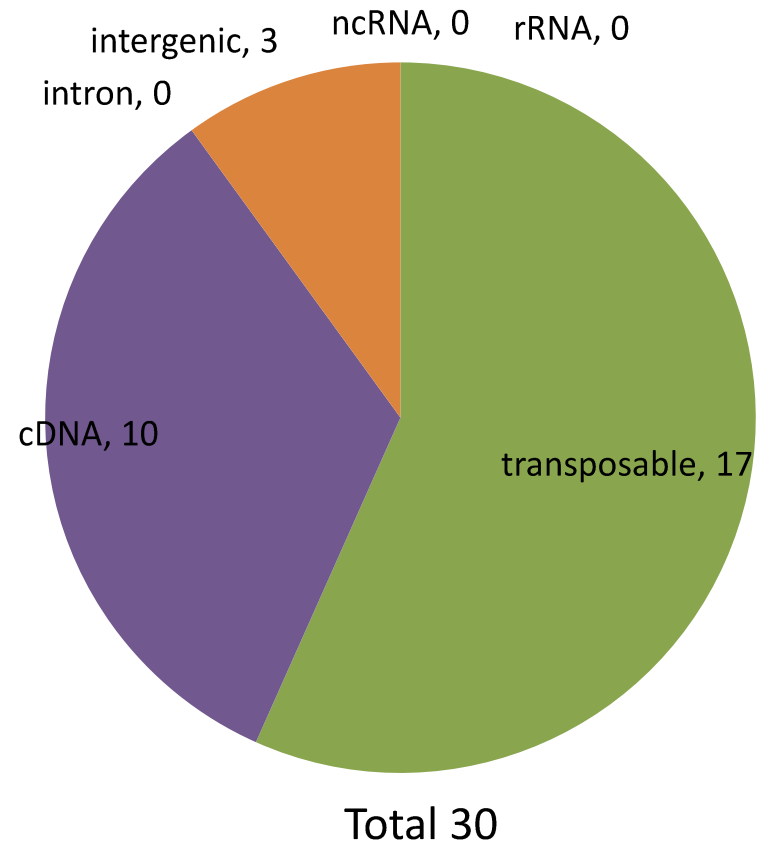


In Δexp5 , a lot of protein-coding related clusters are down regulated, and many transposable elements related clusters are up regulated

WT_vs_ Δexp5 .down



WT_vs_ Δexp5 .up



In Δexp5 , tRNA-lys is downregulated, retro5 clusters are upregulated, and several protein-coding related clusters:

ncRNA	Protein coding	
tRNA-lys	MGG_00246	NADP-dependent alcohol dehydrogenase 6
	MGG_12760	Anthranilate synthase component I; Anthranilate synthase component I, variant
	MGG_10911	Cupin domain-containing protein
	MGG_06868	Acetolactate synthase catalytic subunit
	MGG_02980	DNA-(Apurinic or apyrimidinic site) lyase 2
	MGG_10912	Mycocerosic acid synthase
	MGG_01704	Vacuolar protein-sorting-associated protein 36
	MGG_08072	Cholesterol oxidase
	MGG_10315	Hydrophobin-like protein MPG1
	MGG_01655	Copper radical oxidase
	MGG_15185	Agglutinin isolectin 1
	MGG_05160	Hydrolase
	MGG_08641	Pre-mRNA-splicing factor CWC2
	MGG_02439	Oxidoreductase
	MGG_14897	Polyketide synthase
	MGG_07779	Quinate permease
	MGG_03670	Subtilisin-like proteinase Spm1

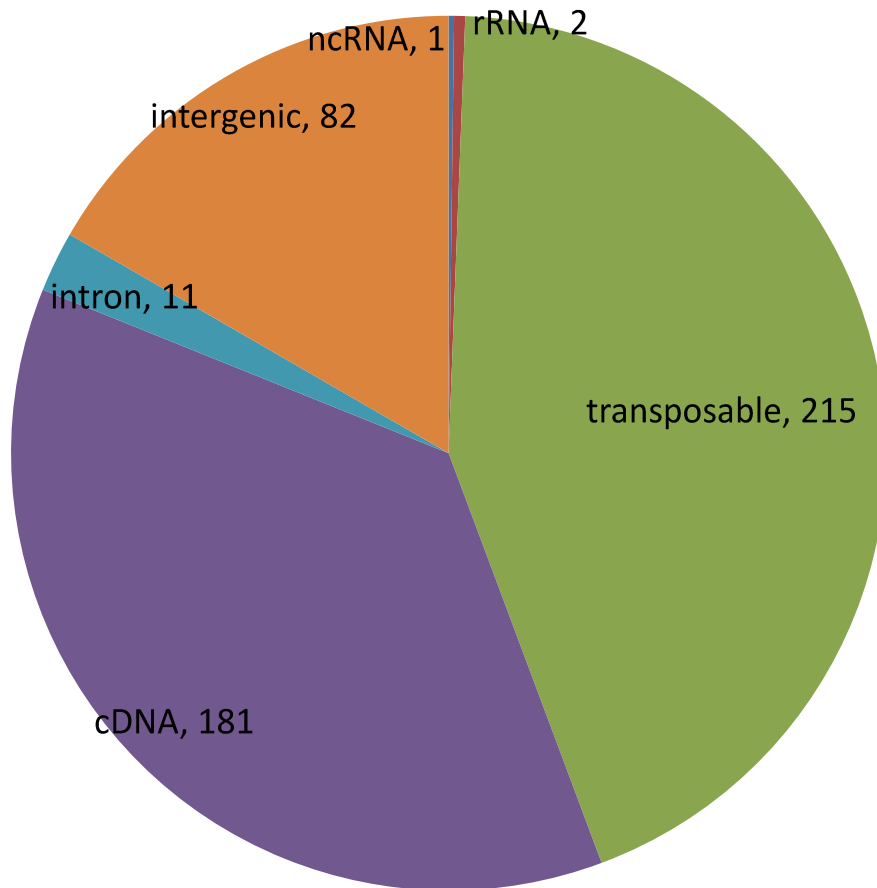
In Δexp5 , retro5 clusters are upregulated, and several protein-coding related clusters:

Transposable element
17 retro5

Protein coding	
MGG_08059	Putative uncharacterized protein
MGG_07571	LysM domain-containing protein
MGG_11176	Putative uncharacterized protein
MGG_08022	Putative uncharacterized protein
MGG_18082	Putative uncharacterized protein
MGG_17196	Putative uncharacterized protein
MGG_06654	Putative uncharacterized protein
MGG_01516	Putative uncharacterized protein

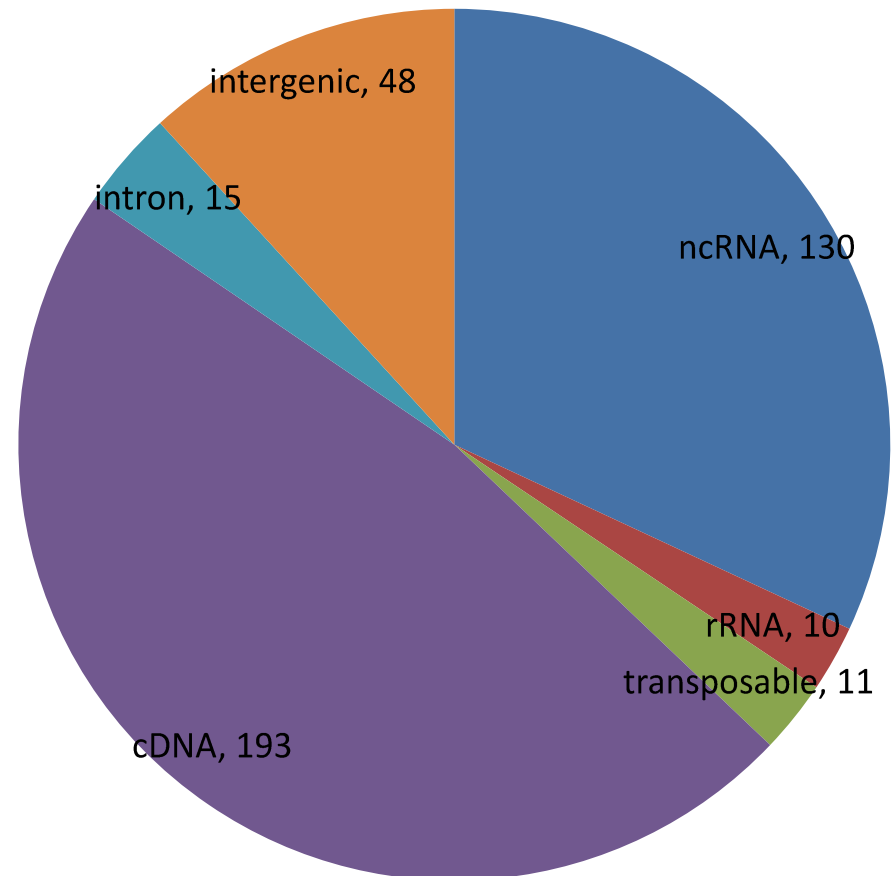
In Δ rbp35, a lot of transposable elements related clusters are down regulated, and many ncRNA related clusters are up regulated

WT_vs_Δrbp35 .down



Total 492

WT_vs_Δrbp35 .up



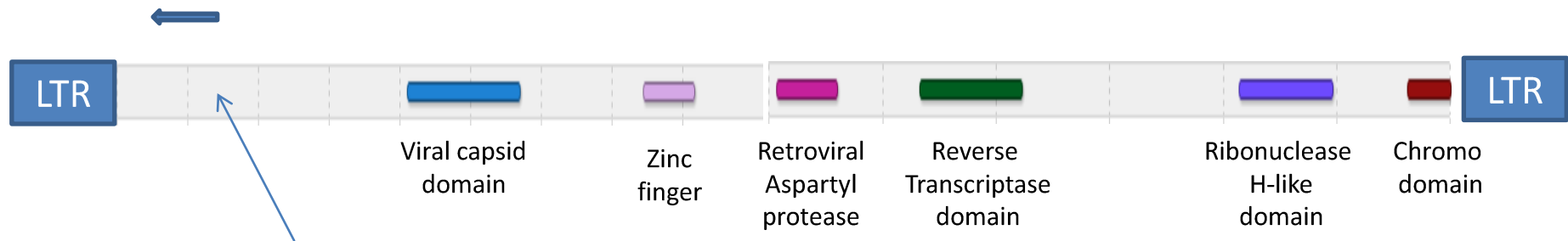
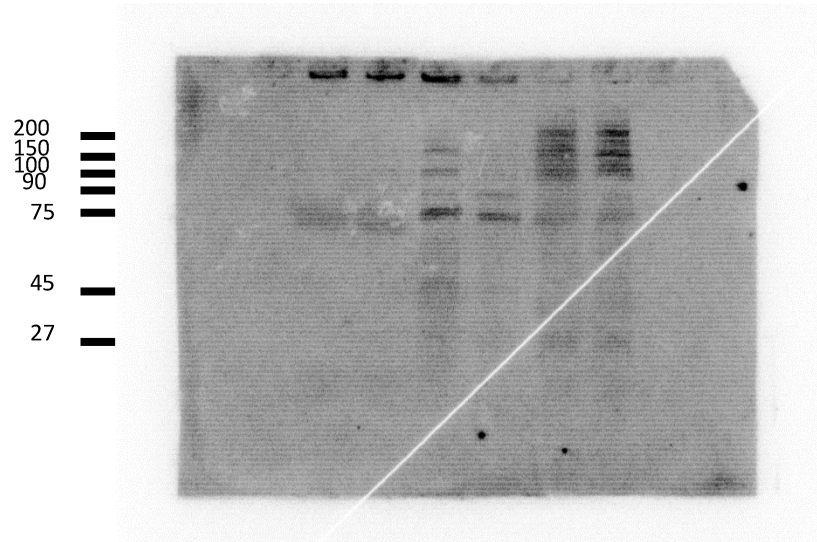
Total 407

In Δ rbp35, a lot of transposable elements (mainly MAGGY and INAGO 1/2) related clusters are down regulated

Transposable element
116 MAGGY
64 INAGO 1
31 INAGO 2
2 Pyret
1 POT2
1 MGRL-3

Protein coding			
MGG_17324	Putative uncharacterized protein		PHI:879
MGG_16535	Putative uncharacterized protein		PHI:871
MGG_04538	Putative uncharacterized protein		PHI:786
MGG_15019	Peroxisomal copper amine oxidase		PHI:2171
MGG_06368	AGC/PKA protein kinase		PHI:2162
MGG_12865	Putative uncharacterized protein		PHI:2131
MGG_05078	Potassium/sodium efflux P-type ATPase		PHI:2098
MGG_03580	Cysteine protease ATG4	ATG4	PHI:2072
MGG_00611	2OG-Fe(II) oxygenase		
MGG_12839	Actin cytoskeleton-regulatory complex protein PAN1		
MGG_15020	Amino acid permease		
MGG_12760	Anthranilate synthase component I; Anthranilate synthase component I, variant		
MGG_04855	ATP-dependent bile acid permease		
MGG_04179	ATP-dependent RNA helicase DBP10	DBP10	
MGG_06335	Calpain-7		
MGG_06327	Candidapepsin-3		
MGG_04407	Cation efflux family protein		
MGG_08072	Cholesterol oxidase		
MGG_09930	Chromodomain-helicase-DNA-binding protein 3		
MGG_07098	Condensin subunit		
MGG_06470	DNA repair helicase RAD25		
MGG_02804	DNA repair protein rad16		

Adriana's Mo-3 is down-regulated in *Δrbp35*, but not in *Δexp5* ;)



MAGGY RETRONTRASPONSON


In Δ rbp35, many ncRNA related clusters are up regulated

ncRNA	Transposable element	Protein coding	
tRNA-Ala	17 MINE	MGG_04582	Putative uncharacterized protein
tRNA-Arg			
tRNA-Asn		MGG_02961	Anucleate primary sterigmata protein A
tRNA-Asp	7 Pyret	MGG_11454	Vacuolar calcium ion transporter
tRNA-Cys		MGG_02074	Potassium/sodium efflux P-type ATPase
tRNA-Gln		MGG_00454	Putative uncharacterized protein
tRNA-Gly	1 MGRL-3		
tRNA-His		MGG_00865	1,3-beta-glucan synthase component FKS1
tRNA-Ile			
tRNA-Leu	1 MGL	MGG_03372	40S ribosomal protein S20
tRNA-Lys		MGG_06657	40S ribosomal protein S25
tRNA-Met		MGG_02392	40S ribosomal protein S30
tRNA-Phe		MGG_02747	40S ribosomal protein S4-A
tRNA-Pro		MGG_06658	40S ribosomal protein S5
tRNA-Pseudo		MGG_06712	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase
tRNA-Ser		MGG_12403	5'-3' exoribonuclease 1
tRNA-Thr		MGG_09194	60S ribosomal protein L17
tRNA-Trp		MGG_04484	60S ribosomal protein L18
tRNA-Undet			
tRNA-Val		MGG_04582	Putative uncharacterized protein
U6			
A. fumigatus snoRNA			
Afu_300			
A. fumigatus snoRNA			
Afu_335			

4 classes of siRNAs were discovered in Mucor

Endogenous short RNAs generated by Dicer 2 and RNA-dependent RNA polymerase 1 regulate mRNAs in the basal fungus *Mucor circinelloides*

Francisco Esteban Nicolas¹, Simon Moxon², Juan P. de Haro³, Silvia Calo³,
Igor V. Grigoriev⁴, Santiago Torres-Martínez³, Vincent Moulton²,
Rosa M. Ruiz-Vázquez^{3,*} and Tamas Dalmay^{1,*}

 Author Affiliations

*To whom correspondence should be addressed. Tel: +34 868 887136; Fax: +34 868 883963; Email: rmruiz@um.es

Correspondence may also be addressed to Tamas Dalmay. Tel: +0044 1603 593221; Fax: +0044 1603 592250; Email: t.dalmay@uea.ac.uk

Received January 10, 2010.
Revision received March 9, 2010.
Accepted April 11, 2010.

Dicer1

Dicer2

RdP1

Rdp2

This is how siRNAs supposedly work...

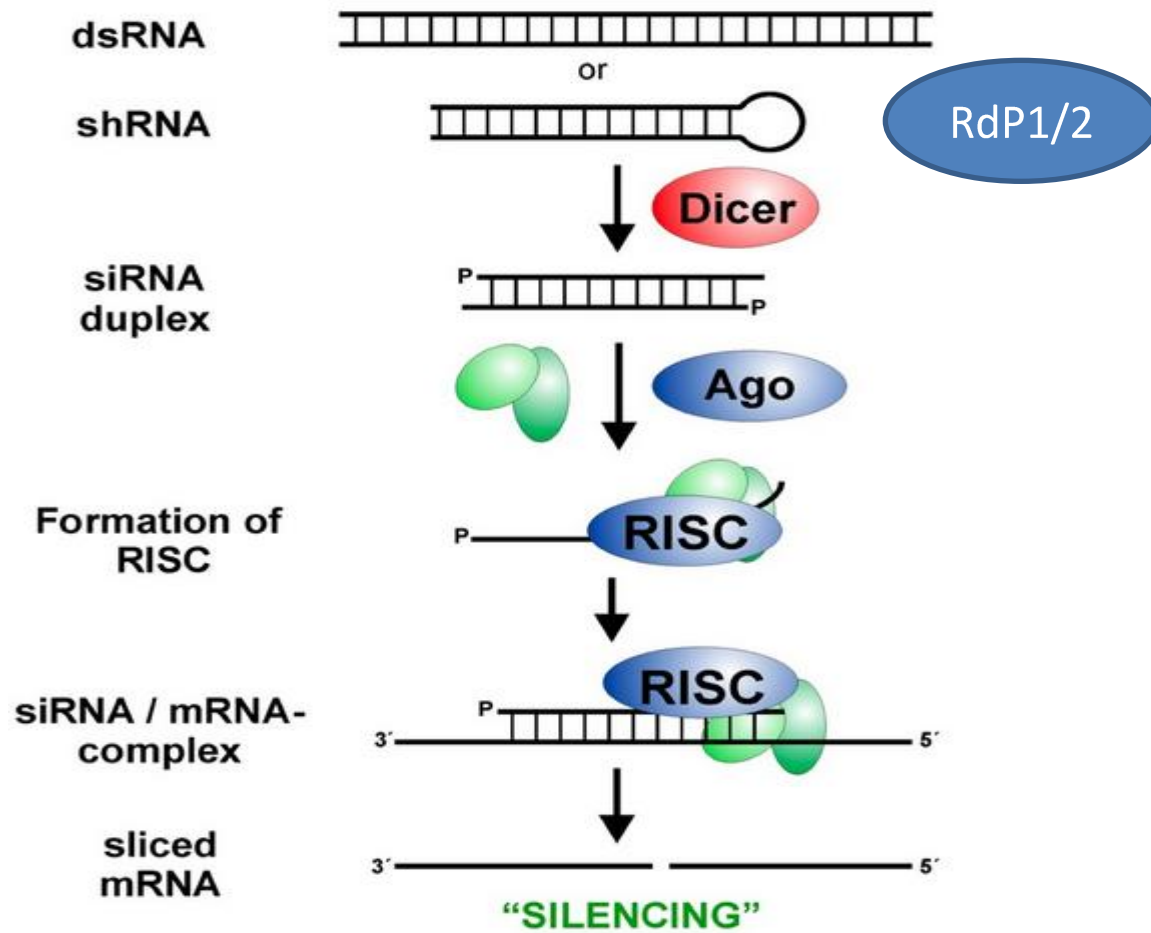
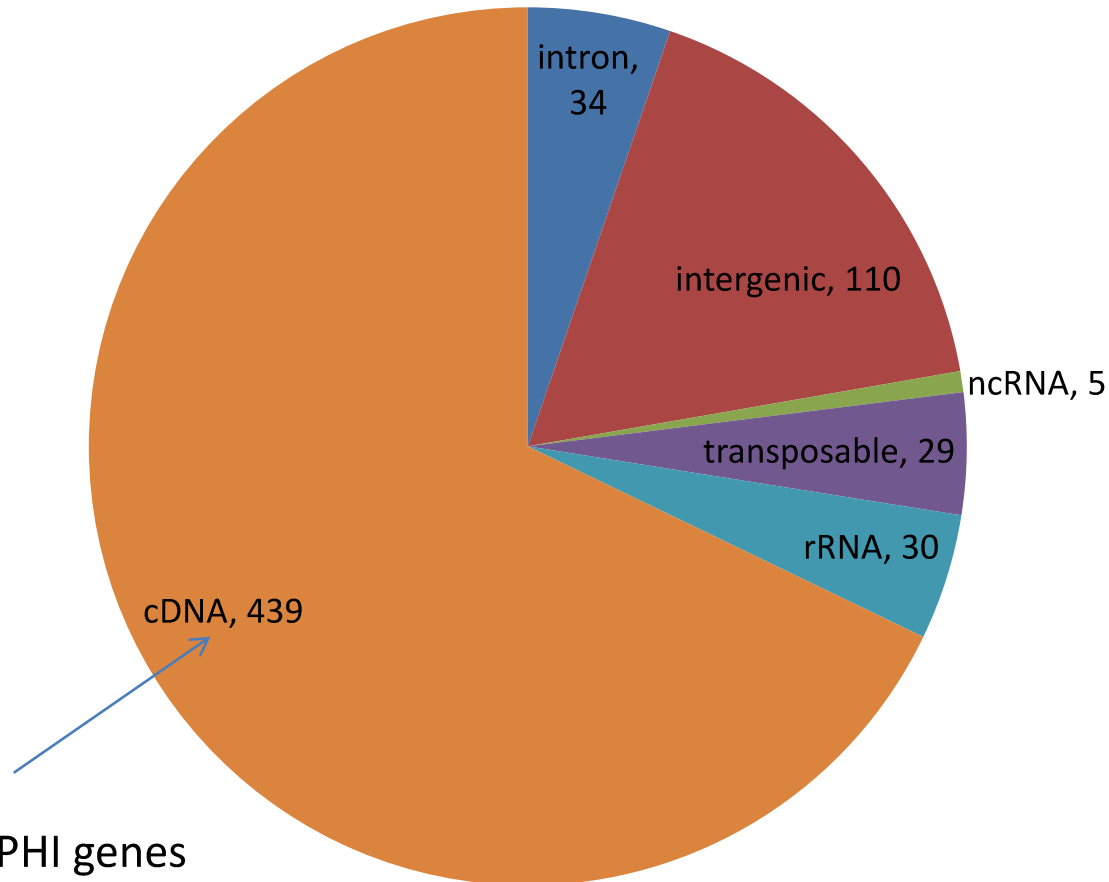


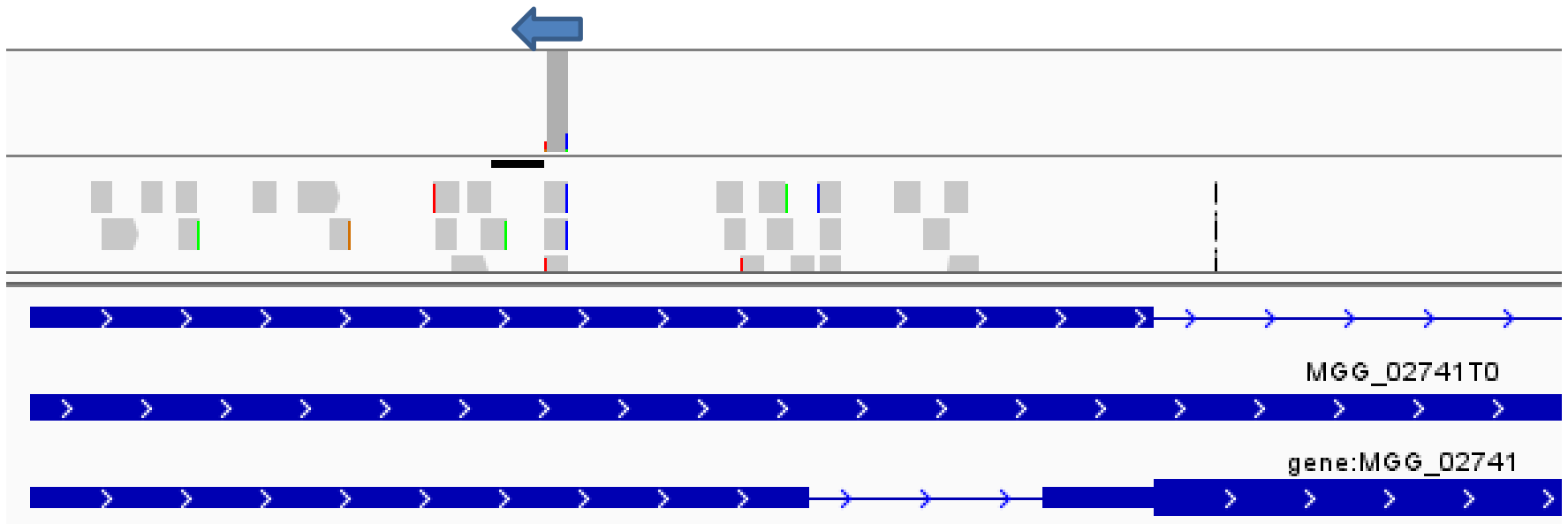
Diagram illustrating the major steps in siRNA biogenesis and subsequent siRNA-mediated gene silencing.

647 siRNAs from the three strains were identified, mostly from protein-coding genes



Including 17 PHI genes

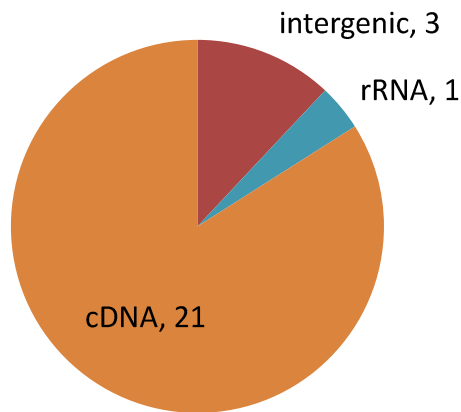
RBP35 itself has a siRNA in its 5'UTR,
before its “famous” intron



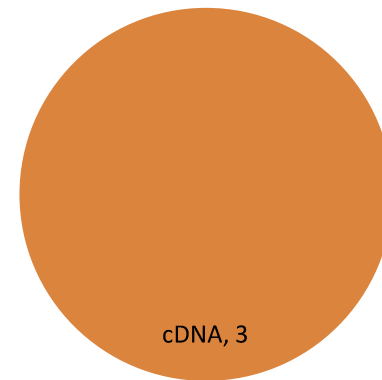
Unlike RBP35, most of siRNAs identified have a SNP, possibly a post transcriptional modification (or some kind of error of mine ;) They are also very short, 18-20 bp.....:-/

27 and 134 siRNA are differentially expressed in $\Delta exp5$ and $\Delta rbp35$, respectively

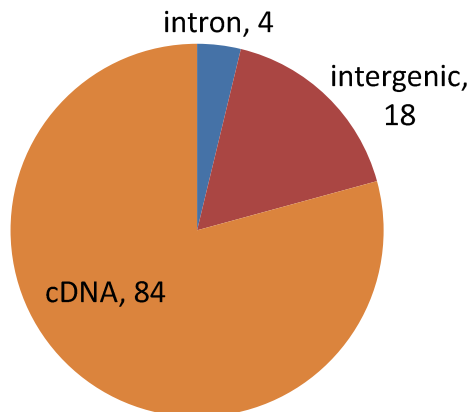
WT_vs_Δexp5 .down



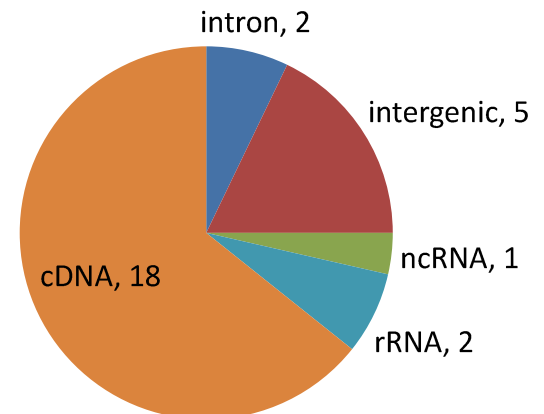
WT_vs_Δexp5 .up



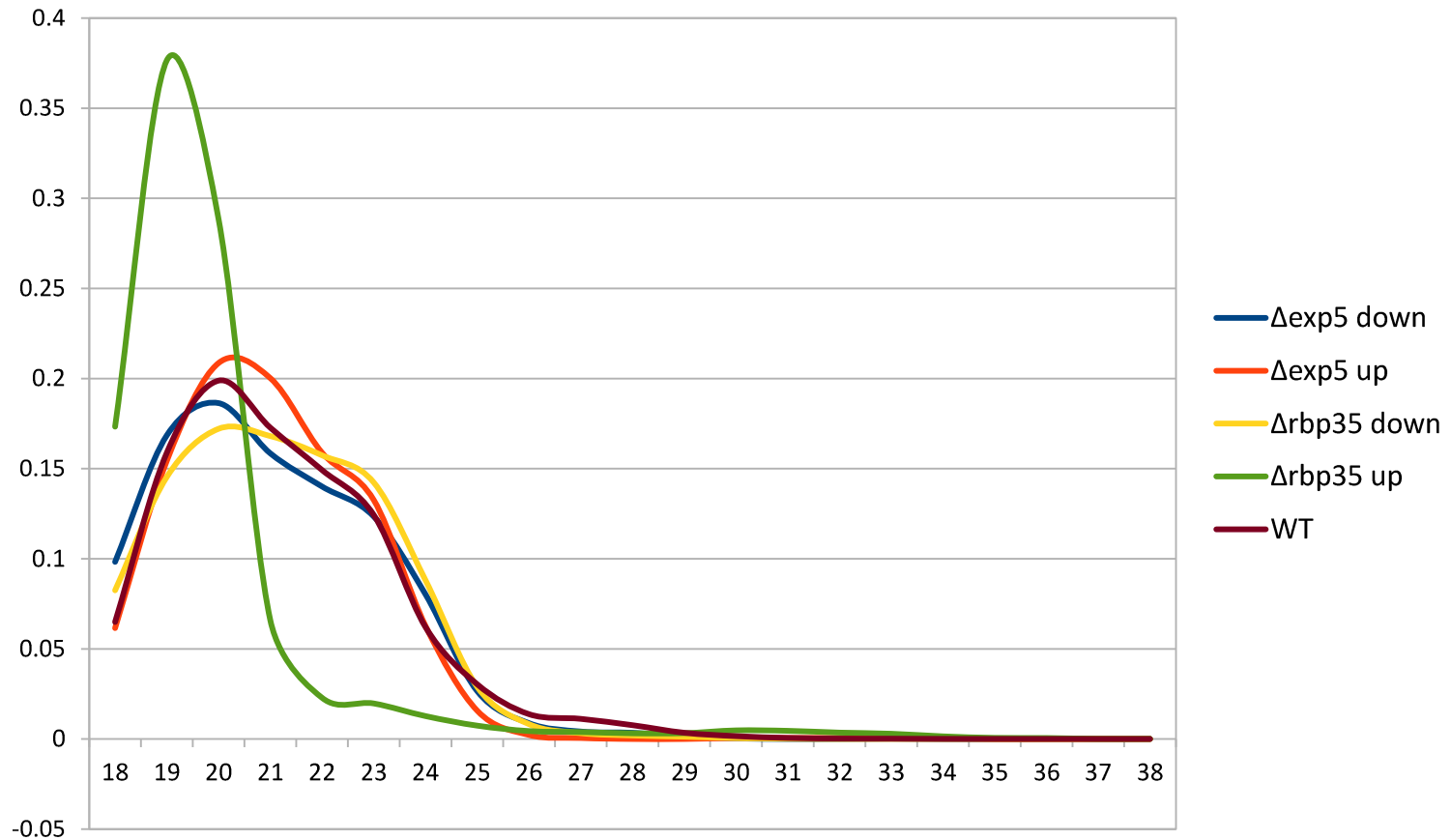
WT_vs_Δrbp35 .down



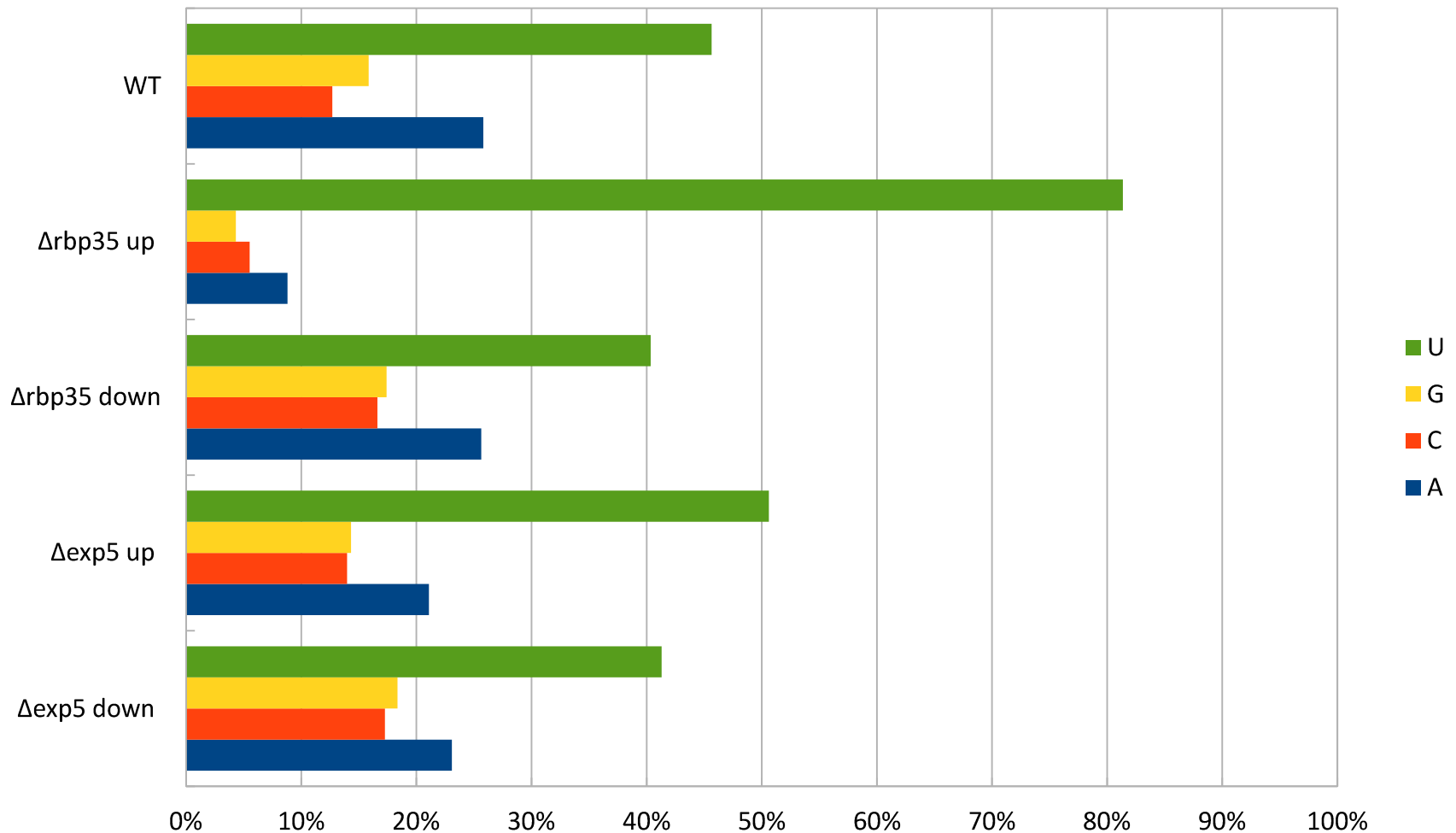
WT_vs_Δrbp35 .up



Δ rbp35 produces shorter smallRNA

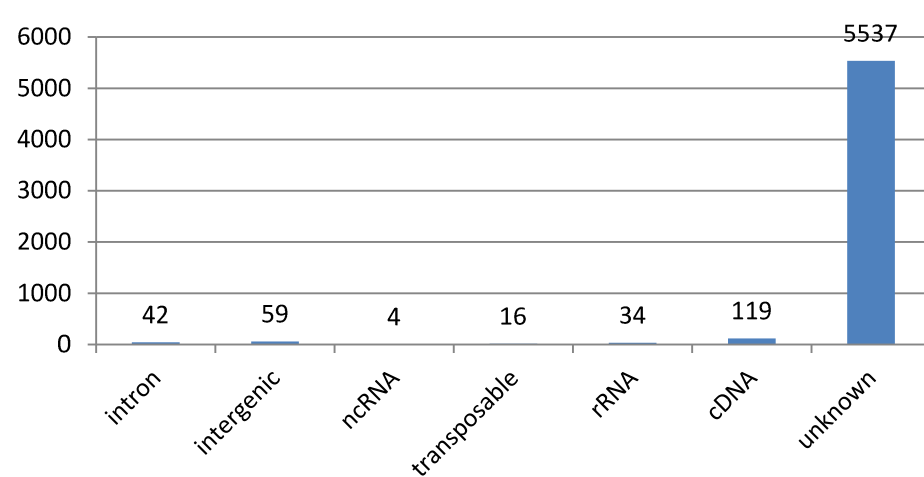


Δ rbp35 produces smallRNA with 1st nucleotide U-preference

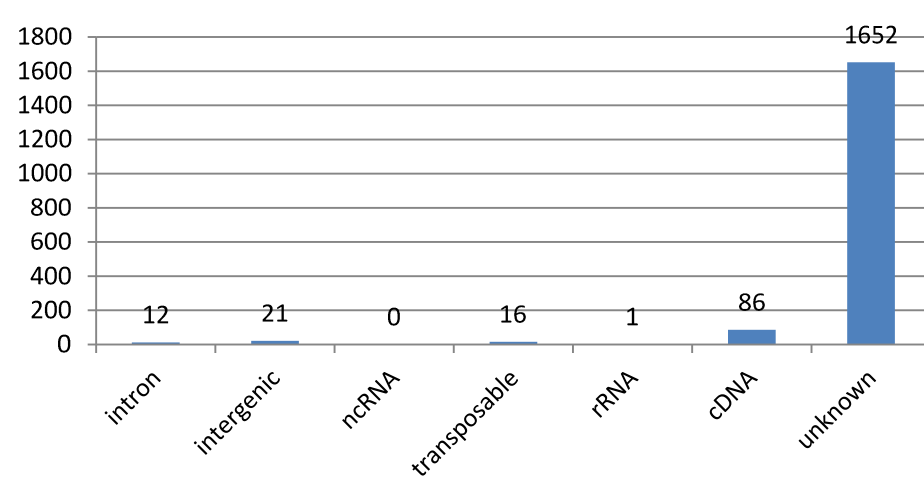


A lot of differentially expressed unique reads are of unknown origin

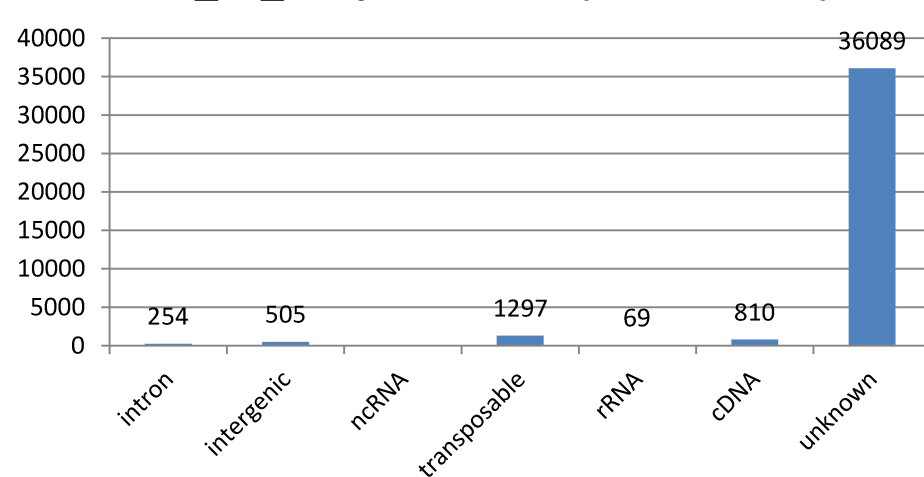
WT_vs_Δexp5 .down (5811 total)



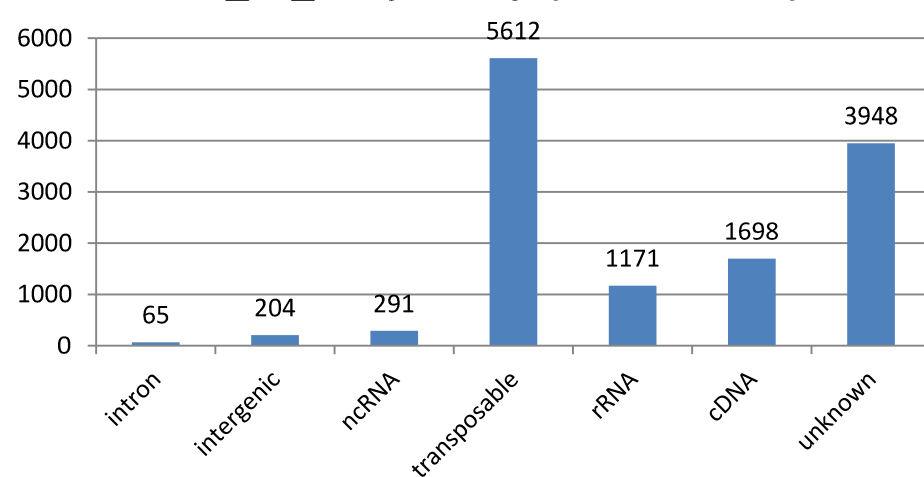
WT_vs_Δexp5 .up (1788 total)



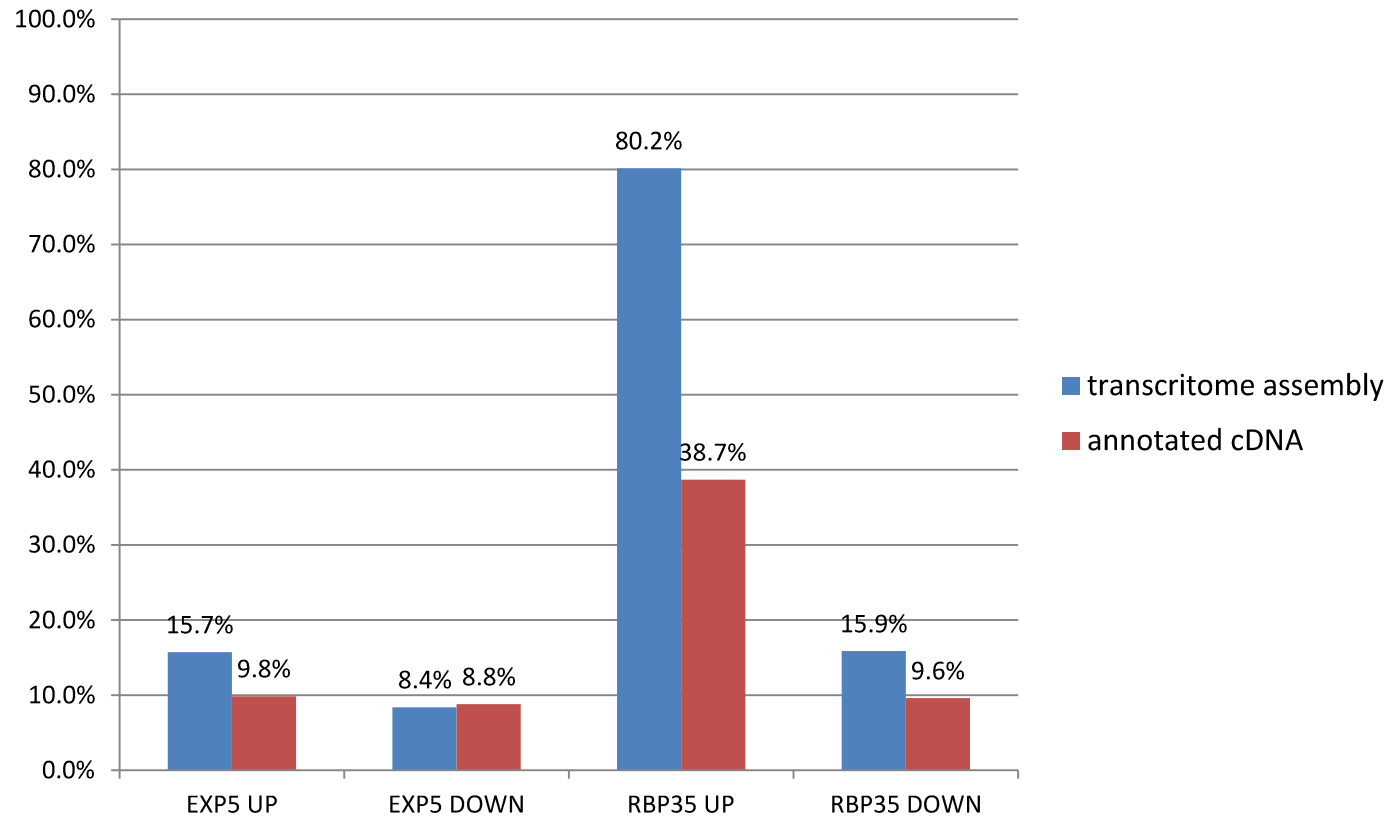
WT_vs_Δrbp35 .down (39024 total)



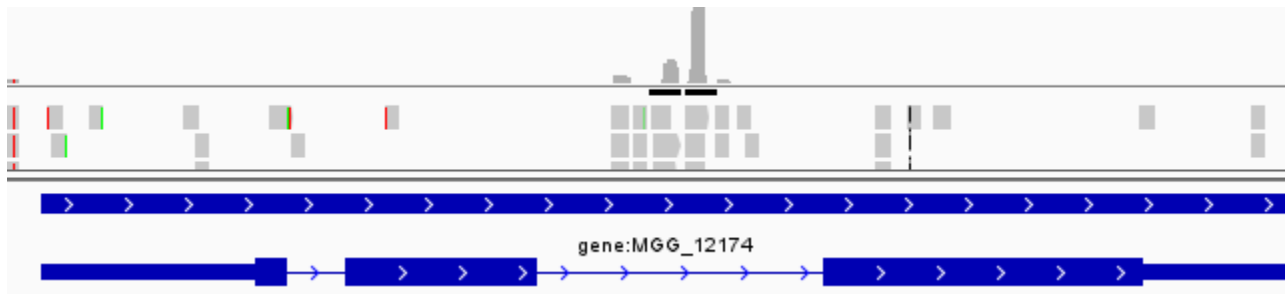
WT_vs_Δrbp35 .up (12989 total)



Is RBP35 involved in alternative splicing?



Nothing notable about Adriana's Mo-2



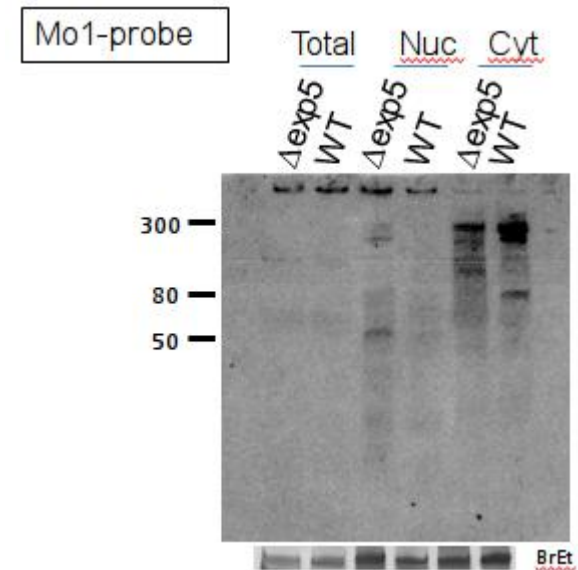
Probably, an intronic snoRNA

Adriana's Mo-1 is belongs to an unknown, but conserved RNA class. Anyway, they don't seem to be affected by the two mutants



OTHER GENES:

Pre-RNA processing factor 39
GET1
Nuclear pore complex protein Nup107
Serine hydroxymethyltransferase
A subunit of the AP-1 complex
A extracellular serine-threonine rich protein



Similar structure conserved in all pezyozyma clade!

Next steps...

- Find out what all those unknown reads are
- Classify better siRNAs and their genes, is the situation conserved? (mucor)
- Analyze the transcriptome as well
- Mix the results with polyadenylation study
- Reads shortening and 1st nucleotide preference in rbp35 is related with anything?
- Redo the analysis on GUY11 genome
- ...