

**[Supplementary Information]**

**The calmodulin fused kinase novel gene family is the major system in plants converting Ca<sup>2+</sup> signals to protein phosphorylation responses**

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**Table S2.** CFKs from *Arabidopsis thaliana* enrolled in pollen development from D

subfamily.

<b>pollen development</b>	<b>myristoylation</b>	<b>palmytoylation</b>	<b>group</b>
At2g17290	y	y	d3
At2g38910	y	y	d3
At3G10660	y	y	d3
At2g35890	y	y	d3
At5G04870	y	y	d3
At4G35310	y	y	d3
At1g18890	y	y	d2
At1g74740	y	y	d2
At2g41860	y	y	d2
At3g57530	y	y	d2
At5g19450	y	y	d2
At5g12480	y	y	d2
At3g51850	y	y	d2
At2g31500	y	y	d2
At5g19360	y	y	d1
At5g12180	y	y	d1
At4g23650	y	y	d1
At3g20410	y	y	d1
At4g04740	y	y	d1
At4g21940	y	y	d1
At4g04710	y	y	d1
At4G04700	y	y	d1

**Figure S1** The details of the phylogenetic tree in Figure 1. The maximum-likelihood local supporting value was produced by FastTree software. Branches are colored to represent the origins from the five eukaryotic supergroups. Green: Archaeplastida, Blue: Opisthokonta, Red: SAR, Black: Amoebozoa, Purple: Excavata.

**Figure S2** The four characteristic insertions in the kinase domain are represented as sequence logo format.

Insertion 1 and 3 each has one amino acid, insertion 2 and 4 each has three amino acids.

**Figure S3** Distribution of X-monophylic members in eukaryotic supergroups.

**Figure S4** X-monophylic members in Eukaryotes other than plants are possibly derived from plant X-monophylic members via horizontal gene transfers.

The plant X-monophylic members are shown in green, and the non-plant X-monophylic members are shown in red. The arrow indicates the possible horizontal gene transfer events.

**Figure S5** The whole maximum-likelihood phylogenetic tree of CFK genes generated with CFKs from all sampled plants.

Abbreviations of species are shown in Table S1.

**Figure S6** Enrichment and expansion of CFKs in the order Brassicales.

(A) The genome density of CFKs amongst green plant representatives. (B) Expansion of CFKs via various forms, and rounds of duplication.

**Figure S7** Expression profile of CFKs in *Arabidopsis thaliana* and a B5 member in *Medicago truncatula*.



Expression profile of AtCFKs includes developmental stages of 8 organs/parts, cold, osmotic stress, salt, drought, genotoxic, oxidative, UV-B, wounding, and heat treatment, pseudomonas, phytophthora, and elicitors treatments. Expression profile of *M. truncatula* CFK includes different organs and developmental stages.

**Figure S8** Functions of CFKs in *Arabidopsis thaliana*.

Domains, motifs, and modifications along with corresponding subcellular localizations are shown. Functions on three aspects (development, abiotic and biotic Stress, Plant-microbe communication) are based on expressional analyses as in Fig. S7 and previous reports (Boudsocq & Sheen, 2013). \* indicates that it was reported by Boudsocq and Sheen (Boudsocq & Sheen, 2013). #\\$ represents reports by Aldous et al. (Aldous, 2014) and Yang et al. (Yang, 2015), respectively.

**Figure S9** Expression profile of four CFKs from *P. patens* under the treatments of salt and drought stresses, respectively.

**Figure S10** The expressional profiles of subfamily D CFKs from *Physcomitrella patens* between the sporophyte stage (mean\_spores) and the gametophyte stage (mean\_gametophore).

Details of the samples and the experiment could be found by Khraiwesh et al. 2015 (Khraiwesh, 2015).

**File S1.** Representative sequences that have innovative gene structure compared to the typical structure composed of a KD and a CaM-LD.

## References

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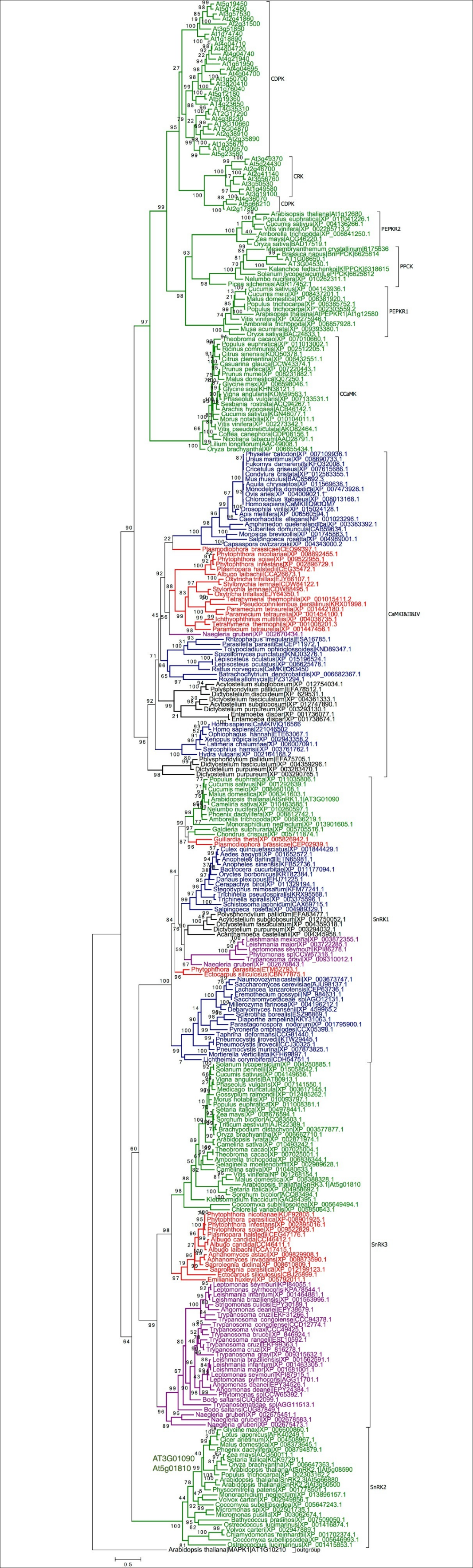
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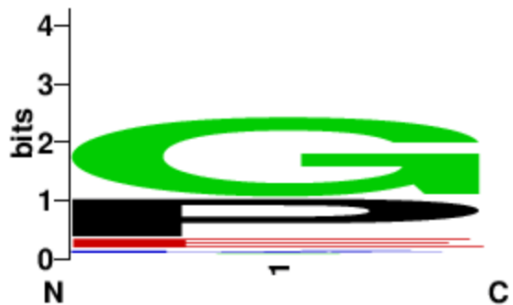
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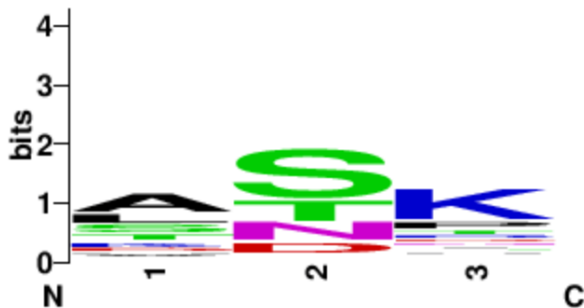
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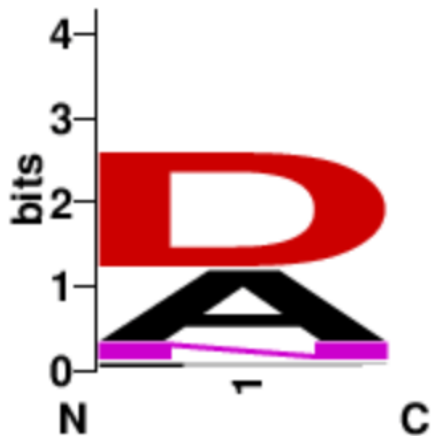




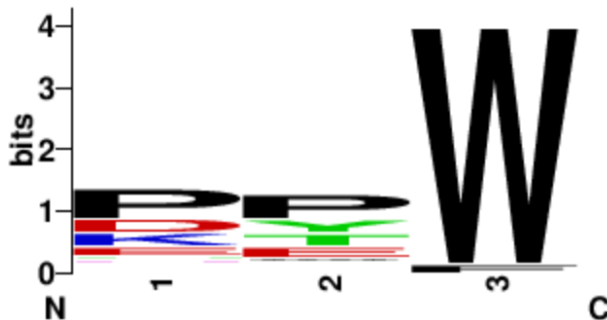
insertion 1



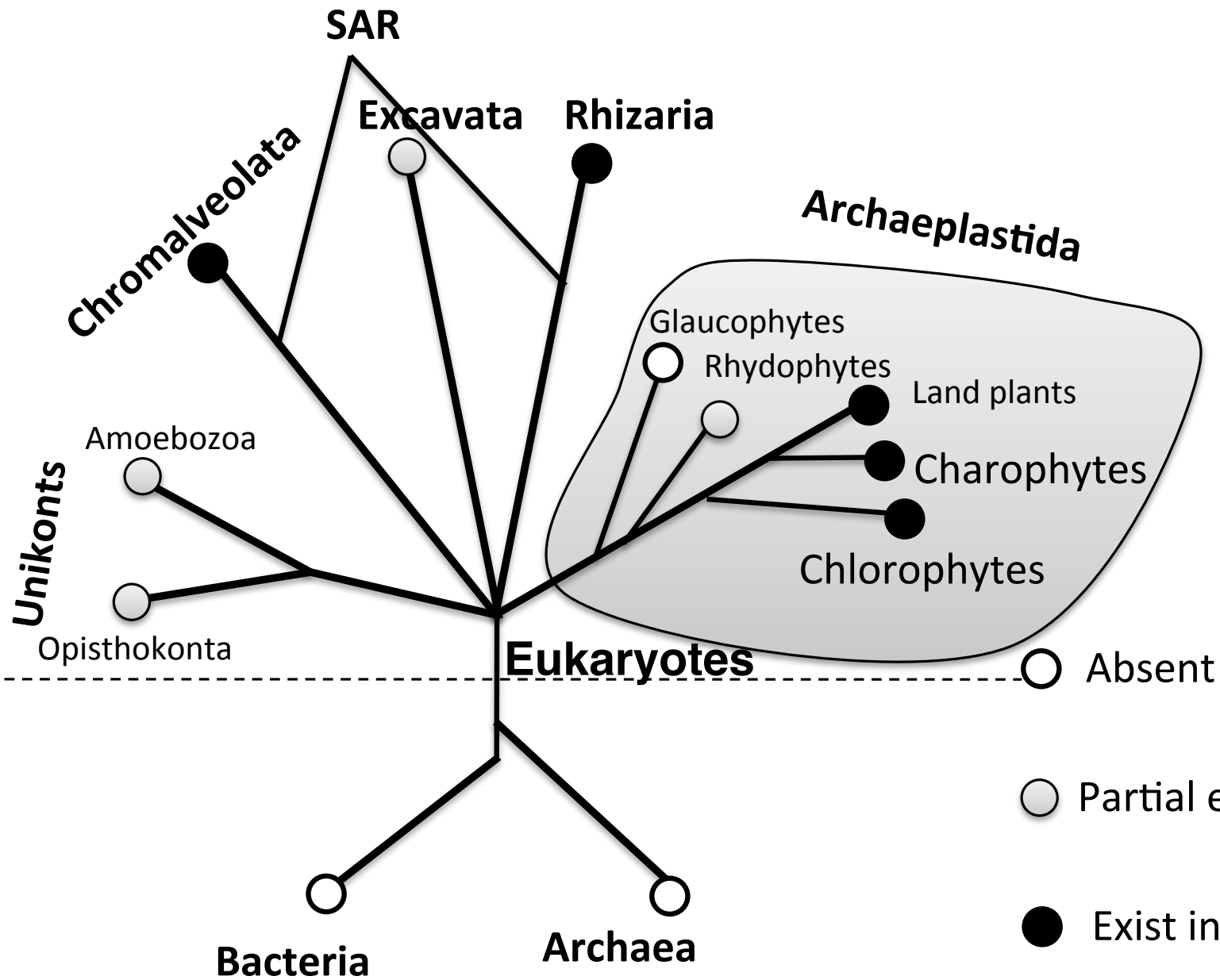
insertion 2



insertion 3



insertion 4



**SAR**

**Chromalveolata**

**Excavata**

**Rhizaria**

**Archaeplastida**

Glaucophytes

Rhodophytes

Land plants

Charophytes

Chlorophytes

**Unikonts**

Amoebozoa

Opisthokonta

**Eukaryotes**

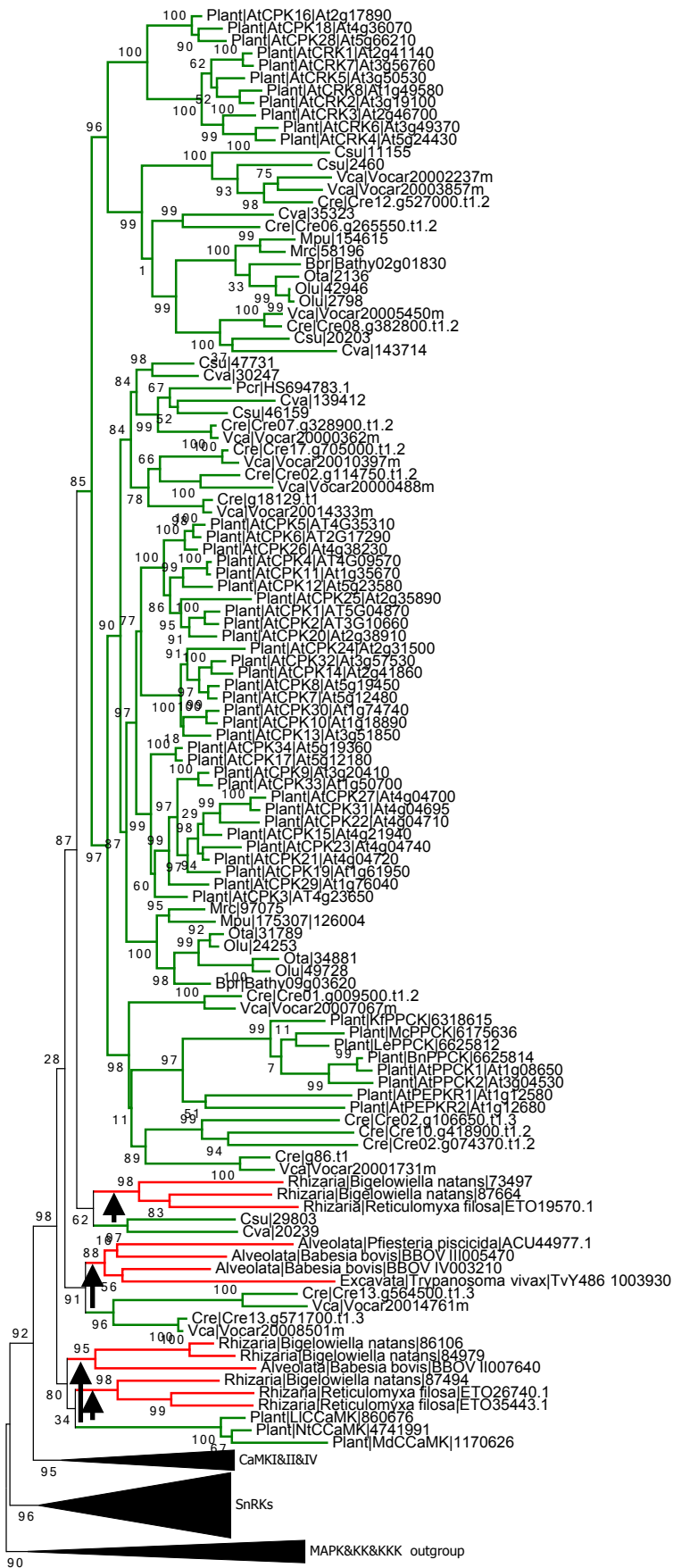
**Bacteria**

**Archaea**

○ Absent

◐ Partial existent

● Exist in all species

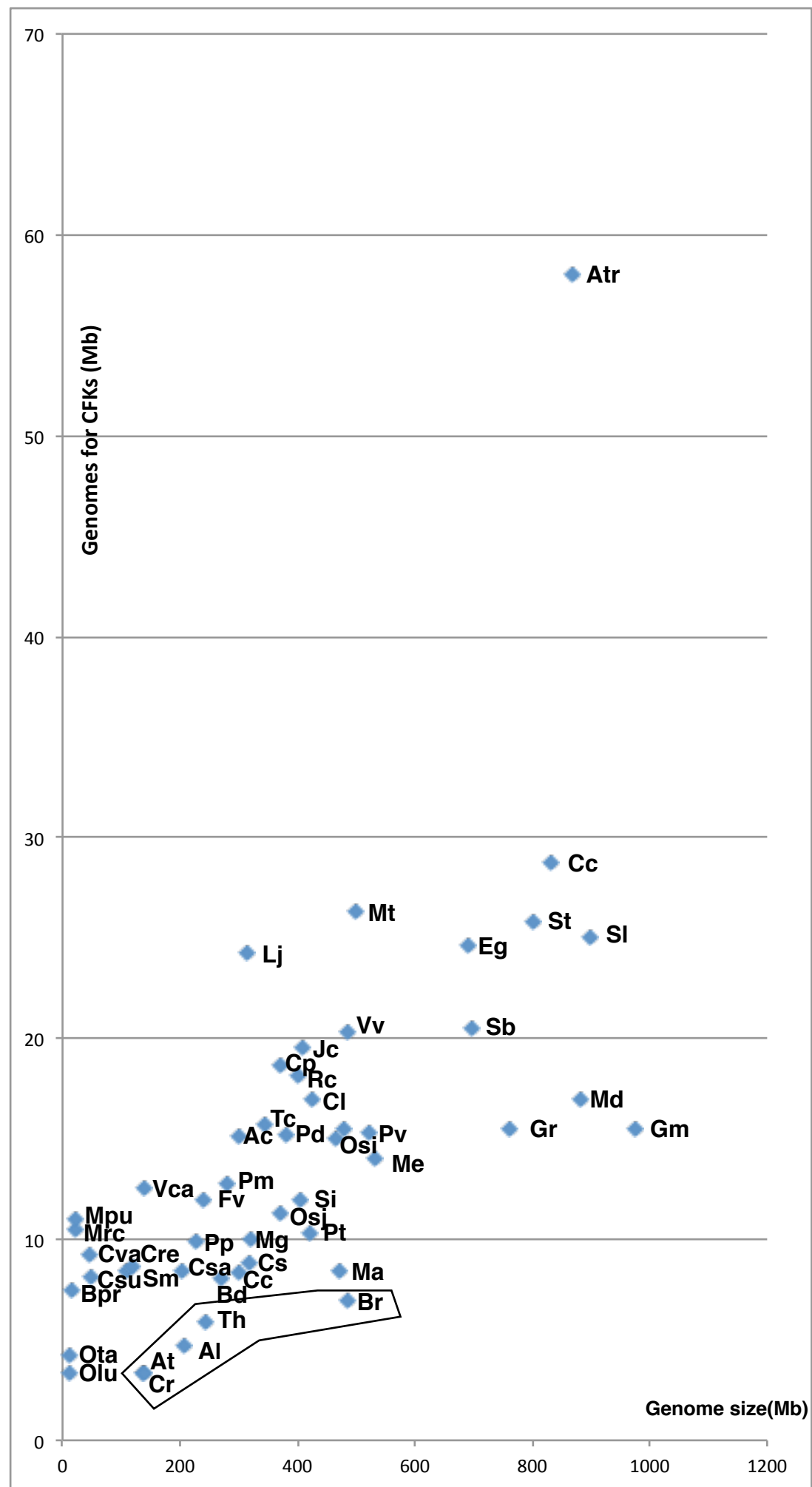
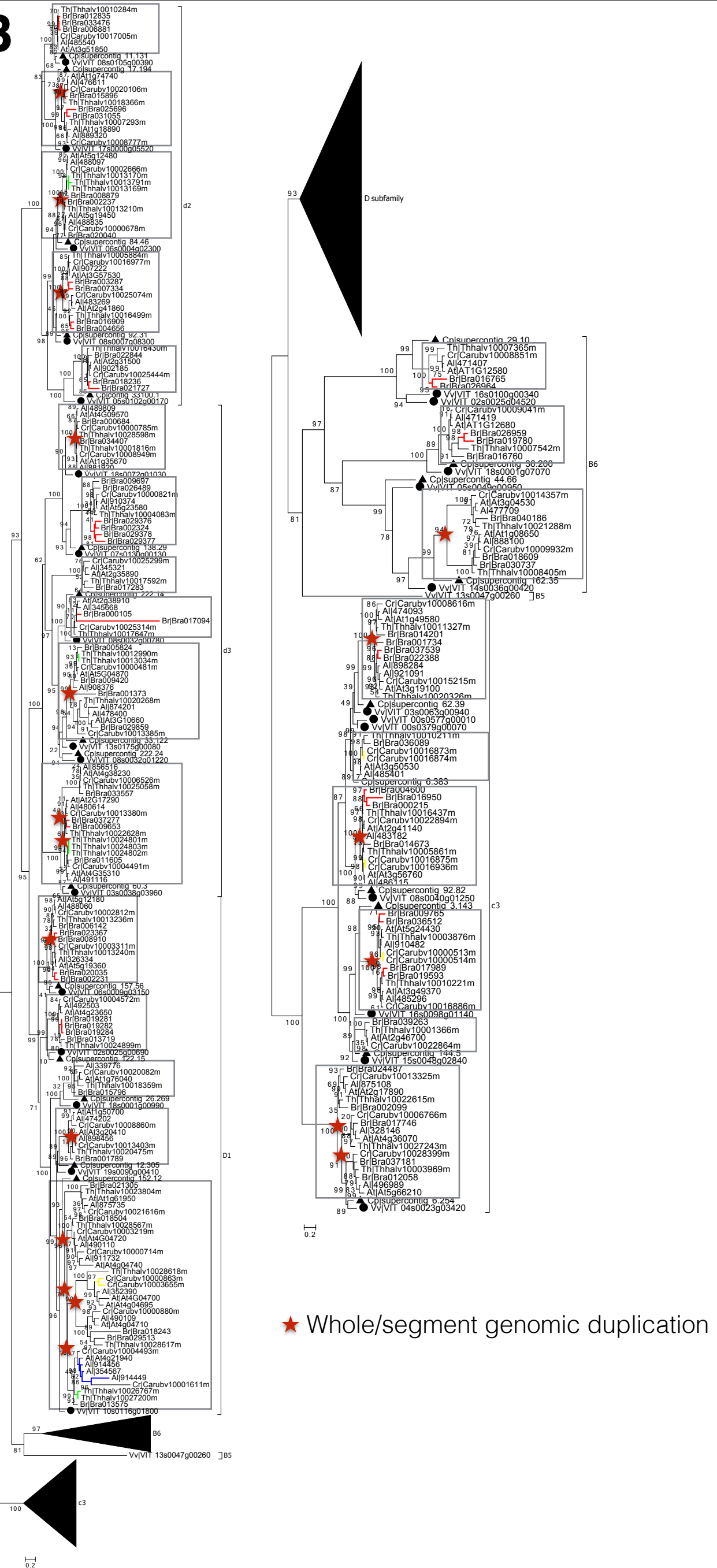


X-monophyly

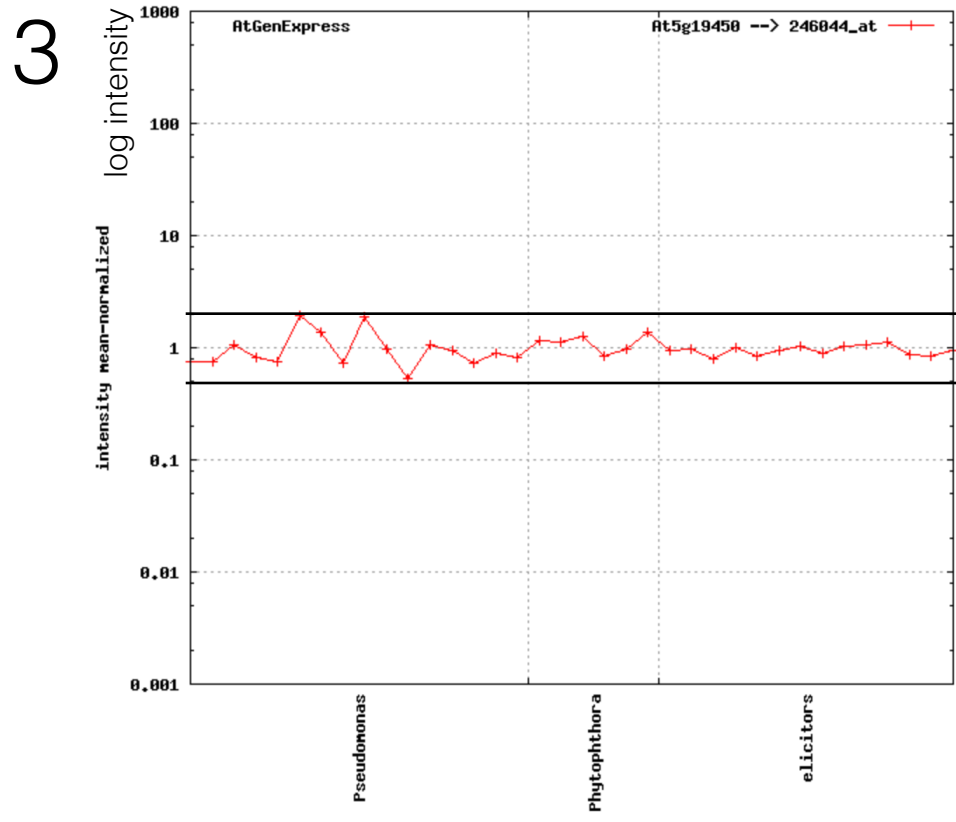
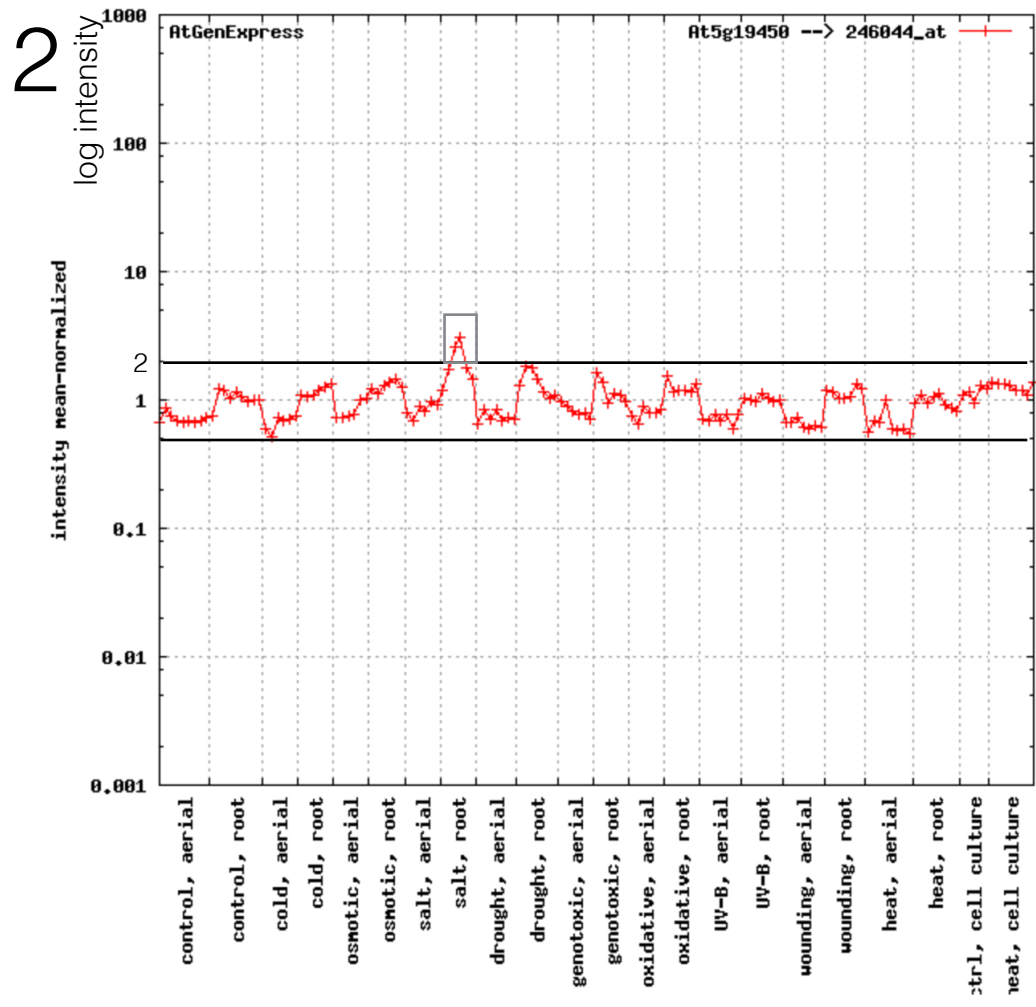
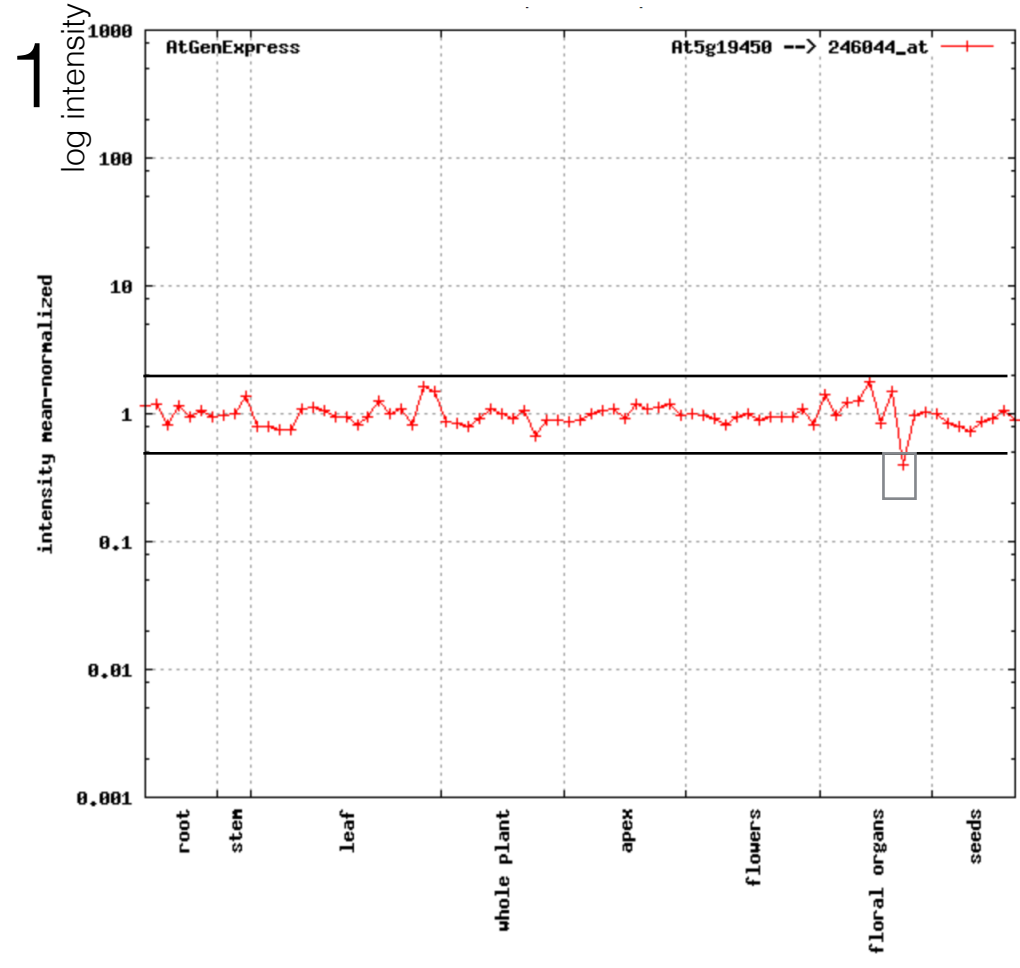
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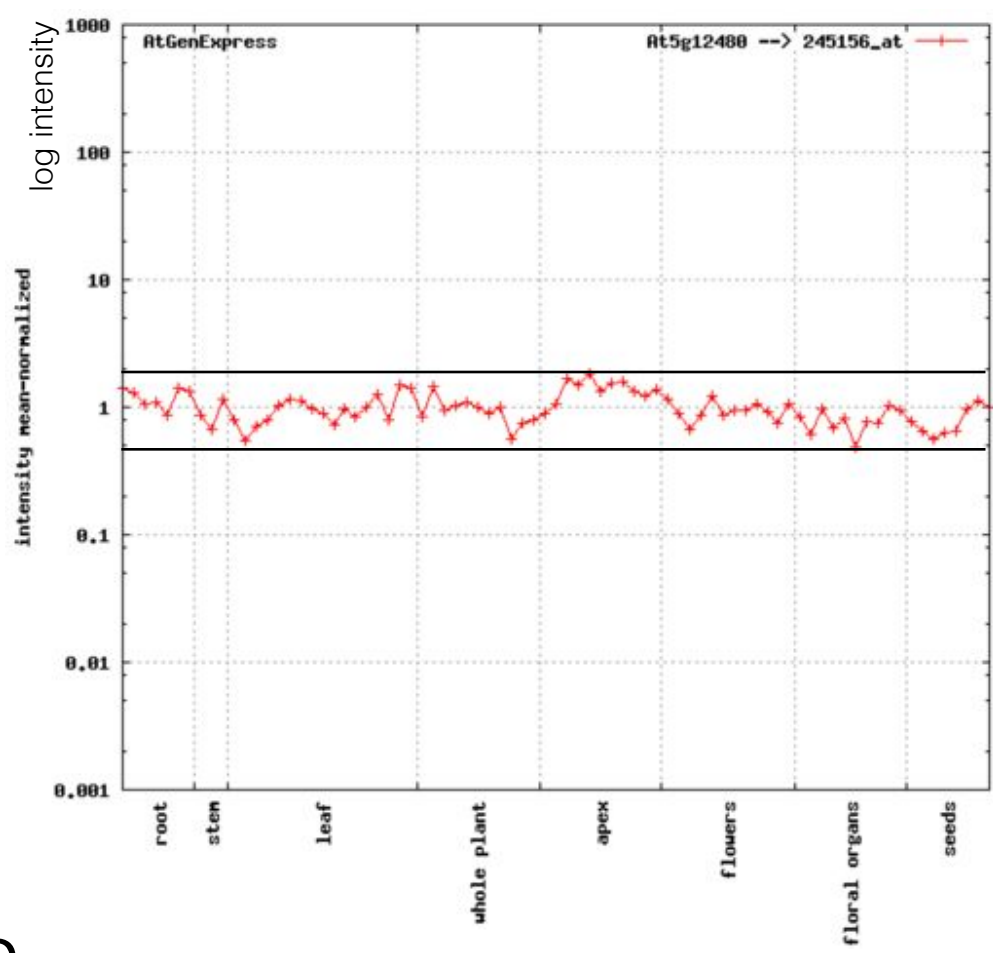


**A****B**

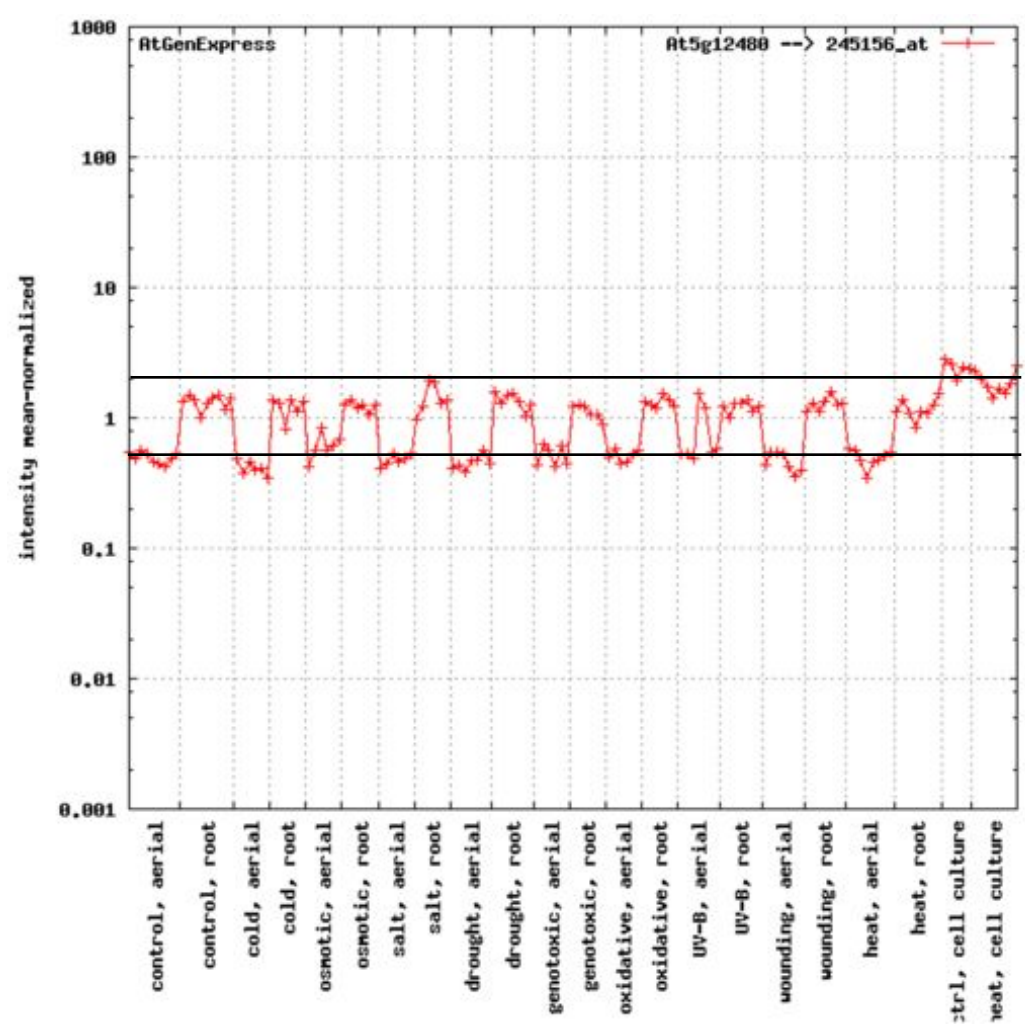




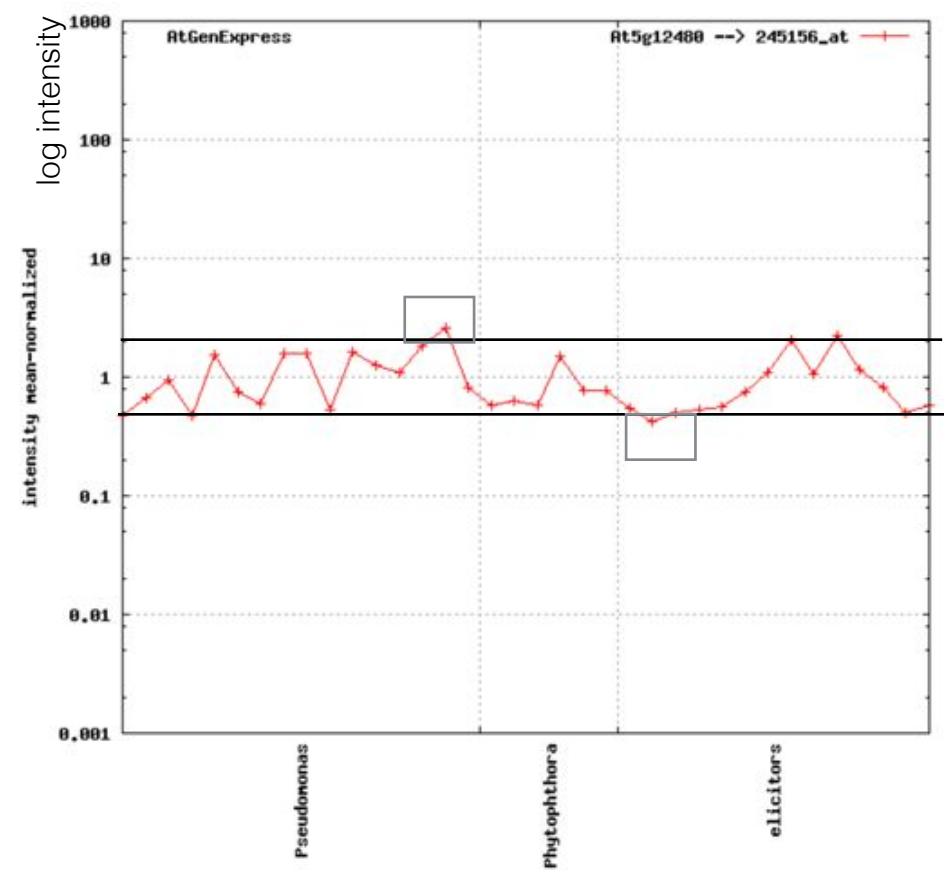
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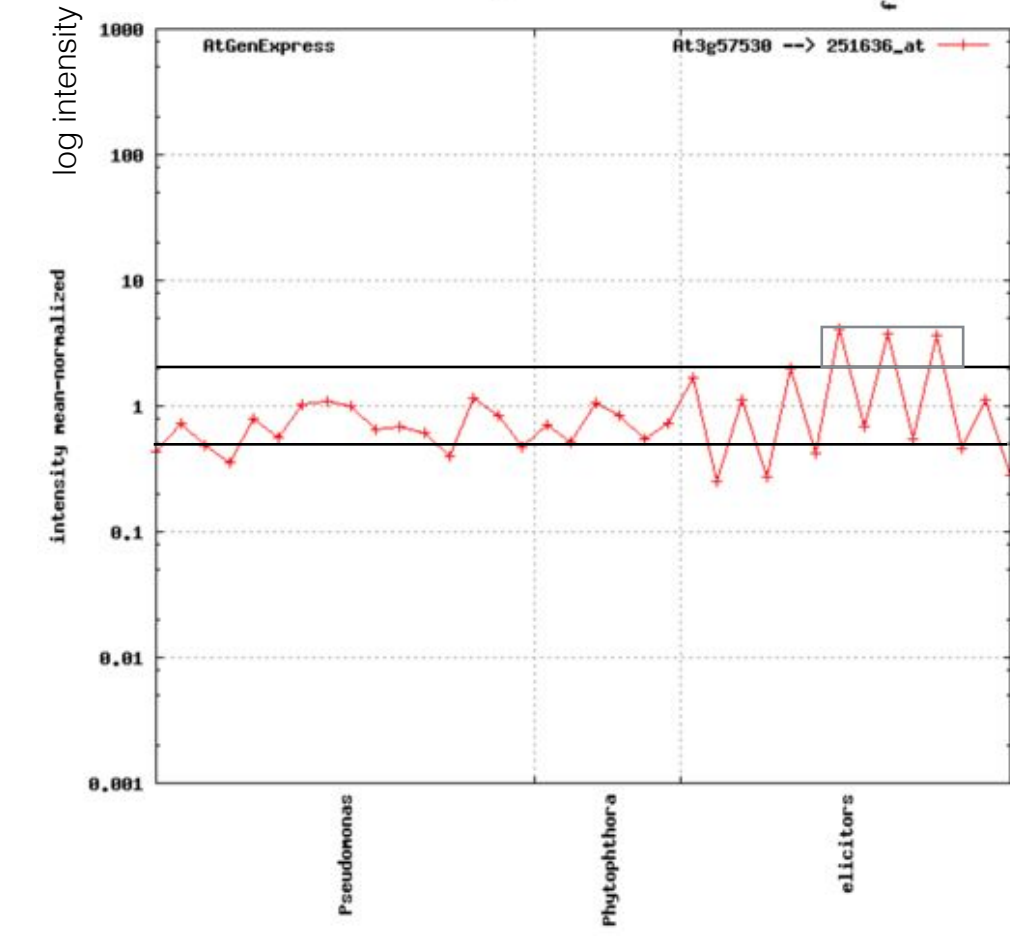
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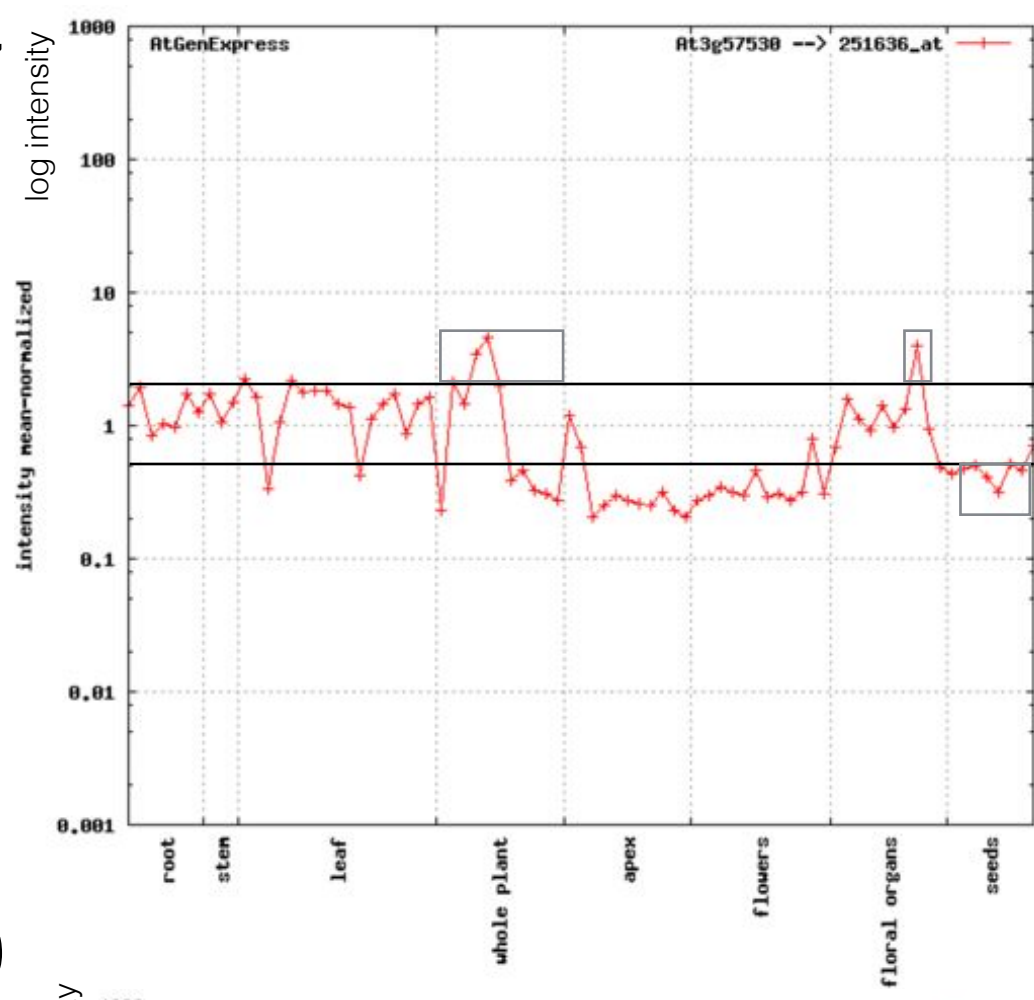
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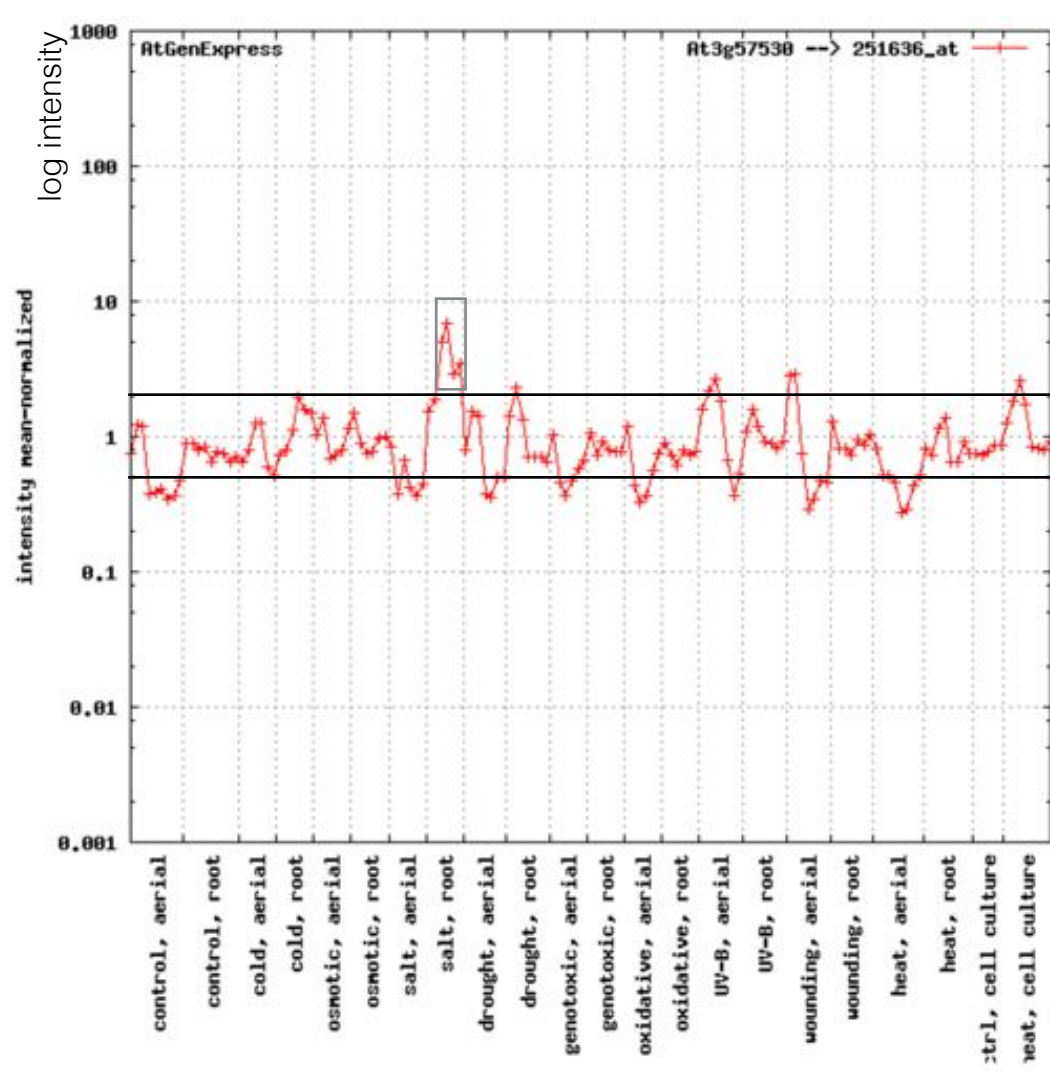
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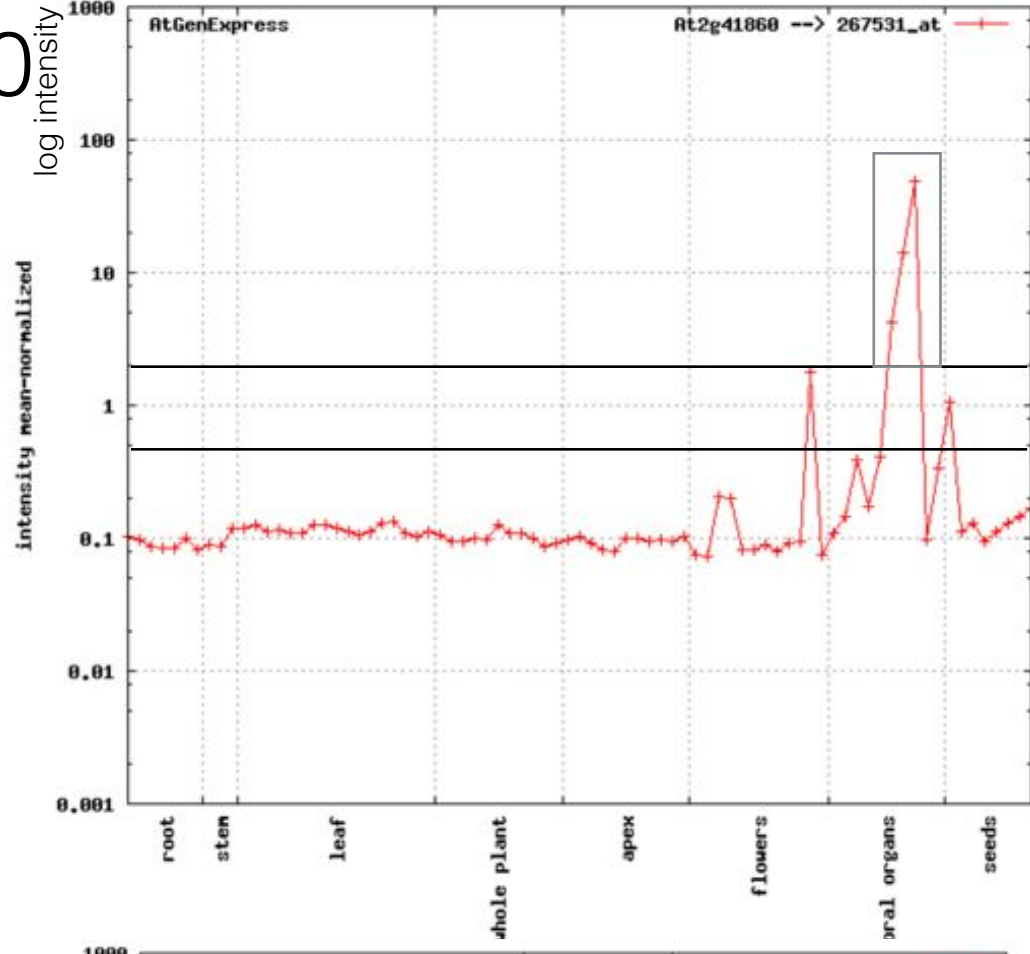
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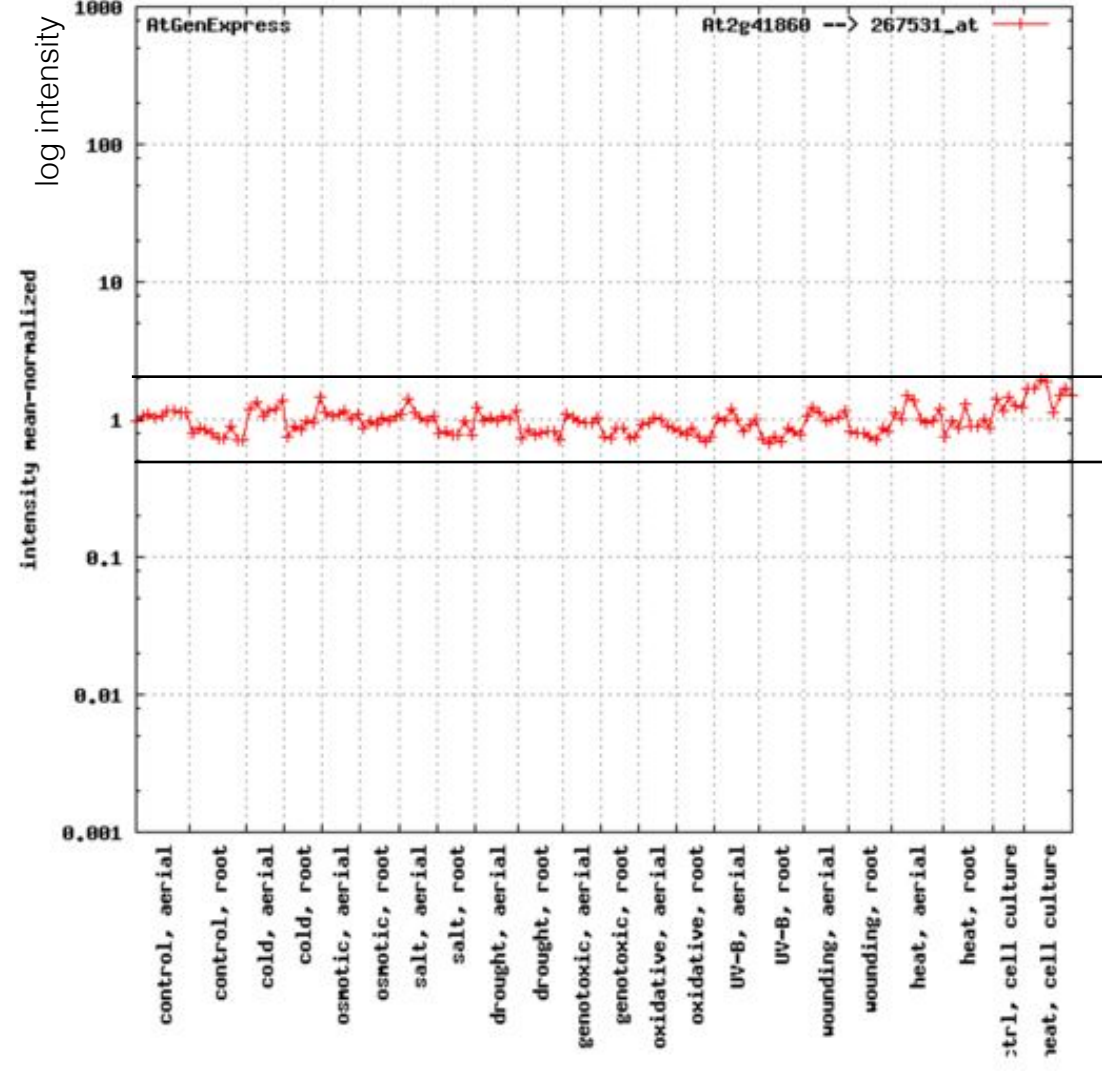
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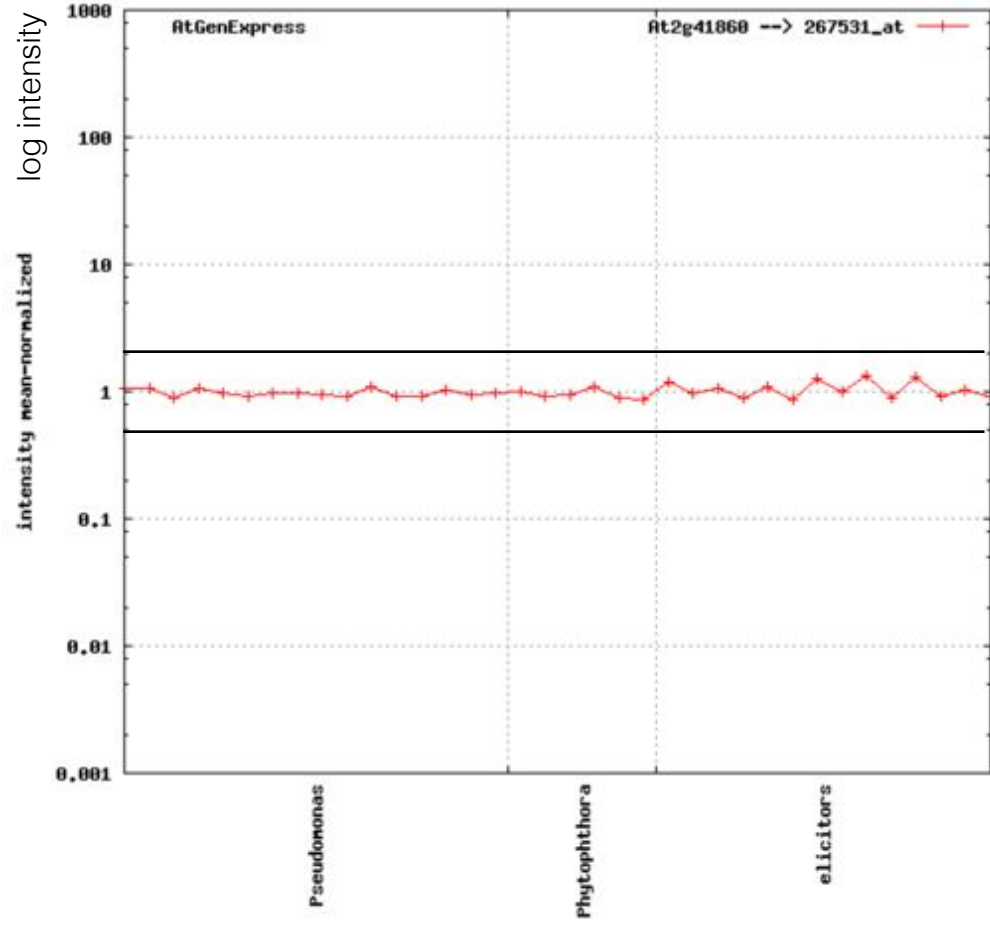
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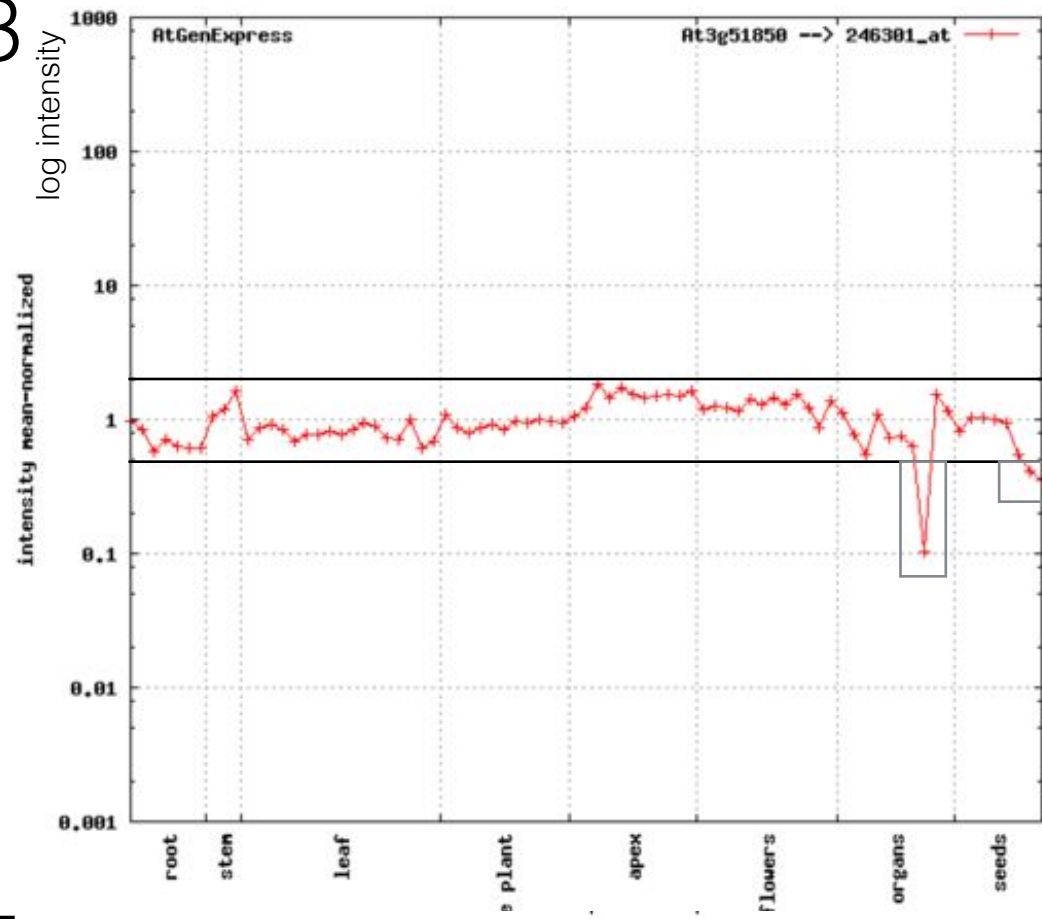


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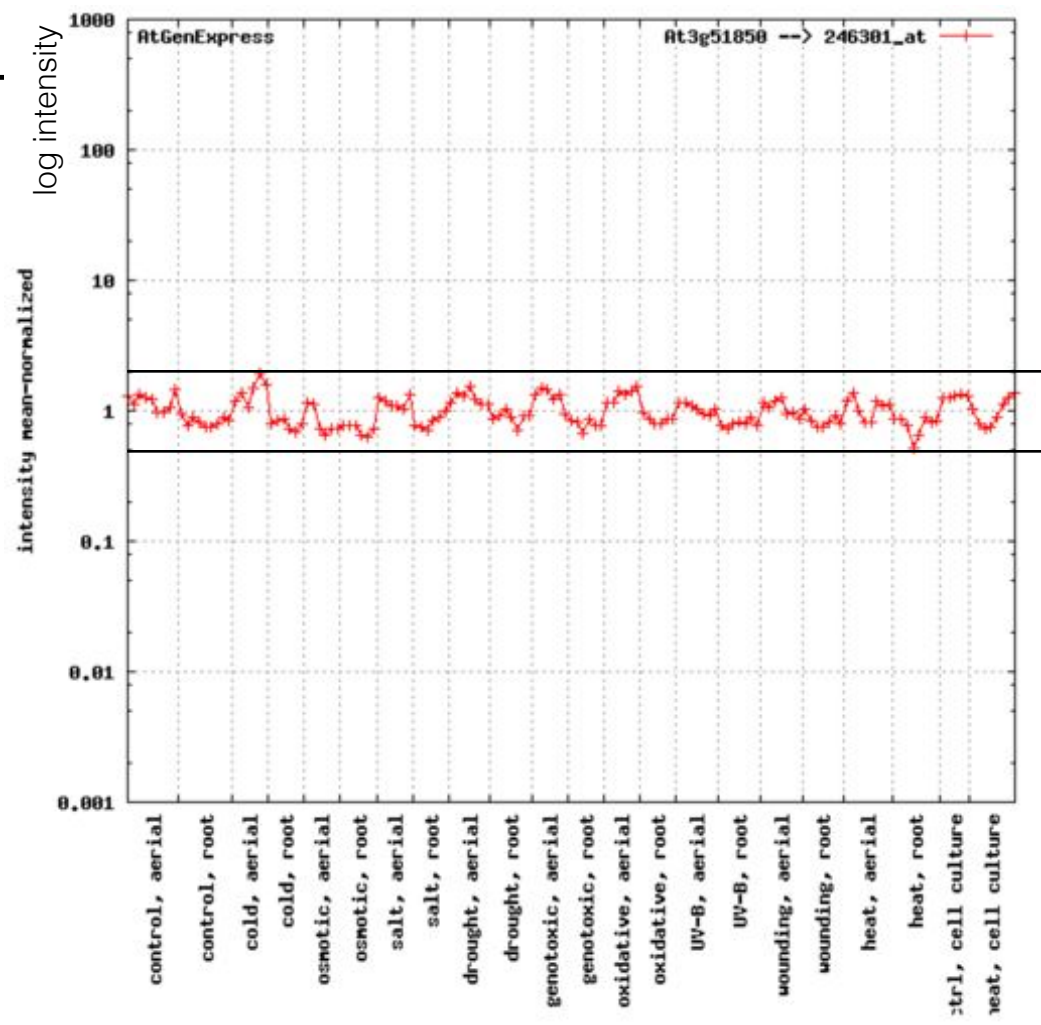




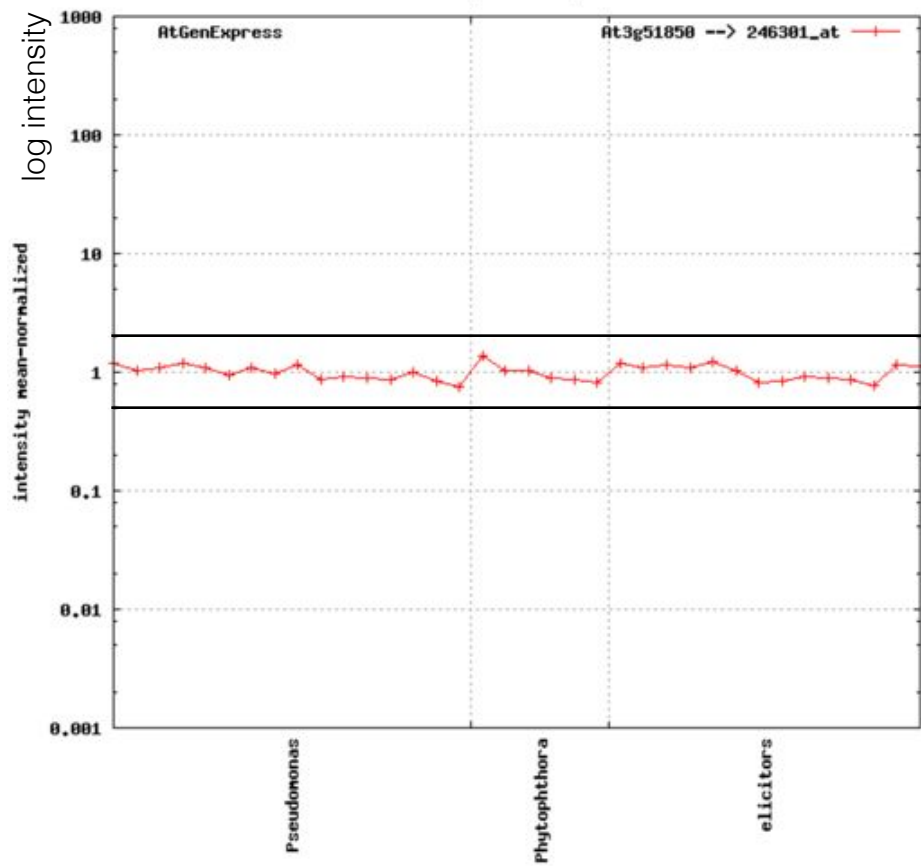
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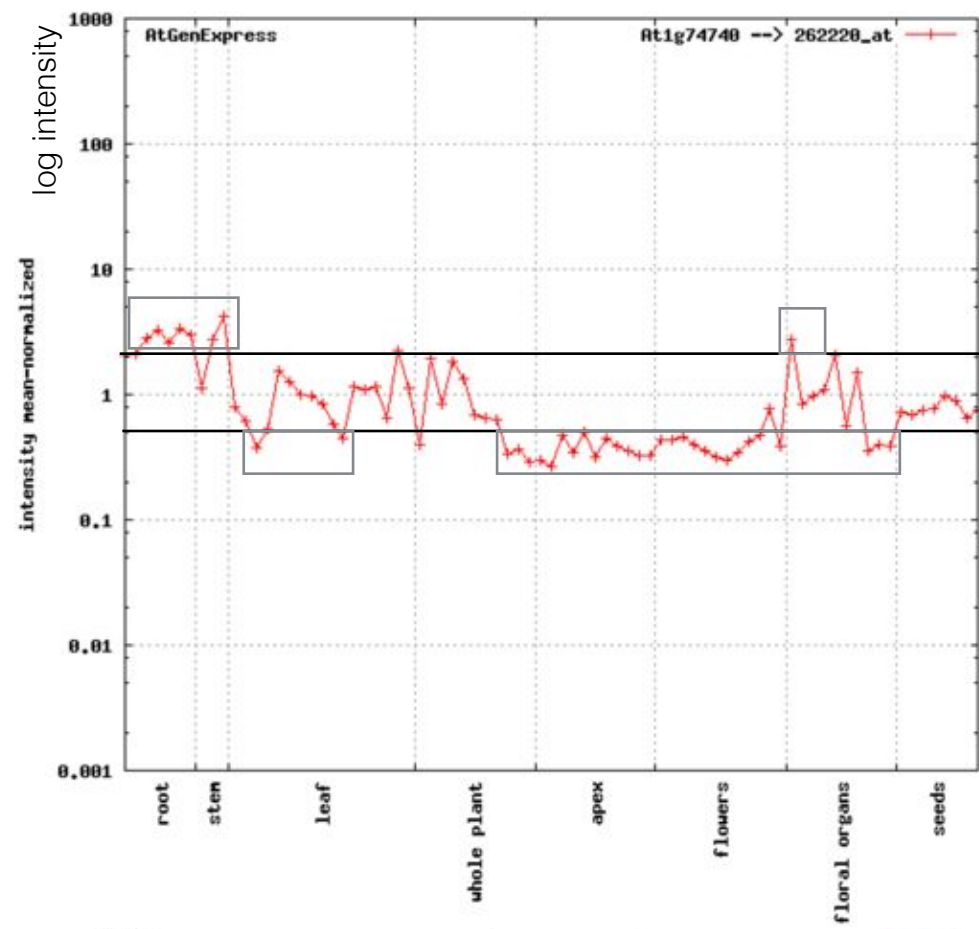
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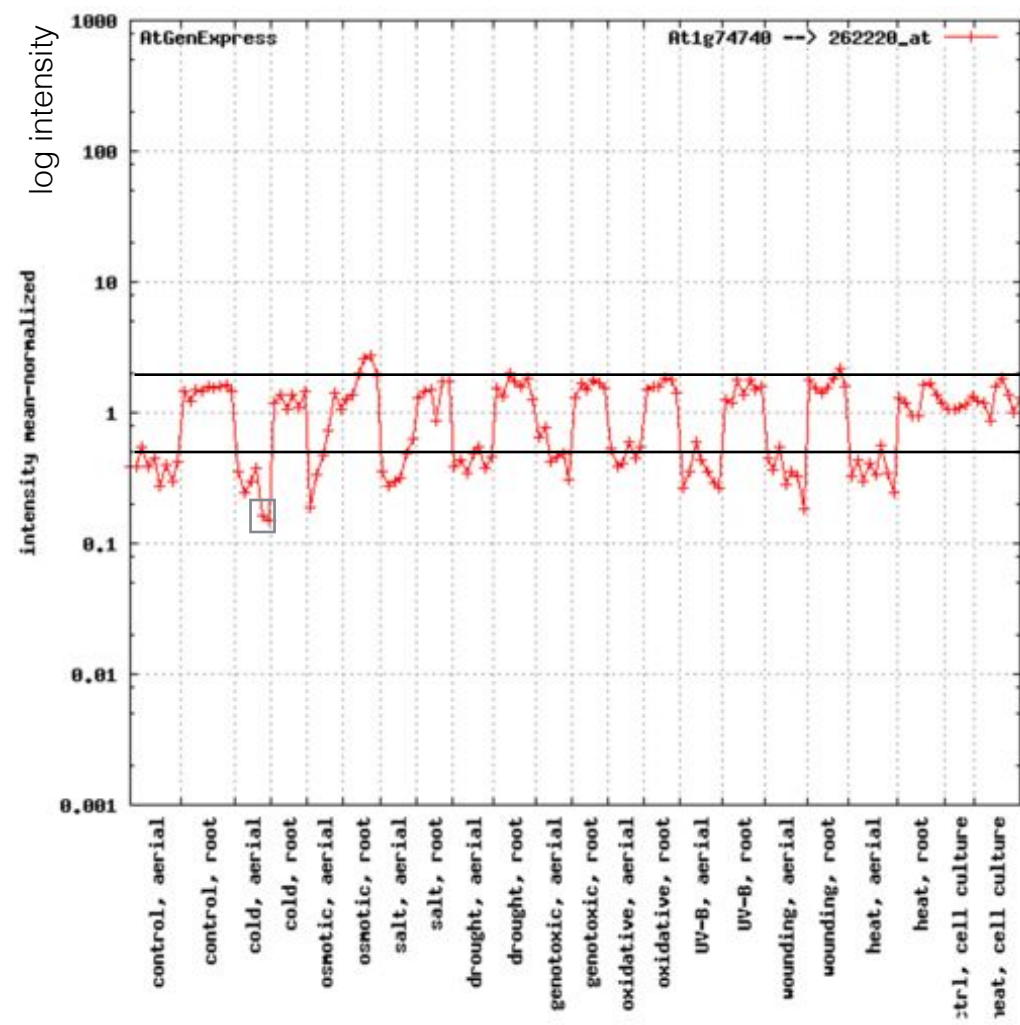
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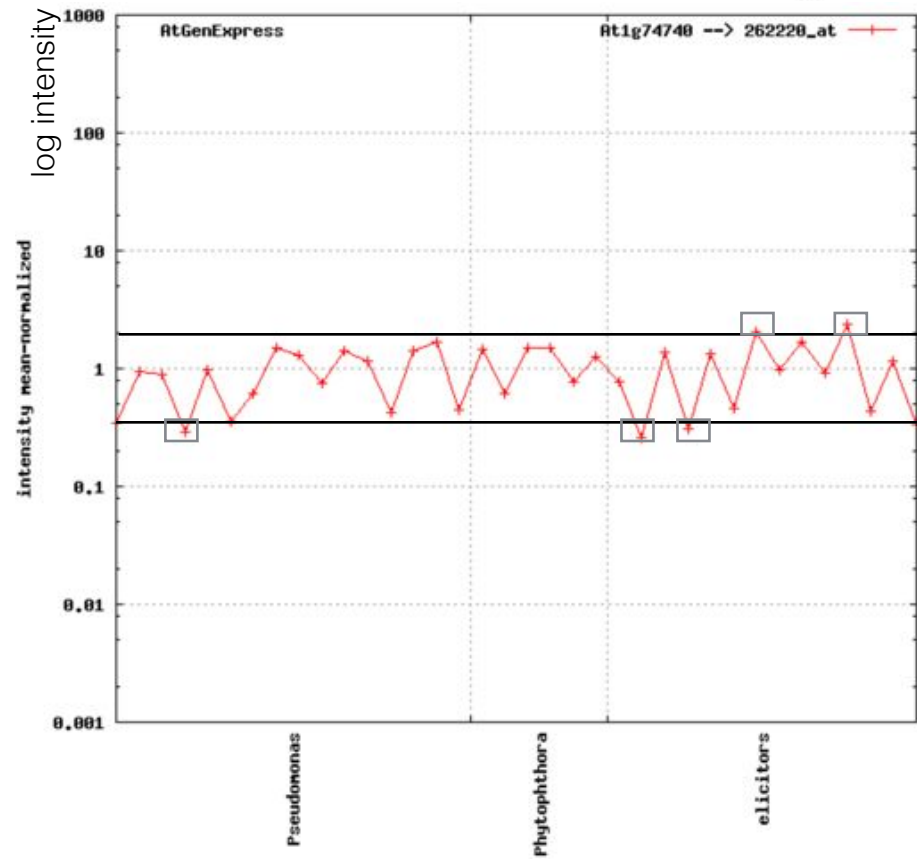
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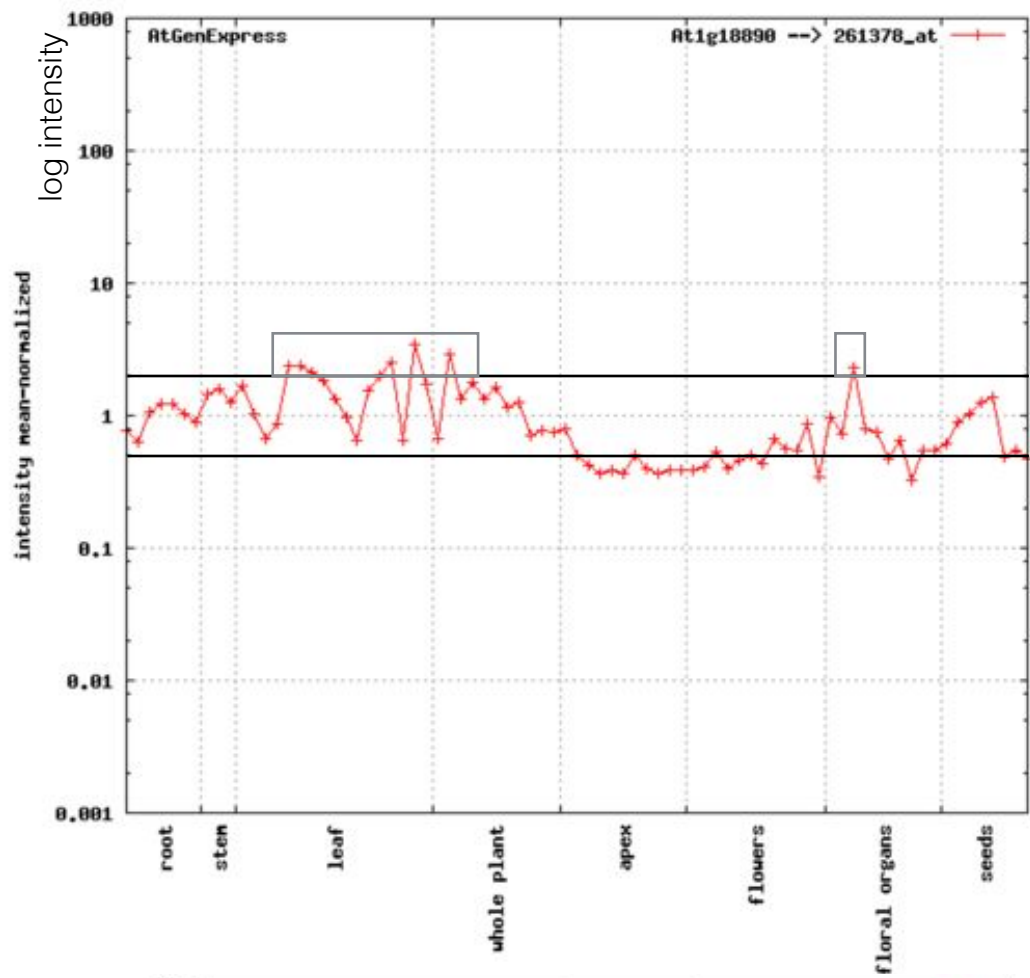
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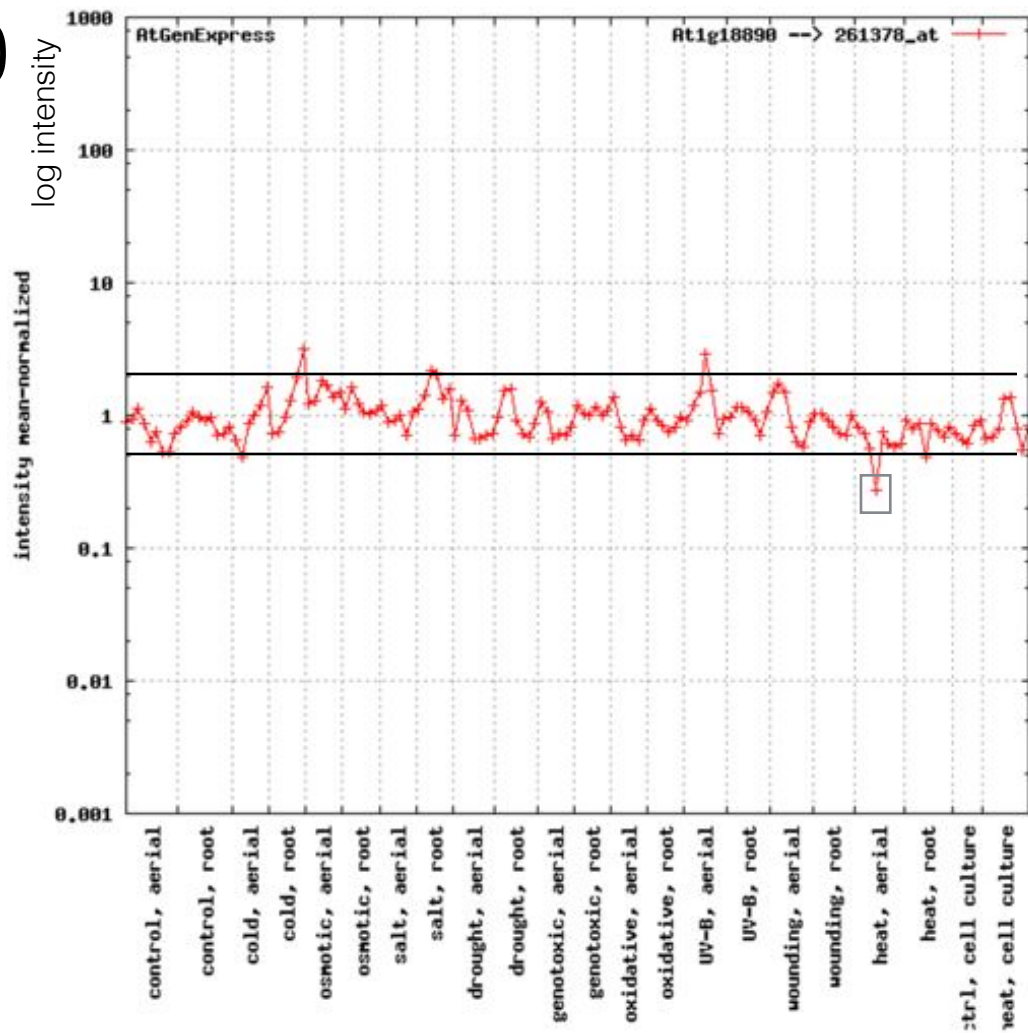
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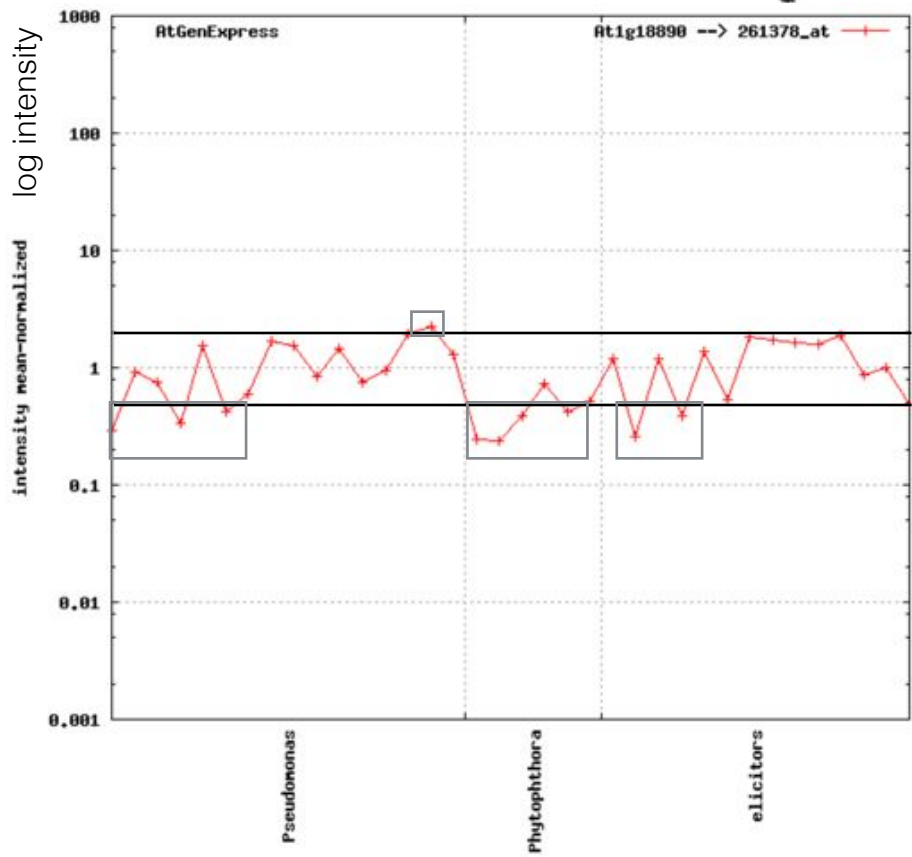
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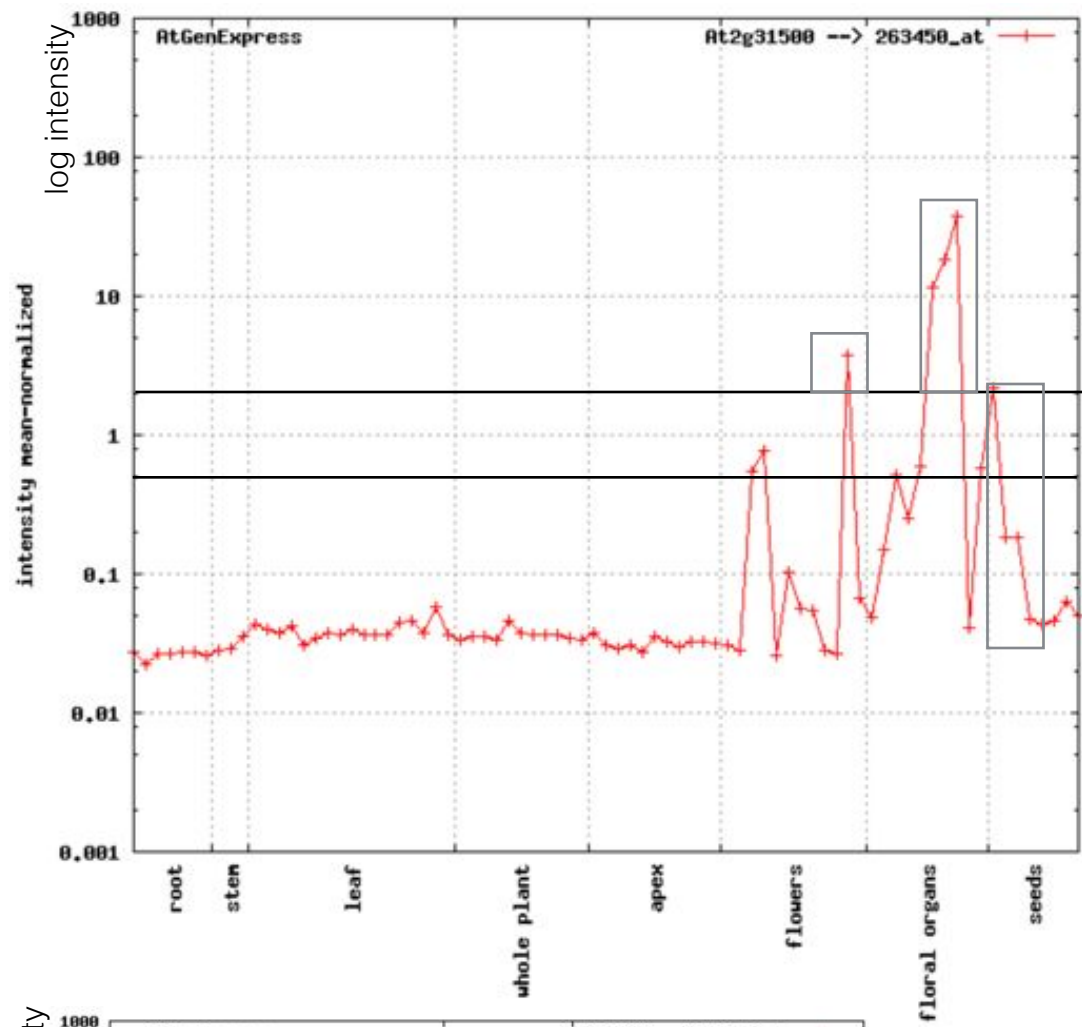


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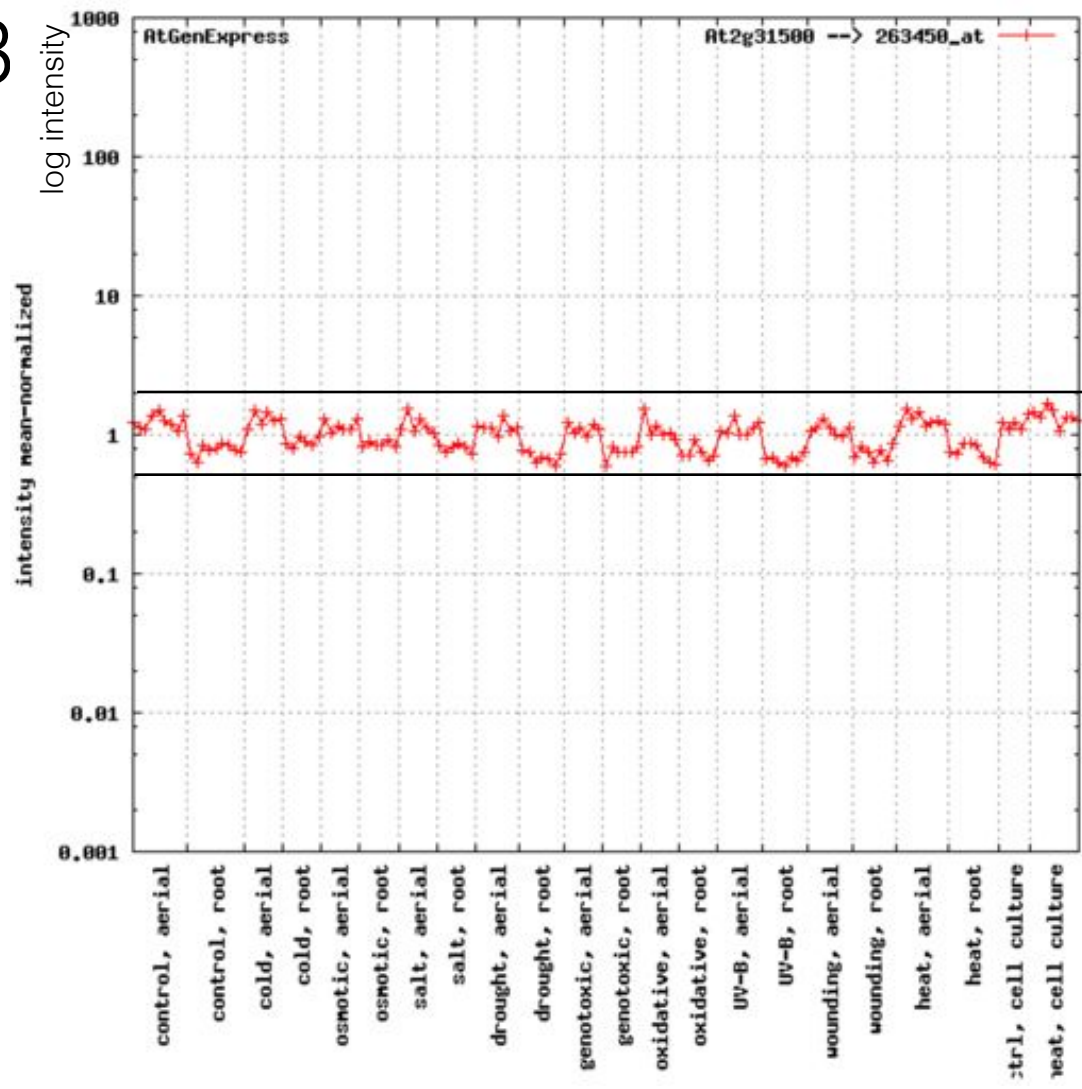




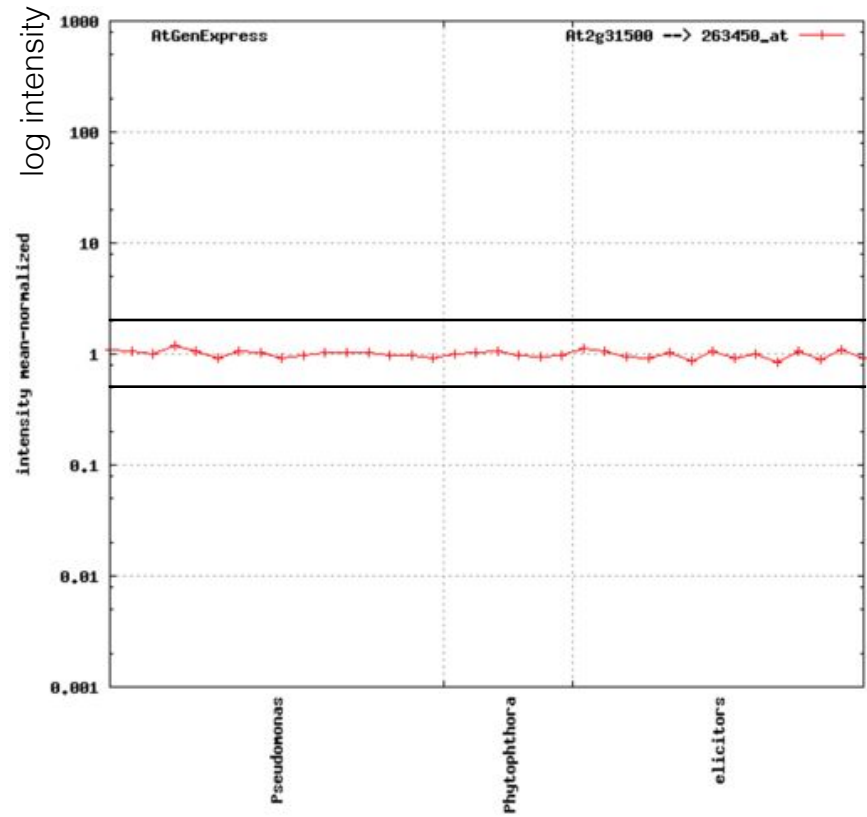
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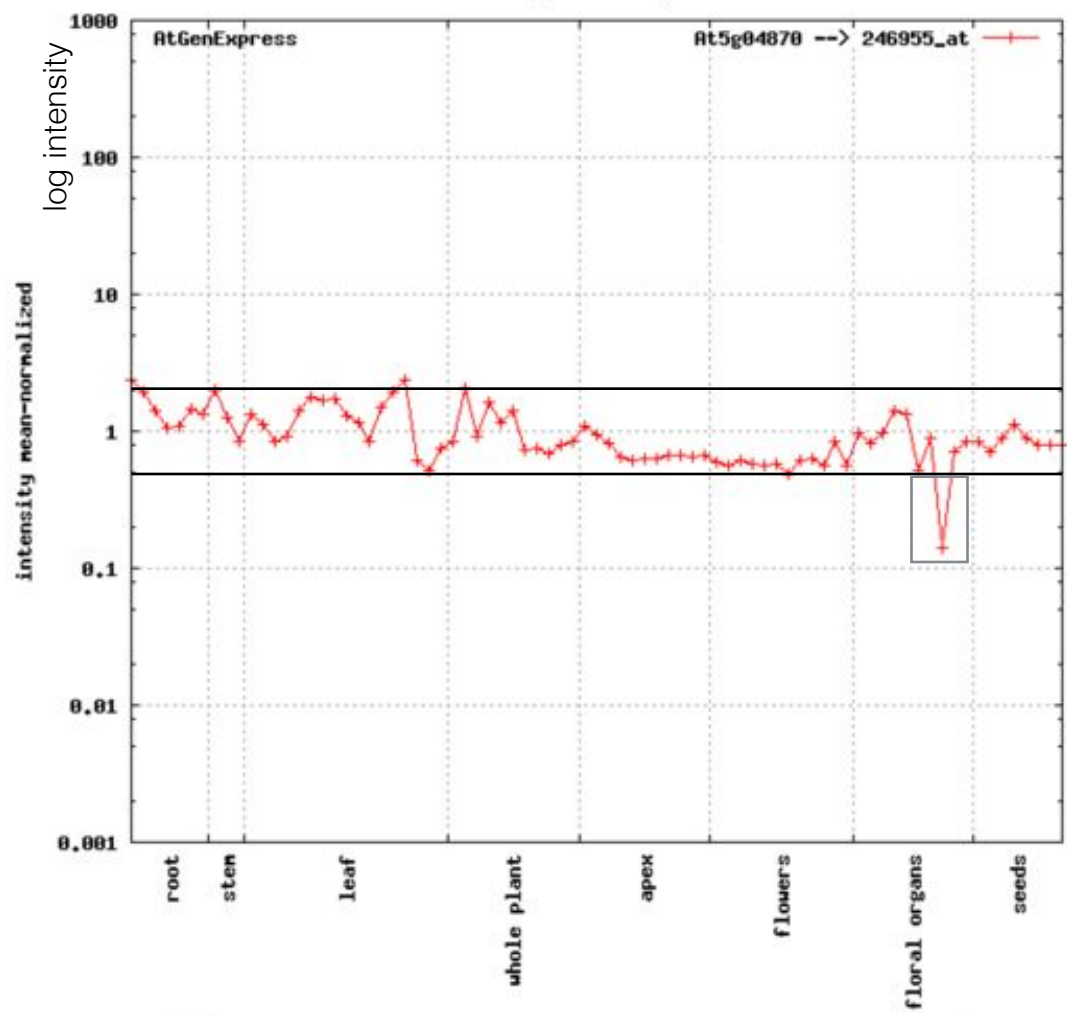


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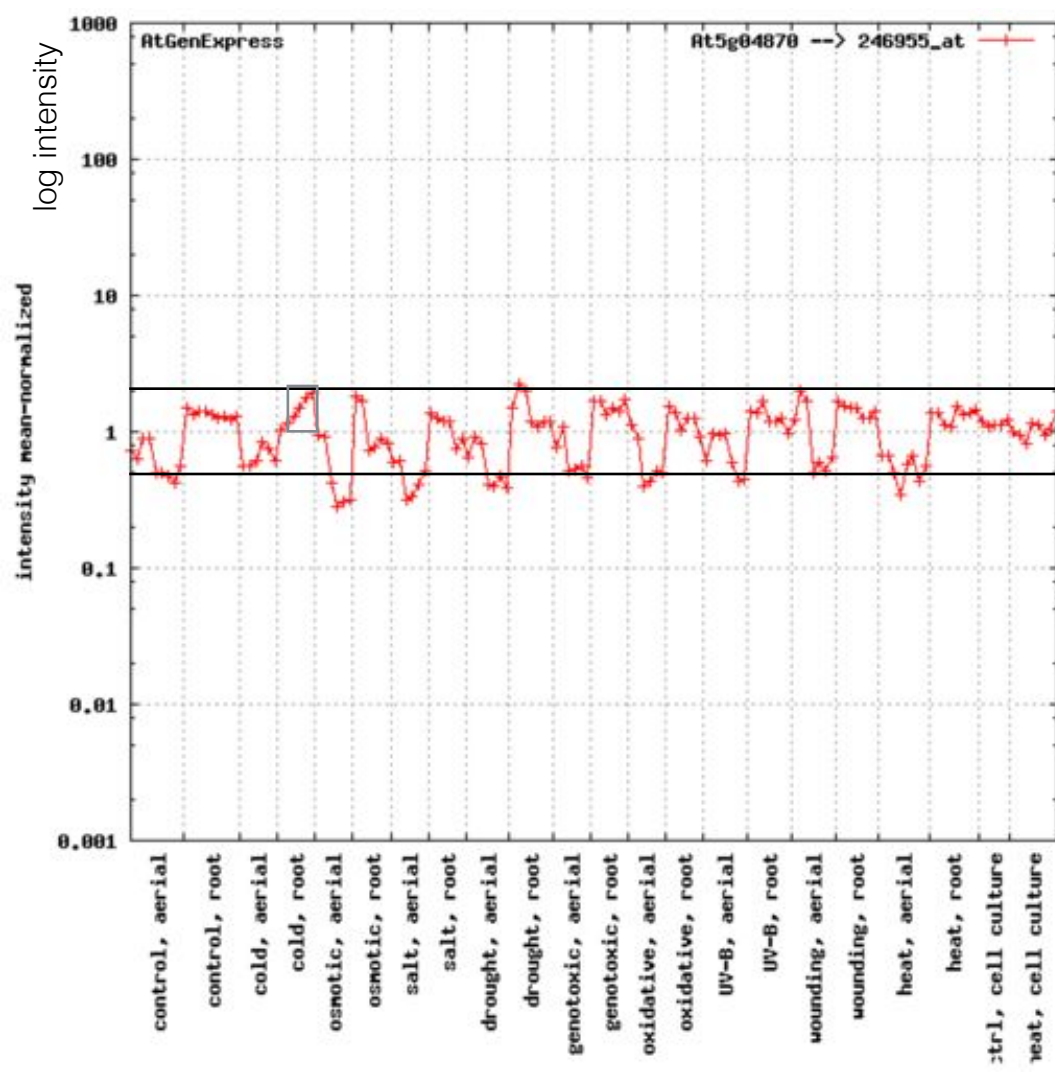




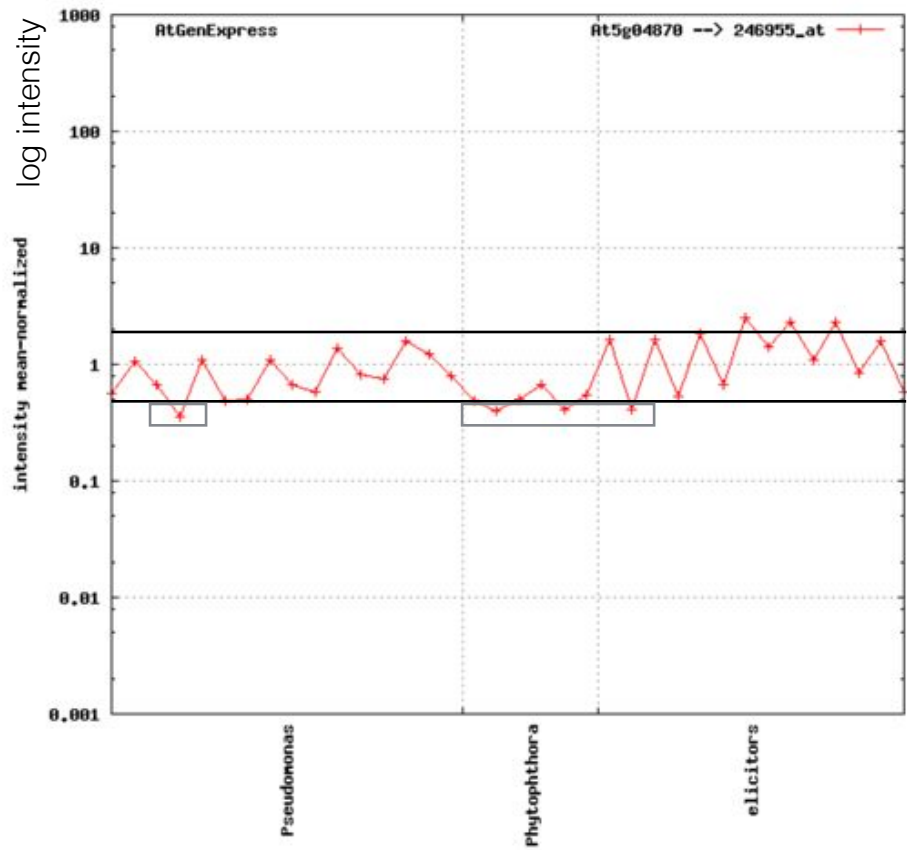
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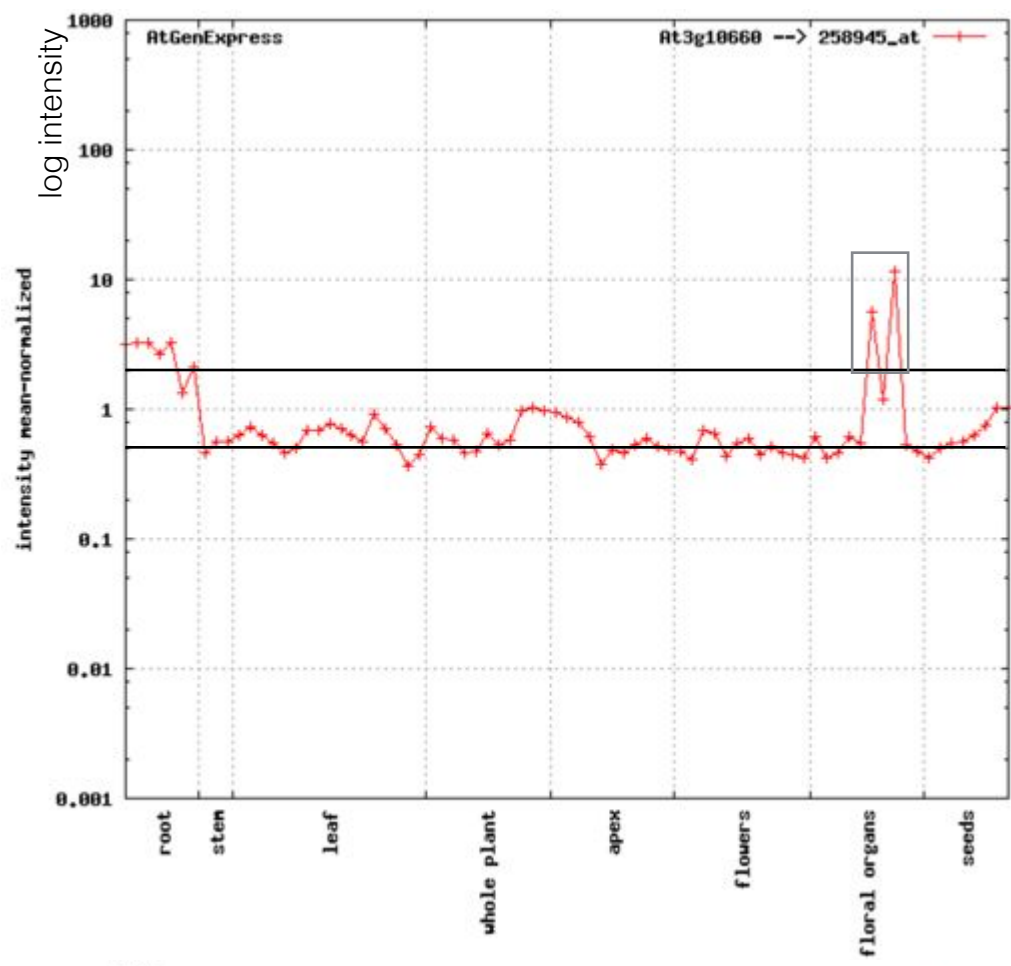
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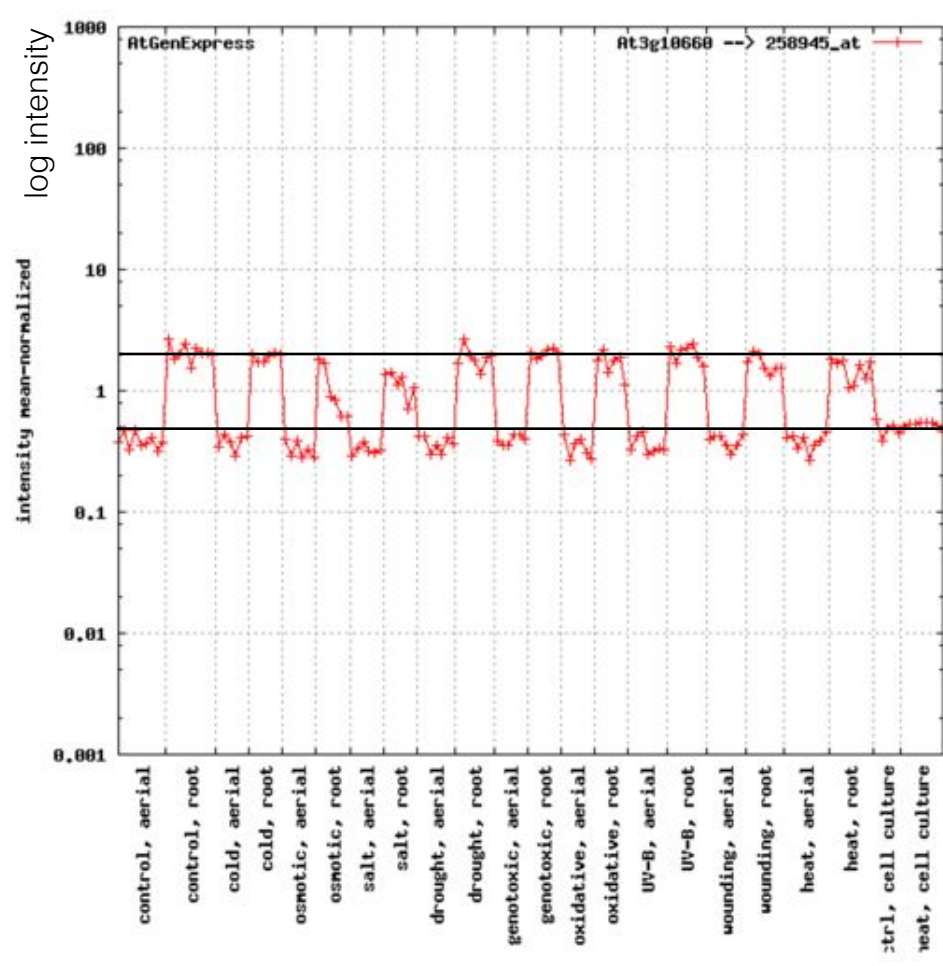
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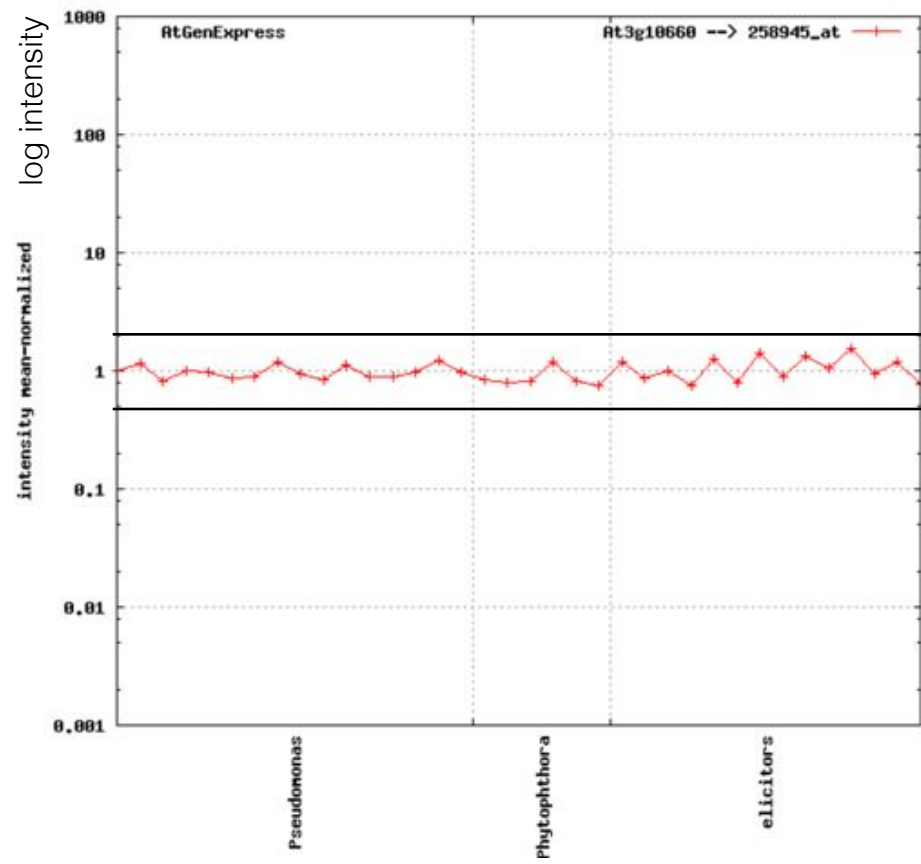
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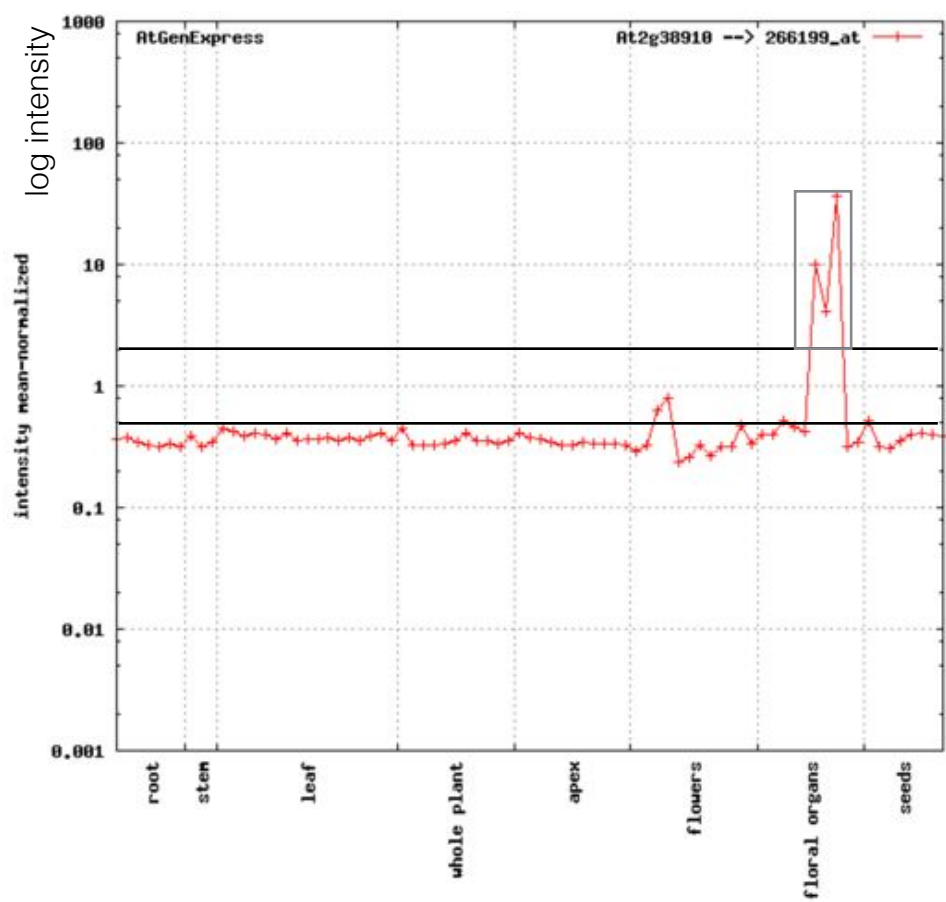
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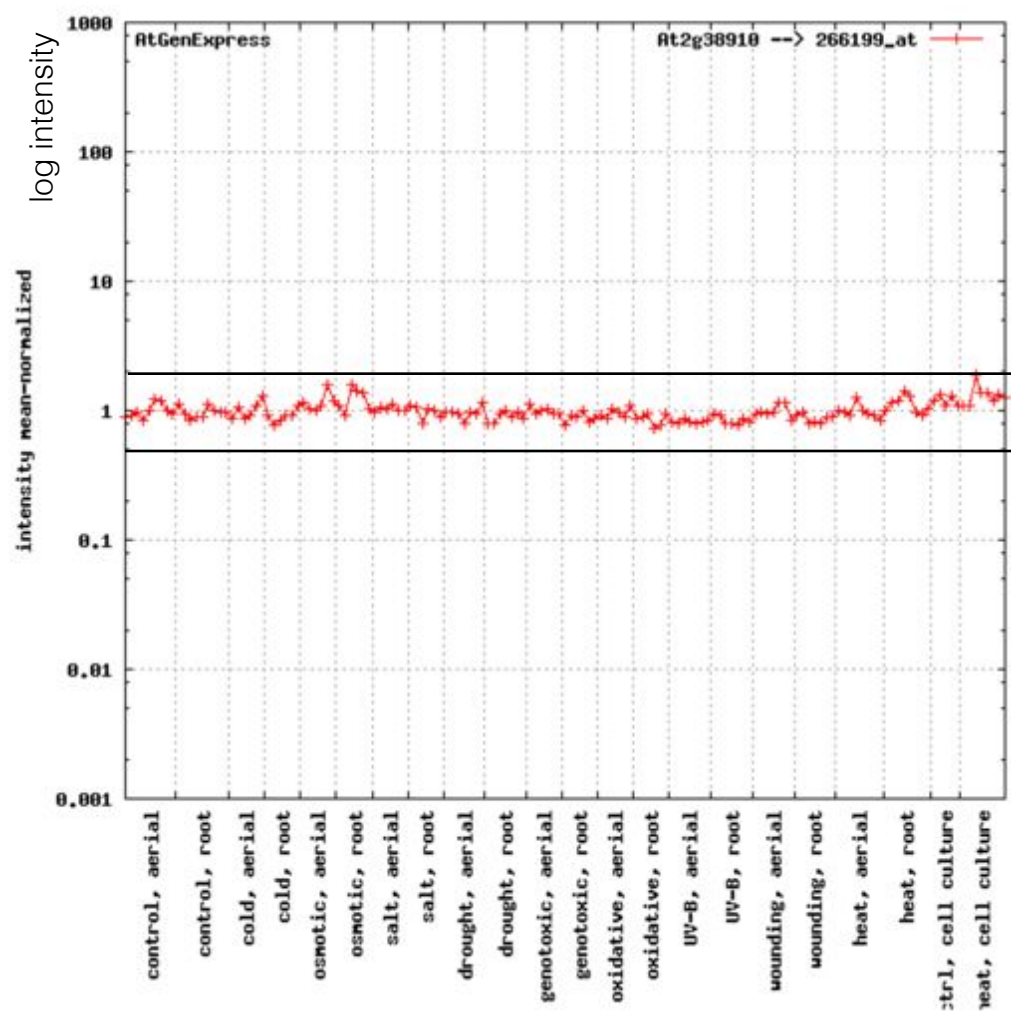
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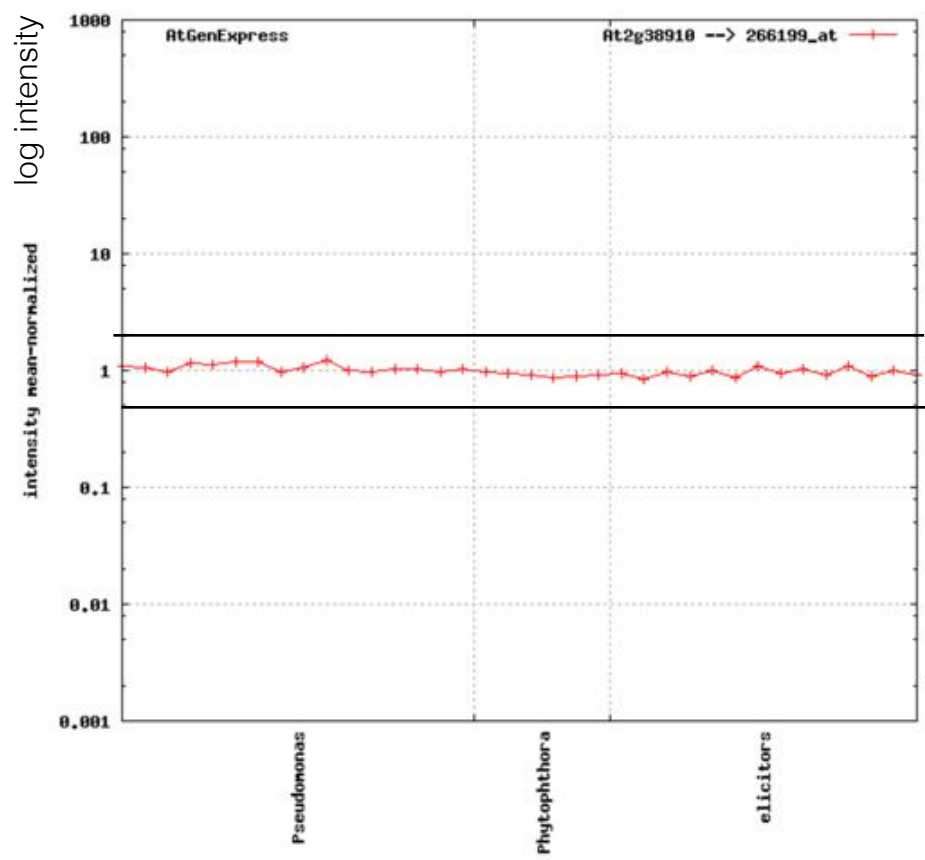
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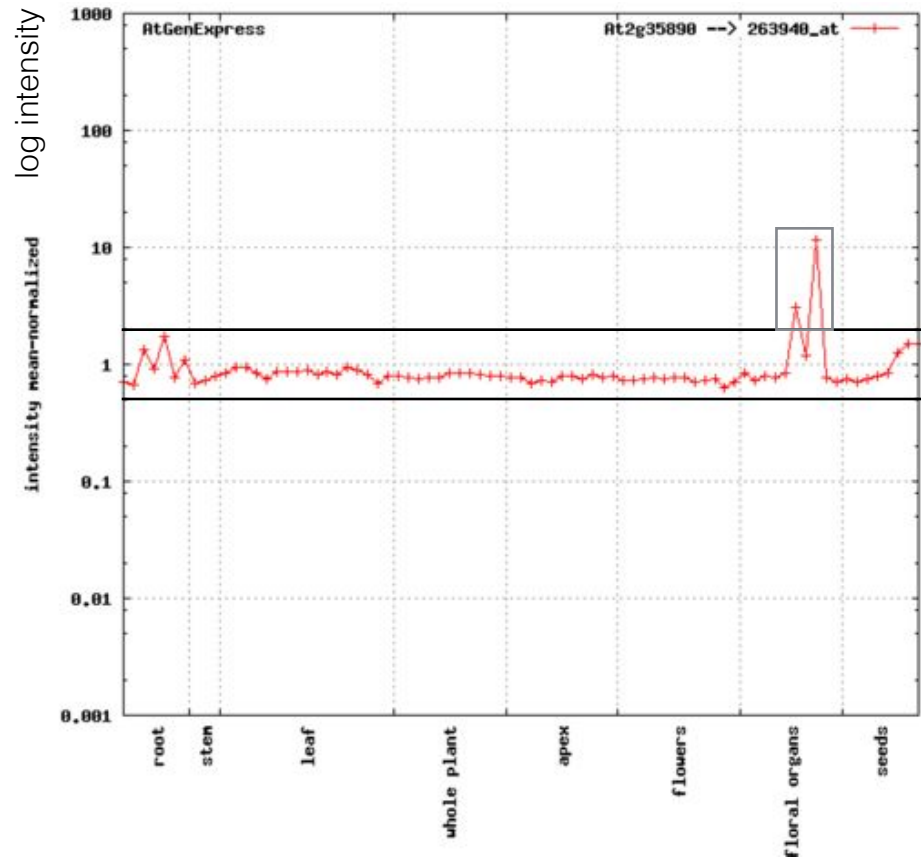


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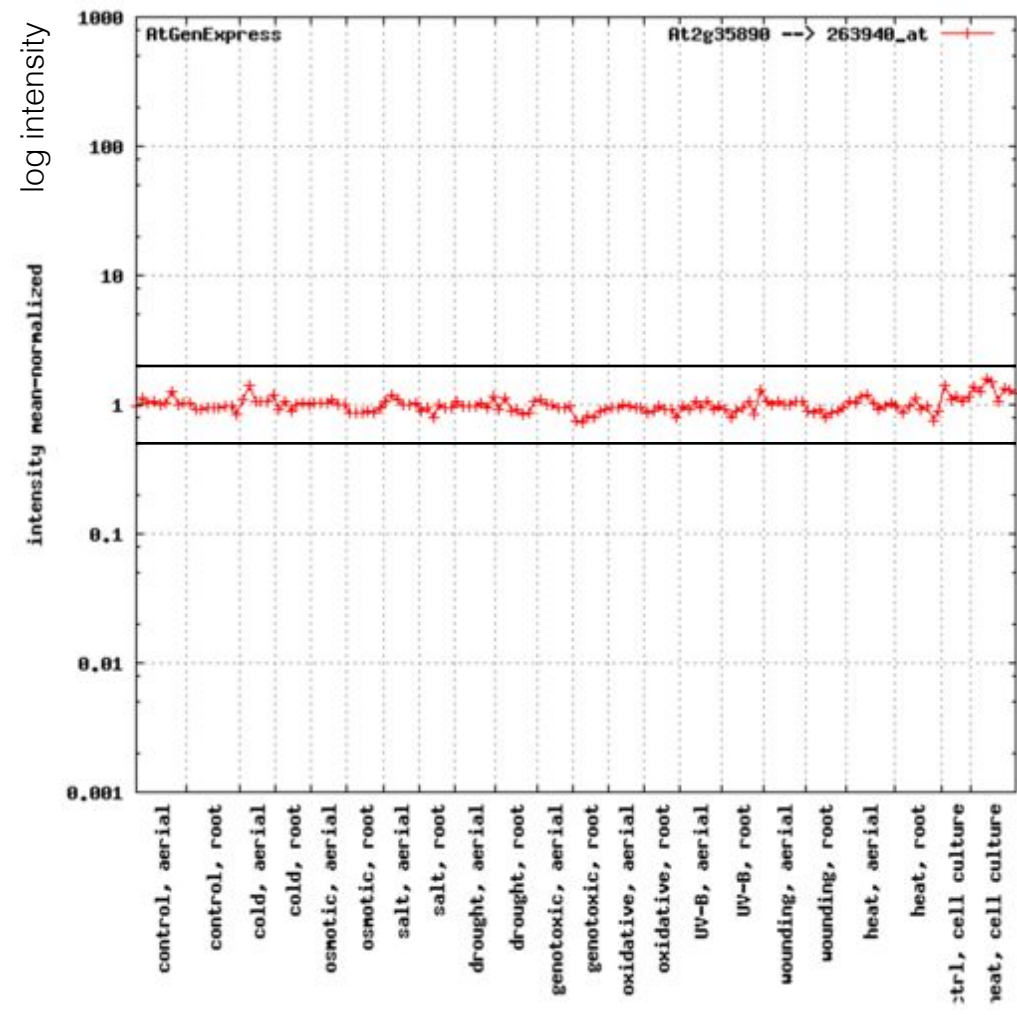




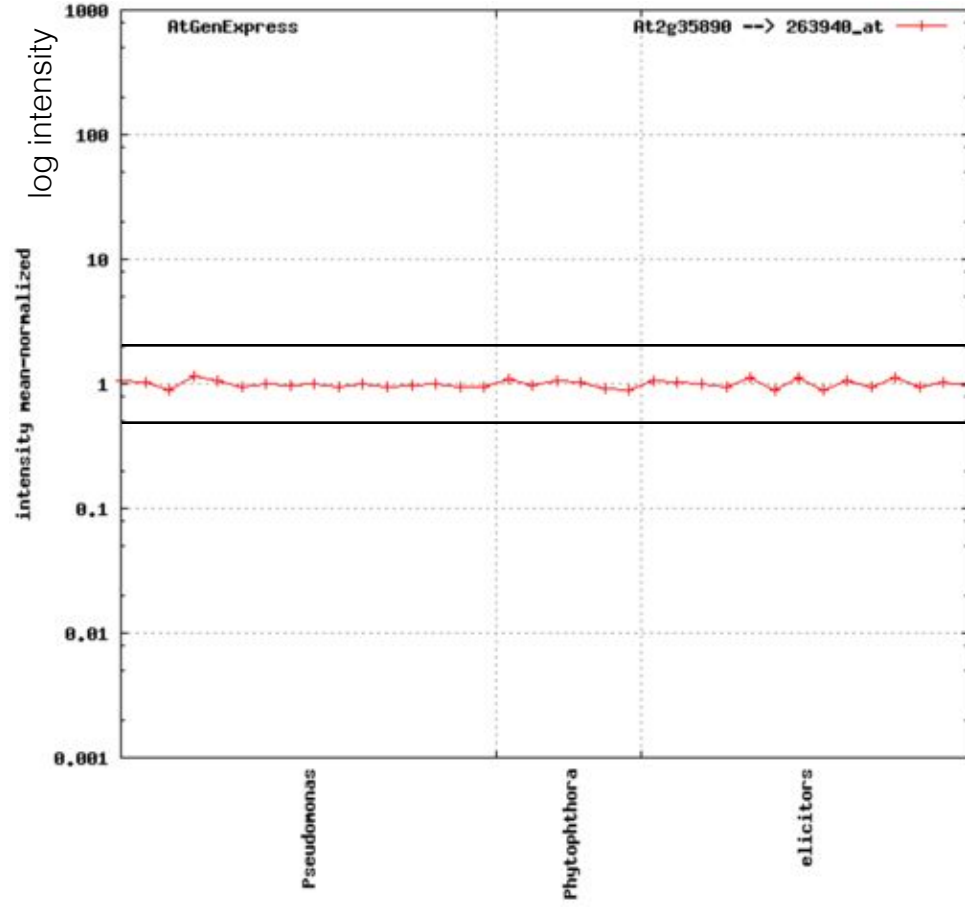
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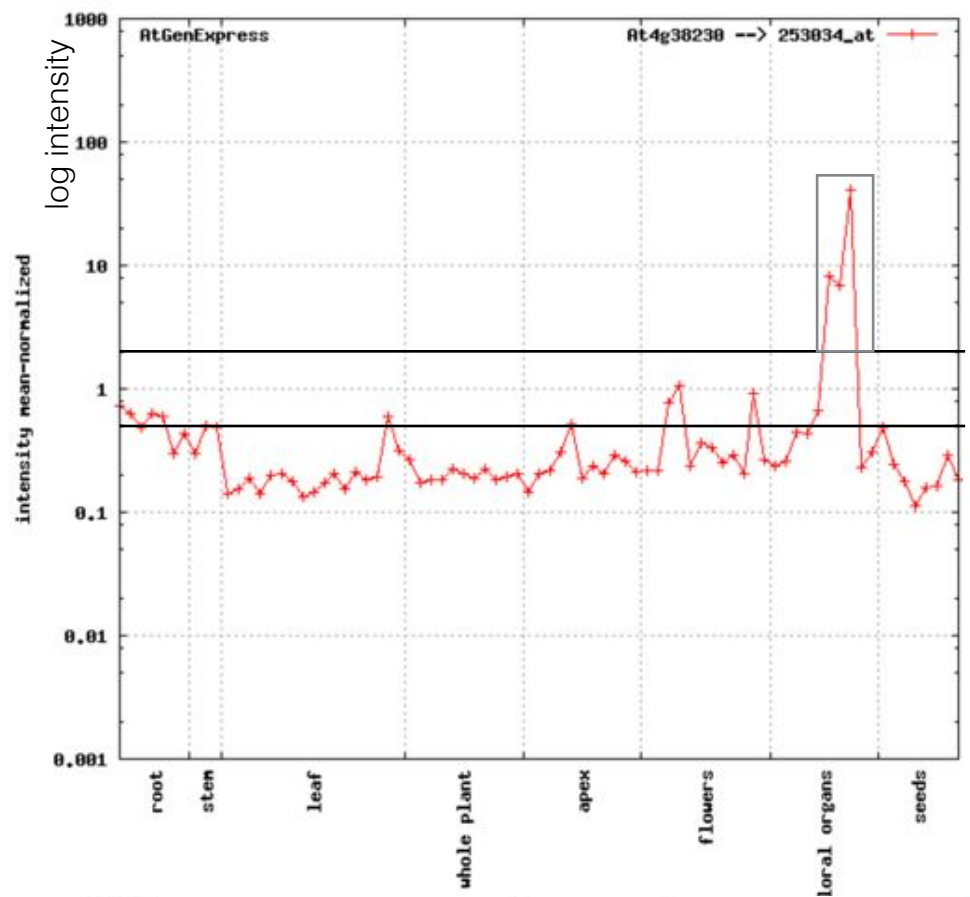
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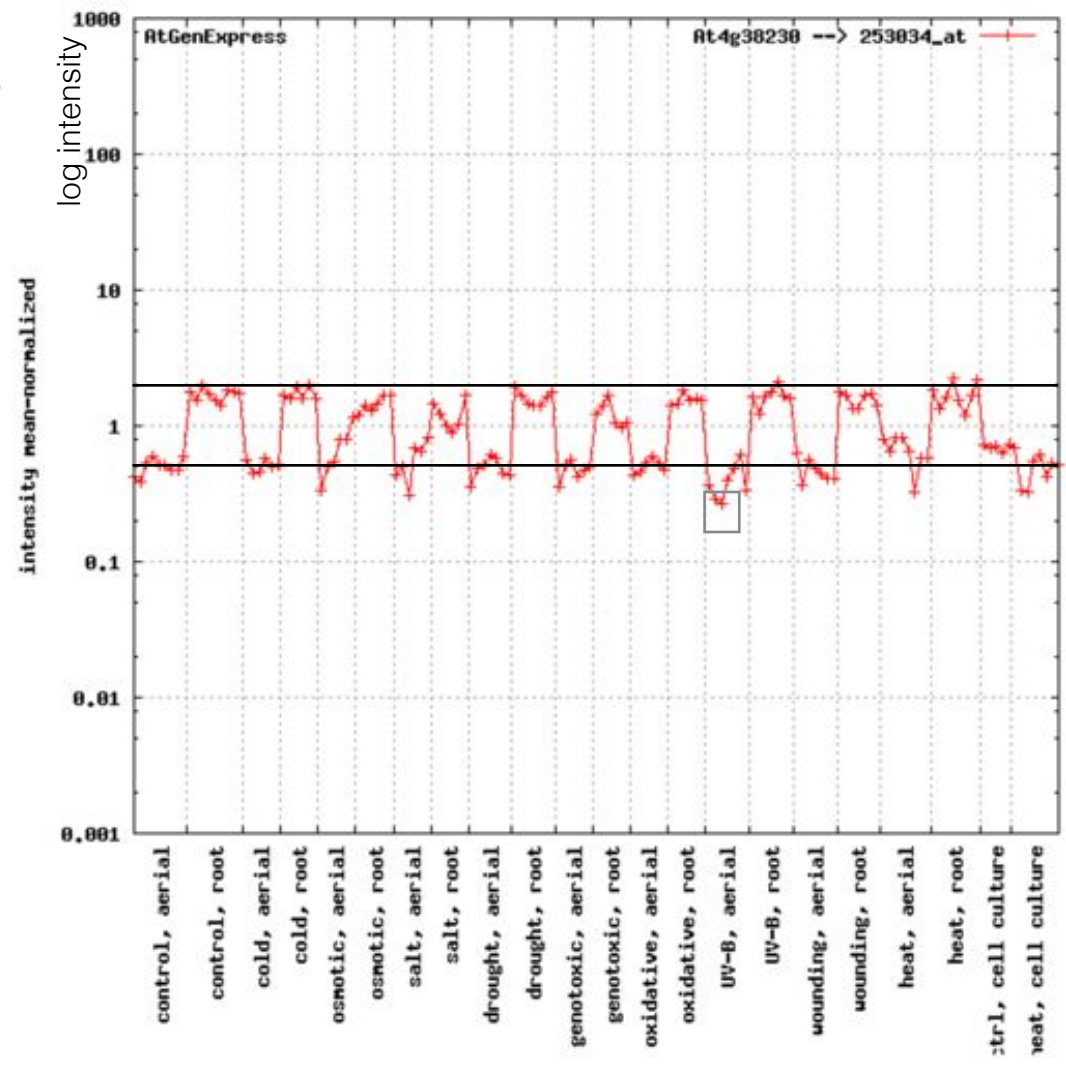
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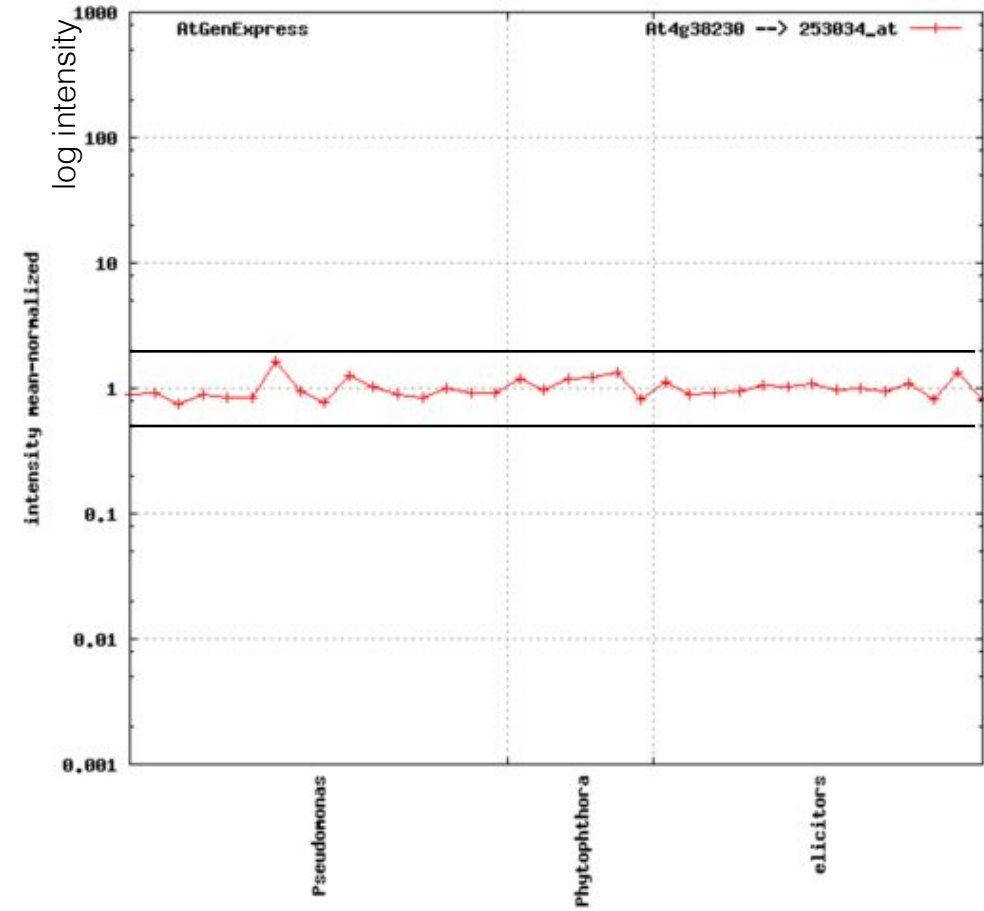
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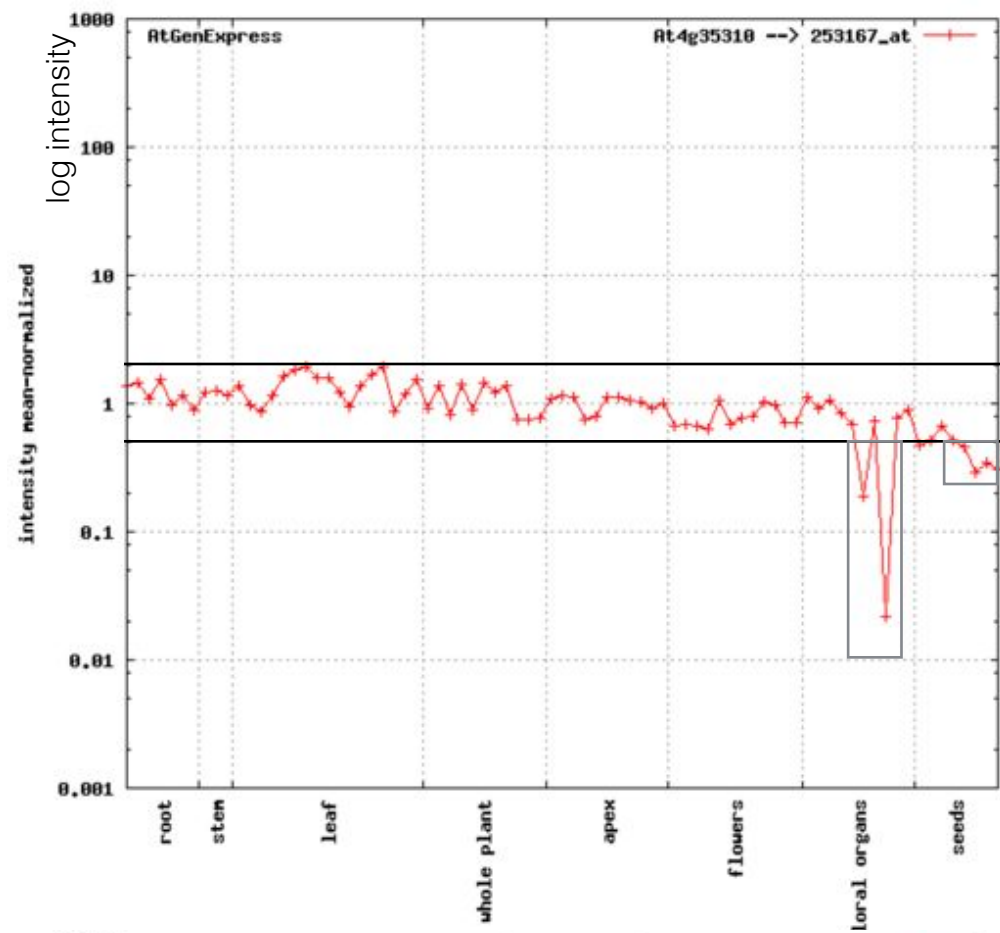
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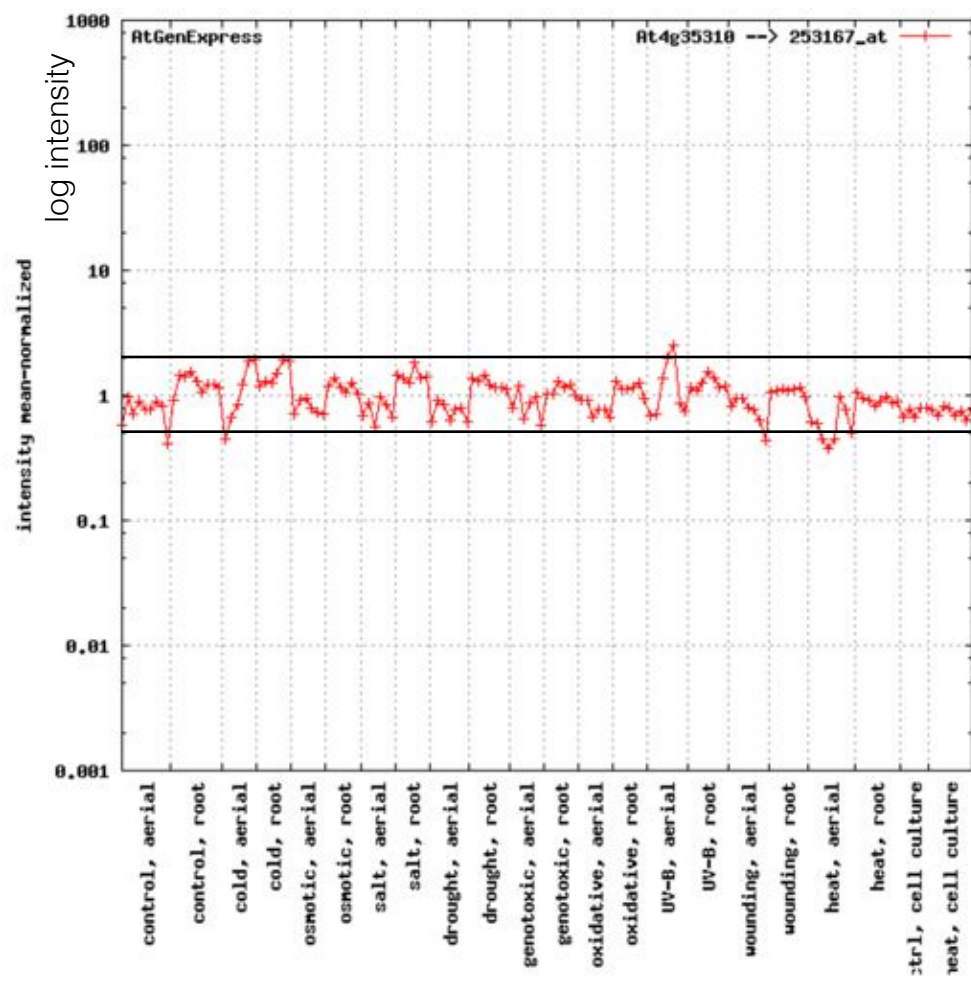
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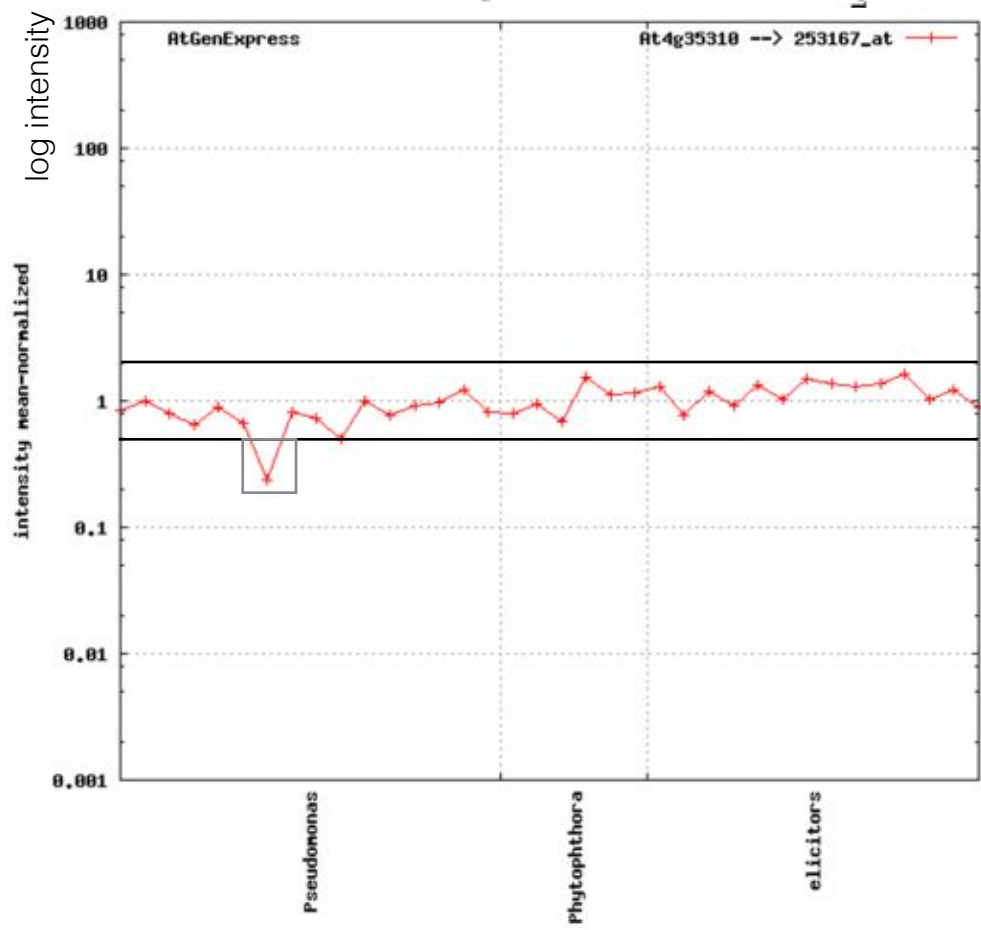
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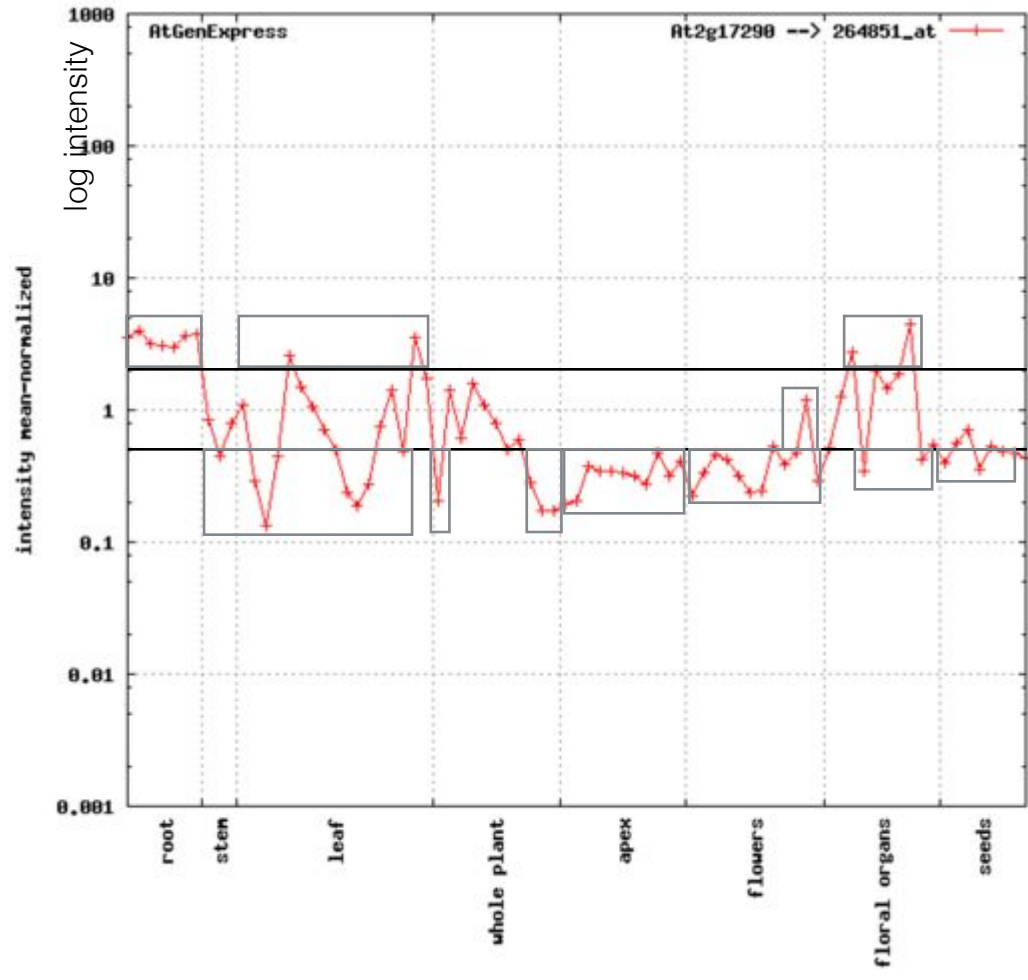


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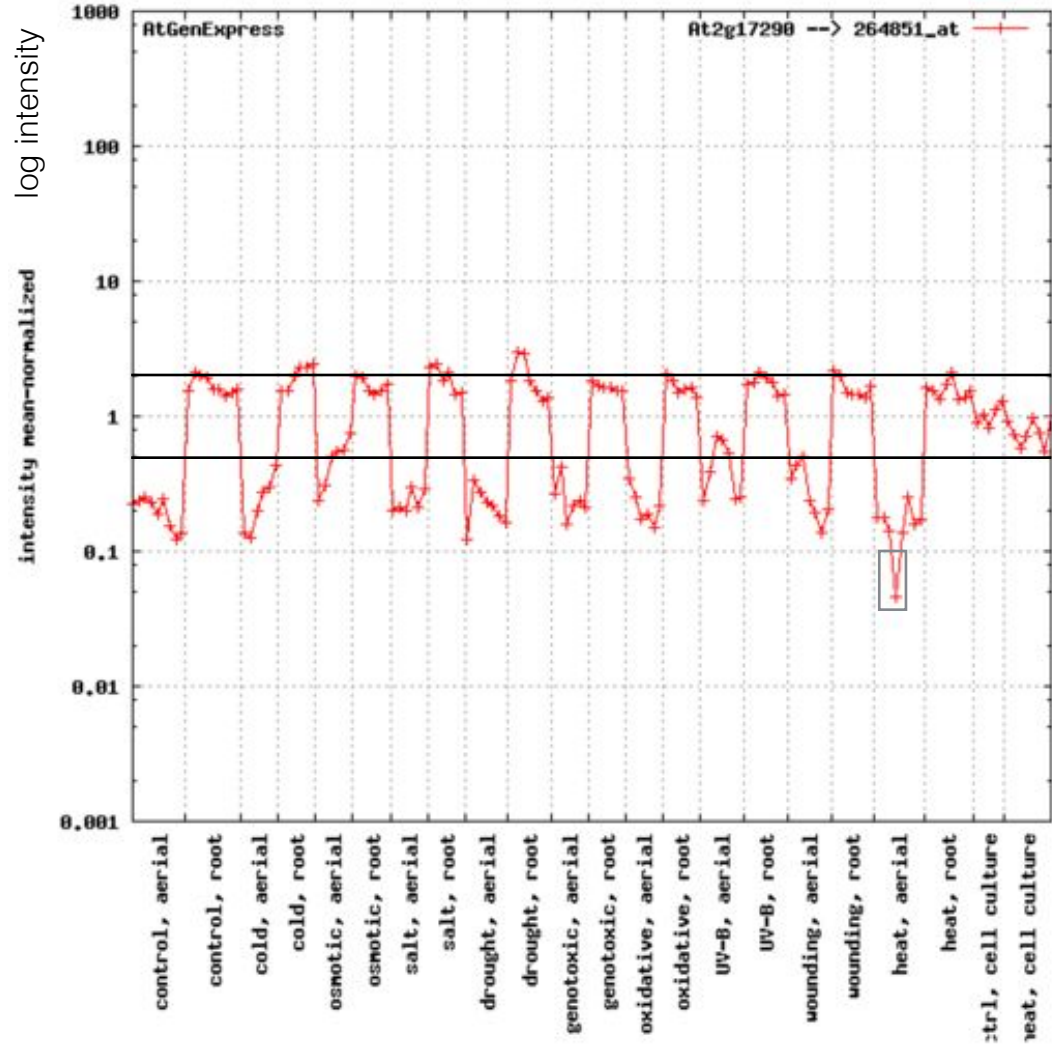




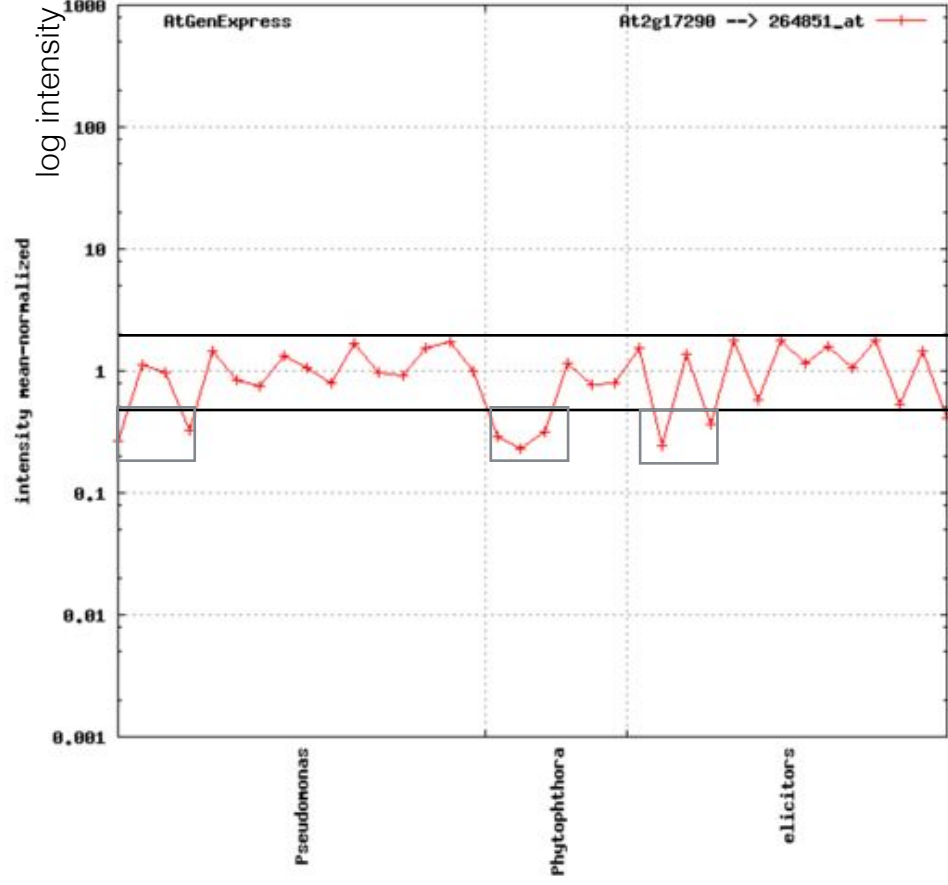
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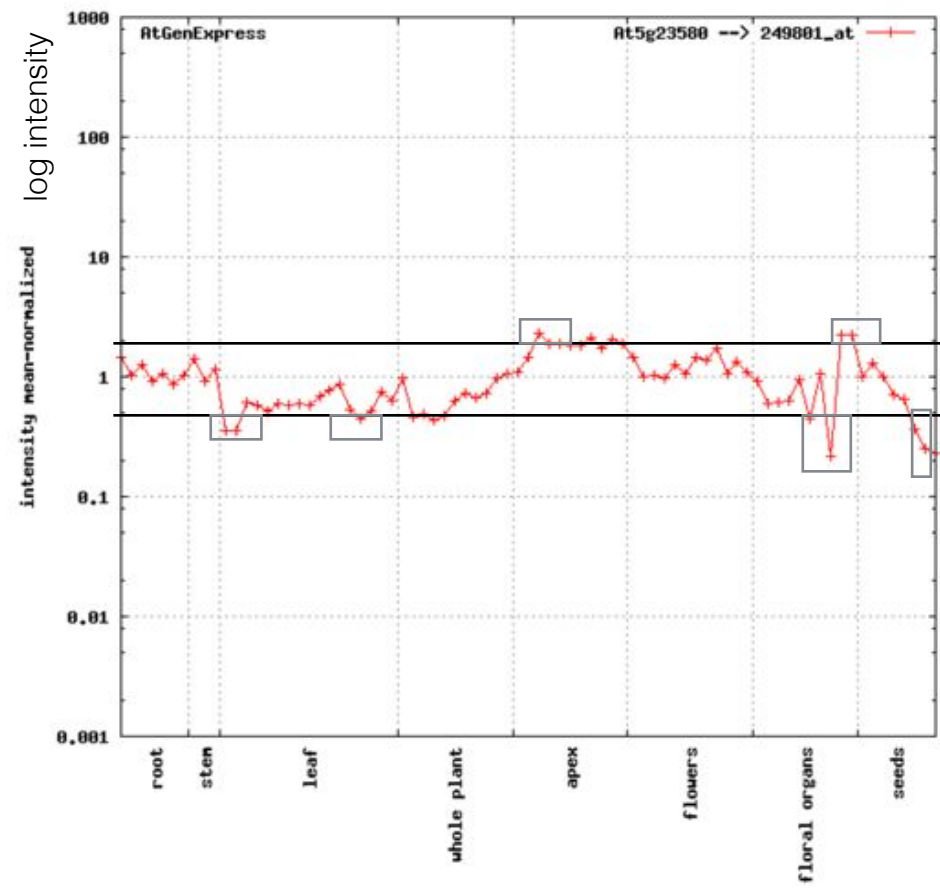
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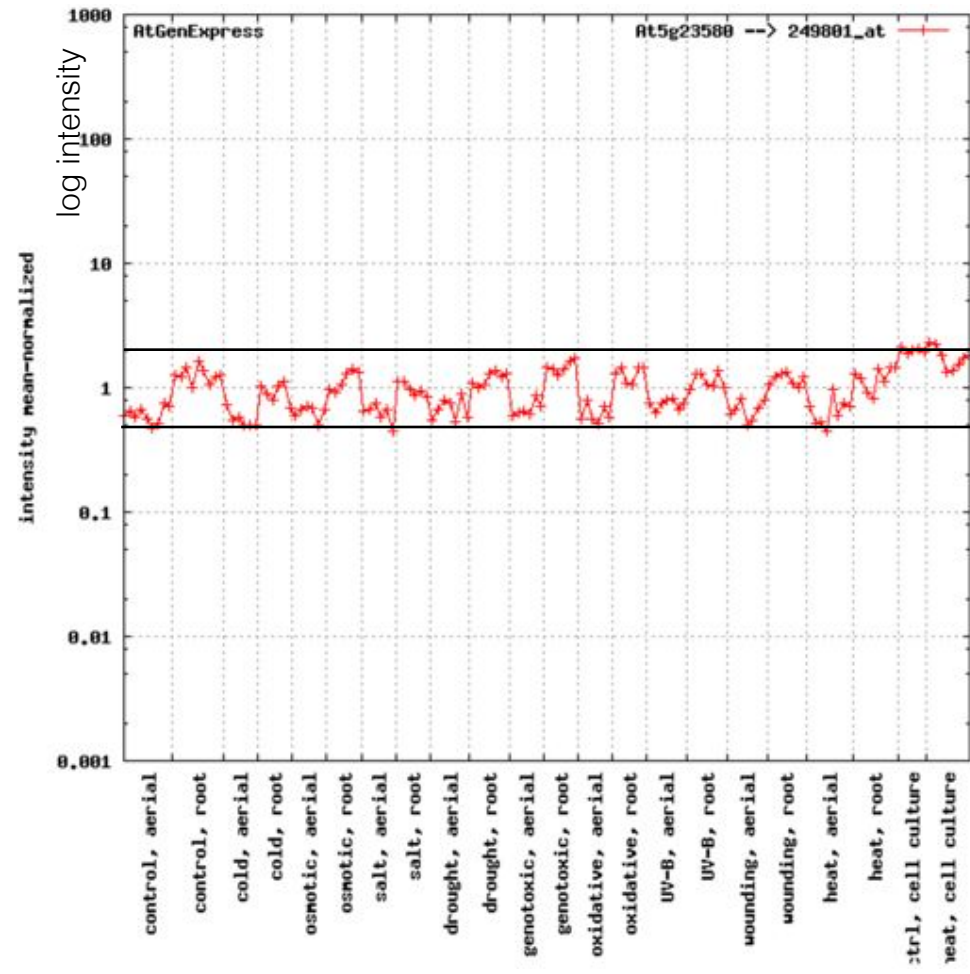
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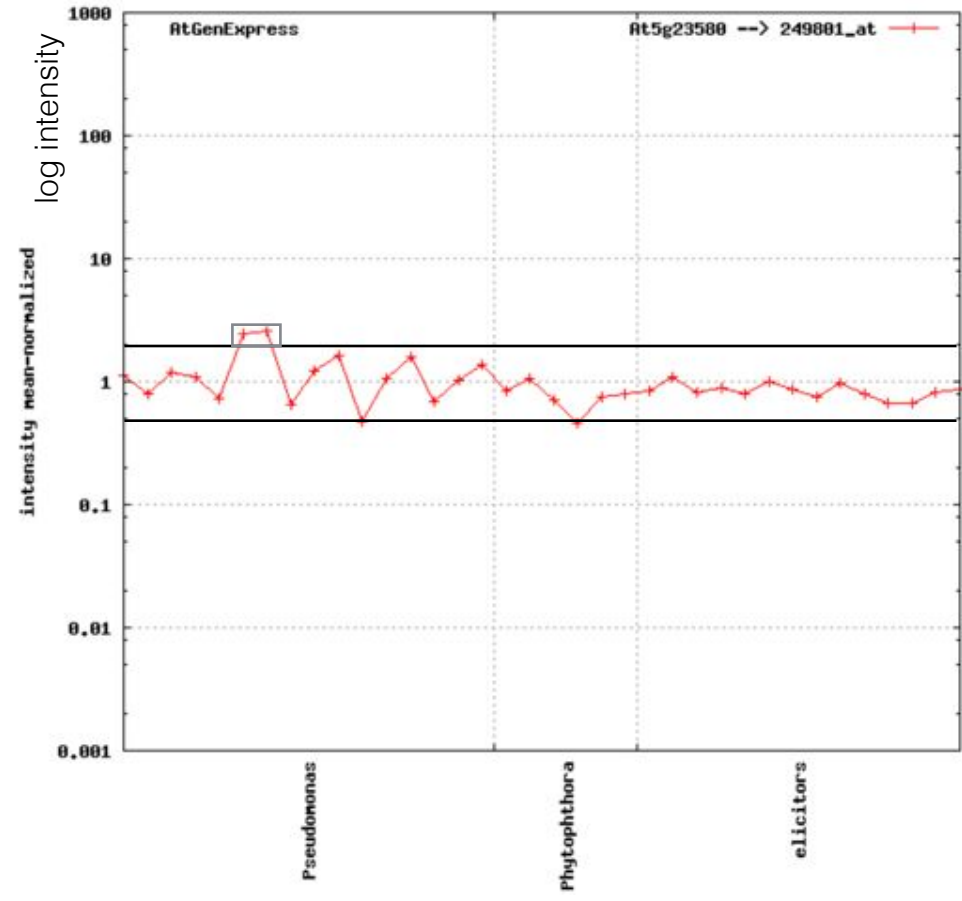
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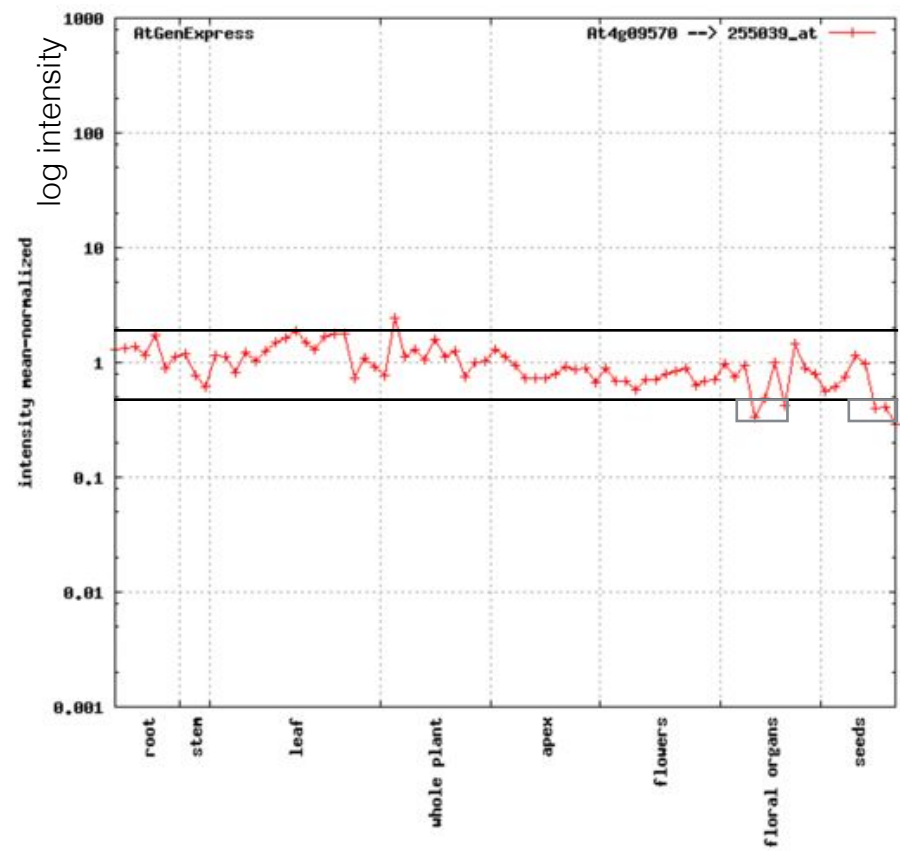


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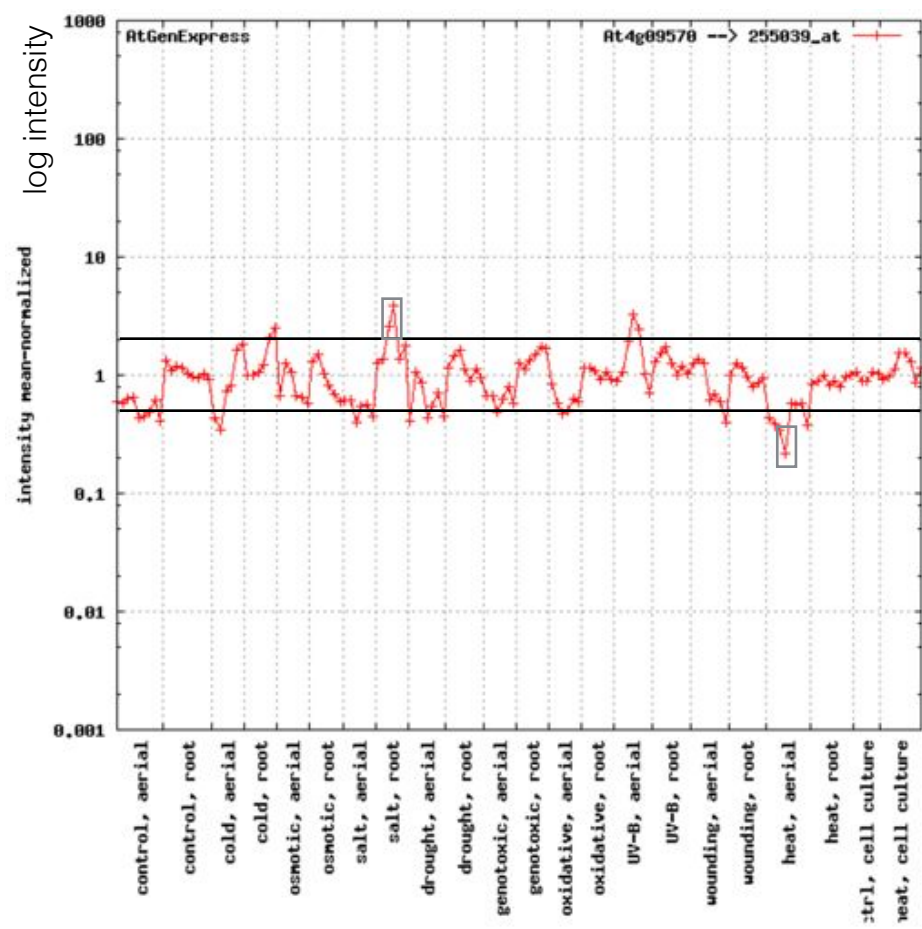




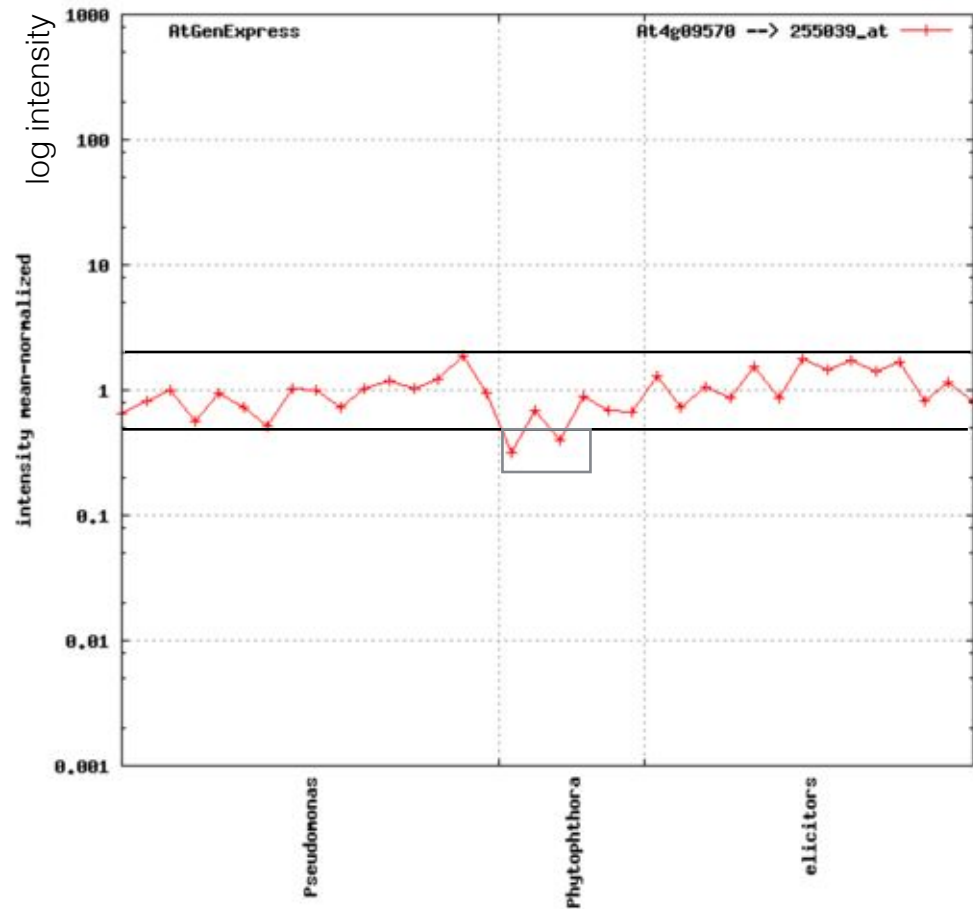
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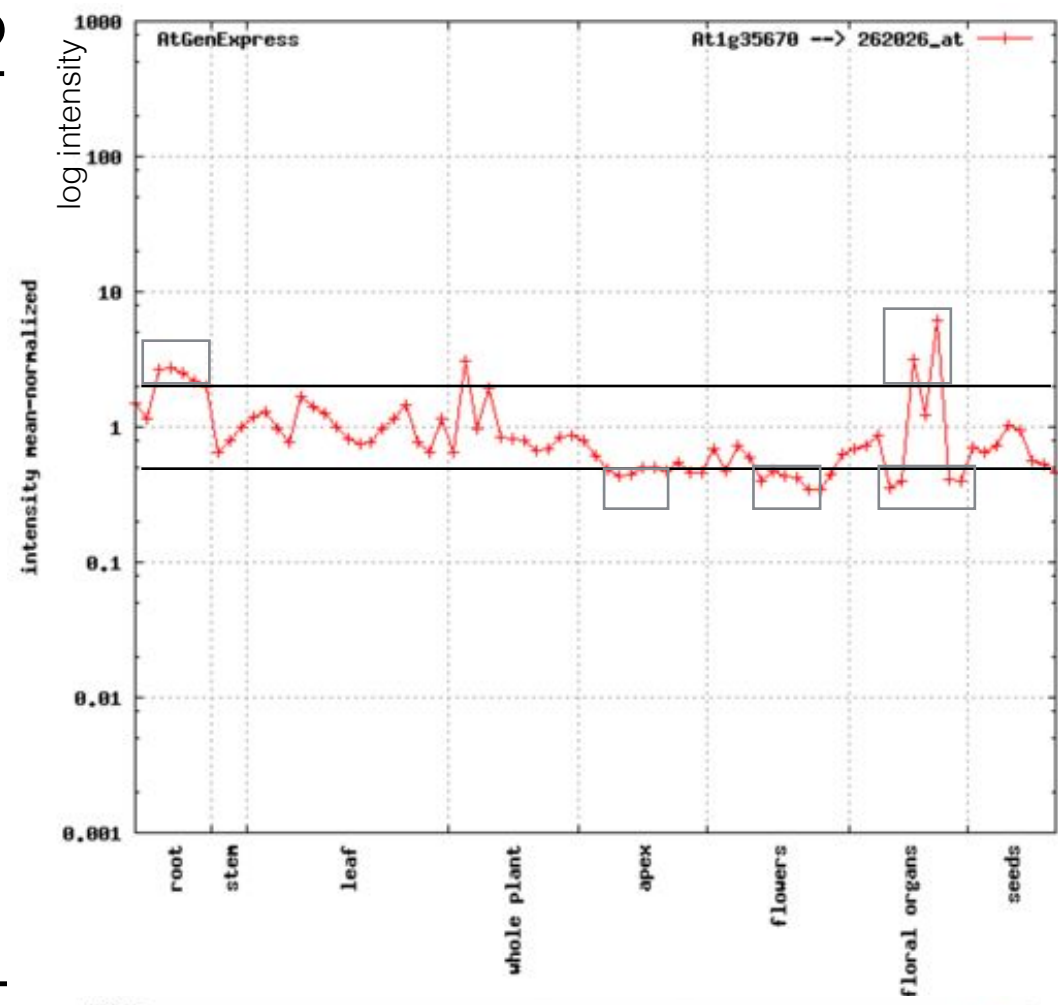
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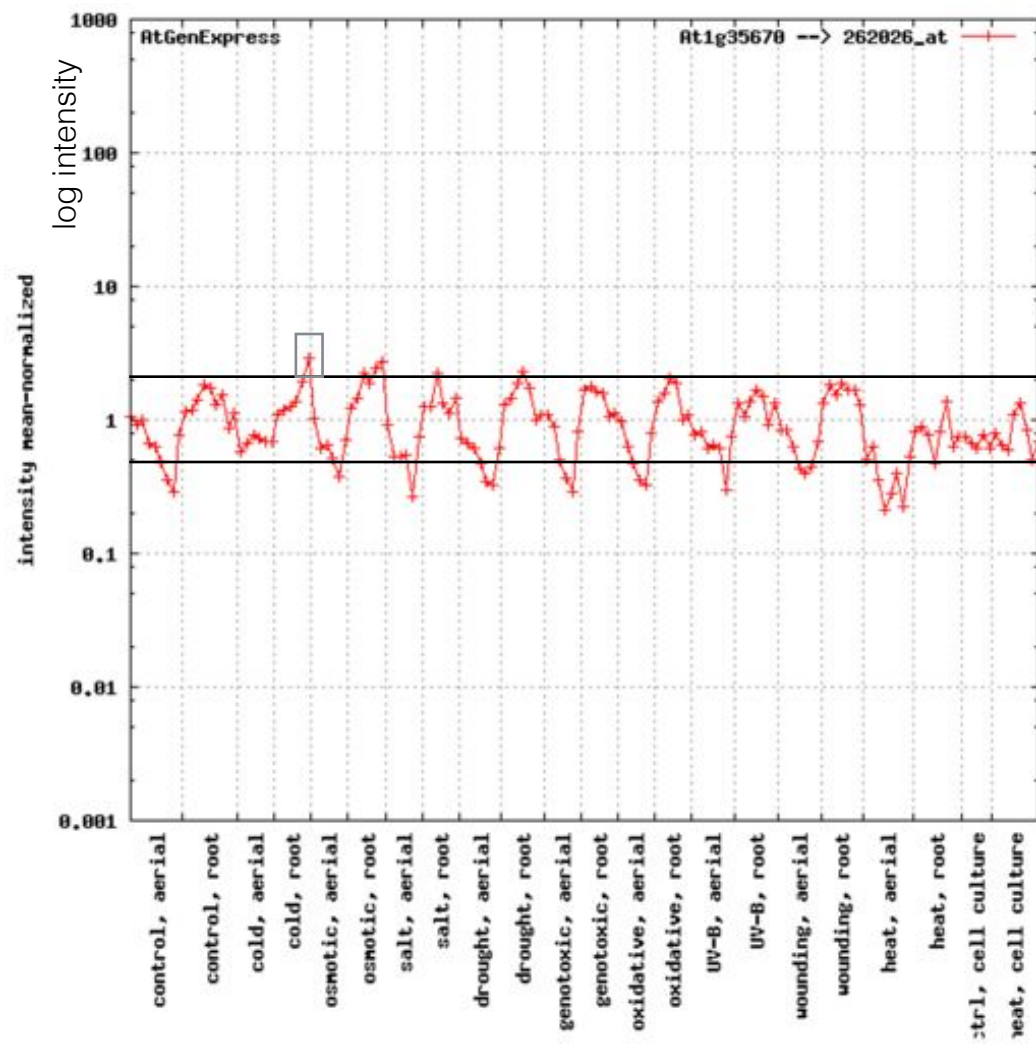
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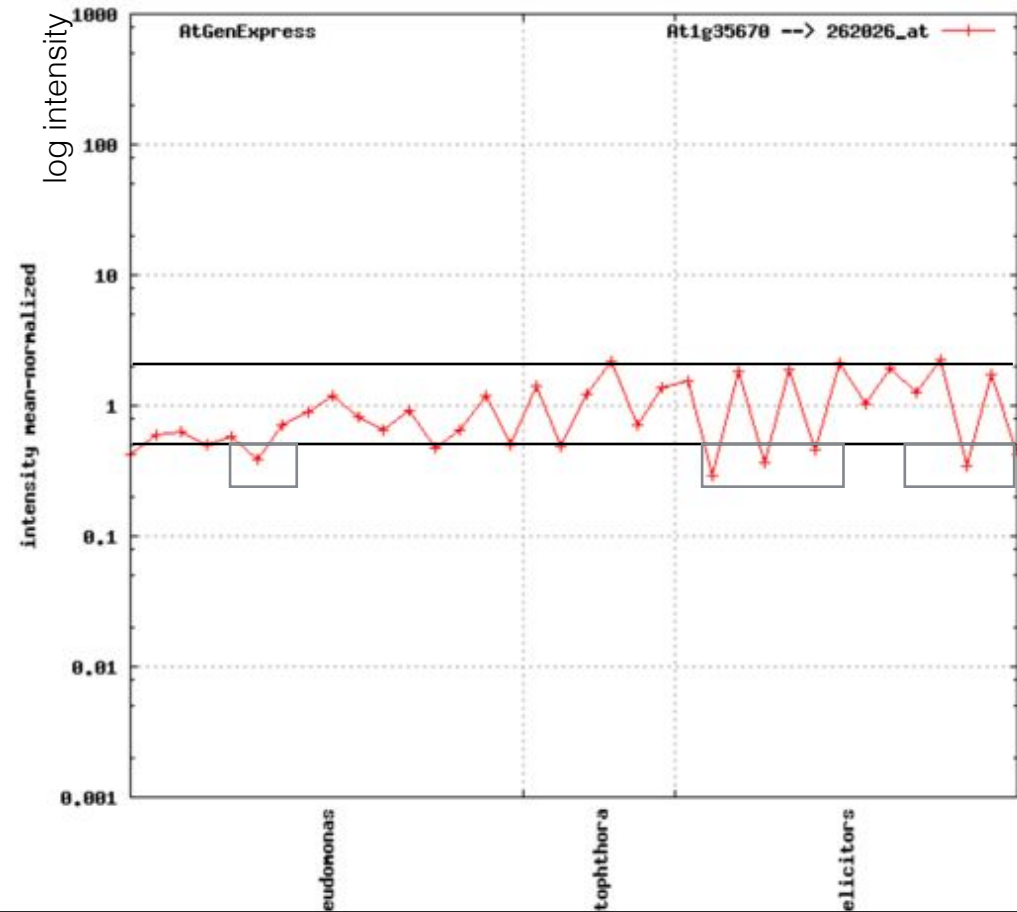
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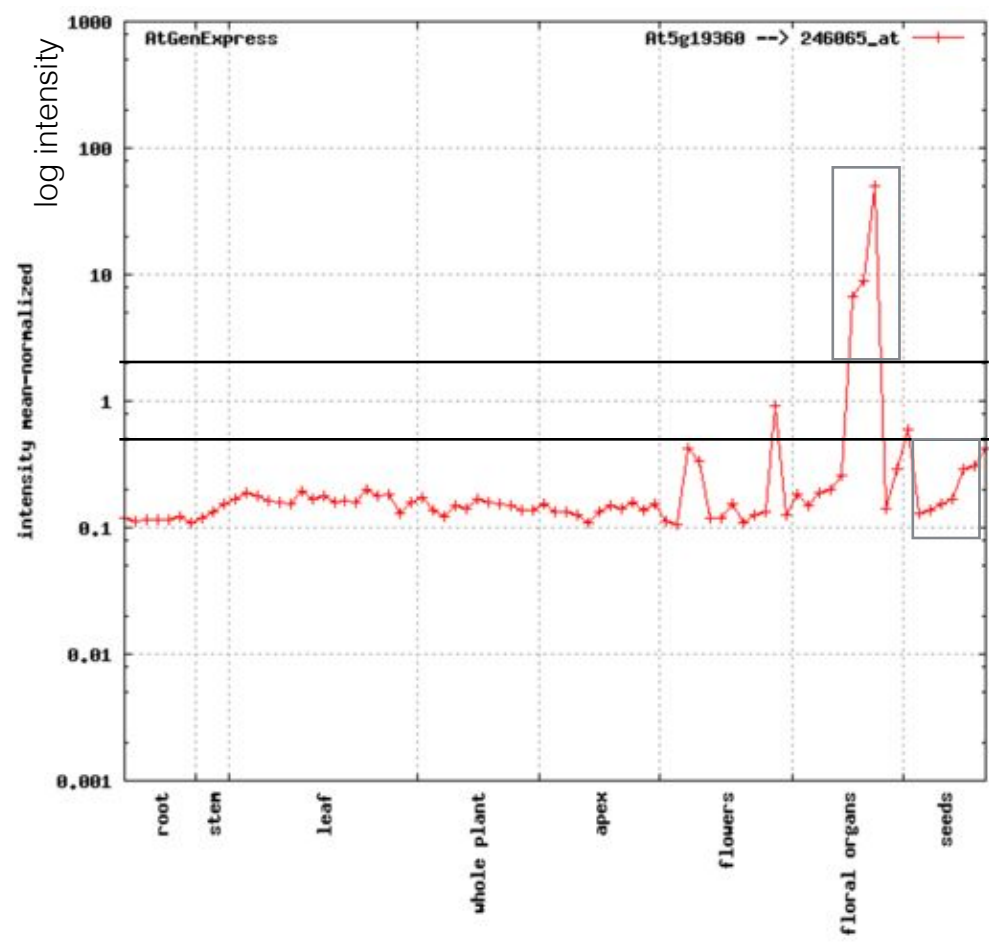
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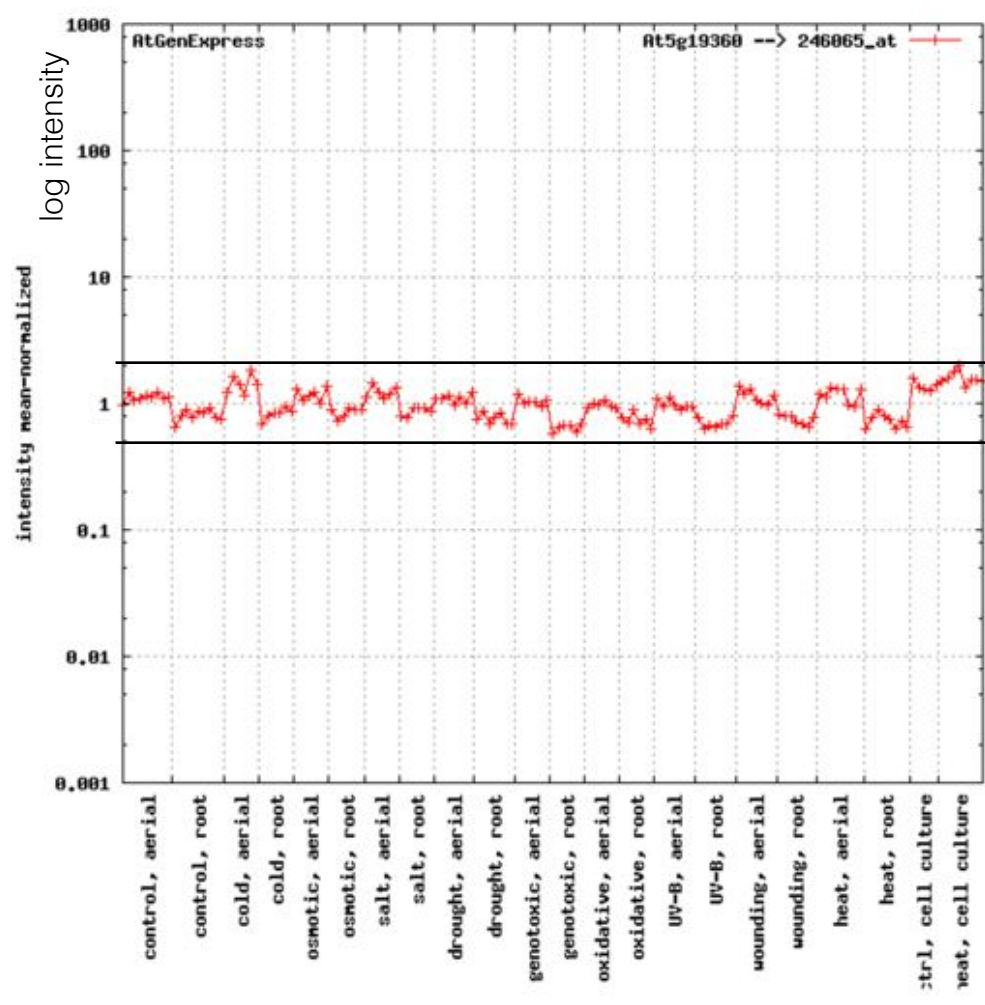
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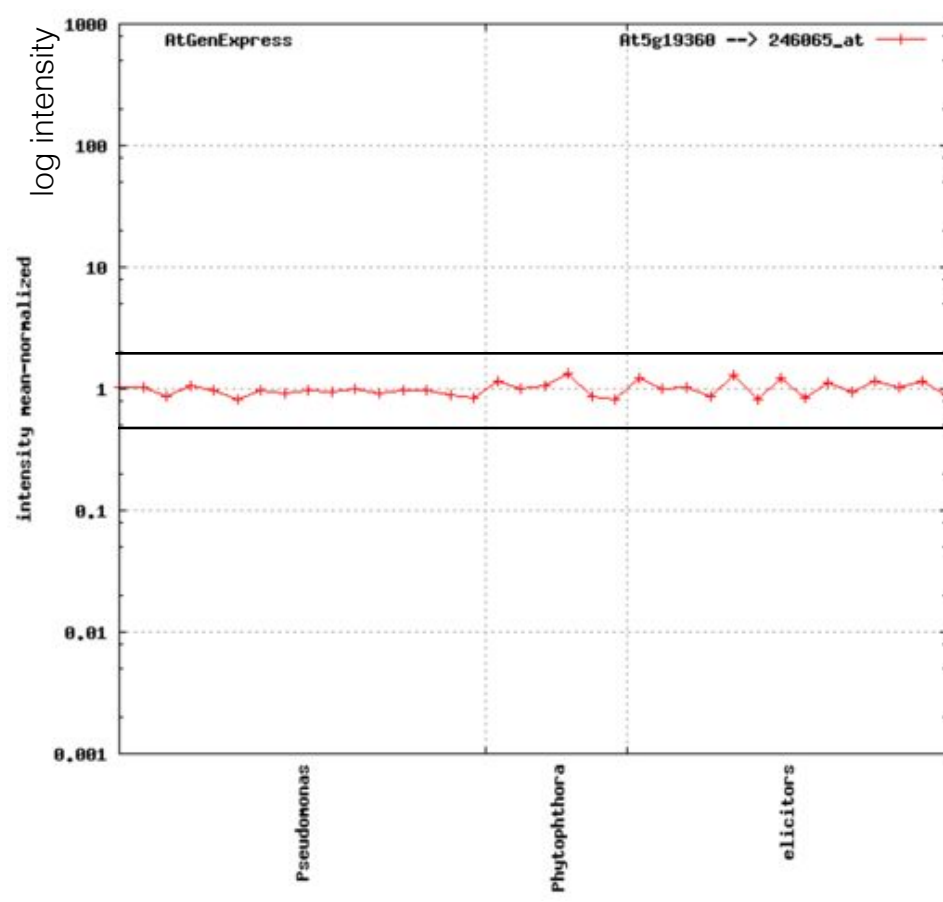
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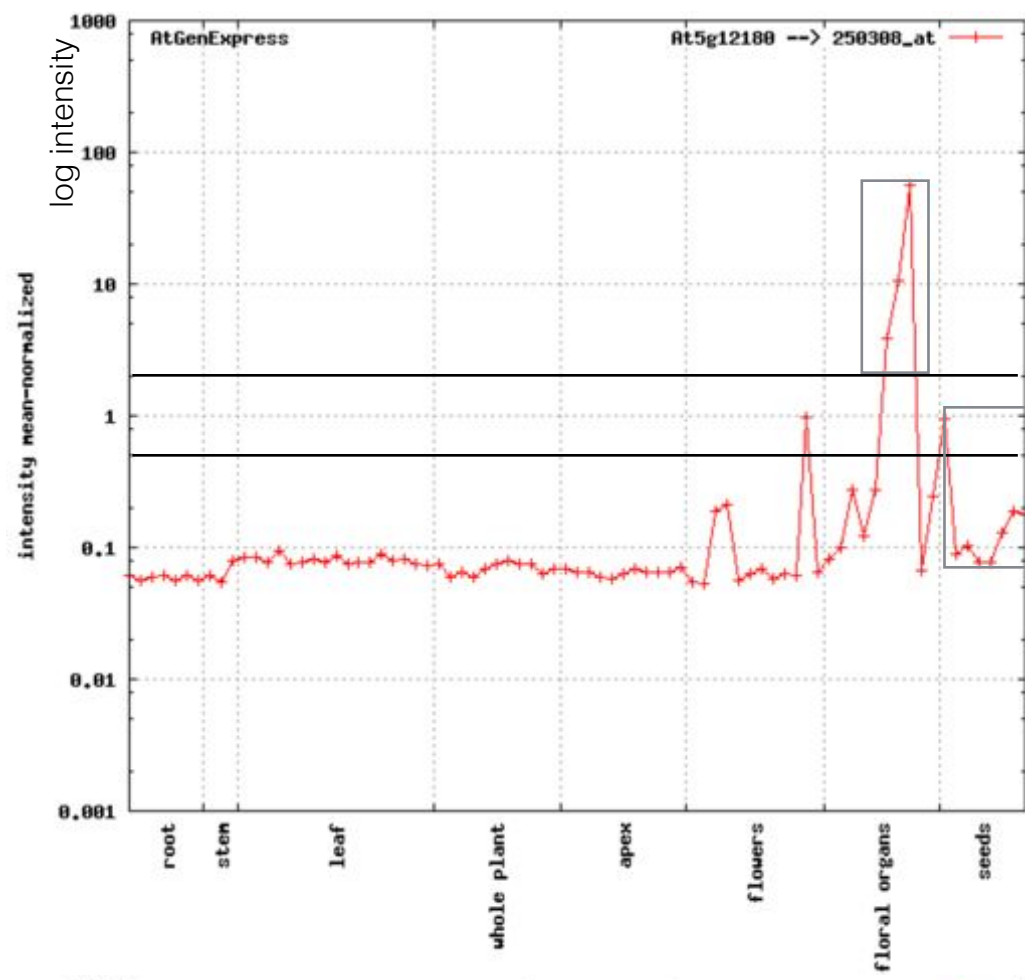


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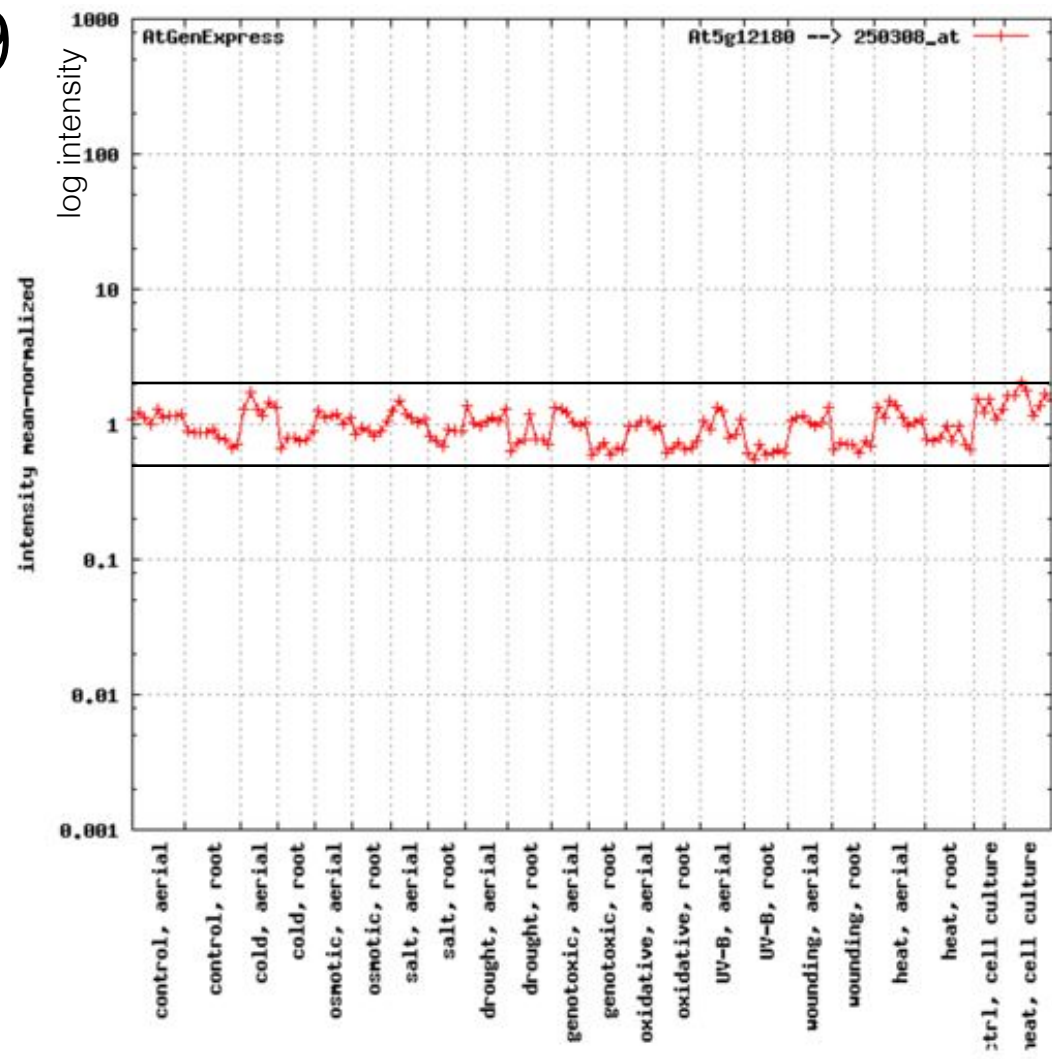




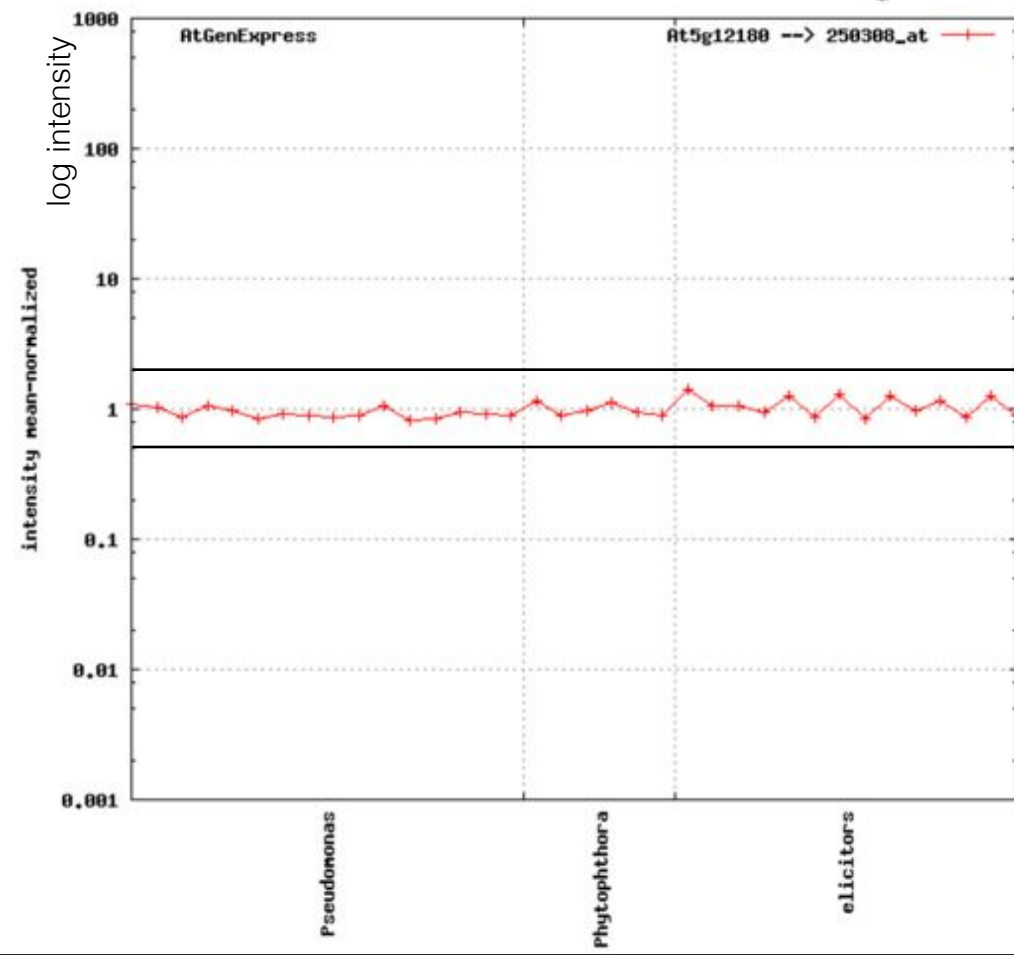
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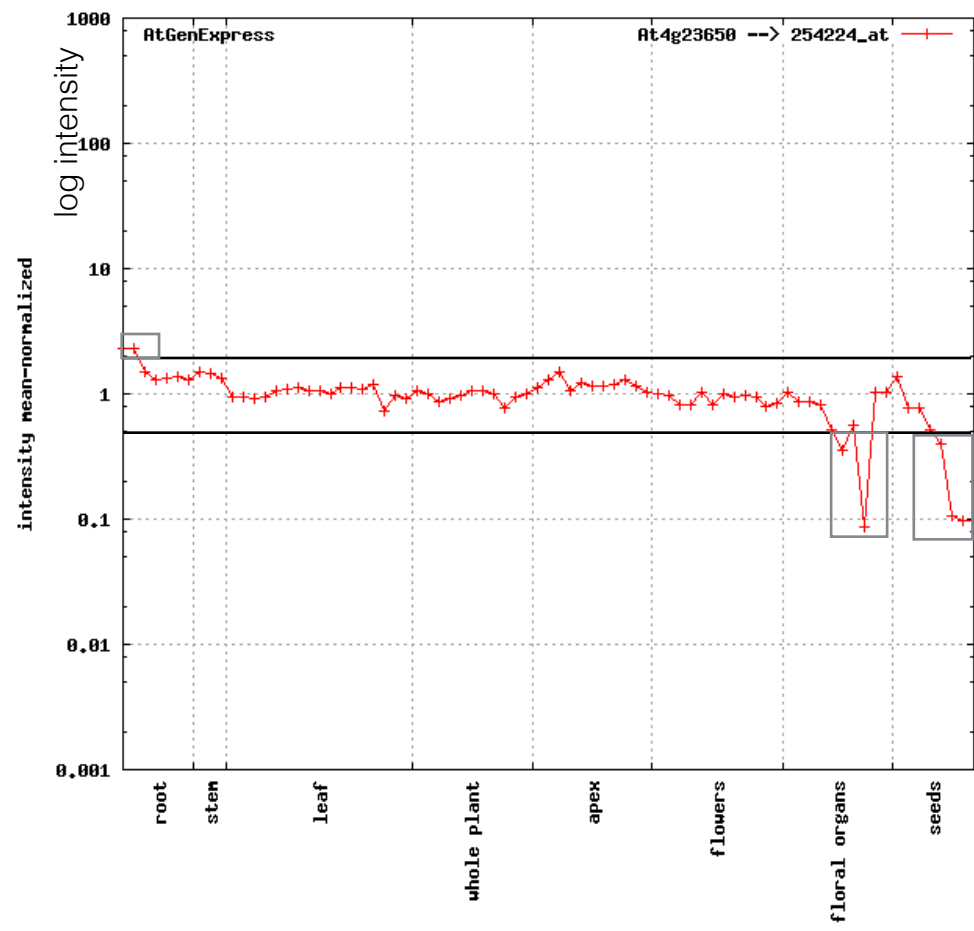
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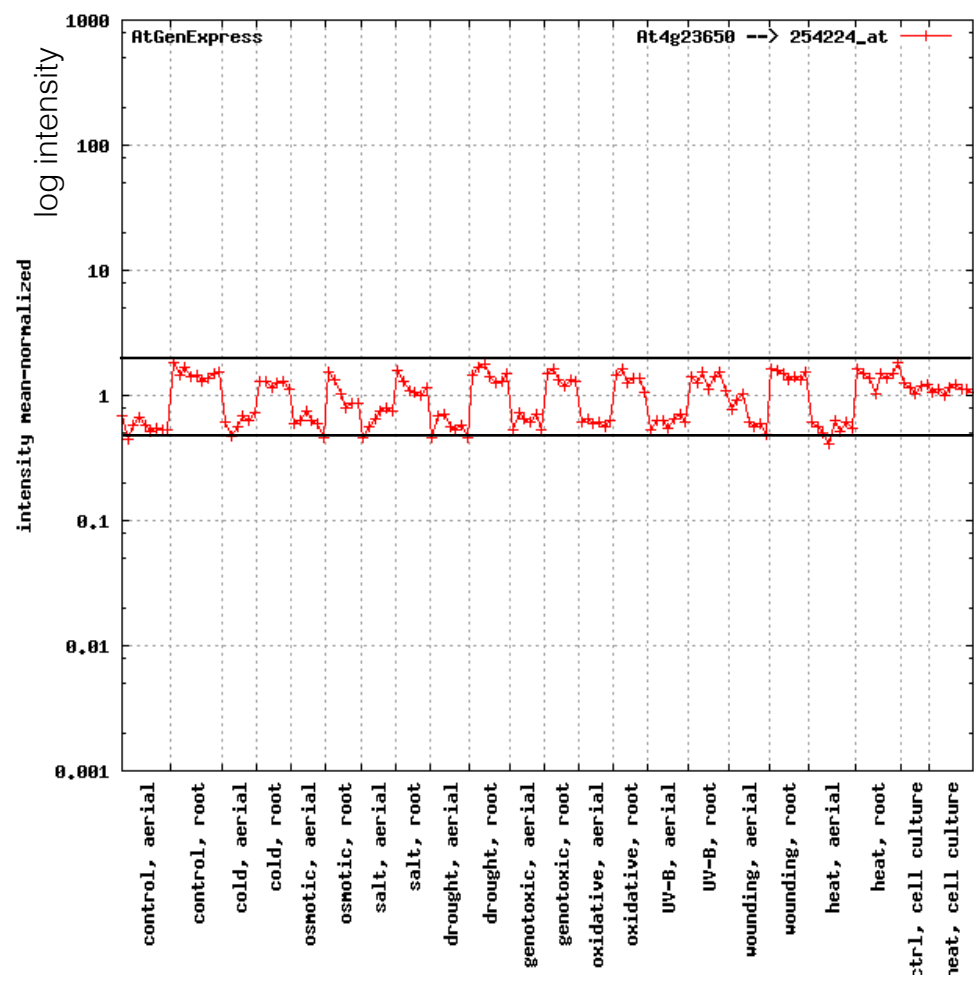
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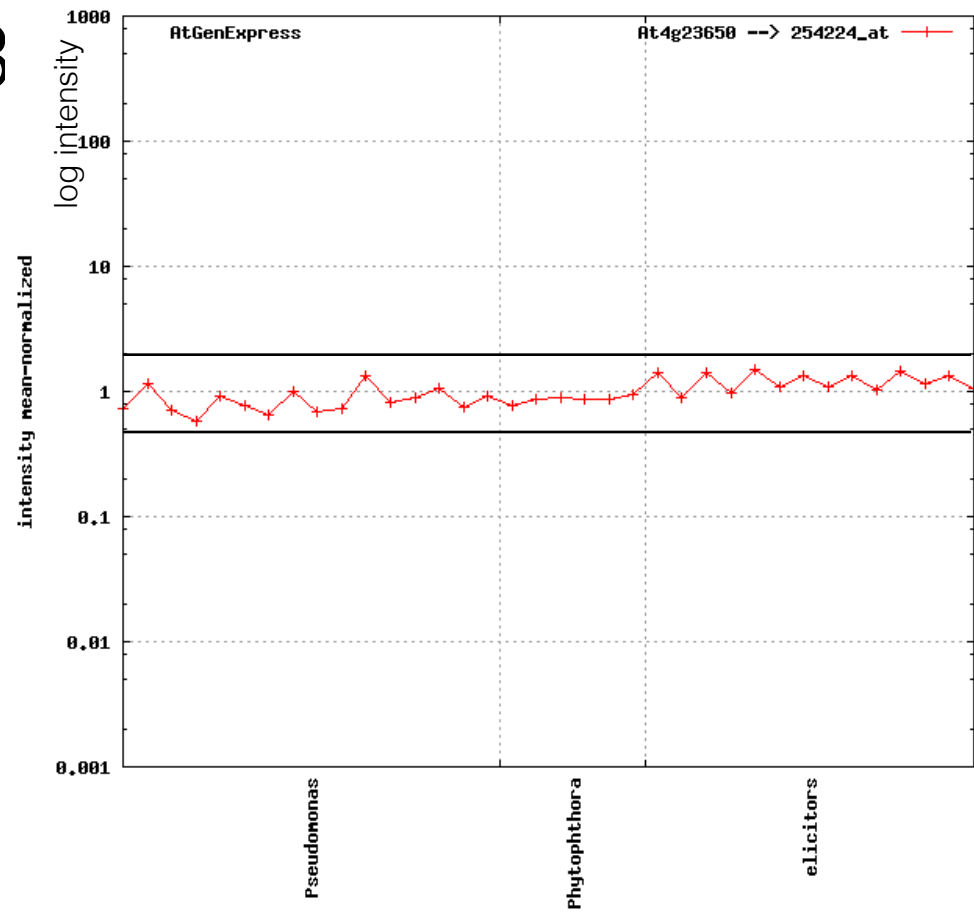
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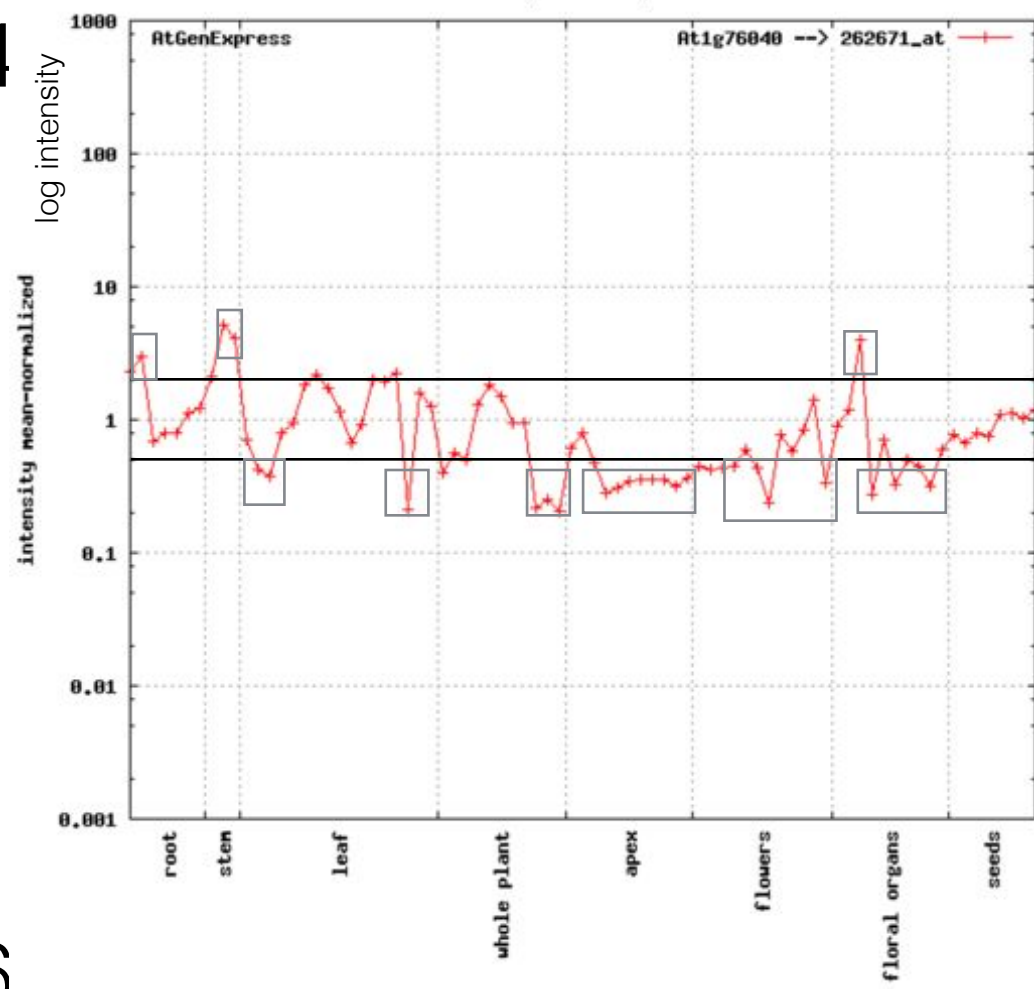
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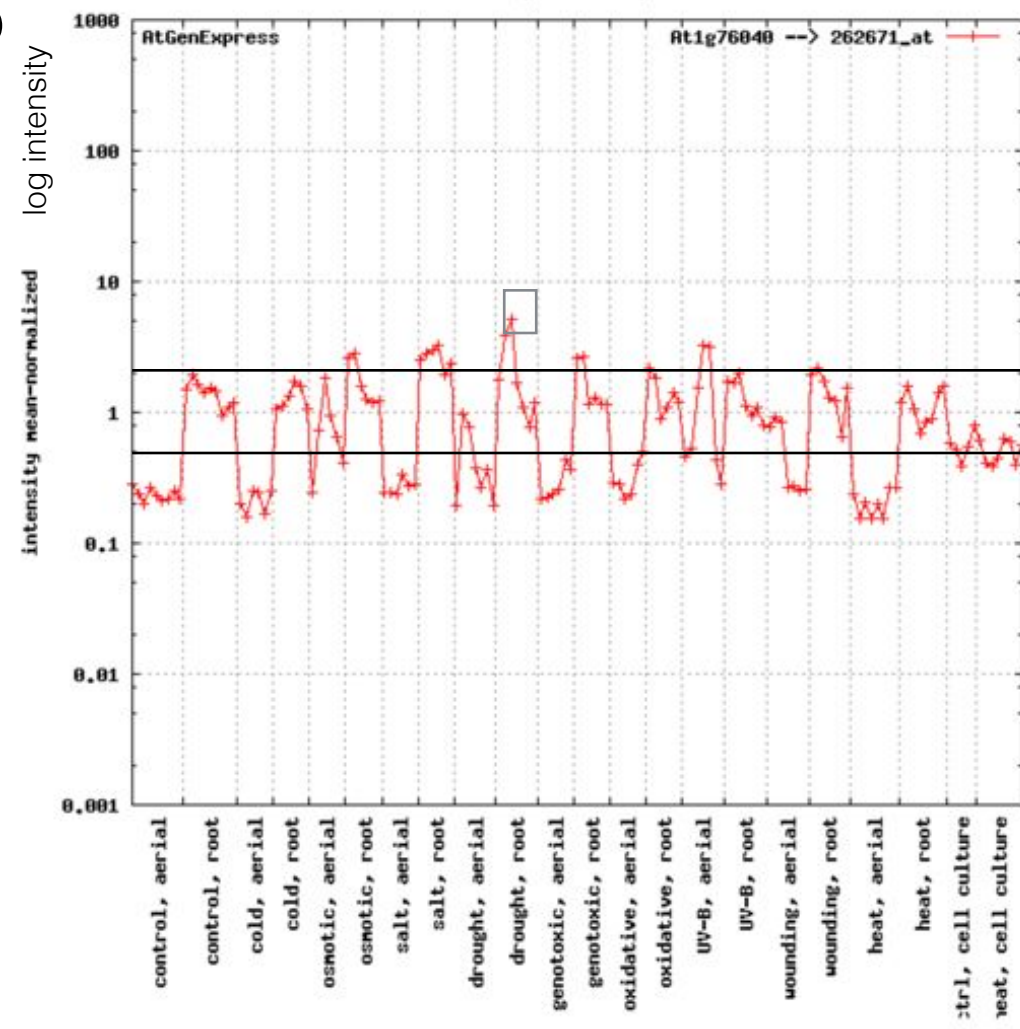
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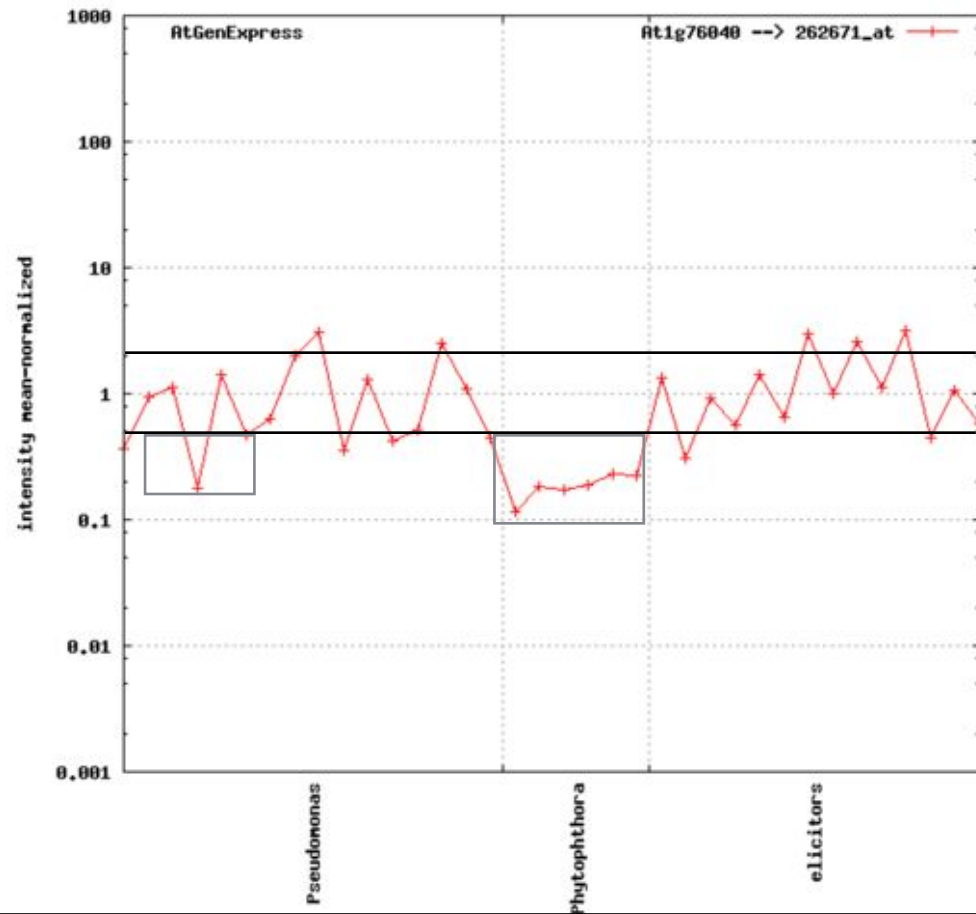
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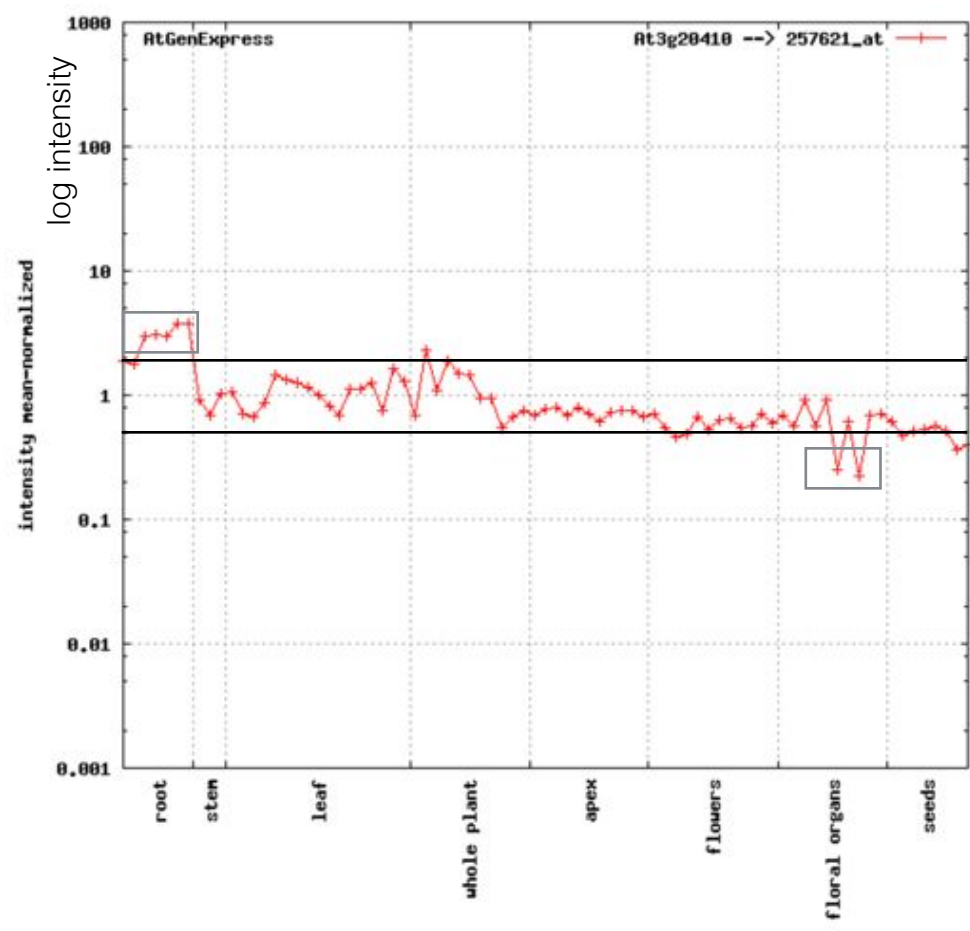
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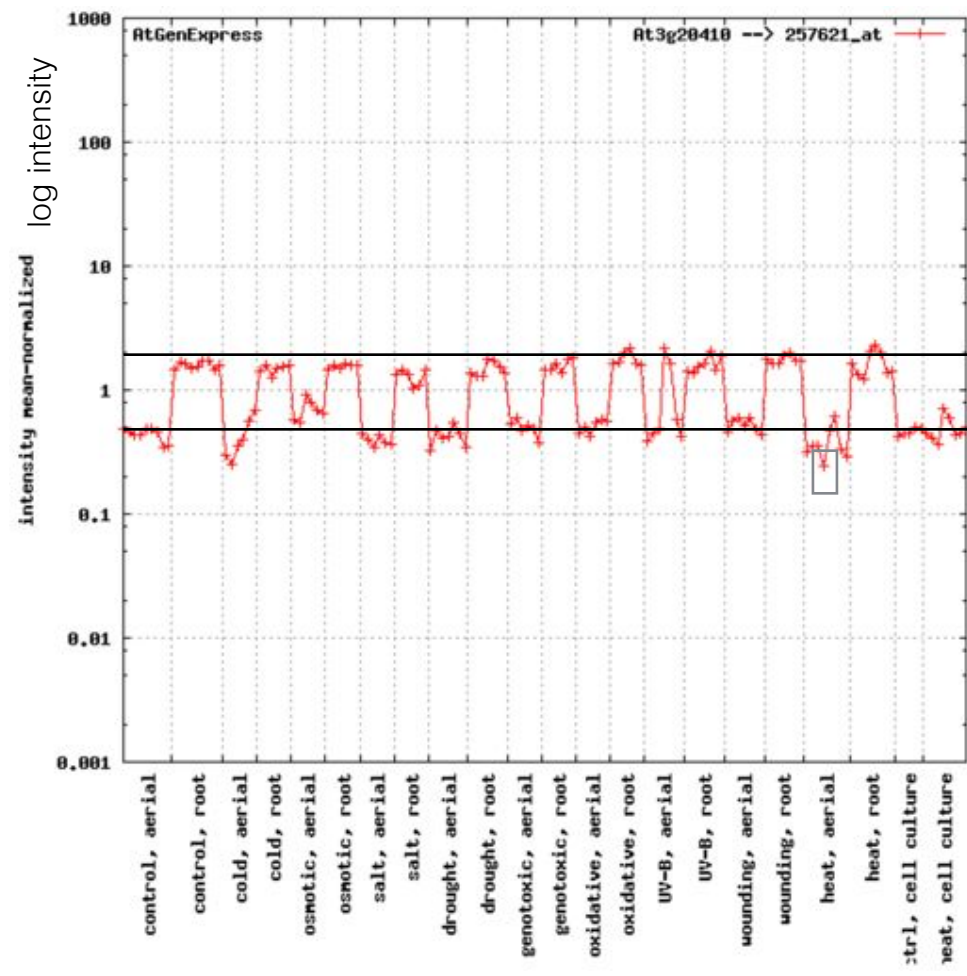
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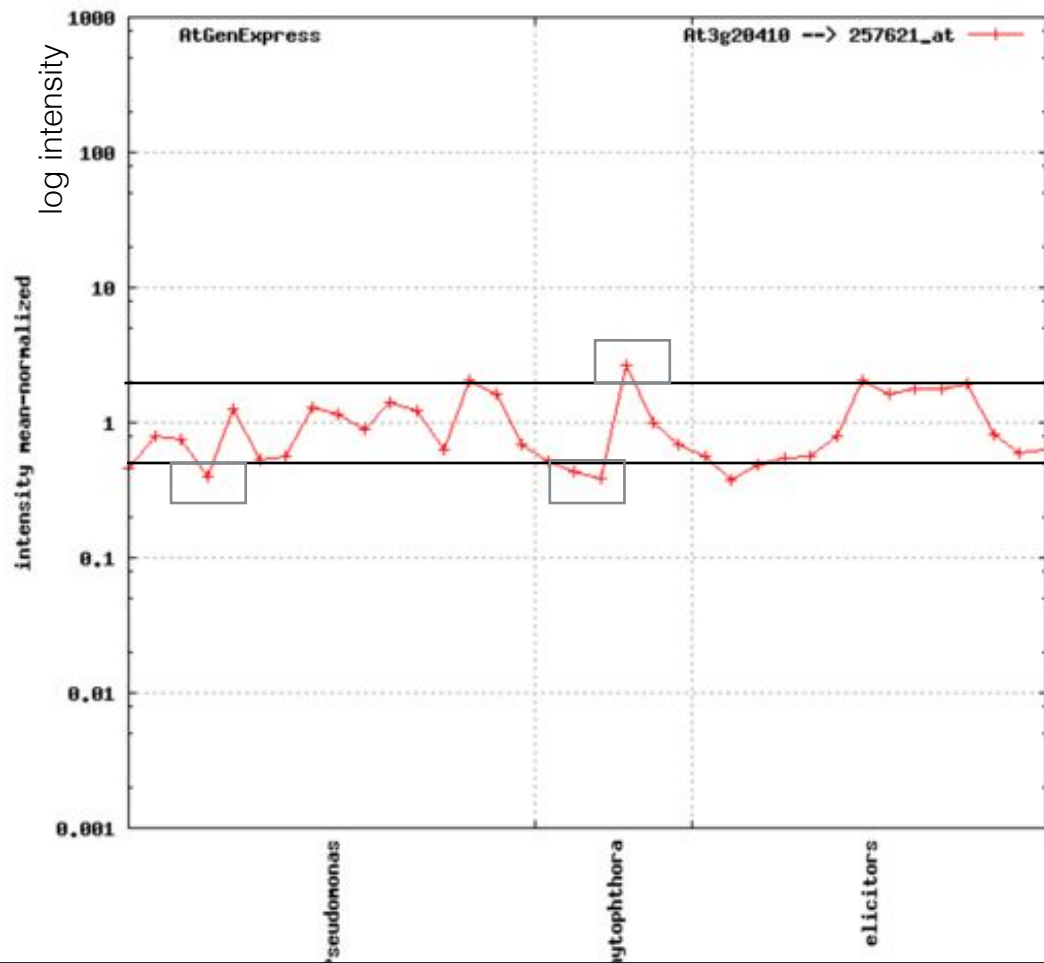
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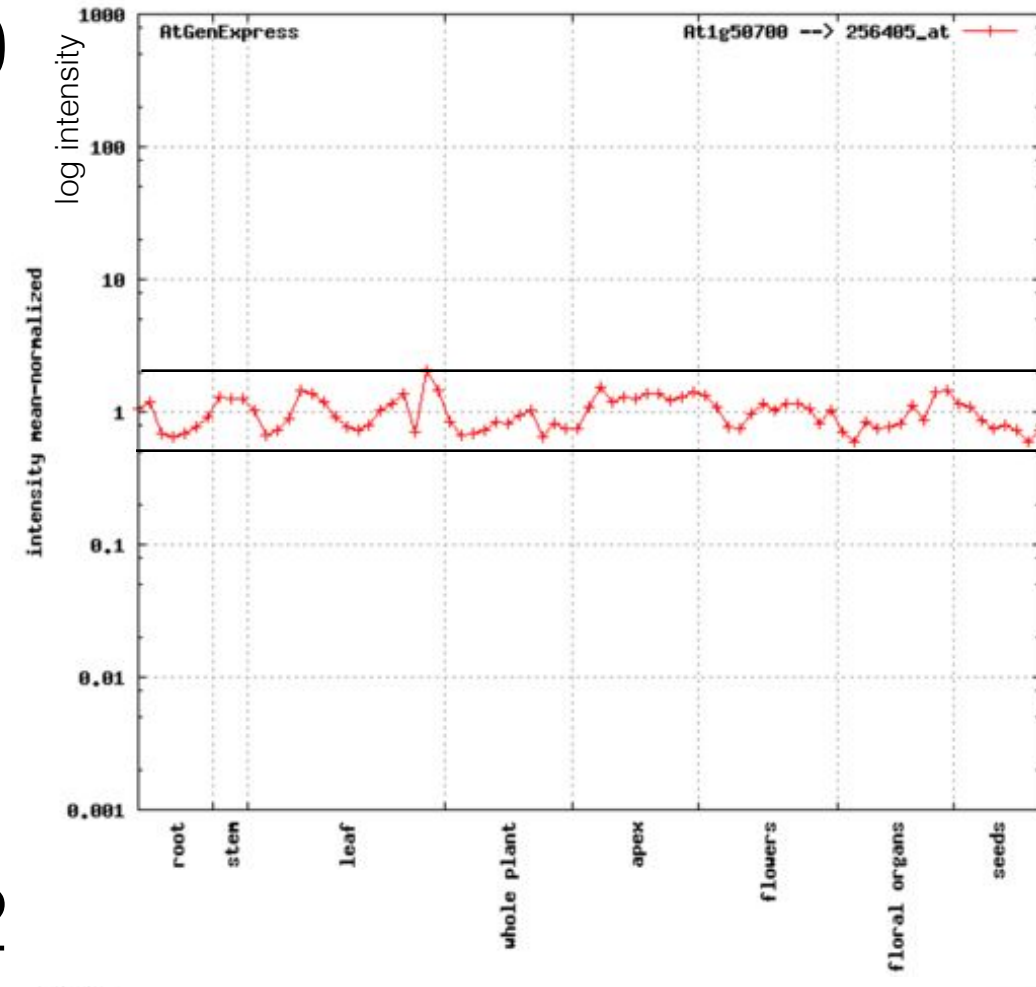


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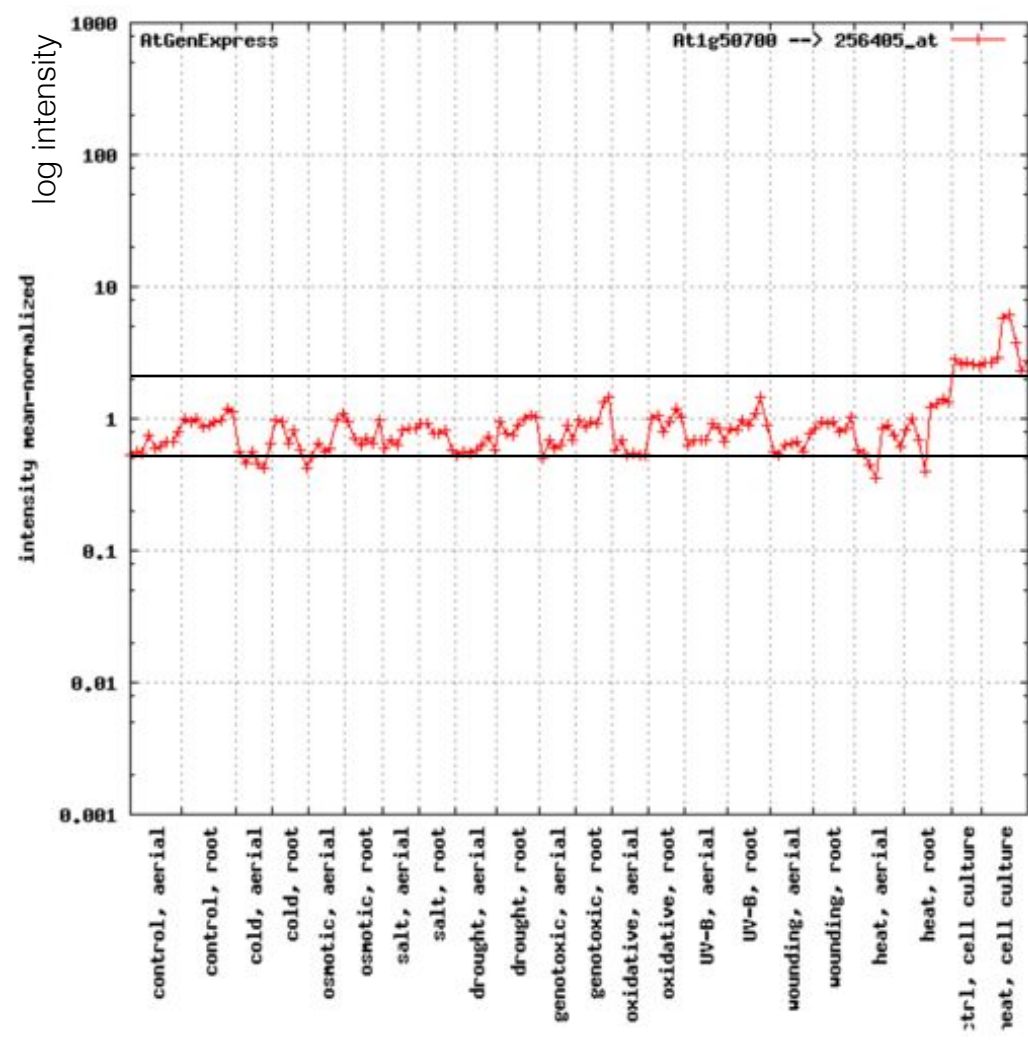




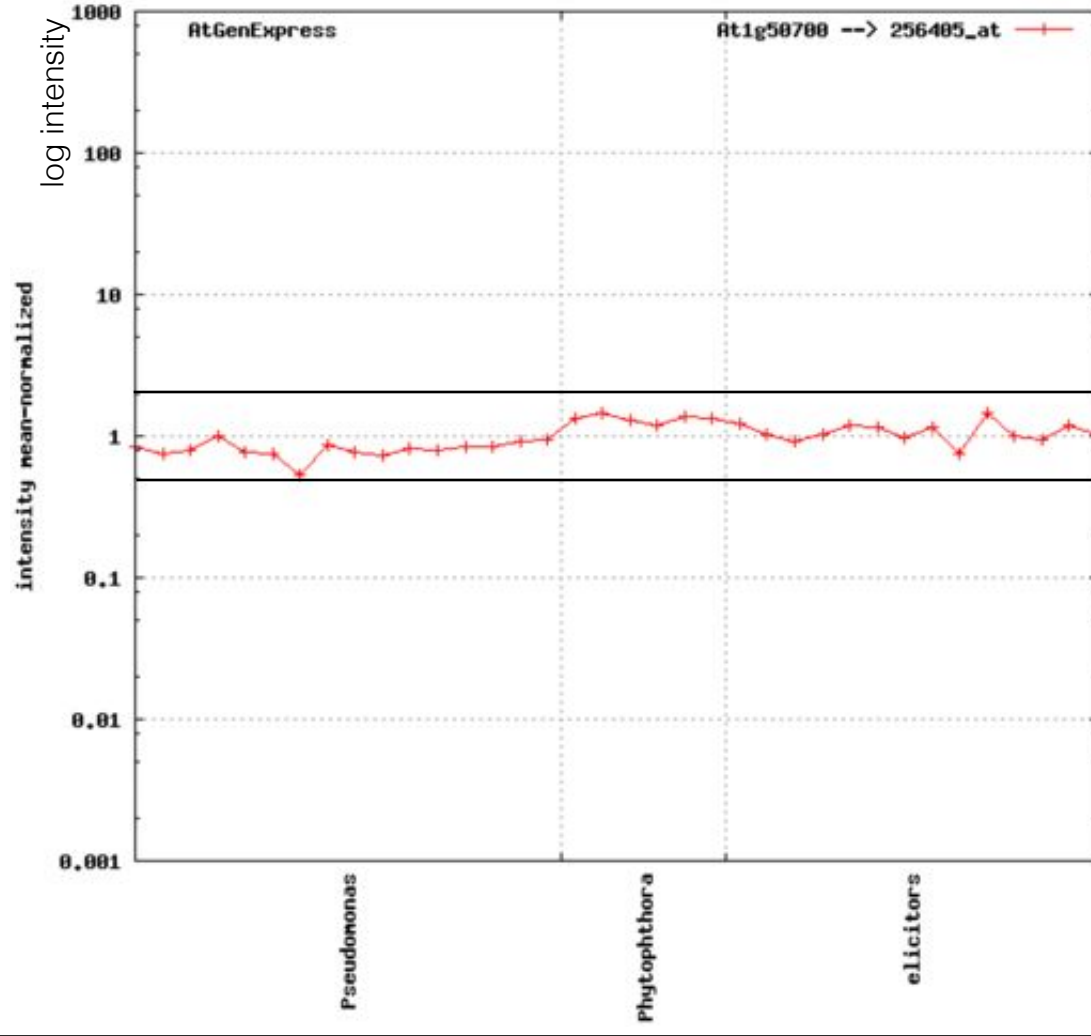
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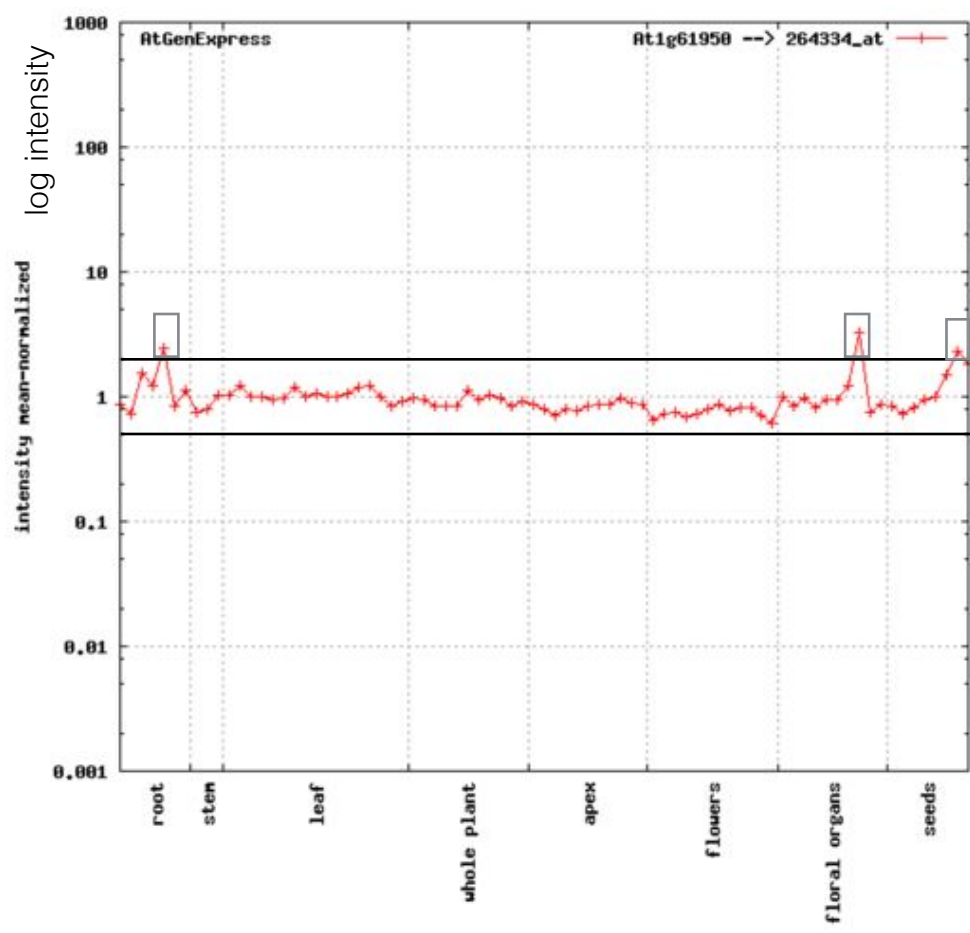


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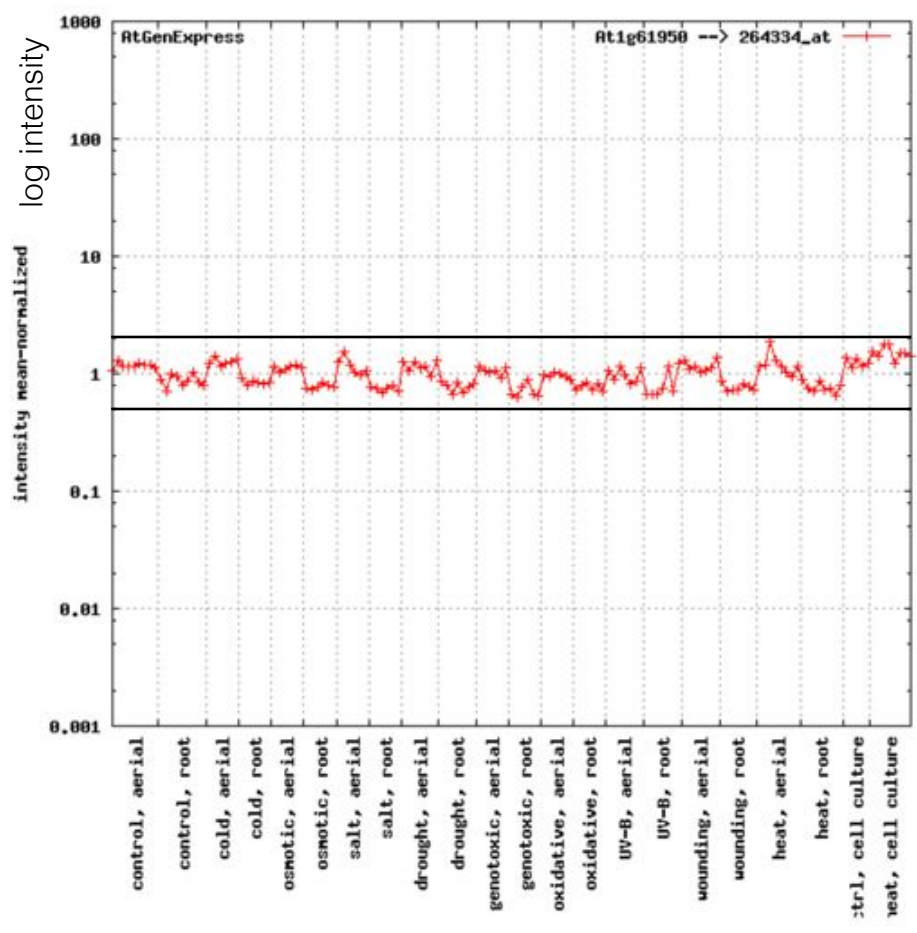




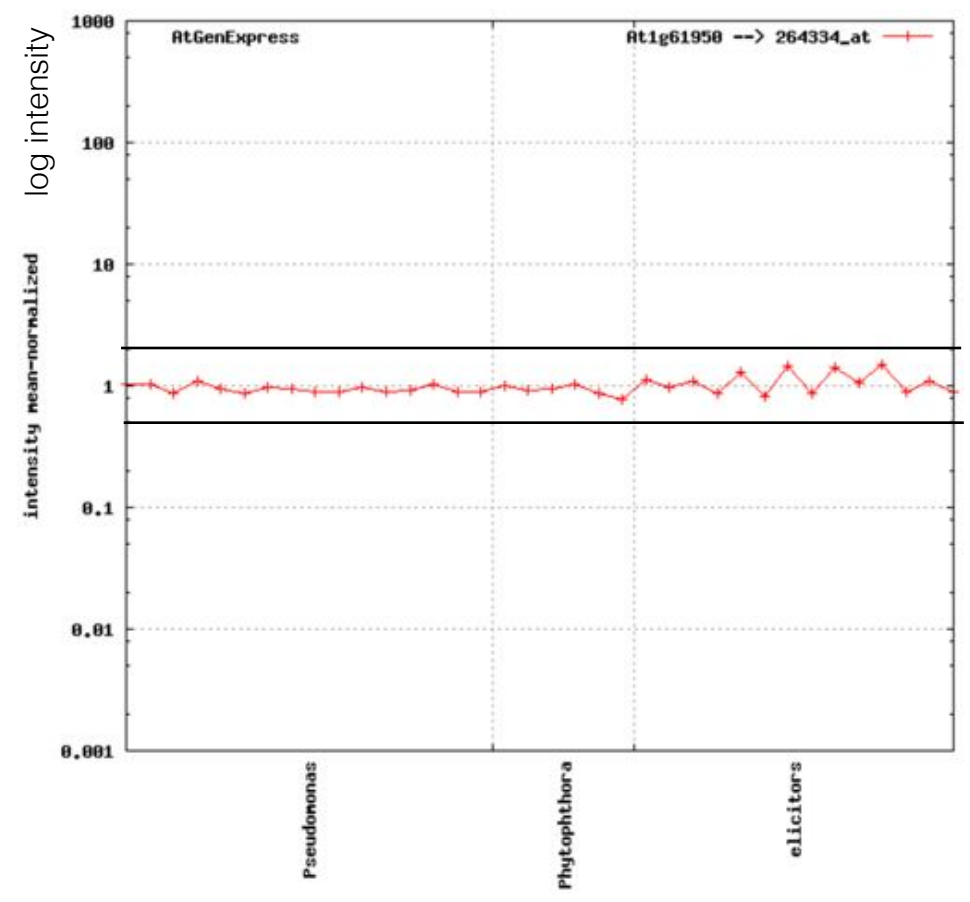
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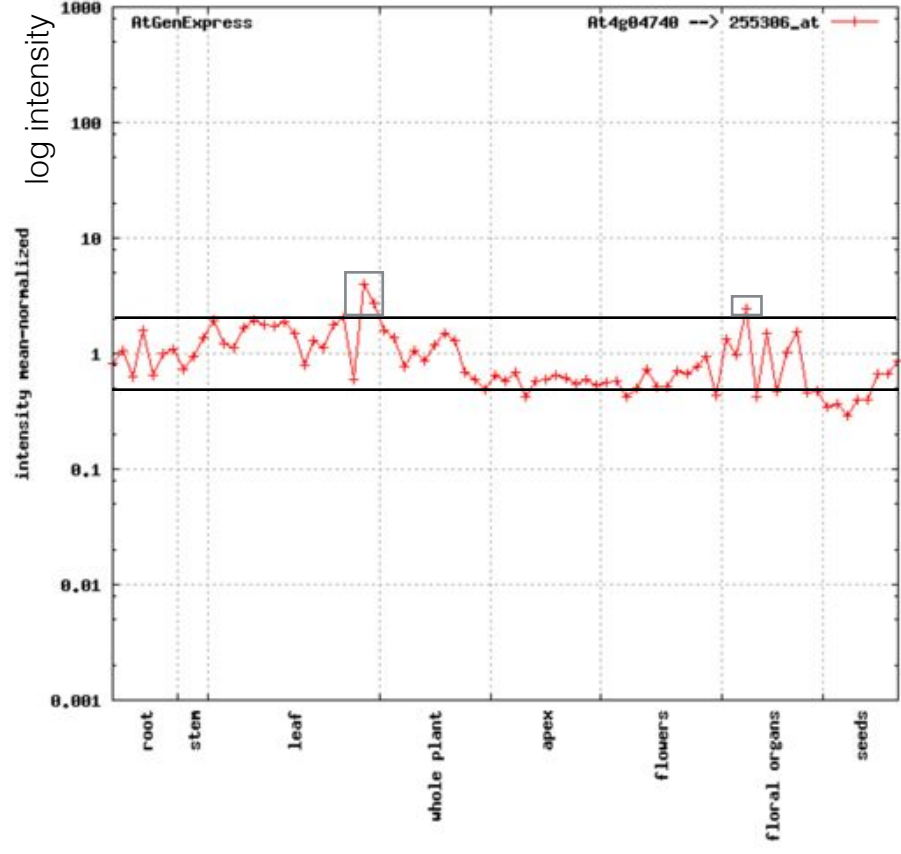
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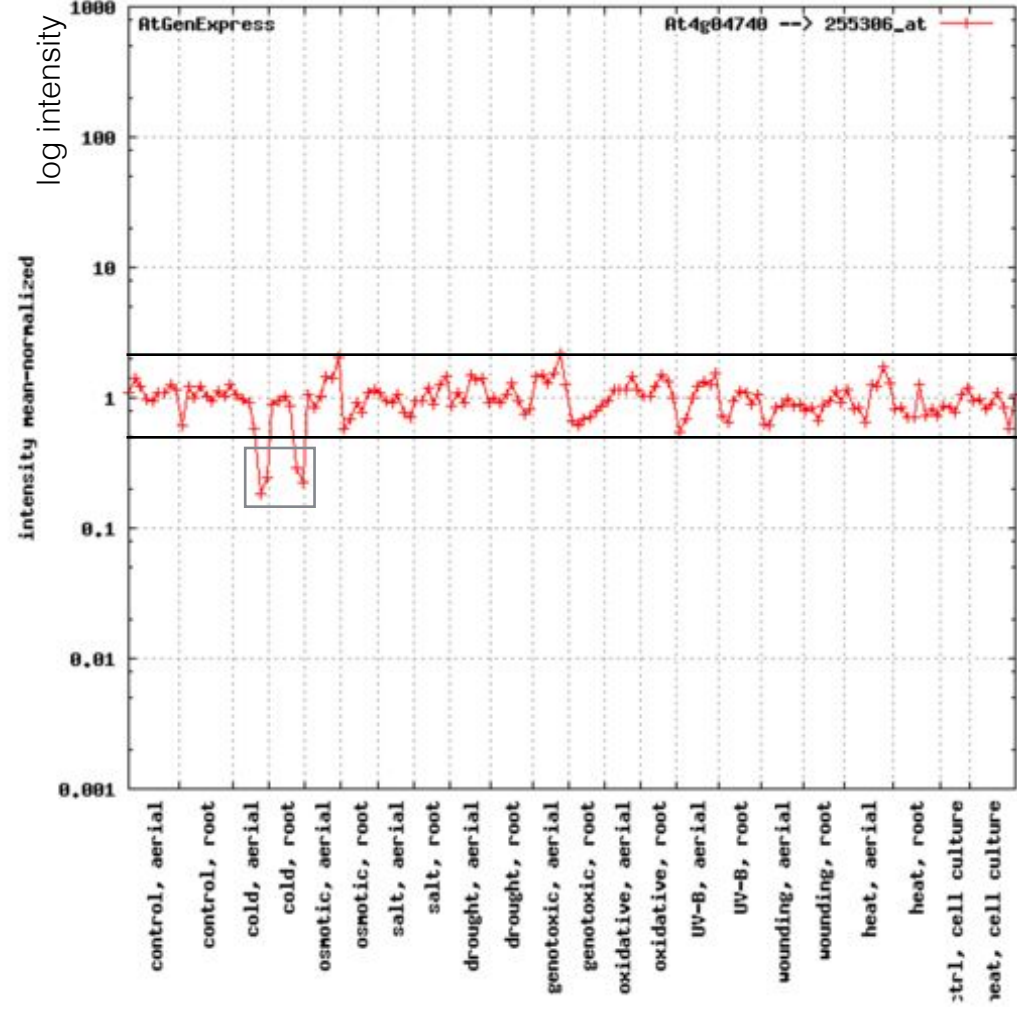
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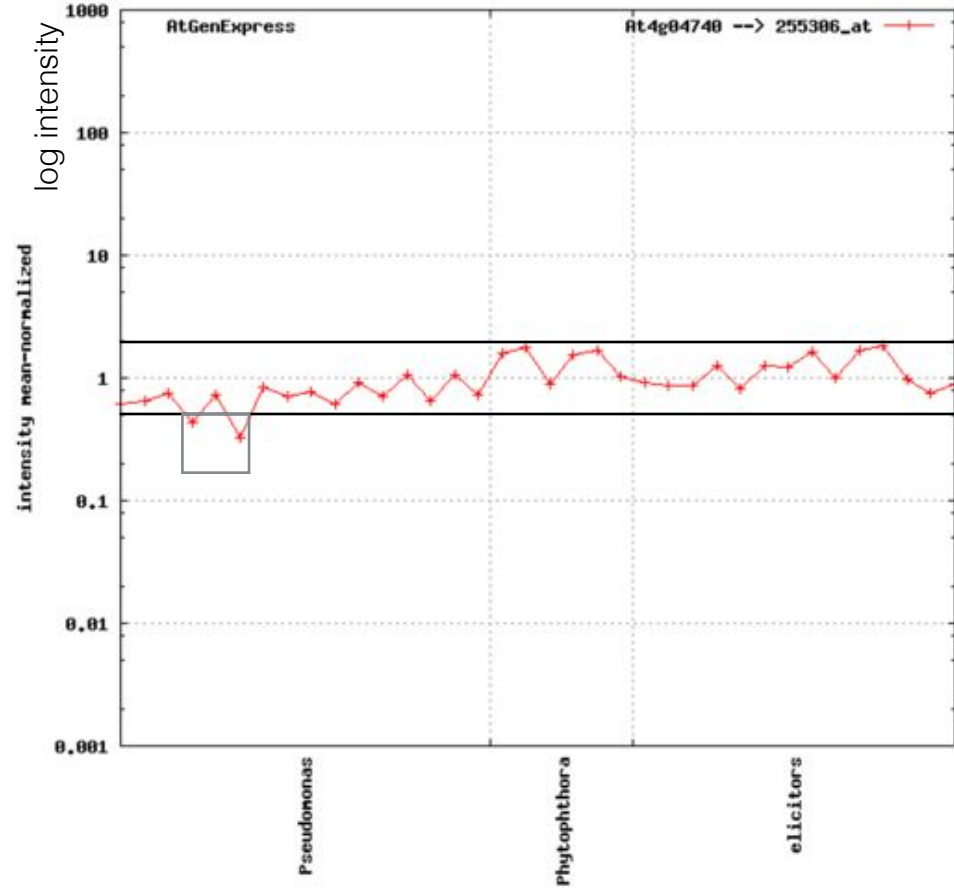
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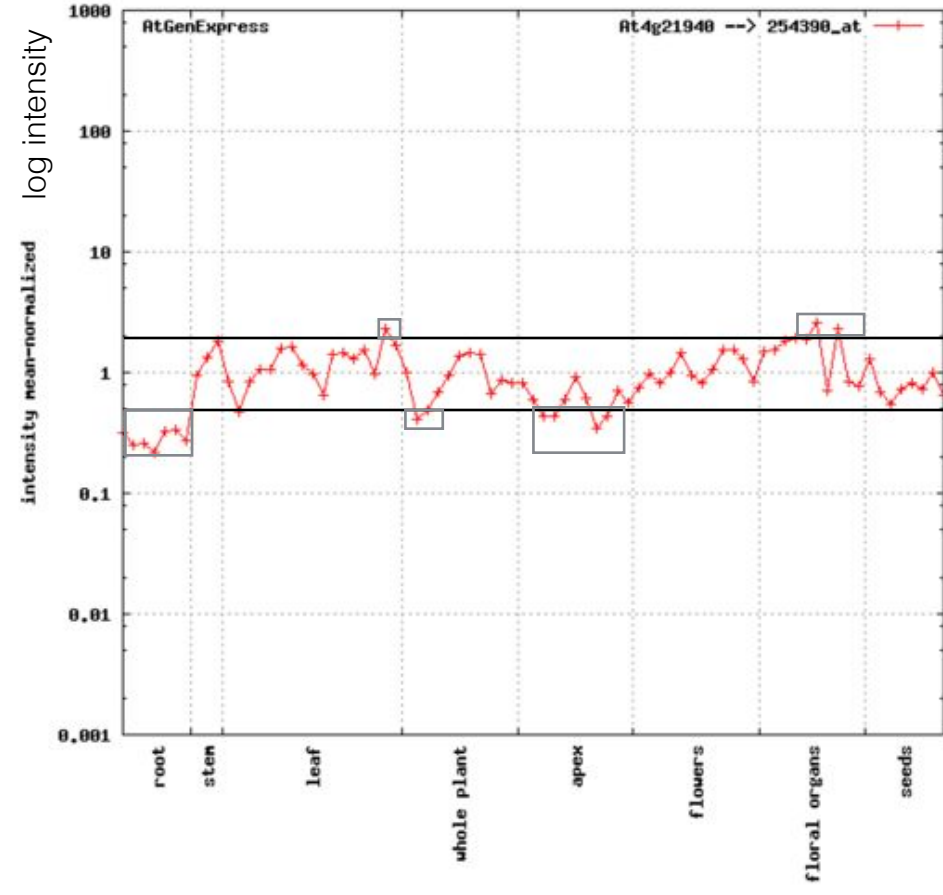
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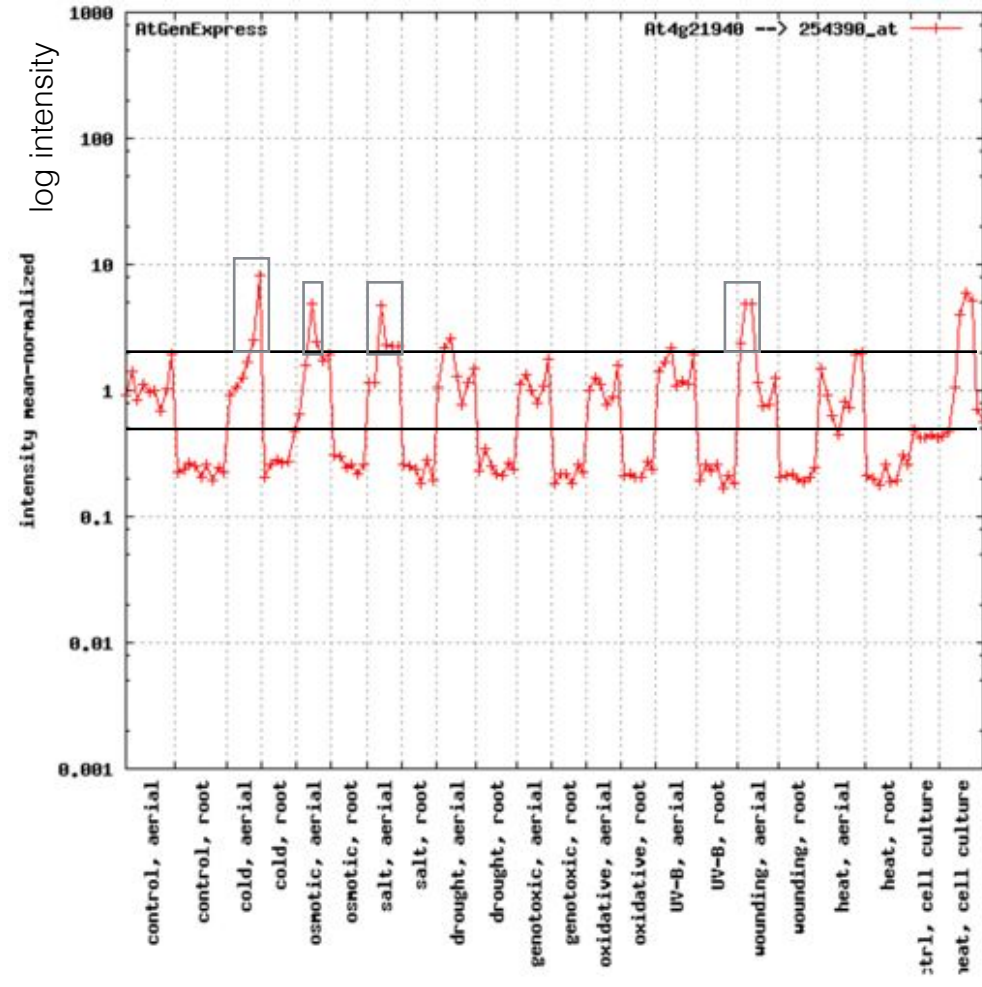
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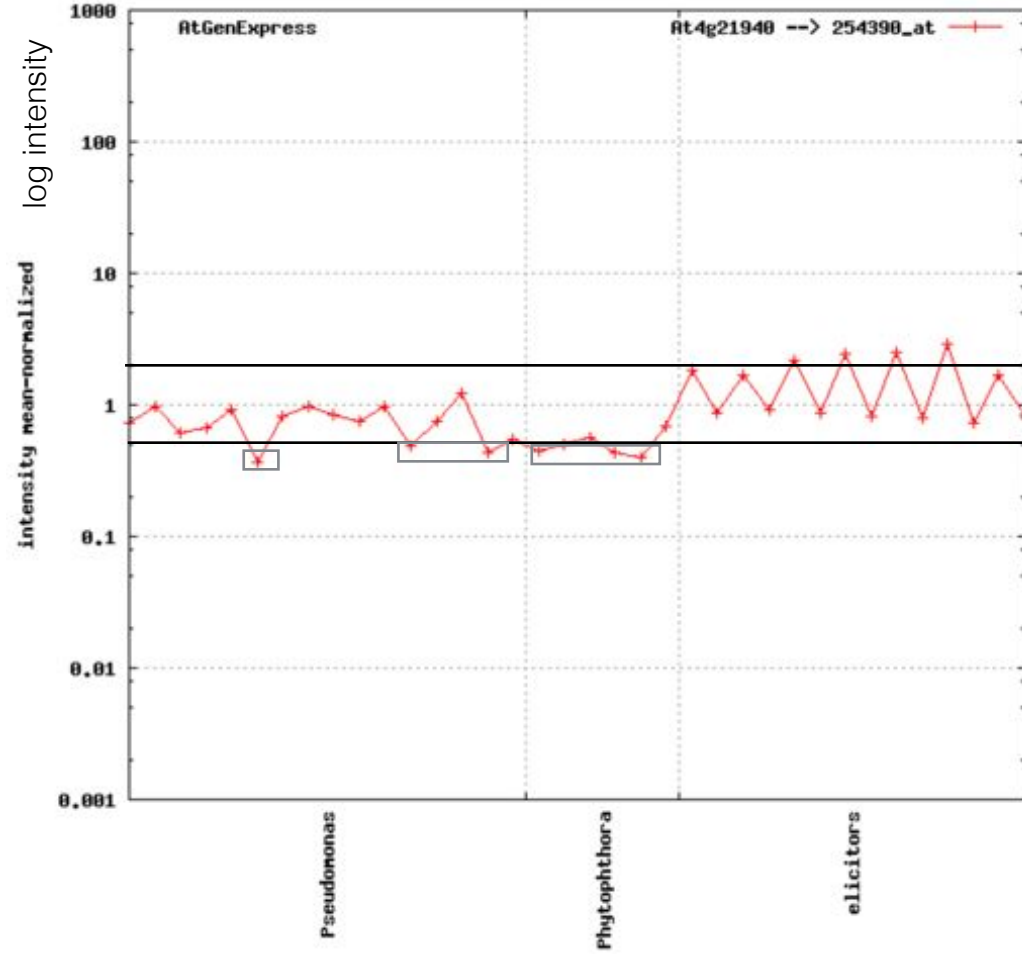
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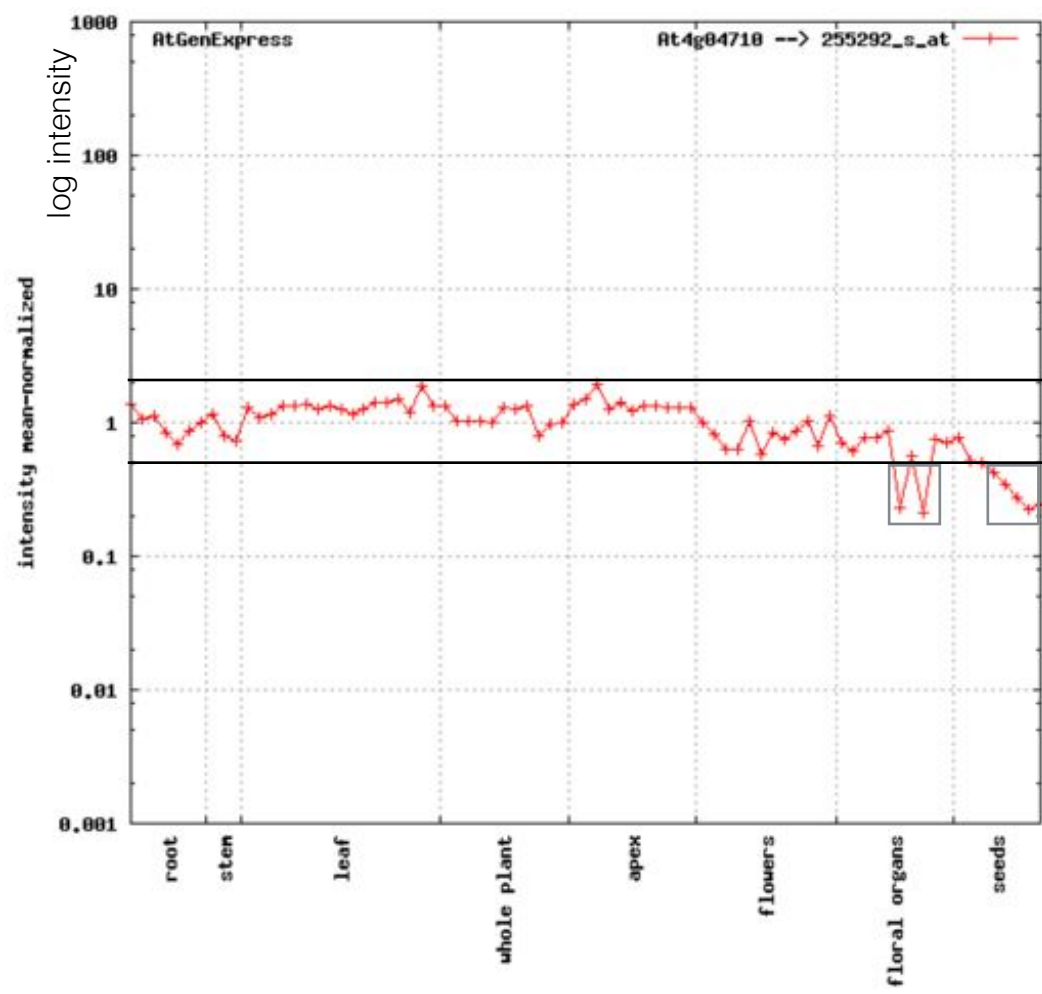


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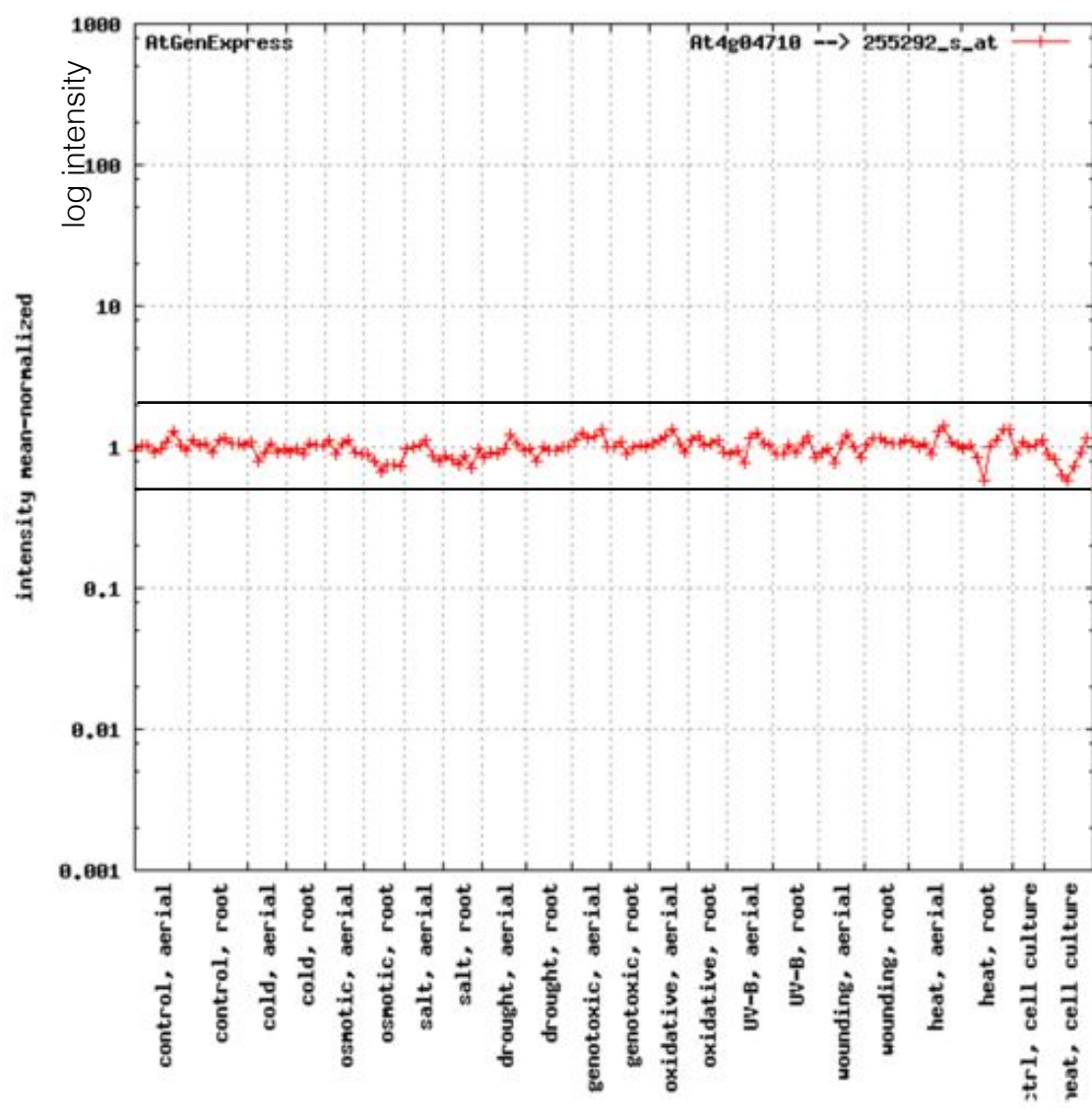




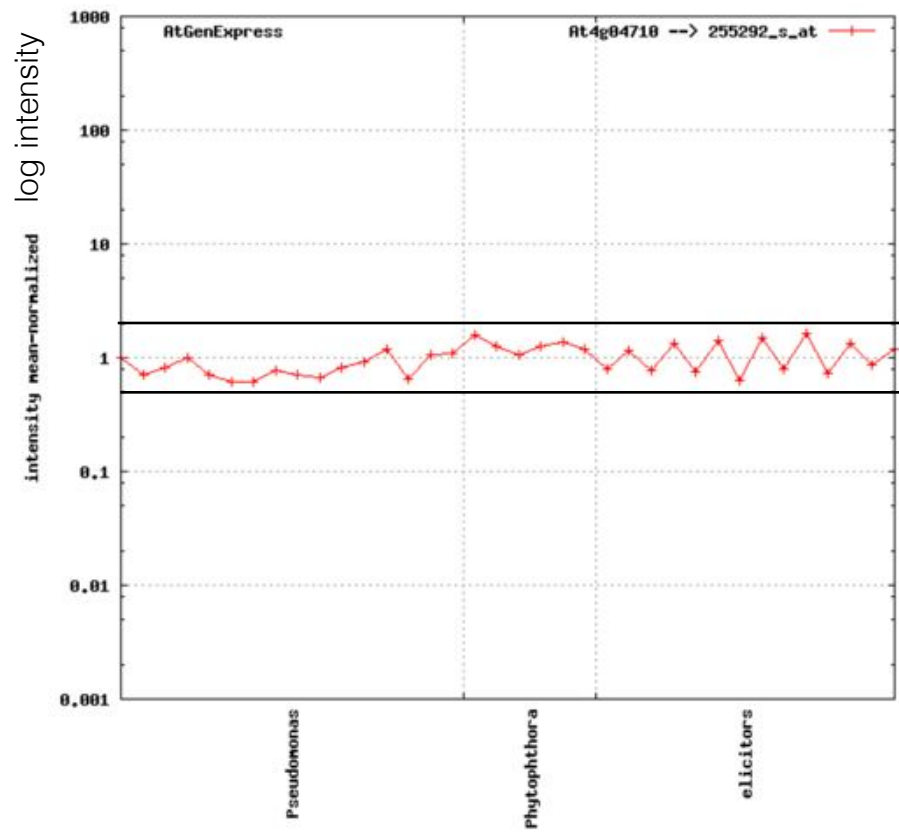
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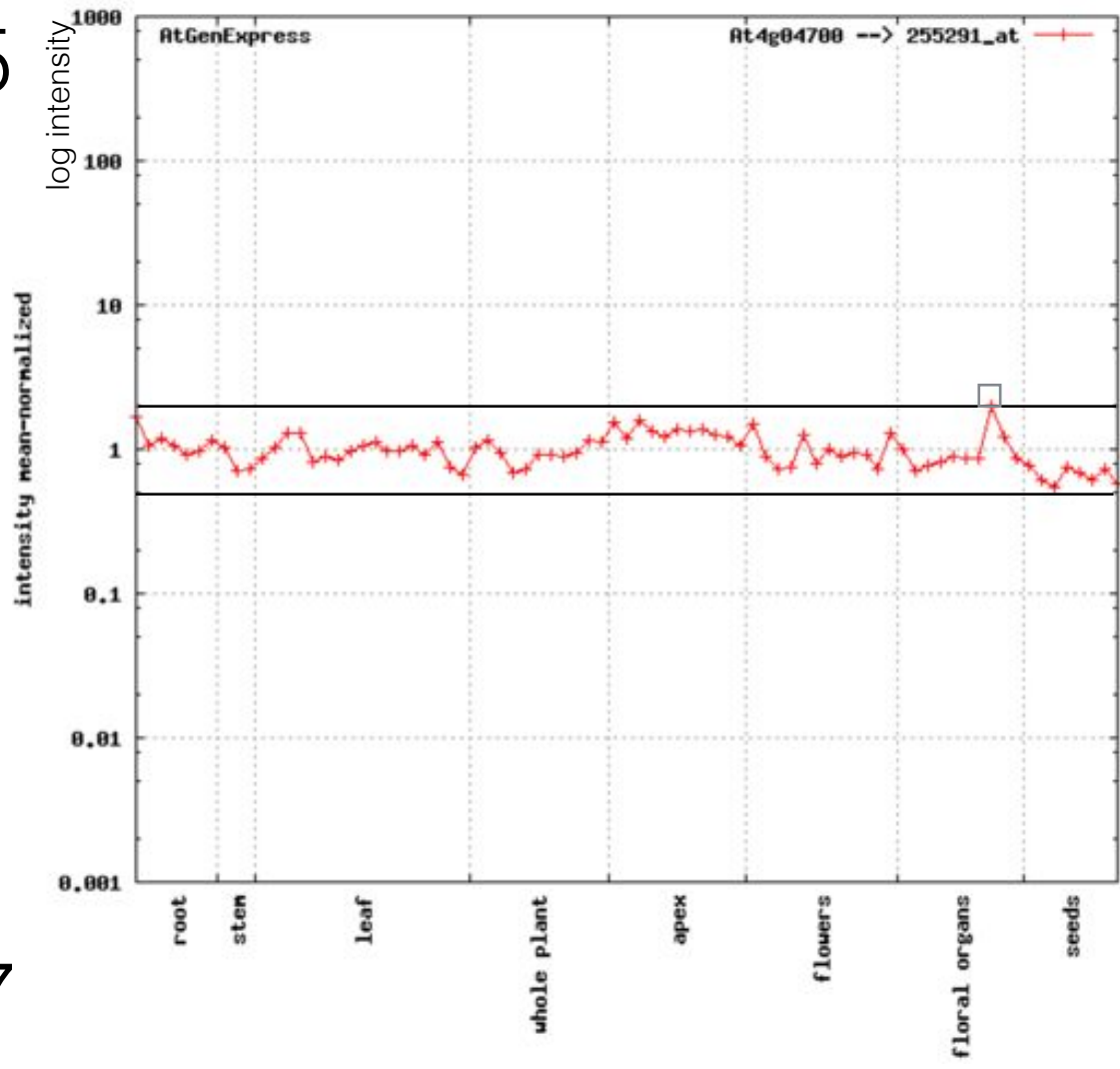
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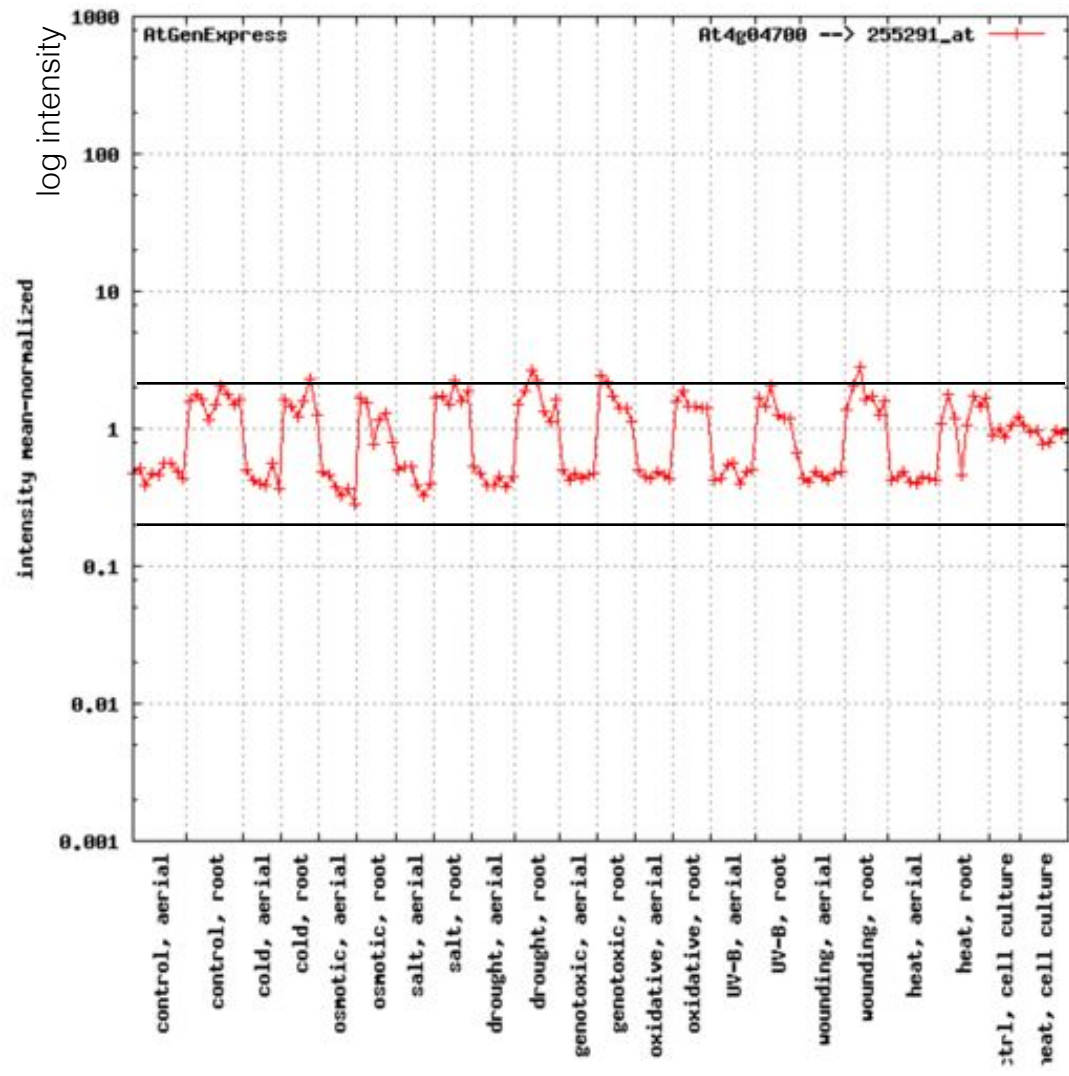
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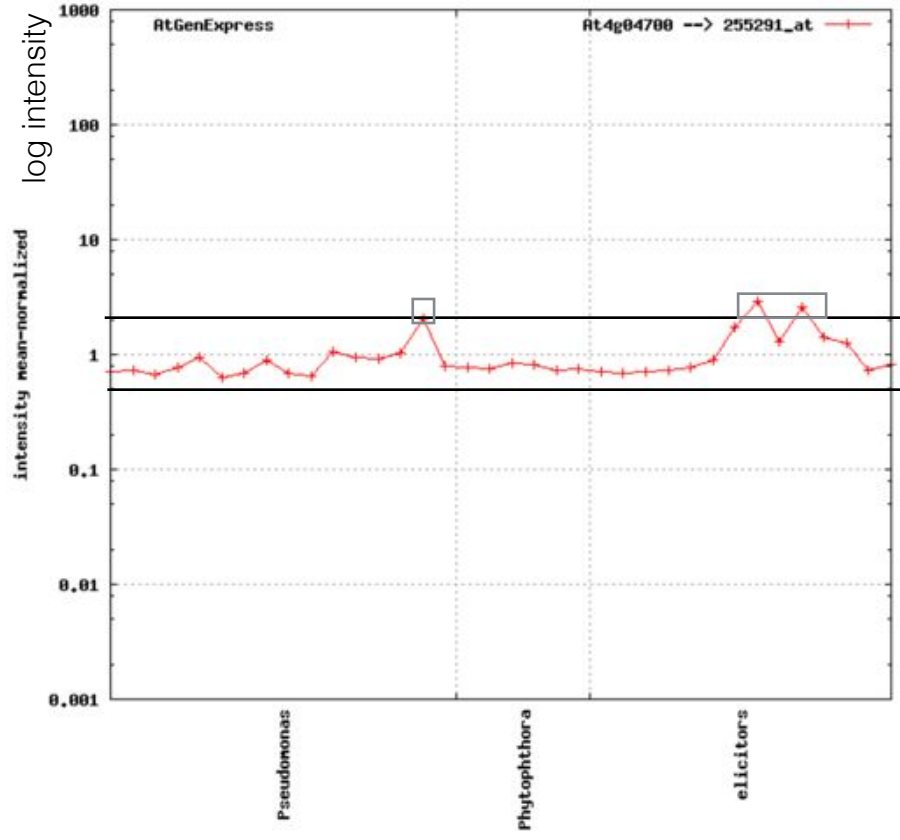
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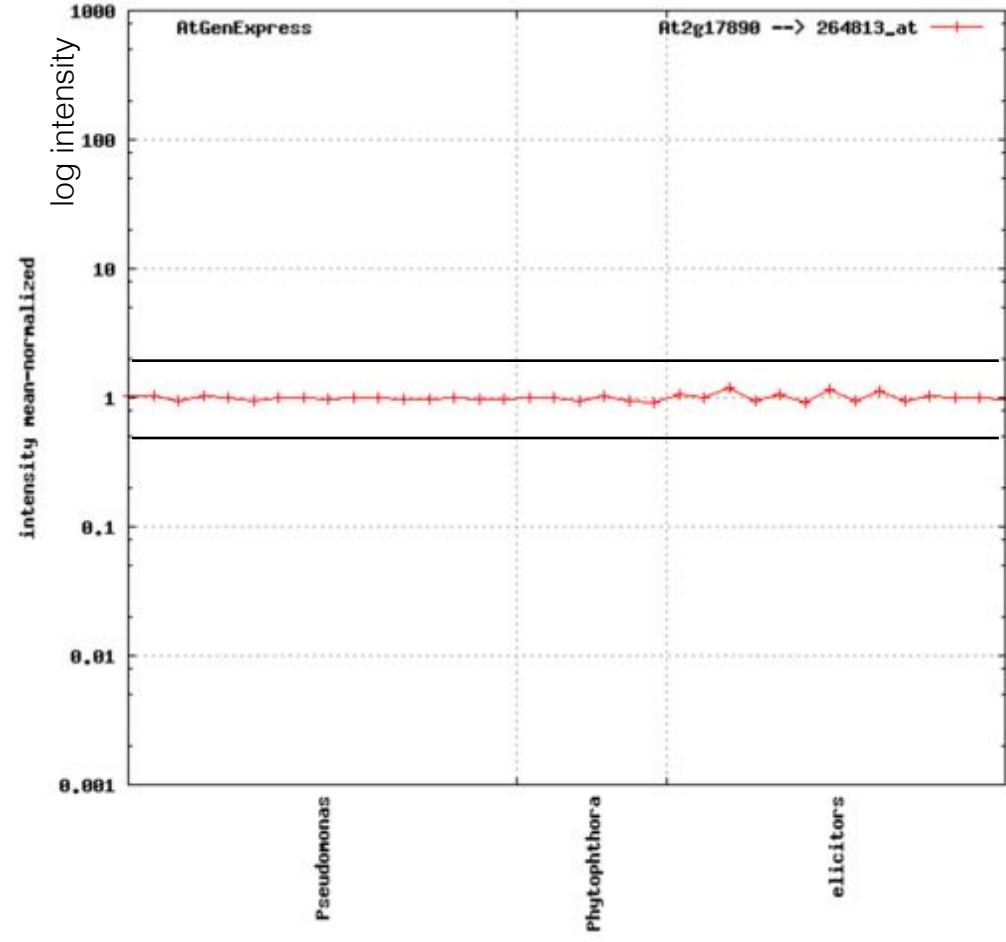
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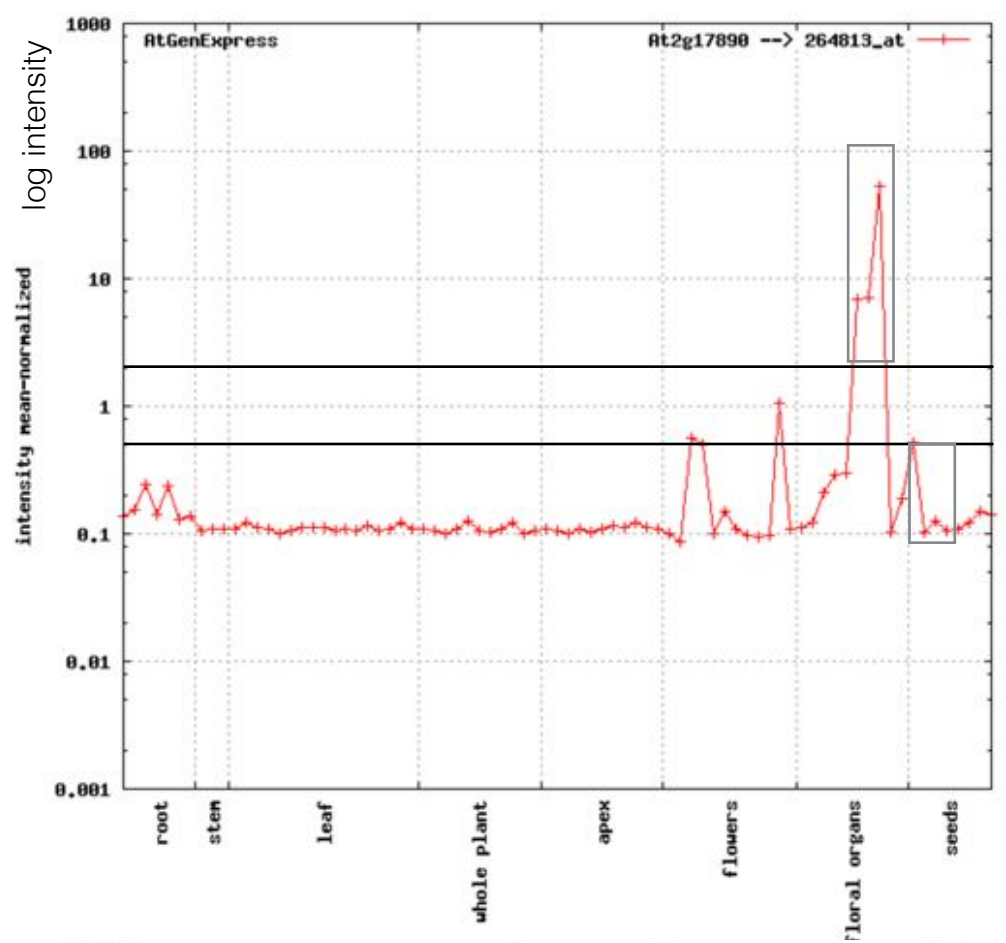
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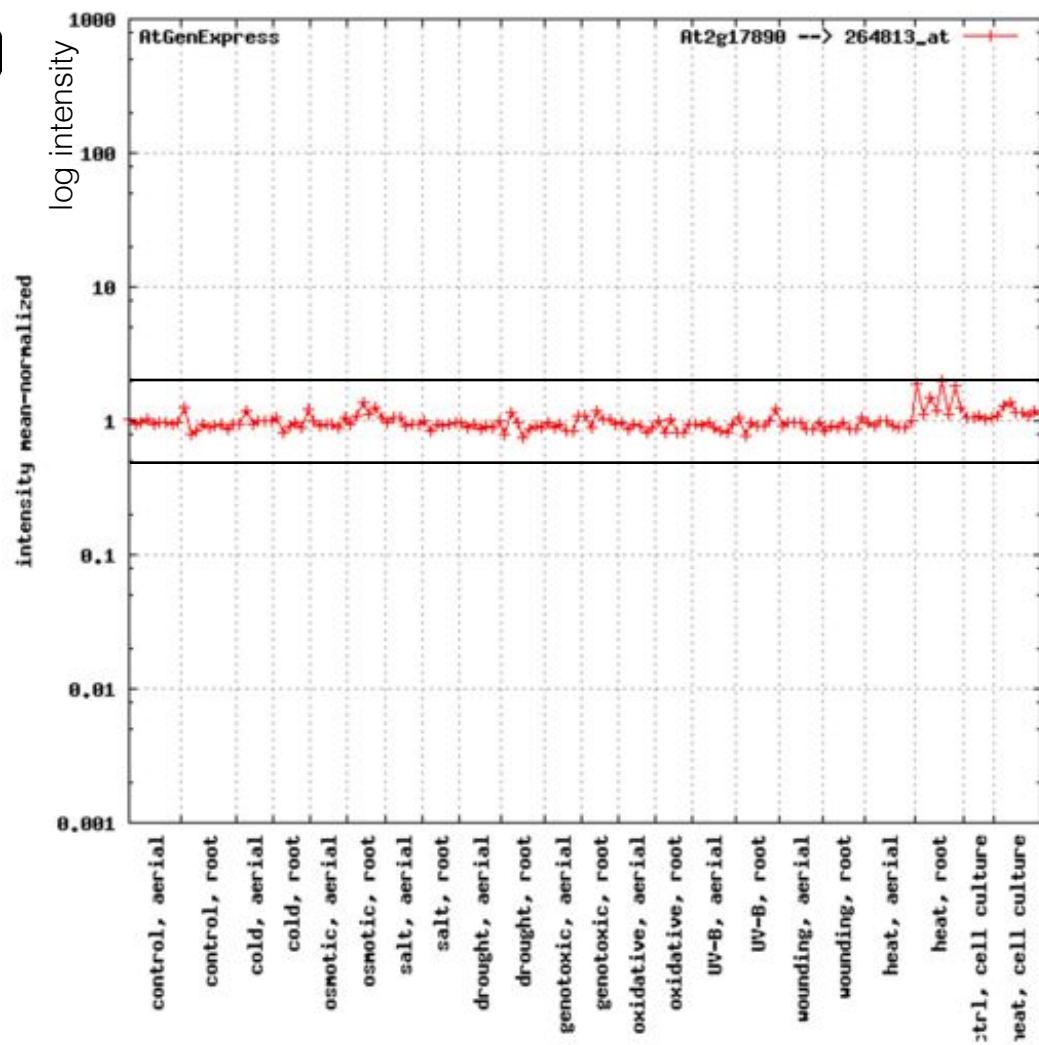
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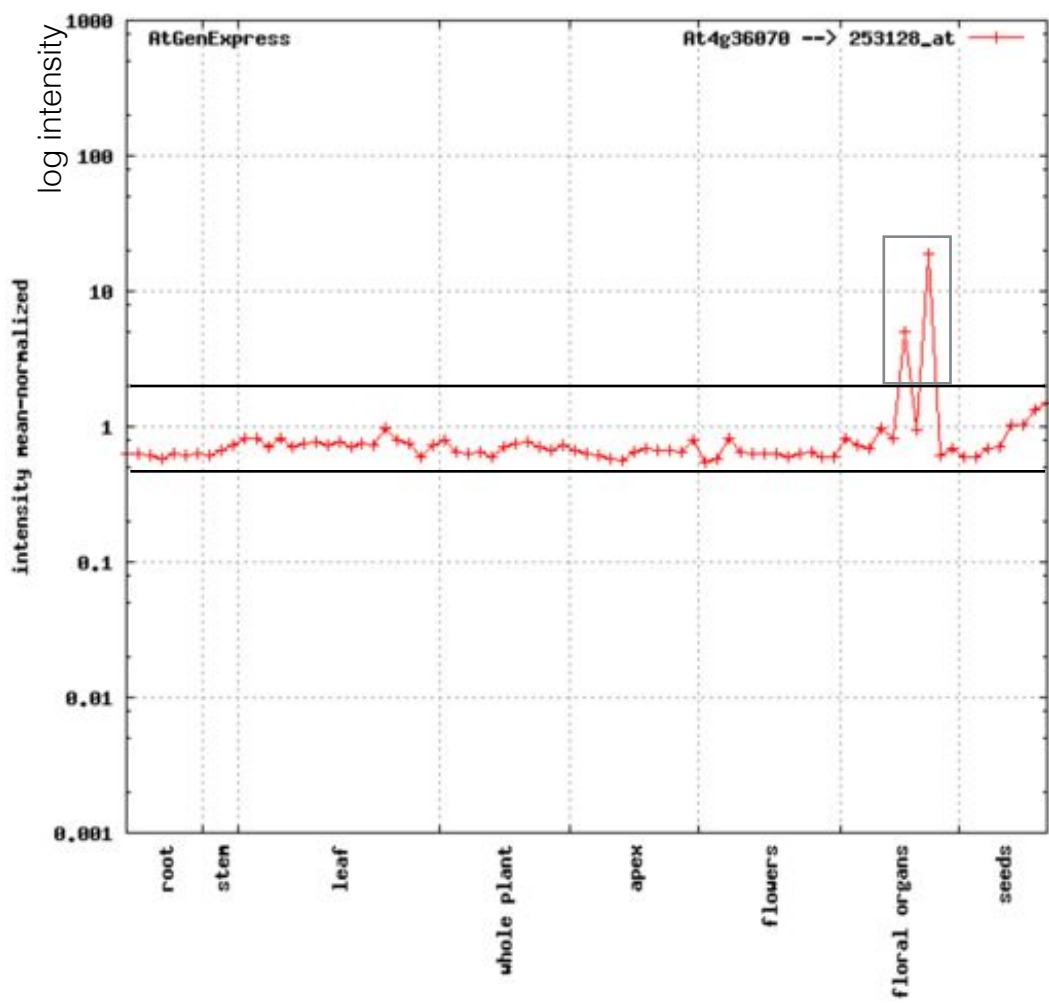


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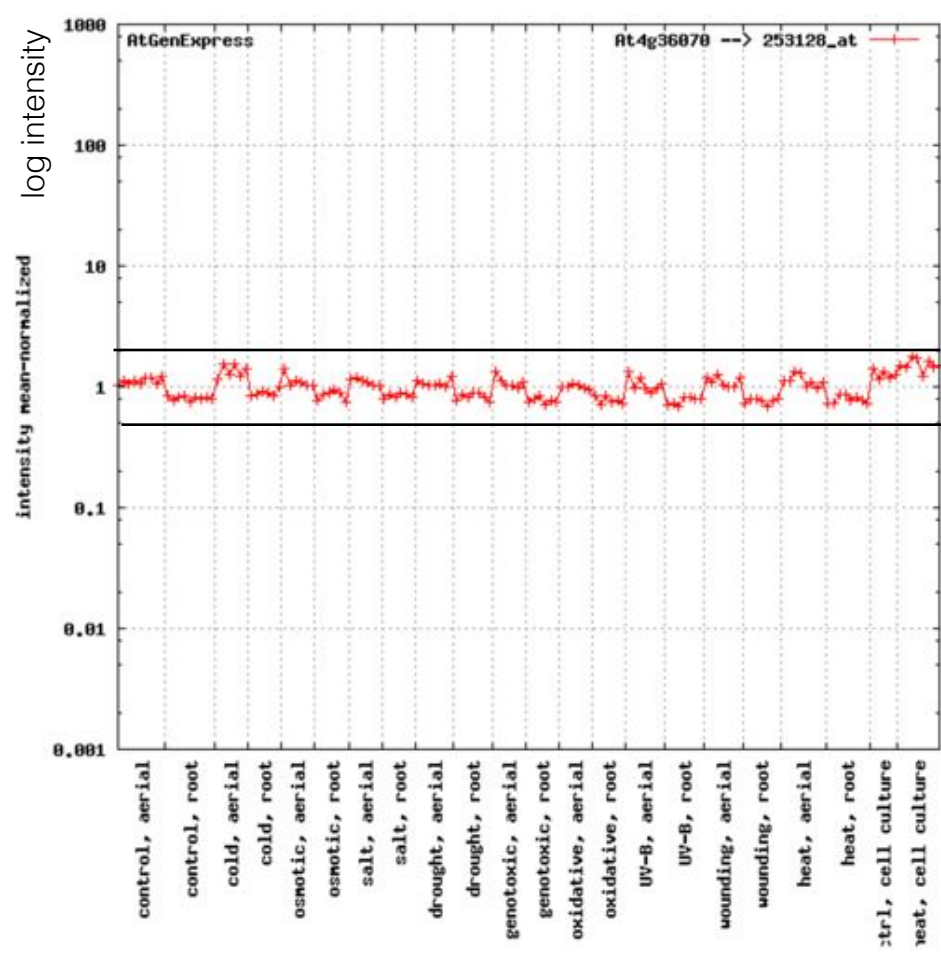




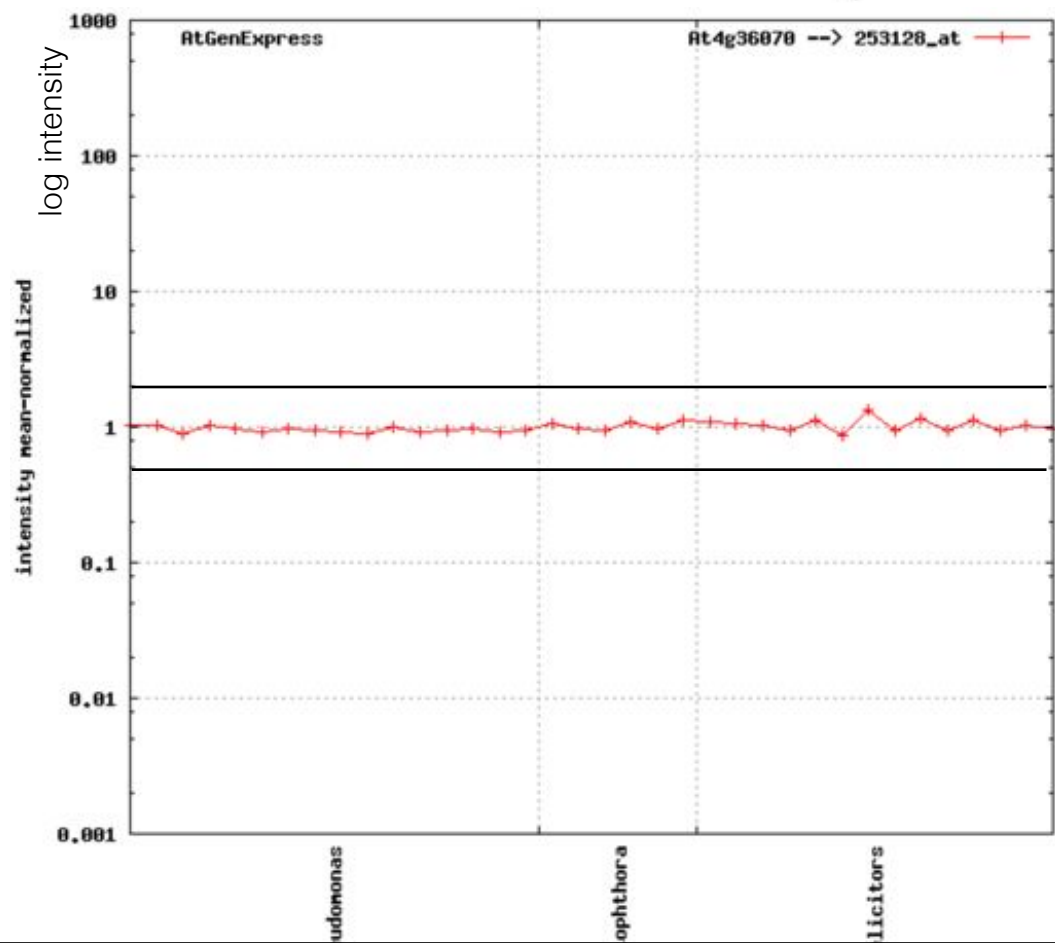
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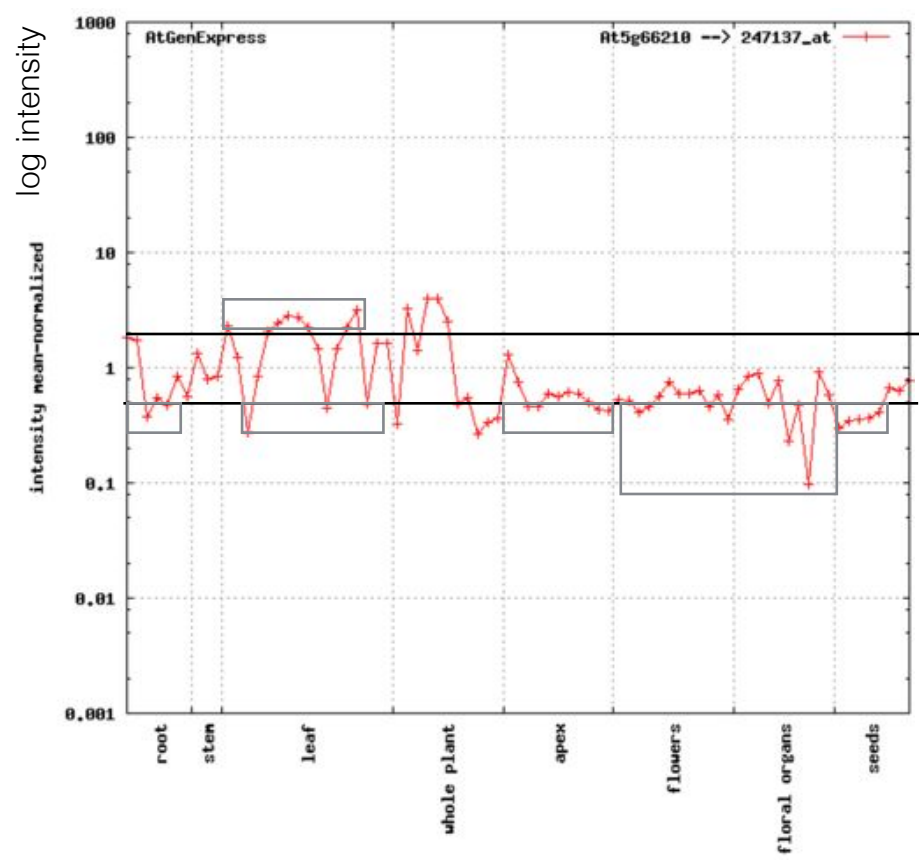
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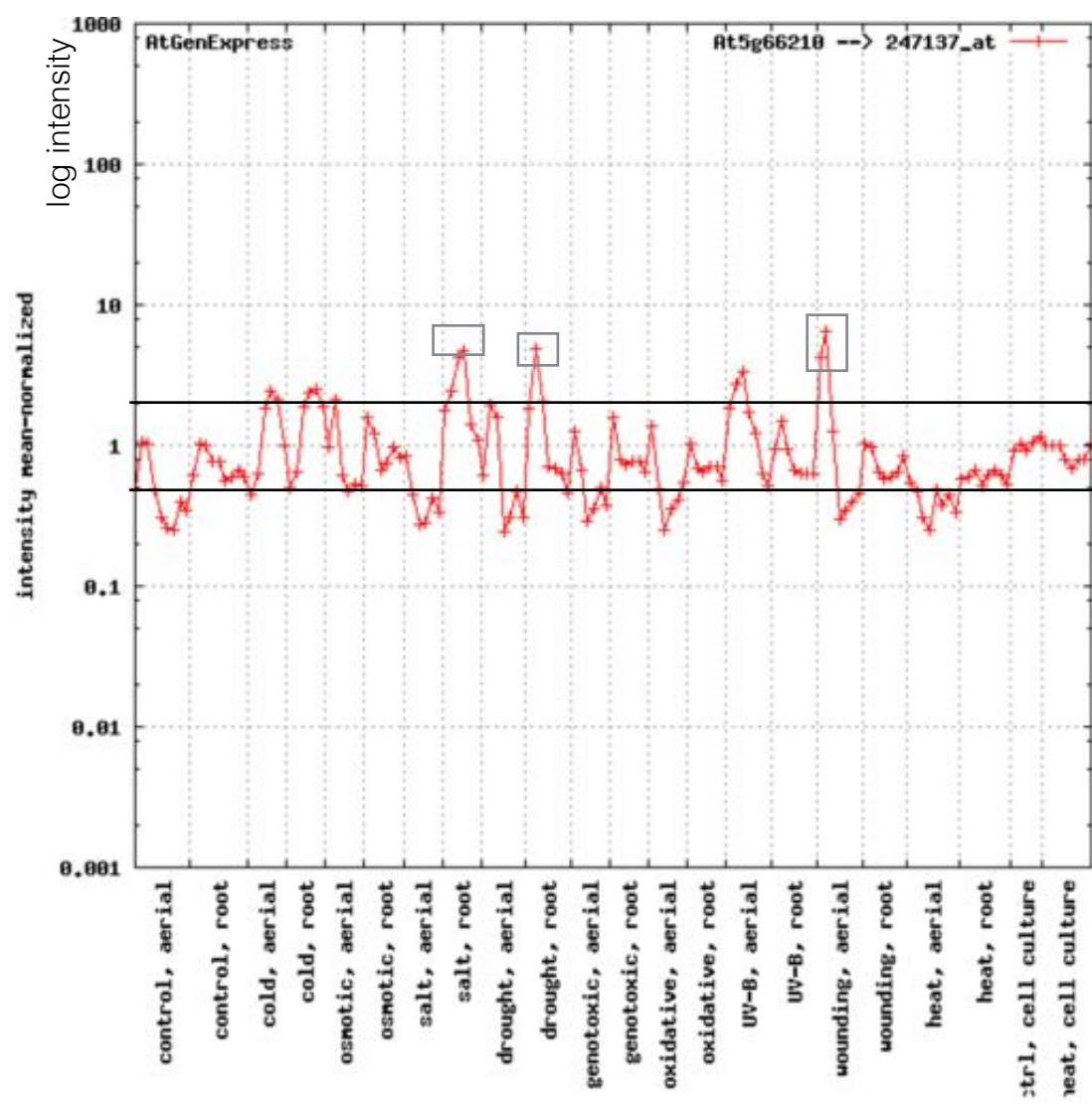
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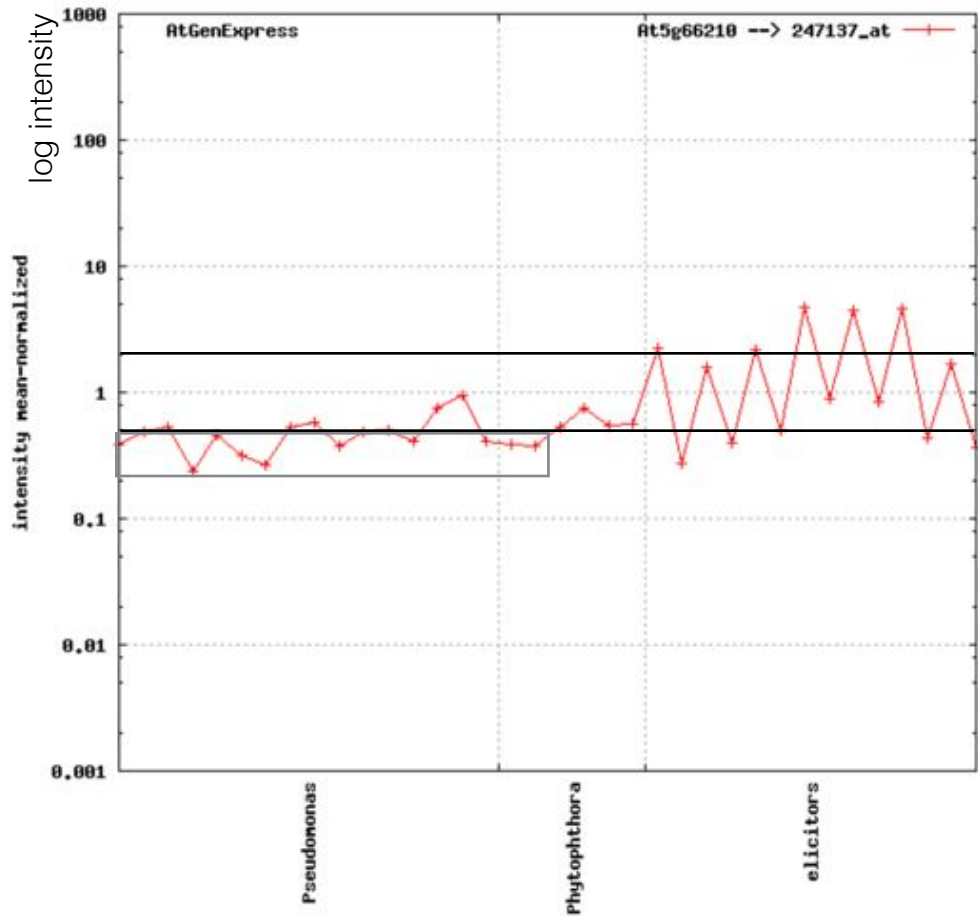
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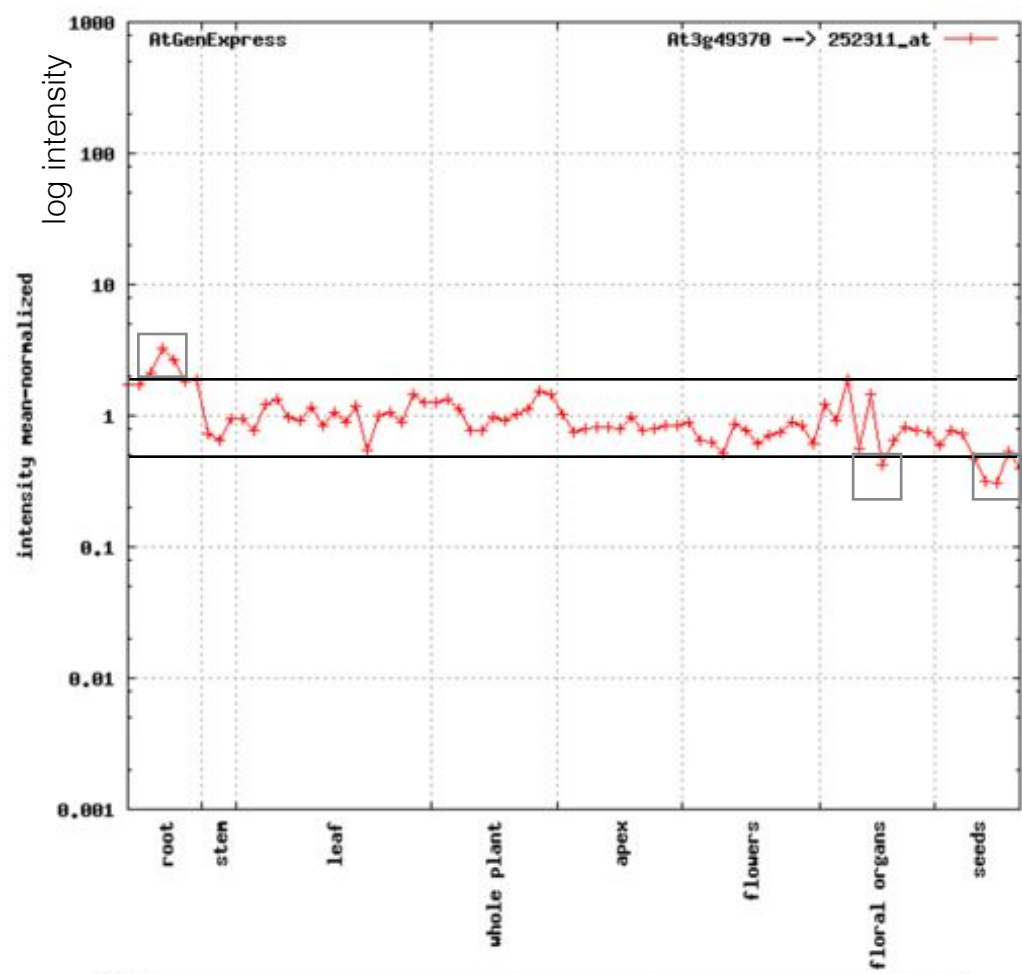


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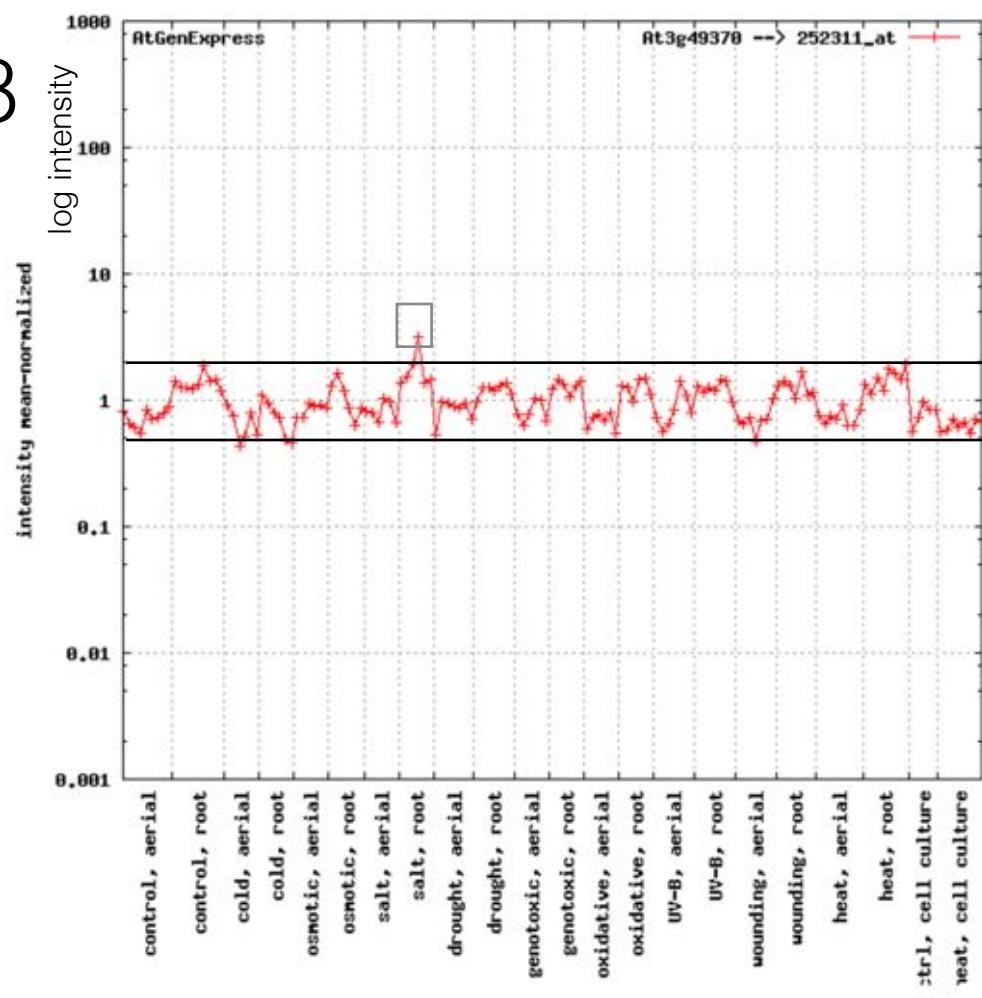




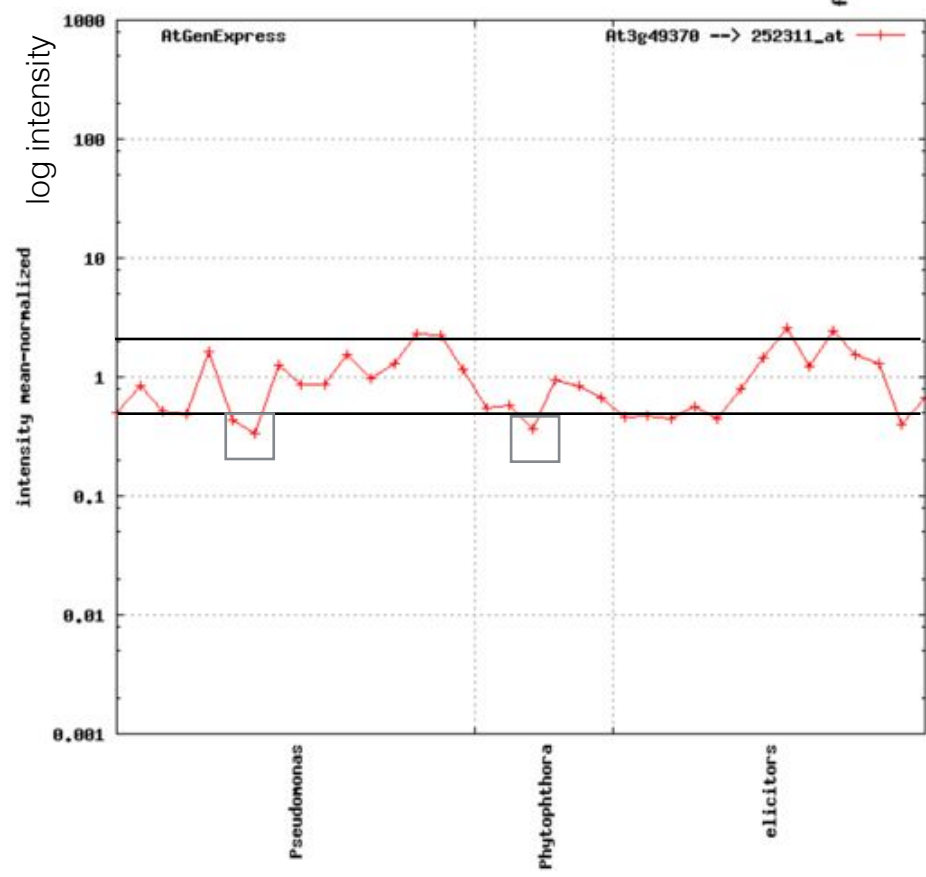
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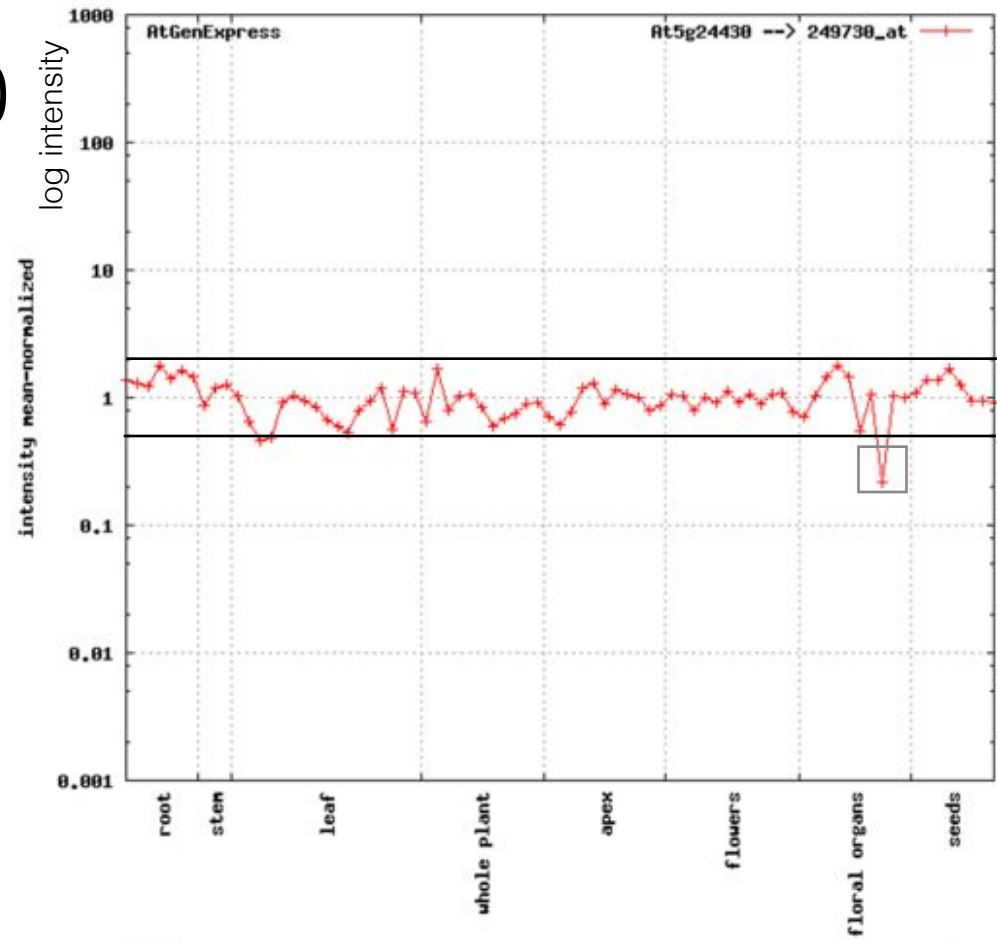
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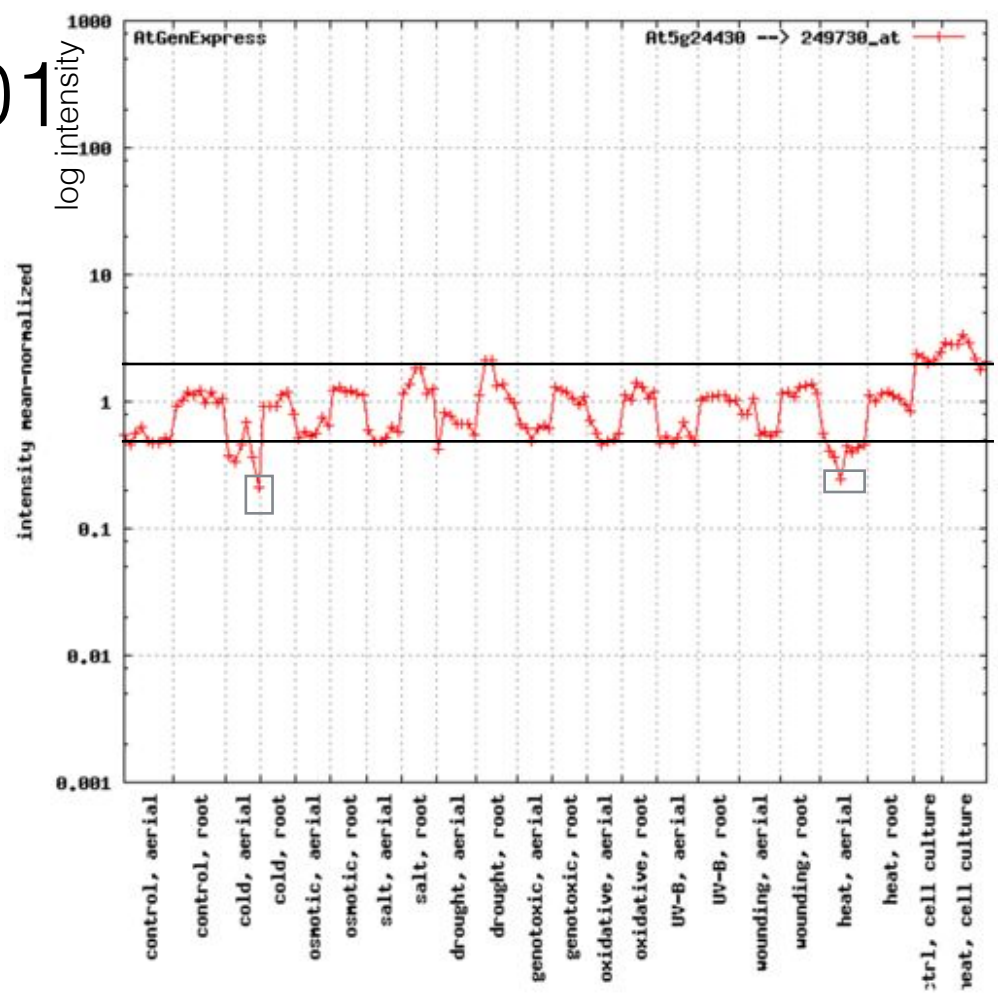
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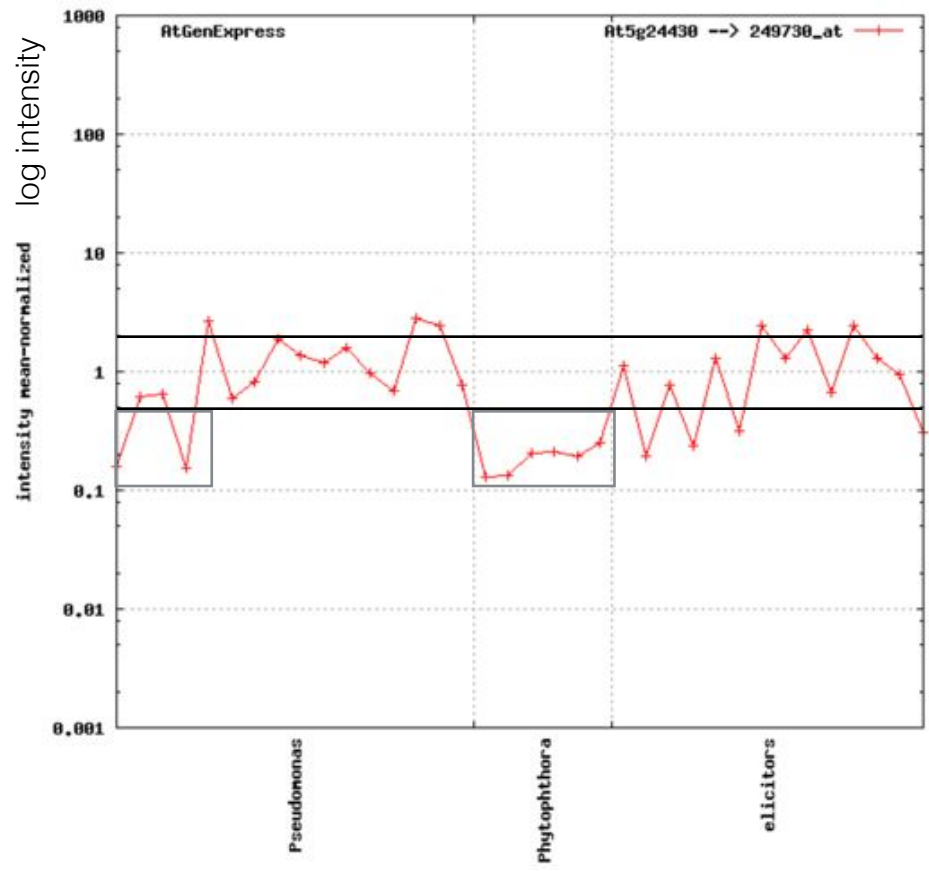
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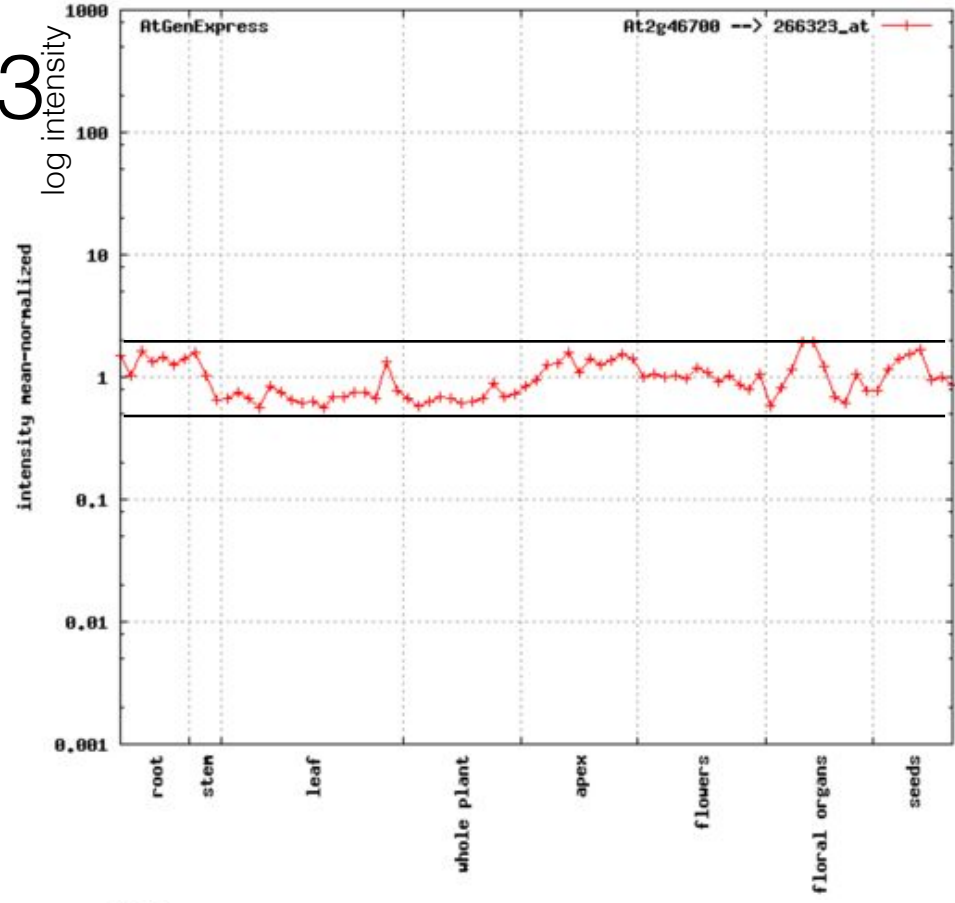
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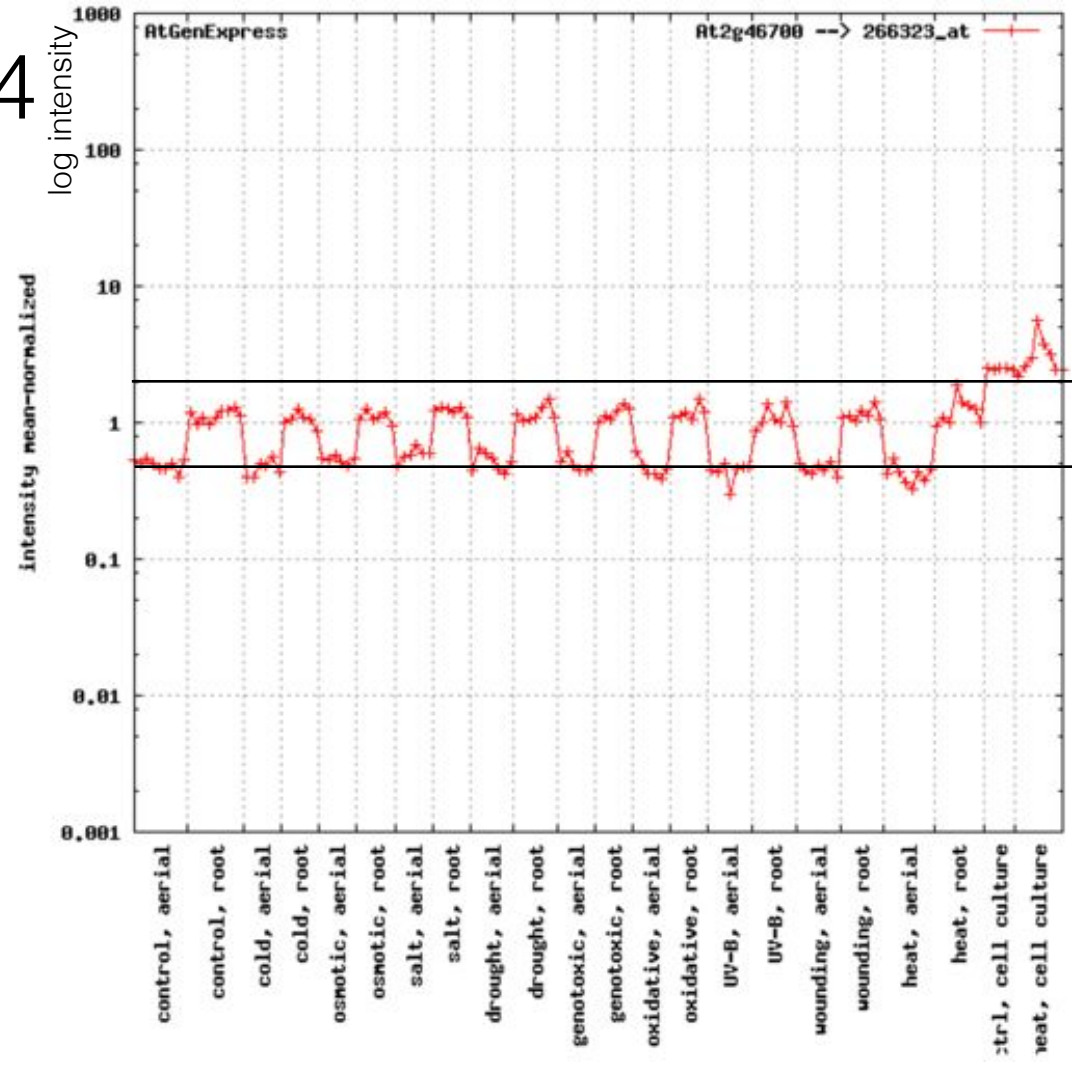
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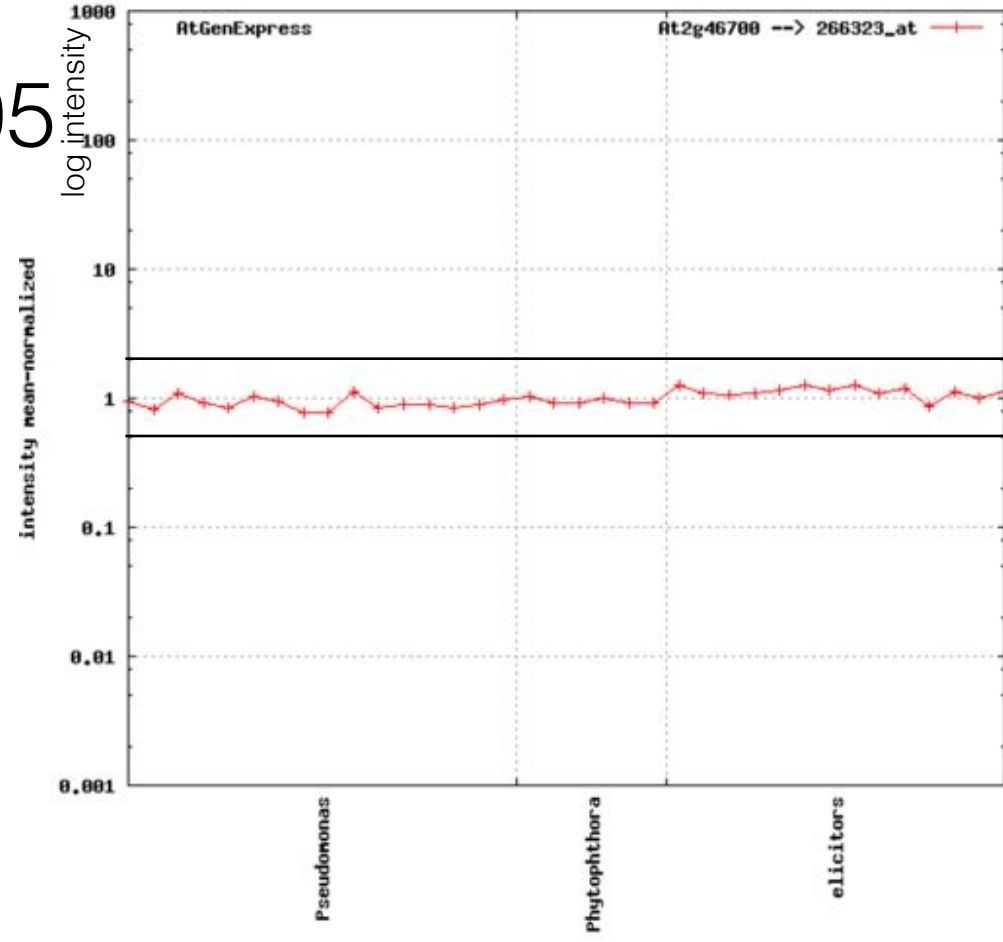
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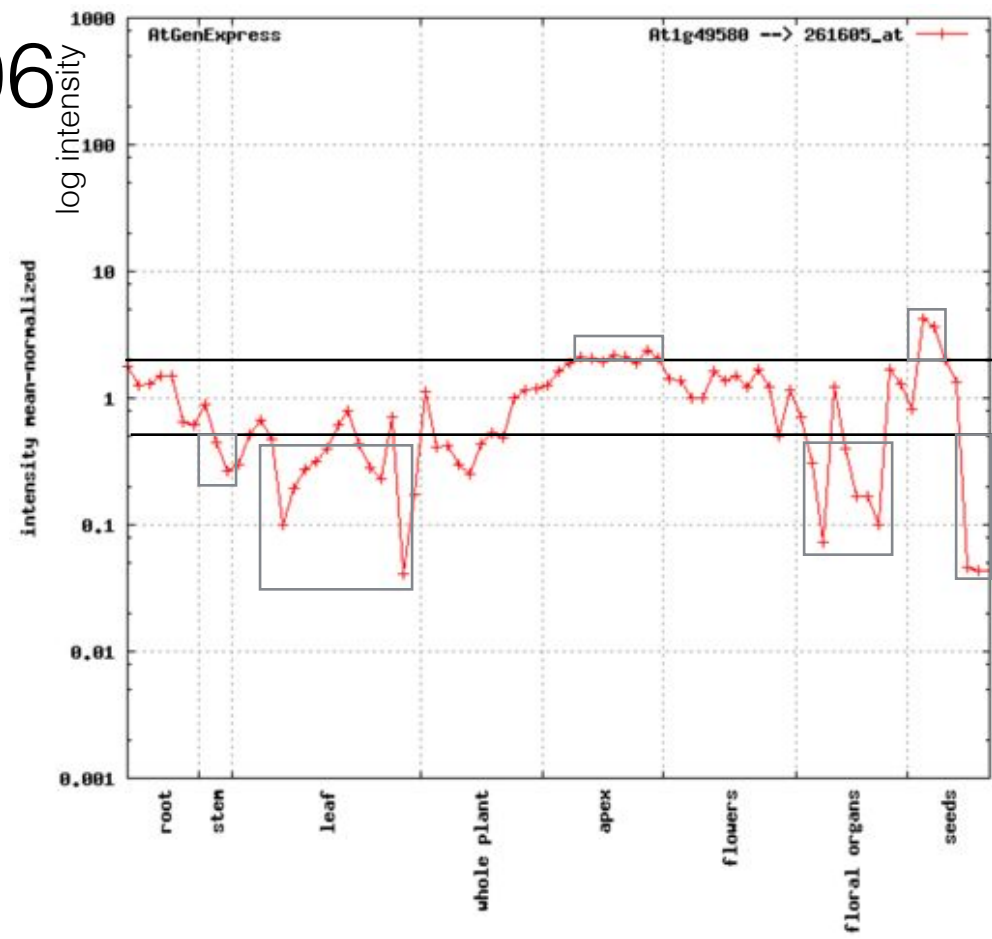


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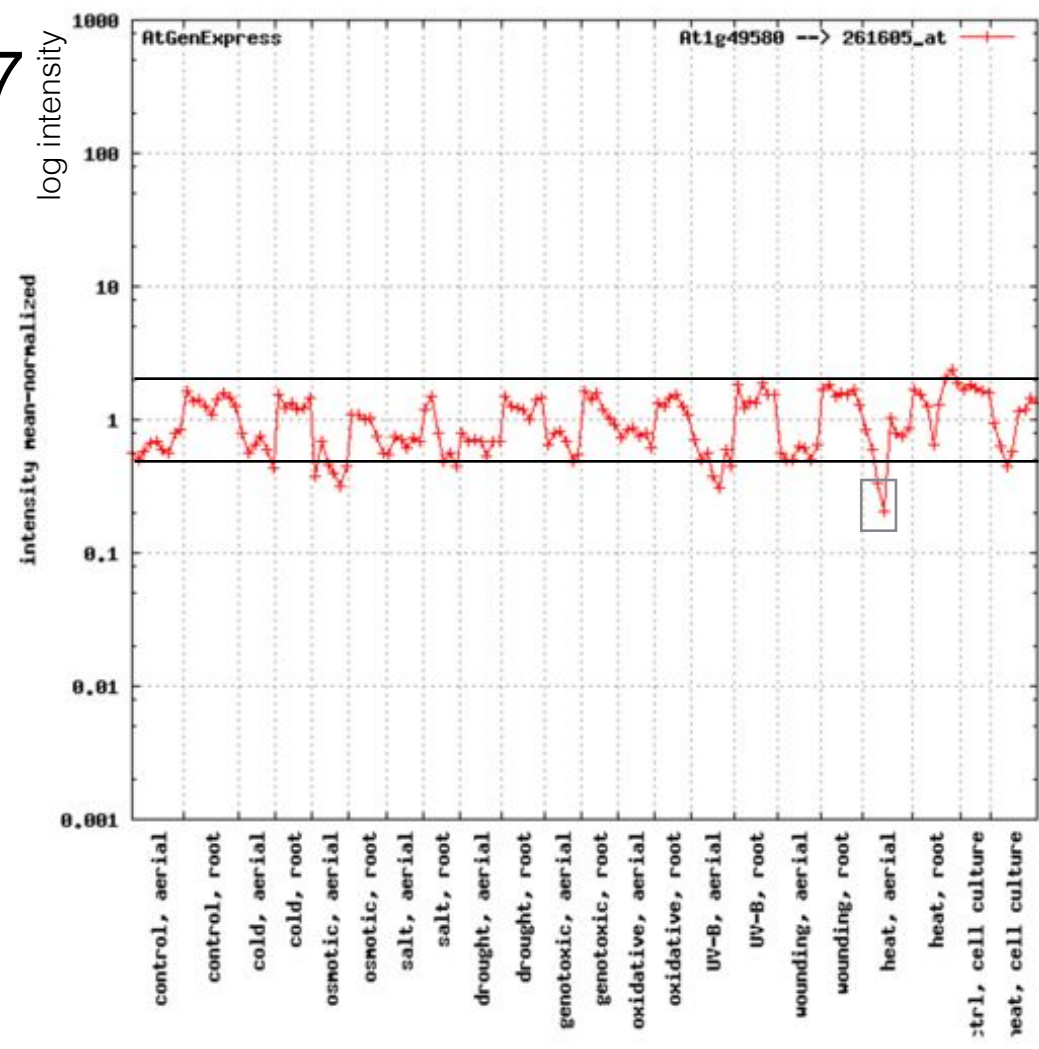




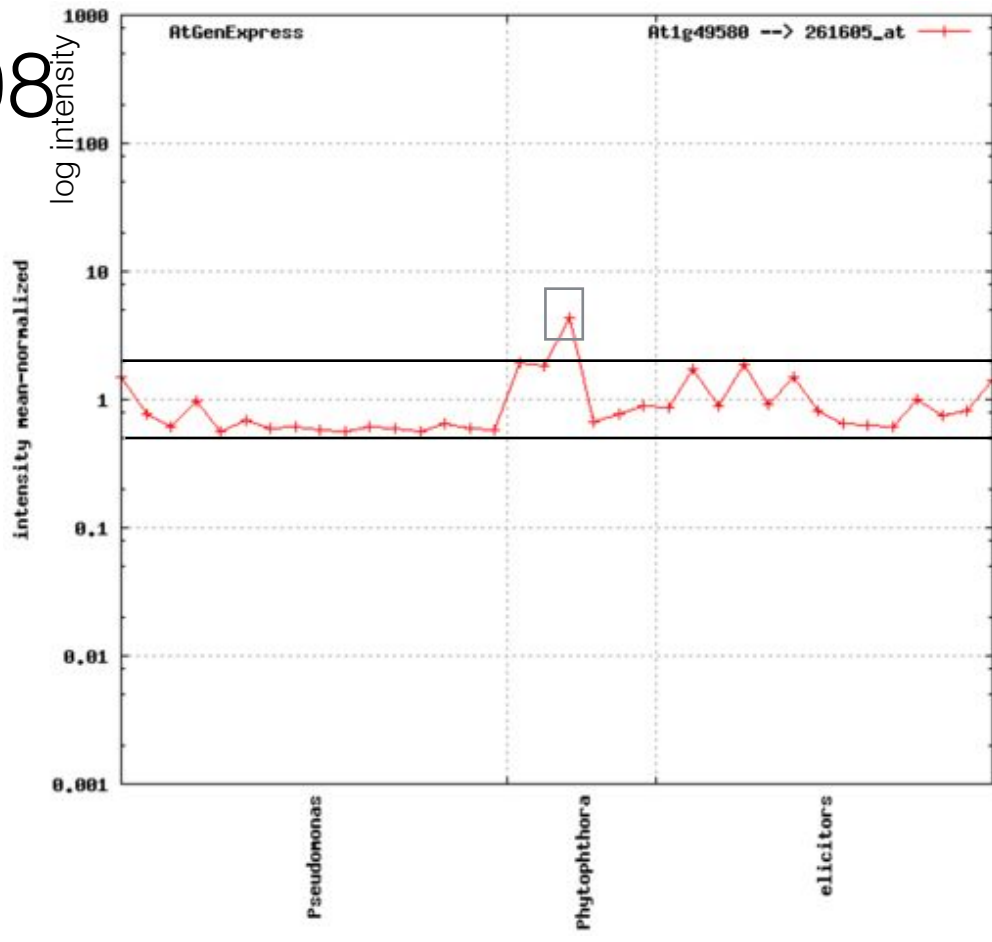
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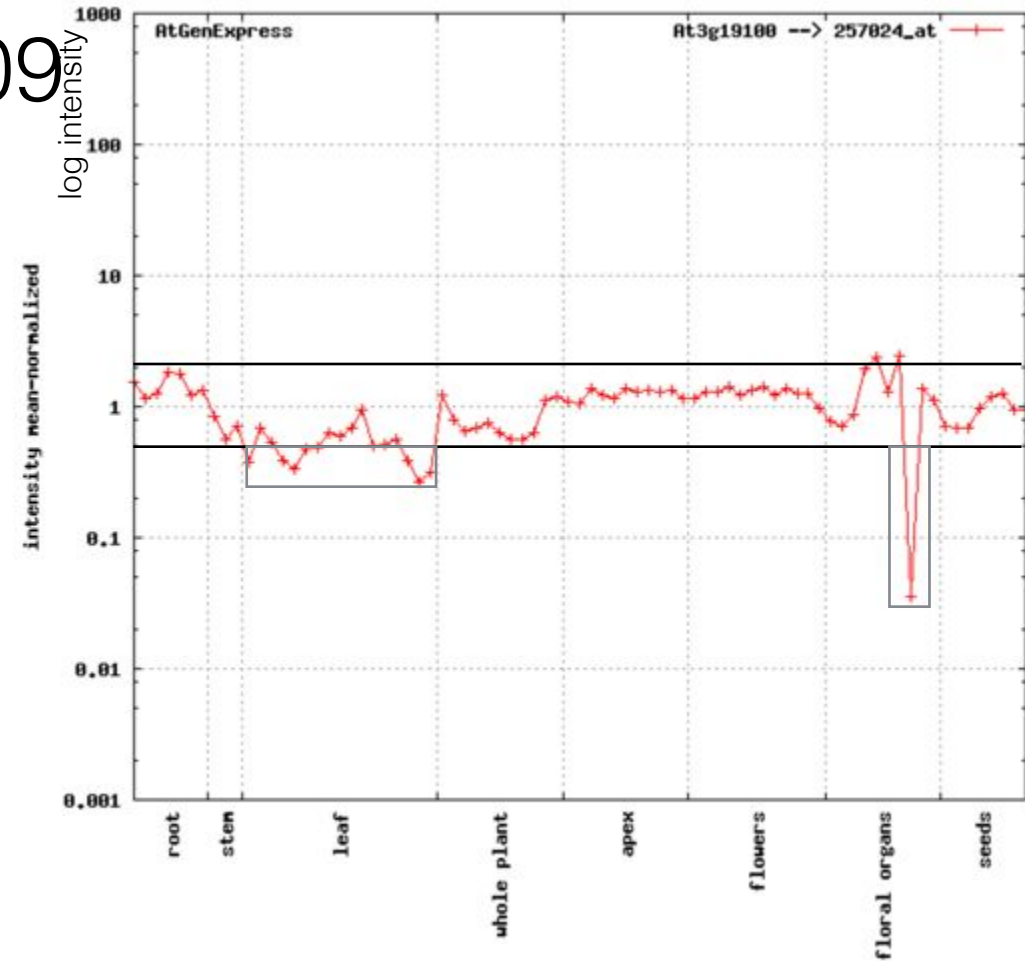


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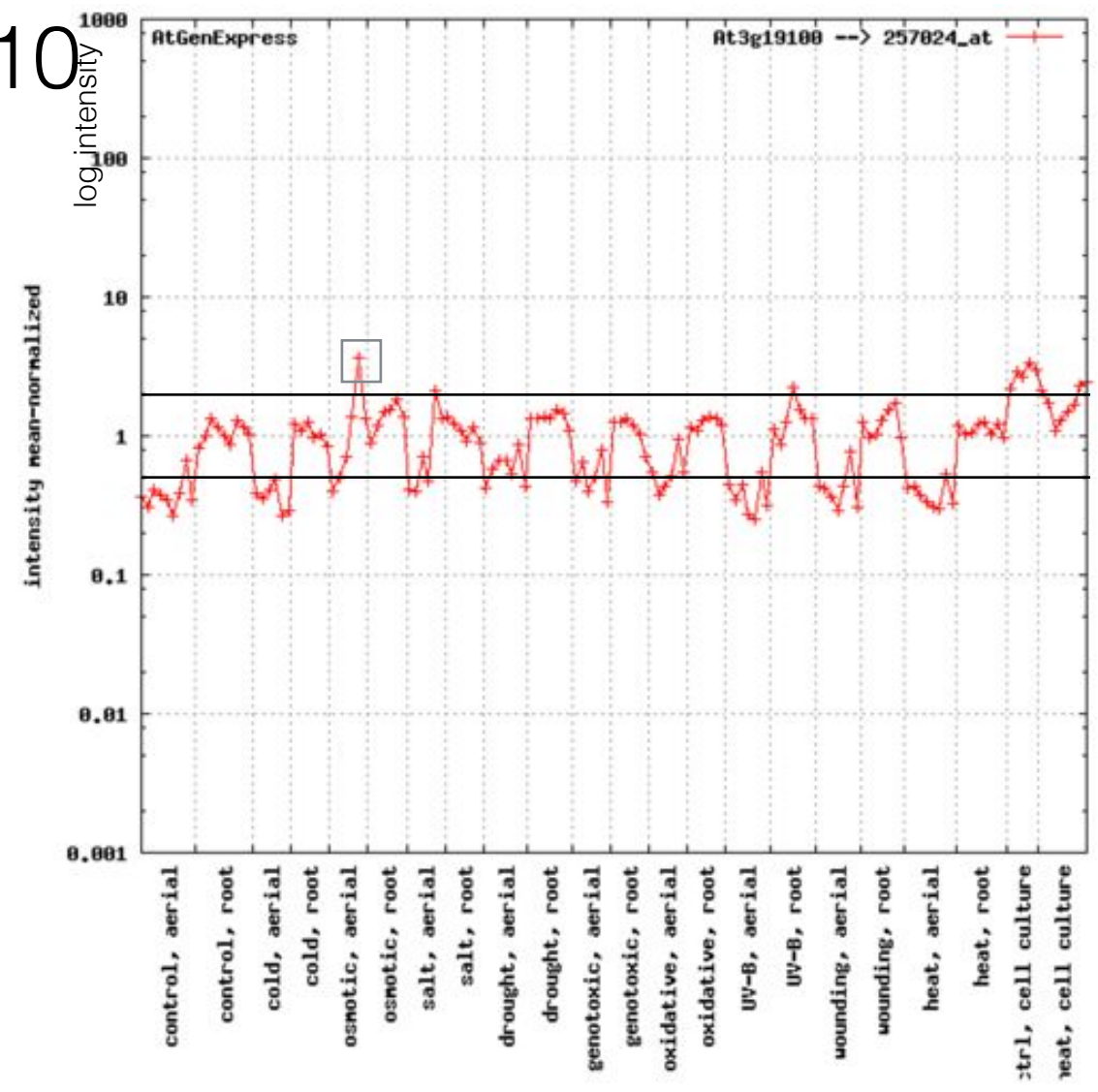




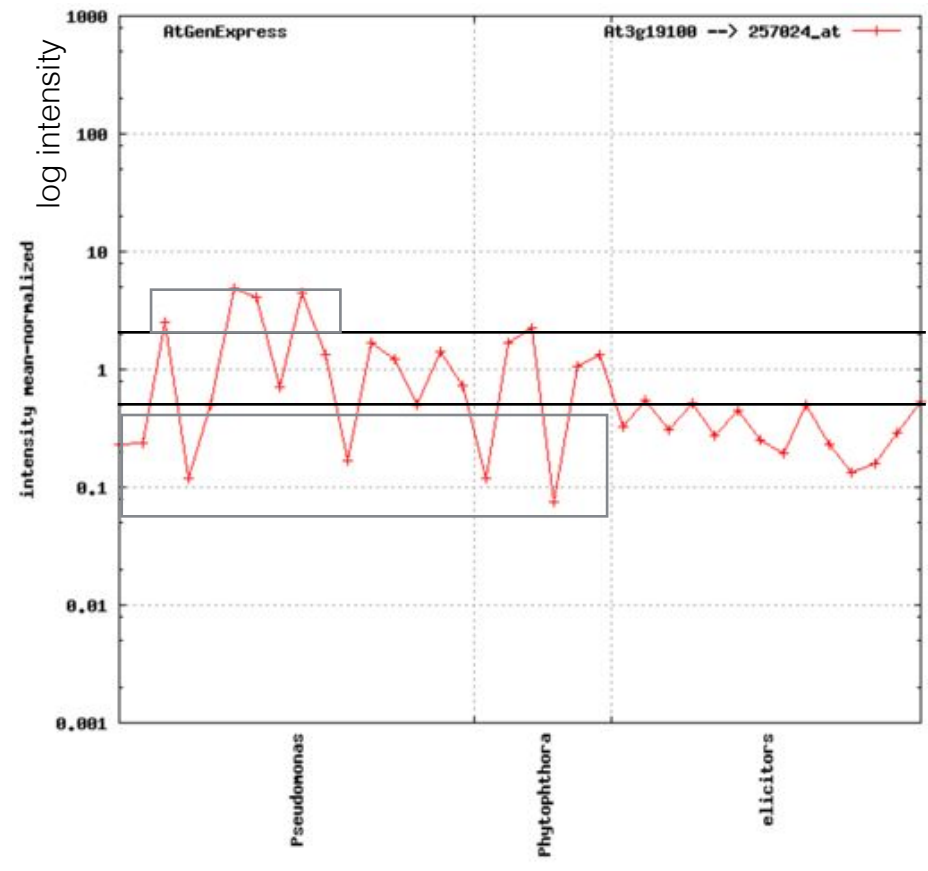
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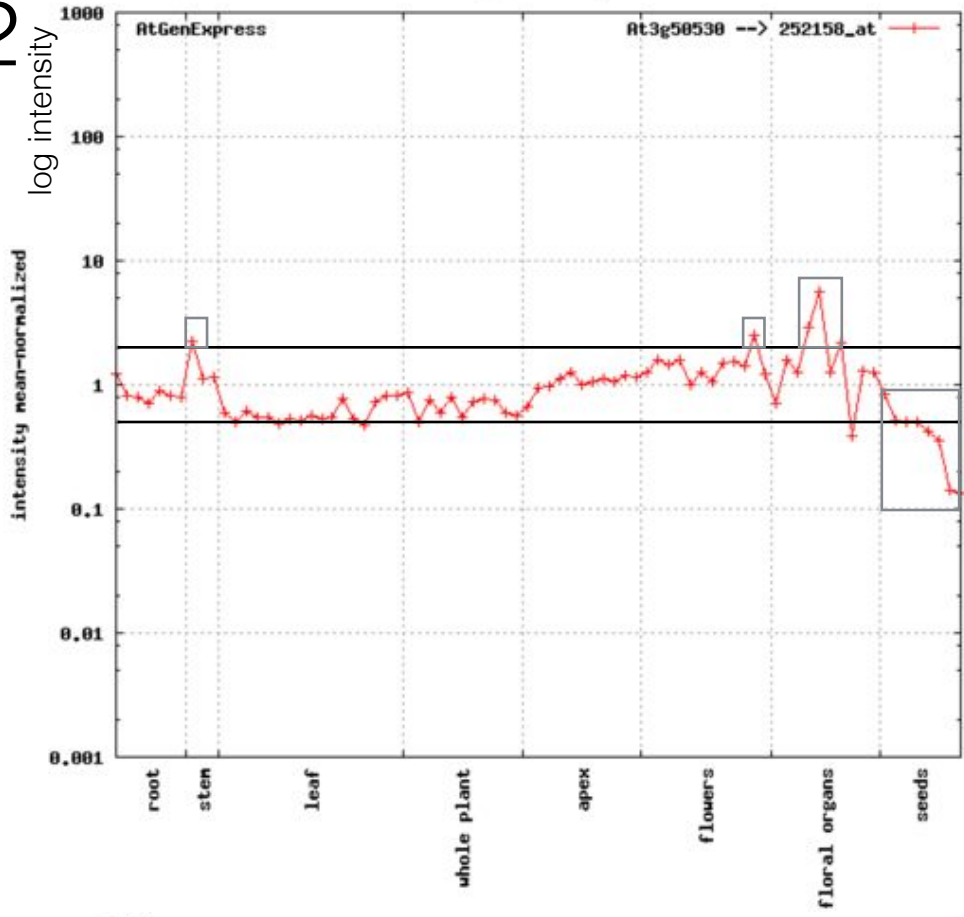
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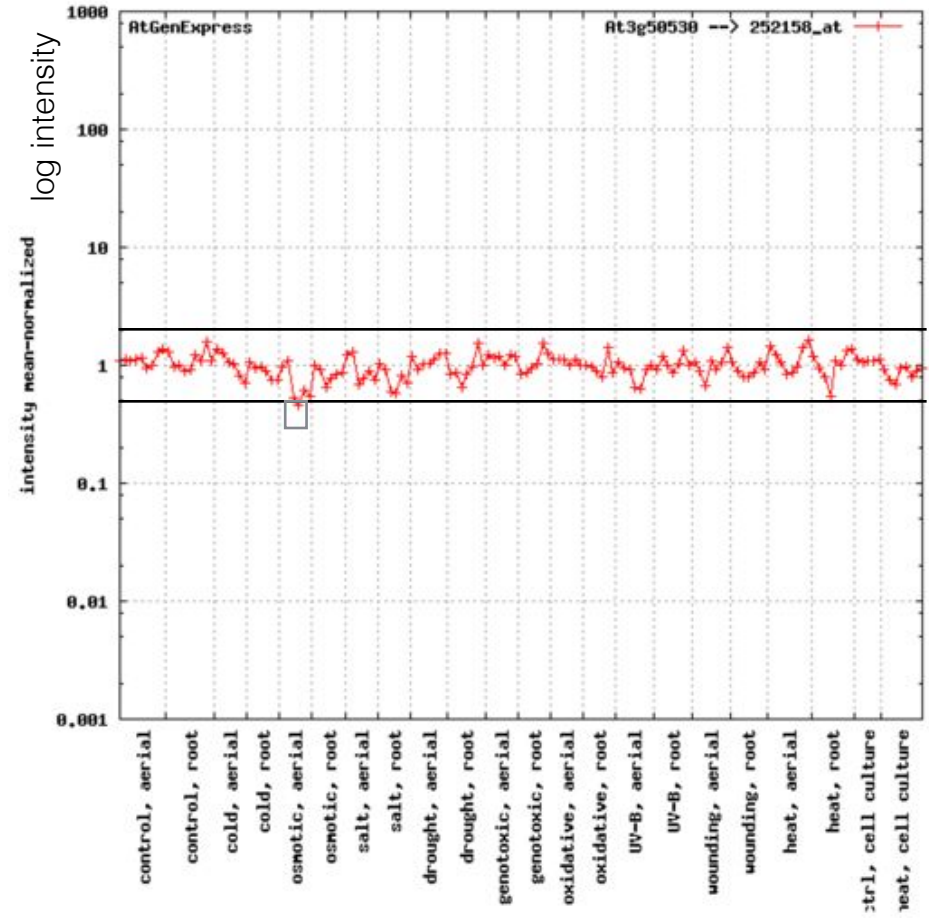
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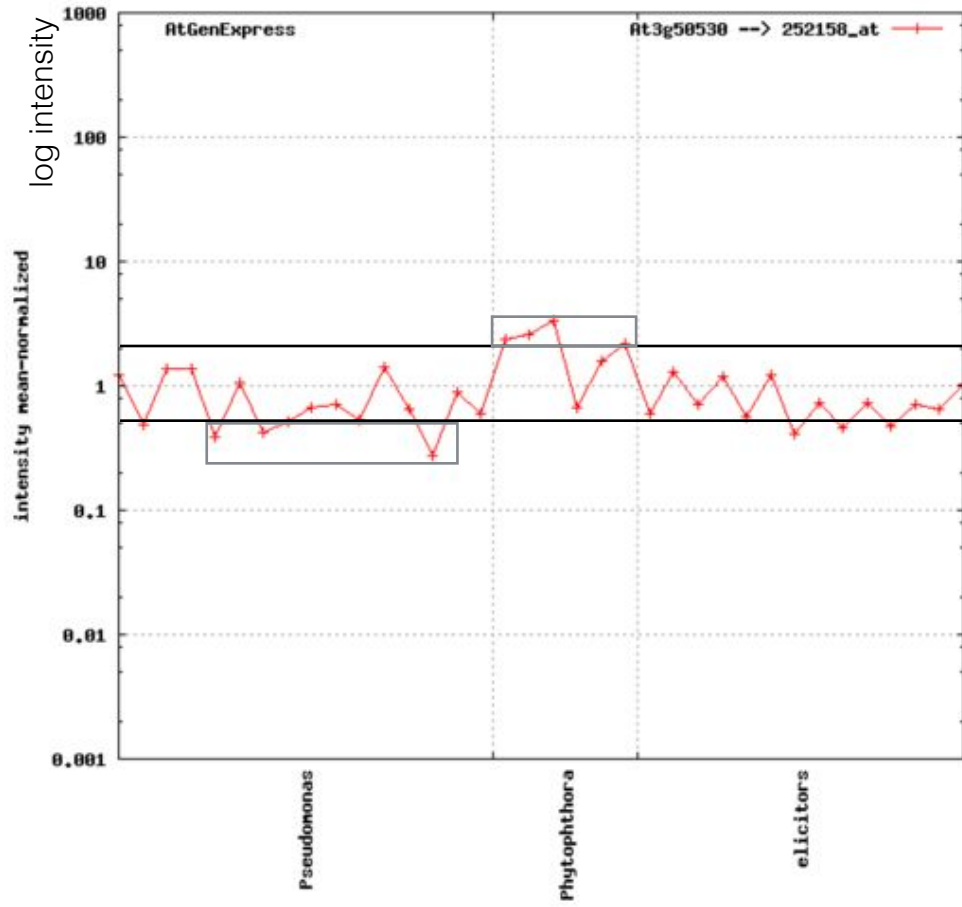
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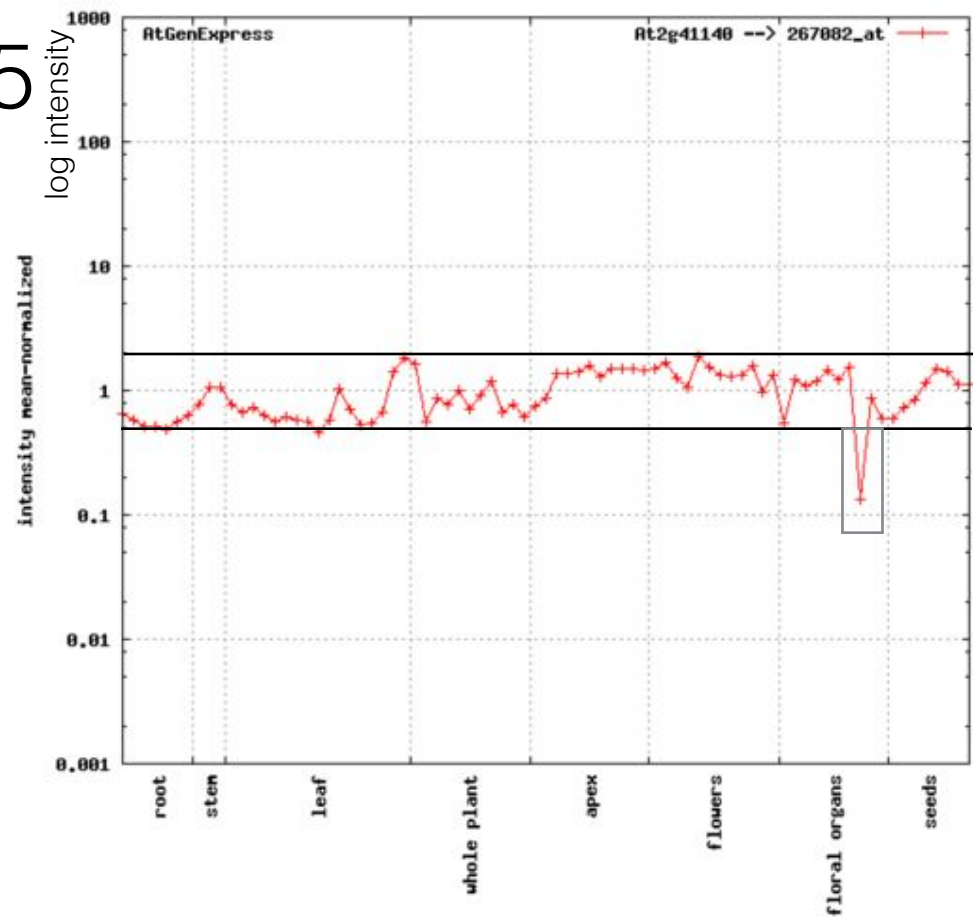
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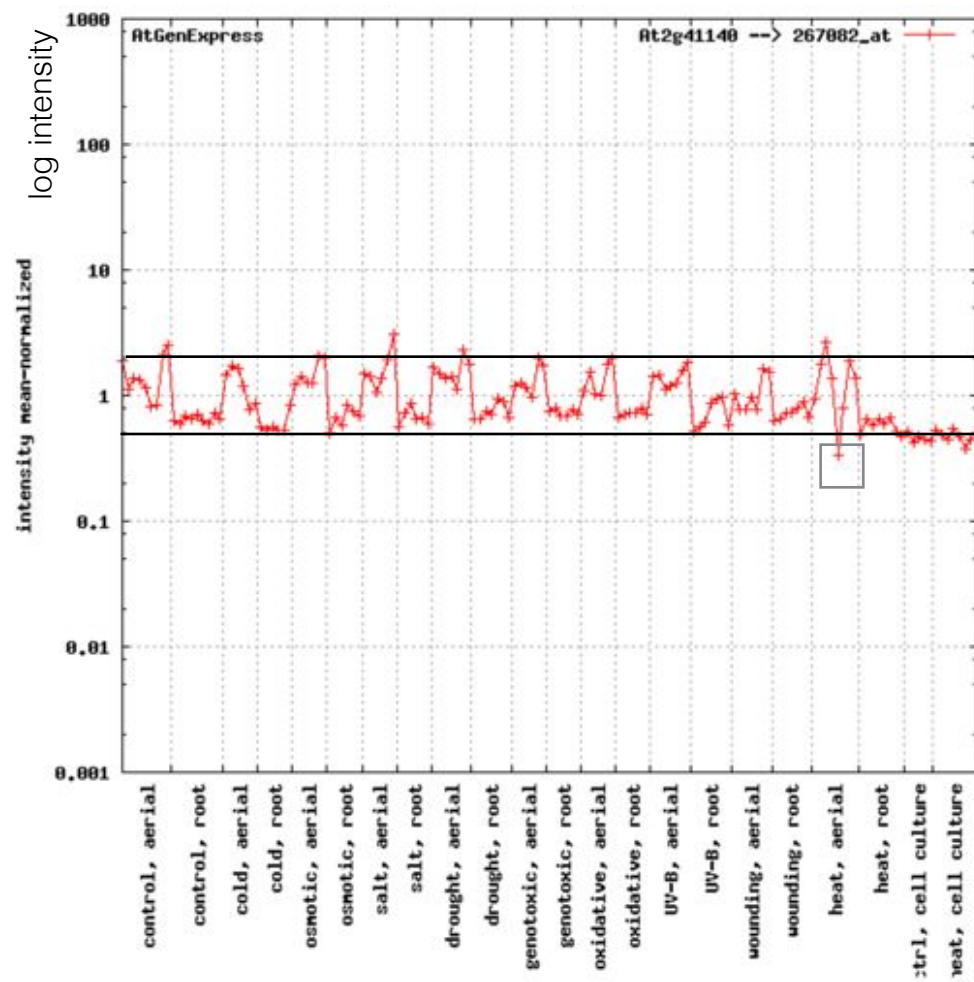
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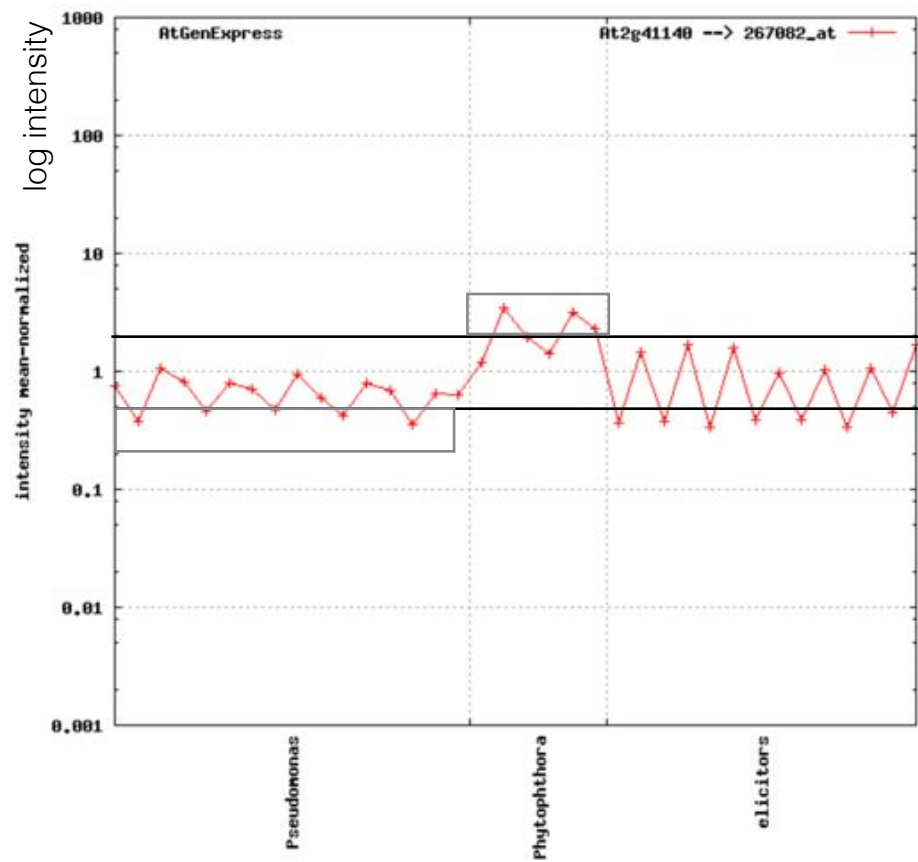
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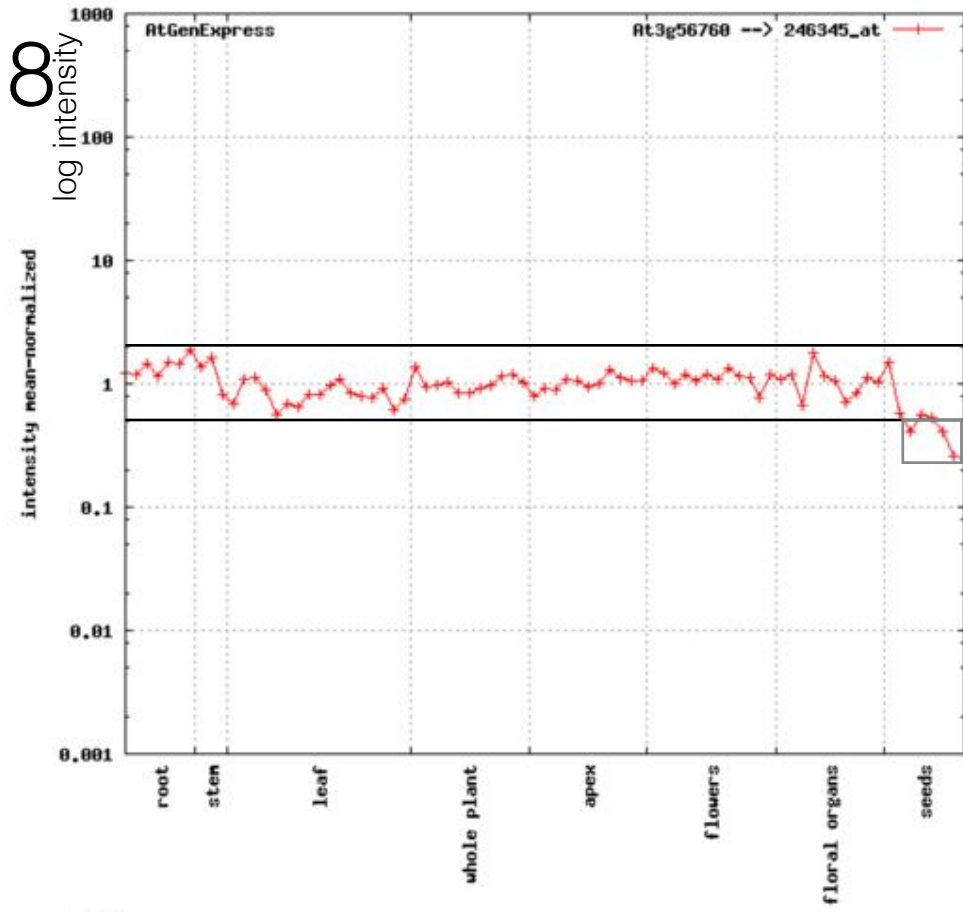


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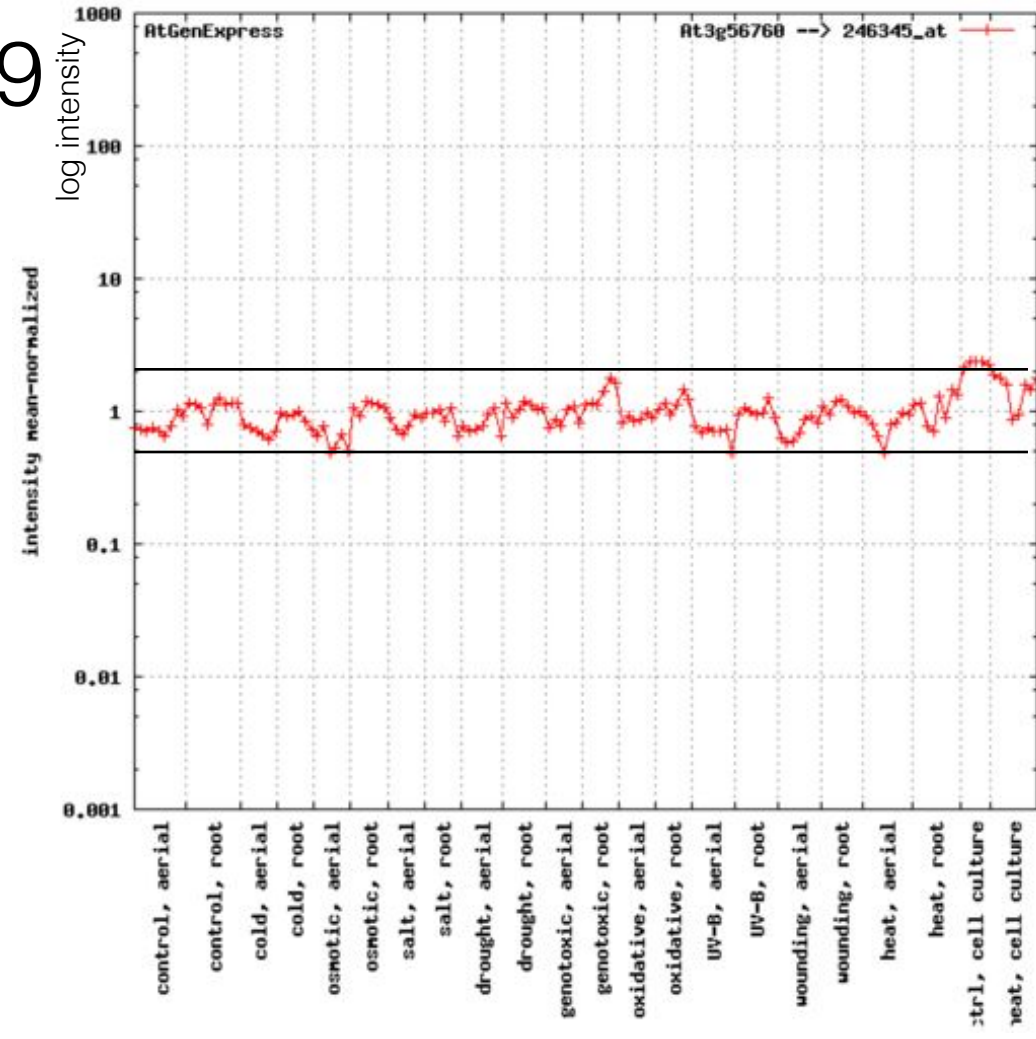




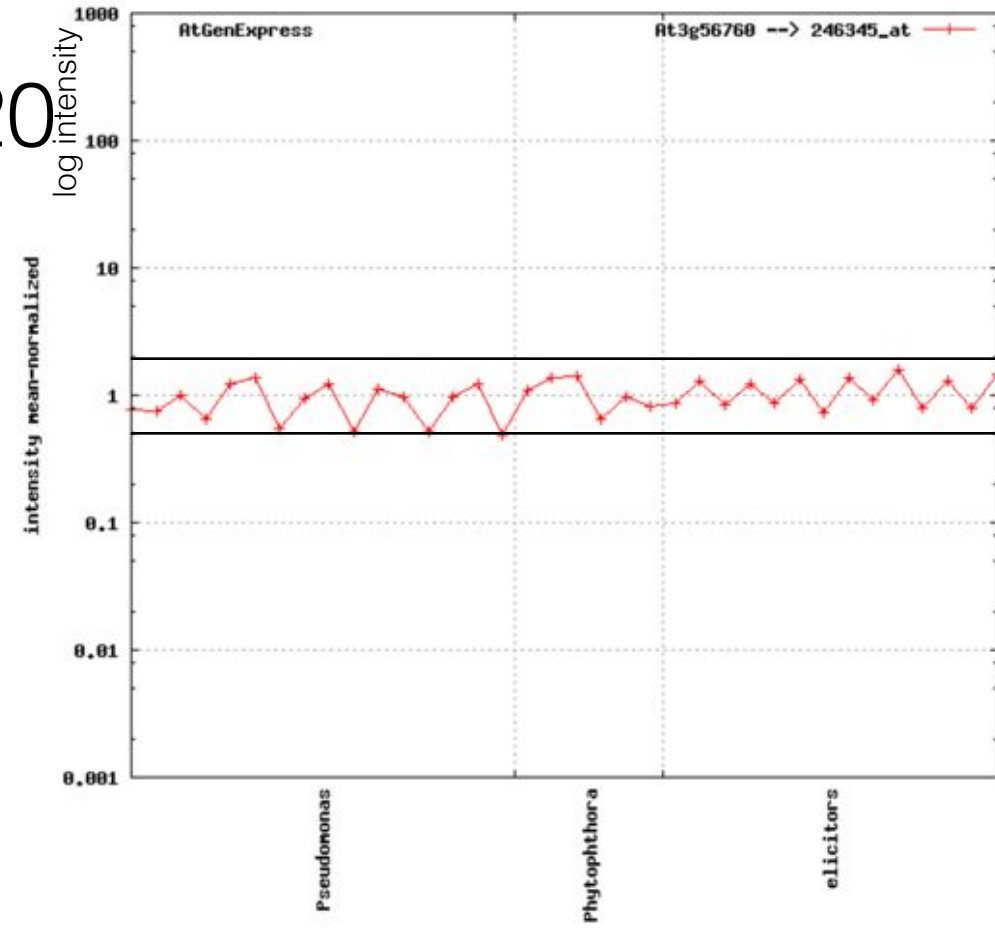
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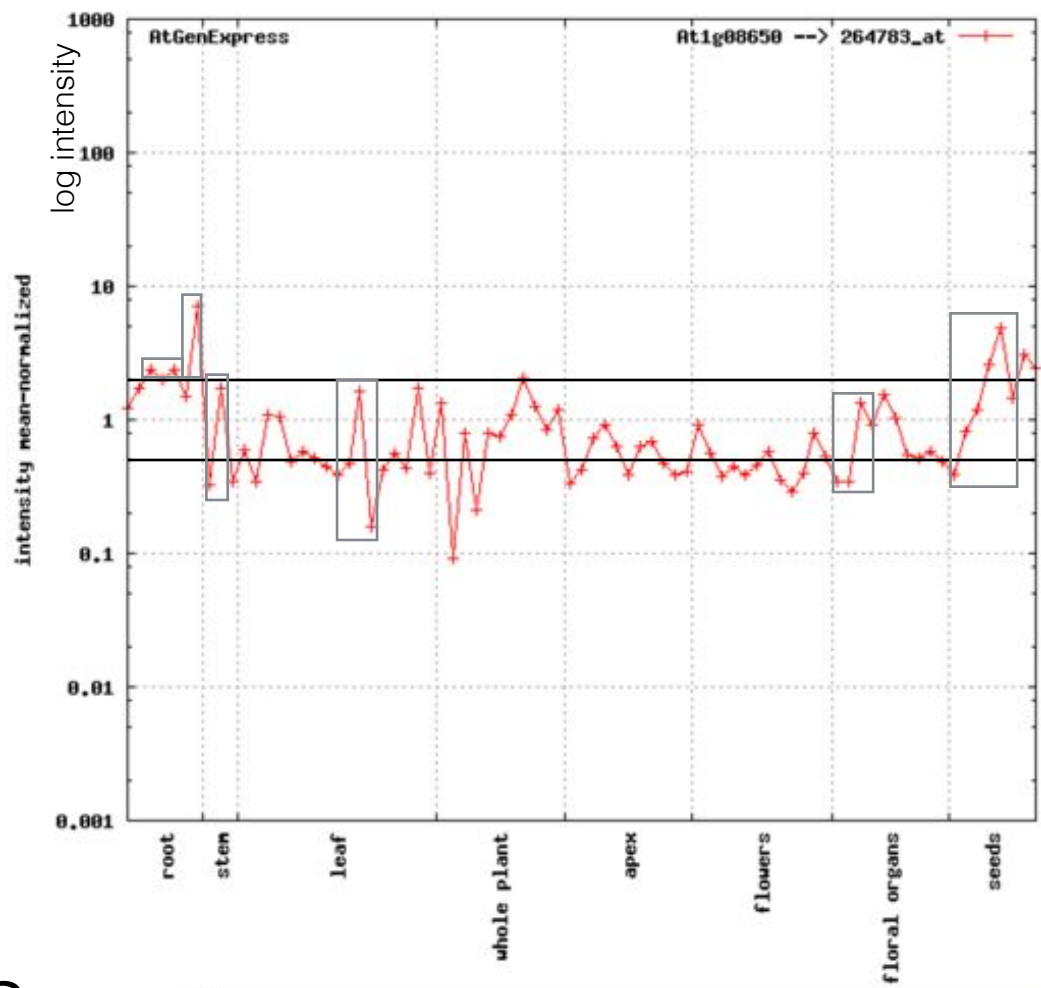


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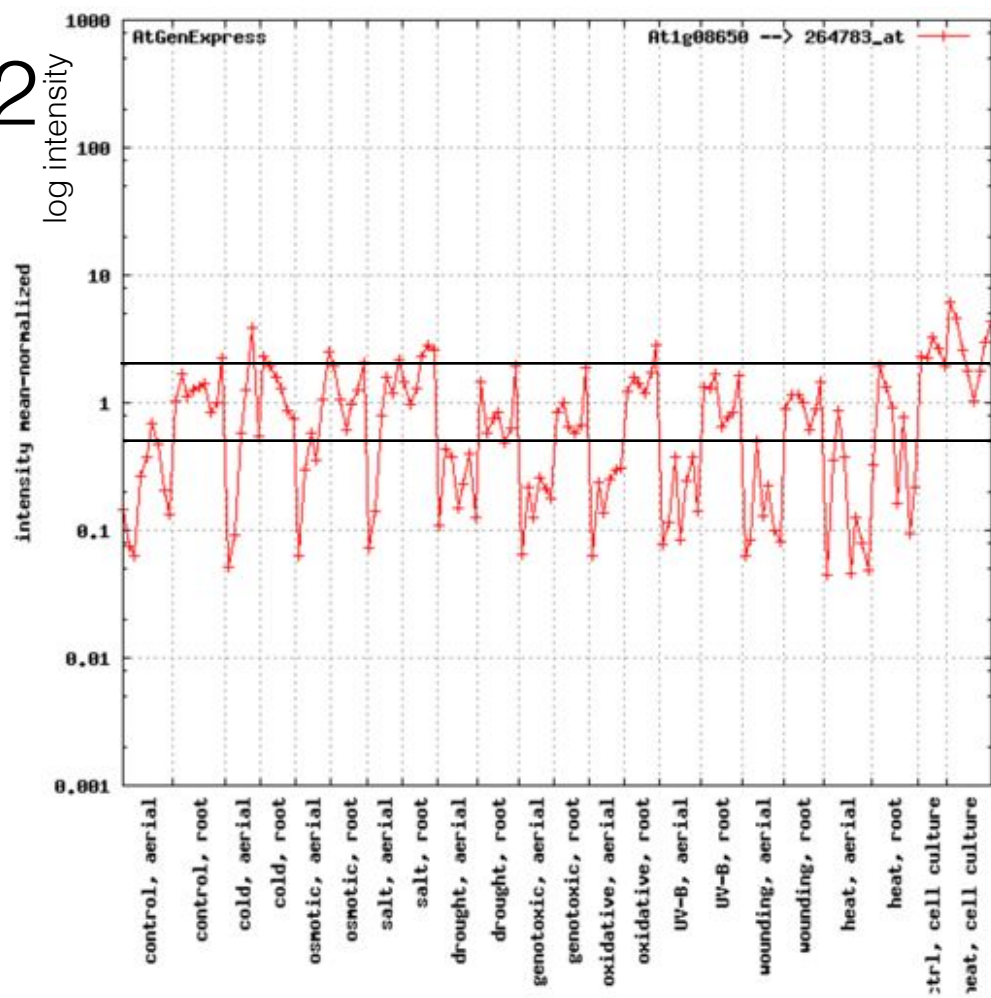




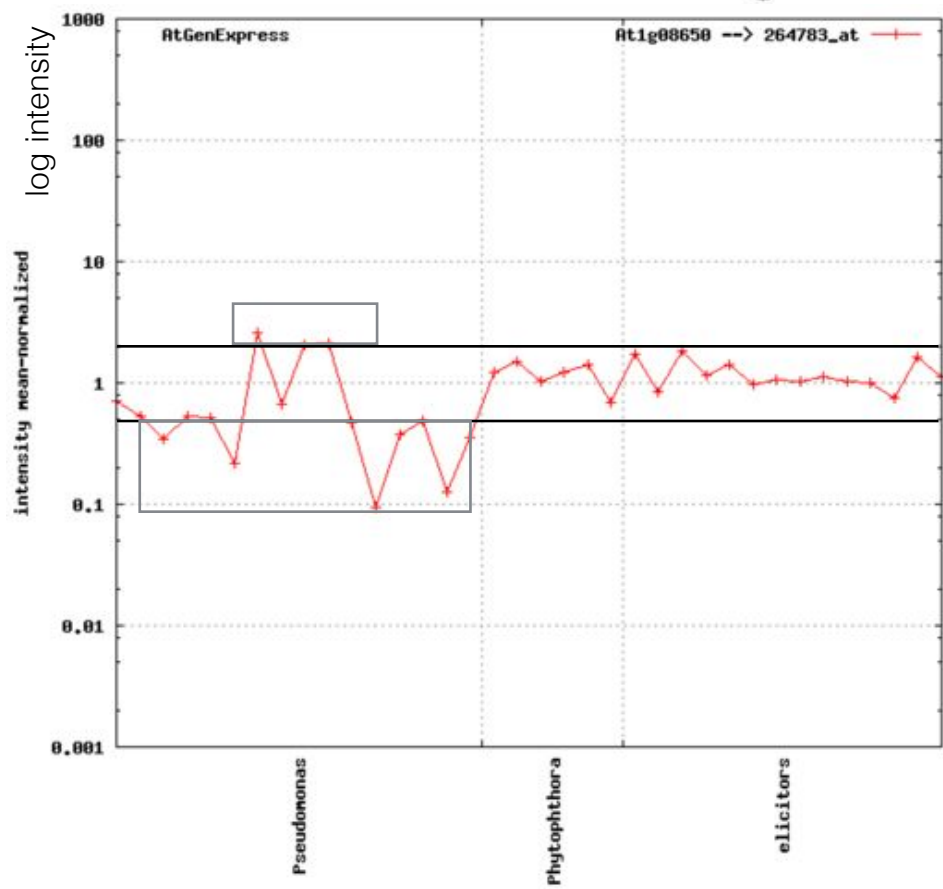
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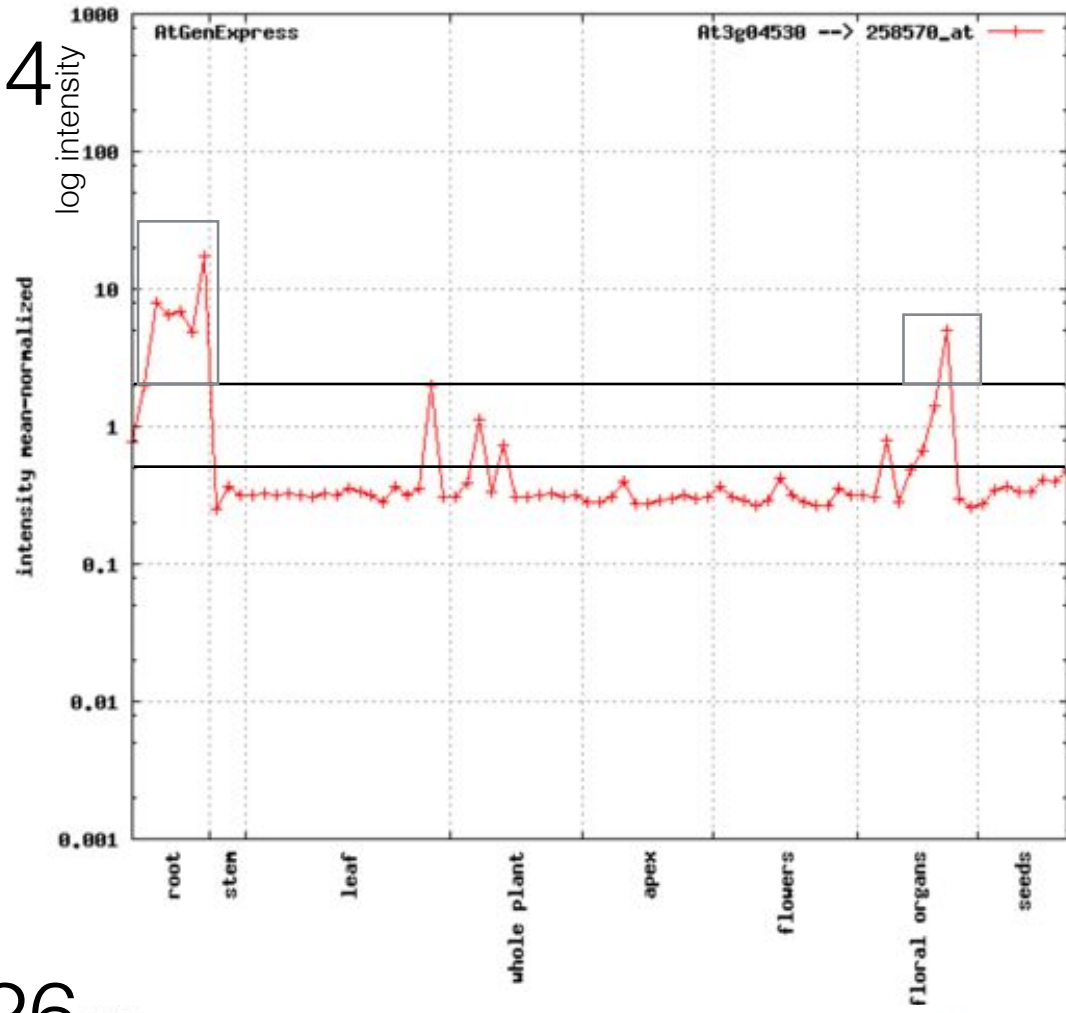
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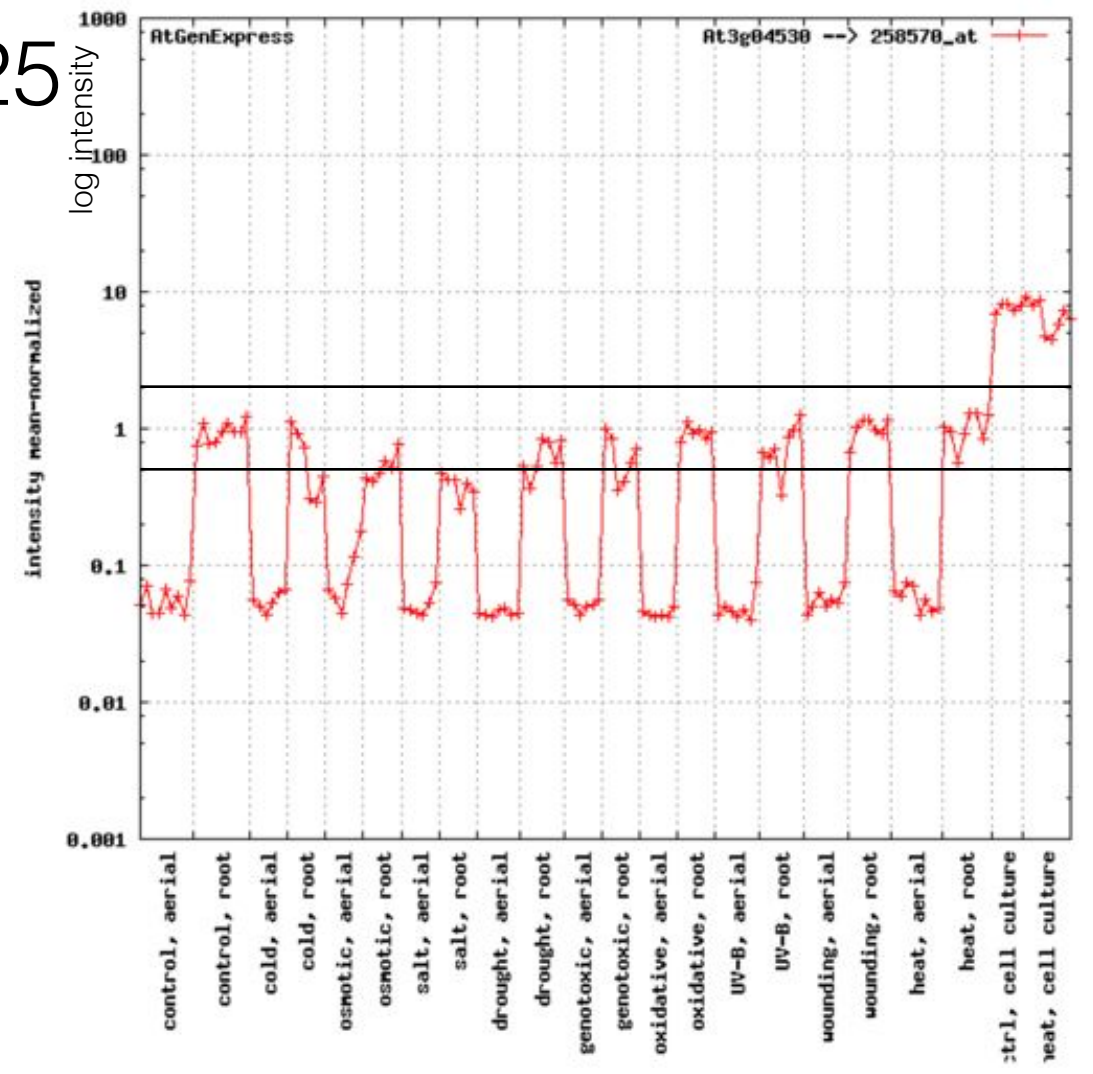
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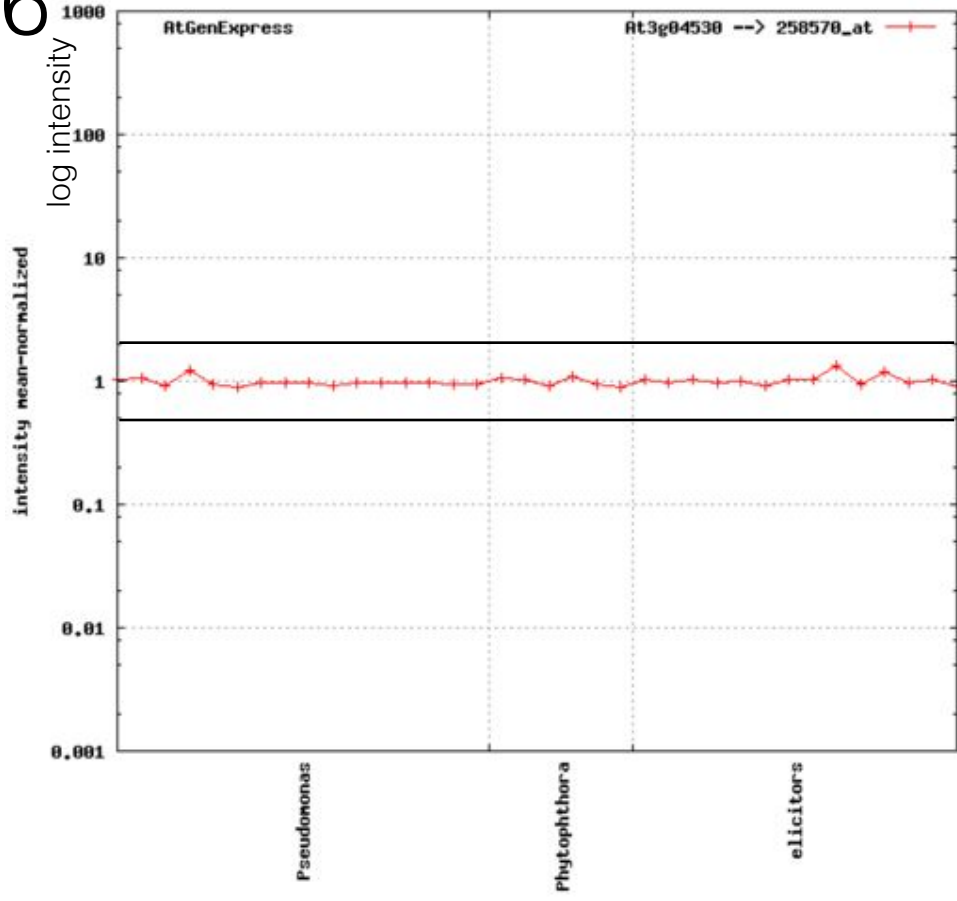
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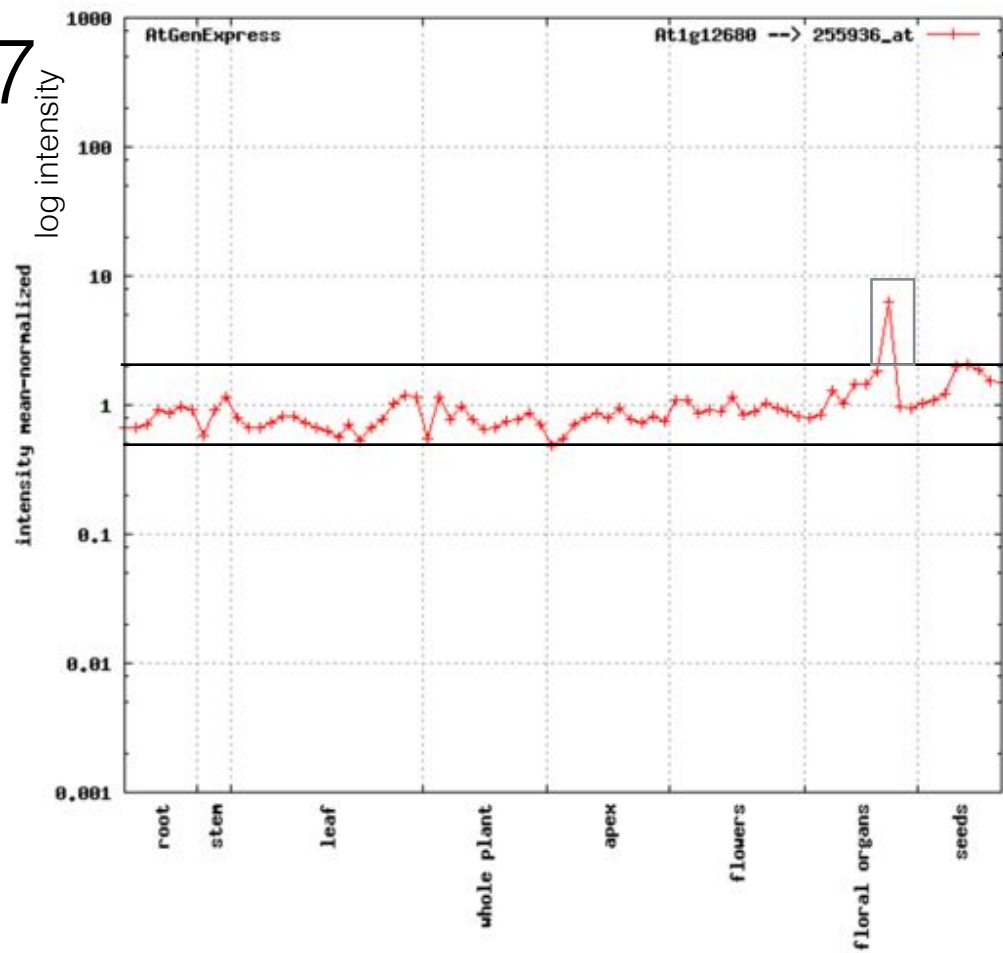
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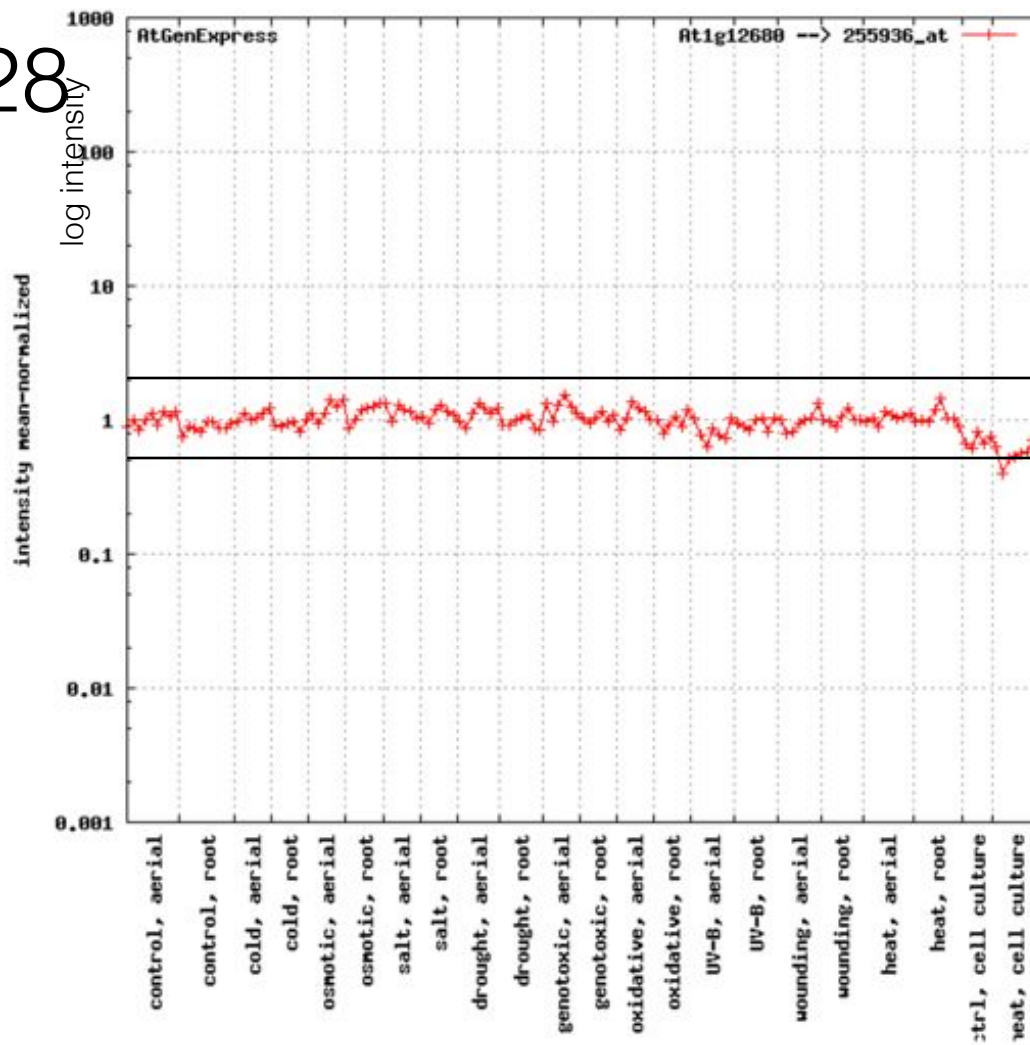
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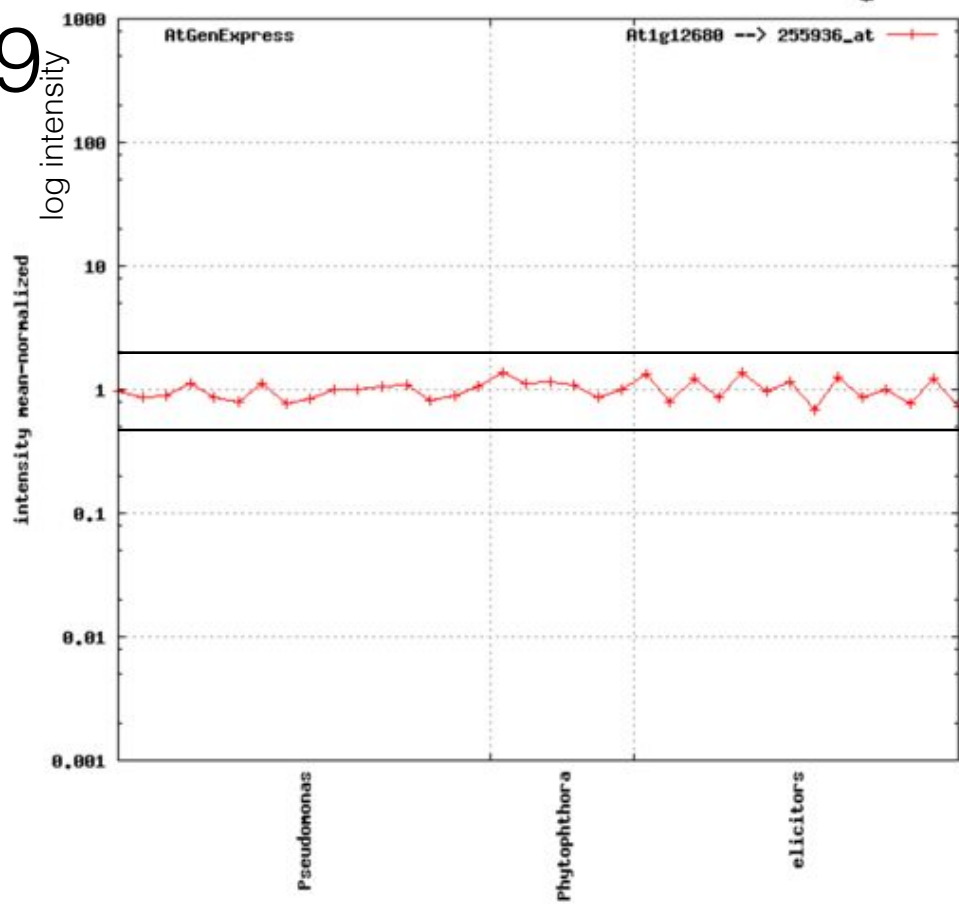
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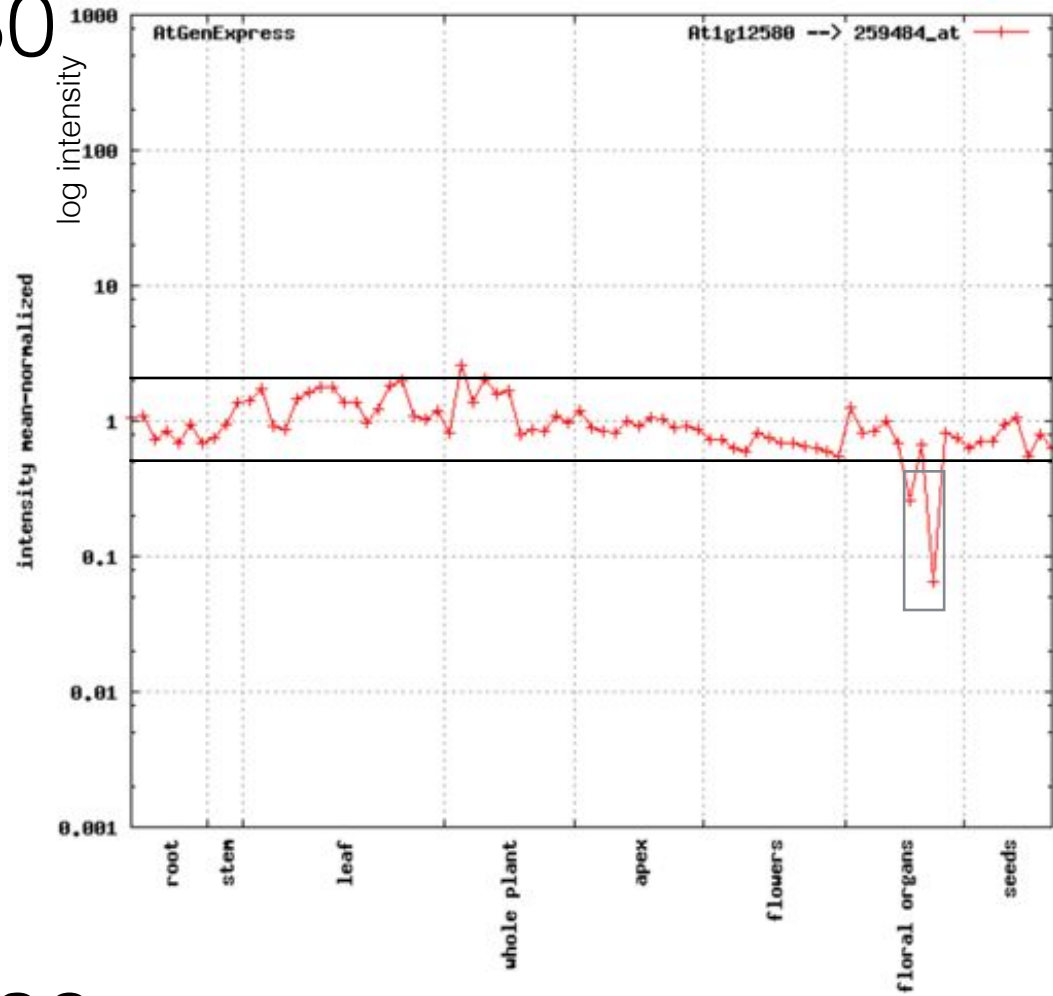


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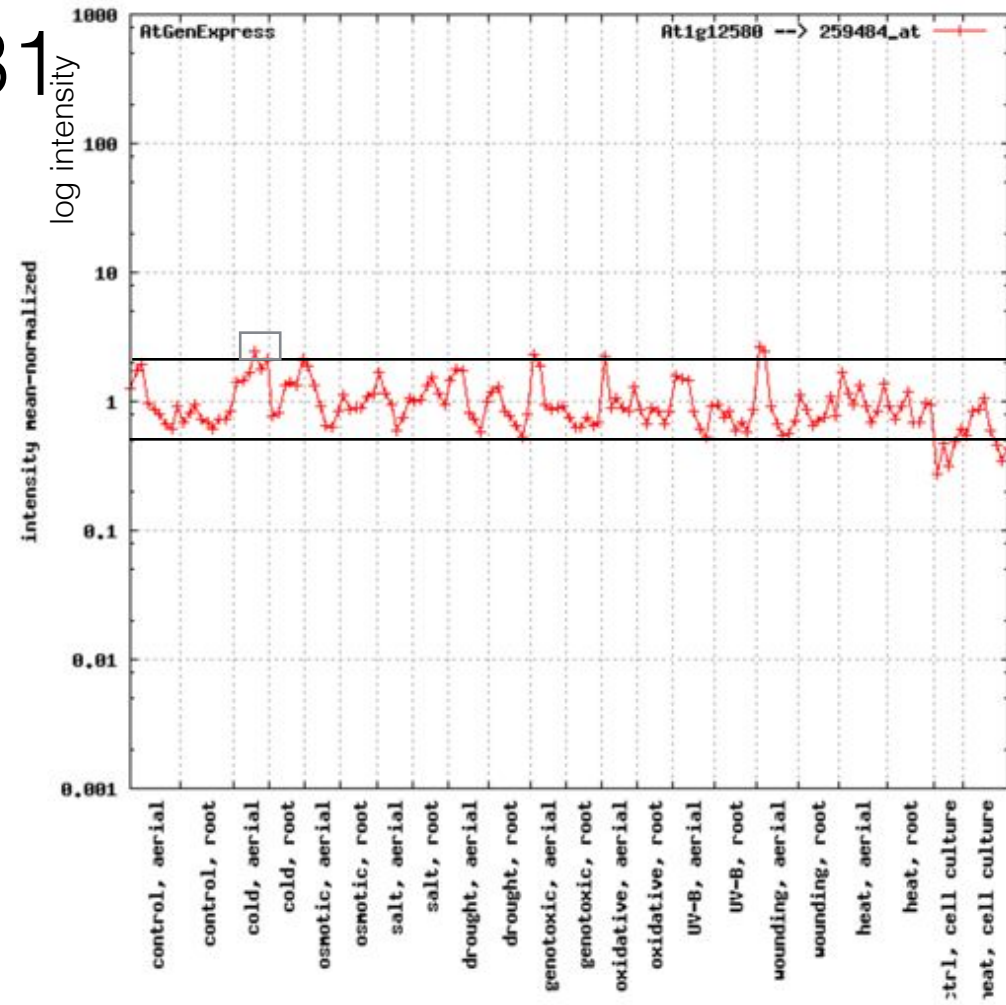




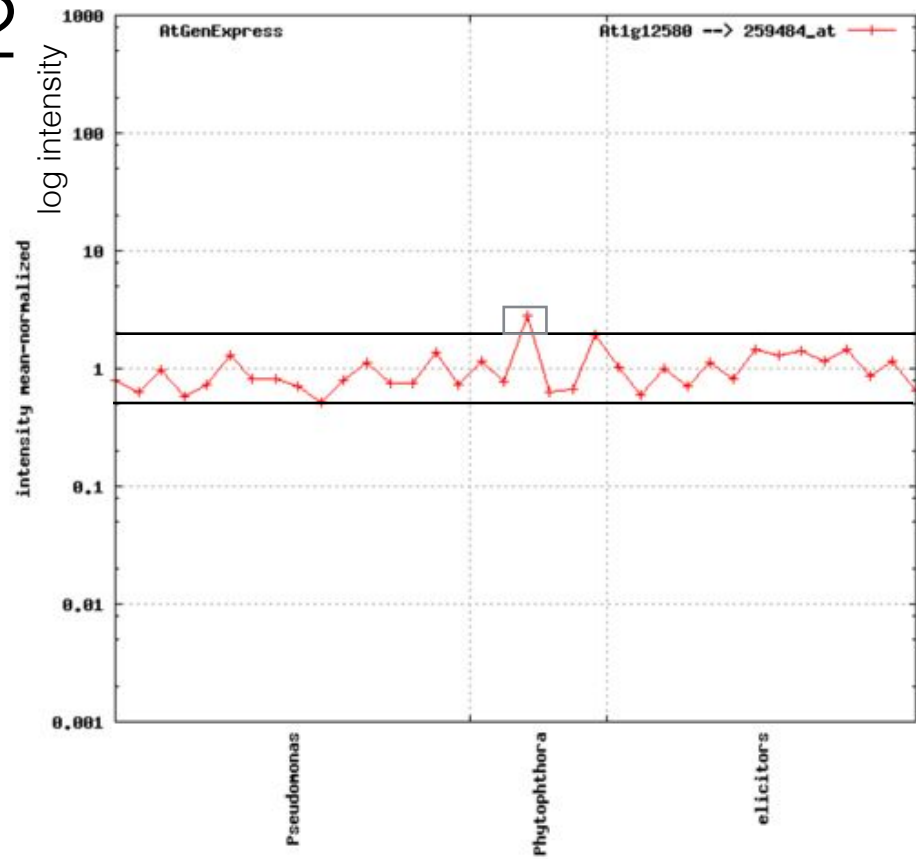
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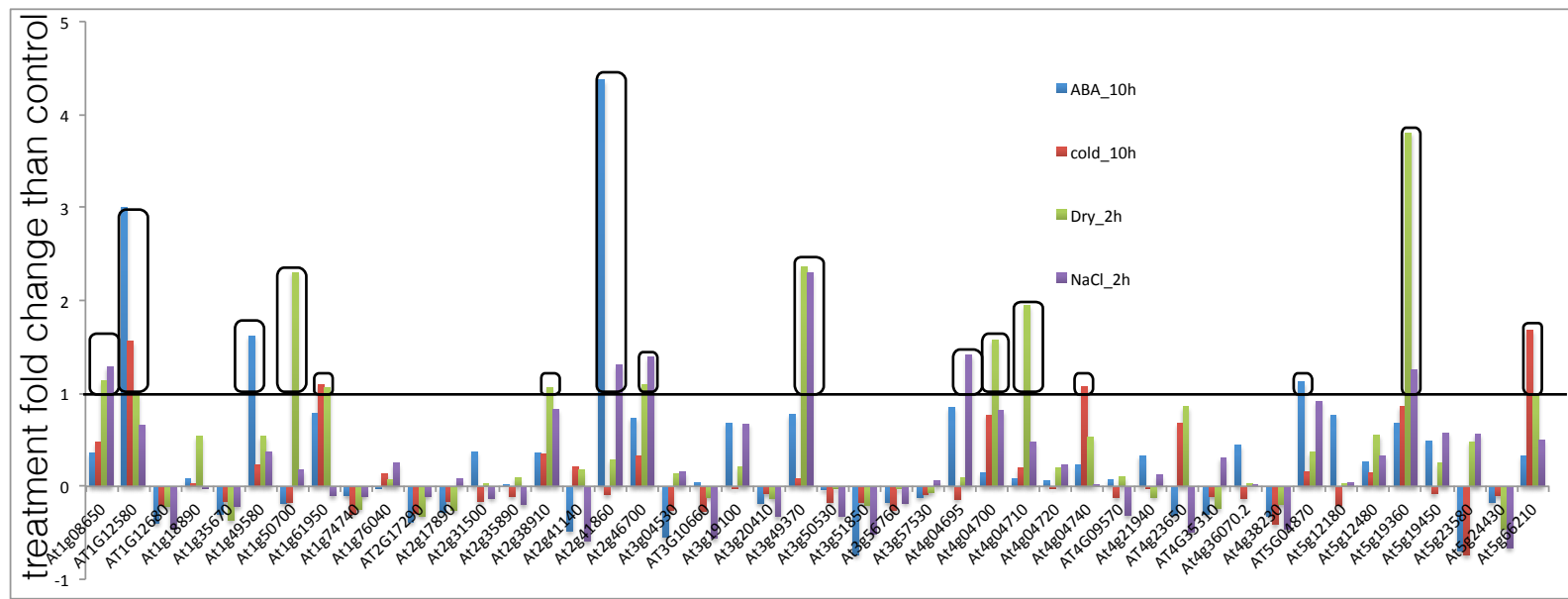


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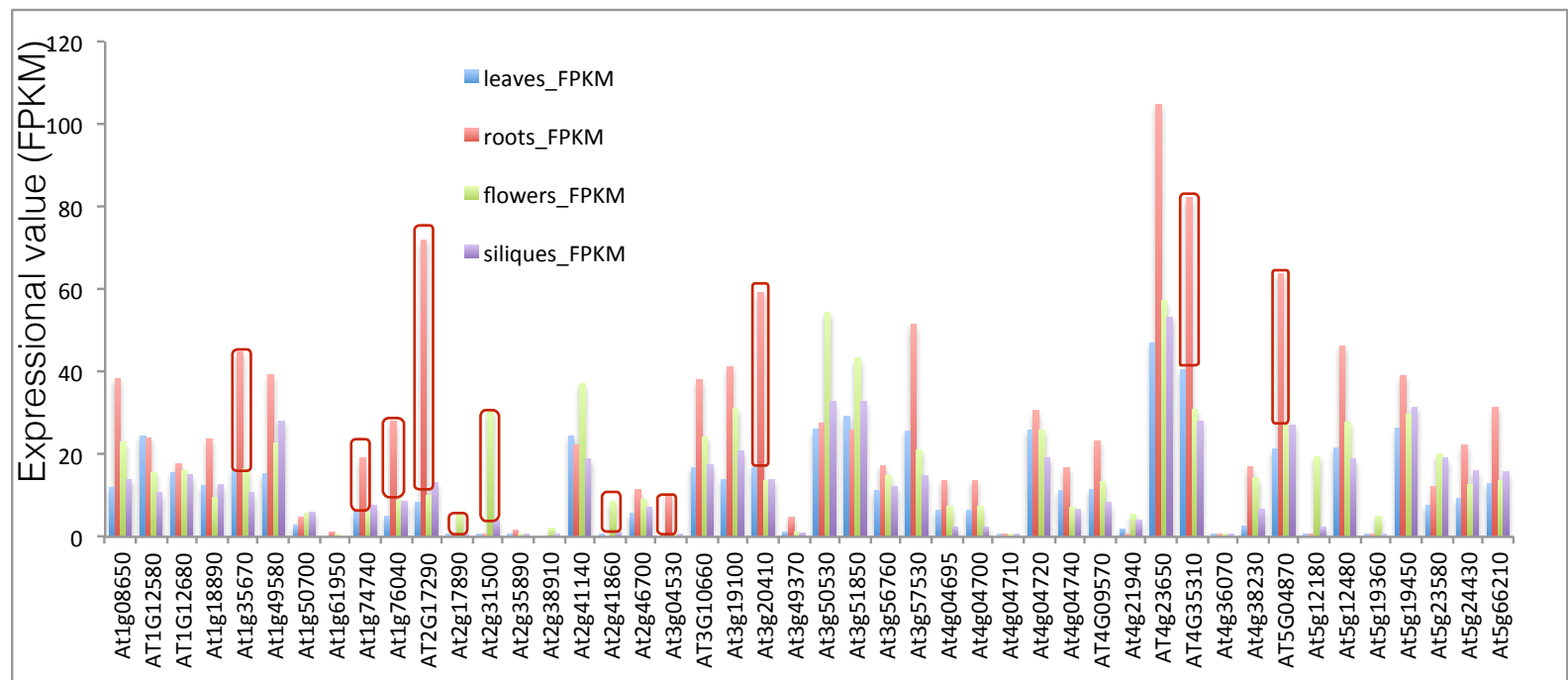




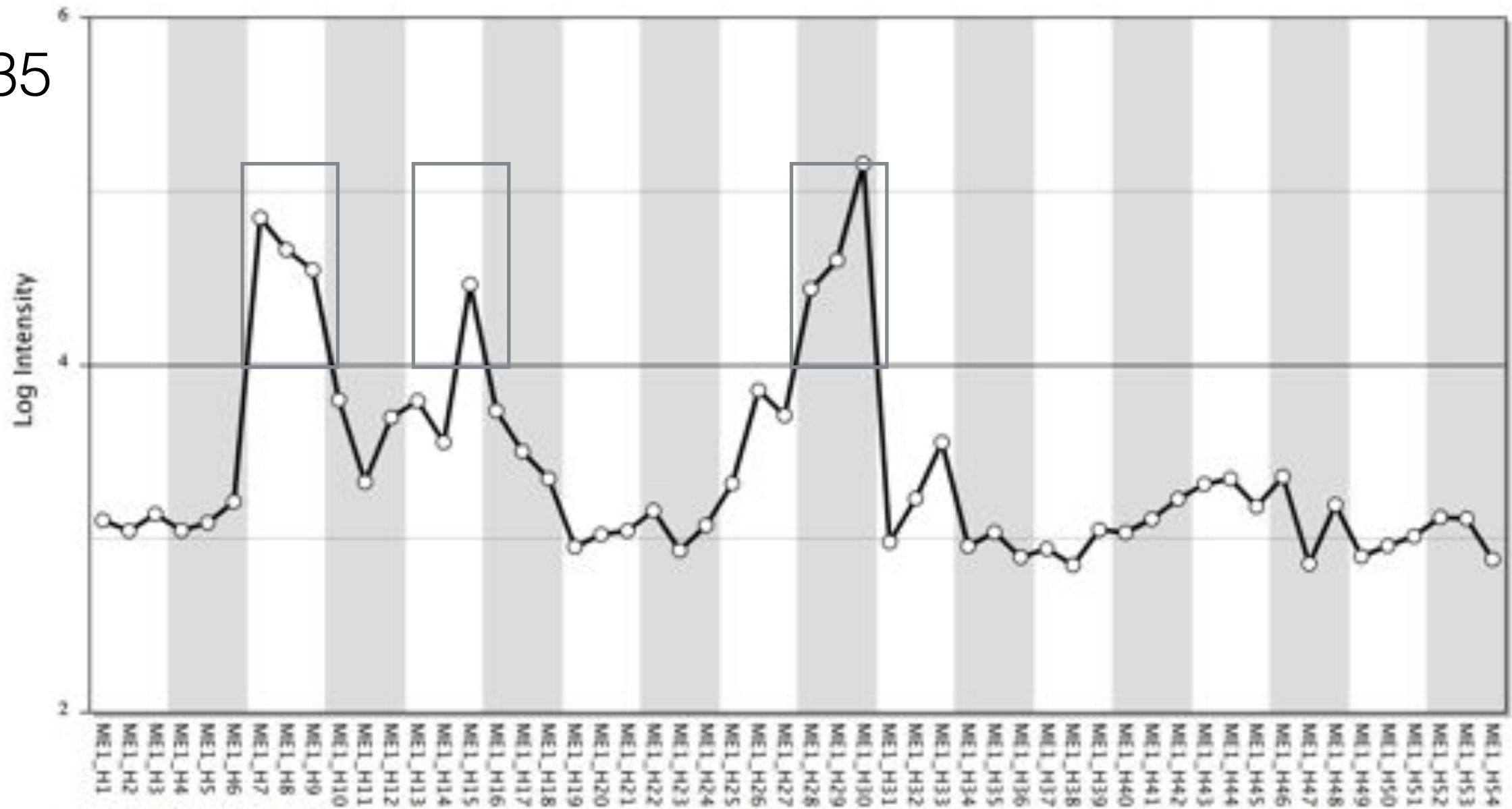
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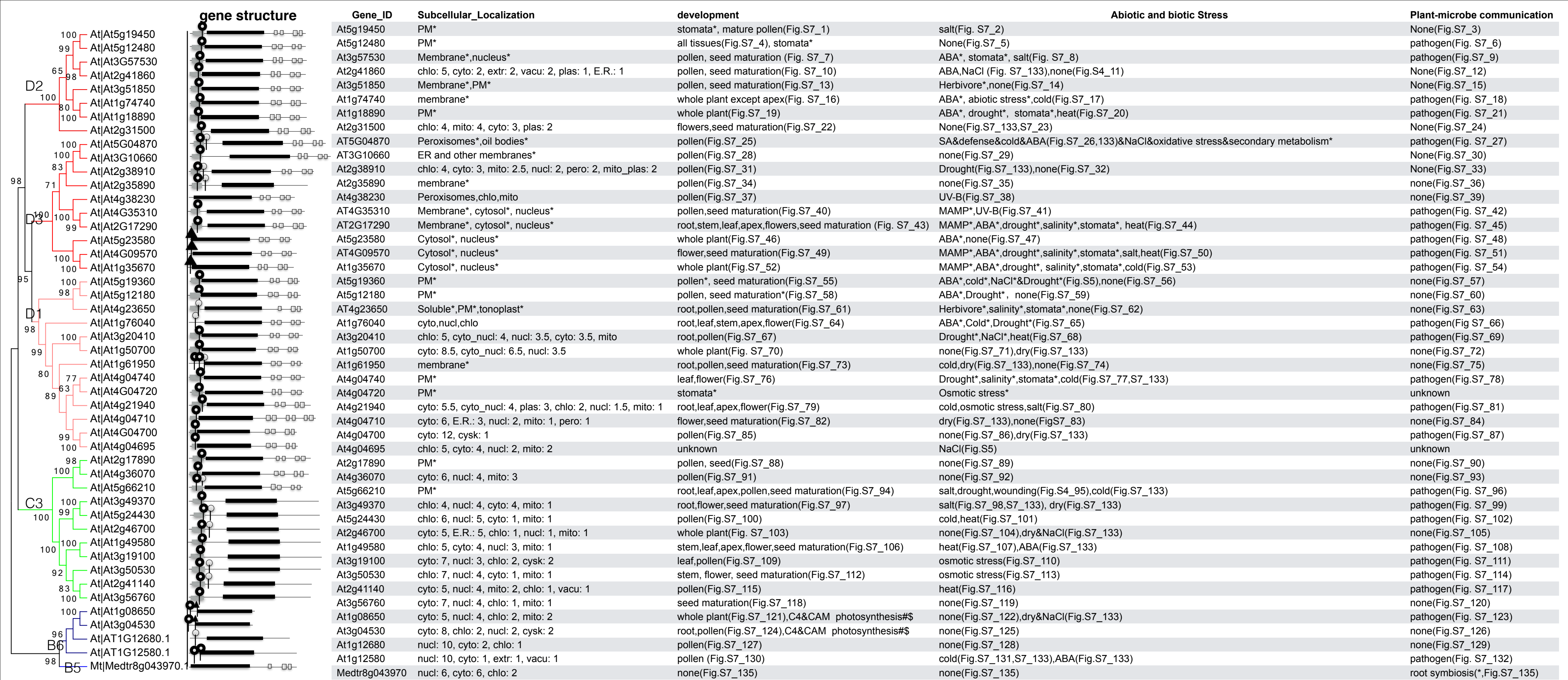
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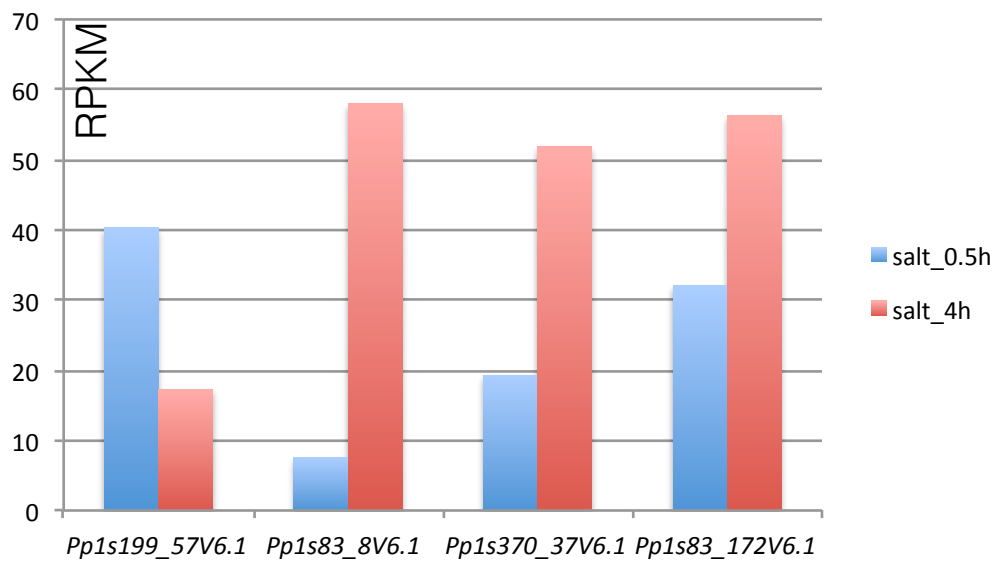
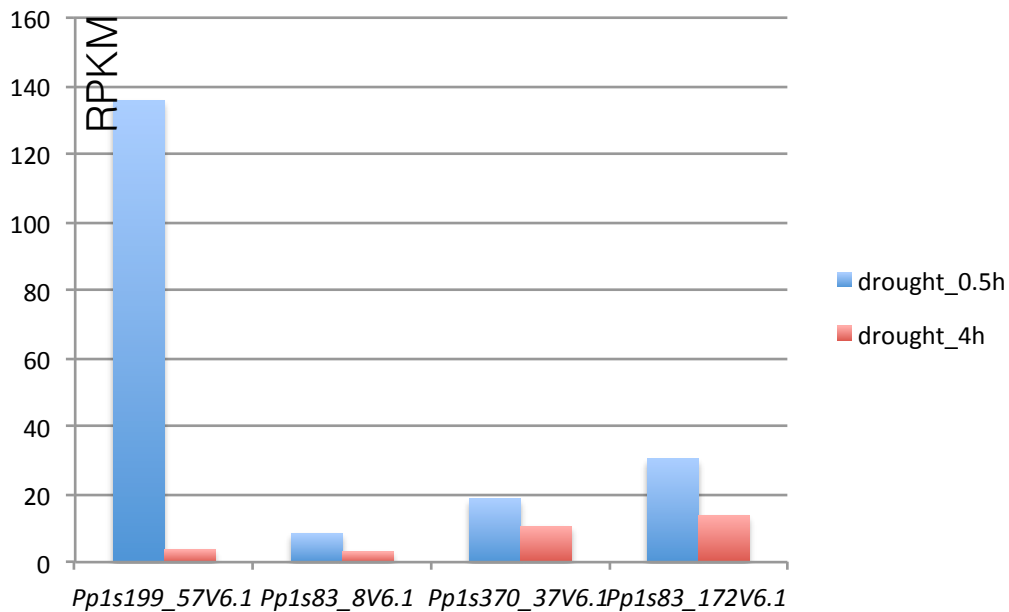


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organism part name	Flower	Leaf	Nod4d	Nod10d	Nod14d	Nodule	Petiole	Pod	Root	Root-0d	Seed10d	Seed12d	Seed16d	Seed20d	Seed24d	Seed36d	Stem	Vegetali
Flower	Flower																	
Nodule						Nodule												
Seed10d											Seed10d							
Seed36d												Seed12d				Seed36d		
		Leaf																
			Nod4d															
				Petiole														
					Pod													
						Root												
							Root-0d											
								Nod10d										
									Seed20d									
										Nod14d								
											Root-0d							
												Seed24d						
													Seed24d					
														Stem				
															Vegetali			

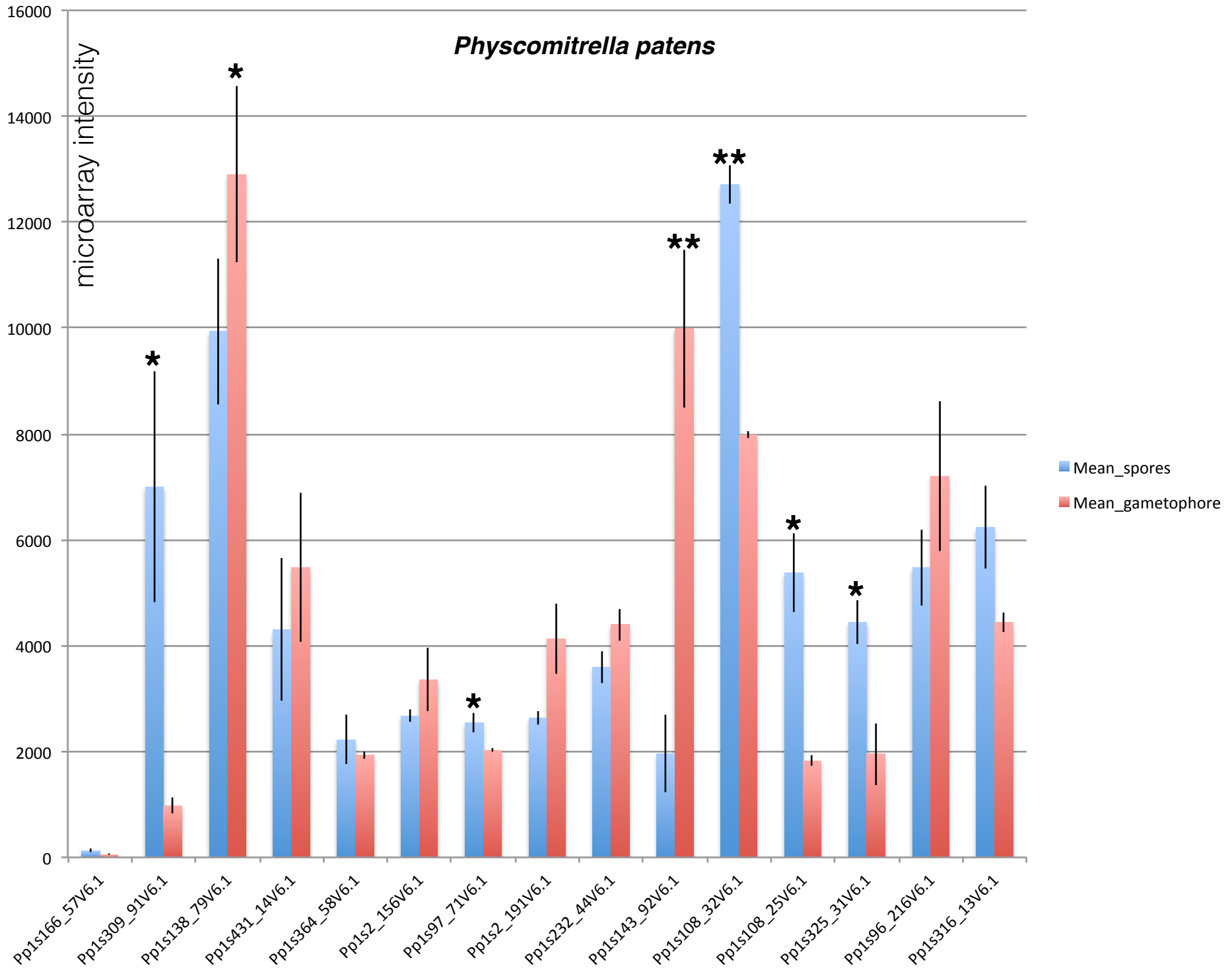


**A****B**



*Physcomitrella patens*

microarray intensity



#####

This text file includes the Supplemental File S1, followed by the references to the supplemental figures.

#####

Supplemental File S1. Representative sequences that have innovative gene structure compared to the typical structure composed of a KD and a CaM-LD.

(1) degenerated kinase domain, distributed in red algae to flowering plants

Pcr|HS694783.1

Vca|Vocar20001731m

Olu|2798

Vitis\_vinifera|VIT\_00s0577g00010

(2) myristoylation at the Nterminal, distributed in Charophytes to flowering plants

Mpu|175307|126004

Cre|Cre13.g564500.t1.3

Kfl|kfl00234\_0080

At|At2G17290

At|At2g41860

At|At5g12180

(3) palmytoylation at the NUTerminal, distributed in seawater algae to flowering plants

Mpu|175307|126004

Cre|Cre13.g564500.t1.3

Kfl|kfl00036\_0390

(4) C2 domain at the NUTerminal, distributed only in freshwater algae

Cre|Cre17.g705000.t1.2

Vca|Vocar20010397m

(5) PEST motif in the NUTerminal, distributed in freshwater algae to flowering plants

Cre|Cre02.g114750.t1.2

Kfl|kfl00013\_0520

Atr|scaffold00018.149

Vv|VIT\_16s0098g01140

(6) Acylation at the NUTerminal, distributed in freshwater algae to flowering plants

Cre|Cre02.g114750.t1.2

Kfl|kfl00013\_0520

Atr|scaffold00018.149

Vv|VIT\_16s0098g01140

(7) DnaJ domain in the NUTerminal, distributed in flowering plants

Br|Bra009697

Fv|mrna09567.1

(8) AAA domain in the NUTerminal, distributed in flowering plants

Br|Bra001373

Br|Bra029859

(9) Two EF hands in the CUTerminal, distributed in red algae to flowering plants

Pcr|HS694783.1

Cva|35323

Cre|Cre06.g265550.t1.2

Cva|20239

Atr|scaffold00073.66

(10) 4 EF hands at the CUTerminal, the typical calmodulinUlike domain, distributed in seawater algae to flowering plants

Mpu|175307|126004

Vca|Vocar20000362m

At|At2G17290

At|At2g41860 (11) three EF hands at the CUTerminal, distributed in seawater algae to flowering plants

Olu|2798

Csu|20203

Atr|scaffold00101.38

At|At4g23650 (12) one EF hands at the CUTerminal, distributed in freshwater

algae to flowering plants

Csu|11155

Csu|2460

Ppa|Pp1s159\_42V6.1

Sm|Sm173444|PACid:15414880

Fv|Fvmrna24077.1

Fv|Fvmrna18654.1

(13) Insertion in the AID, and degeneration of first EF hand, distributed in all streptophytes

Mv|GBSK01009566.1

Pla|ADV78070.1

Atr|scaffold00101.38

Os|LOC\_Os05g41090.1

(14) C-terminal without domain detected

Sm|Sm99940|PACid:15420380

Atr|scaffold00069.118

Vv|VIT\_16s0100g00340

Os|LOC\_Os06g03682.1

(15) no AID nor CaMULD, found in Lycophytes to flowering plants

Sm|Sm99940|PACid:15420380

Atr|scaffold00148.66

At|At3g04530

(16) FBG domain at the C-terminal

Gm|Glyma07g18310.1

Gm|Glyma18g43160.1

Rc|28308.m000065

(17) Transmembrane domain at the C-terminal, found only in flowering plants

Pm|Pm007589

Pm|Pm023987

(18) S4 domain found in the C-terminal, found only in flowering plants

Al|339776

Lu|Lus10005619



Gr|Gorai.009G191500.1

#### Supplemental References

Aldous S. 2014. Evolution of the phosphoenolpyruvate carboxylase protein kinase family in C3 and C4 Flaveria spp. *Plant Physiol.* 165: 1076–1091.

Boudsocq M, Sheen J. 2013. CDPKs in immune and stress signaling. *Trends Plant Sci* 18: 30–40.

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