APPENDICES AND SUPPLEMENTARY DATA

APPENDIX – CHAPTER 3

Table Appx 3.1 Primer pairs, targets and PCR conditions used in the different analyses.

Name	***Primer sequence (5' – 3')****	***Target region***	***Annealing Temperature***	***Amplicon length (bp)***
16S DGGE				
PRUN518r[#]	ATTACCGCGGCTGCTGG	*16S rDNA*	58 ºC	460
pA8f-GC[§]	AGAGTTTGATCCTGGCTCAG			
Standards and nested primers: copy numbers				
leuB_5069F	CAAGAGGGCGCGTAACTAAA	*leuB*	60 ºC	411
leuB_5461R	AACCCCCTGCTGGTTCATA			
leuB_5158R	GAGCGCGCTAATTTAAAAGC		60 ºC	109
trpB_3248F	TACTGCAGCAGGACCACATC	*trpB*	59 ºC	375
trpB_3622R	GCATGTTCTGGACCAACAGA			
trpB_3380R	TCCTCCAACACATGCGATTA		60 ºC	133
***Sequencing:* pleuABCD**				
leuC_6856F	GCTATTGTTGTACCAGGTTCAGG	6856-969	TD[ɖ]	1881
repA1_969R	ACACAGAGCATCAGCCATGA			
repA1_894F	TGTTGATAGACCACATTCATCAGA	894-1830	TD[ɖ]	936
CDS1_1855R	CGTAAAAGATGTACAGAAGATATTGC			
CDS1_1521F	TCTCGTTGGTTRATGTTTCC	1521-2501	TD[ȸ]	982
repA2_2501R	TAAACGTGAAACACGGGTGA			

leuA_3289R	GAAGACATTGCATCACCTGCT	1540-3289	TD^{ɖ}	1749
CDS1_1521F	TCTCGTTGGTTRATGTTTCC			
leuA_3077F	CGTGATGGTGAACAAGCATT	3077-4145	TD^{ɖ}	1068
leuA_4145R	GCCTCTAAATCATAATCAAACACTTG			
leuA_4027F	TTCACGATCAGGAAGAGCTG	4027-6087	TD^{ɖ}	2060
leuC_6087R	TGGGAATCACCACACACAAT			
leuB_5069F	CAAGAGGGCGCGTAACTAAA	5088-6087	TD^{ɖ}	999
leuC_6087R	TGGGAATCACCACACACAAT			
leuC_5957F	CATCAGGTTCAATGGCAAAA	5957-6918	TD^{ȸ}	961
leuC_6918R	CAACCAGGTAAACGCCATTC			
leuC_6856F	GCTATTGTTGTACCAGGTTCAGG	6856-147	TD^{ȸ}	1059
spacer_147R	TGAATTAAACTTTTAAATGCATGTTGT			
leuB_5236F	TGGAGAGAAGTGGTTGAAGAGG	1536-6087	TD^{ɖ}	834
leuC_6087R	TGGGAATCACCACACACAAT			
leuA_4027F	TTCACGATCAGGAAGAGCTG	4046-5169	TD^{ɖ}	1123
leuB_5461R	AACCCCCTGCTGGTTCATA			
leuC_6570F	TTACCTCAAGTTACTTGGGG		TD^{ȸ}	1326
spacer_147R	TGAATTAAACTTTTAAATGCATGTTGT	6589-147		
leuA_3876F	TGCCTATACCTGCTAATAAAGC	3897-5158	TD^{ȸ}	1261
leuB_5158R	GAGCGCGCTAATTTAAAAGC			
repA2_2354F	ATGAACATCGTGCGTGTGC	2372-3289	TD^{ȸ}	917
leuA_3289R	GAAGACATTGCATCACCTGCT			

***Sequencing:* ptrpEG**				
ptrpE_3965F	CCCCTCCTTGATCTCCTACA	7179-1651	TD[φ]	3506
trpE_1674R	CTGAAAATGAGGGAATTCTAAACG			
ptrpE_2260F	TTTGGTGCTTCACCAGAAAG	8115-61	TD[φ]	980
ptrpG_3273R	CTCCAAACCGTCATGATTGA			
ptrpG_3194F	AAGCTTATGGTGGC	14-676	TD[φ]	662
spacer_695R	TCGTTTGGCGACTCATCATA			
spacer_620F	GATCCTGCGCACTCTCAATAG	20-1651	TD[φ]	1631
trpE_1674R	CTGAAAATGAGGGAATTCTAAACG			
trpE_1542F	TCACTCGGTACAACTAACAGCA	1563-2338	TD[ɖ]	775
ptrpE_2357R	ATGTTCCTCCCTCTGGGTCT			

*All primers were designed from Genbank sequences (www.ncbi.nlm.nih.gov) using *Primer3* (Rozen & Skaletsky 2000) and on sequences generated in this study. Total DNA (25 ng) in 25 µL reaction volumes was used for all PCRs, with 0.5 U ExSel High Fidelity DNA polymerase, 1 × reaction buffer with $MgSO_4$ (Southern Cross Biotechnology), 0.4 µM of each primer and 100 µM of each dNTP. GeneAmp 9700 thermocyclers (Applied Biosystems) were used with cycling conditions preceded by an initial denaturation step of 95 ºC for 3 min and followed by a final elongation step of 72 ºC for 10 min. 30 Cycles consisted of 30 sec at 94 ºC, 30 sec at primer annealing temperature and 1 min/1 Kb extension period at 72 ºC. These conditions were standard for all PCR reactions unless otherwise stated. For touchdown PCR: the number of cycles depended on the touchdown range with a cycle increase for each degree change. Normal PCR cycling followed at the lower touchdown temperature with extension times dependent on fragment length (1 min/kb) and a final elongation step at 72 ºC for 10 min. Large fragments were first amplified form the *ptrpEG* plasmid before internal primers were used for sequencing. This was done due to the high homology between the functional and pseudogenes residing on the plasmid. [#] Sequencing primer (Muyzer *et al.* 1993); [§] pA8f-GC contained a 5' 40mer GC-clamp (Fjellbirkeland *et al.* 2001); [ɖ] TD - Touchdown 60-55 ºC (Don *et al.* 1991); [ȸ] TD - Touchdown 55-50 ºC (Don *et al.* 1991); [φ] TD -Touchdown 60-50 ºC (Don *et al.* 1991).

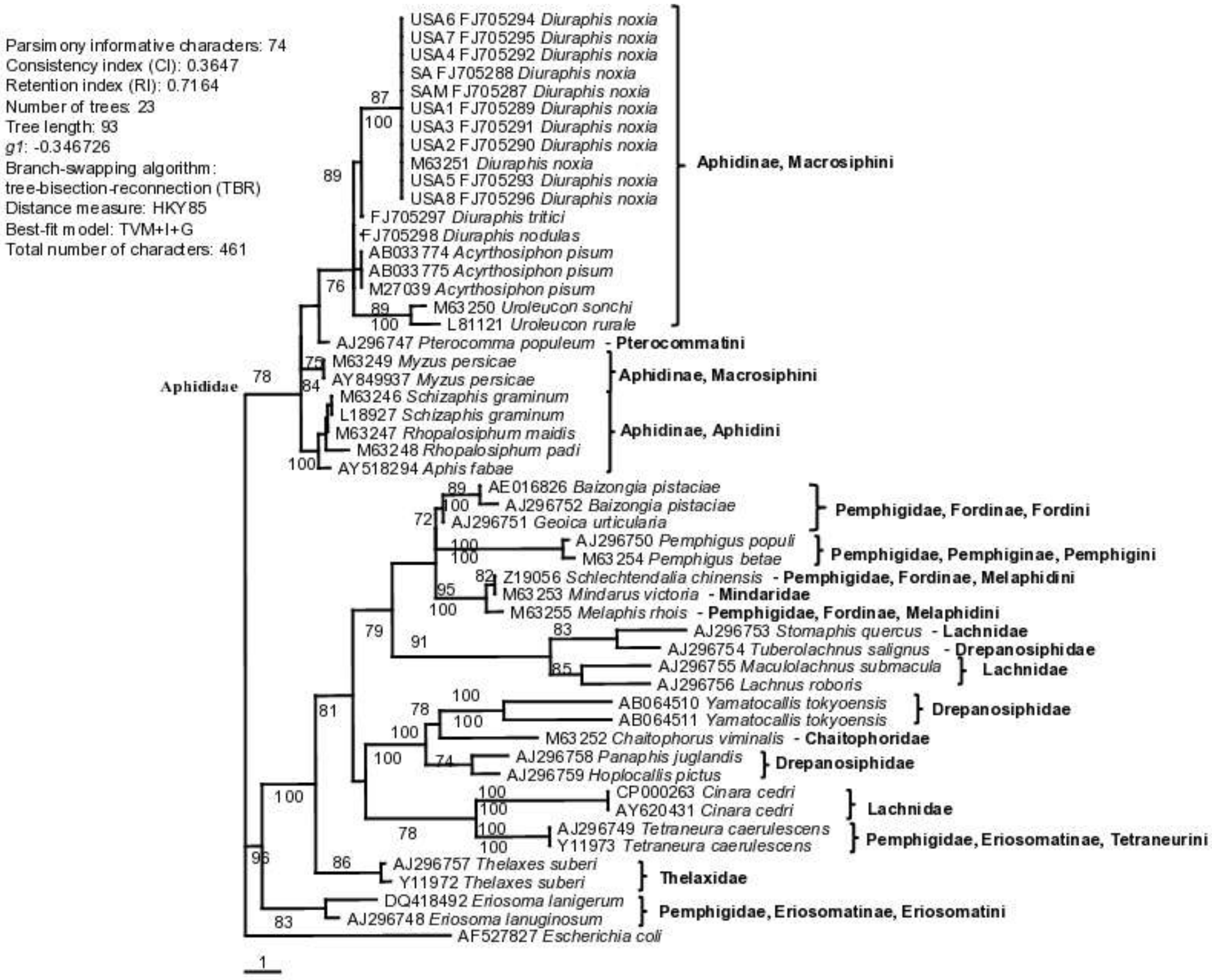

Figure Appx 3.1 Phylogeny of the biotypes to their *16S rDNA* DGGE BLAST results (Genbank). Parsimony topology and branch lengths are shown. Bootstrap percentages from parsimony (70 % and greater, 1000 bootstrap replications) are shown above and Bayesian posterior probability values below branches. The third heuristic tree (out of 23) is presented here. The phylogram had a tree length of 93 and was based on 74 parsimony informative characters with a consistency index of 0.3647 and a retention index of 0.7164. *Escherichia coli* was used as outgroup to root the tree. Branch labels show the Genbank accession, followed by the aphid species name except for the outgroup. Tribes and families for the hosts obtained from Genbank accessions were indicated for the Aphididae. All phylogenetic analyses were done with PAUP 4.0b10 (Swofford 2002) and Bayesian analysis with MrBayes 3.1.1 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). Multiple sequence alignments were done with ClustalW version 2 (Larkin *et al.* 2007) and manually evaluated before further analysis. Ambiguous characters and uninformative nucleotides were excluded from data prior to analysis and all characters were re-weighted to the consistency index. The best statistical model of DNA evolution for each dataset was determined using modeltest 3.7 (Posada & Crandall 1998) and used in subsequent analysis. Heuristic searches using random sequence additions were performed with the tree-bisection-reconnection (TBR) branch-swapping algorithm and MaxTrees set to auto increase. Phylogenetic signal, *i.e.* consistency index (CI) and retention index (RI), was assessed by evaluating the tree length distributions in each dataset after 100 random generated trees. Only groups with a 70 % or more support were retained in bootstrap analyses over a 1000 replicates. MrBayes utilizes a Metropolis-coupled Markov Chain Monte Carlo search algorithm (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). The general time reversal (GTR) evolutionary model was selected with codon site-specific rate variation. One million generations were run for each dataset, using one cold and 3 heated chains. Bayesian inference posterior probabilities were calculated after the appropriate burn in was determined.

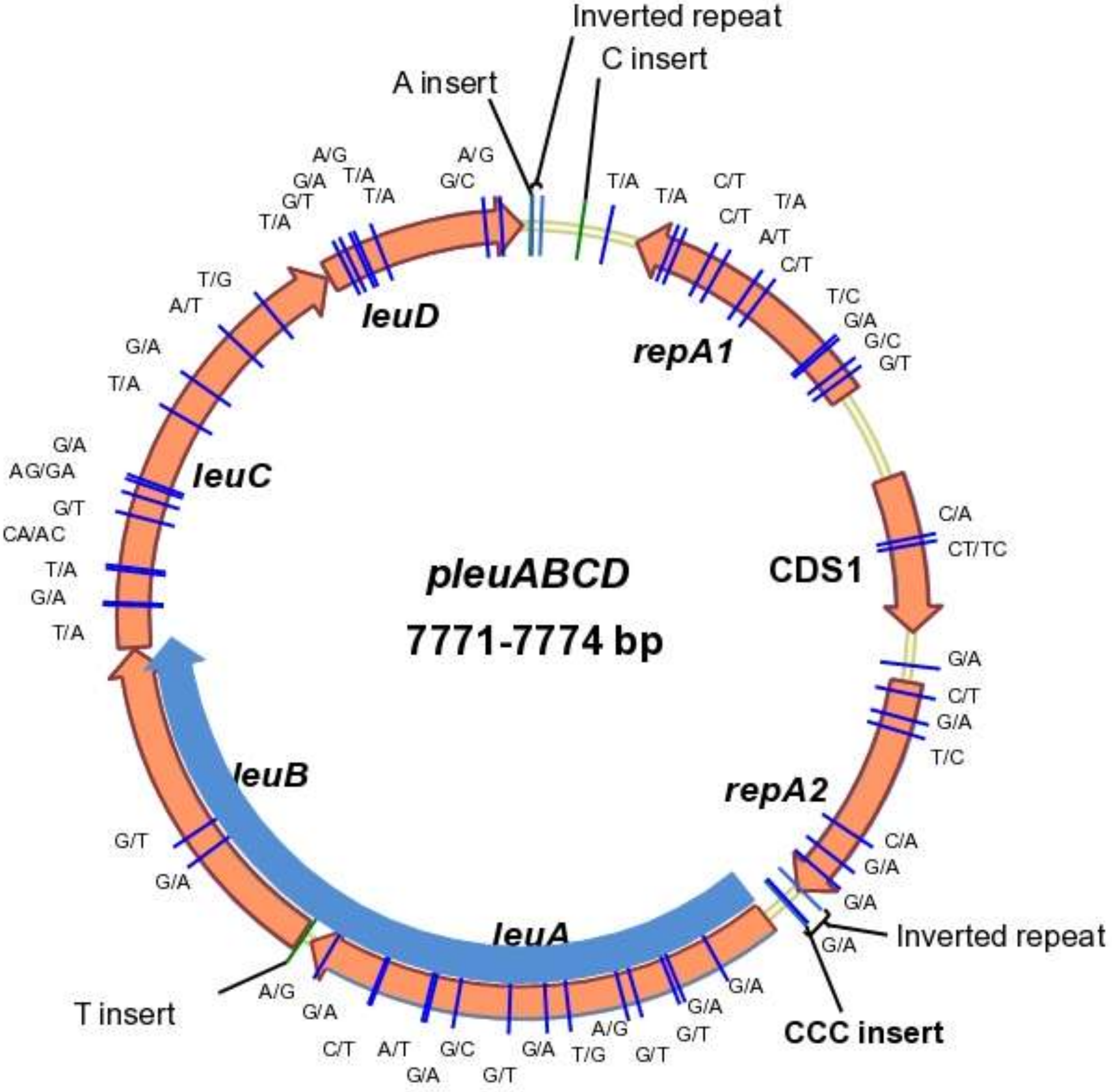

Figure Appx 3.2 Differences between the biotypes (FJ705299-FJ705308) and the Genbank sequences (AF041837, NC001911) for the *pleuABCD* plasmid. The CCC-insert only occurred in the biotypes SA, SAM, USA3 and USA7. A single nucleotide insert, a T-insert, between *leuA* and *leuB* genes, changed *leuB* into the same ORF as the *leuA* gene. The new *leuA*-*leuB* ORF is indicated in blue. The mutations notation used here indicate the nucleotide of the original Genbank sequences, a slash, followed by the changed nucleotide from sequencing results.

Sequences submitted to Genbank

FJ705277 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUHtrpBDnSAM tryptophan synthase large subunit (trpB) gene, partial cds.
ctatcctactattgttcgagaatttcaaaaaattattggagaagaaactaaacaacaaattttagaacaagaaacaaaattaccaaatgcaataatcg
catgtgttggaggaggttctaatgcaattggtattttttcaaattttattaatgataaagaagtaagtttaattggtgttgaacccggggggaagggtat
aaaaacaggtcaacatggtgcaccattaaaacatggtagaactggtatttttttcggaatgaaatctcatttaatgcaagatcaagaaggtcaaattc
aagaatcttggtctatttctgcaggattagactttccatctgttgg

FJ705278 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUHtrpBDnSA tryptophan synthase large subunit (trpB) gene, partial cds.
ctatcctactattgttcgagaatttcaaaaaattattggagaagaaactaaacaacaaattttagaacaagaaacaaaattaccaaatgcaataatcg
catgtgttggaggaggttctaatgcaattggtattttttcaaattttattaatgataaagaagtaagtttaattggtgttgaacccggggggaagggtat
aaaaacaggtcaacatggtgcaccattaaaacatggtagaactggtatttttttcggaatgaaatctcatttaatgcaagatcaagaaggtcaaattc
aagaatcttggtctatttctgcaggattagactttccatctgttgg

FJ705279 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUHtrpBDnUSA1 tryptophan synthase large subunit (trpB) gene, partial cds.
ctatcctactattgttcgagaatttcaaaaaattattggagaagaaactaaacaacaaattttagaacaagaaacaaaattaccaaatgcaataatcg
catgtgttggaggaggttctaatgcaattggtattttttcaaattttattaatgataaagaagtaagtttaattggtgttgaacccggggggaagggtat
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FJ705280 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUHtrpBDnUSA2 tryptophan synthase large subunit (trpB) gene, partial cds.
ctatcctactattgttcgagaatttcaaaaaattattggagaagaaactaaacaacaaattttagaacaagaaacaaaattaccaaatgcaataatcg
catgtgttggaggaggttctaatgcaattggtattttttcaaattttattaatgataaagaagtaagtttaattggtgttgaacccggggggaagggtat
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aagaatcttggtctatttctgcaggattagactttccatctgttgg

FJ705281 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUHtrpBDnUSA3 tryptophan synthase large subunit (trpB) gene, partial cds.
ctatcctactattgttcgagaatttcaaaaaattattggagaagaaactaaacaacaaattttagaacaagaaacaaaattaccaaatgcaataatcg
catgtgttggaggaggttctaatgcaattggtattttttcaaattttattaatgataaagaagtaagtttaattggtgttgaacccggggggaagggtat
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FJ705282 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUHtrpBDnUSA4 tryptophan synthase large subunit (trpB) gene, partial cds.
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catgtgttggaggaggttctaatgcaattggtattttttcaaattttattaatgataaagaagtaagtttaattggtgttgaacccggggggaagggtat

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FJ705283 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUHtrpBDnUSA5 tryptophan synthase large subunit (trpB) gene, partial cds.
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FJ705284 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUHtrpBDnUSA6 tryptophan synthase large subunit (trpB) gene, partial cds.
ctatcctactattgttcgagaatttcaaaaaattattggagaagaaactaaacaacaaattttagaacaagaaacaaaattaccaaatgcaataatcgcatgtgttggaggaggttctaatgcaattggtattttttcaaattttattaatgataaagaagtaagtttaattggtgttgaacccggggggaagggtataaaaacaggtcaacatggtgcaccattaaaacatggtagaactggtatttttttcggaatgaaatctcatttaatgcaagatcaagaaggtcaaattcaagaatcttggtctatttctgcaggattagactttccatctgttgg

FJ705285 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUHtrpBDnUSA7 tryptophan synthase large subunit (trpB) gene, partial cds.
ctatcctactattgttcgagaatttcaaaaaattattggagaagaaactaaacaacaaattttagaacaagaaacaaaattaccaaatgcaataatcgcatgtgttggaggaggttctaatgcaattggtattttttcaaattttattaatgataaagaagtaagtttaattggtgttgaacccggggggaagggtataaaaacaggtcaacatggtgcaccattaaaacatggtagaactggtatttttttcggaatgaaatctcatttaatgcaagatcaagaaggtcaaattcaagaatcttggtctatttctgcaggattagactttccatctgttgg

FJ705286 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUHtrpBDnUSA8 tryptophan synthase large subunit (trpB) gene, partial cds.
ctatcctactattgttcgagaatttcaaaaaattattggagaagaaactaaacaacaaattttagaacaagaaacaaaattaccaaatgcaataatcgcatgtgttggaggaggttctaatgcaattggtattttttcaaattttattaatgataaagaagtaagtttaattggtgttgaacccggggggaagggtataaaaacaggtcaacatggtgcaccattaaaacatggtagaactggtatttttttcggaatgaaatctcatttaatgcaagatcaagaaggtcaaattcaagaatcttggtctatttctgcaggattagactttccatctgttgg

FJ705287 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUH16SDnSAM 16S ribosomal RNA gene, partial sequence.
attgaacgctggcggcaagcctaacacatgcaagtcgagcggcagcgaaagaaagcttgctttcttgtcggcgagcggcaaacgggtgagtaatatctggggatctacccaaaagagggggataactactagaaatggtagctaataccgcataatgttgaaaaaccaaagtgggggacctttttggcctcatgcttatggatgaacccagacgagattagcttgttggtagagtaatagcctaccaaggcgacgatctctagctggtctgagaggataaccagccacactggaactgagacacggtccagactcctacgggaggcagcagtggggaatattgcacaatgggcgaaagcctgatgcagctatgccgcgtgtatgaagaaggccttagggttgtaaagtactttcagcggggaagaaaaaaataaaactaataatttattcctgacgtacc

FJ705288 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUH16SDnSA 16S ribosomal RNA gene, partial sequence.

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FJ705289 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUH16SDnUSA1 16S ribosomal RNA gene, partial sequence.
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FJ705290 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUH16SDnUSA2 16S ribosomal RNA gene, partial sequence.
attgaacgctggcggcaagcctaacacatgcaagtcgagcggcagcgaaagaaagcttgctttcttgtcggcgagcggcaaacgggtgagtaa
tatctggggatctacccaaaagagggggataactactagaaatggtagctaataccgcataatgttgaaaaaccaaagtgggggacctttttggcc
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FJ705291 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUH16SDnUSA3 16S ribosomal RNA gene, partial sequence.
attgaacgctggcggcaagcctaacacatgcaagtcgagcggcagcgaaagaaagcttgctttcttgtcggcgagcggcaaacgggtgagtaa
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FJ705292 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUH16SDnUSA4 16S ribosomal RNA gene, partial sequence.
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FJ705293 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUH16SDnUSA5 16S ribosomal RNA gene, partial sequence.
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FJ705294 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUH16SDnUSA6 16S ribosomal RNA gene, partial sequence.
attgaacgctggcggcaagcctaacacatgcaagtcgagcggcagcgaaagaaagcttgctttcttgtcggcgagcggcaaacgggtgagtaa
tatctggggatctacccaaaagagggggataactactagaaatggtagctaataccgcataatgttgaaaaaccaaagtgggggaccttttggcc
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FJ705295 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUH16SDnUSA7 16S ribosomal RNA gene, partial sequence.
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FJ705296 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUH16SDnUSA8 16S ribosomal RNA gene, partial sequence.
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FJ705297 *Buchnera aphidicola* (*Diuraphis tritici*) clone BUH16SDtri 16S ribosomal RNA gene, partial sequence.
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FJ705298 *Buchnera aphidicola* (*Diuraphis nodulas*) clone BUH16SDnod 16S ribosomal RNA gene, partial sequence.
cctggctcagattgaacgctggcggcaagcctaacacatgcaagtcgagcggcagcgaaagaaagcttgctttcttgtcggcgagcggcaaac
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FJ705299 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pLeu-Dn(SA), complete sequence.
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FJ705300 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pLeu-Dn(SAM), complete sequence.
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FJ705301 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pLeu-Dn(USA1), complete sequence.

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FJ705302 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pLeu-Dn(USA2), complete sequence.

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FJ705303 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pLeu-Dn(USA3), complete sequence.

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FJ705304 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pLeu-Dn(USA4), complete sequence.
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FJ705306 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pLeu-Dn(USA6), complete sequence.

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FJ705307 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pLeu-Dn(USA7), complete sequence.

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FJ705308 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pLeu-Dn(USA8), complete sequence.

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FJ705309 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pTrpEG clone BUHpTrpEGDnSAM anthranilate synthase component II (ptrpG) pseudogene, partial sequence; and anthranilate synthase component I (trpE) gene, partial cds.

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aaagagggagagaaatacaaaaaaaacttgaagcatctttaaactctattcctgtaaggcaagaagtaaaaaatagtatgttaactgcaaatatga
gtgacgaacaatattgttccataataaaaaaattacaaattttaattcgaaaaggtgagatttttcaagttgtaccatctcgaaaatttttttaccctgttc
taatcctttatctgcctatcaaaaattaaaaaaaagcaatcctagtccttatatgttttttatgcaagataaagattttaccttatttggtgcttcaccagaa
agttctttaaaatatgatgacaca

FJ705310 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pTrpEG clone BUHpTrpEGDnSA anthranilate synthase component II (ptrpG) pseudogene, partial sequence; and anthranilate synthase component I (trpE) gene, partial cds.

attgaatatgcaggtgagatattccatggaaaagcctctttaatcaatcatgacggtttggagatgtttaaagatatccctcagccattacctgttgcta
gatatcattcactagtggcatgtaaagatattcctaataatttgattattaattctttttttaaaaaaaccattatgtctatacgaaataataaagatcgagt
gtgtggttttcagtttcatcctgaatctattttaactacatgcggtgatcaaatattaaaacaaataatttattgggcttcgttgcaatatgttatgaagtaa
ccgcaatatattaaatacagtttttattatattgtgatatggttttaattcagcaattttttttaacacccctccgtttgcatgtttgtatatatttctatcaaattgt
tataataactatatatttatctgtatatgttgtgttacttatcagtcatcatttattcttatcatgcatattcataataaaataattttattgttaaaagatatgata
tattgaaaacaaatcaattcacatgtgctcattttattagttgatatttgcatatatcatgatcctgcgcactctcaatagagtggtgatataaagacatg
gcatatatctaggtaatatgaaagaaaatagattatatgatgagtcgccaaacgattaataattttagatcatcattcatataaaatagaagtgcagca
cttataccccatataaaaatttttgttatagactgtttgtcaaatattgttatcatcataatgatacatcaaacaacataaatacaaaatgaattttatatct
caagttatacacagaaattgtggataactttttaaaaatattttttttattataaaaaaaaaaaatgtaacactatgttttaattatatttattctttattttaccat
gaatataatactgttattaacatgtttataagtctggggataagtttggtataatatgcaatgcccctccccacaatataaaaaattagaaaatttgaaat
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tttttttggtttataaaaaaattattagtatcattatatttaacatagtgtgttatttacttatatatattgtattttttctgaaaattatcaaaaatatcgtatgacc
atattgctttaattttgtaaattagttattatttcttctttataaaaatattcttttgacccccctaagagatttgaattatatggaaaaaaagccatacgagatc
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gaaattaacaaaaaaaatgatctagaaagtattatgatcatcgatgctgcgctacgaatttcttctgaaagaaatcactcggtacaactaacagcatt
atctaaaaatggtgaaaatattttatcaattttaaaaagcaatctgaaacaaaaagttcaaatgttcatacaagatacatctattcgtttagaattccctca
ttttcagaaaaatttagatgaagacaaaaaaatttttcattgtctatatttgacacttttagatttattatgaaatttttcaaaaatcgcaataaagtacaga
aagcaatgtttttggtggactattctcctatgatttaatttctaattttgagttattaccaaaattaaaaaaaacacaaaaatgccctcactttttgtttttattt
agcagaaacattactgattgtagatcatcaaaaaaaaacatgtttaattcaaaatagtttatttacgaaaaattcccacgaacagatgagagtagaaa

aaagagggagagaaatacaaaaaaaacttgaagcatctttaaactctattcctgtaaggcaagaagtaaaaaatagtatgttaactgcaaatatga
gtgacgaacaatattgttccataataaaaaaattacaaattttaattcgaaaaggtgagatttttcaagttgtaccatctcgaaaattttttttaccctgttc
taatcctttatctgcctatcaaaaattaaaaaaaagcaatcctagtccttatatgtttttatgcaagataaagattttaccttatttggtgcttcaccagaa
agttctttaaaatatgatgacacaacaagacaagtagaattatatccgattgctgg

FJ705311 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pTrpEG clone BUHpTrpEGDnUSA1 anthranilate synthase component II (ptrpG) pseudogene, partial sequence; and anthranilate synthase component I (trpE) gene, partial cds.

gattgaatatgcaggtgagatattccatggaaaagcctctttaatcaatcatgacggtttggagatgtttaaagatatccctcagccattacctgttgct
agatatcattcactagtggcatgtaaagatattcctaataatttgattattaattctttttttaaaaaaaccattatgtctatacgaaataataaagatcgagt
gtgtggttttcagtttcatcctgaatctattttaactacatgcggtgatcaaatattaaaacaaataatttattgggcttcgttgcaatatgttatgaagtaa
ccgcaatatattaaatacagtttttattatattgtgatatggttttaattcagcaatttttttaacacccctccgtttgcatgtttgtatatatttctatcaaattgt
tataataactatatatttatctgtatatgttgtgttacttatcagtcatcatttattcttatcatgcatattcataataaaataattttattgttaaaagatatgata
tattgaaaacaaatcaattcacatgtgctcattttattagttgatatttgcatatatcatgatcctgcgcactctcaatagagtggtgatataaagacatg
gcatatatctaggtaatatgaaagaaaatagattatatgatgagtcgccaaacgattaataattttagatcatcattcatataaaatagaagtgcagca
cttataccccatataaaaatttttgttatagactgtttgtcaaatattgttatcatcataatgatacatcaaacaacataaatacaaaatgaattttatatct
caagttatacacagaaattgtggataactttttaaaaatattttttttattataaaaaaaaaaaatgtaacactatgttttaattatatttattctttattttaccat
gaatataatactgttattaacatgtttataagtctggggataagtttggtataatatgcaatgcccctccccacaatataaaaaattagaaaatttgaaat
aaaaaatattaaagacaattcaataatctcaaaagataaaatccttaaaagatttaaaaaaaaaagaaatatatgtatataatatttattgttattgttattt
tttttttggtttataaaaaaattattagtatcattatatttaacatagtgtgttatttacttatatatattgtattttttctgaaaattatcaaaaatatcgtatgacc
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aaaatcattcaaaaaaaagcaaagtatcatcctgacccaacaatagtatttaatcatatttgtggatctcaaaaacaaacattgttactagaaacagca
gaaattaacaaaaaaaatgatctagaaagtattatgatcatcgatgctgcgctacgaatttcttctgaaagaaatcactcggtacaactaacagcatt
atctaaaaatggtgaaaatattttatcaattttaaaaagcaatctgaaacaaaaagttcaaatgttcatacaagatacatctattcgtttagaattccctca
ttttcagaaaaatttagatgaagacaaaaaaatttttcattgtctatatttgacacttttagatttattatgaaatttttcaaaaatcgcaataaagtacaga
aagcaatgtttttggtggactattctcctatgatttaatttctaattttgagttattaccaaaattaaaaaaaacacaaaaatgccctcacttttgtttttattt
agcagaaacattactgattgtagatcatcaaaaaaaaacatgtttaattcaaaatagtttatttacgaaaaattcccacgaacagatgagagtagaaa
aaagagggagagaaatacaaaaaaaacttgaagcatctttaaactctattcctgtaaggcaagaagtaaaaaatagtatgttaactgcaaatatga
gtgacgaacaatattgttccataataaaaaaattacaaattttaattcgaaaaggtgagatttttcaagttgtaccatctcgaaaattttttttaccctgttc
taatcctttatctgcctatcaaaaattaaaaaaaagcaatcctagtccttatatgtttttatgcaagataaagattttaccttatttggtgcttcaccagaa
agttctttaaaatatgatgacacaacaagacaagtag

FJ705312 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pTrpEG clone BUHpTrpEGDnUSA2 anthranilate synthase component II (ptrpG) pseudogene, partial sequence; anthranilate synthase component I (trpE) gene, complete cds; and anthranilate synthase component II (trpG) gene, partial cds.

gattgaatatgcaggtgagatattccatggaaaagcctctttaatcaatcatgacggtttggagatgtttaaagatatccctcagccattacctgttgct
agatatcattcactagtggcatgtaaagatattcctaataatttgattattaattctttttttaaaaaaaccattatgtctatacgaaataataaagatcgagt
gtgtggttttcagtttcatcctgaatctattttaactacatgcggtgatcaaatattaaaacaaataatttattgggcttcgttgcaatatgttatgaagtaa
ccgcaatatattaaatacagtttttattatattgtgatatggttttaattcagcaatttttttaacacccctccgtttgcatgtttgtatatatttctatcaaattgt
tataataactatatatttatctgtatatgttgtgttacttatcagtcatcatttattcttatcatgcatattcataataaaataattttattgttaaaagatatgata
tattgaaaacaaatcaattcacatgtgctcattttattagttgatatttgcatatatcatgatcctgcgcactctcaatagagtggtgatataaagacatg

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caagttatacacagaaattgtggataactttttaaaaatattttttttattataaaaaaaaaaaatgtaacactatgttttaattatatttattctttattttaccat
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gaaattaacaaaaaaaatgatctagaaagtattatgatcatcgatgctgcgctacgaatttcttctgaaagaaatcactcggtacaactaacagcatt
atctaaaaatggtgaaaatattttatcaattttaaaaagcaatctgaaacaaaaagttcaaatgttcatacaagatacatctattcgtttagaattccctca
ttttcagaaaaatttagatgaagacaaaaaaatttttcattgtctatatttgacacttttagatttattatgaaatttttcaaaaatcgcaataaagtacaga
aagcaatgttttttggtggactattctcctatgatttaatttctaattttgagttattaccaaaattaaaaaaaacacaaaaatgccctcacttttgtttttattt
agcagaaacattactgattgtagatcatcaaaaaaaaacatgtttaattcaaaatagtttatttacgaaaaattcccacgaacagatgagagtagaaa
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gtgacgaacaatattgttccataataaaaaaattacaaattttaattcgaaaaggtgagatttttcaagttgtaccatctcgaaaatttttttaccctgttc
taatcctttatctgcctatcaaaaattaaaaaaaagcaatcctagtccttatatgttttttatgcaagataaagattttaccttatttggtgcttcaccagaa
agttctttaaaatatgatgacacaacaagacaagtagaattatatccgattgctggcactagacccagagggaggaacatggatggcacattaaat
ctagatttggacagtcgaatcgaacttgaaatgagaaccaatcataaggaactcgccgaacatttaatgttagtagatttagctcgtaacgatttagc
acggatctgtgaaccgggatcaagatatgtctcggatttggttagagtcgacaaatatcctcatgtcatgcatttagtctctagagtcgtaggaacgtt
aaaaccagagttagatgcattacatgcttatgcagcttgcatgaatatgggtacattaactggtgctcctaaaattcgcgctatggaattgattgctga
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agaggacaatattgcaaccattcaatcaggatcaggtattgtttataattccatacccgaagacgaagtcaaagaaggtatcaataaagcgaaacc
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cattttaatgttatctccagggccgagtatcccccaatatgctggatgtatgttacccttgataaaaaaagtgaaaggttacctacctataataggtattt
gtttaggtcatcaggcgatagtagaagcttatggtggc

FJ705313 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pTrpEG clone BUHpTrpEGDnUSA3 anthranilate synthase component II (ptrpG) pseudogene, partial sequence; and anthranilate synthase component I (trpE) gene, partial cds.

gattgaatatgcaggtgagatattccatggaaaagcctctttaatcaatcatgacggtttggagatgtttaaagatatccctcagccattacctgttgct
agatatcattcactagtggcatgtaaagatattcctaataatttgattattaattcttttttaaaaaaaccattatgtctatacgaaataataaagatcgagt
gtgtggttttcagtttcatcctgaatctattttaactacatgcggtgatcaaatattaaaacaaataatttattgggcttcgttgcaatatgttatgaagtaa
ccgcaatatattaaatacagtttttattatattgtgatatggttttaattcagcaattttttaacacccctccgtttgcatgtttgtatatatttctatcaaattgt
tataataactatatatttatctgtatatgttgtgttacttatcagtcatcatttattcttatcatgcatattcataataaaataattttattgttaaaagatatgata
tattgaaaacaaatcaattcacatgtgctcattttattagttgatatttgcatatatcatgatcctgcgcactctcaatagagtggtgatataaagacatg
gcatatatctaggtaatatgaaagaaaatagattatatgatgagtcgccaaacgattaataattttagatcatcattcatataaaatagaagtgcagca
cttataccccatataaaaatttttgttatagactgtttgtcaaatattgttatcatcataatgatacatcaaacaacataaatacaaaatgaattttatatct
caagttatacacagaaattgtggataactttttaaaaatattttttttattataaaaaaaaaaaatgtaacactatgttttaattatatttattctttattttaccat
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aaaaaatattaaagacaattcaataatctcaaaagataaaatccttaaaagatttaaaaaaaaaagaaatatatgtatataatatttattgttattgttattt
tttttggtttataaaaaaattattagtatcattatatttaacatagtgtgttatttacttatatatattgtattttttctgaaaattatcaaaaatatcgtatgacc

atattgctttaattttgtaaattagttattatttcttctttataaaaatattcttttgacccccctaagagatttgaattatatggaaaaaaagccatacgagatc
aaaatcattcaaaaaaaagcaaagtatcatcctgacccaacaatagtatttaatcatatttgtggatctcaaaaacaaacattgttactagaaacagca
gaaattaacaaaaaaaatgatctagaaagtattatgatcatcgatgctgcgctacgaatttcttctgaaagaaatcactcggtacaactaacagcatt
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ttttcagaaaaatttagatgaagacaaaaaaatttttttcattgtctatatttgacacttttagatttattatgaaatttttcaaaaatcgcaataaagtacaga
aagcaatgtttttttggtggactattctcctatgatttaatttctaattttgagttattaccaaaattaaaaaaaacacaaaaatgccctcacttttgtttttattt
agcagaaacattactgattgtagatcatcaaaaaaaaacatgtttaattcaaaatagtttatttacgaaaaattcccacgaacagatgagagtagaaa
aaagagggagagaaatacaaaaaaaacttgaagcatctttaaactctattcctgtaaggcaagaagtaaaaaatagtatgttaactgcaaatatga
gtgacgaacaatattgttccataataaaaaaattacaaattttaattcgaaaaggtgagatttttcaagttgtaccatctcgaaaatttttttttaccctgttc
taatcctttatctgcctatcaaaaattaaaaaaaagcaatcctagtccttatatgtttttttatgcaagataaagattttaccttatttggtgcttcaccagaa
agttctttaaaatatgatgacacaacaagacaagtagaa

FJ705314 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pTrpEG clone BUHpTrpEGDnUSA4 anthranilate synthase component II (ptrpG) pseudogene, partial sequence; and anthranilate synthase component I (trpE) gene, partial cds.

gattgaatatgcaggtgagatattccatggaaaagcctctttaatcaatcatgacggtttggagatgtttaaagatatccctcagccattacctgttgct
agatatcattcactagtggcatgtaaagatattcctaataatttgattattaattctttttttaaaaaaaccattatgtctatacgaaataataaagatcgagt
gtgtggttttcagtttcatcctgaatctattttaactacatgcggtgatcaaatattaaaacaaataatttattgggcttcgttgcaatatgttatgaagtaa
ccgcaatatattaaatacagtttttattatattgtgatatggttttaattcagcaatttttttaacacccctccgtttgcatgtttgtatatatttctatcaaattgt
tataataactatatatttatctgtatatgttgtgttacttatcagtcatcatttattcttatcatgcatattcataataaaataattttattgttaaaagatatgata
tattgaaaacaaatcaattcacatgtgctcattttattagttgatatttgcatatatcatgatcctgcgcactctcaatagagtggtgatataaagacatg
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cttataccccatataaaaatttttgttatagactgtttgtcaaatattgttatcatcataatgatacatcaaacaacataaatacaaaatgaattttatatct
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gaatataatactgttattaacatgtttataagtctggggataagtttggtataatatgcaatgccccctccccacaatataaaaaattagaaaatttgaaat
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ttttttggtttataaaaaaattattagtatcattatatttaacatagtgtgttatttacttatatatattgtattttttctgaaaattatcaaaaatatcgtatgacc
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gaaattaacaaaaaaaatgatctagaaagtattatgatcatcgatgctgcgctacgaatttcttctgaaagaaatcactcggtacaactaacagcatt
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agttctttaaaatatgatgacacaacaagacaagtagaattatatccgattgctgg

FJ705315 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pTrpEG clone BUHpTrpEGDnUSA5 anthranilate synthase component II (ptrpG) pseudogene, partial sequence; and anthranilate synthase component I (trpE) gene, partial cds.

tgaatatgcaggtgagatattccatggaaaagcctctttaatcaatcatgacggtttggagatgtttaaagatatccctcagccattacctgttgctag
atatcattcactagtggcatgtaaagatattcctaataatttgattattaattctttttttaaaaaaaccattatgtctatacgaaataataaagatcgagtgt
gtggttttcagtttcatcctgaatctattttaactacatgcggtgatcaaatattaaaacaaataatttattgggcttcgttgcaatatgttatgaagtaacc
gcaatatattaaatacagtttttattatattgtgatatggttttaattcagcaattttttaacacccctccgtttgcatgtttgtatatatttctatcaaattgtta
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ttgaaaacaaatcaattcacatgtgctcattttattagttgatatttgcatatatcatgatcctgcgcactctcaatagagtggtgatataaagacatggc
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ctttaaaatatgatgacacaacaagacaagtagaat

FJ705316 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pTrpEG clone BUHpTrpEGDnUSA6 anthranilate synthase component II (ptrpG) pseudogene, partial sequence; and anthranilate synthase component I (trpE) gene, partial cds.

tgaatatgcaggtgagatattccatggaaaagcctctttaatcaatcatgacggtttggagatgtttaaagatatccctcagccattacctgttgctag
atatcattcactagtggcatgtaaagatattcctaataatttgattattaattctttttttaaaaaaaccattatgtctatacgaaataataaagatcgagtgt
gtggttttcagtttcatcctgaatctattttaactacatgcggtgatcaaatattaaaacaaataatttattgggcttcgttgcaatatgttatgaagtaacc
gcaatatattaaatacagtttttattatattgtgatatggttttaattcagcaattttttaacacccctccgtttgcatgtttgtatatatttctatcaaattgtta
taataactatatatttatctgtatatgttgtgttacttatcagtcatcatttattcttatcatgcatattcataataaaataattttattgttaaaagatatgatata
ttgaaaacaaatcaattcacatgtgctcattttattagttgatatttgcatatatcatgatcctgcgcactctcaatagagtggtgatataaagacatggc
atatatctaggtaatatgaaagaaaatagattatatgatgagtcgccaaacgattaataattttagatcatcattcatataaaatagaagtgcagcactt
ataccccccatataaaaatttttgttatagactgtttgtcaaatattgttatcatcataatgatacatcaaacaacataaatacaaaatgaattttatatctca
agttatacacagaaattgtggataactttttaaaaatattttttttattataaaaaaaaaaaatgtaacactatgttttaattatatttattctttattttaccatga
atataatactgttattaacatgtttataagtctggggataagtttggtataatatgcaatgcccctccccacaatataaaaaattagaaaatttgaaataa
aaaatattaaagacaattcaataatctcaaaagataaaatccttaaaagatttaaaaaaaaaagaaatatatgtatataatatttattgttattgttattttttt
tttggtttataaaaaaattattagtatcattatatttaacatagtgtgttatttacttatatatattgtattttttctgaaaattatcaaaaatatcgtatgaccata
ttgctttaattttgtaaattagttattatttcttctttataaaaatattcttttgacccccctaagagatttgaattatatggaaaaaaagccatacgagatcaaa
atcattcaaaaaaaagcaaagtatcatcctgacccaacaatagtatttaatcatatttgtggatctcaaaaacaaacattgttactagaaacagcaga
aattaacaaaaaaaatgatctagaaagtattatgatcatcgatgctgcgctacgaatttcttctgaaagaaatcactcggtacaactaacagcattatc

taaaaatggtgaaaatattttatcaattttaaaaagcaatctgaaacaaaaagttcaaatgttcatacaagatacatctattcgtttagaattccctcatttt
cagaaaaatttagatgaagacaaaaaaattttttcattgtctatatttgacacttttagatttattatgaaatttttcaaaaatcgcaataaagtacagaaa
gcaatgtttttggtggactattctcctatgatttaatttctaattttgagttattaccaaaattaaaaaaaacacaaaaatgccctcacttttgtttttatttag
cagaaacattactgattgtagatcatcaaaaaaaaacatgtttaattcaaaatagtttatttacgaaaaattcccacgaacagatgagagtagaaaaa
agagggagagaaatacaaaaaaaacttgaagcatctttaaactctattcctgtaaggcaagaagtaaaaaatagtatgttaactgcaaatatgagtg
acgaacaatattgttccataataaaaaaattacaaattttaattcgaaaaggtgagatttttcaagttgtaccatctcgaaaatttttttaccctgttctaat
cctttatctgcctatcaaaaattaaaaaaaagcaatcctagtccttatatgtttttatgcaagataaagattttaccttatttggtgcttcaccagaaagtt
ctttaaaatatgatgacacaacaagacaagtagaa

FJ705317 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pTrpEG clone BUHpTrpEGDnUSA7 anthranilate synthase component II (ptrpG) pseudogene, partial sequence; and anthranilate synthase component I (trpE) gene, partial cds.
gcaggtgagatattccatggaaaagcctctttaatcaatcatgacggtttggagatgtttaaagatatccctcagccattacctgttgctagatatcatt
cactagtggcatgtaaagatattcctaataatttgattattaattctttttttaaaaaaaccattatgtctatacgaaataataaagatcgagtgtgtggtttt
cagtttcatcctgaatctattttaactacatgcggtgatcaaatattaaaacaaataatttattgggcttcgttgcaatatgttatgaagtaaccgcaatat
attaaatacagtttttattatattgtgatatggttttaattcagcaattttttaacacccctccgtttgcatgtttgtatatatttctatcaaattgttataataact
atatatttatctgtatatgttgtgttacttatcagtcatcatttattcttatcatgcatattcataataaaataattttattgttaaaagatatgatatattgaaaac
aaatcaattcacatgtgctcattttattagttgatatttgcatatatcatgatcctgcgcactctcaatagagtggtgatataaagacatggcatatatcta
ggtaatatgaaagaaaatagattatatgatgagtcgccaaacgattaataattttagatcatcattcatataaaatagaagtgcagcacttatacccccc
atataaaaatttttgttatagactgtttgtcaaatattgttatcatcataatgatacatcaaacaacataaatacaaaatgaattttatatctcaagttataca
cagaaattgtggataactttttaaaaatattttttttattataaaaaaaaaaaatgtaacactatgttttaattatatttattctttattttaccatgaatataatac
tgttattaacatgtttataagtctggggataagtttggtataatatgcaatgcccctccccacaatataaaaaattagaaaatttgaaataaaaaatatta
aagacaattcaataatctcaaaagataaaatccttaaaagatttaaaaaaaaaagaaatatatgtatataatatttattgttattgttattttttttttggtttat
aaaaaaattattagtatcattatatttaacatagtgtgttatttacttatatatattgtatttttttctgaaaattatcaaaaatatcgtatgaccatattgctttaat
tttgtaaattagttattatttcttctttataaaaatattcttttgacccccctaagagatttgaattatatggaaaaaaagccatacgagatcaaaatcattcaa
aaaaaagcaaagtatcatcctgacccaacaatagtatttaatcatatttgtggatctcaaaaacaaacattgttactagaaacagcagaaattaacaa
aaaaaatgatctagaaagtattatgatcatcgatgctgcgctacgaatttcttctgaaagaaatcactcggtacaactaacagcattatctaaaaatgg
tgaaaatattttatcaattttaaaaagcaatctgaaacaaaaagttcaaatgttcatacaagatacatctattcgtttagaattccctcattttcagaaaaa
tttagatgaagacaaaaaaattttttcattgtctatatttgacacttttagatttattatgaaatttttcaaaaatcgcaataaagtacagaaagcaatgttttt
tggtggactattctcctatgatttaatttctaattttgagttattaccaaaattaaaaaaaacacaaaaatgccctcacttttgtttttatttagcagaaacat
tactgattgtagatcatcaaaaaaaaacatgtttaattcaaaatagtttatttacgaaaaattcccacgaacagatgagagtagaaaaaagagggag
agaaatacaaaaaaaacttgaagcatctttaaactctattcctgtaaggcaagaagtaaaaaatagtatgttaactgcaaatatgagtgacgaacaat
attgttccataataaaaaaattacaaattttaattcgaaaaggtgagatttttcaagtt

FJ705318 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pTrpEG clone BUHpTrpEGDnUSA8 anthranilate synthase component II (ptrpG) pseudogene, partial sequence; and anthranilate synthase component I (trpE) gene, partial cds.
tgaatatgcaggtgagatattccatggaaaagcctctttaatcaatcatgacggtttggagatgtttaaagatatccctcagccattacctgttgctag
atatcattcactagtggcatgtaaagatattcctaataatttgattattaattctttttttaaaaaaaccattatgtctatacgaaataataaagatcgagtgt
gtggttttcagtttcatcctgaatctattttaactacatgcggtgatcaaatattaaaacaaataatttattgggcttcgttgcaatatgttatgaagtaacc
gcaatatattaaatacagtttttattatattgtgatatggttttaattcagcaattttttaacacccctccgtttgcatgtttgtatatatttctatcaaattgtta
taataactatatatttatctgtatatgttgtgttacttatcagtcatcatttattcttatcatgcatattcataataaaataattttattgttaaaagatatgatata

ttgaaaacaaatcaattcacatgtgctcattttattagttgatatttgcatatatcatgatcctgcgcactctcaatagagtggtgatataaagacatggc
atatatctaggtaatatgaaagaaaatagattatatgatgagtcgccaaacgattaataattttagatcatcattcatataaaatagaagtgcagcactt
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agttatacacagaaattgtggataactttttaaaaatatttttttattataaaaaaaaaaaatgtaacactatgttttaattatatttattctttattttaccatga
atataatactgttattaacatgtttataagtctggggataagtttggtataatatgcaatgcccctccccacaatataaaaaattagaaaatttgaaataa
aaaatattaaagacaattcaataatctcaaaagataaaatccttaaaagatttaaaaaaaaaagaaatatatgtatataatatttattgttattgttattttttt
tttggtttataaaaaaattattagtatcattatatttaacatagtgtgttatttacttatatatattgtattttttctgaaaattatcaaaaatatcgtatgaccata
ttgctttaattttgtaaattagttattatttcttctttataaaaatattcttttgacccccctaagagatttgaattatatggaaaaaaagccatacgagatcaaa
atcattcaaaaaaaagcaaagtatcatcctgacccaacaatagtatttaatcatatttgtggatctcaaaaacaaacattgttactagaaacagcaga
aattaacaaaaaaaatgatctagaaagtattatgatcatcgatgctgcgctacgaatttcttctgaaagaaatcactcggtacaactaacagcattatc
taaaaatggtgaaaatattttatcaattttaaaaagcaatctgaaacaaaaagttcaaatgttcatacaagatacatctattcgtttagaattccctcatttt
cagaaaaatttagatgaagacaaaaaaatttttcattgtctatatttgacacttttagatttattatgaaatttttcaaaaatcgcaataaagtacagaaa
gcaatgtttttggtggactattctcctatgatttaatttctaattttgagttattaccaaaattaaaaaaaacacaaaaatgccctcacttttgtttttatttag
cagaaacattactgattgtagatcatcaaaaaaaaacatgtttaattcaaaatagtttatttacgaaaaattcccacgaacagatgagagtagaaaaa
agagggagagaaatacaaaaaaaacttgaagcatctttaaactctattcctgtaaggcaagaagtaaaaaatagtatgttaactgcaaatatgagtg
acgaacaatattgttccataataaaaaaattacaaattttaattcgaaaaggtgagatttttcaagttgtaccatctcgaaaattttttttaccctgttctaat
cctttatctgcctatcaaaaattaaaaaaaagcaatcctagtccttatatgtttttatgcaagataaagattttaccttatttggtgcttcaccagaaagtt
ctttaaaatatgatgacacaacaagacaagtagaattatatccg

APPENDIX – CHAPTER 4

Sequences submitted to Genbank

GU145279 5'UTR from mRNA, leader sequence for leuA from *B. aphidicola* (*D. noxia*, SA biotype) plasmid pLeu-Dn(SA), primary endosymbiont.
1tataaatttattttttaataaaacatctataatttatagtgagaaaatttttatgagttctaaagttattatttttgataccaccttacg

GU145280 5'UTR from mRNA, leader sequence for leuA from *B. aphidicola* (*D. noxia*, USA biotype) plasmid pLeu-Dn(USA), primary endosymbiont.
tttattttttaataaaacatctataatttatagtgagaaaatttttatgagttctaaagttattatttttgataccaccttacg

GU145281 repA, leuA and inverted repeat region of *Buchnera aphidicola* plasmid pLeuABCD, primary endosymbiont of *Diuraphis mexicana*, partial.
tcaatcacccgagcttcacgtttaataaatgatttttttagaaccaatgggttttattagatgtaaaaaaataaaaagtaaatctgttagtaattatatacct
aaaaaaatatttttaacaccaatgttttttatgttatttaacatatcacaatcaaaaataaatagttatttatctaagtcaatgaaaatatctcaaaaattaaa
gatttcagaaaaaaaaatatttatttctttttcagatattaaggtaatgtcaaagttagatgaaaaatctgttagaaaaaaaattttaaatgttttaattaatt
attatacagcaagtgaactgacaaaaataggccctaagggcctaaagaaacgaatagatgttgaatataataatttatgtaaattgtttaaaaaaatta
aaaaataaatttatacaataaaaatttcataagacatgaaggtacaaatttttgtaccttccatgtcttatgaaatttttattgtataaatttatttttttaaaaaa
aacattcaaaatctatagtgagaaaaatttttatgagttctaaagttattatctttgataccaccctacgtgatggtgaacaggc

GU145282 repA, leuA and inverted repeat region of *Buchnera aphidicola* plasmid pLeuABCD, primary endosymbiont of *Diuraphis tritici*, partial.
Atgcggcttatctactttttccattctggtaataaatctatcacccgtgcttcacgtttaataaatgatttttttagaaccgatggggtttgttaaatgcaaa
aaaataaacagtgaatctgttagtaattatatacctaaaaaaatatttttaacaccaatgttctttatgttgtttaacataccacaatcaaaaataaatagtt
atttatctaagtcaatgaacatgtctcaaaagttaaaaataacagaaaaaaaaatgtttatttctttttcagatattaaggtaatgtcaaaattagatgaaa
aatctgttagaaaaaaaattttaaatgctttaattaattattatacagcaagtgaactaacaaaaataggccctaagggcctaaaacaaagaatagat
gttgaatataataatttatgtaaattatttaaaaaaattaaaaaataagttgatacaataaaaatttcataagacatgtaggtacacaattttgtacctacat
gtcttatgaaatttttttagtgtatccttatttttttttaaaaacaactttttataatctttaaaaaggggagaaatttttttatgagttctaaagttattatctttgata
ccaccttacgtgatggtgaacaagcattacaagcaagtttgagcgttaaagaaaaattacaaatcgcattatcttctagaaaaatgtgggatagatat
ttattgagtagcttttcttatttcatcacctgg

GU145283 repA, leuA and inverted repeat region of *Buchnera aphidicola* plasmid pLeuABCD, endosymbiont of *Macrosiphum rosae* (MF), partial.
aaattggtccgaaaggtcttaaaaaaaagatagatattgaatataataatttatgtaatttgcataaaaaaaaaatcataaaataaatttatacaacaaaa
tttcataagacatcatatacaaaaaacatccttgtatatgatgtcttatgaaattttgttgtataaatttattttattggtataaaacattctatttcctaatcga
gaaaaattttatgaattcgaaagttattatttttgatacaactttgcgggatggtgaccaagcattaggat

GU145284 repA, leuA and inverted repeat region of *Buchnera aphidicola* plasmid pLeuABCD, endosymbiont of *Myzus persicae*, partial.
cgaaaattggtccgaaaggtcttaaaaaaaagatagatattgaatatcataatttatgtaaattatacaaaagaaaataataaaataaatttatacacaaaaatttcataagacgtaaaaatgcaatacatgtgtttttaatgtcttatgaaatttttgtgtataaatttattttatttaataaatcattttatttattgtcgagaaaatttttatgaactctaaagttattatttttgatacaa

GU145285 repA, leuA and inverted repeat region of *Buchnera aphidicola* plasmid pLeuABCD, endosymbiont of *Uroleucon sonchi* (ACAM), partial.
gaattaactaaaattggtcctcaaggtctgaaaaaaaagatagatatcgaatataataatttatgtaagttaaacaaacataaataacatcatattatacaataaaaatttcataagacatcgttgtacaaaaatcatttttgtacaacgatgtcttatgaaatttttattgtataatacgatgttatttaacaaacattcattttatcggaataattttatgaattctaaagttattatttttgatacaactctacgtgatggtgatcaagcattaggatac

GU145286 repA, leuA and inverted repeat region of *Buchnera aphidicola* plasmid pLeuABCD, endosymbiont of *Hyalopterus pruni* (MF), partial.
gaattaacgaaaattggtccgaagggtcttaaaaaaaagatagatattgaatatggtaaattatgtaaattatataaaaaaaaatattagataaattatacaataaaaatttcataaagcagaaagatgcatttttaaaaatgcatctttctgttttatgaaatttttattgtataatttatctaatttaaacaaactattttttaaaattagcgagaaaaaatttatgaattcaaaaattattatttttgatacgactctacgagatggggaccaagcattaggatctagta

GU145287 repA, leuA and inverted repeat region of *Buchnera aphidicola* plasmid pLeuABCD, endosymbiont of *Hyalopterus pruni* (MF), partial.
gaattaacaaaaataggtcctcaaggtcttaaaaaaaagatagatattgaatatggtaaattatgtaaattatataaaaaaaaatattagataaattatacaataaaaatttcataaaacagaaagatgcatttttaaaaatgcatctttctgttttatgagatttttattgtataatttatctaatttaaacaaactattttttaaaattagcgagaaaaaatttatgaattcaaaaattattatttttgatacgactctacgggatggagaccaagcattaggatacagaatgg

GU145288 repA, leuA and inverted repeat region of *Buchnera aphidicola* plasmid pLeuABCD, endosymbiont of *Brevicoryne brassicae* (MF), partial.
aatagatattgaatataataatttatgtaaattatttaacaaaattaaaaaataaatttacacaataaaaatttcataagacatcaaggtacaaaattttgtaccttgatgtcttatgaaatttttattgtgtaaatttattttattcaaaaacatttttattttttaaaagatgagaaaaatttttatgaattctaaagttattattttt

GU145289 repA, leuA and inverted repeat region of *Buchnera aphidicola* plasmid pLeuABCD, endosymbiont of *Brevicoryne brassicae* (MF), partial.
gaattaacgaaaataggtcctaaagggcttaaaaaacaaatagatattgaatataataatttatgtaaattatttaacaaaattaaaaaataaatttacacaataaaaatttcataagacatcaaggtacaaaattttgtaccttgatgtcttatgaaatttttattgtgtaaatttattttattcaaaaacatttttattttttaaaagatgagaaaaatttttatgaattctaaagttattatttttgataccaccttacgcgatggagatcaagcattaggatac

APPENDIX – CHAPTER 5

Table Appx 5.1 Scripts, codes and files used and/or produced during the data analyses of the different Affymetrix experiments.

Script, code or filename	***Description***
Script 1 Data in, normalization & quality check	Read all the **.Cel* files and *Target.txt* files in and normalizes with RMA, PLM, GCRMA, VSN and MAS5.0. Provides various figures to investigate the quality of the slides, *i.e.* raw data (*Figure 5.2*).
Script 2 Quality control analysis	Uses the **.RData* files generated with script 1 and produce graphs for the different normalized datasets (*Figure 5.2*).
Script 3 Getting differentially expressed genes	Identifying differentially expressed genes after normalization with RMA, GCRMA, VSN, PLM and MAS5, using LIMMA and BA packages for 95, 99 and 99.9 % confidence. The FDR is determined using Bonferonni and Benjamini-Hochberg FDR methods and written to excel files and different folders (*Figure 5.2*).
Target.txt	A summary, tab delimited file that contains sample and slide names, filenames, replicates, *etc.* Data in this file is used for the figure legends.
Convert	*MS Excel 2007* macro that copies all the worksheets, *i.e.* **.csv* files, of one or more workbooks in the same directory as the workbook in which the macro is run, to that workbook. Note that the worksheets must be named differently.

Note: Script 1, 2 and 3, Target and Convert follows this table via the links above.

Script 1 Data in, normalization & quality check

```
#Name:   Script 1 Data in, normalization & quality check.R
#Author  Z.H. Swanevelder, Department of Genetics, University of Pretoria, RSA
#Ref:    Please cite this script as: Z.H. Swanevelder (2010). Aphid-plant
         interactions and the possible role of an endosymbiont in aphid biotype
         development. Ph.D. Thesis, University of Pretoria.
#Aim:    Read the *.Cel files in and normalizes with RMA, GCRMA, PLM, VSN and
         MAS5.0
#Usage:  Run script in directory containing the *.CEL & Target.txt files
#Notes:  1. Target.txt is a file with phenotypic data like: Names (sample
            names), CEL Filenames, etc. in tab delimited format. Labels in the
            Target.txt file are used in figures (see example attached at the end
            of these scripts)
#        2. Running the script twice in the same directory may give a warning
            that the directories exist - it can be ignored HOWEVER files WILL BE
            overwritten
#        3. Output RData files are saved into a newly made directory called "Raw
            and Normalized R datasets"
#        4. Figures of the raw data is saved into the directory "/R
            results/Quality control raw data/" and a summary of the raw data is
            given in the file "Summary of all data.jpg". Subfolders: "Slide
            Images raw/"; "Log2 plots/" & "MA plots/" contains raw data slide
            comparisons and slide images


#1.LOAD LIBRARIES
print("Loading libraries....")
library(affy)
library(gcrma)
library(limma)
library(affyPLM)
library(marray)
library(annaffy)
library(geneplotter)
library(made4)
library(multtest)
library(vsn)
library(graphics)
library(IDPmisc)
print("Libraries Loaded")

#2.READ DATA FROM .CEL FILES INTO VARIABLES
base<-getwd()
DATA<-paste(base,"/Raw and Normalized R datasets/",sep=""); DATA
dir.create(DATA,showWarnings=TRUE,recursive=FALSE)
if (file.exists("Target.txt"))
     {
     print("Target.txt exist. Reading targets and other info in")
     pd<-read.AnnotatedDataFrame("Target.txt",header=TRUE,row.names=1)
     data<-ReadAffy(filenames=pData(pd)$FileName)
     data@phenoData<-pd
     save(data,file=paste(DATA,"Rawdata.RData",sep=""))
 } else {
     print("Target.txt DOESN'T exist. NO extra phenoData will be added!!")
     data<-ReadAffy()
```

```
     save(data,file=paste(DATA,"Rawdata.RData",sep=""))
     }

setwd("Raw and Normalized R datasets")
print("Running Background and Normalization scripts....")
esetRMA<-rma(data); print("RMA DONE...")
save(esetRMA,file="esetRMA.RData"); print("RMA FILE DONE...")
esetVSN<-vsnrma(data); print("VSN DONE...")
save(esetVSN,file="esetVSN.RData"); print("VSN FILE DONE...")
esetGCRMA<-gcrma(data); print("GCRMA DONE...")
save(esetGCRMA,file="esetGCRMA.RData"); print("GCRMA FILE DONE...")
esetPLM<-fitPLM(data); print("PLM DONE...")
save(esetPLM,file="esetPLM.RData"); print("PLM FILE DONE...")
esetMAS<-mas5(data); print("MAS5 DONE...")
save(esetMAS,file="esetMAS.RData"); print("MAS5 FILE DONE...")
setwd(base)

#SETTING GRAPHICS LEGEND
print("Setting graphics")
fil<-(2:(length(data)+1))
lgnd<-(is.null(data$Name))
if (lgnd)
     {
     print("Target.txt doesn't exist. Default samplenames!")
     legend<-(sampleNames(data))
  } else {
     print("Setting the legend according Target.txt Name")
     legend<-(data$Name)
     }
print("Legend set...done")

#DIRECTORIES
base<-getwd()
RESULTS<-paste(base,"/R results/Quality control raw data/",sep=""); RESULTS
dir.create(RESULTS,showWarnings=TRUE,recursive=TRUE)
setwd(base)
print("Directory Results rawdata analysis made")

#ASSESSMENT OF RAW DATA
print("ASSESSMENT OF RAW DATA: IMAGE ANALYSIS")

#PLOTTING RAW IMAGES OF ARRAYS
print("Single slide comparisons")
RAWIMAG<-paste(RESULTS,"Slide Images raw/",sep=""); RAWIMAG
dir.create(RAWIMAG, showWarnings = TRUE, recursive = FALSE)
number<-length(data)
lgnd<-(is.null(data$Name))
if (lgnd)
    {
    print("No target.txt file!")
    for (x in seq(1,number,1))
      {
      filename<-paste(RAWIMAG,sampleNames(data[,x]),"_raw.jpg",sep="")
      filename2<-paste(RAWIMAG,sampleNames(data[,x]),"_log2_raw.jpg",sep="")
      jpeg(filename)
      par(mfrow=c(1,1))
      image(data[,x],transfo=I)
      dev.off()
```

```
      jpeg(filename2)
      par(mfrow=c(1,1))
      image(data[,x],transfo=log2)
      dev.off()
      }
      print("Raw Images DONE")
   } else {
      print("Name from target.txt file is used!")
      for (x in seq(1,number,1))
        {
        filename<-paste(RAWIMAG,data$Name[x],"_raw.jpg",sep="")
        filename2<-paste(RAWIMAG,data$Name[x],"_log2_raw.jpg",sep="")
        jpeg(filename)
        par(mfrow=c(1,1))
        image(data[,x])
        dev.off()
        jpeg(filename2)
        par(mfrow=c(1,1))
        image(data[,x],transfo=log2)
        dev.off()
        print(paste(data$Name[x],"...done",sep=""))
        }
      print("Raw Images DONE")
      }

#EXPRESSION SET OF LOG2 FOR SLIDE COMPARISONS
print("Log2 plot comparing replicates in an treatment: RAW data")
LOGP<-paste(RESULTS,"Log2 plots/",sep=""); LOGP
dir.create(LOGP, showWarnings = TRUE, recursive = FALSE)
exprs(data) <- log2(exprs(data))
number<-length(data)
lgnd<-!(is.null(data$Name))
if (lgnd)
    {
    for (x in seq(1,number,3))
      {
      y<-(x+1)
      z<-(x+2)
      filename<-paste(LOGP,data$Name[x],"_vs_",data$Name[y],"_log2.jpg",sep="")
      jpeg(filename)
      par(mfrow=c(1,1))
      xlable<-paste("Log2 expression on array:",data$Name[x],sep=" ")
      ylable<-paste("Log2 expression on array:",data$Name[y],sep=" ")
      plot(2^exprs(data)[,x],2^exprs(data)[,y],log="xy",xlab=xlable,ylab=ylable,
      cex=.25,pch=16,yaxt="n")
      lines(2^seq(-2,16),2^seq(-1,17),col="red")
      lines(2^seq(-1,17),2^seq(-2,16),col="red")
      dev.off()
      filename<-paste(LOGP,data$Name[x],"_vs_",data$Name[z],"_log2.jpg",sep="")
      jpeg(filename)
      par(mfrow=c(1,1))
      xlable<-paste("Log2 expression on array:",data$Name[x],sep=" ")
      ylable<-paste("Log2 expression on array:",data$Name[z],sep=" ")
      plot(2^exprs(data)[,x],2^exprs(data)[,z],log="xy",xlab=xlable,ylab=ylable,
      cex=.25,pch=16,yaxt="n")
      lines(2^seq(-2,16),2^seq(-1,17),col="red")
      lines(2^seq(-1,17),2^seq(-2,16),col="red")
      dev.off()
```

```
      filename<-paste(LOGP,data$Name[y],"_vs_",data$Name[z],"_log2.jpg",sep="")
      jpeg(filename)
      par(mfrow=c(1,1))
      xlable<-paste("Log2 expression on array:",data$Name[y],sep=" ")
      ylable<-paste("Log2 expression on array:",data$Name[z],sep=" ")
      plot(2^exprs(data)[,y],2^exprs(data)[,z],log="xy",xlab=xlable,ylab=ylable,
      cex=.25,pch=16,yaxt="n")
      lines(2^seq(-2,16),2^seq(-1,17),col="red")
      lines(2^seq(-1,17),2^seq(-2,16),col="red")
      dev.off()
      }
    }

#MA PLOT COMPARISONS
print("MA plot comparing replicates in an treatment ")
MAPLOTS<-paste(RESULTS,"MA plots/",sep="")
MAPLOTS
dir.create(MAPLOTS, showWarnings = TRUE, recursive = FALSE)
number<-length(data)
lgnd<-(is.null(data$Name))
if (lgnd)
    {
    print("No target.txt file!")
    for (x in seq(1,number,3))
      {
      y<-(x+1)
      z<-(x+2)
      filename<-
      paste(MAPLOTS,sampleNames(data[,x]),"_vs_",sampleNames(data[,y]),"_raw.jpg
      ",sep="")
      jpeg(filename)
      par(mfrow=c(1,1))
      MAplot(data,which=c(x,y),ref=x)
      dev.off()
      print(filename)
      filename<-
      paste(MAPLOTS,sampleNames(data[,x]),"_vs_",sampleNames(data[,z]),"_raw.jpg
      ",sep="")
      jpeg(filename)
      par(mfrow=c(1,1))
      MAplot(data,which=c(x,z),ref=x)
      dev.off()
      print(filename)
      filename<-
      paste(MAPLOTS,sampleNames(data[,y]),"_vs_",sampleNames(data[,z]),"_raw.jpg
      ",sep="")
      jpeg(filename)
      par(mfrow=c(1,1))
      MAplot(data,which=c(y,z),ref=y)
      dev.off()
      print(filename)
      }
    print("MA comparisons DONE");
   }else {
    for (x in seq(1,number,3))
      {
      y<-(x+1)
      z<-(x+2)
```

```
      filename<-
paste(MAPLOTS,data$Name[x],"_vs_",data$Name[y],"_raw.jpg",sep="")
      jpeg(filename)
      par(mfrow=c(1,1))
      MAplot(data,which=c(x,y),ref=x,identify=TRUE)
      dev.off()
      filename<-
paste(MAPLOTS,data$Name[x],"_vs_",data$Name[z],"_raw.jpg",sep="")
      jpeg(filename)
      par(mfrow=c(1,1))
      MAplot(data,which=c(x,z),ref=x,identify=TRUE)
      dev.off()
      filename<-
paste(MAPLOTS,data$Name[y],"_vs_",data$Name[z],"_raw.jpg",sep="")
      jpeg(filename)
      par(mfrow=c(1,1))
      MAplot(data,which=c(y,z),ref=y,identify=TRUE)
      dev.off()
      }
    print("MA & Hexbin comparisons DONE")
   }

#SUMMARY OF RAW DATA
filename<-paste(RESULTS,"Summary of all data.jpg",sep="")
jpeg(filename)
par(mfrow=c(1,1))
overview(data)
dev.off()

print("Analysis done! Hit enter to return to shell")
```

Script 2 Quality control analysis

```
#Name:    Script 3 Quality control analysis.R
#Author:  Z.H. Swanevelder, Department of Genetics, University of Pretoria, RSA
#Ref:     Please cite this script as: Z.H. Swanevelder (2010). Aphid-plant
          interactions and the possible role of an endosymbiont in aphid biotype
          development. Ph.D. Thesis, University of Pretoria.
#Aim:     Generating graphs for normalized datasets
#Usage:   Run script after and in the same directory as script 1. It uses the
          normalized data in the directory: "Raw and Normalized R datasets"
#Notes:   1. Labels in the Target.txt file are used in figures
#         2. Running the script twice in the same directory may give a warning
             that the directories exist - it can be ignored HOWEVER files WILL BE
             overwritten
#         3. Output: Files are saved into the directory "/R results/" under the
             sub-directories: Graphs of raw and normalized data/"; "Graphs of raw
             and normalized data/Log2 plots/" & "Graphs of raw and normalized
             data/MA plots/"

#FUNCTION: COMPARE REPLICATES WITH EACH OTHER
compare<-function(slidenum_x,slidenum_y,data_obj,direct,method)
   {
  head<-
  paste(pData(data_obj)$Name[slidenum_x],"_vs_",pData(data_obj)$Name[slidenum_y]
  ,sep="")
```

```
  print(head)
  filename<-paste(direct,"MA plots/",method,"_",head,".jpg",sep="")
  jpeg(filename,width=1000,height=1200,pointsize=12)
  par(mfrow=c(1,1))
  MAplot(data_obj,which=c(slidenum_x,slidenum_y),ref=slidenum_x)
  dev.off()
  filename<-paste(direct,"Log2 plots/",method,"_",head,"_log2.jpg",sep="")
  jpeg(filename)
  par(mfrow=c(1,1))
  xlable<-paste("Log2 expression ",pData(data_obj)$Name[slidenum_x],sep=" ")
  ylable<-paste("Log2 expression ",pData(data_obj)$Name[slidenum_y],sep=" ")
  plot(2^exprs(data_obj)[,slidenum_x],2^exprs(data_obj)[,slidenum_y],log="xy",xl
  ab=xlable,ylab=ylable,cex=.25,pch=16,yaxt="n")
  lines(2^seq(-2,16),2^seq(-1,17),col="red")
  lines(2^seq(-1,17),2^seq(-2,16),col="red")
  dev.off()
  }

comparePLM<-function(slidenum_x,slidenum_y,data_obj,direct,method)
  {
  head<-
  paste(pData(data_obj)$Name[slidenum_x],"_vs_",pData(data_obj)$Name[slidenum_y]
  ,sep="")
  print(head)
  filename<-paste(direct,"MA plots/",method,"_",head,".jpg",sep="")
  jpeg(filename,width=1000,height=1200,pointsize=12)
  par(mfrow=c(1,1))
  MAplot(data_obj,which=c(slidenum_x,slidenum_y),ref=slidenum_x)
  dev.off()
  }

#LOAD LIBRARIES
print("Loading libraries....")
library(affy)
library(gcrma)
library(limma)
library(affyPLM)
library(marray)
library(annaffy)
library(geneplotter)
library(made4)
library(multtest)
library(IDPmisc)
library(vsn)
library(graphics)
print("Libraries loaded")

#LOADING R OBJECT FILES
base<-getwd()
setwd("Raw and Normalized R datasets")
print("Loading ...")
load("Rawdata.RData"); print("Raw data in ...")
load("esetRMA.RData"); print("RMA data in ...")
load("esetGCRMA.RData"); print("GCRMA data in ...")
load("esetMAS.RData"); print("MAS data in ...")
load("esetVSN.RData"); print("VSN data in ...")
load("esetPLM.RData"); print("PLM data in ...")
setwd(base)
```

```
#READING OBJECTS INTO LIST
x<-1
normList<-
list("rawdata"=x,"esetRMA"=x,"esetVSN"=x,"esetGCRMA"=x,"esetMAS"=x,"esetPLM"=x)
if(is.object(data)) {normList$rawdata<-data} else {print("RAW DATA NOT LOADED")}
if(is.object(esetRMA)) {normList$esetRMA<-esetRMA} else {print("RMA DATA NOT
LOADED")}
if(is.object(esetGCRMA)) {normList$esetGCRMA<-esetGCRMA} else {print("GCRMA DATA
NOT LOADED")}
if(is.object(esetMAS)) {normList$esetMAS<-esetMAS} else {print("MAS DATA NOT
LOADED")}
if(is.object(esetVSN)) {normList$esetVSN<-esetVSN} else {print("VSN DATA NOT
LOADED")}
if(is.object(esetPLM)) {normList$esetPLM<-esetPLM} else {print("PLM DATA NOT
LOADED")}

#MAKING THE BASE DIRECTORIES
base<-getwd()
RESULTS<-paste(base,"/R results/",sep=""); RESULTS
dir.create(RESULTS,showWarnings=TRUE,recursive=FALSE)
IMAG<-paste(RESULTS,"Graphs of raw and normalized data/",sep=""); IMAG
dir.create(IMAG, showWarnings = TRUE, recursive = FALSE)
LOGG<-paste(RESULTS,"Graphs of raw and normalized data/Log2 plots/",sep="");
IMAG
dir.create(LOGG, showWarnings = TRUE, recursive = FALSE)
LOGG<-paste(RESULTS,"Graphs of raw and normalized data/MA plots/",sep=""); IMAG
dir.create(LOGG, showWarnings = TRUE, recursive = FALSE)

#GENERATING PLOTS
number2<-length(normList)
print("Number of objects to be analized:"); number2
name<-names(normList)
for (d in seq(1,number2,1))
   {
   if (is.object(normList[[d]]))
      {
      print("Analysing")
      print(name[d])
      nar<-normList[[name[d]]]
      number<-length(pData(nar)$Name)
      fil<-(2:(length(pData(nar)$Name)+1))
      legend<-pData(nar)$Name

      #BOXPLOTS
      print("Drawing the boxplot")
      filename<-paste(IMAG,"Boxplot_",name[d],".jpg",sep="")
      jpeg(filename,width=2000,height=1000,pointsize=16)
      par(mfrow=c(1,1))
      boxplot(nar,col=fil,names=legend)
      dev.off()
      print("Boxplot done!")

      if (d != 6)
         {
         #MA PLOT COMPARISONS IN AN TREATMENT
         print("Doing MA-Plots ...")
         for (x in seq(1,number,3))
```

```
            {
            y<-(x+1)
            z<-(x+2)
            Compared<-compare(x,y,nar,IMAG,name[d])
            Compared<-compare(x,z,nar,IMAG,name[d])
            Compared<-compare(y,z,nar,IMAG,name[d])
            }

          #HISTOGRAM OF DENSITY VS LOG INTENSITIES
          print("Drawing histogram")
          filename<-paste(IMAG,"Histogram_",name[d],".jpg",sep="")
          jpeg(filename,width=800,height=1000,pointsize=16)
          par(mfrow=c(1,1))
          print(nar)
          hist(nar,col=fil,names=pData(nar)$Name)
          legend("topright",legend,inset=.02,col=fil,lty=fil,bg="white")
          dev.off()
          print("Histogram done")
        } else {
          print("PLM being done")
          print("Doing MA-Plots ...")
          for (x in seq(1,number,3))
            {
            y<-(x+1)
            z<-(x+2)
            Compared<-comparePLM(x,y,nar,IMAG,name[d])
            Compared<-comparePLM(x,z,nar,IMAG,name[d])
            Compared<-comparePLM(y,z,nar,IMAG,name[d])
            }
          }
      }
   }
print("Analysis done! Hit enter to return to shell")
```

Script 3 Getting differentially expressed genes

```
#Name:    Script 3 Getting differentially expressed genes.R
#Author:  Z.H. Swanevelder, Department of Genetics, University of Pretoria, RSA
#Ref:     Please cite this script as: Z.H. Swanevelder (2010). Aphid-plant
          interactions and the possible role of an endosymbiont in aphid biotype
          development. Ph.D. Thesis, University of Pretoria.
#Aim:     Identifying differentially expressed genes after normalization with
          RMA, GCRMA, VSN, PLM and MAS5, using LIMMA and BA for 95, 99 and 99.9%
          confidence. The FDR is determined using Bonferroni and Benjamini-
          Hochberg FDR methods.
#Usage:   Run script in the same directory and after script 1 & 2.
#Notes:   1. Labels in the Target.txt file are used in figures
#         2. Running the script twice in the same directory may give a warning
            that the directories exist - it can be ignored HOWEVER files WILL BE
            overwritten
#         3. Output: Generating *.csv excel files with differentially expressed
            genes in the directory “/R results/Regulated genes/” & the sub-
            directories: “Differential genes 95/”; “Differential genes 99/” &
            “Differential genes 99.9/”. The latter 3 directories are again
            divided into the different methods used for the normalization which
```

```
            contains the files before FDR corrections, as well as the
            directories for the FDR correction methods.
#         4. Please change the ‘model.matrix’ layout, ‘colnames’ and ‘contrast
            matrix’ to suite your dataset

#LOAD LIBRARIES
print("Loading libraries....")
library(affy)
library(gcrma)
library(limma)
library(affyPLM)
library(marray)
library(annaffy)
library(vsn)
print("Libraries Loaded")

#LOAD FILES
base<-getwd()
setwd("Raw and Normalized R datasets")
print("Loading ...")
load("esetRMA.RData"); print("RMA data in ...")
load("esetGCRMA.RData"); print("GCRMA data in ...")
load("esetMAS.RData"); print("MAS data in ...")
load("esetVSN.RData"); print("VSN data in ...")
load("esetPLM.RData"); print("PLM data in ...")
setwd(base)

#WRITING NORMALIZED DATASETS TO A LIST FOR EASY ACCESS
x<-1
normList<-list("esetPLM"=x,"esetRMA"=x,"esetGCRMA"=x,"esetMAS"=x,"esetVSN"=x)
if(is.object(esetRMA))  {normList$esetRMA<-esetRMA} else {print("RMA DATA NOT
LOADED")}
if(is.object(esetGCRMA)){normList$esetGCRMA<-esetGCRMA} else {print("GCRMA DATA
NOT LOADED")}
if(is.object(esetMAS))  {normList$esetMAS<-esetMAS} else {print("MAS DATA NOT
LOADED")}
if(is.object(esetVSN))  {normList$esetVSN<-esetVSN} else {print("VSN DATA NOT
LOADED")}
if(is.object(esetPLM))  {normList$esetPLM<-esetPLM} else {print("PLM DATA NOT
LOADED")}

#CREATING OUTPUT DIRECTORIES
counter<-1
pvalueList<-c(99.9, 99, 95)
for (c in seq(1,3,1))
   {
  base<-getwd()
  RESULT<-paste(base,"/R results/Regulated genes/",sep="")
  dir.create(RESULT, showWarnings = TRUE, recursive = FALSE)
  DIFF<-paste(RESULT,"Differential genes ",pvalueList[c],"/",sep="")
  dir.create(DIFF, showWarnings = TRUE, recursive = FALSE)
  setwd(base)

  #RUNNING NORMALIZED DATA THROUGH LIMMA AND EBAYES
  number2<-length(normList)
  for (d in seq(1,number2,1))
      {
      if (is.object(normList[[d]]))
```

```
{
pval<-((100-pvalueList[c])/100)
pval
nam<-names(normList)
print(nam[d])
RESULT<-paste(DIFF,nam[d],"/",sep="")
dir.create(RESULT, showWarnings = TRUE, recursive = FALSE)
BON<-paste(RESULT,"Bonferonni/",sep="")
dir.create(BON, showWarnings = TRUE, recursive = FALSE)
BENHOCH<-paste(RESULT," Benjamini-Hochberg/",sep="")
dir.create(BENHOCH, showWarnings = TRUE, recursive = FALSE)
nar<-normList[[nam[d]]]
design<-model.matrix(~-1+factor(c(1,1,1,2,2,2,3,3,3,4,4,4)))
colnames(design)<-c("Tug","TuD","Tu2","Tu5")
print("Doing LIMMA")
fit<-lmFit(nar,design)
contrast.matrix<-makeContrasts(TuD-Tug,Tu2-Tug,Tu5-Tug,Tu2-TuD,Tu5-
TuD,Tu5-Tu2,levels=design)
fit2<-contrasts.fit(fit,contrast.matrix)
print("To eBayes")
fit2<-eBayes(fit2)
results<-decideTests(fit2,method="nestedF",p.value=pval,lfc=1)
if (nam[d] == "esetPLM")
    {
    summary(fit2$F.p.value)
  } else {
    index<-grep("AFFX",featureNames(nar))
    summary(fit2$F.p.value[index])
    }
results<-classifyTestsF(fit2,p.value=pval)
res<-summary(results)
filename<-paste(RESULT,nam[d],"_summary.csv",sep="")
write.table(res,file=filename,sep=",",row.names=FALSE,quote=FALSE)
options(digits=3)
UpTotal<-0
DownTotal<-0
compar<-dim(results)
compar<-compar[2]
for (comp in seq(1,compar,1))
   {
   UpReg<-results[results[,comp]==1,]
   DownReg<-results[results[,comp]==-1,]
   UpTotal<-rbind(UpTotal,UpReg)
   DownTotal<-rbind(DownTotal,DownReg)
   }
UpNDown<-UpTotal
filename1<-paste(RESULT,nam[d],"_UP-regulated.csv",sep="")
for (UD in seq(1,2,1))
   {
   UpNDownLength<-dim(UpNDown)
   UpNDownLength<-(UpNDownLength[1])
   UpNDown<-UpNDown[2:UpNDownLength,]
   write.table(UpNDown,file=filename1,sep=",",col.names=NA)
   UpNDown<-DownTotal
   filename1<-paste(RESULT,nam[d],"_DOWN-regulated.csv",sep="")
   }

#Getting the p-values with Toptable
```

```
          comparisons<-c("TuD-Tug","Tu2-Tug","Tu5-Tug","Tu2-TuD","Tu5-TuD","Tu5-
Tu2")
          numberComp<-length(comparisons)
          for (UD in seq(1,numberComp,1))
            {
            name<-comparisons[UD]
            topBon<-
topTable(fit2,coef=UD,number=30000,sort.by="P",adjust="holm")
            #STEP-DOWN BONFERRONI
            filenameB<-paste(BON,name,"_topBon.csv",sep="")
            write.table(topBon,file=filenameB,sep=",",col.names=NA)
            topBenHoch<-
topTable(fit2,coef=UD,number=30000,sort.by="P",adjust="fdr")
            #STEP-DOWN BENJAMINI-HOCHBERG
            filenameB<-paste(BENHOCH,name,"_topBenHoch.csv",sep="")
            write.table(topBenHoch,file=filenameB,sep=",",col.names=NA)
            }
          }
      }
  }
print("Analysis done! Hit enter to return to shell")
```

Target.txt

```
SlideNumber     FileName        Target  TechRep BioRep  Name
1            TUG 1.CEL    1      0      1      Tug1
2            TUG 2.CEL    1      0      2      Tug2
3            TUG 3.CEL    1      0      3      Tug3
4            TUG DN1_1.CEL      2      0      1      TugDn_1
5            TUG DN1_2.CEL      2      0      2      TugDn_2
6            TUG DN1_3.CEL      2      0      3      TugDn_3
7            TUG DN2-2.CEL      3      1      1      TugDn2_1
8            TUG DN2-3.CEL      3      1      1      TugDn2_2
9            TUG DN2-4.CEL      3      0      2      TugDn2_3
10           TUG DN5-1.CEL      4      0      1      TugDn5_1
11           TUG DN5-2.CEL      4      0      2      TugDn5_2
12           TUG DN5-3.CEL      4      0      3      TugDn5_3
```

Convert

```
Option Explicit
Sub Convert()

Dim ws As Worksheet
Dim myDir As String
Dim fn As String
Dim Dirr As String
myDir = ThisWorkbook.Path & "\"
Dirr = (myDir & "*.csv")
fn = Dir(Dirr)

If fn = "" Then
 MsgBox "no file"
Exit Sub
End If
```

```
Do While fn <> ""
 With Workbooks.Open(myDir & fn)
  For Each ws In .Worksheets
   ActiveSheet.Copy Before:=ThisWorkbook.Sheets(1)
  Next
 Close False
End With
 fn = Dir
Loop

End Sub
```

Data visualization before normalization and background correcting

The data in this subsection, produced by Script 1 (*Table Appx 5.1*), can be found in the folder: */R results/Quality control raw data/* and its sub folders.

Figure Appx 5.1 **- 12 Slides**

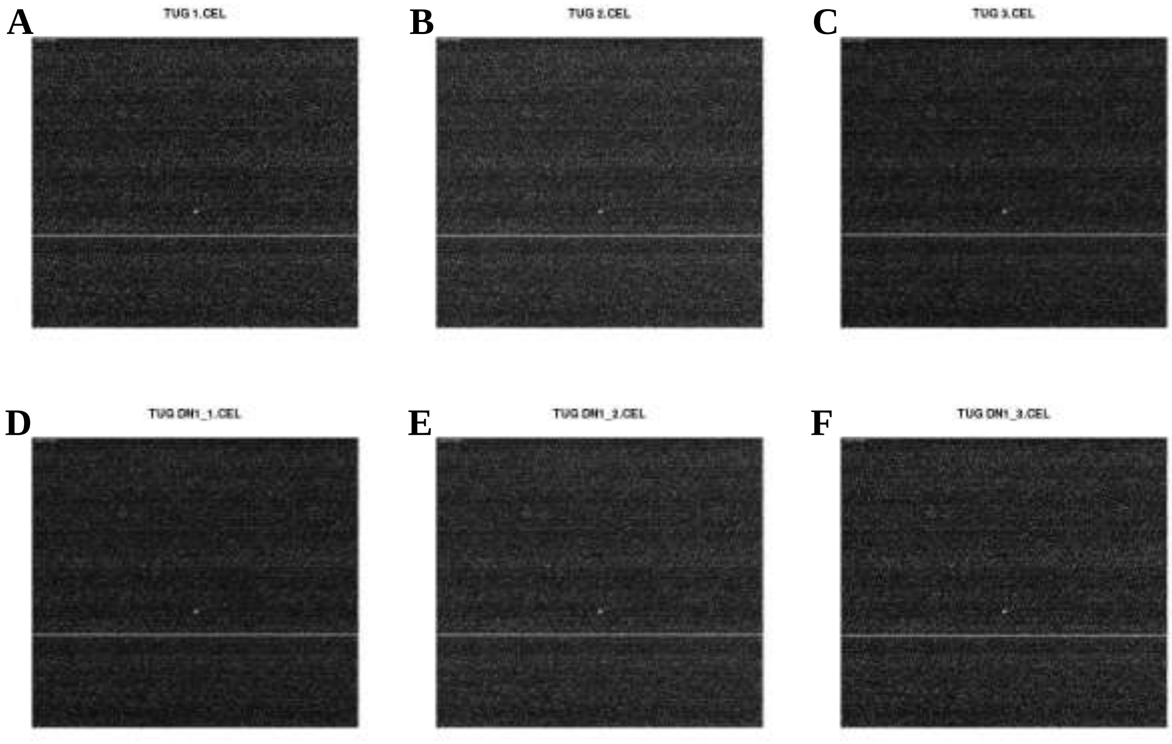


Figure Appx 5.1 **- 12 Slides cont.**

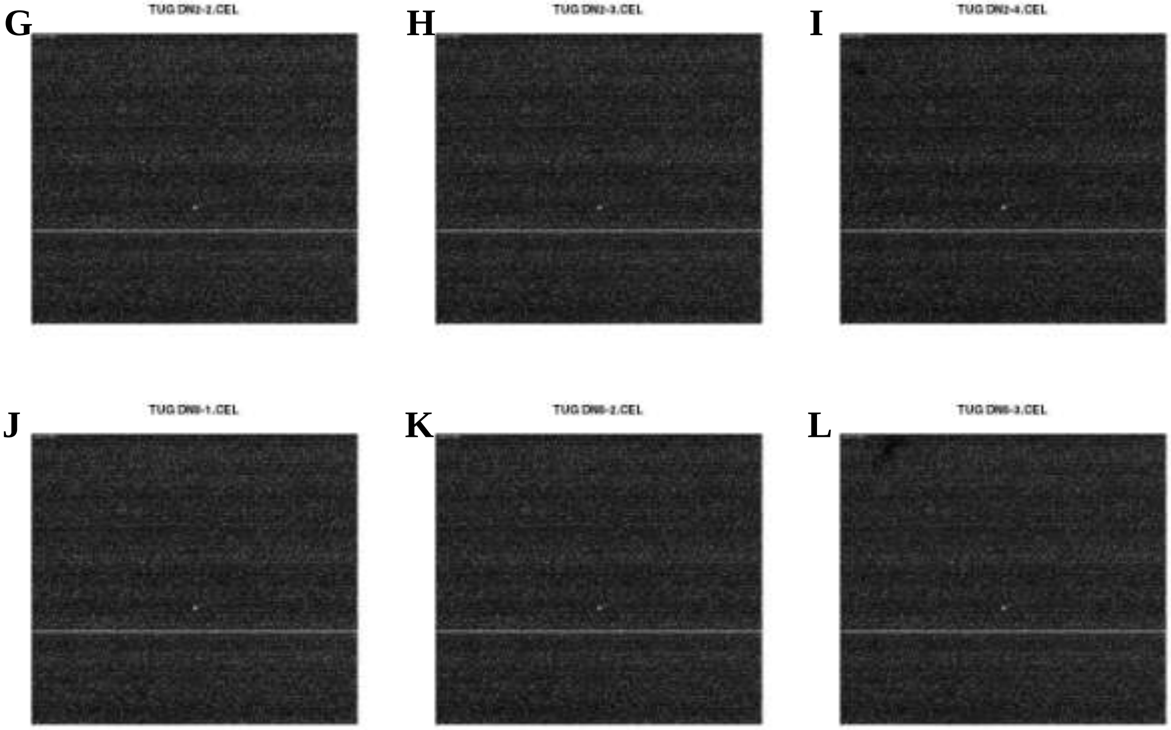


Figure Appx 5.1 **- 18 Slides**

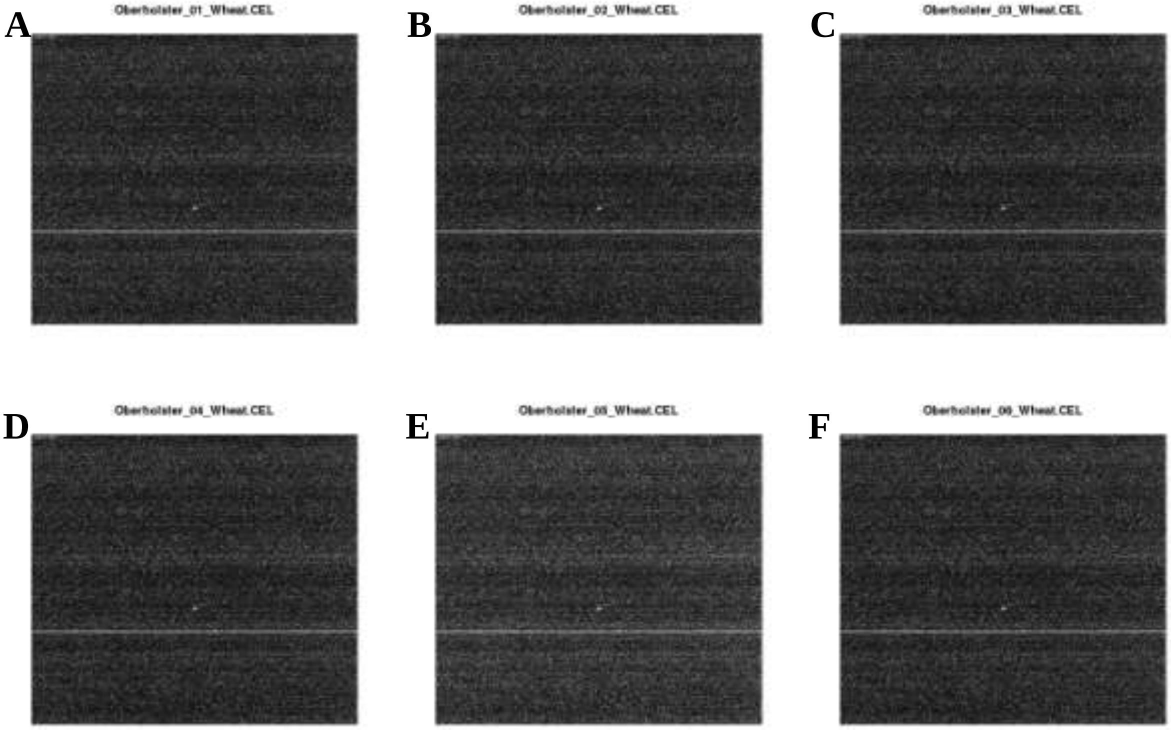


Figure Appx 5.1 - 18 Slides cont.

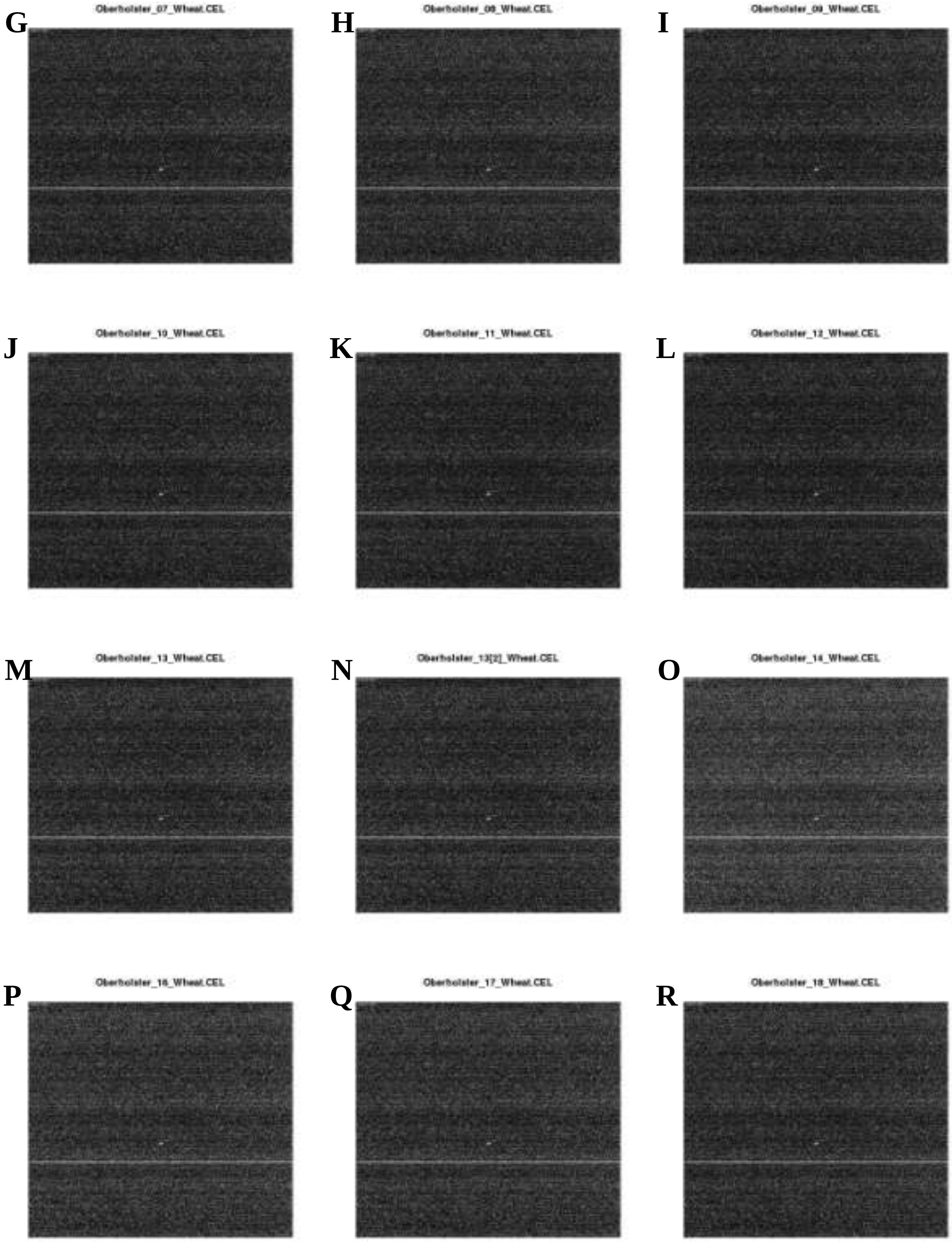


Figure Appx 5.1 Images of the scanned Affymetrix slides after hybridization with the different treatments. The images were produced from the *CEL* files. The 12 slides images were the originally produced *CEL* images, while the 18 slides images are the $\log_2$ derivatives of the original 18 slides images. Both types were produced by script 1 and saved in */R results/Quality control raw data/Slide Images raw/*. The filenames for the 12 slides are: (A) Tug1_raw.jpg, (B) Tug2_raw.jpg, (C) Tug3_raw.jpg, (D) TugDn_1_raw.jpg, (E) TugDn_2_raw.jpg, (F) TugDn_3_raw.jpg, (G) TugDn2_1_raw.jpg, (H) TugDn2_2_raw.jpg, (I) TugDn2_3_raw.jpg, (J) TugDn5_1_raw.jpg, (K) TugDn5_2_raw.jpg, and (L) TugDn5_3_raw.jpg.

The $\log_2$ derivatives filenames for the 18 slides are: (A) RWA1_GS_3_log2_raw.jpg, (B) RWA1_GS_2_log2_raw.jpg, (C) RWA1_GS_1_log2_raw.jpg, (D) RWA2_GS_1_log2_raw.jpg, (E) RWA2_GS_2_log2_raw.jpg, (F) RWA2_GS_3_log2_raw.jpg, (G) RWA1_GR_1_log2_raw.jpg, (H) RWA1_GR_2_log2_raw.jpg, (I) RWA1_GR_3_log2_raw.jpg, (J) RWA2_GR_1_log2_raw.jpg, (K) RWA2_GR_2_log2_raw.jpg, (L) RWA2_GR_3_log2_raw.jpg, (M) Gam_S_1_log2_raw.jpg, (N) Gam_S_2_log2_raw.jpg, (O) Gam_S_3_log2_raw.jpg, (P) Gam_R_1_log2_raw.jpg, (Q) Gam_R_2_log2_raw.jpg, (R) Gam_R_3_log2_raw.jpg.

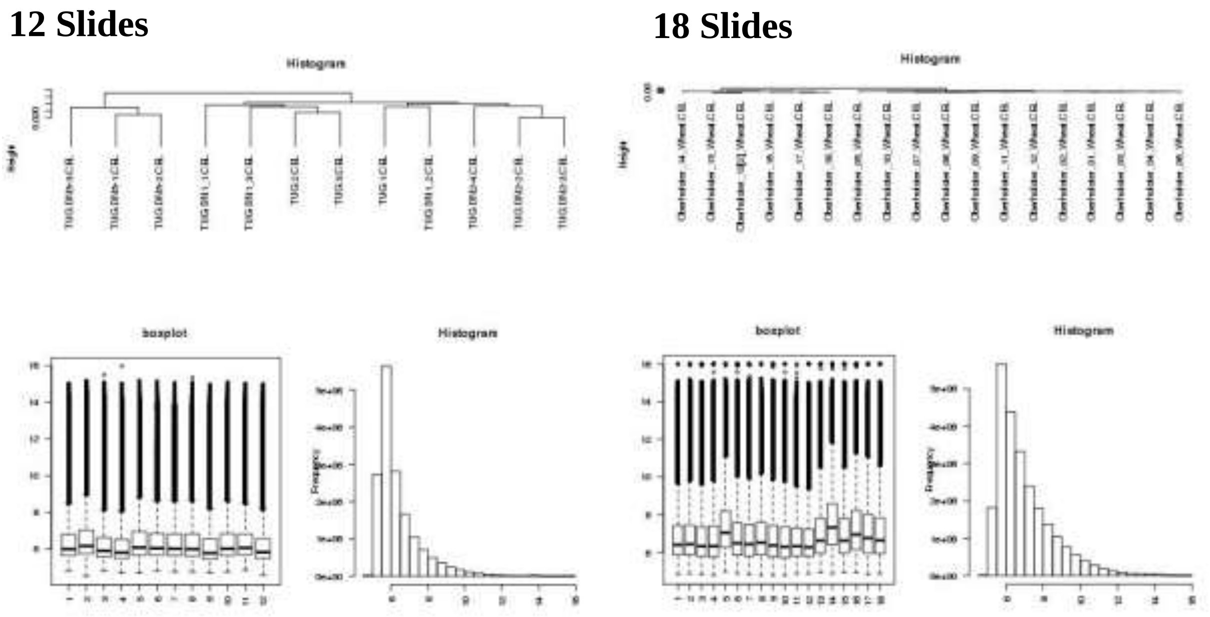


Figure Appx 5.2 Summary figures, including histograms and boxplots, of all the slide data before normalization for the 12 slide and 18 slide experiment. These figures were produced with script 1 and saved as *Summary of all data.jpg.*

Figure Appx 5.3 - **12 Slides**

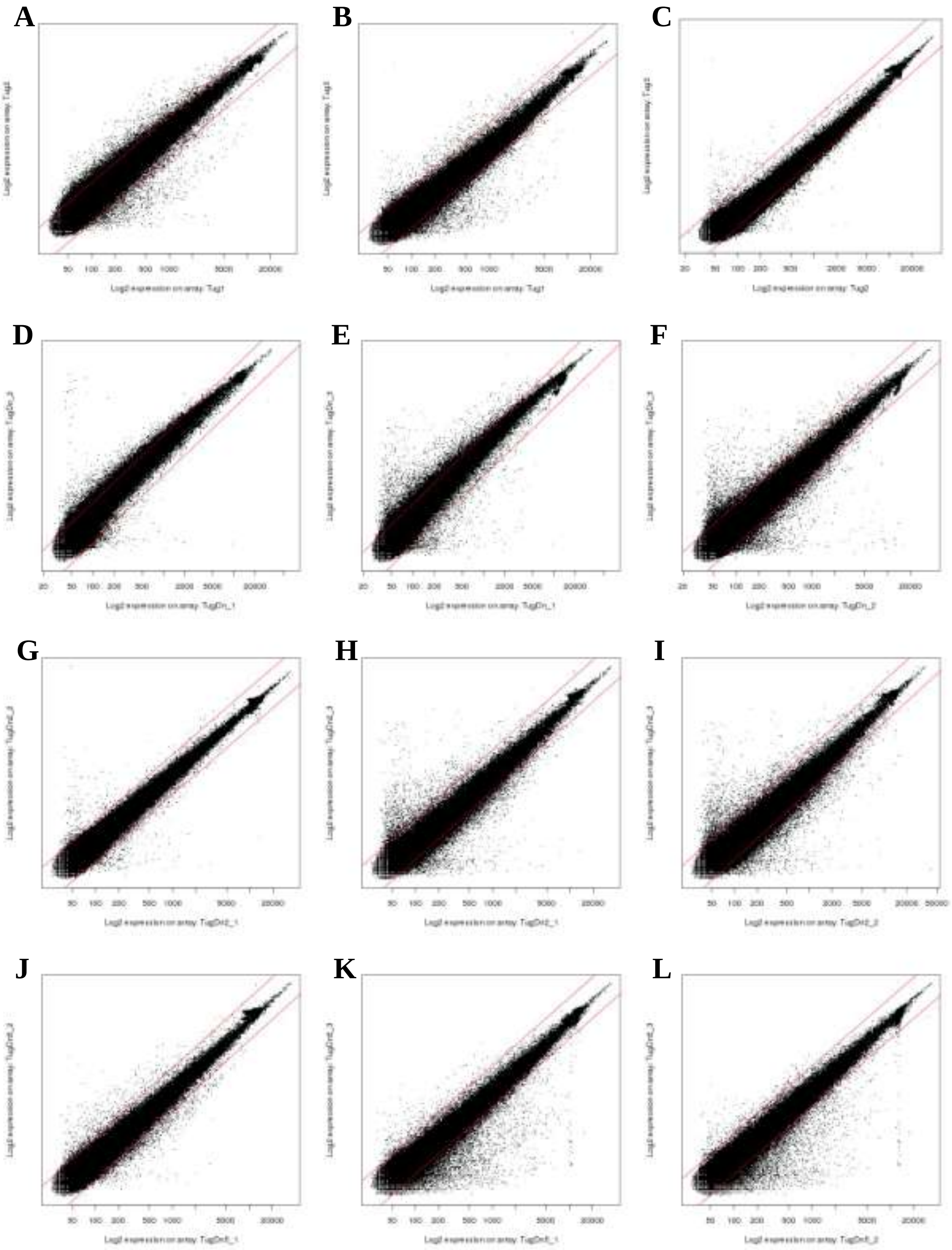


Figure Appx 5.3 - 18 Slides

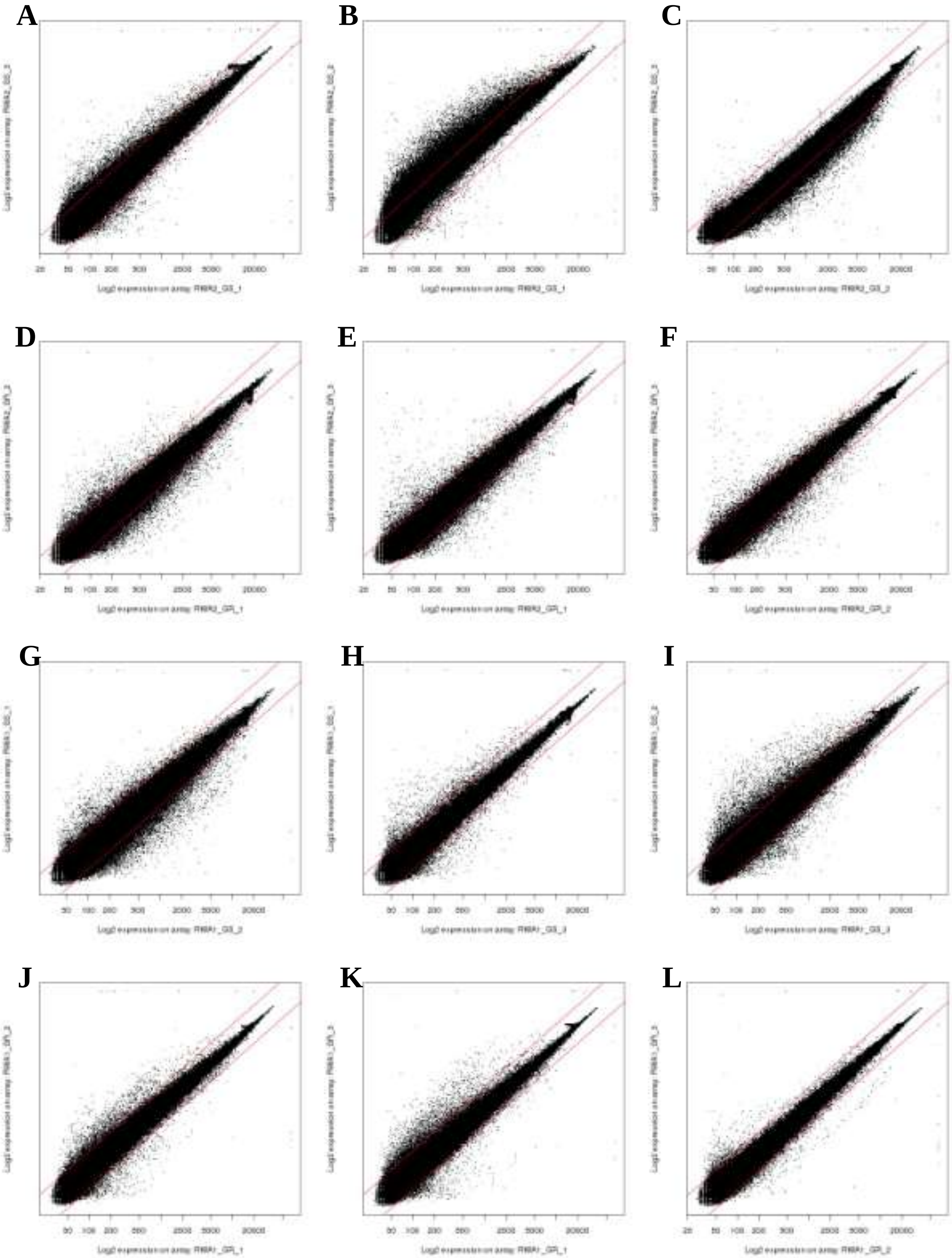


Figure Appx 5.3 **- 18 Slides cont.**

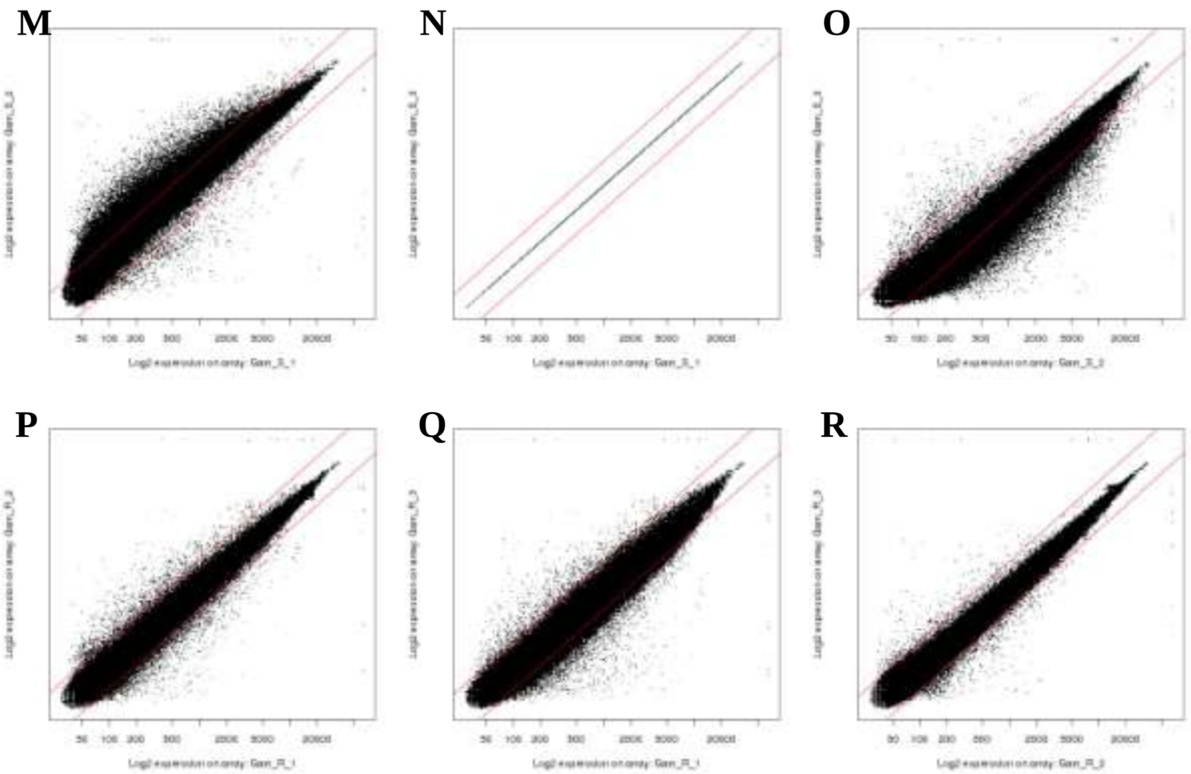


Figure Appx 5.3 The $\log_2$ expression comparisons of the raw, pre-normalized data within treatments. The 12 slides files are: (A) Tug1_vs_Tug2_log2.jpg, (B) Tug1_vs_Tug3_log2.jpg, (C) Tug2_vs_Tug3_log2.jpg, (D) TugDn_1_vs_TugDn_2_log2.jpg, (E) TugDn_1_vs_TugDn_3_log2.jpg, (F) TugDn_2_vs_TugDn_3_log2.jpg, (G) TugDn2_1_vs_TugDn2_2_log2.jpg, (H) TugDn2_1_vs_TugDn2_3_log2.jpg, (I) TugDn2_2_vs_TugDn2_3_log2.jpg, (J) TugDn5_1_vs_TugDn5_2_log2.jpg, (K) TugDn5_1_vs_TugDn5_3_log2.jpg, (L) TugDn5_2_vs_TugDn5_3_log2.jpg.

The 18 slides files are: (A) RWA2_GS_1_vs_RWA2_GS_2_log2.jpg, (B) RWA2_GS_1_vs_RWA2_GS_3_log2.jpg, (C) RWA2_GS_2_vs_RWA2_GS_3_log2.jpg, (D) RWA2_GR_1_vs_RWA2_GR_2_log2.jpg, (E) RWA2_GR_1_vs_RWA2_GR_3_log2.jpg, (F) RWA2_GR_2_vs_RWA2_GR_3_log2.jpg, (G) RWA1_GS_2_vs_RWA1_GS_1_log2.jpg, (H) RWA1_GS_3_vs_RWA1_GS_1_log2.jpg, (I) RWA1_GS_3_vs_RWA1_GS_2_log2.jpg, (J) RWA1_GR_1_vs_RWA1_GR_2_log2.jpg, (K) RWA1_GR_1_vs_RWA1_GR_3_log2.jpg, (L) RWA1_GR_2_vs_RWA1_GR_3_log2.jpg, (M) Gam_S_1_vs_Gam_S_2_log2.jpg, (N) Gam_S_1_vs_Gam_S_3_log2.jpg, (O) Gam_S_2_vs_Gam_S_3_log2.jpg, (P) Gam_R_1_vs_Gam_R_2_log2.jpg, (Q) Gam_R_1_vs_Gam_R_3_log2.jpg, (R) Gam_R_2_vs_Gam_R_3_log2.jpg.

Figure Appx 5.4 - 12 Slides

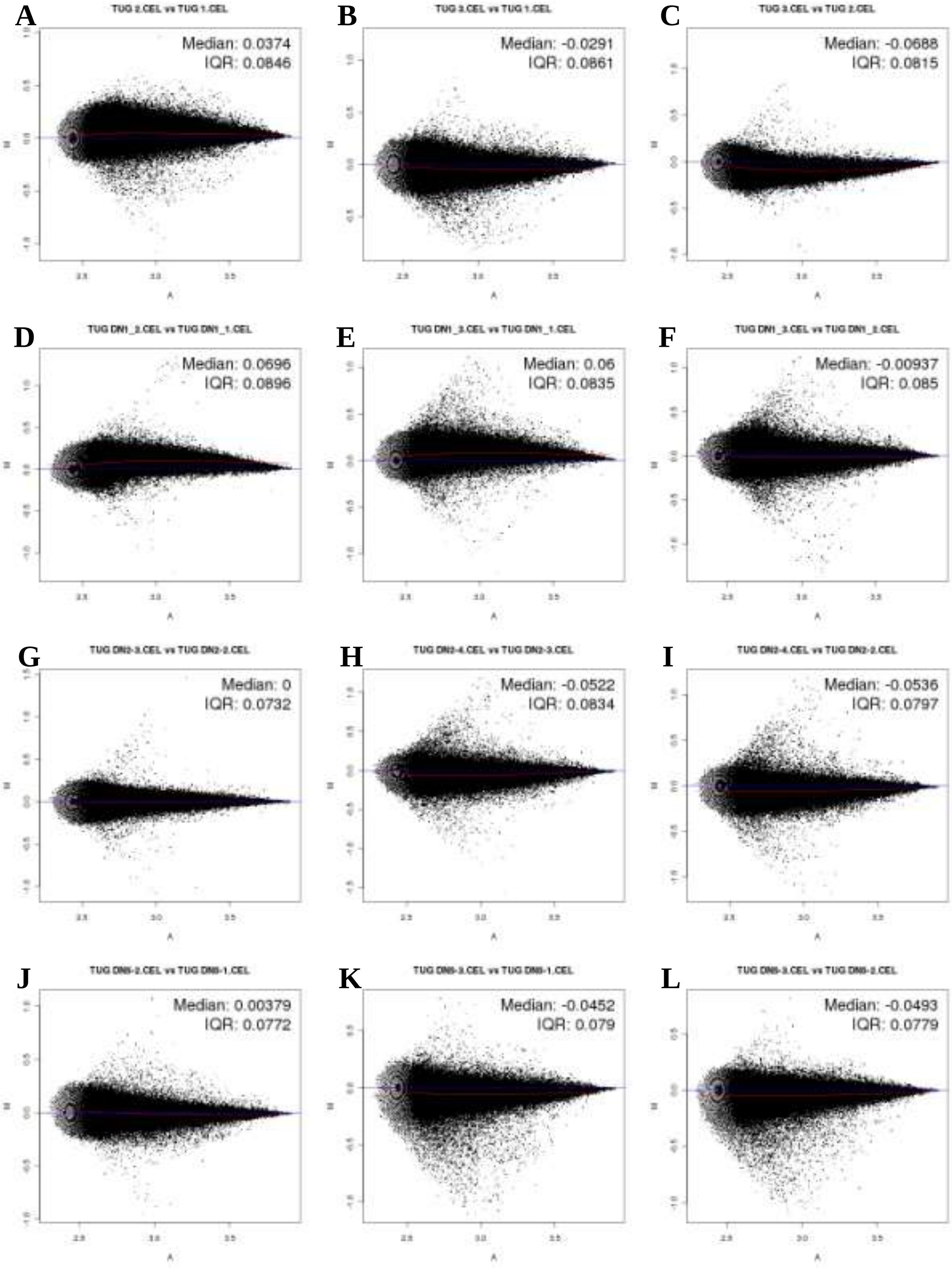


***Figure Appx 5.4* - 18 Slides**

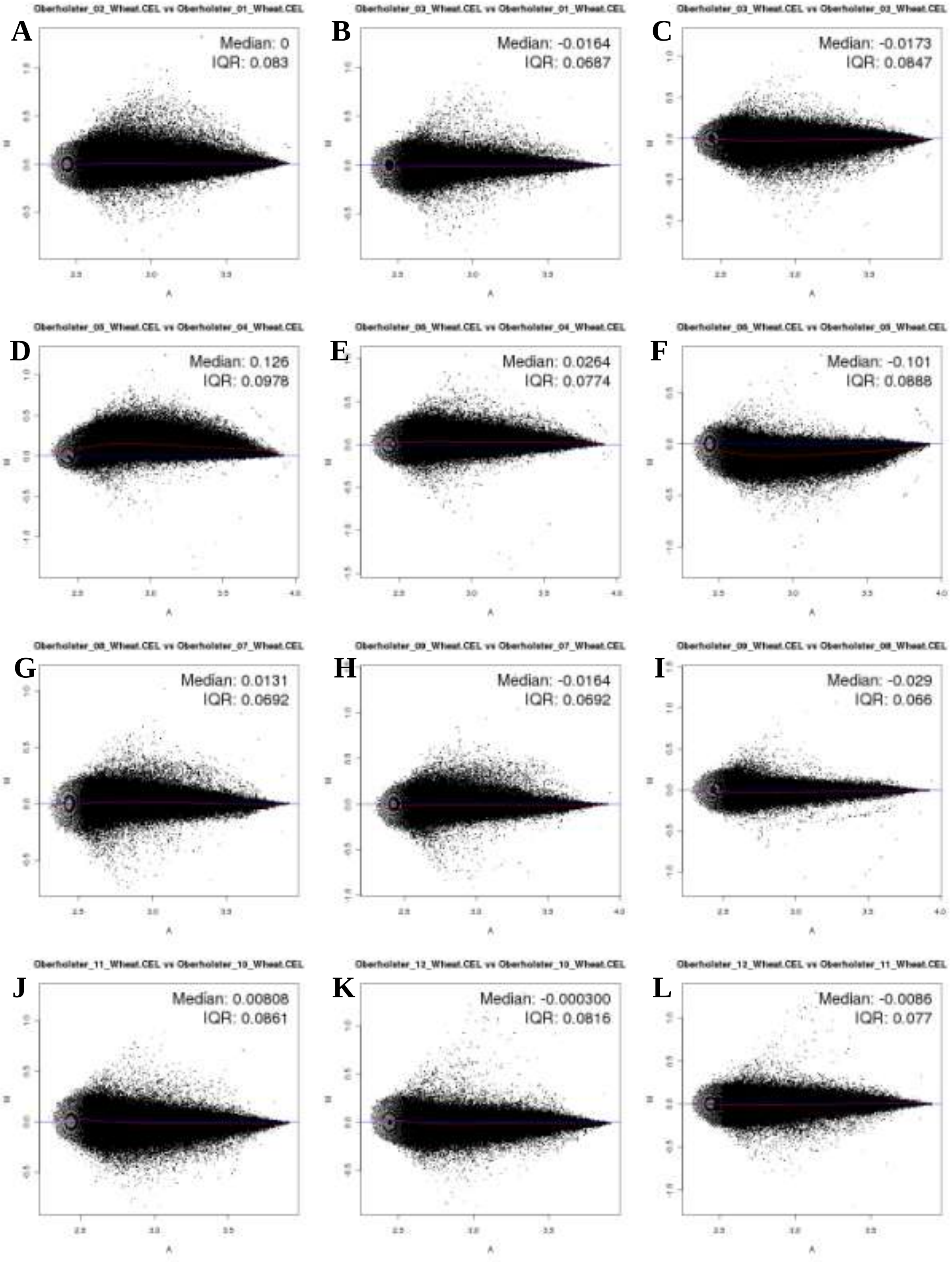


Figure Appx 5.4 **- 18 Slides cont.**

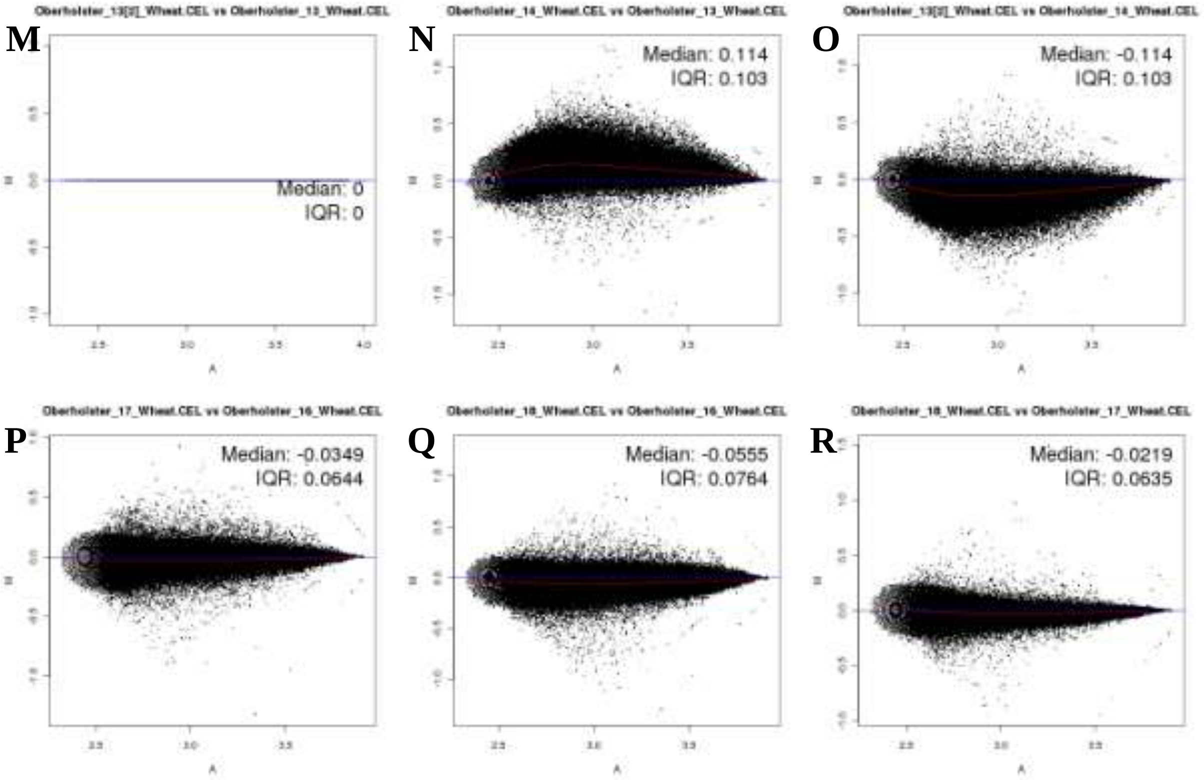
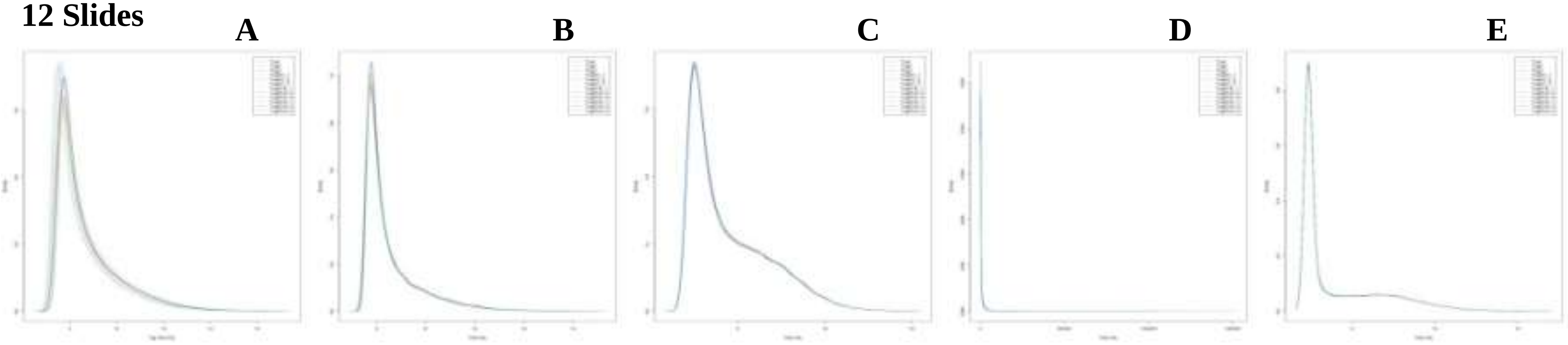


Figure Appx 5.4 MA-plots of the raw, pre-normalized slide comparisons within treatments. The 12 slides files are: (A) Tug1_vs_Tug2_raw.jpg, (B) Tug1_vs_Tug3_raw.jpg, (C) Tug2_vs_Tug3_raw.jpg, (D) TugDn_1_vs_TugDn_2_raw.jpg, (E) TugDn_1_vs_TugDn_3_raw.jpg, (F) TugDn_2_vs_TugDn_3_raw.jpg, (G) TugDn2_1_vs_TugDn2_2_raw.jpg, (H) TugDn2_1_vs_TugDn2_3_raw.jpg, (I) TugDn2_2_vs_TugDn2_3_raw.jpg, (J) TugDn5_1_vs_TugDn5_2_raw.jpg, (K) TugDn5_1_vs_TugDn5_3_raw.jpg, (L) TugDn5_2_vs_TugDn5_ 3_raw.jpg.

The 18 slides files are: (A) RWA2_GS_1_vs_RWA2_GS_2_raw.jpg, (B) RWA2_GS_1_vs_RWA2_GS_3_raw.jpg, (C) RWA2_GS_2_vs_RWA2_GS_3_raw.jpg, (D) RWA2_GR_1_vs_RWA2_GR_2_raw.jpg, (E) RWA2_GR_1_vs_RWA2_GR_3_raw.jpg, (F) RWA2_GR_2_vs_RWA2_GR_3_raw.jpg, (G) RWA1_GR_1_vs_RWA1_GR_2_raw.jpg, (H) RWA1_GR_1_vs_RWA1_GR_3_raw.jpg, (I) RWA1_GR_2_vs_RWA1_GR_3_raw.jpg, (J) RWA1_GS_2_vs_RWA1_GS_1_raw.jpg, (K) RWA1_GS_3_vs_RWA1_GS_1_raw.jpg, (L) RWA1_GS_3_vs_RWA1_GS_2_raw.jpg, (M) Gam_S_1_vs_Gam_S_2_raw.jpg, (N) Gam_S_1_vs_Gam_S_3_raw.jpg, (O) Gam_S_2_vs_Gam_S_3_raw.jpg, (P) Gam_R_1_vs_Gam_R_2_raw.jpg, (Q) Gam_R_1_vs_Gam_R_3_raw.jpg, (R) Gam_R_2_vs_Gam_ R_3_raw.jpg.

Data visualization after background correcting and normalization

12 Slides



18 Slides

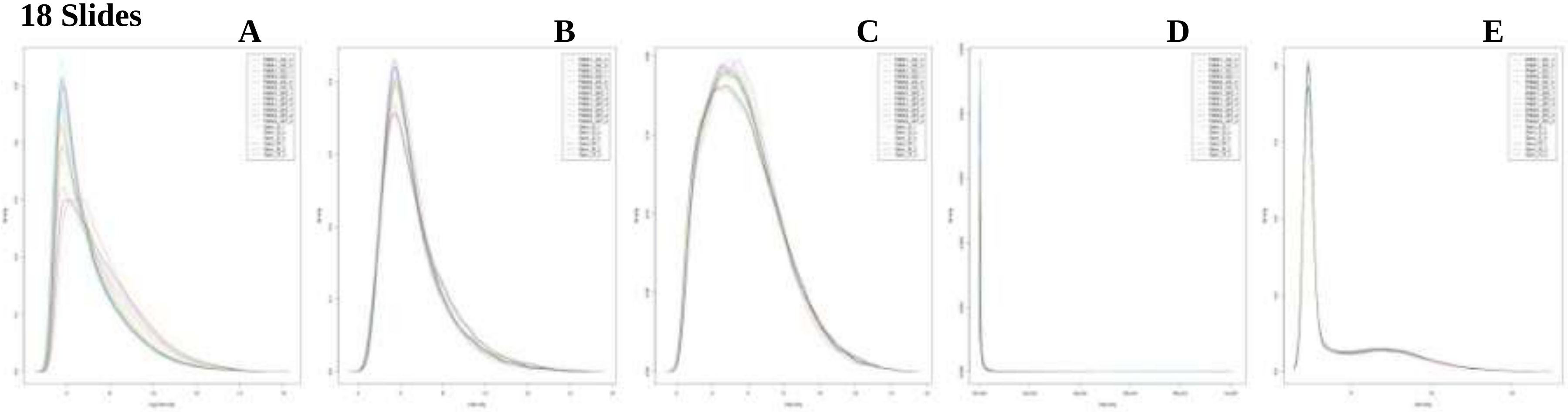


Figure Appx 5.5 Histograms of the slides before ((A) Histogram_rawdata.jpg) and after normalization ((B) Histogram_esetVSN.jpg, (C) Histogram_esetRMA.jpg, (D) Histogram_esetMAS.jpg, (E) Histogram_eset GCRMA.jpg) for the 12 Slide and 18 Slide experiments.

18 Slides

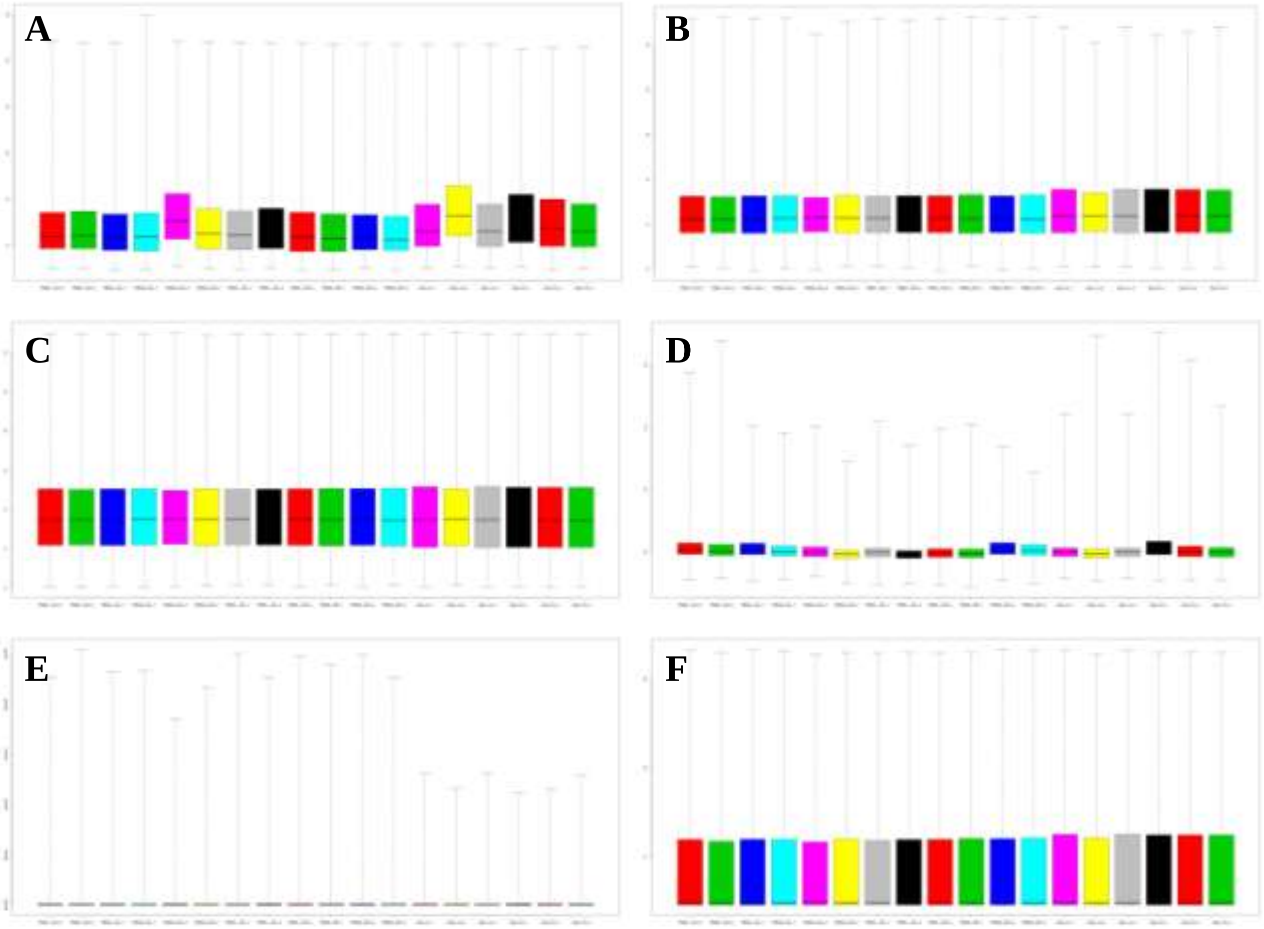


Figure Appx 5.6 Box plots of the $\log_2$(PM) values, before ((A) Boxplot_rawdata.jpg) and after normalization ((B) Boxplot_esetVSN.jpg, (C) Boxplot_esetRMA.jpg, (D) Boxplot_esetPLM.jpg, (E) Boxplot_esetMAS.jpg, (F) Boxplot_esetGCRMA.jpg) for the 12 Slide (see *Figure 5.4*) and the 18 Slide experiment using different statistical approaches. All the sample slides occur in the same order in the various graphs in their corresponding triplicate slides, and are: RWA1_GS_3, RWA1_GS_2, RWA1_GS_1, RWA2_GS_1, RWA2_GS_2, RWA2_GS_3, RWA1_GR_1, RWA1_GR_2, RWA1_GR_3, RWA2_GR_1, RWA2_GR_2, RWA2_GR_3, Gam_S_1, Gam_S_2, Gam_S_3, Gam_R_1, Gam_R_2, Gam_R_3. The Y-axis differs in accordance to the method used.

Figure Appx 5.7 **- 12 Slides**

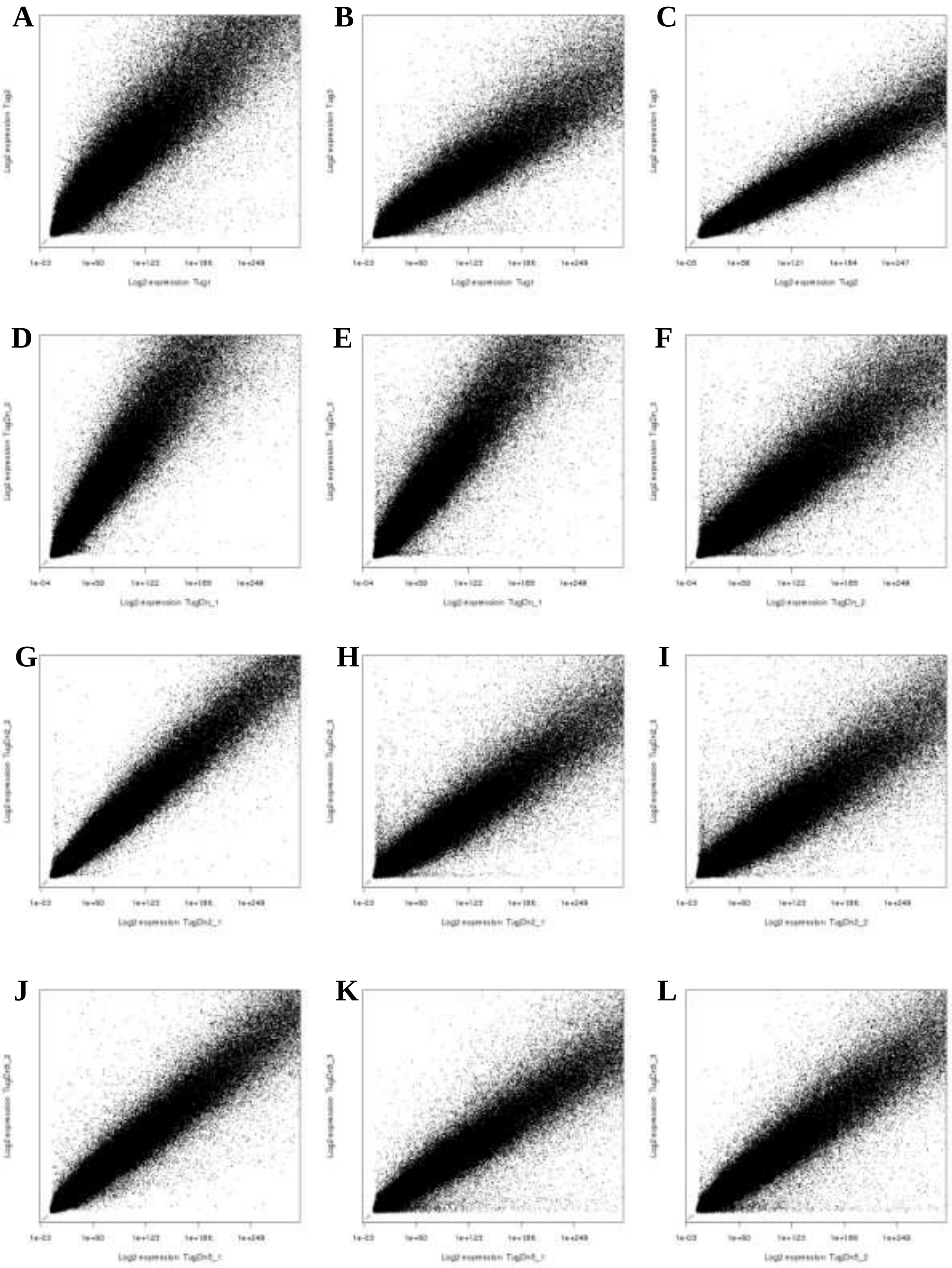


Figure Appx 5.7 - 12 Slides cont.

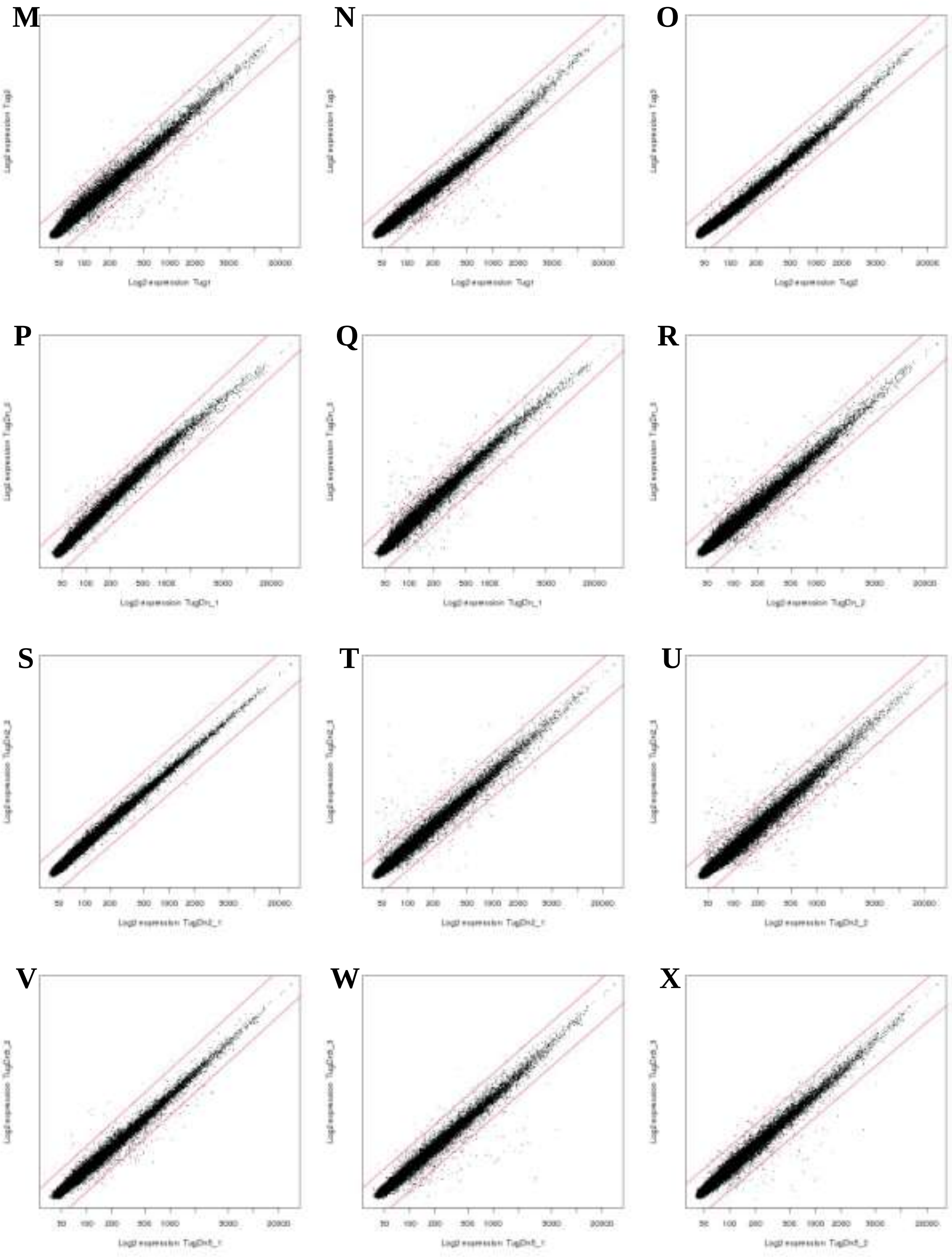


Figure Appx 5.7 **- 12 Slides cont.**

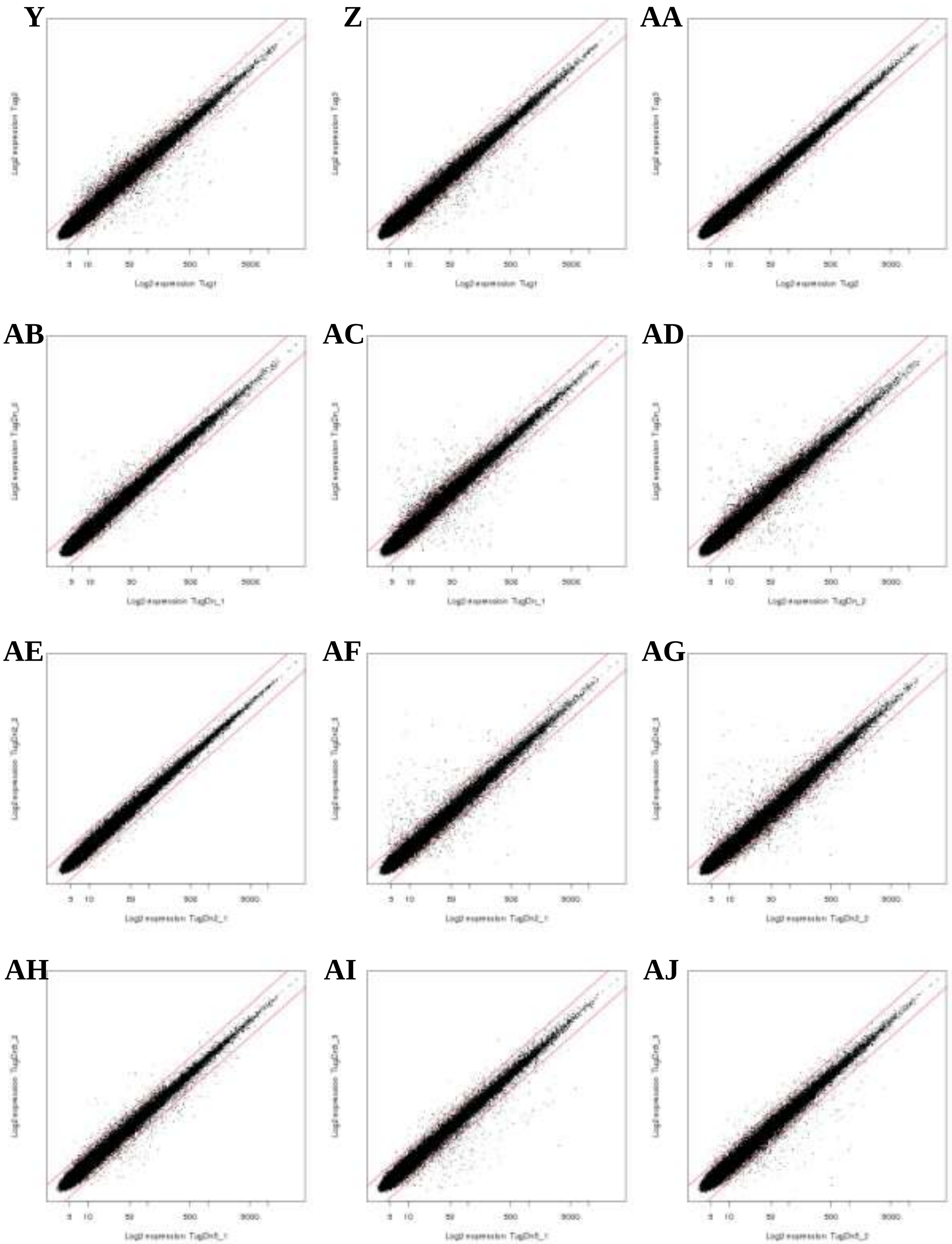


Figure Appx 5.7 - 12 Slides cont.

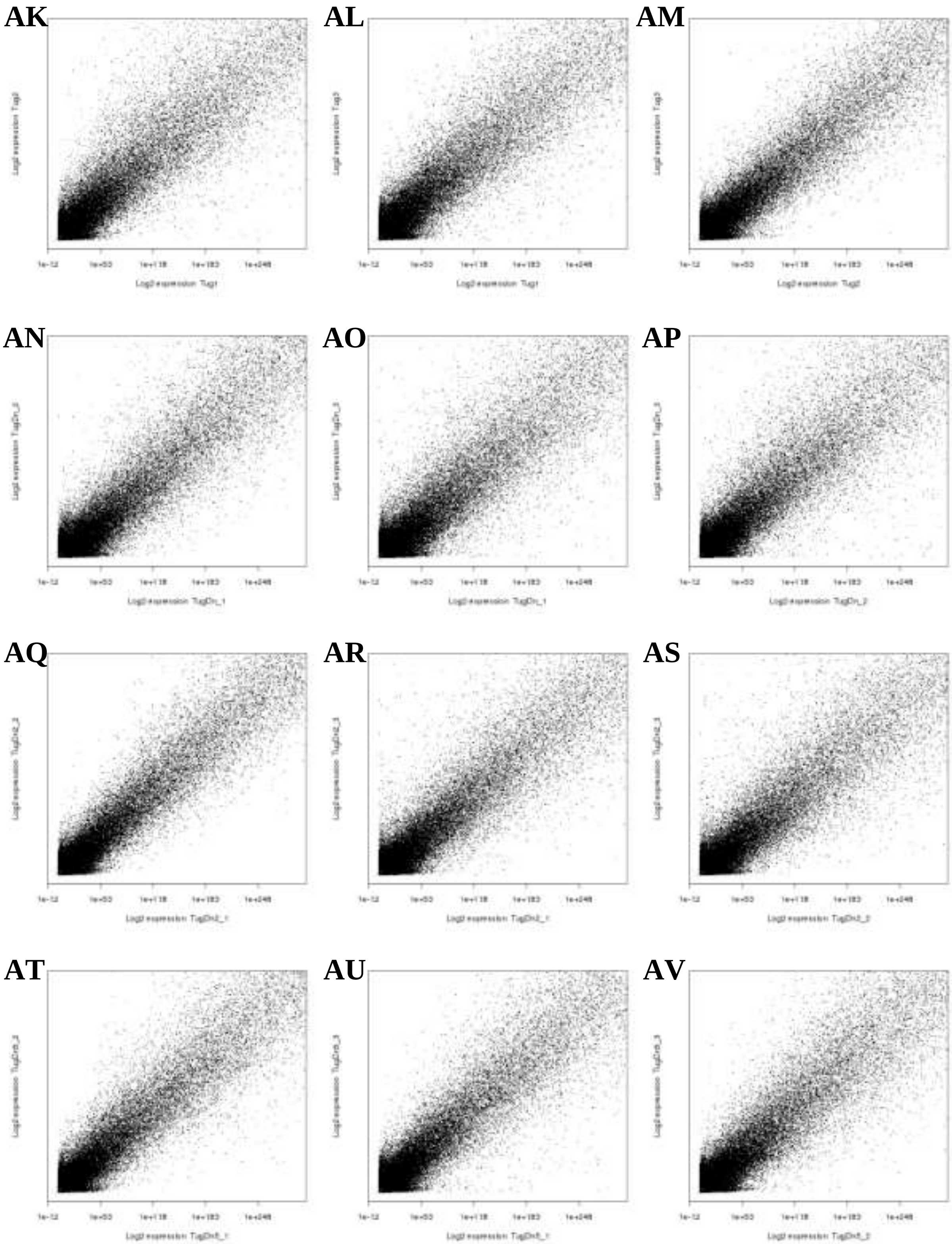


Figure Appx 5.7 **- 12 Slides cont.**

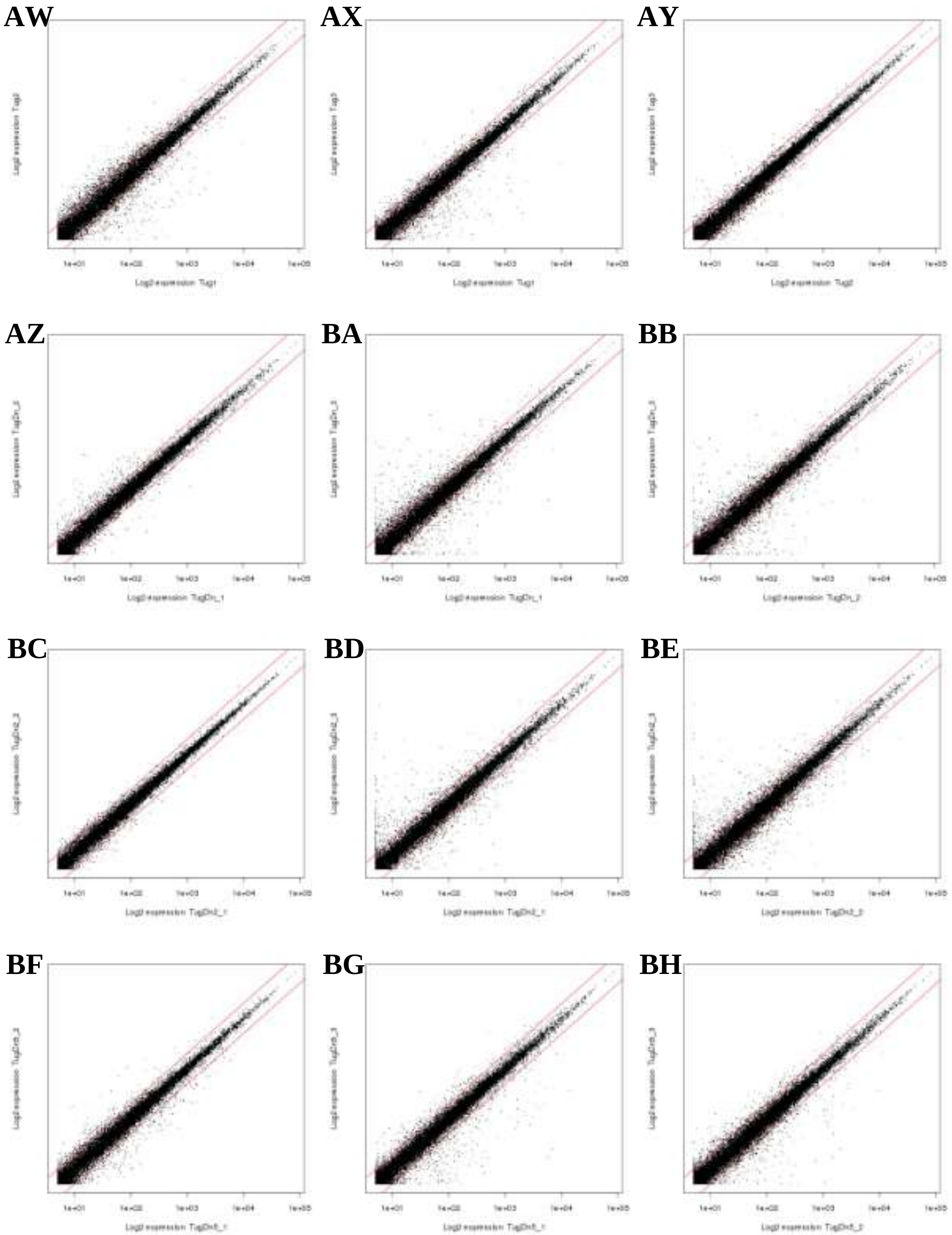


Figure Appx 5.7 **- 18 Slides**

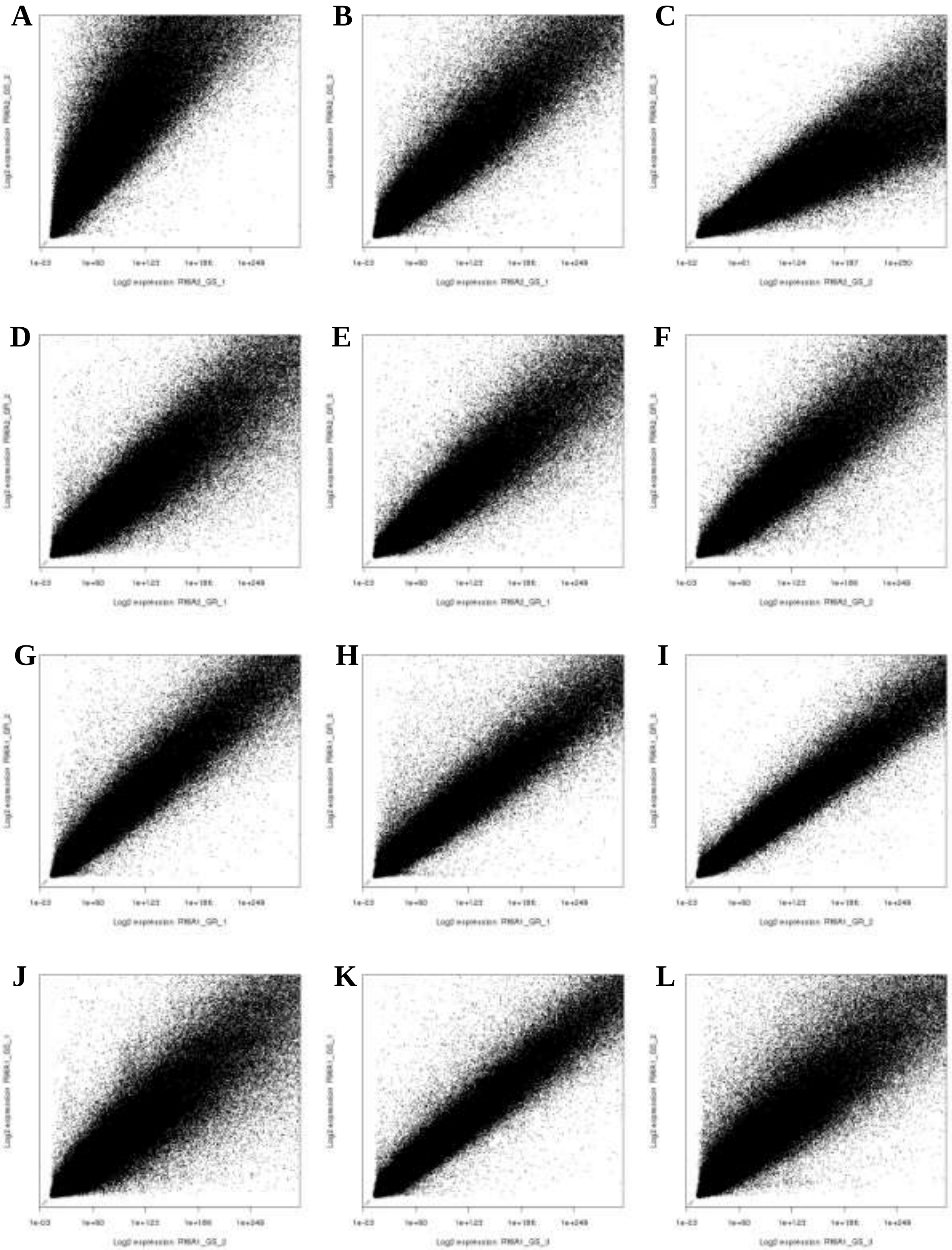


Figure Appx 5.7 - 18 Slides cont.

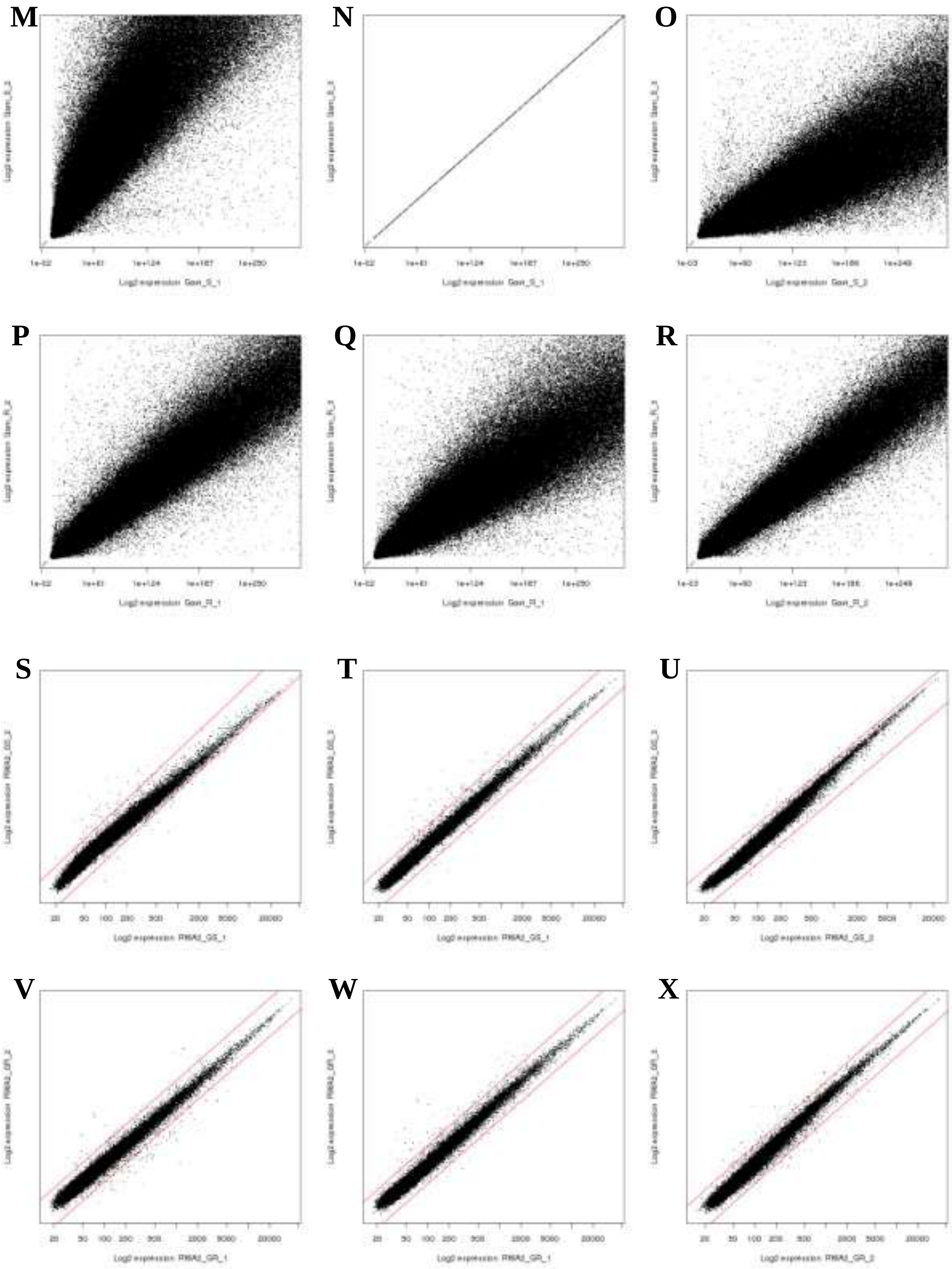


Figure Appx 5.7 **- 18 Slides cont.**

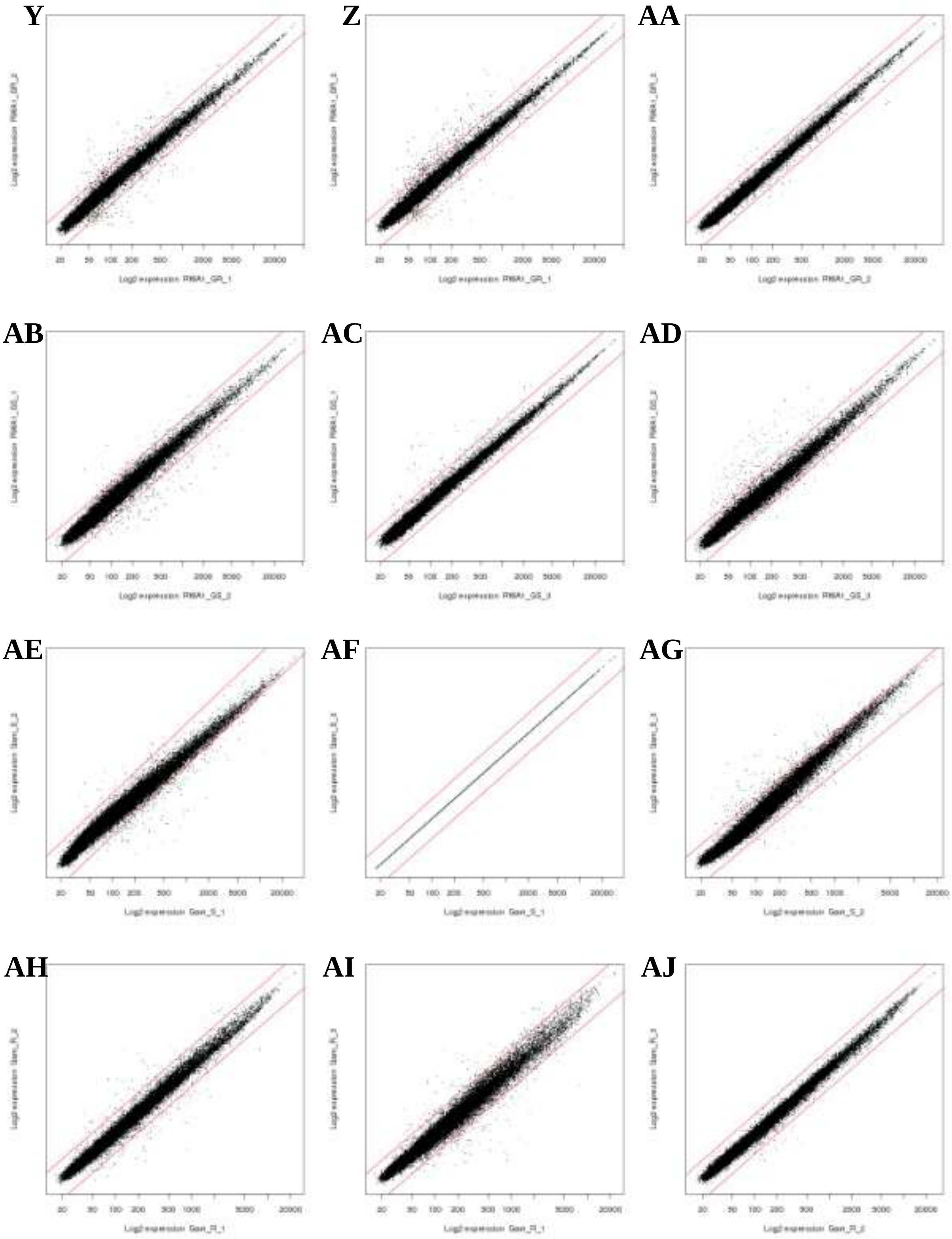


***Figure Appx 5.7* - 18 Slides cont.**

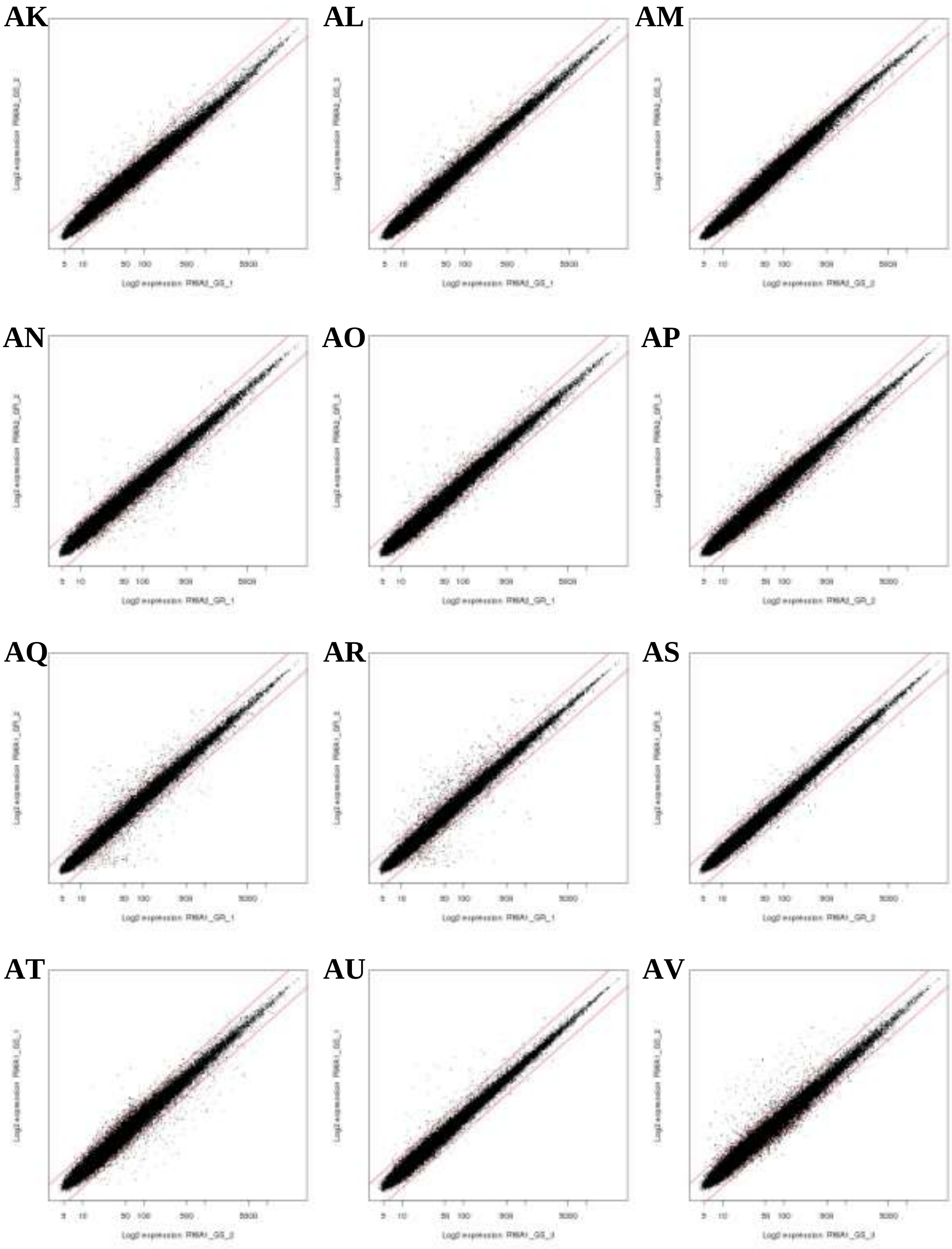


Figure Appx 5.7 - 18 Slides cont.

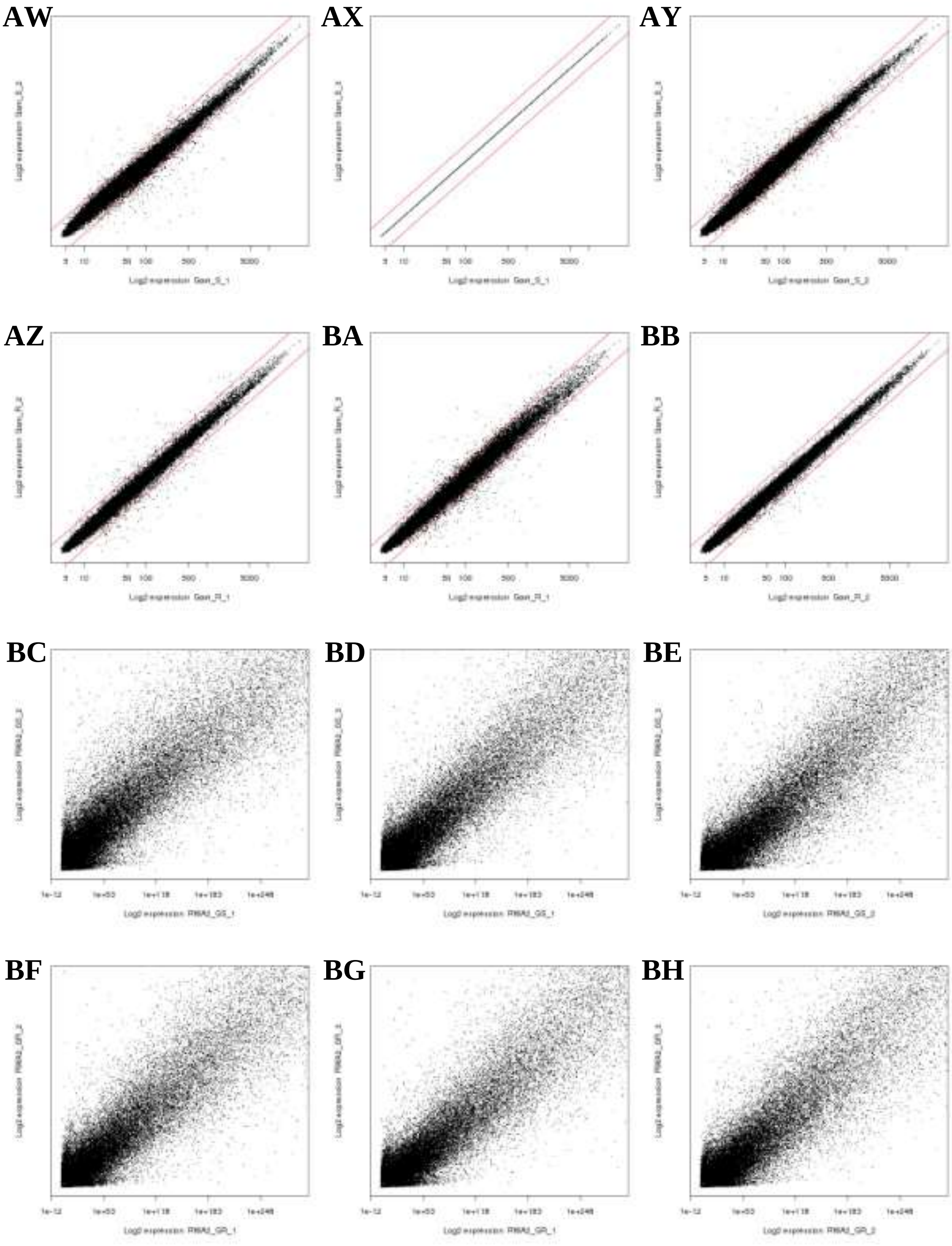


Figure Appx 5.7 - 18 Slides cont.

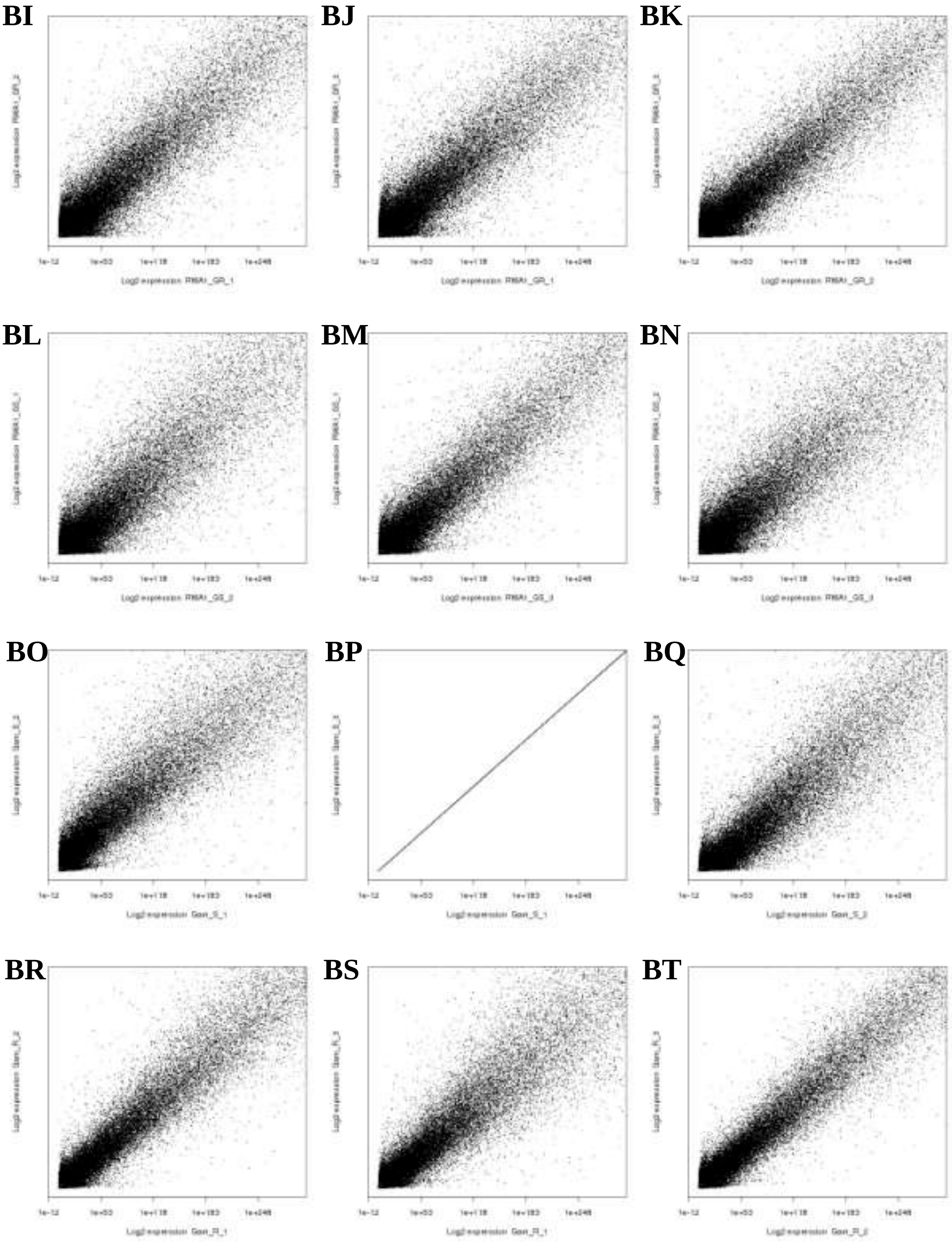


Figure Appx 5.7 - **18 Slides cont.**

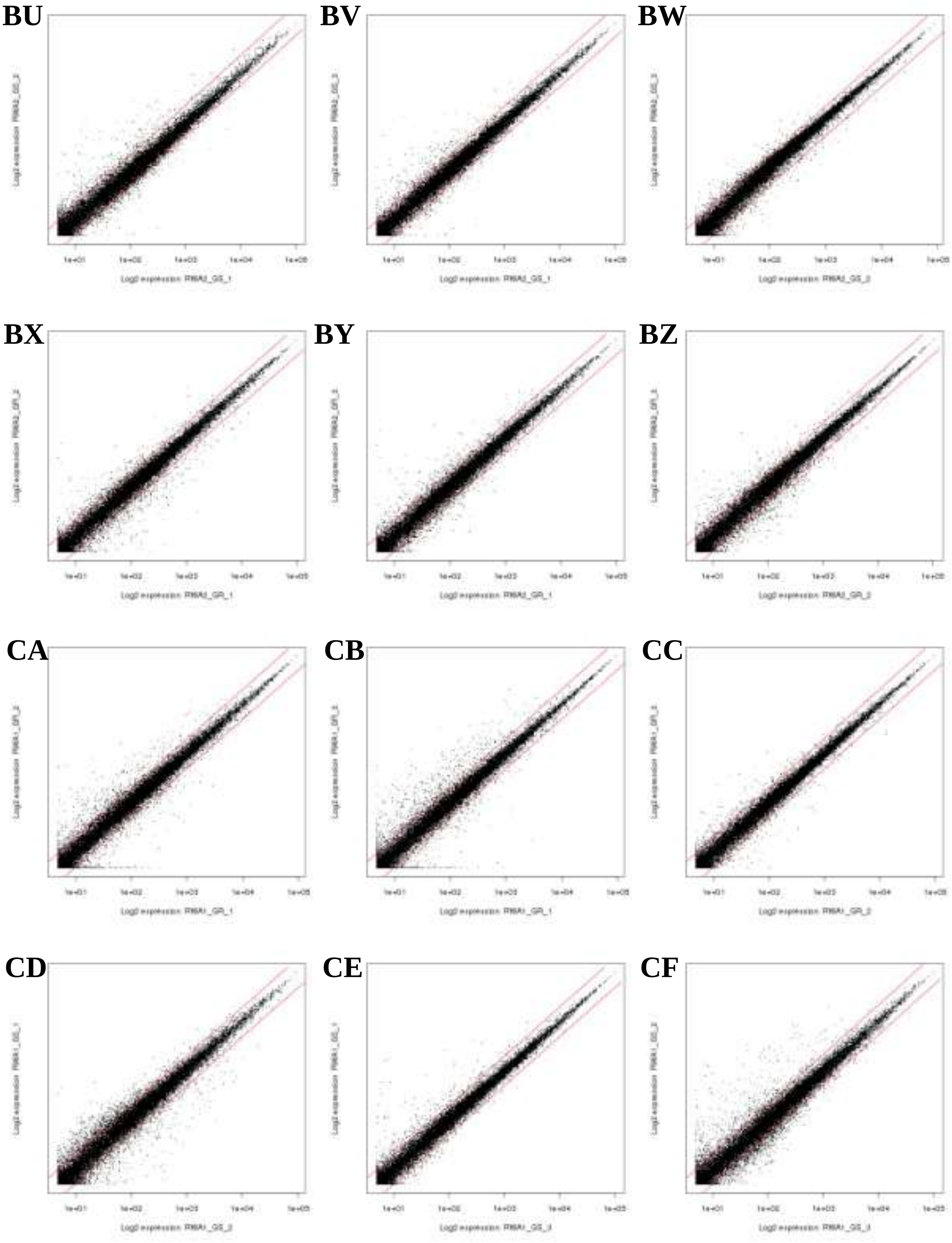


Figure Appx 5.7 **- 18 Slides cont.**

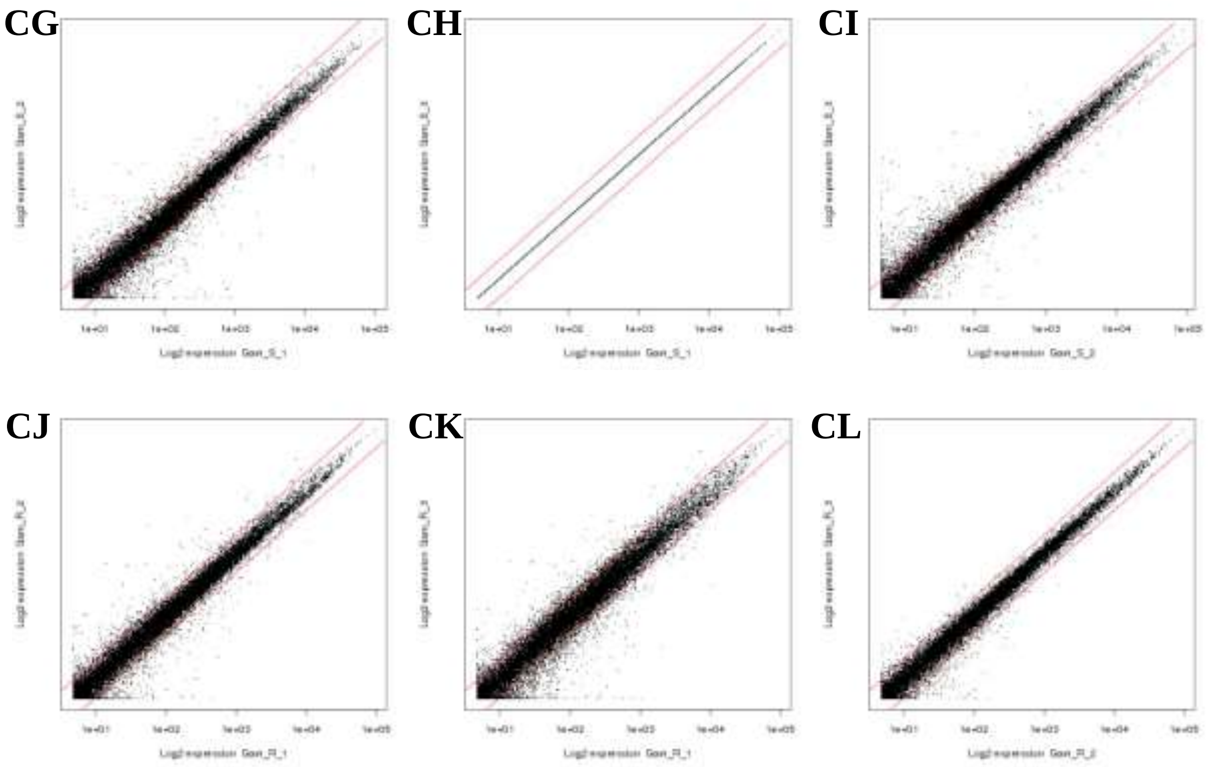


Figure Appx 5.7 The $\log_2$ expression slide comparison plots for all the normalized datasets. The raw/pre-normalized dataset is also produced and included for comparison. The files are written to the folder */R results/Graphs of raw and normalized data/Log2 plots/*. The 12 Slides files are: (A) rawdata_Tug1_vs_Tug2_log2.jpg, (B) rawdata_Tug1_vs_Tug3_log2.jpg, (C) rawdata_Tug2_vs_Tug3_log2.jpg, (D) rawdata_TugDn_1_vs_TugDn_2_log2.jpg, (E) rawdata_TugDn_1_vs_TugDn_3_log2.jpg, (F) rawdata_TugDn_2_vs_TugDn_3_log2.jpg, (G) rawdata_TugDn2_1_vs_TugDn2_2_log2.jpg, (H) rawdata_TugDn2_1_vs_TugDn2_3_log2.jpg, (I) rawdata_TugDn2_2_vs_TugDn2_3_log2.jpg, (J) rawdata_TugDn5_1_vs_TugDn5_2_log2.jpg, (K) rawdata_TugDn5_1_vs_TugDn5_3_log2.jpg, (L) rawdata_TugDn5_2_vs_TugDn5_3_log2.jpg, (M) esetVSN_Tug1_vs_Tug2_log2.jpg, (N) esetVSN_Tug1_vs_Tug3_log2.jpg, (O) esetVSN_Tug2_vs_Tug3_log2.jpg, (P) esetVSN_TugDn_1_vs_TugDn_2_log2.jpg, (Q) esetVSN_TugDn_1_vs_TugDn_3_log2.jpg, (R) esetVSN_TugDn_2_vs_TugDn_3_log2.jpg, (S) esetVSN_TugDn2_1_vs_TugDn2_2_log2.jpg, (T) esetVSN_TugDn2_1_vs_TugDn2_3_log2.jpg, (U) esetVSN_TugDn2_2_vs_TugDn2_3_log2.jpg, (V) esetVSN_TugDn5_1_vs_TugDn5_2_log2.jpg, (W) esetVSN_TugDn5_1_vs_TugDn5_3_log2.jpg, (X) esetVSN_TugDn5_2_vs_TugDn5_3_log2.jpg, (Y) esetRMA_Tug1_vs_Tug2_log2.jpg, (Z) esetRMA_Tug1_vs_Tug3_log2.jpg, (AA) esetRMA_Tug2_vs_Tug3_log2.jpg, (AB) esetRMA_TugDn_1_vs_TugDn_2_log2.jpg, (AC) esetRMA_TugDn_1_vs_TugDn_3_log2.jpg, (AD) esetRMA_-

TugDn_2_vs_TugDn_3_log2.jpg, (AE) esetRMA_TugDn2_1_vs_TugDn2_2_log2.jpg, (AF) esetRMA_TugDn2_1_vs_TugDn2_3_log2.jpg, (AG) esetRMA_TugDn2_2_vs_TugDn2_3_log2.jpg, (AH) esetRMA_TugDn5_1_vs_TugDn5_2_log2.jpg, (AI) esetRMA_TugDn5_1_vs_TugDn5_3_log2.jpg, (AJ) esetRMA_TugDn5_2_vs_TugDn5_3_log2.jpg, (AK) esetMAS_Tug1_vs_Tug2_log2.jpg, (AL) esetMAS_Tug1_vs_Tug3_log2.jpg, (AM) esetMAS_Tug2_vs_Tug3_log2.jpg, (AN) esetMAS_TugDn_1_ vs_TugDn_2_log2.jpg, (AO) esetMAS_TugDn_1_vs_TugDn_3_log2.jpg, (AP) esetMAS_TugDn_2_vs_TugDn_3_log2.jpg, (AQ) esetMAS_TugDn2_1_vs_TugDn2_2_log2.jpg, (AR) esetMAS_TugDn2_1_vs_TugDn2_3_log2.jpg, (AS) esetMAS_TugDn2_2_vs_TugDn2_3_log2.jpg, (AT) esetMAS_TugDn5_1_vs_TugDn5_2_log2.jpg, (AU) esetMAS_TugDn5_1_vs_TugDn5_3_log2.jpg, (AV) esetMAS_TugDn5_2_vs_TugDn5_3_log2.jpg, (AW) esetGCRMA_Tug1_vs_Tug2_log2.jpg, (AX) esetGCRMA_Tug1_vs_Tug3_log2.jpg, (AY) esetGCRMA_Tug2_vs_Tug3_log2.jpg, (AZ) esetGCRMA_TugDn_1_vs_TugDn_2_log2.jpg, (BA) esetGCRMA_TugDn_1_vs_TugDn_3_log2.jpg, (BB) esetGCRMA_TugDn_2_vs_TugDn_3_log2.jpg, (BC) esetGCRMA_TugDn2_1_vs_TugDn2_2_log2.jpg, (BD) esetGCRMA_TugDn2_1_vs_TugDn2_3_log2.jpg, (BE) esetGCRMA_TugDn2_2_vs_TugDn2_3_log2.jpg, (BF) esetGCRMA_TugDn5_1_vs_TugDn5_2_log2.jpg, (BG) esetGCRMA_TugDn5_1_vs_TugDn5_3_log2.jpg, (BH) esetGCRMA_TugDn5_2_vs_TugDn5_3_log2.jpg.

The 18 Slides files are: (A) rawdata_RWA2_GS_1_vs_RWA2_GS_2_log2.jpg, (B) rawdata_RWA2_GS_1_vs_RWA2_GS_3_log2.jpg, (C) rawdata_RWA2_GS_2_vs_RWA2_GS_3_log2.jpg, (D) rawdata_RWA2_GR_1_vs_RWA2_GR_2_log2.jpg, (E) rawdata_RWA2_GR_1_vs_RWA2_GR_3_log2.jpg, (F) rawdata_RWA2_GR_2_vs_RWA2_GR_3_log2.jpg, (G) rawdata_RWA1_GR_1_vs_RWA1_GR_2_log2.jpg, (H) rawdata_RWA1_GR_1_vs_RWA1_GR_3_log2.jpg, (I) rawdata_RWA1_GR_2_vs_RWA1_GR_3_log2.jpg, (J) rawdata_RWA1_GS_2_vs_RWA1_GS_1_log2.jpg, (K) rawdata_RWA1_GS_3_vs_RWA1_GS_1_log2.jpg, (L) rawdata_RWA1_GS_3_vs_RWA1_GS_2_log2.jpg, (M) rawdata_Gam_S_1_vs_Gam_S_2_log2.jpg, (N) rawdata_Gam_S_1_vs_Gam_S_3_log2.jpg, (O) rawdata_Gam_S_2_vs_Gam_S_3_log2.jpg, (P) rawdata_Gam_R_1_vs_Gam_R_2_log2.jpg, (Q) rawdata_Gam_R_1_vs_Gam_R_3_log2.jpg, (R) rawdata_Gam_R_2_vs_Gam_R_3_log2.jpg, (S) esetVSN_RWA2_GS_1_vs_RWA2_GS_2_log2.jpg, (T) esetVSN_RWA2_GS_1_vs_RWA2_GS_3_log2.jpg, (U) esetVSN_RWA2_GS_2_vs_RWA2_GS_3_log2.jpg, (V) esetVSN_RWA2_GR_1_vs_RWA2_GR_2_log2.jpg, (W) esetVSN_RWA2_GR_1_vs_RWA2_GR_3_log2.jpg, (X) esetVSN_RWA2_GR_2_vs_RWA2_GR_3_log2.jpg, (Y) esetVSN_RWA1_GR_1_vs_RWA1_GR_2_log2.jpg, (Z) esetVSN_RWA1_GR_1_vs_RWA1_GR_3_log2.-

jpg, (AA) esetVSN_RWA1_GR_2_vs_RWA1_GR_3_log2.jpg, (AB) esetVSN_RWA1_GS_2_vs_RWA1_GS_1_log2.jpg, (AC) esetVSN_RWA1_GS_3_vs_RWA1_GS_1_log2.jpg, (AD) esetVSN_RWA1_GS_3_vs_RWA1_GS_2_log2.jpg, (AE) esetVSN_Gam_S_1_vs_Gam_S_2_log2.jpg, (AF) esetVSN_Gam_S_1_vs_Gam_S_3_log2.jpg, (AG) esetVSN_Gam_S_2_vs_Gam_S_3_log2.jpg, (AH) esetVSN_Gam_R_1_vs_Gam_R_2_log2.jpg, (AI) esetVSN_Gam_R_1_vs_Gam_R_3_log2.jpg, (AJ) esetVSN_Gam_R_2_vs_Gam_R_3_log2.jpg, (AK) esetRMA_RWA2_GS_1_vs_RWA2_GS_2_log2.jpg, (AL) esetRMA_RWA2_GS_1_vs_RWA2_GS_3_log2.jpg, (AM) esetRMA_RWA2_GS_2_vs_RWA2_GS_3_log2.jpg, (AN) esetRMA_RWA2_GR_1_vs_RWA2_GR_2_log2.jpg, (AO) esetRMA_RWA2_GR_1_vs_RWA2_GR_3_log2.jpg, (AP) esetRMA_RWA2_GR_2_vs_RWA2_GR_3_log2.jpg, (AQ) esetRMA_RWA1_GR_1_vs_RWA1_GR_2_log2.jpg, (AR) esetRMA_RWA1_GR_1_vs_RWA1_GR_3_log2.jpg, (AS) esetRMA_RWA1_GR_2_vs_RWA1_GR_3_log2.jpg, (AT) esetRMA_RWA1_GS_2_vs_RWA1_GS_1_log2.jpg, (AU) esetRMA_RWA1_GS_3_vs_RWA1_GS_1_log2.jpg, (AV) esetRMA_RWA1_GS_3_vs_RWA1_GS_2_log2.jpg, (AW) esetRMA_Gam_S_1_vs_Gam_S_2_log2.jpg, (AX) esetRMA_Gam_S_1_vs_Gam_S_3_log2.jpg, (AY) esetRMA_Gam_S_2_vs_Gam_S_3_log2.jpg, (AZ) esetRMA_Gam_R_1_vs_Gam_R_2_log2.jpg, (BA) esetRMA_Gam_R_1_vs_Gam_R_3_log2.jpg, (BB) esetRMA_Gam_R_2_vs_Gam_R_3_log2.jpg, (BC) esetMAS_RWA2_GS_1_vs_RWA2_GS_2_log2.jpg, (BD) esetMAS_RWA2_GS_1_vs_RWA2_GS_3_log2.jpg, (BE) esetMAS_RWA2_GS_2_vs_RWA2_GS_3_log2.jpg, (BF) esetMAS_RWA2_GR_1_vs_RWA2_GR_2_log2.jpg, (BG) esetMAS_RWA2_GR_1_vs_RWA2_GR_3_log2.jpg, (BH) esetMAS_RWA2_GR_2_vs_RWA2_GR_3_log2.jpg, (BI) esetMAS_RWA1_GR_1_vs_RWA1_GR_2_log2.jpg, (BJ) esetMAS_RWA1_GR_1_vs_RWA1_GR_3_log2.jpg, (BK) esetMAS_RWA1_GR_2_vs_RWA1_GR_3_log2.jpg, (BL) esetMAS_RWA1_GS_2_vs_RWA1_GS_1_log2.jpg, (BM) esetMAS_RWA1_GS_3_vs_RWA1_GS_1_log2.jpg, (BN) esetMAS_RWA1_GS_3_vs_RWA1_GS_2_log2.jpg, (BO) esetMAS_Gam_S_1_vs_Gam_S_2_log2.jpg, (BP) esetMAS_Gam_S_1_vs_Gam_S_3_log2.jpg, (BQ) esetMAS_Gam_S_2_vs_Gam_S_3_log2.jpg, (BR) esetMAS_Gam_R_1_vs_Gam_R_2_log2.jpg, (BS) esetMAS_Gam_R_1_vs_Gam_R_3_log2.jpg, (BT) esetMAS_Gam_R_2_vs_Gam_R_3_log2.jpg, (BU) esetGCRMA_RWA2_GS_1_vs_RWA2_GS_2_log2.jpg, (BV) esetGCRMA_RWA2_GS_1_vs_RWA2_GS_3_log2.jpg, (BW) esetGCRMA_RWA2_GS_2_vs_RWA2_GS_3_log2.jpg, (BX) esetGCRMA_RWA2_GR_1_vs_RWA2_GR_2_log2.jpg, (BY) esetGCRMA_RWA2_GR_1_vs_RWA2_GR_3_log2.jpg, (BZ) esetGCRMA_RWA2_GR_2_vs_RWA2_GR_3_log2.jpg, (CA) esetGCRMA_RWA1_GR_1_vs_RWA1_GR_2_log2.jpg, (CB) esetGCRMA_RWA1_GR_1_vs_

RWA1_GR_3_log2.jpg, (CC) esetGCRMA_RWA1_GR_2_vs_RWA1_GR_3_log2.jpg, (CD) esetGCRMA_RWA1_GS_2_vs_RWA1_GS_1_log2.jpg, (CE) esetGCRMA_RWA1_GS_3_vs_RWA1_GS_1_log2.jpg, (CF) esetGCRMA_RWA1_GS_3_vs_RWA1_GS_2_log2.jpg, (CG) esetGCRMA_Gam_S_1_vs_Gam_S_2_log2.jpg, (CH) esetGCRMA_Gam_S_1_vs_Gam_S_3_log2.jpg, (CI) esetGCRMA_Gam_S_2_vs_Gam_S_3_log2.jpg, (CJ) esetGCRMA_Gam_R_1_vs_Gam_R_2_log2.jpg, (CK) esetGCRMA_Gam_R_1_vs_Gam_R_3_log2.jpg, (CL) esetGCRMA_Gam_R_2_vs_Gam_R_3_log2.jpg.

Figure Appx 5.8 **- 12 Slides**

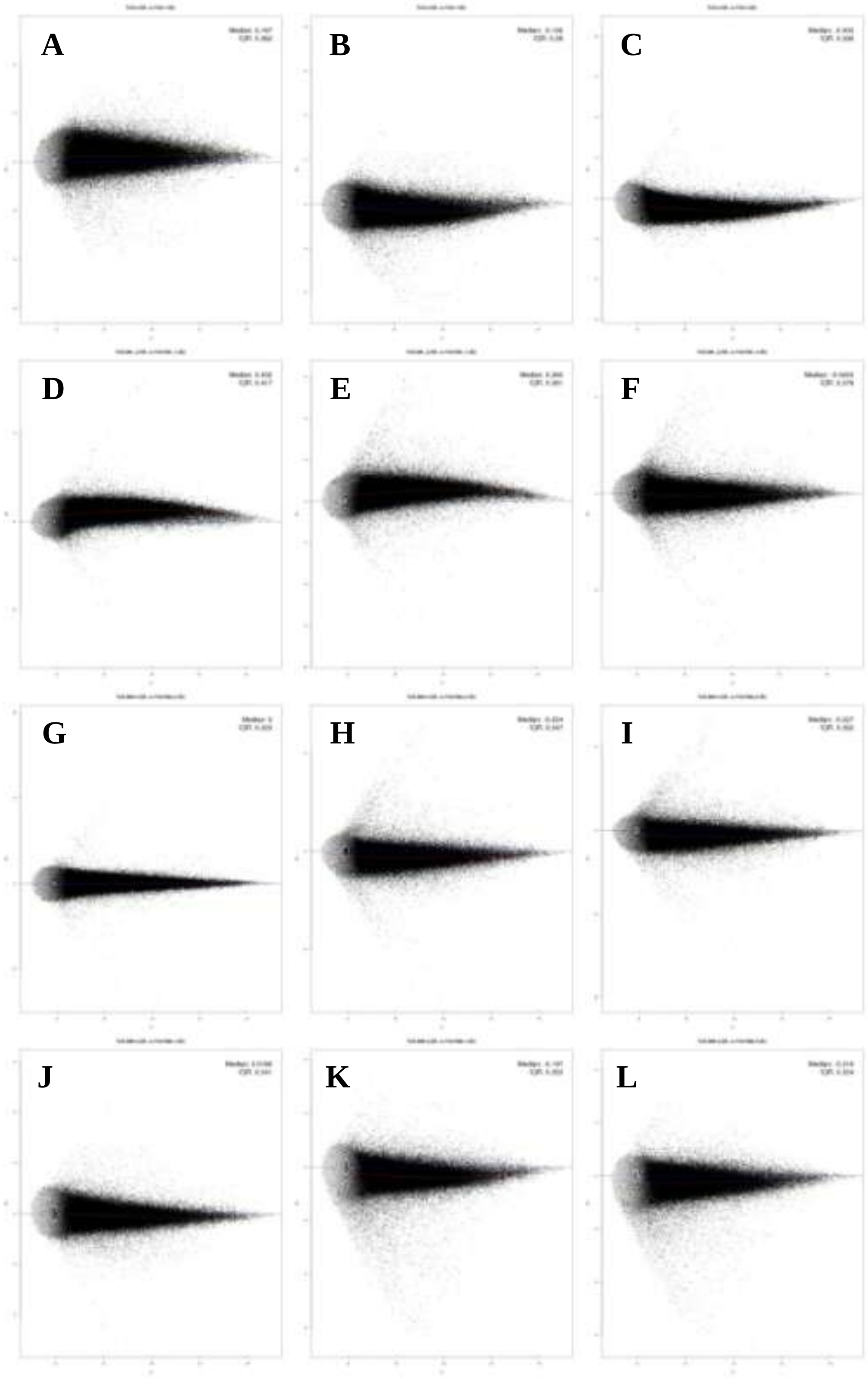


Figure Appx 5.8 **- 12 Slides cont.**

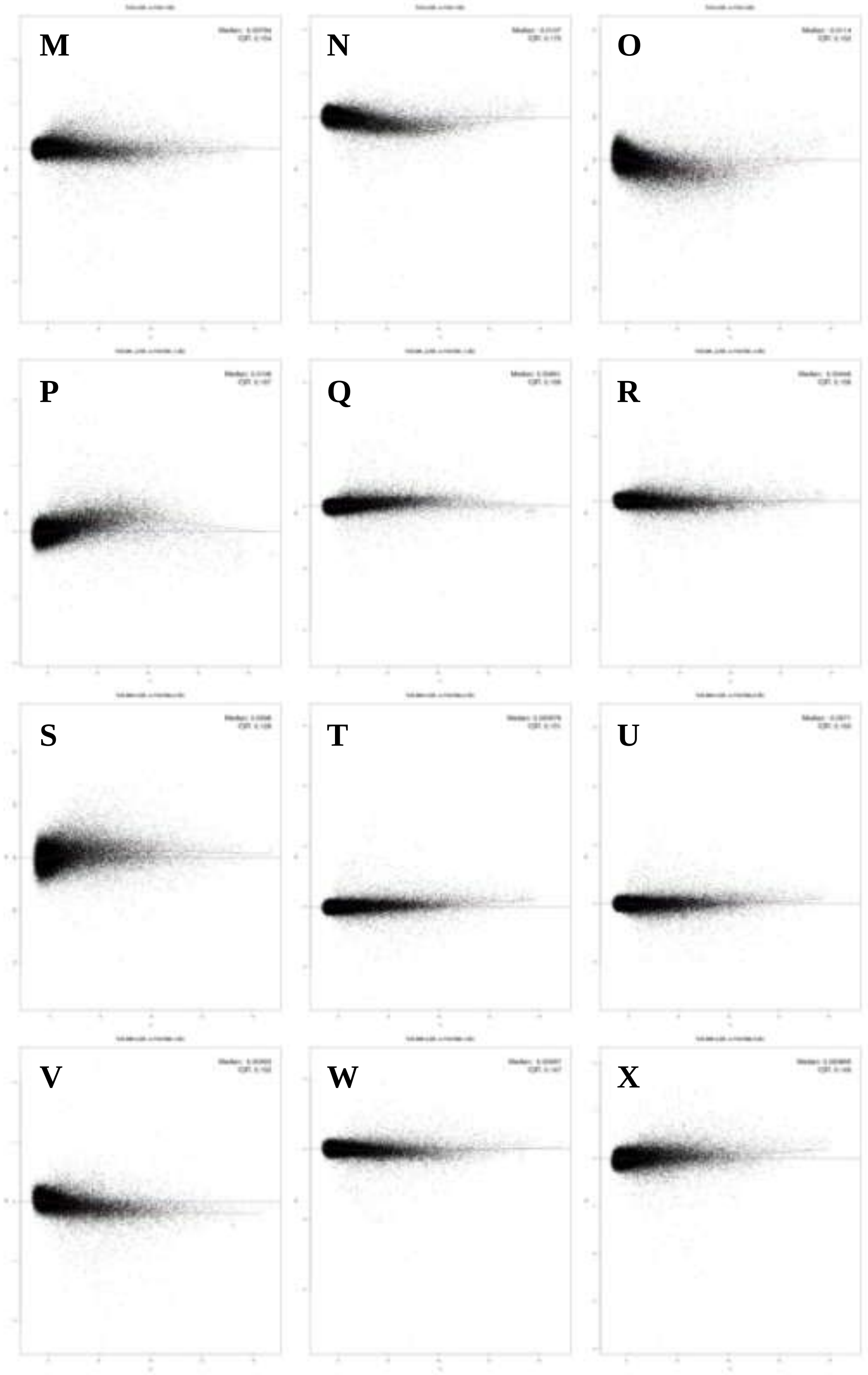


Figure Appx 5.8 **- 12 Slides cont.**

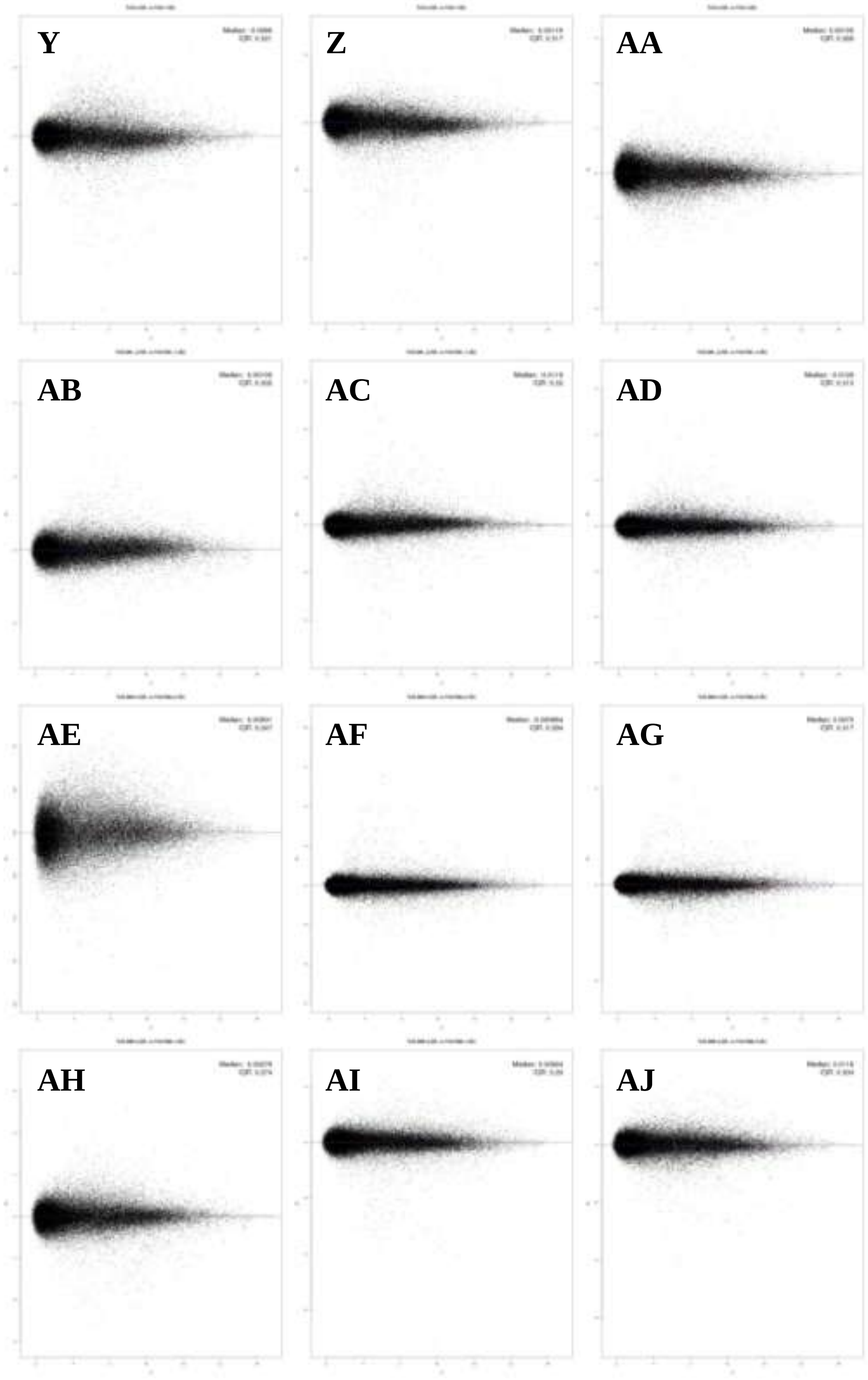


***Figure Appx 5.8* - 12 Slides cont.**

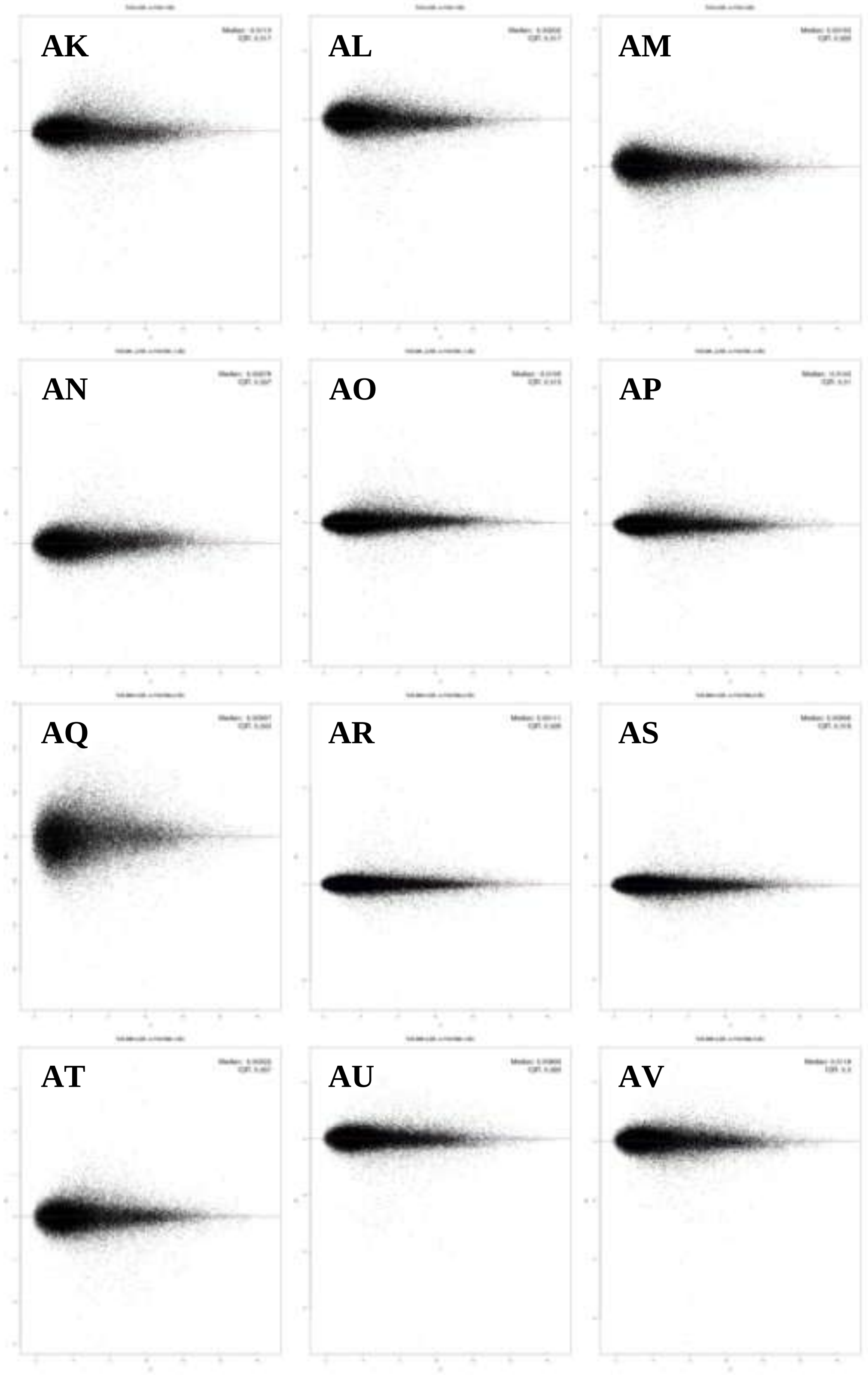


***Figure Appx 5.8* - 12 Slides cont.**

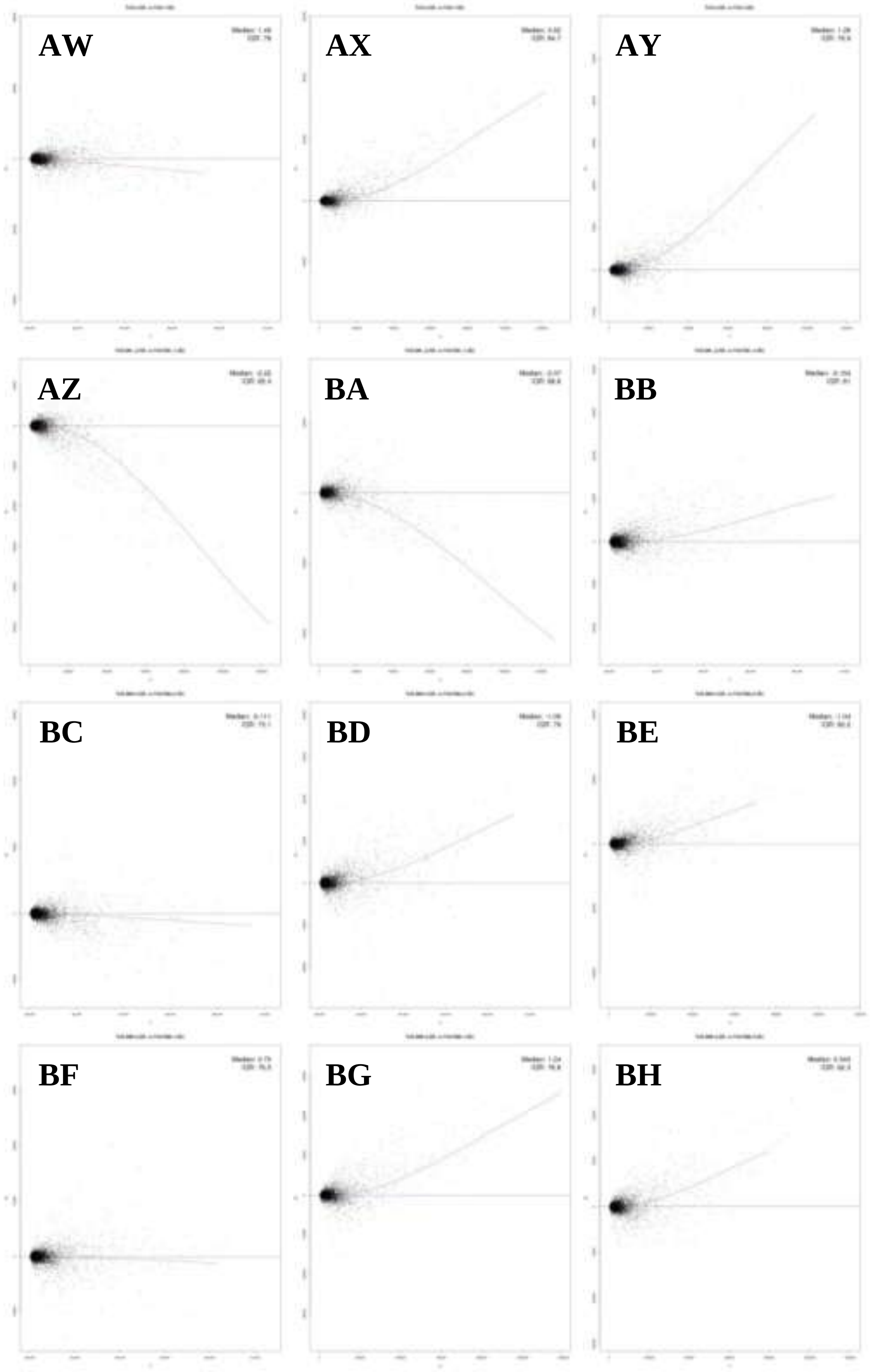


Figure Appx 5.8 - 12 Slides cont.

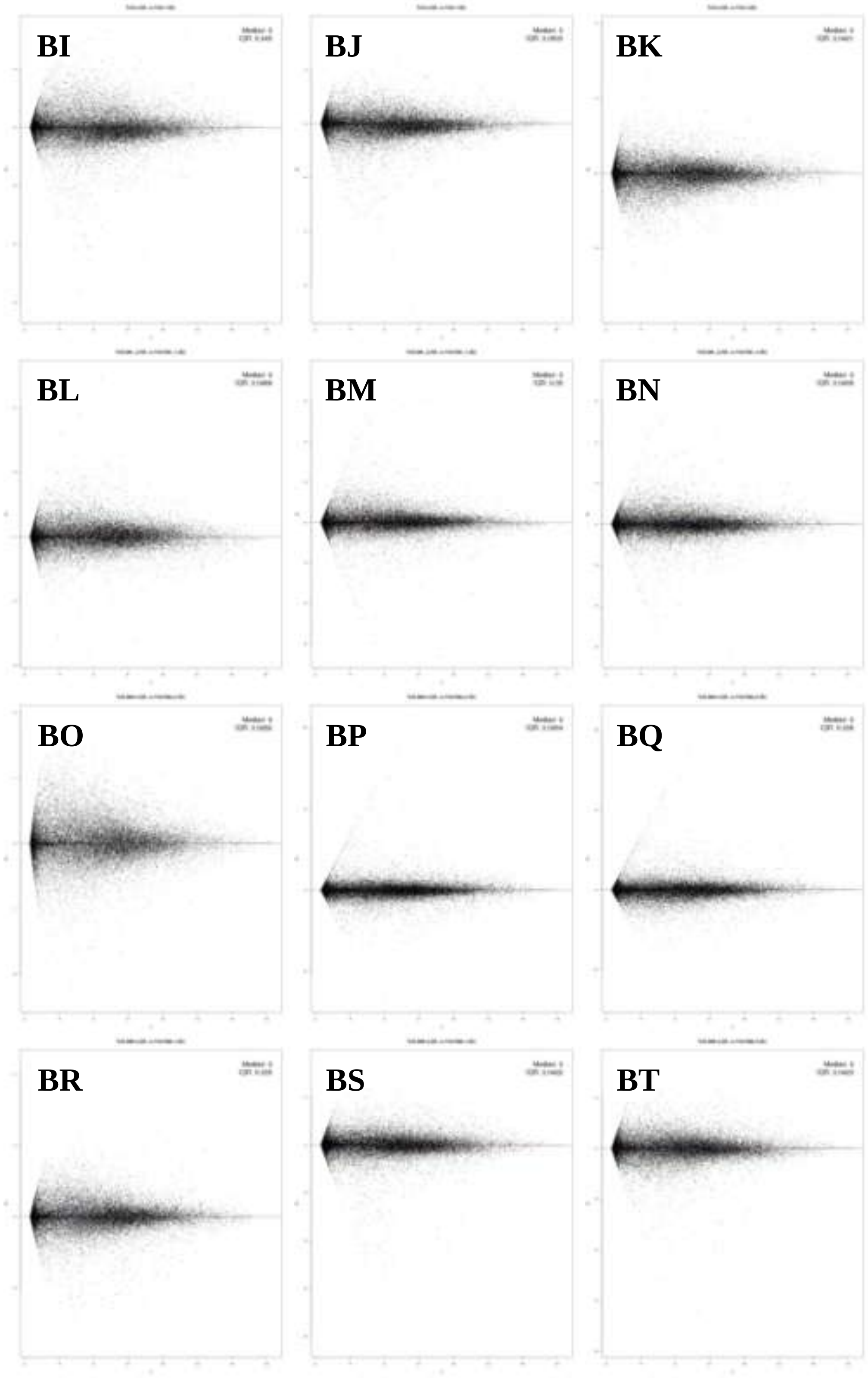


Figure Appx 5.8 **- 18 Slides**

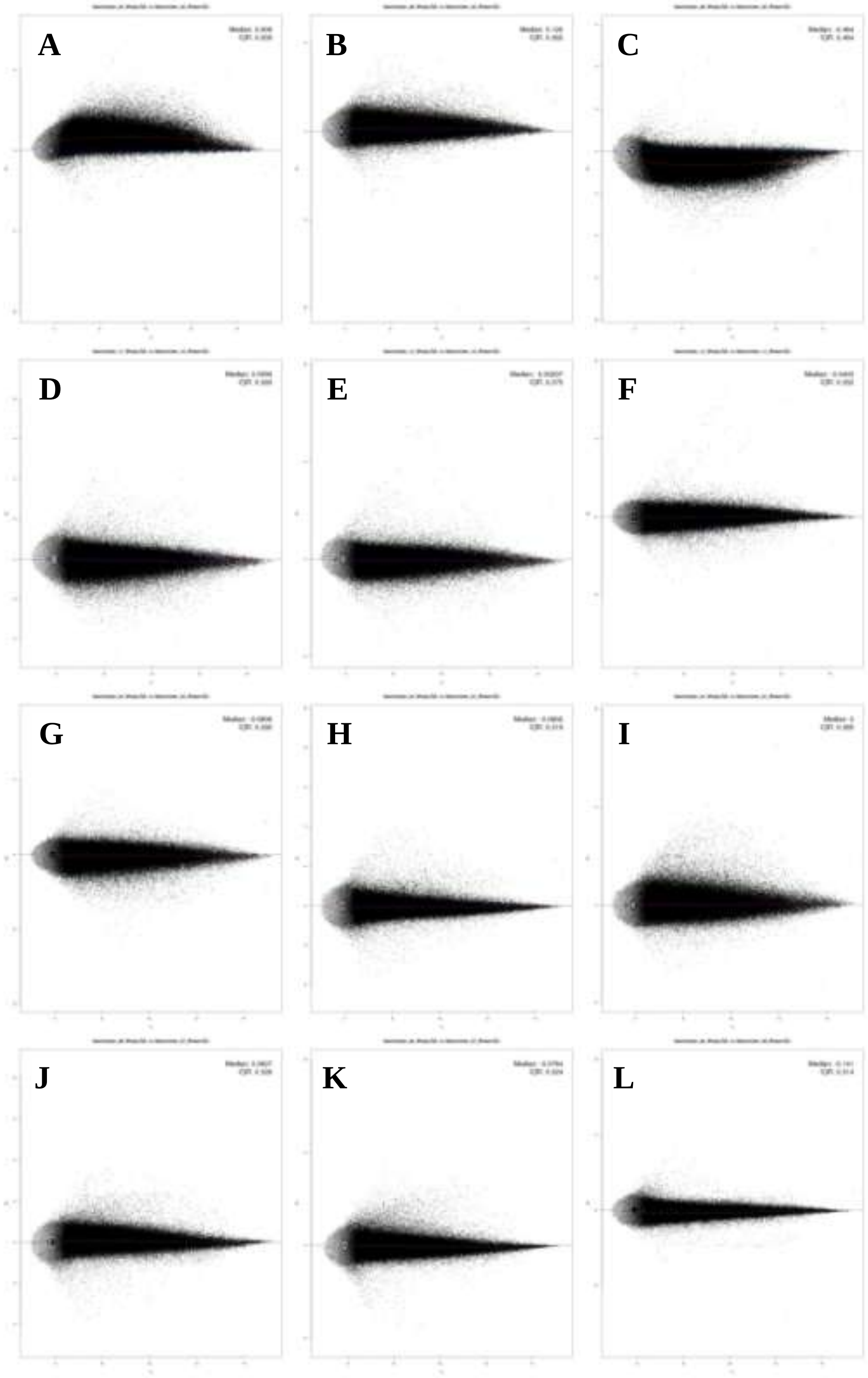


Figure Appx 5.8 - **18 Slides cont.**

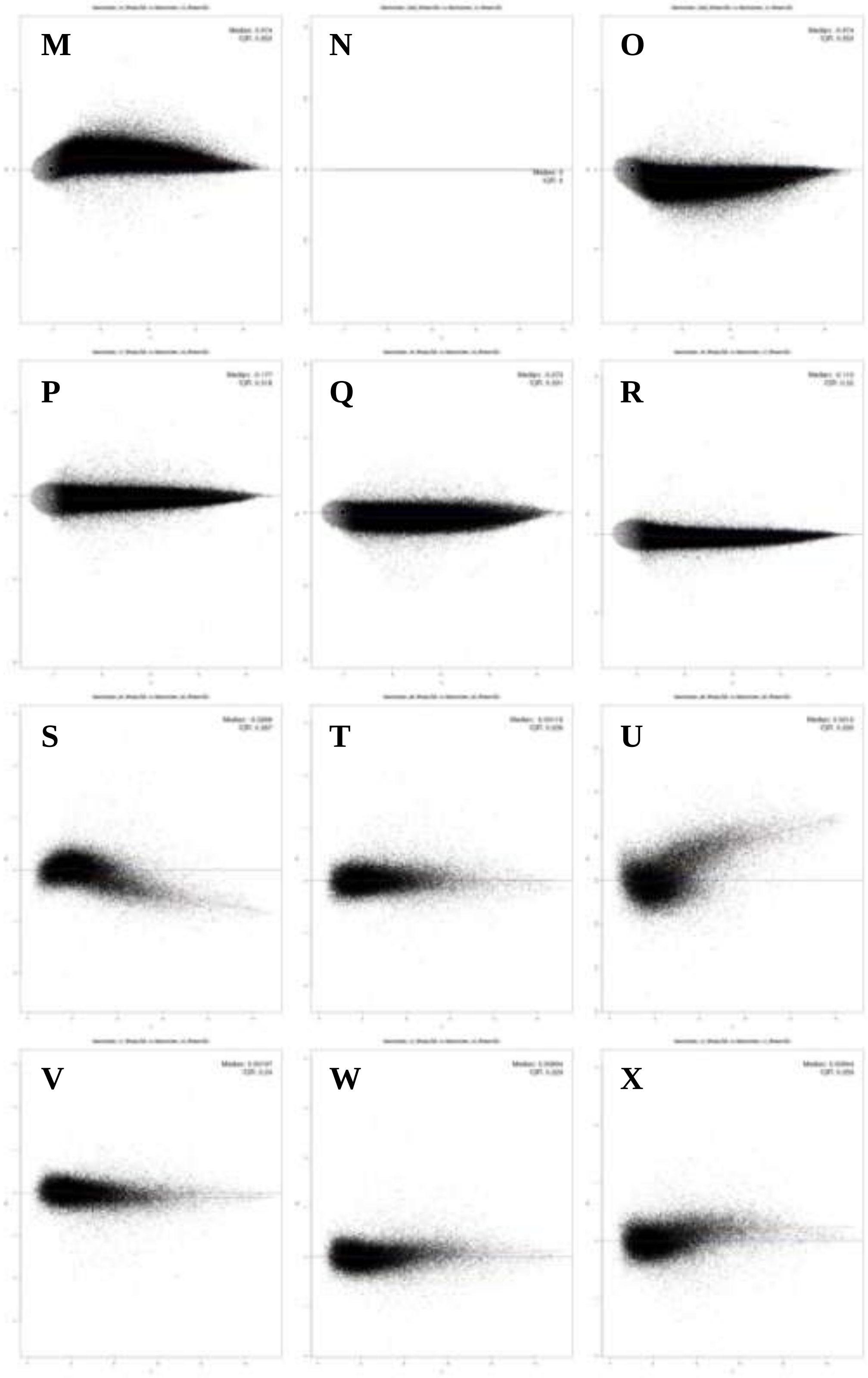


Figure Appx 5.8 - **18 Slides cont.**

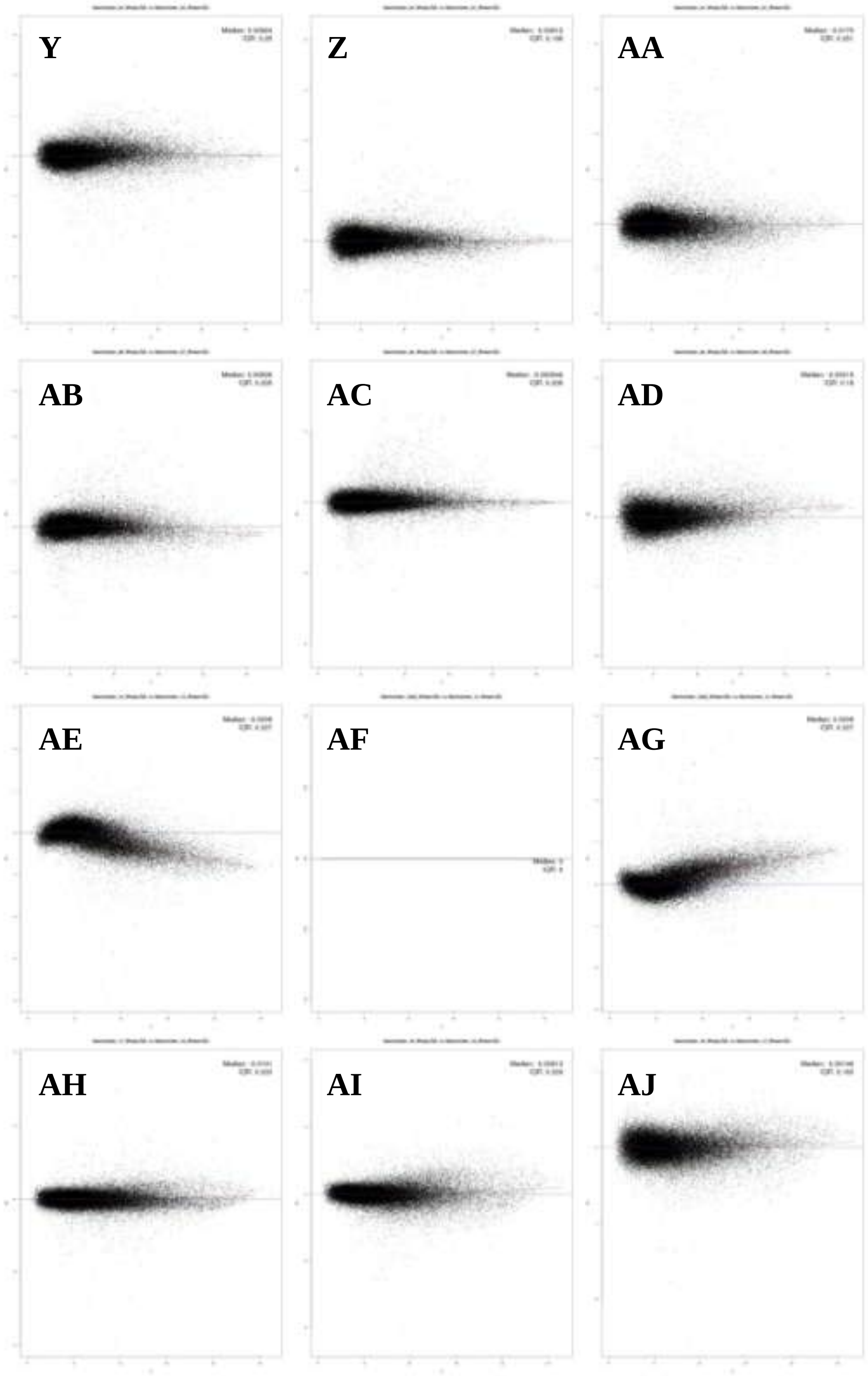


Figure Appx 5.8 **- 18 Slides cont.**

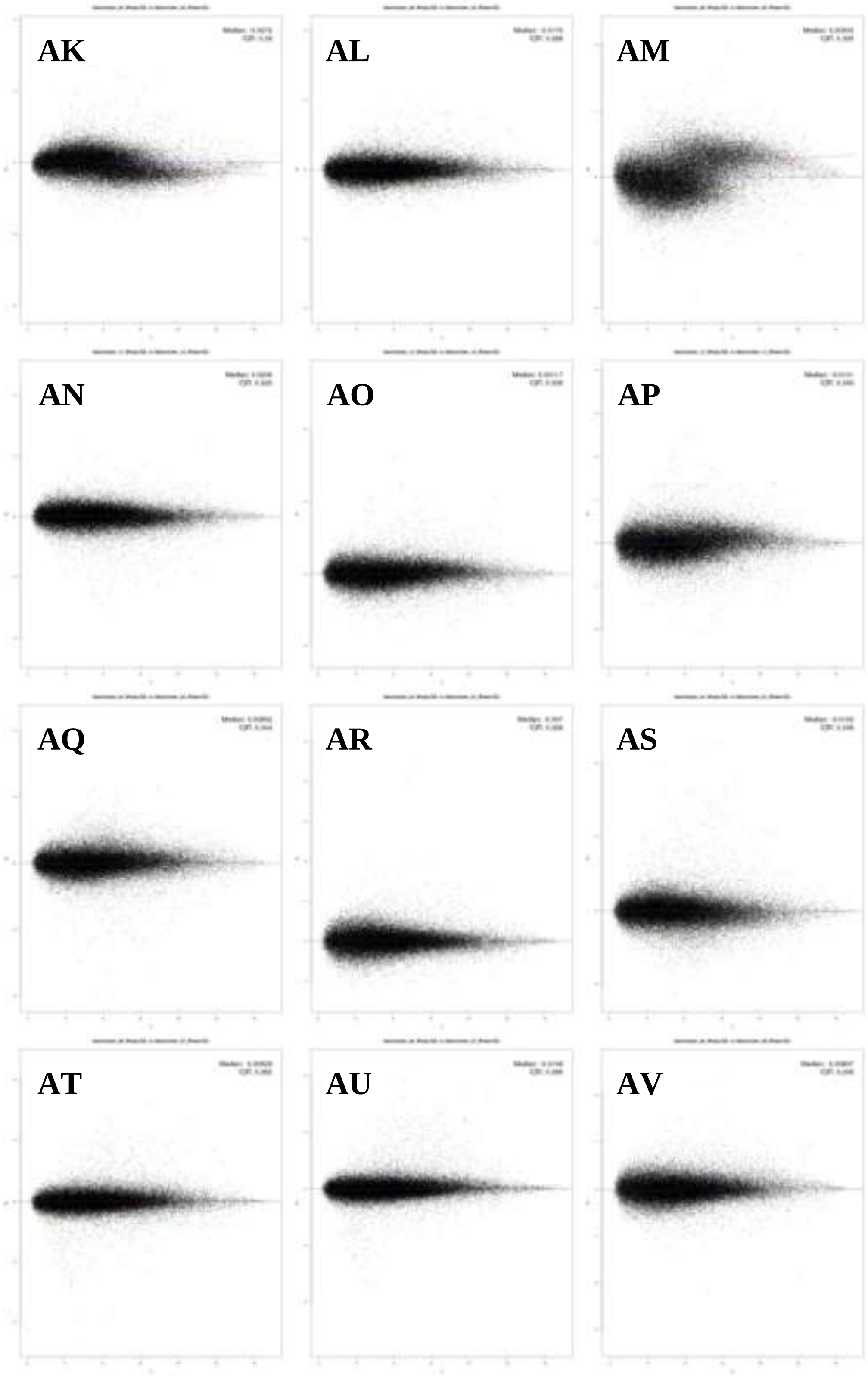


Figure Appx 5.8 **- 18 Slides cont.**

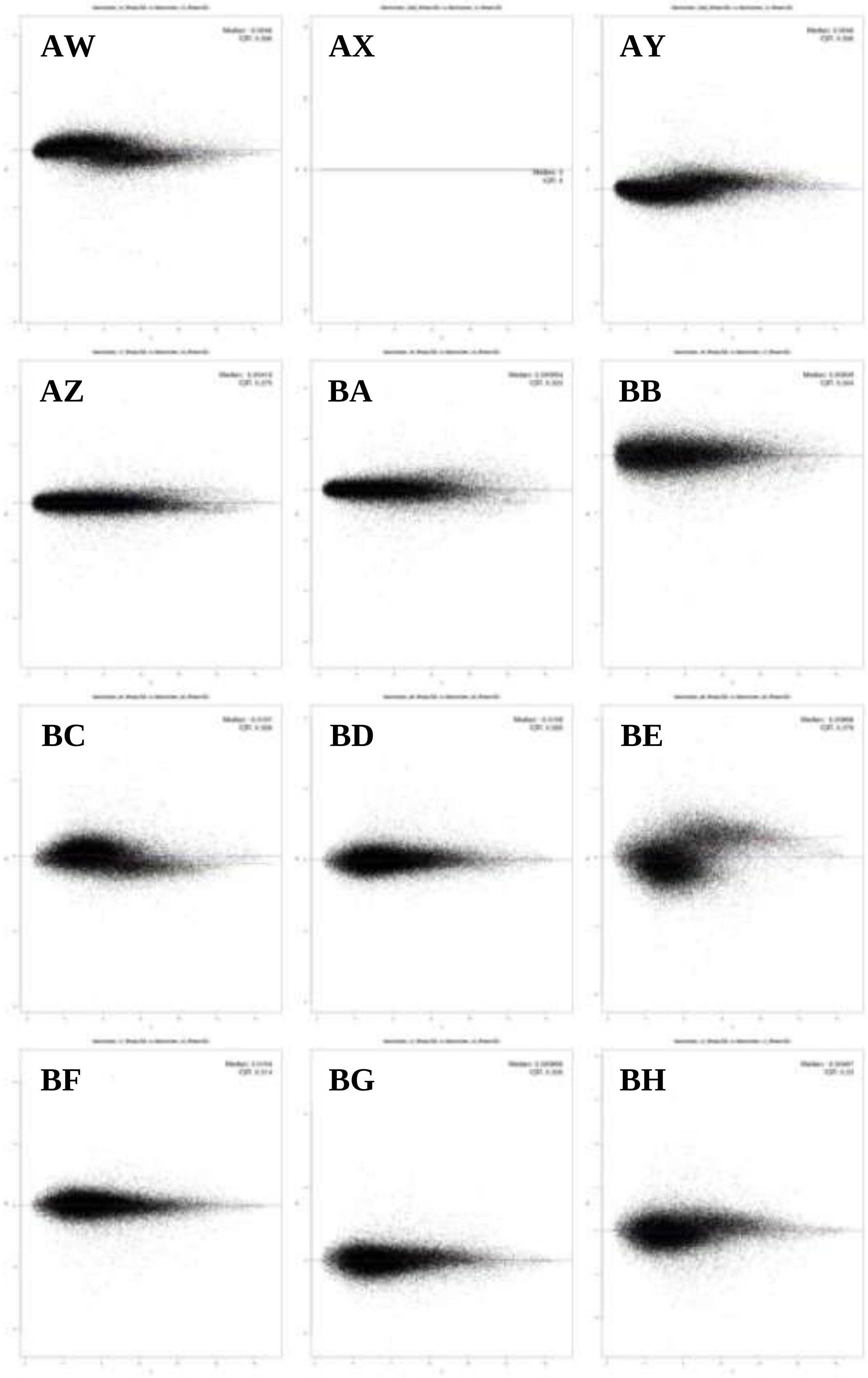


Figure Appx 5.8 **- 18 Slides cont.**

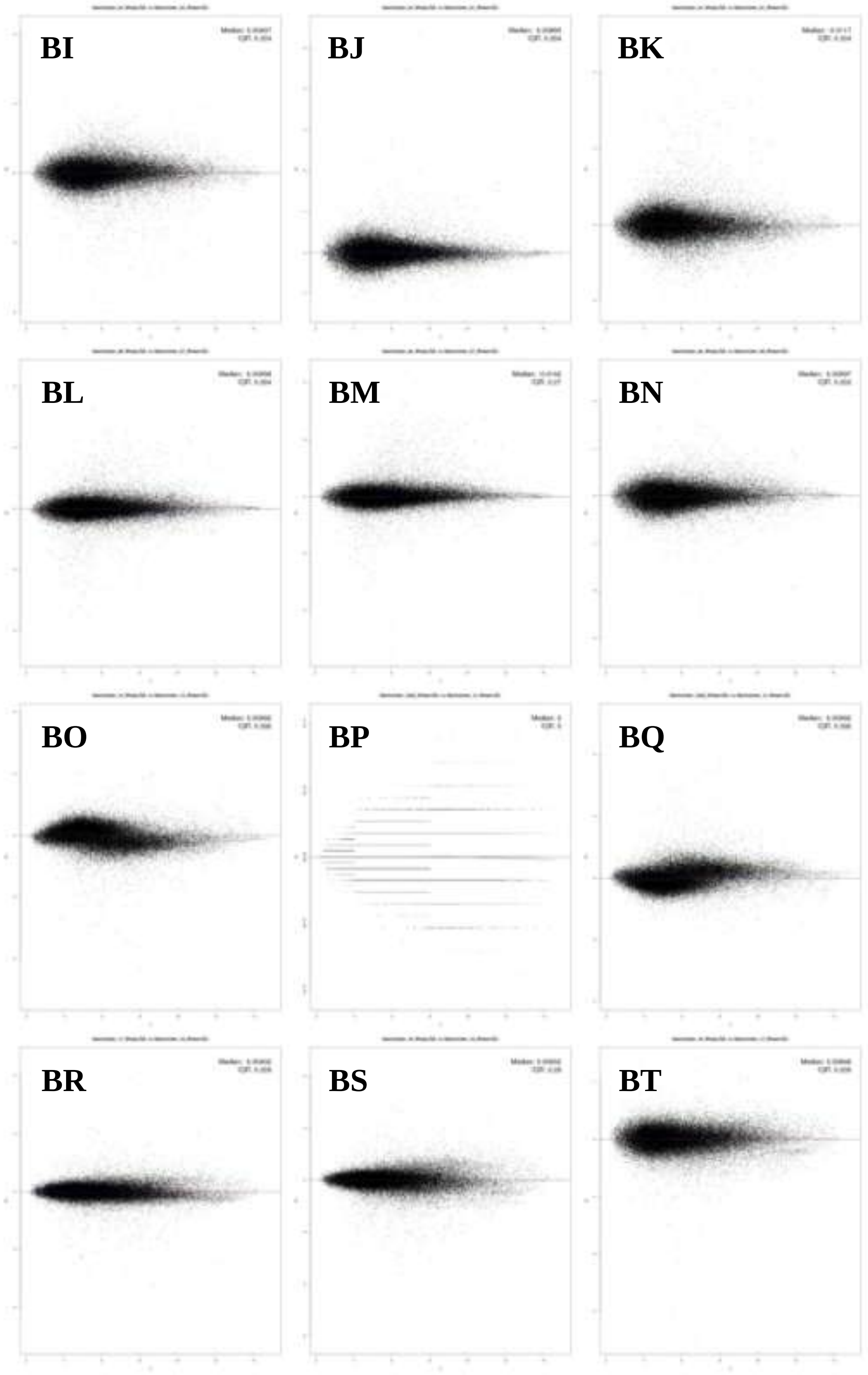


Figure Appx 5.8 - **18 Slides cont.**

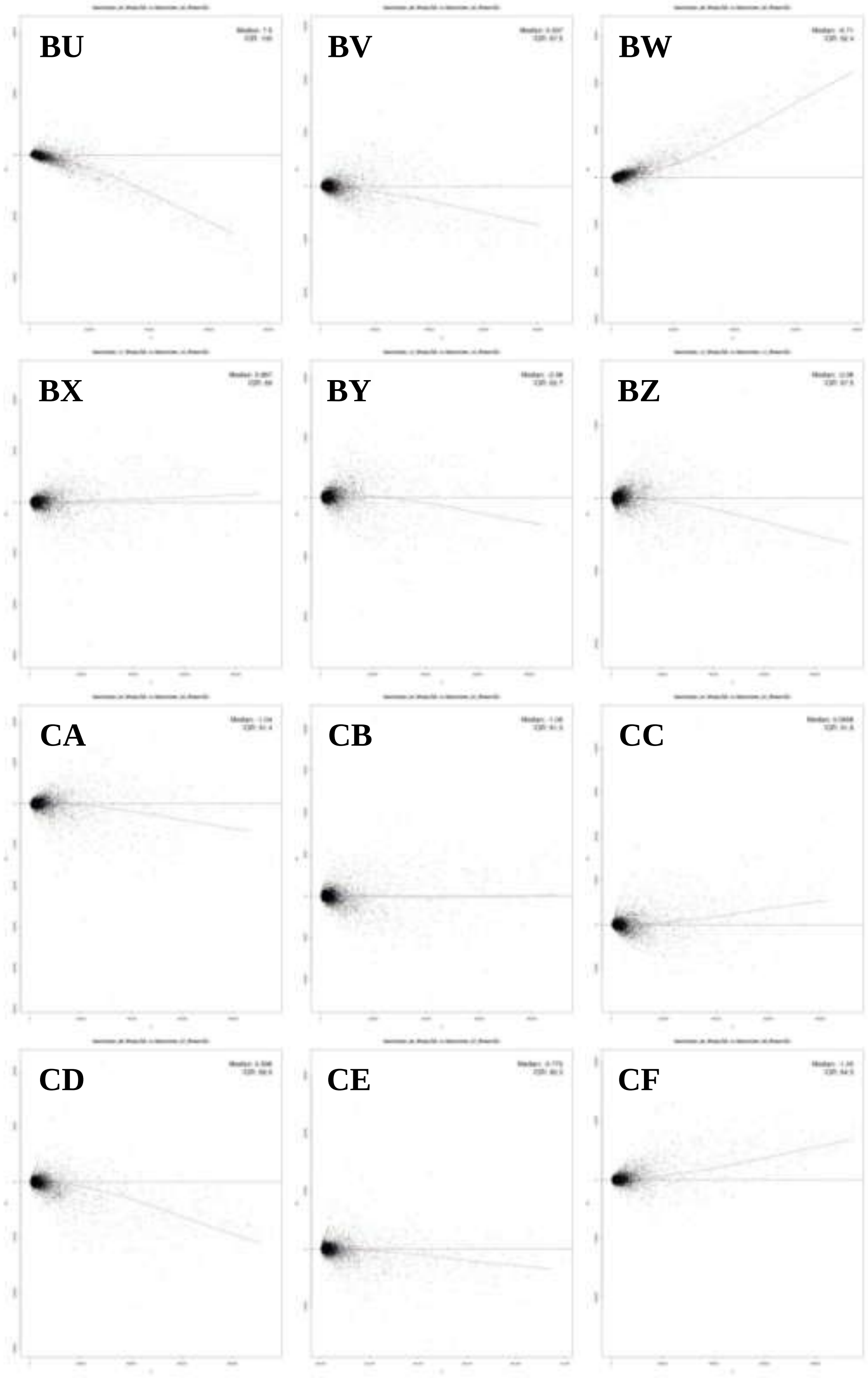


Figure Appx 5.8 - **18 Slides cont.**

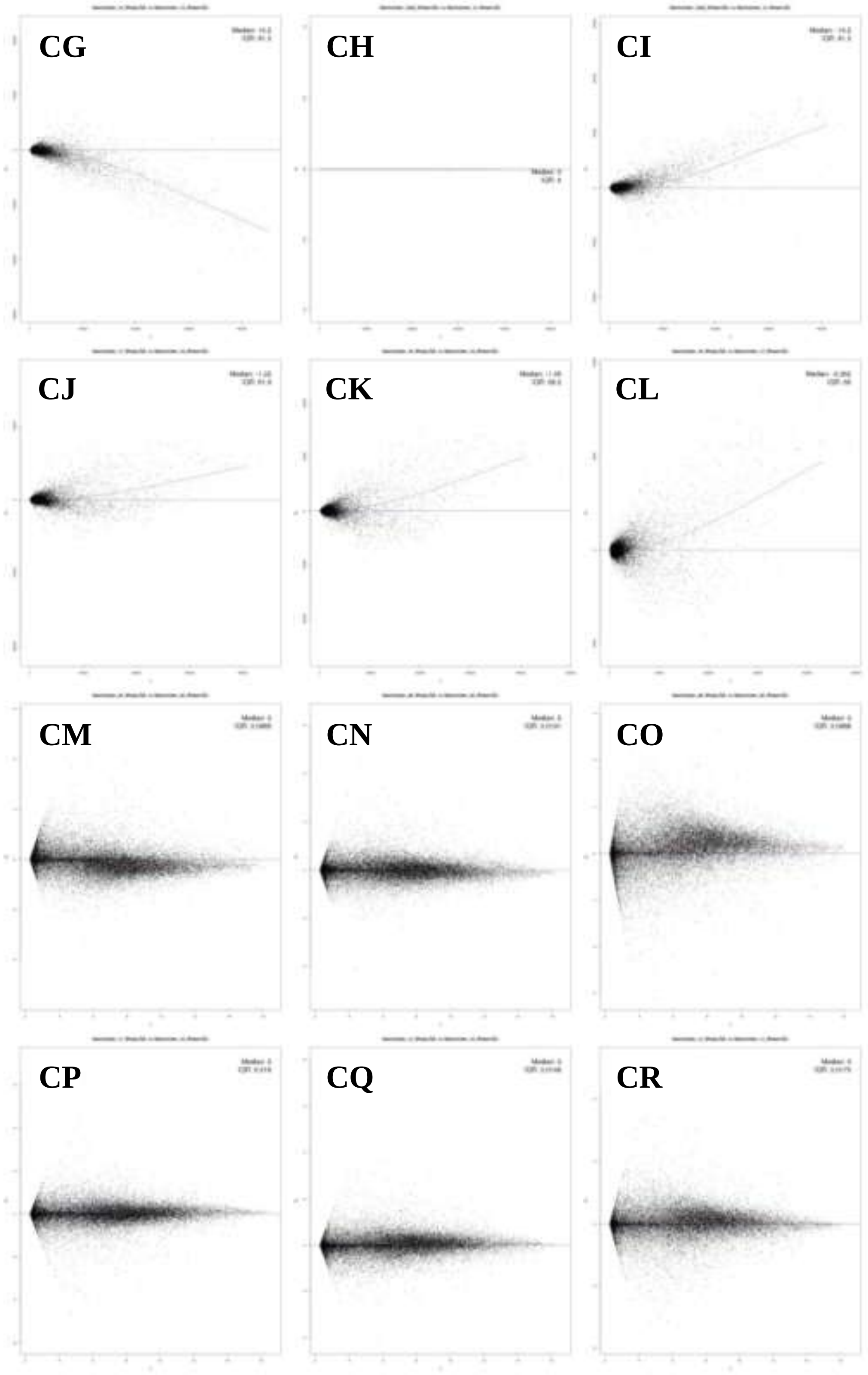


***Figure Appx 5.8* - 18 Slides cont.**

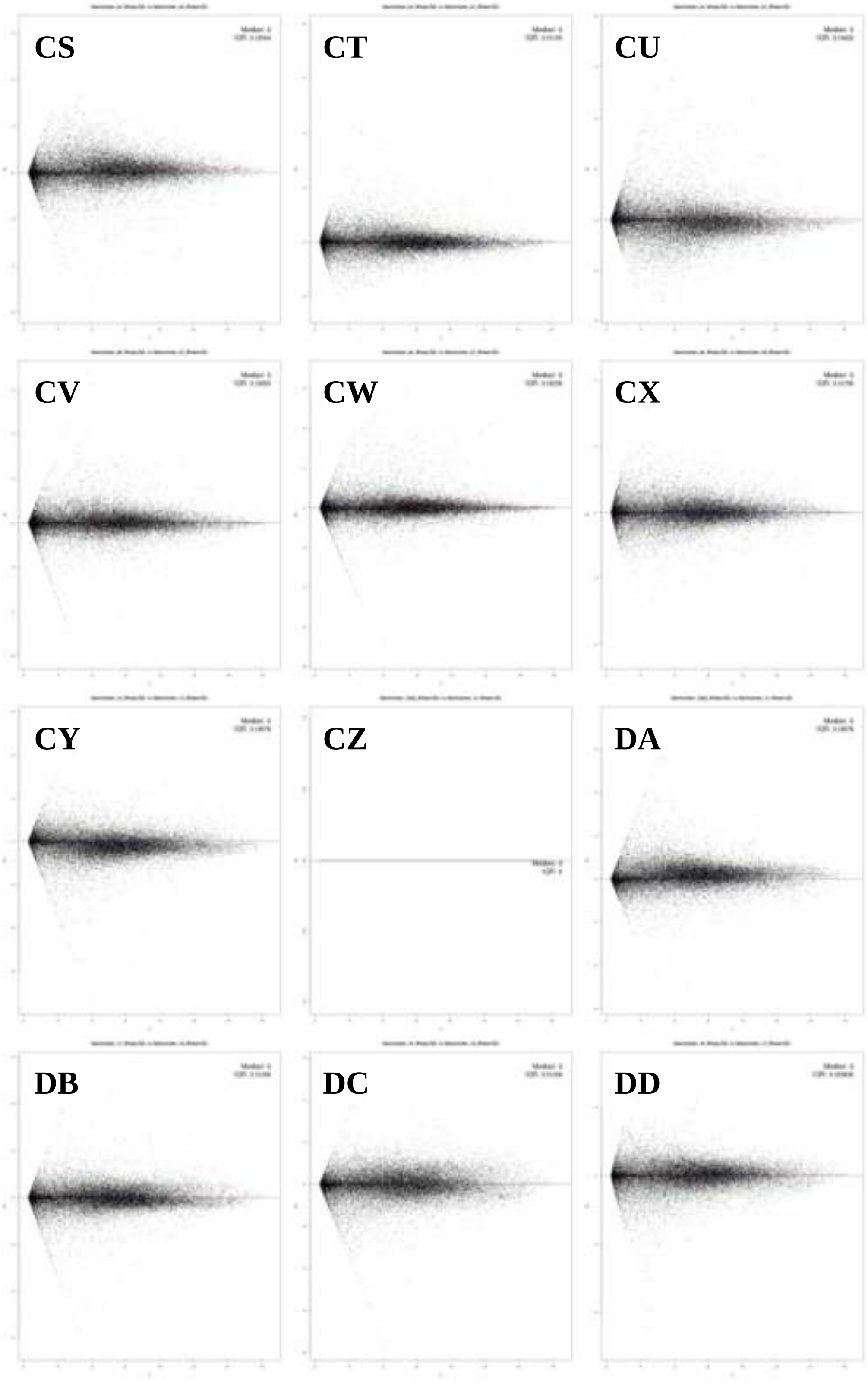


Figure Appx 5.8 The MA-plot comparisons of the different slides after normalization by the different methods. The raw/pre-normalized plots are also included for comparison. The files are written to the subfolder */R results/Graphs of raw and normalized data/MA plots/*. The 12 slides files are: (A) rawdata_Tug1_vs_Tug2.jpg, (B) rawdata_Tug1_vs_Tug3.jpg, (C) rawdata_Tug2_vs_Tug3.jpg, (D) rawdata_TugDn_1_vs_TugDn_2.jpg, (E) rawdata_TugDn_1_vs_TugDn_3.jpg, (F) rawdata_TugDn_2_vs_TugDn_3.jpg, (G) rawdata_TugDn2_1_vs_TugDn2_2.jpg, (H) rawdata_TugDn2_1_vs_TugDn2_3.jpg, (I) rawdata_TugDn2_2_vs_TugDn2_3.jpg, (J) rawdata_TugDn5_1_vs_TugDn5_2.jpg, (K) rawdata_TugDn5_1_vs_TugDn5_3.jpg, (L) rawdata_TugDn5_2_vs_TugDn5_3.jpg, (M) esetVSN_Tug1_vs_Tug2.jpg, (N) esetVSN_Tug1_vs_Tug3.jpg, (O) esetVSN_Tug2_vs_Tug3.jpg, (P) esetVSN_TugDn_1_vs_TugDn_2.jpg, (Q) esetVSN_TugDn_1_vs_TugDn_3.jpg, (R) esetVSN_TugDn_2_vs_TugDn_3.jpg, (S) esetVSN_TugDn2_1_vs_TugDn2_2.jpg, (T) esetVSN_TugDn2_1_vs_TugDn2_3.jpg, (U) esetVSN_TugDn2_2_vs_TugDn2_3.jpg, (V) esetVSN_TugDn5_1_vs_TugDn5_2.jpg, (W) esetVSN_TugDn5_1_vs_TugDn5_3.jpg, (X) esetVSN_TugDn5_2_vs_TugDn5_3.jpg, (Y) esetRMA_Tug1_vs_Tug2.jpg, (Z) esetRMA_Tug1_vs_Tug3.jpg, (AA) esetRMA_Tug2_vs_Tug3.jpg, (AB) esetRMA_TugDn_1_vs_TugDn_2.jpg, (AC) esetRMA_TugDn_1_vs_TugDn_3.jpg, (AD) esetRMA_TugDn_2_vs_TugDn_3.jpg, (AE) esetRMA_TugDn2_1_vs_TugDn2_2.jpg, (AF) esetRMA_TugDn2_1_vs_TugDn2_3.jpg, (AG) esetRMA_TugDn2_2_vs_TugDn2_3.jpg, (AH) esetRMA_TugDn5_1_vs_TugDn5_2.jpg, (AI) esetRMA_TugDn5_1_vs_TugDn5_3.jpg, (AJ) esetRMA_TugDn5_2_vs_TugDn5_3.jpg, (AK) esetPLM_Tug1_vs_Tug2.jpg, (AL) esetPLM_Tug1_vs_Tug3.jpg, (AM) esetPLM_Tug2_vs_Tug3.jpg, (AN) esetPLM_TugDn_1_ vs_TugDn_2.jpg, (AO) esetPLM_TugDn_1_vs_TugDn_3.jpg, (AP) esetPLM_TugDn_2_vs_TugDn_3.jpg, (AQ) esetPLM_TugDn2_1_vs_TugDn2_2.jpg, (AR) esetPLM_TugDn2_1_vs_TugDn2_3.jpg, (AS) esetPLM_TugDn2_2_vs_TugDn2_3.jpg, (AT) esetPLM_TugDn5_1_vs_TugDn5_2.jpg, (AU) esetPLM_TugDn5_1_vs_TugDn5_3.jpg, (AV) esetPLM_TugDn5_2_vs_TugDn5_3.jpg, (AW) esetMAS_Tug1_vs_Tug2.jpg, (AX) esetMAS_Tug1_vs_Tug3.jpg, (AY) esetMAS_Tug2_vs_Tug3.jpg, (AZ) esetMAS_TugDn_1_vs_TugDn_2.jpg, (BA) esetMAS_TugDn_1_vs_TugDn_3.jpg, (BB) esetMAS_TugDn_2_vs_TugDn_3.jpg, (BC) esetMAS_TugDn2_1_vs_TugDn2_2.jpg, (BD) esetMAS_TugDn2_1_vs_TugDn2_3.jpg, (BE) esetMAS_TugDn2_2_vs_TugDn2_3.jpg, (BF) esetMAS_TugDn5_1_vs_TugDn5_2.jpg, (BG) esetMAS_TugDn5_1_vs_TugDn5_3.jpg, (BH) esetMAS_TugDn5_2_vs_TugDn5_3.jpg, (BI) esetGCRMA_Tug1_vs_Tug2.jpg, (BJ) esetGCRMA_Tug1_vs_Tug3.jpg, (BK) esetGCRMA_Tug2_vs_Tug3.jpg, (BL) esetGCRMA_TugDn_1_vs_TugDn_2.jpg, (BM) esetGCRMA_TugDn_1_vs_TugDn_3.jpg, (BN) esetGCRMA_TugDn_2_vs_TugDn_3.jpg, (BO) esetGCRMA_TugDn2_1_vs_TugDn2_2.jpg, (BP) esetGCRMA_TugDn2_1_vs_TugDn2_3.jpg, (BQ) esetGCRMA_

TugDn2_2_vs_TugDn2_3.jpg, (BR) esetGCRMA_TugDn5_1_vs_TugDn5_2.jpg, (BS) esetGCRMA_TugDn5_1_vs_TugDn5_3.jpg, (BT) esetGCRMA_TugDn5_2_vs_TugDn5_3.jpg.

The 18 Slides files are: (A) rawdata_RWA2_GS_1_vs_RWA2_GS_2.jpg, (B) rawdata_RWA2_GS_1_vs_RWA2_GS_3.jpg, (C) rawdata_RWA2_GS_2_vs_RWA2_GS_3.jpg, (D) rawdata_RWA2_GR_1_vs_RWA2_GR_2.jpg, (E) rawdata_RWA2_GR_1_vs_RWA2_GR_3.jpg, (F) rawdata_RWA2_GR_2_vs_RWA2_GR_3.jpg, (G) rawdata_RWA1_GR_1_vs_RWA1_GR_2.jpg, (H) rawdata_RWA1_GR_1_vs_RWA1_GR_3.jpg, (I) rawdata_RWA1_GR_2_vs_RWA1_GR_3.jpg, (J) rawdata_RWA1_GS_2_vs_RWA1_GS_1.jpg, (K) rawdata_RWA1_GS_3_vs_RWA1_GS_1.jpg, (L) rawdata_RWA1_GS_3_vs_RWA1_GS_2.jpg, (M) rawdata_Gam_S_1_vs_Gam_S_2.jpg, (N) rawdata_Gam_S_1_vs_Gam_S_3.jpg, (O) rawdata_Gam_S_2_vs_Gam_S_3.jpg, (P) rawdata_Gam_R_1_vs_Gam_R_2.jpg, (Q) rawdata_Gam_R_1_vs_Gam_R_3.jpg, (R) rawdata_Gam_R_2_vs_Gam_R_3.jpg, (S) esetVSN_RWA2_GS_1_vs_RWA2_GS_2.jpg, (T) esetVSN_RWA2_GS_1_vs_RWA2_GS_3.jpg, (U) esetVSN_RWA2_GS_2_vs_RWA2_GS_3.jpg, (V) esetVSN_RWA2_GR_1_vs_RWA2_GR_2.jpg, (W) esetVSN_RWA2_GR_1_vs_RWA2_GR_3.jpg, (X) esetVSN_RWA2_GR_2_vs_RWA2_GR_3.jpg, (Y) esetVSN_RWA1_GR_1_vs_RWA1_GR_2.jpg, (Z) esetVSN_RWA1_GR_1_vs_RWA1_GR_3.jpg, (AA) esetVSN_RWA1_GR_2_vs_RWA1_GR_3.jpg, (AB) esetVSN_RWA1_GS_2_vs_RWA1_GS_1.jpg, (AC) esetVSN_RWA1_GS_3_vs_RWA1_GS_1.jpg, (AD) esetVSN_RWA1_GS_3_vs_RWA1_GS_2.jpg, (AE) esetVSN_Gam_S_1_vs_Gam_S_2.jpg, (AF) esetVSN_Gam_S_1_vs_Gam_S_3.jpg, (AG) esetVSN_Gam_S_2_vs_Gam_S_3.jpg, (AH) esetVSN_Gam_R_1_vs_Gam_R_2.jpg, (AI) esetVSN_Gam_R_1_vs_Gam_R_3.jpg, (AJ) esetVSN_Gam_R_2_vs_Gam_R_3.jpg, (AK) esetRMA_RWA2_GS_1_vs_RWA2_GS_2.jpg, (AL) esetRMA_RWA2_GS_1_vs_RWA2_GS_3.jpg, (AM) esetRMA_RWA2_GS_2_vs_RWA2_GS_3.jpg, (AN) esetRMA_RWA2_GR_1_vs_RWA2_GR_2.jpg, (AO) esetRMA_RWA2_GR_1_vs_RWA2_GR_3.jpg, (AP) esetRMA_RWA2_GR_2_vs_RWA2_GR_3.jpg, (AQ) esetRMA_RWA1_GR_1_vs_RWA1_GR_2.jpg, (AR) esetRMA_RWA1_GR_1_vs_RWA1_GR_3.jpg, (AS) esetRMA_RWA1_GR_2_vs_RWA1_GR_3.jpg, (AT) esetRMA_RWA1_GS_2_vs_RWA1_GS_1.jpg, (AU) esetRMA_RWA1_GS_3_vs_RWA1_GS_1.jpg, (AV) esetRMA_RWA1_GS_3_vs_RWA1_GS_2.jpg, (AW) esetRMA_Gam_S_1_vs_Gam_S_2.jpg, (AX) esetRMA_Gam_S_1_vs_Gam_S_3.jpg, (AY) esetRMA_Gam_S_2_vs_Gam_S_3.jpg, (AZ) esetRMA_Gam_R_1_vs_Gam_R_2.jpg, (BA) esetRMA_Gam_R_1_vs_Gam_R_3.jpg, (BB) esetRMA_Gam_R_2_vs_Gam_R_3.jpg, (BC) esetPLM_RWA2_GS_1_vs_RWA2_GS_2.jpg, (BD) esetPLM_RWA2_GS_1_vs_RWA2_GS_3.jpg, (BE) esetPLM_RWA2_GS_2_vs_RWA2_GS_3.jpg, (BF) esetPLM_RWA2_GR_1_vs_RWA2_GR_2.jpg, (BG) esetPLM_RWA2_GR_1_vs_RWA2_GR_3.jpg, (BH) esetPLM_RWA2_GR_2_vs_RWA2_GR_3.jpg, (BI) esetPLM_RWA1_GR_1_vs_RWA1_GR_2.jpg, (BJ) esetPLM_RWA1_GR_1_vs_RWA1_GR_3.jpg, (BK) esetPLM_RWA1_GR_2_vs_RWA1_GR_3.jpg, (BL) esetPLM_RWA1_GS_2_vs_RWA1_GS_1.jpg, (BM) esetPLM_RWA1_GS_3_vs_RWA1_GS_1.jpg, (BN) esetPLM_RWA1_GS_3_vs_RWA1_GS_2.jpg, (BO) esetPLM_Gam_S_1_vs_Gam_S_2.jpg, (BP) esetPLM_Gam_S_1_vs_Gam_S_3.jpg, (BQ) esetPLM_Gam_S_2_vs_Gam_S_3.jpg, (BR) esetPLM_Gam_R_1_vs_Gam_R_2.jpg, (BS) esetPLM_Gam_R_1_vs_Gam_R_3.jpg, (BT) esetPLM_Gam_R_2_vs_Gam_R_3.jpg, (BU) esetMAS_RWA2_GS_1_vs_RWA2_GS_2.jpg, (BV) esetMAS_RWA2_GS_1_vs_RWA2_GS_3.jpg, (BW) esetMAS_RWA2_GS_2_vs_RWA2_GS_3.jpg, (BX) esetMAS_RWA2_GR_1_vs_RWA2_GR_2.jpg, (BY) esetMAS_RWA2_GR_1_vs_RWA2_GR_3.jpg, (BZ) esetMAS_RWA2_GR_2_vs_RWA2_GR_3.jpg, (CA) esetMAS_RWA1_GR_1_vs_RWA1_GR_2.jpg, (CB) esetMAS_RWA1_GR_1_vs_RWA1_GR_3.jpg, (CC) esetMAS_RWA1_GR_2_vs_RWA1_GR_3.jpg, (CD) esetMAS_RWA1_GS_2_vs_RWA1_GS_1.jpg, (CE) esetMAS_RWA1_GS_3_vs_RWA1_GS_1.jpg, (CF) esetMAS_RWA1_GS_3_vs_RWA1_GS_2.jpg, (CG) esetMAS_Gam_S_1_vs_Gam_S_2.jpg, (CH) esetMAS_Gam_S_1_vs_Gam_S_3.jpg, (CI) esetMAS_Gam_S_2_vs_Gam_S_3.jpg, (CJ) esetMAS_Gam_R_1_vs_Gam_R_2.jpg, (CK) esetMAS_Gam_R_1_vs_Gam_R_3.jpg, (CL) esetMAS_Gam_R_2_vs_Gam_R_3.jpg, (CM) esetGCRMA_RWA2_GS_1_vs_RWA2_GS_2.jpg, (CN) esetGCRMA_RWA2_GS_1_vs_RWA2_GS_3.jpg, (CO) esetGCRMA_RWA2_GS_2_vs_RWA2_GS_3.jpg, (CP) esetGCRMA_RWA2_GR_1_vs_RWA2_GR_2.jpg, (CQ) esetGCRMA_RWA2_GR_1_vs_RWA2_GR_3.jpg, (CR) esetGCRMA_RWA2_GR_2_vs_RWA2_GR_3.jpg, (CS) esetGCRMA_RWA1_GR_1_vs_RWA1_GR_2.jpg, (CT) esetGCRMA_RWA1_GR_1_vs_RWA1_GR_3.jpg, (CU) esetGCRMA_RWA1_GR_2_vs_RWA1_GR_3.jpg, (CV) esetGCRMA_RWA1_GS_2_vs_RWA1_GS_1.jpg, (CW) esetGCRMA_RWA1_GS_3_vs_RWA1_GS_1.jpg, (CX) esetGCRMA_RWA1_GS_3_vs_RWA1_GS_2.jpg, (CY) esetGCRMA_Gam_S_1_vs_Gam_S_2.jpg, (CZ) esetGCRMA_Gam_S_1_vs_Gam_S_3.jpg, (DA) esetGCRMA_Gam_S_2_vs_Gam_S_3.jpg, (DB) esetGCRMA_Gam_R_1_vs_Gam_R_2.jpg, (DC) esetGCRMA_Gam_R_1_vs_Gam_R_3.jpg, (DD) esetGCRMA_Gam_R_2_vs_Gam_R_3.jpg.

Table Appx 5.2 Selected GeneIDs/probe sets shown to be up regulated (12 slide experiment), either generally or for specific slide comparisons (TuD-Tug, Tu2-Tug, *etc*.). Values under the different normalization methods (VSN, RMA, *etc*.) indicate the number of times the specific probe set was obtained across the six slide comparisons within that specific normalization method, *i.e.* 6 indicates that the geneID was obtained in all six slide comparisons. The slide comparisons indicate the number of times the geneID/probe set was obtained after normalization with the five methods, for that specific comparison, *i.e.* a 5 indicates that all five normalization methods detected that probe set for the specific slide comparison. Values summed for the normalization methods equals the values summed for the slide comparisons and are given by the "occurrence" (Occ.) value at specific confidences. *Table 5.1* shows examples of up regulated GeneIDs/probe sets.

Probe set ID	***VSN***	***RMA***	***PLM***	***MAS5***	***GCRMA***	***Occ. 95%***	***TuD - Tug***	***Tu2 - Tug***	***Tu5 - Tug***	***Tu2 - TuD***	***Tu5 - TuD***	***Tu5 - Tu2***	***Occ. 99%***	***Occ. 99.9%***
TaAffx.110208.1.S1_at	6	6	6	6	6	**30**	5	5	5	5	5	5	29	22
TaAffx.26346.1.S1_at	5	6	5	5	6	**27**	5	5	5	5	5	2	25	18
Ta.4593.1.A1_at	6	5	5	5	5	**26**	5	5	5	5	5	1	22	15
Ta.10581.1.A1_at	1	1	1	1	1	**5**	5	0	0	0	0	0	4	1
Ta.11261.1.S1_at	1	1	1	1	1	**5**	5	0	0	0	0	0	4	2
Ta.11832.1.S1_at	1	1	1	1	1	**5**	5	0	0	0	0	0	4	1
Ta.7584.1.S1_s_at	1	2	1	1	1	**6**	0	5	0	1	0	0	3	0
Ta.21925.1.S1_at	1	2	2	1	1	**7**	0	5	0	2	0	0	2	0
Ta.7361.1.A1_at	2	1	1	1	2	**7**	0	5	0	2	0	0	0	0
Ta.27788.2.S1_a_at	2	2	2	1	1	**8**	0	0	5	0	0	3	0	0
TaAffx.29630.1.S1_at	2	2	2	1	1	**8**	0	0	5	0	0	3	5	0
Ta.2107.1.S1_s_at	2	2	2	2	2	**10**	0	0	5	0	0	5	3	0
Ta.10008.1.A1_at	1	1	1	1	1	**5**	0	0	0	5	0	0	3	0
Ta.10426.1.A1_at	1	1	1	1	1	**5**	0	0	0	5	0	0	4	0
Ta.10552.1.S1_at	1	1	1	1	1	**5**	0	0	0	5	0	0	0	0
Ta.1040.1.S1_at	1	1	1	1	1	**5**	0	0	0	0	5	0	0	0
Ta.22932.1.S1_x_at	2	1	2	1	1	**7**	0	0	0	0	5	2	2	0
Ta.959.1.S1_at	2	2	1	1	1	**7**	0	0	0	0	5	2	2	0
Ta.10520.1.S1_at	1	1	1	1	1	**5**	0	0	0	0	0	5	1	0
Ta.10883.2.S1_at	1	1	1	1	1	**5**	0	0	0	0	0	5	3	0
Ta.10891.1.S1_at	1	1	1	1	1	**5**	0	0	0	0	0	5	3	0

Table Appx 5.3 GeneIDs/probe sets, selected in *Table 5.1*, that were still shown to be differentially regulated after FDR (Benjamini-Hochberg method) or FWER (Bonferroni) correction. Values under the different normalization methods (VSN, RMA, *etc*.) indicate the number of times the specific probe set was obtained across the six slide comparisons (TuD-Tug, Tu2-Tug, *etc*.) within that specific normalization method, *i.e.* 6 indicates that the geneID was obtained in all six slide comparisons. The slide comparisons indicate the number of times the geneID/probe set was obtained after normalization with the five methods, for that specific comparison, *i.e.* a 5 indicates that all five normalization methods detected that probe set for the specific slide comparison. Values summed for the normalization methods equals the values summed for the slide comparisons and are given by the "occurrence" (Occ.) value at specific confidences. *Table 5.2* shows examples of differentially up regulated GeneIDs/probe sets after FDR/FWER correction.

Probe set ID	***VSN***	***RMA***	***PLM***	***MAS5***	***GCRMA***	***Occ. 95%***	***TuD - Tug***	***Tu2 - Tug***	***Tu5 - Tug***	***Tu2 - TuD***	***Tu5 - TuD***	***Tu5 - Tu2***	***Occ. 99%***	***Occ. 99.9%***
After FDR														
TaAffx.110208.1.S1_at	3	4	5	3	4	**19**	0	3	5	1	5	5	16	11
TaAffx.26346.1.S1_at	3	3	3	3	2	**14**	0	4	5	0	5	0	8	3
Ta.4593.1.A1_at	3	3	3	3	1	**13**	0	4	5	0	4	0	7	4
TaAffx.29630.1.S1_at	0	1	1	0	0	**2**	0	0	2	0	0	0	0	0
Ta.2107.1.S1_s_at	0	0	1	0	0	**1**	0	0	1	0	0	0	0	0
Ta.22932.1.S1_x_at	1	0	1	0	0	**2**	0	0	0	0	2	0	0	0
Ta.959.1.S1_at	1	1	0	0	0	**2**	0	0	0	0	2	0	0	0
Ta.10883.2.S1_at	1	1	1	0	0	**3**	0	0	0	0	0	3	0	0
Ta.10891.1.S1_at	1	0	1	0	0	**2**	0	0	0	0	0	2	0	0
After FWER														
TaAffx.110208.1.S1_at	1	1	3	1	1	**7**	0	0	5	0	1	1	5	2
TaAffx.26346.1.S1_at	0	1	0	0	0	**1**	0	0	1	0	0	0	0	0

CURRICULUM VITAE

Zacharias Hendrik Swanevelder was born June 1st, 1978 in Ventersdorp, North-West Province. He attended the High School Verwoerdburg where he matriculated with five distinctions in 1996. He obtained his *Baccalaureus Scientiae* degree *cum laude* at the University of South Africa in 1999, majoring in Botany and Biochemistry. The degree *Baccalaureus Scientiae Honores*, with specialisation in Plant Physiology, was awarded *cum laude* in the Department of Botany, University of Pretoria in 2000. For his achievements he was awarded Academic Colours by the University of Pretoria and received the *Margaretha Mess*-medal for the best BSc (Hons) Botany student (2000).

In his *Magister Scientiae* entitled, Diversity and population structure of *Clivia miniata* Lindl. (Amaryllidaceae): Evidence from molecular genetics and ecology, he focused on the development of molecular markers, the ecology and taxonomy of the indigenous genus *Clivia*, and conducted a molecular marker analysis of the genus. The degree was obtained *cum laude* in 2003, where after he again was bestowed Academic Colours from the University of Pretoria. His MSc produced four scientific papers in peer review journals, entitled: A new species of *Clivia* (Amaryllidaceae) endemic to the Pondoland Centre of Endemism, South Africa; Amaryllidaceae: a new variety in the genus *Clivia*; Amaryllidaceae: a new variety of *Clivia robusta* and Amaryllidaceae: a natural hybrid in the genus *Clivia*, as well as other articles in popular journals. He is also the recipient of a NRF PhD Prestigious Bursary.

In his PhD, entitled: Aphid-Plant interactions and the possible role of an endosymbiont in aphid biotype development, he investigates essential amino acid biosynthetic plasmid genes of the aphid endosymbiont, *Buchnera aphidicola*, from different Russian wheat aphid biotypes and the possible role of this bacterium in the development of the new aphid biotypes. The aphid-plant interaction, from a plant's perspective, is also investigated on a molecular level with special focus on the influences that statistical normalization methods have on identifying differentially regulated genes after Russian wheat aphid infestation. The study resulted in the peer reviewed paper: Limited endosymbiont variation in *Diuraphis noxia* (Hemiptera: Aphididae) biotypes from the USA and South Africa, and also contributed to two additional papers entitled: Deciphering defense strategies that are elucidated in wheat containing different *Dn* resistance genes, and Transcript profiling of wheat genes expressed during feeding by two different biotypes of *Diuraphis noxia*. In his PhD he presented at various international and national research meetings, where he also received a special award for a presentation in 2008 at the Joint Meeting of the WERA66 (Integrated Management of Russian Wheat Aphid and other Cereal Arthropod Pests) and IPRI (International Plant Resistance to Insects Workshop). In 2009, he was the author of a book entitled: *Clivias*: Nature and Nurture.