

## APPENDICES AND SUPPLEMENTARY DATA

## APPENDIX – CHAPTER 3

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

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

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

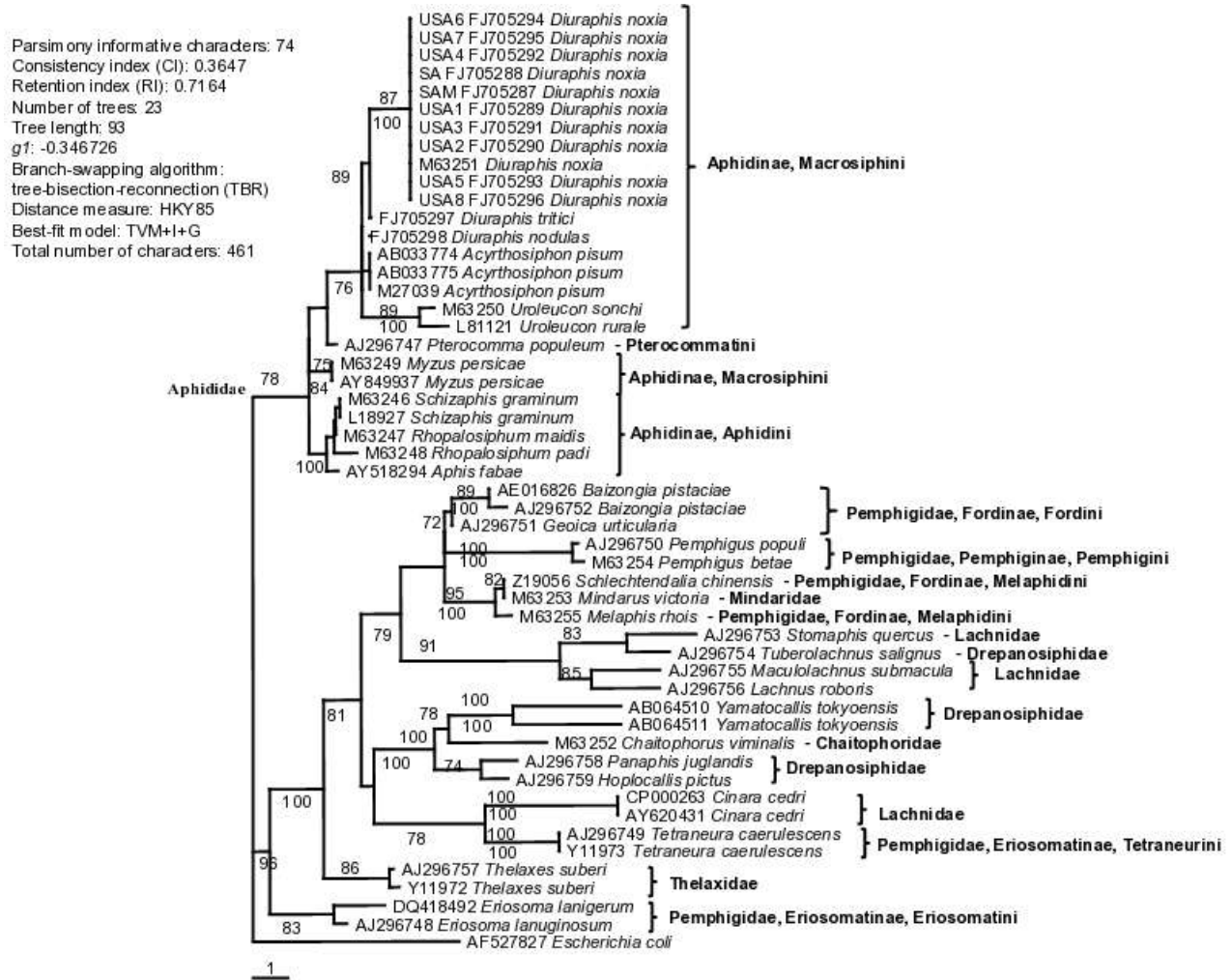
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**Sequencing: ptrpEG**

<i>ptrpE_3965F</i>	CCCCTCCTTGATCTCCTACA	7179-1651	TD <sup>φ</sup>	3506
<i>trpE_1674R</i>	CTGAAAATGAGGGAATTCTAAACG			
<i>ptrpE_2260F</i>	TTTGGTGCTTCACCAGAAAG	8115-61	TD <sup>φ</sup>	980
<i>ptrpG_3273R</i>	CTCCAAACCGTCATGATTGA			
<i>ptrpG_3194F</i>	AAGCTTATGGTGGC	14-676	TD <sup>φ</sup>	662
<i>spacer_695R</i>	TCGTTTGGCGACTCATCATA			
<i>spacer_620F</i>	GATCCTGCGCACTCTCAATAG	20-1651	TD <sup>φ</sup>	1631
<i>trpE_1674R</i>	CTGAAAATGAGGGAATTCTAAACG			
<i>trpE_1542F</i>	TCACTCGGTACAACAAACAGCA	1563-2338	TD <sup>d</sup>	775
<i>ptrpE_2357R</i>	ATGTTCCCTCCCTCTGGGTCT			

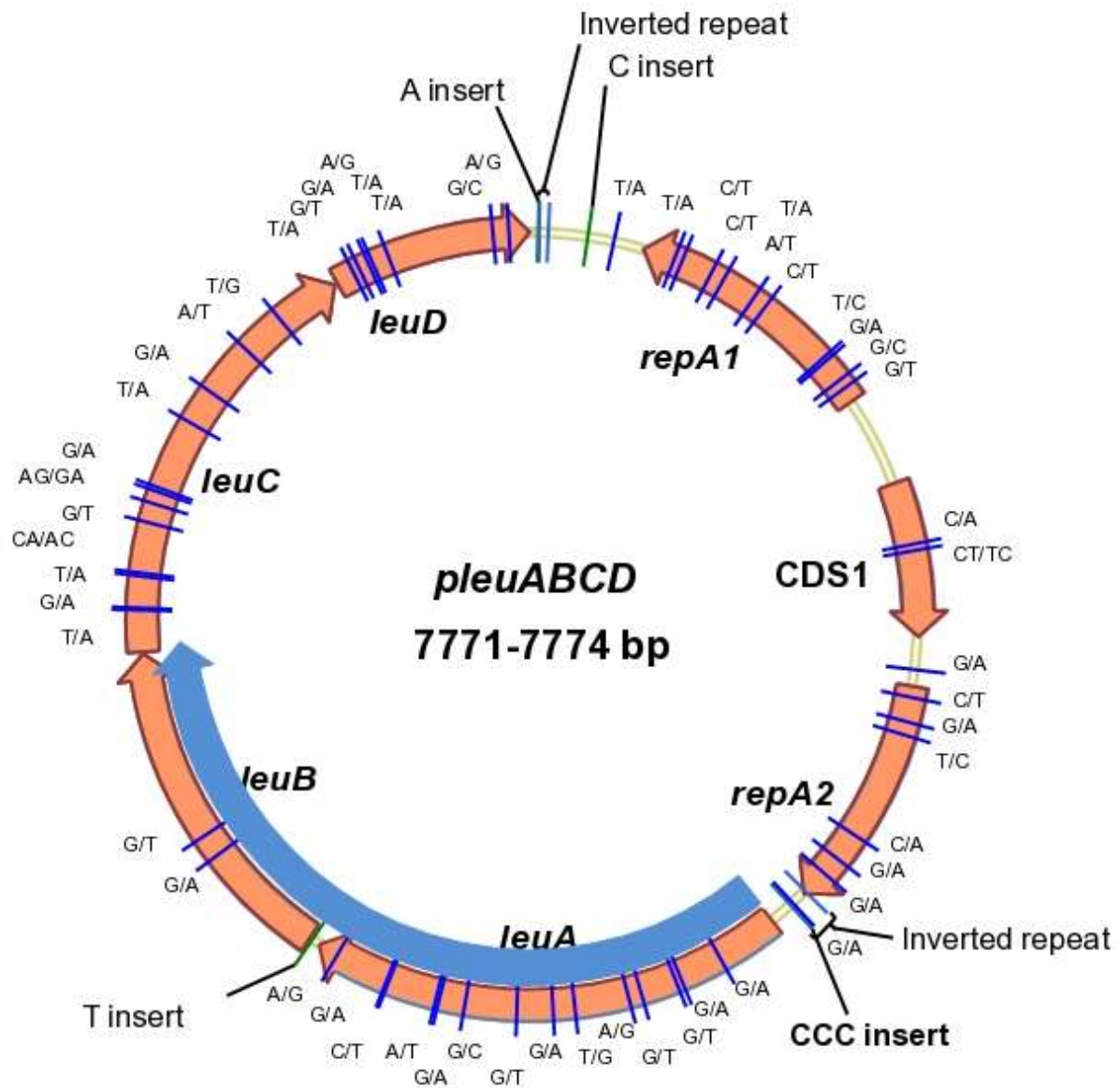
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\*All primers were designed from Genbank sequences ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) using *Primer3* (Rozen & Skaletsky 2000) and on sequences generated in this study. Total DNA (25 ng) in 25 µL reaction volumes was used for all PCRs, with 0.5 U ExSel High Fidelity DNA polymerase, 1 × reaction buffer with MgSO<sub>4</sub> (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 °C for 3 min and followed by a final elongation step of 72 °C for 10 min. 30 Cycles consisted of 30 sec at 94 °C, 30 sec at primer annealing temperature and 1 min/1 Kb extension period at 72 °C. 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 °C for 10 min. Large fragments were first amplified from 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. <sup>#</sup> Sequencing primer (Muyzer *et al.* 1993); <sup>§</sup> pA8f-GC contained a 5' 40mer GC-clamp (Fjellbirkeland *et al.* 2001); <sup>d</sup> TD - Touchdown 60-55 °C (Don *et al.* 1991); <sup>b</sup> TD - Touchdown 55-50 °C (Don *et al.* 1991); <sup>φ</sup> TD - Touchdown 60-50 °C (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.

```
ctatcctactattgttcgagaattcaaaaaattattggagaagaaactaacaacaaatTTtagaacaagaaacaaaattaccaaataatcg  
catgtgtggaggaggttctaatacaattggatTTTTTcaattttattaatgataaagaagtaagttaattgggtttgaaccggggggaagggtat  
aaaaacaggtcaacatggtgcaccattaaacatggtagaactggtatttttcggaatgaaatctcatttaataatgcaagatcaagaagggtcaaatc  
aagaatcttgggtctatttctgcaggattagactttccatctgttgg
```

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

```
ctatcctactattgttcgagaattcaaaaaattattggagaagaaactaacaacaaatTTtagaacaagaaacaaaattaccaaataatcg  
catgtgtggaggaggttctaatacaattggatTTTTTcaattttattaatgataaagaagtaagttaattgggtttgaaccggggggaagggtat  
aaaaacaggtcaacatggtgcaccattaaacatggtagaactggtatttttcggaatgaaatctcatttaataatgcaagatcaagaagggtcaaatc  
aagaatcttgggtctatttctgcaggattagactttccatctgttgg
```

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

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aaaaacaggtcaacatggtgcaccattaaacatggtagaactggtatttttcggaatgaaatctcatttaataatgcaagatcaagaagggtcaaatc  
aagaatcttgggtctatttctgcaggattagactttccatctgttgg
```

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

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aaaaacaggtcaacatggtgcaccattaaacatggtagaactggtatttttcggaatgaaatctcatttaataatgcaagatcaagaagggtcaaatc  
aagaatcttgggtctatttctgcaggattagactttccatctgttgg
```

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

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catgtgtggaggaggttctaatacaattggatTTTTTcaattttattaatgataaagaagtaagttaattgggtttgaaccggggggaagggtat  
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aagaatcttgggtctatttctgcaggattagactttccatctgttgg
```

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

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ctatcctactattgttcgagaattcaaaaaattattggagaagaaactaacaacaaatTTtagaacaagaaacaaaattaccaaataatcg  
catgtgtggaggaggttctaatacaattggatTTTTTcaattttattaatgataaagaagtaagttaattgggtttgaaccggggggaagggtat
```



aaaaacaggtcaacatggtgcaccattaaaacatggtagaactggtatTTTTTCggaatgaaatctcatttaatgcaagatcaagaaggtcaaattc  
aagaatcttggctatttctgcaggattagactttccatctgttgg

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

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aagaatcttggctatttctgcaggattagactttccatctgttgg

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

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aagaatcttggctatttctgcaggattagactttccatctgttgg

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

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

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

attgaacgctggcggcaagcctaacacatgcaagtcgagcggcagcgaagaaagcttcttctgtcggcagcggcaaacgggtgagtaa  
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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.

attgaacgctggcggcaagcctaacacatgcaagtcgagcggcagcgaagaaagcttgctttctgtcggcgagcggcaaacgggtgagtaa  
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FJ705291 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUH16SDnUSA3 16S ribosomal RNA gene, partial sequence.

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FJ705292 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUH16SDnUSA4 16S ribosomal RNA gene, partial sequence.

attgaacgctggcggcaagcctaacacatgcaagtcgagcggcagcgaagaaagcttgctttctgtcggcgagcggcaaacgggtgagtaa  
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FJ705293 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUH16SDnUSA5 16S ribosomal RNA gene, partial sequence.

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gtgatgaagaaggccttaggggtgtaaagtactttcagcgggggaagaaaaataaaactaataatttattcctgacgtacc

FJ705294 *Buchnera aphidicola* (*Diuraphis noxia*) clone BUH16SDnUSA6 16S ribosomal RNA gene, partial sequence.

attgaacgctggcggcaagcctaacacatgcaagtcgagcggcagcgaaagaaagcttgctttctgtcggcgagcggcaaacgggtgagtaa  
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cactggaactgagacacggtccagactcctacgggagggcagcagtggggaatattgcacaatggcgaaagcctgatgcagctatgccgc  
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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.

attgaacgctggcggcaagcctaacacatgcaagtcgagcggcagcgaaagaaagcttgctttctgtcggcgagcggcaaacgggtgagtaa  
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gtgatgaagaaggccttaggggtgtaaagtactttcagcgggggaagaaaaataaaactaataatttattcctgacgtacc

FJ705297 *Buchnera aphidicola* (*Diuraphis tritici*) clone BUH16SDtri 16S ribosomal RNA gene, partial sequence.

cctggctcagattgaacgctggcggcaagcctaacacatgcaagtcgagcggcagcgaaagaaagcttgctttctgtcggcgagcggcaaac  
gggtgagtaatatctgggatctgccccaaaagagggggataactactagaaatggtagctaataccgcataatgtgaaaaccaaagtggggg  
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FJ705298 *Buchnera aphidicola* (*Diuraphis nodulas*) clone BUH16SDnod 16S ribosomal RNA gene, partial sequence.

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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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FJ705305 *Buchnera aphidicola* (*Diuraphis noxia*) plasmid pLeu-Dn(USA5), 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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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.

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aagcaatgtttttggtggactattctctatgatttaatttcaatgttgatttaccaaaataaaaaaacacaaaaatgcctcactttgtttttatt  
agcagaacattactgattgtagatcatcaaaaaaacatgtttaattcaaaatagtttttacgaaaaattcccagaacagatgagagtagaaa

aaagaggagagaaatacaaaaaaacttgaagcatctttaaactctattcctgtaaggcaagaagtaaaaaatagatgtaactgcaaatatga  
gtgacgaacaatattgtccataataaaaaaattacaaatttaattcgaaggtgagattttcaagttgtaccatctcgaaaatTTTTTaccctgttc  
taatcctttatctgcctatcaaaaftaaaaaaagcaatcctagtccttatatgTTTTTatgcaagataaagattttaccttatttggtgcttcaccagaa  
agttctttaaaatgatgatgacacaacaagacaagtagaattatatccgattgctgg

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.

gattgaatatgcaggtgagatattccatggaaaagcctttaaataatcatgacggttggagatgttaaagatatccctcagccattacctgttgc  
agatatcattcactagtggtcatgtaaagatattcctaataattgattattaattctTTTTTaaaaaaccattatgtctatacgaataataaagatcgagt  
gtgtggtttcagttcatcctgaatctatttaactacatgcggtgatcaaatataaaacaataattttattgggcttcgttgcfaatatggtatgaagtaa  
ccgcaatatattaatacagttttattatattgtgatatggttttaattcagcaatttttaaacaccctccggttgcattgttatatttctatcaaatgt  
tataaactatataattatctgtatattgtgttacttatcagtcatttattcttatcatgcatattcataaaaaataattttattgttaaagatatgata  
tattgaaaacaataattcattcatgtctcttttattagttgatattgcatatcatgatcctgcgcactctcaatagagtggtgatataaagacatg  
gcatatatctagtgtaatatgaaagaaaatagattatgatgagtcgccaacgattaataattttgatcattcatataaaaatagaagtgcagca  
cttataccccatataaaaaattttgttatagactggttgcfaatattgttatcatcataatgatacatcaaaacaataaaatacaaaaatgaattttatct  
caagttatacacagaaattgtggataactTTTTTaaaaatTTTTTattataaaaaaaatgtaaacactatgttttaattatatttattctttaccat  
gaatataactgttattaacatgtttataagtctgggataagttggataaatgcaatgccccccccacaataaaaaaattagaaaattgaaat  
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aaaatcattcaaaaaaagcaagtatcatcctgacccaacaatagtttaataatattgtggatctcaaaaacaacattgttactagaacagca  
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tttcagaaaaattgatgaagacaaaaaatttttcattgtctatattgacacttttagatttattatgaaattttcaaaaatcgaataaagtacaga  
aagcaatgTTTTTggtgactattctctatgatttaattctaatTTTgattattaccaaaattaaaaaacacaaaaatgcctcacttttTTTTTatt  
agcagaacattactgattgtgatcatcaaaaaaacatgtttaatcaaaaatagttatttacgaaaattcccagaacagatgagagtagaaa  
aaagaggagagaaatacaaaaaaacttgaagcatctttaaactctattcctgtaaggcaagaagtaaaaaatagatgtaactgcaaatatga  
gtgacgaacaatattgtccataataaaaaaattacaaatttaattcgaaggtgagattttcaagttgtaccatctcgaaaatTTTTTaccctgttc  
taatcctttatctgcctatcaaaaftaaaaaaagcaatcctagtccttatatgTTTTTatgcaagataaagattttaccttatttggtgcttcaccagaa  
agttctttaaaatgatgatgacacaacaagacaagtag

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.

gattgaatatgcaggtgagatattccatggaaaagcctttaaataatcatgacggttggagatgttaaagatatccctcagccattacctgttgc  
agatatcattcactagtggtcatgtaaagatattcctaataattgattattaattctTTTTTaaaaaaccattatgtctatacgaataataaagatcgagt  
gtgtggtttcagttcatcctgaatctatttaactacatgcggtgatcaaatataaaacaataattttattgggcttcgttgcfaatatggtatgaagtaa  
ccgcaatatattaatacagttttattatattgtgatatggttttaattcagcaatttttaaacaccctccggttgcattgttatatttctatcaaatgt  
tataaactatataattatctgtatattgtgttacttatcagtcatttattcttatcatgcatattcataaaaaataattttattgttaaagatatgata  
tattgaaaacaataattcattcatgtctcttttattagttgatattgcatatcatgatcctgcgcactctcaatagagtggtgatataaagacatg



gcataatctaggtaatgaaagaaaatagattatgatgagtcgccaaacgattaataattttagatcatcattcatataaaaatagaagtgcagca  
cttatacccccataaaaaatgtttatagactgtttgtcaaatattgttatcatcataatgatacatcaacaacataaaatacaaaatgaattttatct  
caagttatacacagaaatgtggataacttttttaaaaatattttttattataaaaaaaatgtaacactatgttttaattatatttattttttaccat  
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ttttttgtttataaaaaatatttagtatcattatatttaacatagtggttatttacttatatatattgtattttctgaaaattatcaaaaatcgtatgacc  
atattgcttaattttgtaaattagttattttctttttataaaaaatattcttttgacccttaagagattgaattatggaaaaaagccatacagagac  
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gaaattaacaaaaaaatgatctagaagattatgatcatcgcgctcgaatttctctgaaagaaatcactcggtaactaacagcatt  
atctaaaaatggtgaaaatatttatcaattttaaaagcaatctgaacaaaaagtcaaatgtcatacaagatacatctattcgtttagaattccctca  
ttttcagaaaaatttagatgaagacaaaaaaatttttcattgtctatattgacacttttagatttattatgaaattttcaaaaatcgcaataaagtacaga  
aagcaatgtttttgtggactattctctatgatttaatttcaattttgagtattaccaaaaataaaaaacacaaaaatgccctcactttgtttttatt  
agcagaacattactgattgtatcatcaaaaaaacatgtttaattcaaaatagtttttacgaaaaatcccacgaacagatgagagtagaaa  
aaagaggagagaaatacaaaaaaaactgaaagcatttaactctattcctgtaaggcaagaagtaaaaaatagtagttaactgcaaatatga  
gtgacgaacaatattgtccataataaaaaaattacaatttaattcgaaggtgagattttcaagttgtaccatctcgaatattttttaccctgttc  
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agttctttaaaatagtagacacaacaagacaagtagaattatccgattgtcgcactagaccagaggaggaaacatggatggcacattaat  
ctagatttggacagtcgaatcgaactgaaatgagaaccaatcataaggaactcggcaacattaatgtagtagatttagctcgtaacgatttagc  
acggatctgtgaaccgggatcaagatatgtctcggattgttagagtcgacaatctctcatgcatgatttagtcttagagtcgtaggaacgtt  
aaaaccagagtttagatgattacatgcttatgcagcttgcataatgggtacattaactgggtcctcaaaatcgcgctatggaattgattgctga  
atatgaaatggaacagagggcgagttacgggtggtgccataggatattttactgatttagaaatttagacacatgcattacaatacgttcggcttatgt  
agaggacaatattgcaaccattcaatcaggatcaggtattgtttataattccataccgaagacgaagtcaagaaggtatcaataaagcgaaac  
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ttgtagaacaactgaggaatcaaaacaatcaagtttaattatcgtaatcggtagatattgaggtcatttttgacgctttaaagaatataaaacc  
cattttaatgttatccagggccgagatcccccaatagctggatgtatgtacccttgataaaaaagtgaaaggttacctacctataataggtatt  
gtttaggtcatcaggcagatagaagcttatggtggc

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.

gattgaatatgcagtgagatattccatgaaaagcctttaatcaatcatgacggttggagatgttaaagatatccctcagccattacctgttgc  
agatatcactactagtgcatgaaagatattcctaataattgattataattcttttttaaaaaaccattatgtctatacgaataataaagatcgagt  
gtgtggtttcagtttcatcctgaatctatttaactacatcgggtgatcaaatataaaacaataattttattgggcttcgttgcaaatatgttatgaagtaa  
ccgcaatataataaacagttttattatattgtgatattgttttaattcagcaatttttaaacaccctccgtttgcatgtttgtatatatttctatcaaatgt  
tataaactatataattatctgtatatgttggttacttatcagtcatttattcttatcatgcatattcataaaaaataattttattgttaaaagatatgata  
tattgaaaacaatcaattcacatgtgctcattttattagttgatattgcatatatcatgatcctcgcgactctcaatagagtggtgatataaagacatg  
gcataatctaggtaatgaaagaaaatagattatgatgagtcgccaaacgattaataattttagatcatcattcatataaaaatagaagtgcagca  
cttatacccccataaaaaatgtttatagactgtttgtcaaatattgttatcatcataatgatacatcaacaacataaaatacaaaatgaattttatct  
caagttatacacagaaatgtggataacttttttaaaaatattttttattataaaaaaaatgtaacactatgttttaattatatttattttttaccat  
gaatataactgttattaacatgtttataagtctggggataagttgggataaatgcaatgccctccccacaataaaaaattagaaaattgaaat  
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atattgctttaatgtgtaaattagttattttcttctttataaaaatattctttgaccccctaagagattgaattatatggaaaaagccatacagagatc  
aaaatcattcaaaaaaaagcaaagtatcatcctgacccaacaatagtttaaatcatattgtggatctcaaaaacaacattgttactagaacagca  
gaaattaacaaaaaaatgatctagaagattatgatcatcgcgatcgaatttcttgaaagaaatcactcggtaactaacagcatt  
atctaaaaatggtgaaaatatttatcaattttaaaagcaatctgaacaaaaagttcaaatgttcatacaagatacatctattcgtttagaattccctca  
tttcagaaaaatttagatgaagacaaaaaatttttcattgtctatattgacacttttagatttattatgaaattttcaaaaatcgcaataaagtacaga  
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agcagaacattactgattgtagatcatcaaaaaaacatgtttaattcaaaatagtttttacgaaaaattcccacgaacagatgagagtagaaa  
aaagaggagagaaatacaaaaaaactggaagcatcttaaaactctattcctgtaaggcaagaagtaaaaaatagtatgtaactgcaaatatga  
gtgacgaacaatattgtccataataaaaaattacaatttaattcgaaggtgagattttcaagttgtaccatctcgaaaattttttaccctgttc  
taatcctttatctgcctatcaaaaftaaaaaaagcaatcctagctcttatatgtttttatgcaagataaagattttaccttatttggtgcttcaccagaa  
agttctttaaaatgatgacacaacaagacaagtagaaa

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.

gattgaatatgcaggtgagatattccatggaaaagcctcttaaatcaatcatgacggtttgagatgtttaaagatatccctcagccattacctgttgc  
agatatcactactagtgcatgaaagatattcctaataatttgatttaattcttttttaaaaaaccattatgtctatacgaataataaagatcgagt  
gtgtggtttcagttcactcctgaatctatttaactacatcgggtgatcaaatataaaacaataattttattgggcttcgttgcaatatgttatgaagtaa  
ccgcaatatataaatacagttttattatattgtgatatggttttaattcagcaatttttaacaccctccgtttgcatgtttgtatatatttctatcaattgt  
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tattgaaaacaatcaattcacatgtgctcattttattagttgatattgcatatatcatgacctcgcgactcctcaatagagtggtgatataaagacatg  
gcataatctaggtaatgaaagaaaatagattatgatgagtcgccaacgattaataatttttagatcatcattcatataaaaatagaagtgcagca  
cttataccccatataaaaattttgttatagactgtttgtcaaatattgttatcatcataatgatacatcaacaacataaatacaaaaatgaattttatct  
caagttatacacagaaattgtggataacttttttaaaaatattttttattataaaaaaaaatgtaactatgttttaattatatttattctttttaccat  
gaataataactgttattaacatgtttataagctcgggataagtttggtataatgcaatgccctccccacaataataaaaaattagaaaattgaaat  
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ttttttggttataaaaaattattagatcattatatttaacatagtggttatttacttatatattgtatttttctgaaaattatcaaaaatcgtatgacc  
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aaaatcattcaaaaaaaagcaaagtatcatcctgacccaacaatagtttaaatcatattgtggatctcaaaaacaacattgttactagaacagca  
gaaattaacaaaaaaatgatctagaagattatgatcatcgcgatcgaatttcttgaaagaaatcactcggtaactaacagcatt  
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tttcagaaaaatttagatgaagacaaaaaatttttcattgtctatattgacacttttagatttattatgaaattttcaaaaatcgcaataaagtacaga  
aagcaatgttttggtggactattctcctatgatttaatttcaattttgagtattaccaaaattaaaaaacacaaaaatgcctcactttgtttttatt  
agcagaacattactgattgtagatcatcaaaaaaacatgtttaattcaaaatagtttttacgaaaaattcccacgaacagatgagagtagaaa  
aaagaggagagaaatacaaaaaaactggaagcatcttaaaactctattcctgtaaggcaagaagtaaaaaatagtatgtaactgcaaatatga  
gtgacgaacaatattgtccataataaaaaaattacaatttaattcgaaggtgagattttcaagttgtaccatctcgaaaattttttaccctgttc  
taatcctttatctgcctatcaaaaftaaaaaaagcaatcctagctcttatatgtttttatgcaagataaagattttaccttatttggtgcttcaccagaa  
agttctttaaaatgatgacacaacaagacaagtagaattatccgattgctgg

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.

tgaatatgcaggtgagatattccatggaaaagcctctttaatcaatcatgacggttggagatgtttaaagatatccctcagccattacctgttgctag  
atatcattcactagtgcatgtaaagatattcctaataatttgattattaattcttttttaaaaaaacattatgtctatacgaataataaagatcgagtg  
gtggtttcagttcactcgaatctatttaactacatgcgggatcaaatatataaaacaataattttgggcttcgttgcaatatgttatgaagtaacc  
gcaatatattaatacagttttattatattgtagatggtttaattcagcaatttttaacaccctccggttgcatggttgatatatttctatcaaatgta  
taataactatatttctgtatatgttggttacttatcagtcatttattctatcatgcataatcataaaaaataattttattgtaaaagatatgatata  
ttgaaaacaaatcaatcacatgtgctcattttattagttgatatttgcataatcatgatcctgcgcactctcaatagagtggtgatataaagacatggc  
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atacccccataaaaaattttgttagactgttgtcaaatattgttatcatcataatgatacatcaacaacataaaatacaaatgaattttatctca  
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gcaatgtttttggtgactattctctatgatttaatttctaattttgagttattaccaaaatataaaaaaacacaaaaatgccctcactttgttttttag  
cagaacattactgattgtagatcaaaaaaaacatgtttaattcaaaatagttattttacgaaaaattcccacgaacagatgagtagaaaaa  
agaggagagaaatacaaaaaaacttgaagcatcttaaaacttattcctgtaaggcaagtaaaaaatagatgtaactgcaaatatgagtg  
acgaacaatattgtccataataaaaaaattacaatttaattcgaagggtagattttcaagttgaccatctcgaattttttaccctgttctaat  
cctttatctgcctatcaaaaaatataaaaaaagcaatctagtccttatattttttatgcaagataaagattttacattttggtgcttcaccagaaagtt  
ctttaaaatgatgacacaacaagacaagtagaat

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.

tgaatatgcaggtgagatattccatggaaaagcctctttaatcaatcatgacggttggagatgtttaaagatatccctcagccattacctgttgctag  
atatcattcactagtgcatgtaaagatattcctaataatttgattattaattcttttttaaaaaaacattatgtctatacgaataataaagatcgagtg  
gtggtttcagttcactcgaatctatttaactacatgcgggatcaaatatataaaacaataattttgggcttcgttgcaatatgttatgaagtaacc  
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ttgaaaacaaatcaatcacatgtgctcattttattagttgatatttgcataatcatgatcctgcgcactctcaatagagtggtgatataaagacatggc  
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atcattcaaaaaaagcaagtatcctgacccaacaatgatttaacatattgtggtatcctcaaaaacaacattgttactagaacagcaga  
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taaaaatggtgaaaatattttatcaattttaaaaagcaatctgaacaaaaagttcaaatgttcatacaagatacatctattcgtttagaattccctcatttt  
cagaaaaatttagatgaagacaaaaaaatttttcattgtctatatttgacacttttagatttattatgaaattttcaaaaatcgcaataaagtagacagaaa  
gcaatgtttttggtggactattctctatgatttaatttctaattttgagttattaccaaaatataaaaaaacacaaaaatgccctcacttttgttttatttag  
cagaaacattactgattgtagatcatcaaaaaaaacatgtttaattcaaaaatagttatttacgaaaaattcccacgaacagatgagagtagaaaaa  
agagggagagaaaatacaaaaaaaacttgaagcatctttaaactctattcctgtaaggcaagaagtaaaaaatagtagttaactgcaaatatgagtg  
acgaacaatattgtccataataaaaaaattacaattttaattcgaaaaggtgagattttcaagttgtaccatctcgaaaattttttaccctgttctaat  
cctttatctgcctatcaaaaataaaaaaaagcaatcctagtccttatatgtttttatgcaagataaagattttaccttatttgggtgcttcaccagaaagtt  
ctttaaaatgatgacacaacaagacaagtagaa

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.

gcaggtgagatattccatggaaaagcctcttaataatcaatcatgacggttggagatgtttaaagatatccctcagccattacctgttgctagatattcatt  
cactagtggcatgtaaagatattcctaataatttgattattaattcttttttaaaaaaacattatgtctatatacgaataataaagatcgagtggtggtttt  
cagtttcatctgaatctattttaaactacatgcgggtgatcaaatataaaacaataattttattgggcttcgttgcaaatatgttatgaagtaaccgcaatat  
attaaatacagttttattatattgtgatattggttttaattcagcaatttttaacaccctccggttgcatgtttgtatataatttctatcaaatgttataataact  
atataattatctgtatattgtgtgttacttatcagtcatttattcttatcatgcatattcataataaaataattttattgttaaaagatatgatattgaaaac  
aaatcaattcacatgtgctcattttattagttgatattgcatatataatgatcctgcgcactctcaatagagtggtgatataaagacatggcatatatac  
ggtaatatgaaagaaaatagattatattgatgagtcgccaacgattaataatttttagatcatcattcatataaaatagaagtgacgacttatcccc  
atataaaaattttgttatagactgtttgtcaaatattgttatcatcataatgatacatcaaacataaaatacaaaaatgaattttatctcaagttataca  
cagaaattgtggataacttttttaaaaatattttttattataaaaaaaatgaacactatgttttaattatatttattctttattttaccatgaatataaac  
tggtattaacatgtttataagctctgggataagtttggtataatgcaatgccctcccacaatataaaaaatagaaaattgaaataaaaaatatta  
aagacaattcaataatctcaaaagataaaatccttaaaagatttaaaaaaaagaaatataatgtatataatattattgttattgtttttttttggtttat  
aaaaaaatttagtatcattatattaacatagtggttatttacttatataatattgtattttctgaaaattatcaaaaatcgtatgaccatattgctttaat  
tttgtaaattagttattttcttctttataaaaaatattcttttgaccctaaagagatttgaattatattgaaaaaaagccatacagatcaaaatcattcaa  
aaaaagcaagtatcctgacccaacaatagtttaatacattttgtggatctcaaaaacaacattgttactagaacagcagaaattaacaa  
aaaaatgatctagaagattatgatcatcgtgctgcgtacgaattctctgaagaaatcactcgttacaactaacagcattatctaaaatgg  
tgaanaattttatcaatttttaaaaagcaatctgaacaaaaagttcaaatgttcatacaagatacatctattcgtttagaattccctcattttcagaaaaa  
tttagatgaagacaaaaaaatttttcattgtctatatttgacacttttagatttattatgaaattttcaaaaatcgcaataaagtagacagaaagcaatgtttt  
tggtggactattctctatgatttaatttctaattttgagttattaccaaaatataaaaaaacacaaaaatgccctcacttttgttttatttagcagaaacat  
tactgattgtagatcatcaaaaaaaacatgtttaattcaaaaatagttatttacgaaaaattcccacgaacagatgagagtagaaaaaagaggggag  
agaaatacaaaaaaaacttgaagcatctttaaactctattcctgtaaggcaagaagtaaaaaatagtagttaactgcaaatatgagtgacgaacaat  
attgtccataataaaaaaattacaattttaattcgaaaaggtgagattttcaagtt

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.

tgaatatgcaggtgagatattccatggaaaagcctcttaataatcaatcatgacggttggagatgtttaaagatatccctcagccattacctgttgctag  
atatcattcactagtggcatgtaaagatattcctaataatttgattattaattcttttttaaaaaaacattatgtctatatacgaataataaagatcgagtg  
gtggttttcagtttcatctgaatctattttaaactacatgcgggtgatcaaatataaaacaataattttattgggcttcgttgcaaatatgttatgaagtaacc  
gcaatataaaatacagttttattatattgtgatattggttttaattcagcaatttttaacaccctccggttgcatgtttgtatataatttctatcaaatgtta  
taataactatataattatctgtatattgtgtgttacttatcagtcatttattcttatcatgcatattcataataaaataattttattgttaaaagatatgata

ttgaaaacaaatcaattcacatgtgctcattttattagttgatatttgcataatcatgatcctgcgcactctcaatagagtggatataaagacatggc  
atataatctaggtaatatgaaagaaaatagattatatgatgagtcgccaacgattaataattttagatcatcattcatataaaaatagaagtgcagcactt  
ataccccatataaaaattttggatagactgttgcaaatattgttatcatcataatgatacatcaaacataaaatacaaaaatgaatttatatctca  
agttatacacagaaattgtggataactttttaaaaatattttttattataaaaaaaaaaatgtaacactatgtttaattatatttattttttaccatga  
atataatactgttattaacatgtttataagtctggggataagtttggtataatgcaatgcccccacataataaaaaattagaaaaattgaaataa  
aaaatattaaagacaattcaataatctcaaaagataaaatccttaaaagatttaaaaaaaaagaaatataatgtatataatattattgttattgtttttt  
tttggtttataaaaaattattagatcattatatttaacatagtggttatttacttatatatattgtatttttctgaaaattatcaaaaatcgtatgaccata  
ttgctttaatttgtaaattagttattttctttttataaaaaatattcttttgaccccctaaagagatttgaattatatggaaaaaagccatacagatcaaa  
atcattcaaaaaaagcaaagtatcctgacccaacaatagtttaacatatttgggatctcaaaaacaacattgttactagaaacagcaga  
aattaacaaaaaaatgatctagaagattatgatcctgatgctgcgctacgaatttctctgaaagaatcactcggtaacaactaacagcattatc  
taaaaatggtgaaaatttttatcaattttaaaaagcaatctgaaacaaaaagttcaaatgttcatacaagatacatctattcgtttagaattccctcatttt  
cagaaaaattagatgaagacaaaaaaattttctattgtctatattgacacttttagatttattatgaaattttcaaaaatcgcaataaagtacagaaa  
gcaatgtttttggtggactattctctatgatttaatttctaattttgagttattacaaaataaaaaaacacaaaaatgccctcacttttgtttttatttag  
cagaaacattactgattgtagatcatcaaaaaaacatgtttaattcaaaaatagtttatttacgaaaaattcccacgaacagatgagagtagaaaaa  
agagggagagaaatacaaaaaaacttgaagcatcttaaaacttattcctgtaaggcaagaagtaaaaaatagtatgtaactgcaaatatgagtg  
acgaacaatattgtccataataaaaaaattacaaattttaattcgaaaagggtgagattttcaagttgtaccatctcgaaaattttttaccctgttctaat  
ctttatctgcctatcaaaaatfaaaaaaagcaatcctagtccttatatgtttttatgcaagataaagattttaccttatttggtgcttcaccagaaagtt  
ctttaaataatgatgacacaacaagacaagtagaattatatccg

## 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.

I t a t a a t t t t t t t a a t a a a c a t c t a t a t t t a t a g t g a g a a a t t t t a t g a g t t c t a a a g t t a t t t t t g a t a c c a c t t a c g

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

t t t a t t t t t a a t a a a c a t c t a t a t t t a t a g t g a g a a a t t t t a t g a g t t c t a a a g t t a t t t t t g a t a c c a c t t a c g

GU145281 repA, leuA and inverted repeat region of *Buchnera aphidicola* plasmid pLeuABCD, primary endosymbiont of *Diuraphis mexicana*, partial.

t c a a t c a c c g a g c t t c a c g t t t a a t a a a t g a t t t t t a g a a c c a a t g g g t t t a t t a g a t g t a a a a a a t a a a a g t a a a t c t g t t a g t a a t t a t a c c t  
a a a a a a t a t t t t a a c c a a t g t t t t t a t g t t a t t t a a c a t a t c a a a t a a a a t a g t t a t t a t c t a a g t c a a t g a a a a t a t c t c a a a a t a a a  
g a t t c a g a a a a a a a t a t t t a t t c t t t c a g a t a t t a a g g t a a t g t c a a a g t t a g a t g a a a a t c t g t t a g a a a a a a t t t a a t g t t t a a t t a a t t  
a t t a t a c a g c a a g t g a a c t g a c a a a a t a g g c c c t a a g g g c c t a a a g a a c g a a t a g a t g t t g a a t a t a a a t t t a t g t a a a t t t t a a a a a a t t a  
a a a a a a a a t t a t a c a a a a a t t c a t a a g a c a t g a a g g t a c a a t t t t g t a c c t c c a t g t c t t a g a a t t t t a t t g t a t a a t t t t t t a a a a a  
a a c a t t c a a a a t c t a t a g t g a g a a a a t t t a t g a g t t c t a a a g t t a t t a t c t t g a t a c c a c c t a c g t g a t g g t g a a c a g g c

GU145282 repA, leuA and inverted repeat region of *Buchnera aphidicola* plasmid pLeuABCD, primary endosymbiont of *Diuraphis tritici*, partial.

A t g c g g c t t a c t a c t t t t c a t t c t g g t a a a a t c t a t c a c c g t g t t c a c g t t t a a t a a a t g a t t t t t a g a a c c g a t g g g g t t g t t a a t g c a a a  
a a a a a a c a g t g a a t c t g t t a g t a a t t a t a c c t a a a a a a t t t t t a c c a a t g t t c t t a t g t t g t t a a c a t a c c a a t c a a a a a a a t a g t t  
a t t a t c t a a g t c a a t g a a c a t g t c t c a a a a g t t a a a a t a a c a g a a a a a a a t g t t a t t c t t t t c a g a t a t t a a g g t a a t g t c a a a a t t a g a t g a a a  
a a t c t g t t a g a a a a a a t t t a a t g c t t t a a t t a a t t a t a t a c a g c a a g t g a a c t a c a a a a a t a g g c c t a a g g c c t a a a c a a a g a a t a g a t  
g t t g a a t a t a a a t t a t g t a a a t t t a a a a a a a a a a a a a a g t t g a t a c a a a a a a t t c a t a a g a c a t g t a g g t a c a c a a t t t t g a c c a t a c a t  
g t c t t a t g a a a t t t t a g t g t a t c t t a t t t t t t t a a a a c a a c t t t t a a t c t t t a a a a g g g g a g a a a t t t t a t g a g t t c t a a a g t t a t t a t c t t t g a t a  
c c a c c t a c g t g a t g g t g a c a a g c a t t a c a a g c a a g t t g a c g t t a a g a a a a t t a c a a a t c g a t t a t c t t a g a a a a t g t g g g a t a g a t a t  
t a t t g a g t a g c t t t t e t t a t t c a t c a c c t g g

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

a a a t t g t c c g a a a g g t c t t a a a a a a a g a t a g a t a t t g a a t a t a a t t t a t g t a a t t g c a t a a a a a a a a t c a t a a a a a a t t t a t a c a c a a a a  
t t c a t a a g a c a t c a t a t a c a a a a a c a t c t t g t a t a t g a t g t c t t a t g a a a t t t g t t g t a a a t t t t a t t t a t t g g t a t a a a c a t t c t a t t t c t a t c g a  
g a a a a t t t a t g a a t t c g a a a g t t a t t t t t t g a t a c a a c t t t g c g g g a t g g t g a c c a a g c a t t a g g a t

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

cgaaaattggtccgaaaggtcttaaaaaaagatagatattgaatatacatttatgtaattatacaaaagaaaataaaaaataaatttatacacia  
aaatttcataagacgtaaaaatgcaatacatgtgttttaagtcttatgaaattttgtgtataaatttttttaataaatcattttattttgtcagaaa  
aatttttatgaactctaaagttatttttgatacaa

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

gaattaactaaaattggtcctcaaggtctgaaaaaaagatagatcgaatataaatttatgtaagttaacaaacataaataacatcatattatac  
aataaaaatttcataagacatcggtgacaaaaatcattttttgtacaacgatgtcttatgaaattttattgtataatcagatgttattaacaacattcatt  
ttatcggaataattttatgaattctaaagttattttttgatacaactctacgtgatgggatcaagcattaggatac

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

gaattaacgaaaattggtccgaaggtcttaaaaaaagatagatattgaatattgtaattatgtaattataaaaaaaatattagataaattata  
caataaaaatttcataaaagcagaaagatgcatttttaaaaatgcactttctgttttatgaaattttattgtataatttatctaattaacaaactatttttaa  
aattagcgagaaaaatttatgaattcaaaaattattttttgatacactctacgagatggggaccaagcattaggatctagta

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

gaattaacaaaaatggtcctcaaggtcttaaaaaaagatagatattgaatattgtaattatgtaattataaaaaaaatattagataaattata  
caataaaaatttcataaaacagaaagatgcatttttaaaaatgcactttctgttttatgagattttattgtataatttatctaattaacaaactatttttaa  
aattagcgagaaaaatttatgaattcaaaaattattttttgatacactctacgggatggagaccaagcattaggatcacagaatgg

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

aatagatattgaatataaatttatgtaatttttaacaaaattaaaaataaatttacacaataaaaatttcataagacatcaaggtacaaaattttgta  
ccttgatgtcttatgaaattttattgtgtaatttttttcaaaaacatttttttttaaaagatgagaaaaatttttatgaattctaaagttatttttt

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

gaattaacgaaaatggtcctaaagggtcttaaaaaacaaatagatattgaatataaatttatgtaatttttaacaaaattaaaaataaatttaca  
caataaaaatttcataagacatcaaggtacaaaattttgtaccttgatgtcttatgaaattttattgtgtaatttttttcaaaaacatttttttttaa  
aagatgagaaaaatttttatgaattctaaagttattttttgataccaccttacgcatggagatcaagcattaggatac

## APPENDIX – CHAPTER 5

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

<i>Script, code or filename</i>	<i>Description</i>
<i>Script 1 Data in, normalization &amp; quality check</i>	Read all the *. <i>Cel</i> files and <i>Target.txt</i> files in and normalizes with RMA, PLM, GCRMA, VSN and MAS5.0. Provides various figures to investigate the quality of the slides, <i>i.e.</i> raw data ( <i>Figure 5.2</i> ).
<i>Script 2 Quality control analysis</i>	Uses the *. <i>RData</i> files generated with script 1 and produce graphs for the different normalized datasets ( <i>Figure 5.2</i> ).
<i>Script 3 Getting differentially expressed genes</i>	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 ( <i>Figure 5.2</i> ).
<i>Target.txt</i>	A summary, tab delimited file that contains sample and slide names, filenames, replicates, <i>etc.</i> Data in this file is used for the figure legends.
<i>Convert</i>	<i>MS Excel 2007</i> macro that copies all the worksheets, <i>i.e.</i> *. <i>csv</i> 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(multttest)
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(filenamees=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",xlab=xlable,ylabel=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 analyzed:"); 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

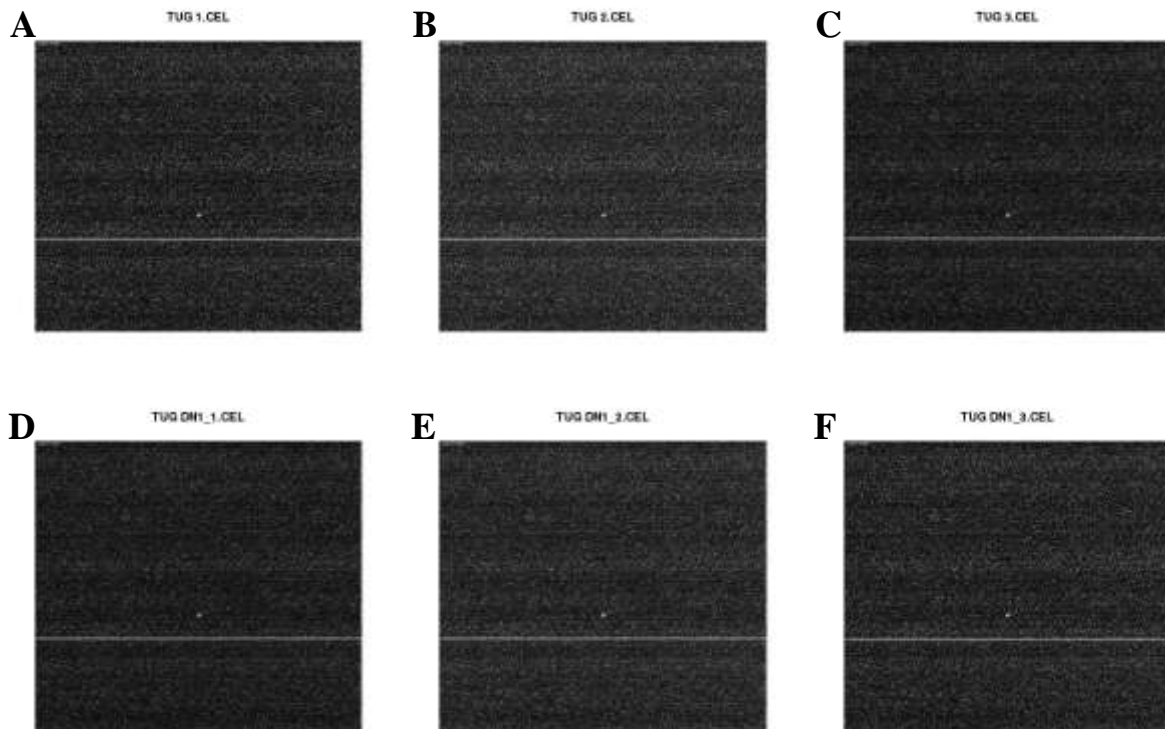
End If

```
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
```

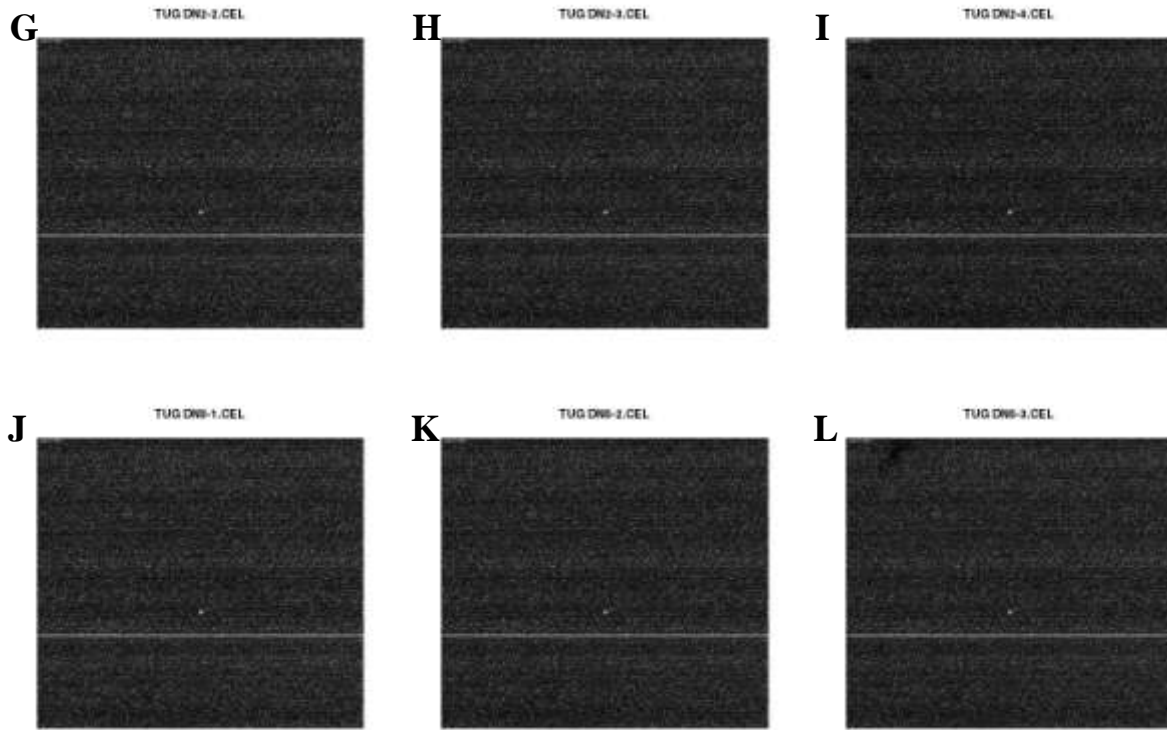
### Data visualization before normalization and background correcting

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.

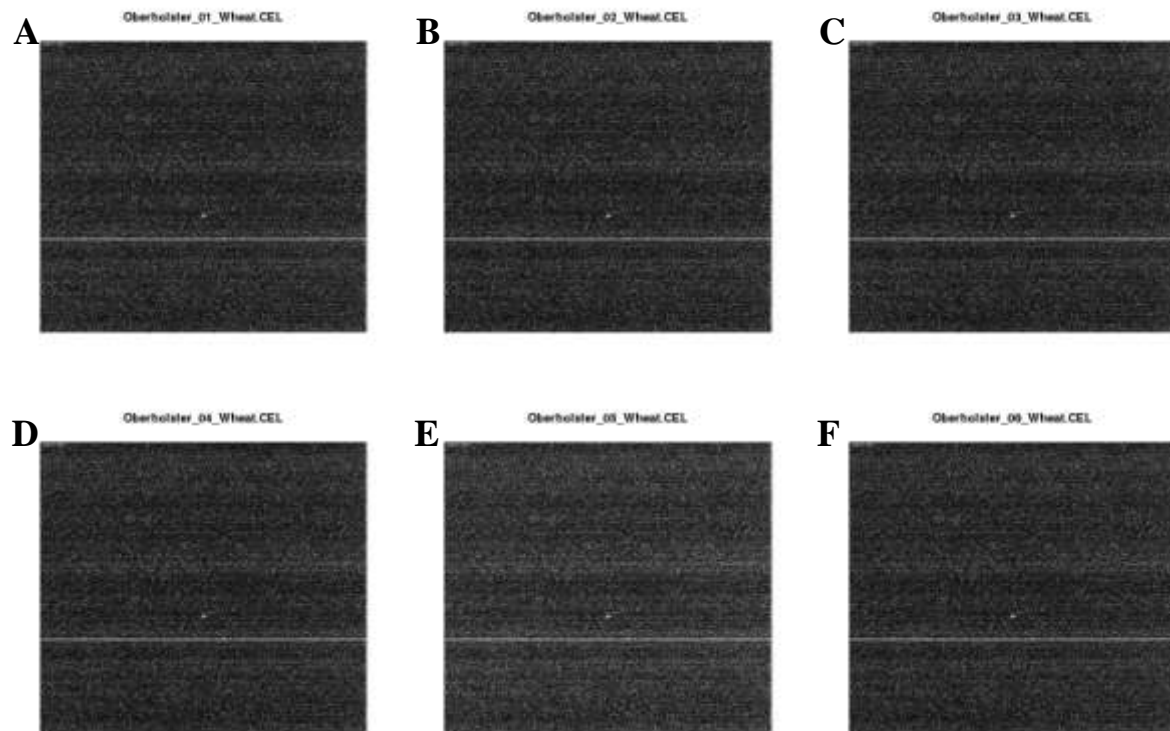
**Figure Appx 5.1 - 12 Slides**



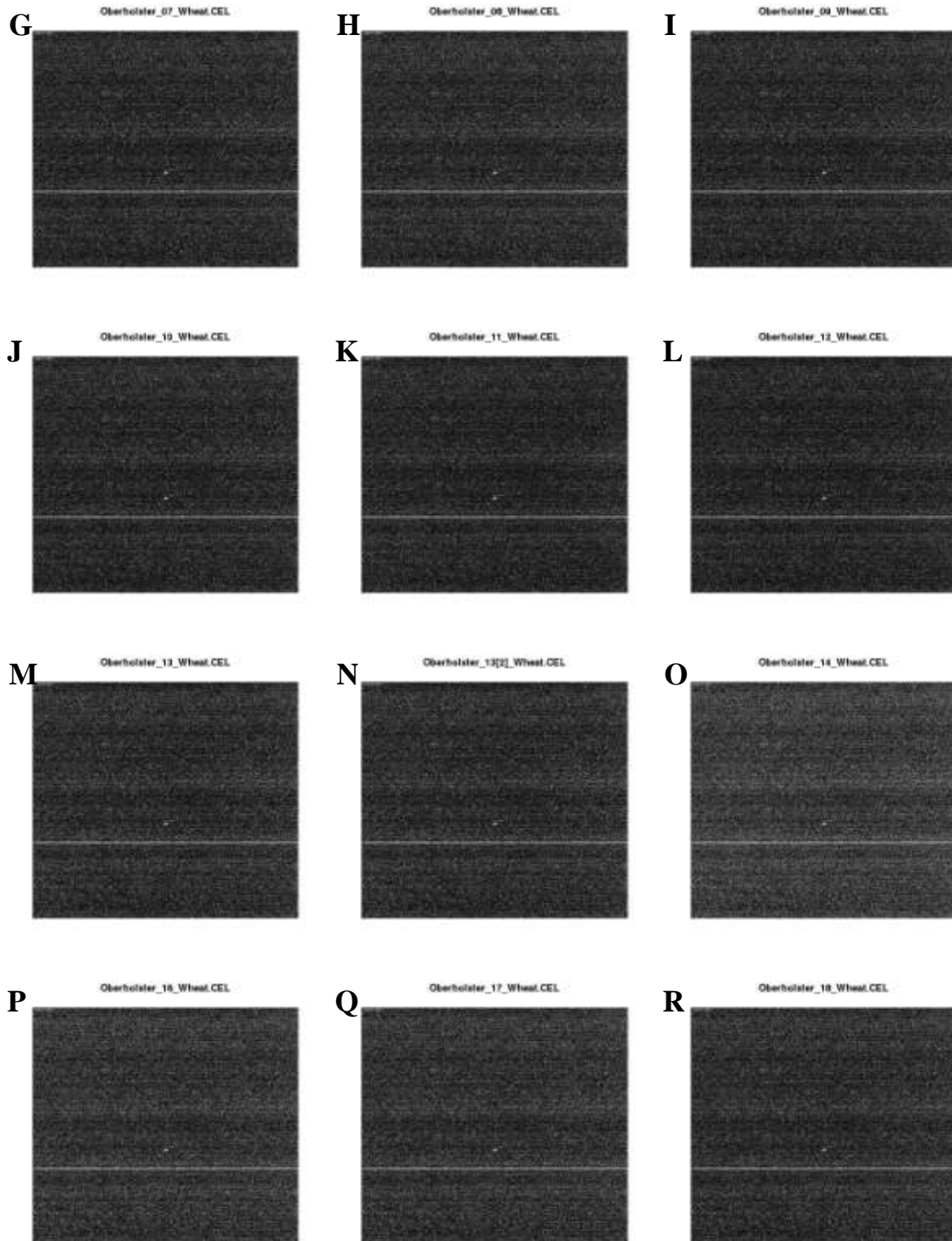
*Figure Appx 5.1 - 12 Slides cont.*



*Figure Appx 5.1 - 18 Slides*



**Figure Appx 5.1 - 18 Slides cont.**



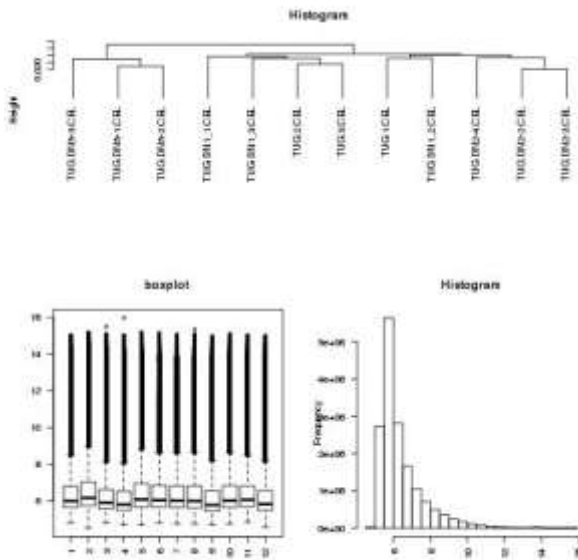
**Figure Appx 5.1** Images of the scanned Affymetrix slides after hybridization with the different treatments. The images were produced from the *CEL* files. The 12 slides images were the originally produced *CEL* images, while the 18 slides images are the  $\log_2$  derivatives of the original 18 slides



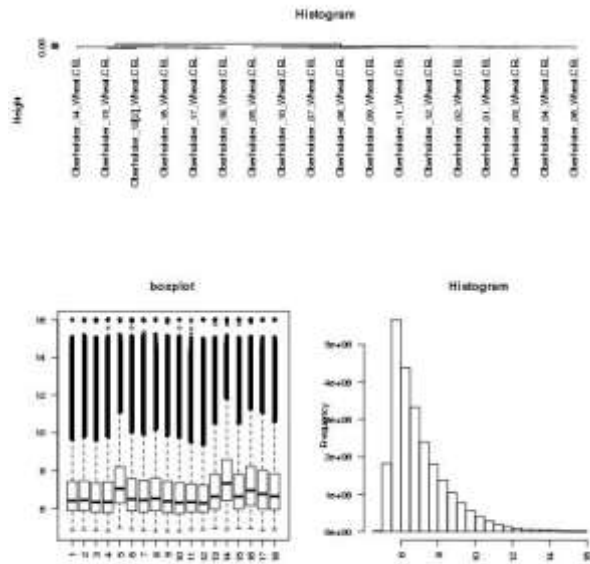
images. Both types were produced by script 1 and saved in */R results/Quality control raw data/Slide Images raw/*. The filenames for the 12 slides are: (A) Tug1\_raw.jpg, (B) Tug2\_raw.jpg, (C) Tug3\_raw.jpg, (D) TugDn\_1\_raw.jpg, (E) TugDn\_2\_raw.jpg, (F) TugDn\_3\_raw.jpg, (G) TugDn2\_1\_raw.jpg, (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<sub>2</sub> 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.

## 12 Slides



## 18 Slides



**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

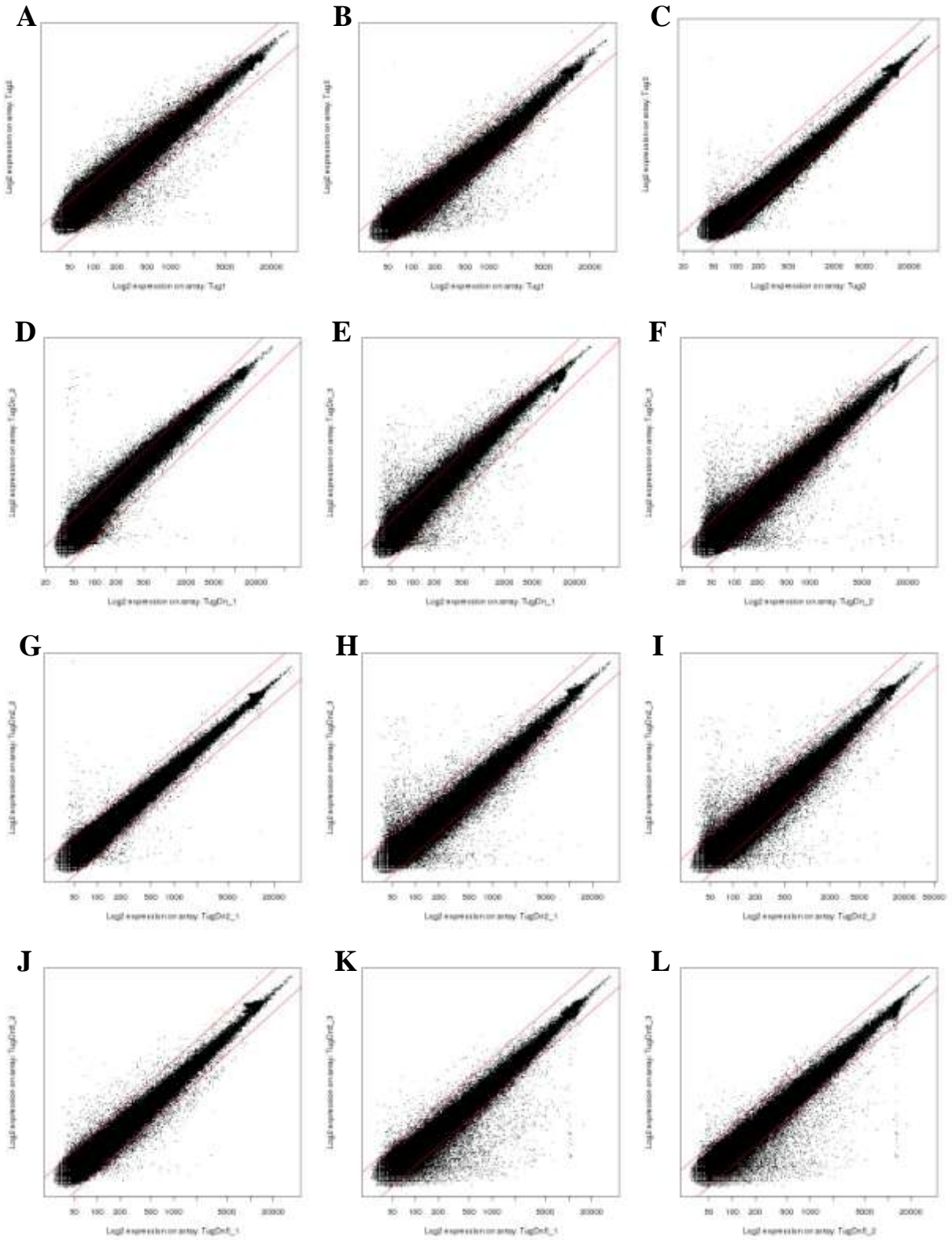
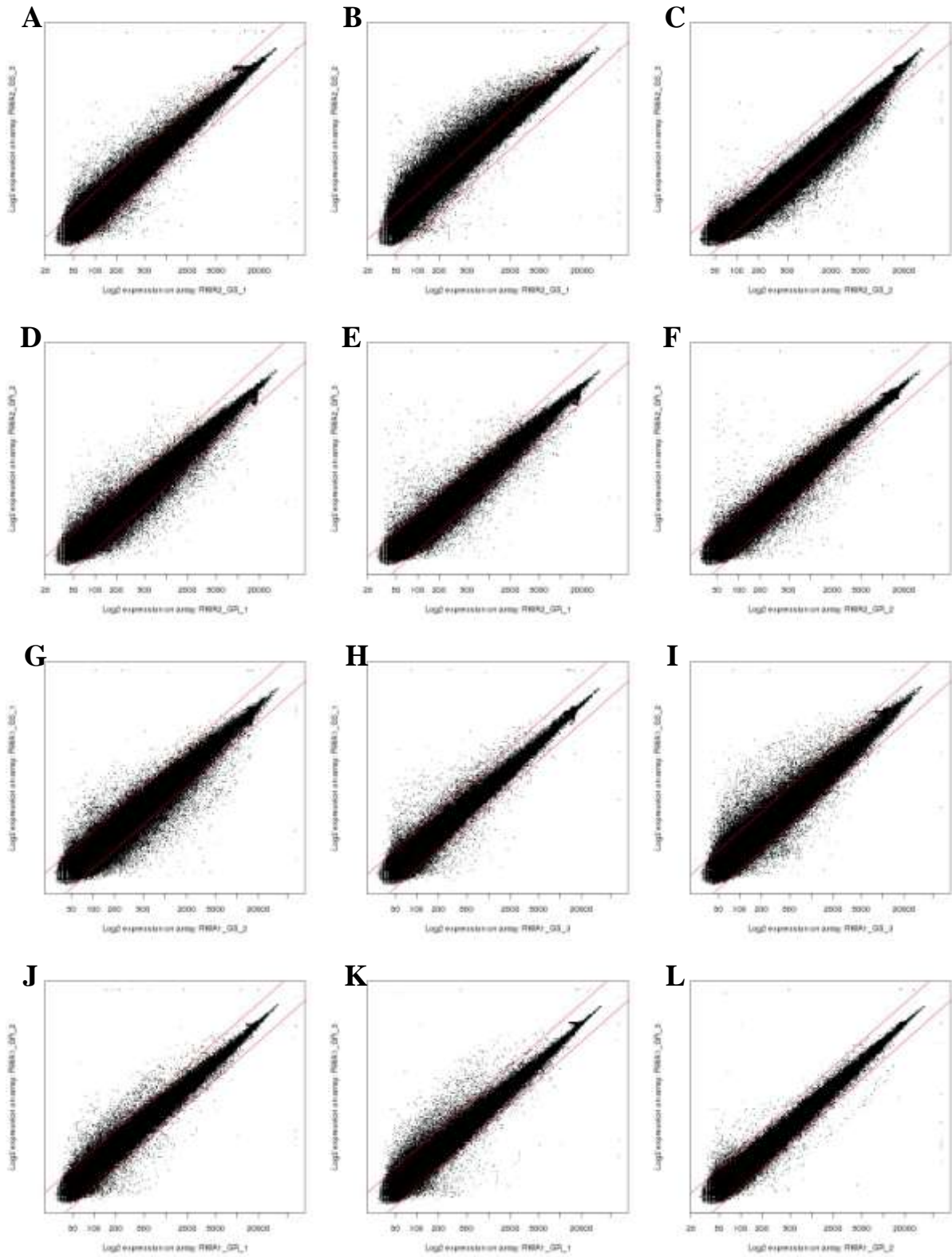
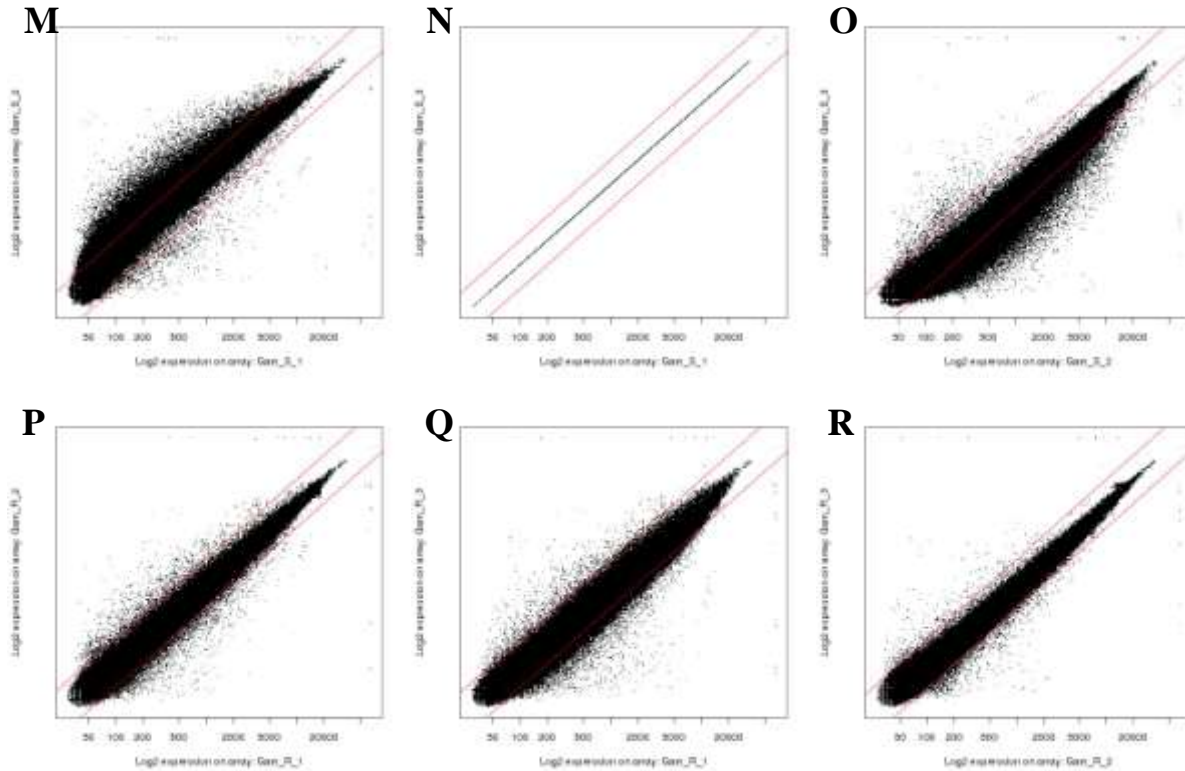




Figure Appx 5.3 - 18 Slides



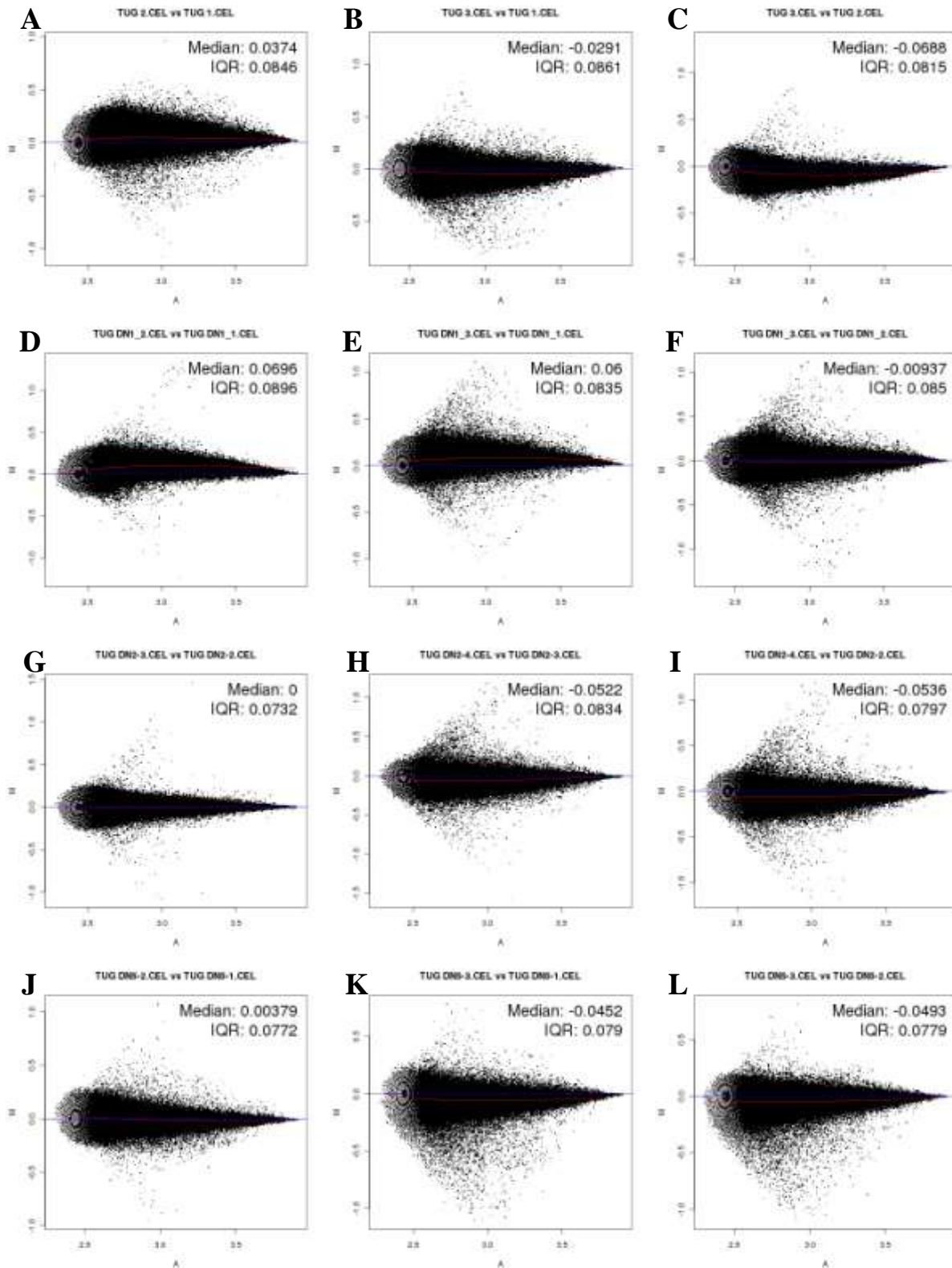
**Figure Appx 5.3 - 18 Slides cont.**



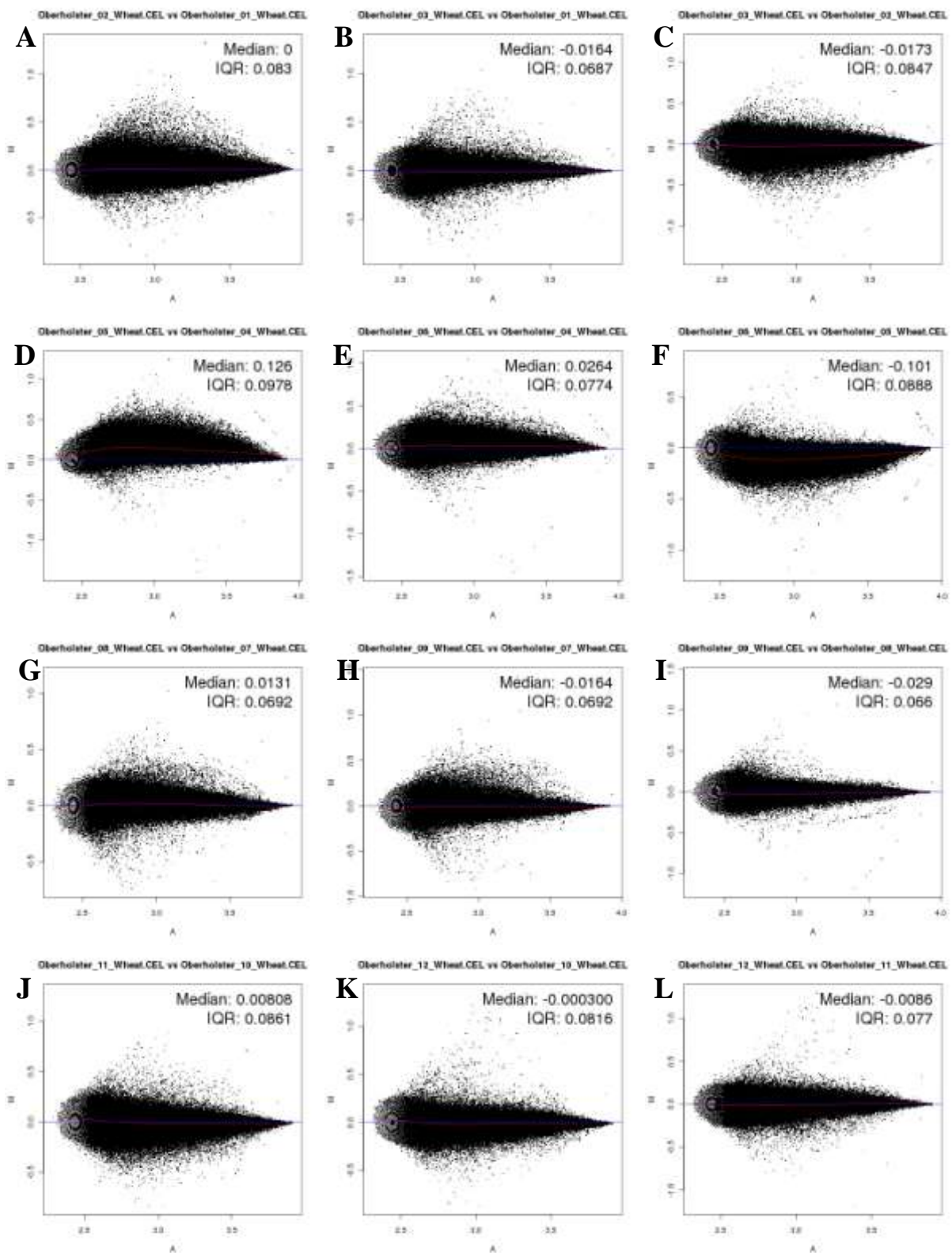
**Figure Appx 5.3** The  $\log_2$  expression comparisons of the raw, pre-normalized data within treatments. The 12 slides files are: (A) Tug1\_vs\_Tug2\_log2.jpg, (B) Tug1\_vs\_Tug3\_log2.jpg, (C) Tug2\_vs\_Tug3\_log2.jpg, (D) TugDn\_1\_vs\_TugDn\_2\_log2.jpg, (E) TugDn\_1\_vs\_TugDn\_3\_log2.jpg, (F) 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

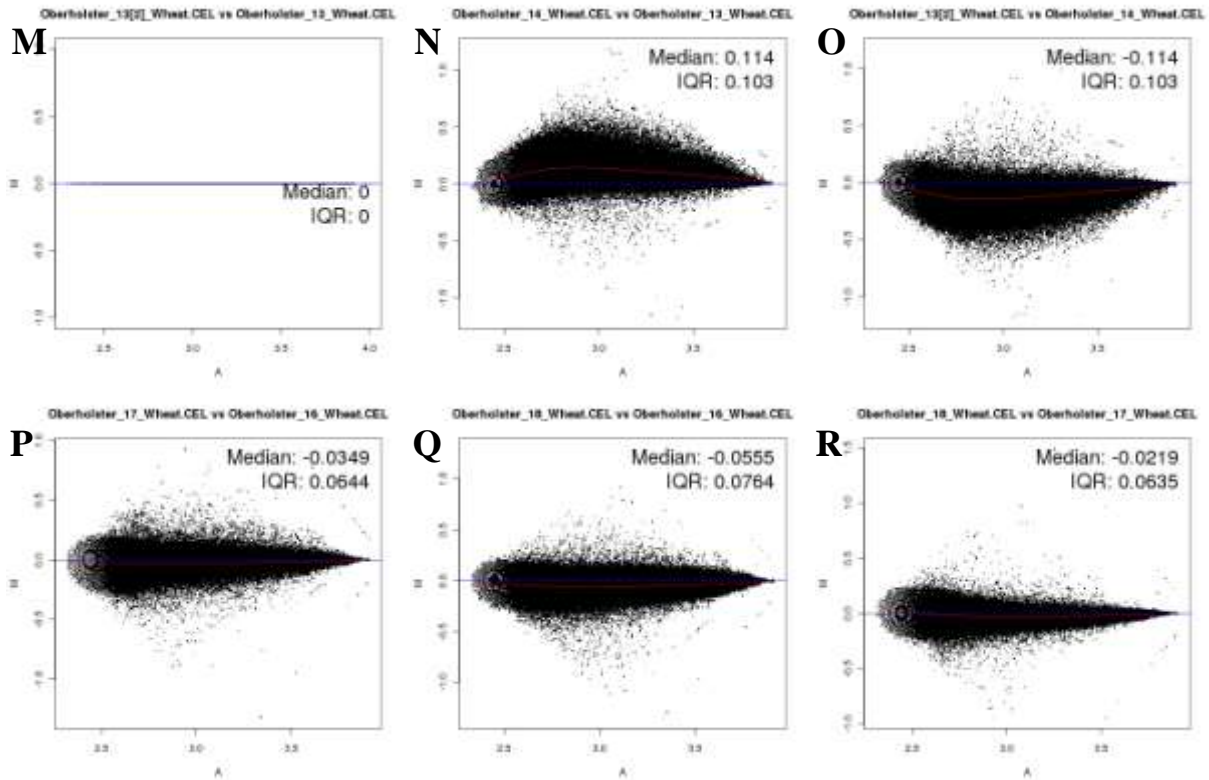


**Figure Appx 5.4 - 18 Slides**





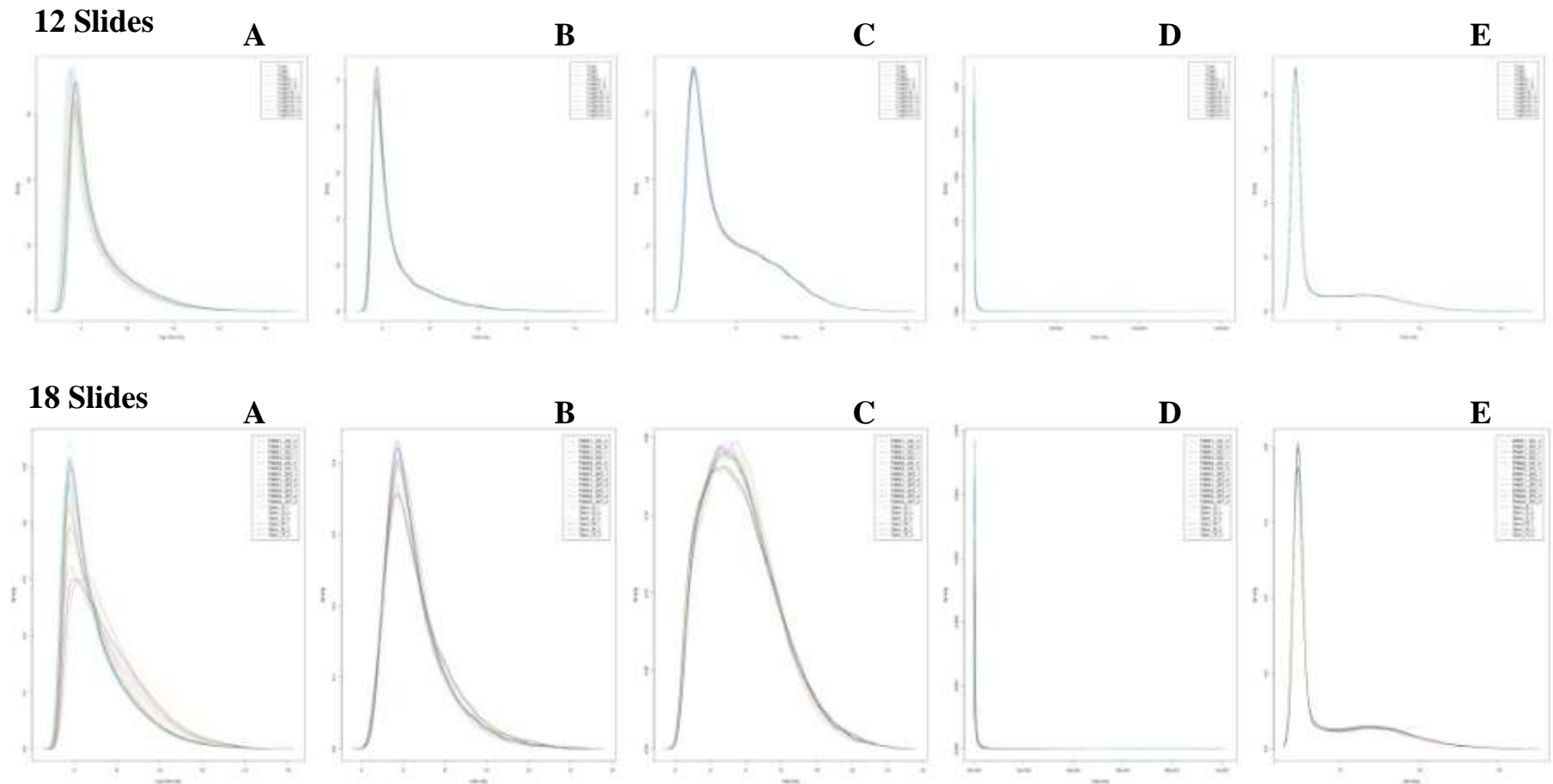
**Figure Appx 5.4 - 18 Slides cont.**



**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.

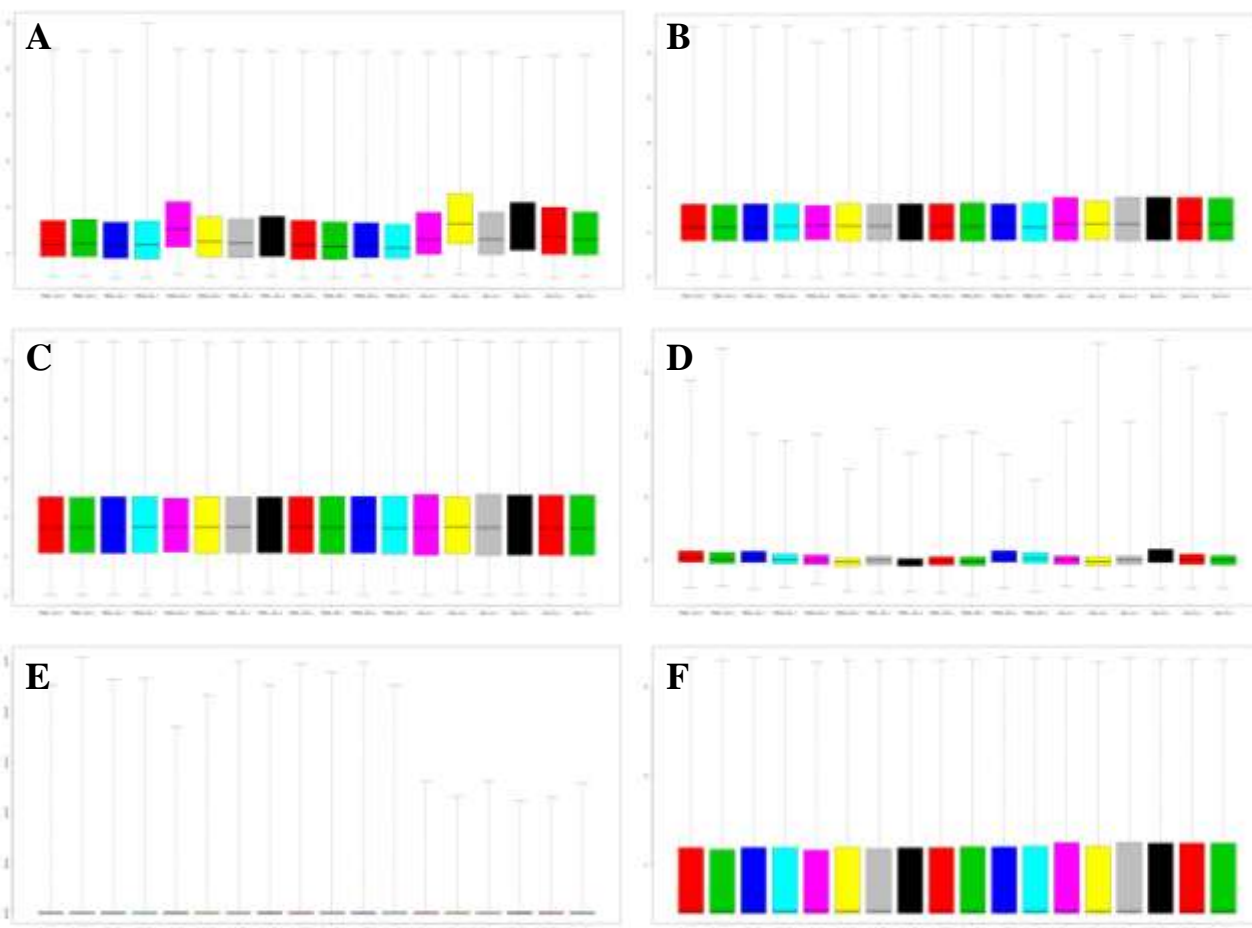
The 18 slides files are: (A) RWA2\_GS\_1\_vs\_RWA2\_GS\_2\_raw.jpg, (B) RWA2\_GS\_1\_vs\_RWA2\_GS\_3\_raw.jpg, (C) RWA2\_GS\_2\_vs\_RWA2\_GS\_3\_raw.jpg, (D) RWA2\_GR\_1\_vs\_RWA2\_GR\_2\_raw.jpg, (E) RWA2\_GR\_1\_vs\_RWA2\_GR\_3\_raw.jpg, (F) RWA2\_GR\_2\_vs\_RWA2\_GR\_3\_raw.jpg, (G) RWA1\_GR\_1\_vs\_RWA1\_GR\_2\_raw.jpg, (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**



**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  $\log_2(\text{PM})$  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

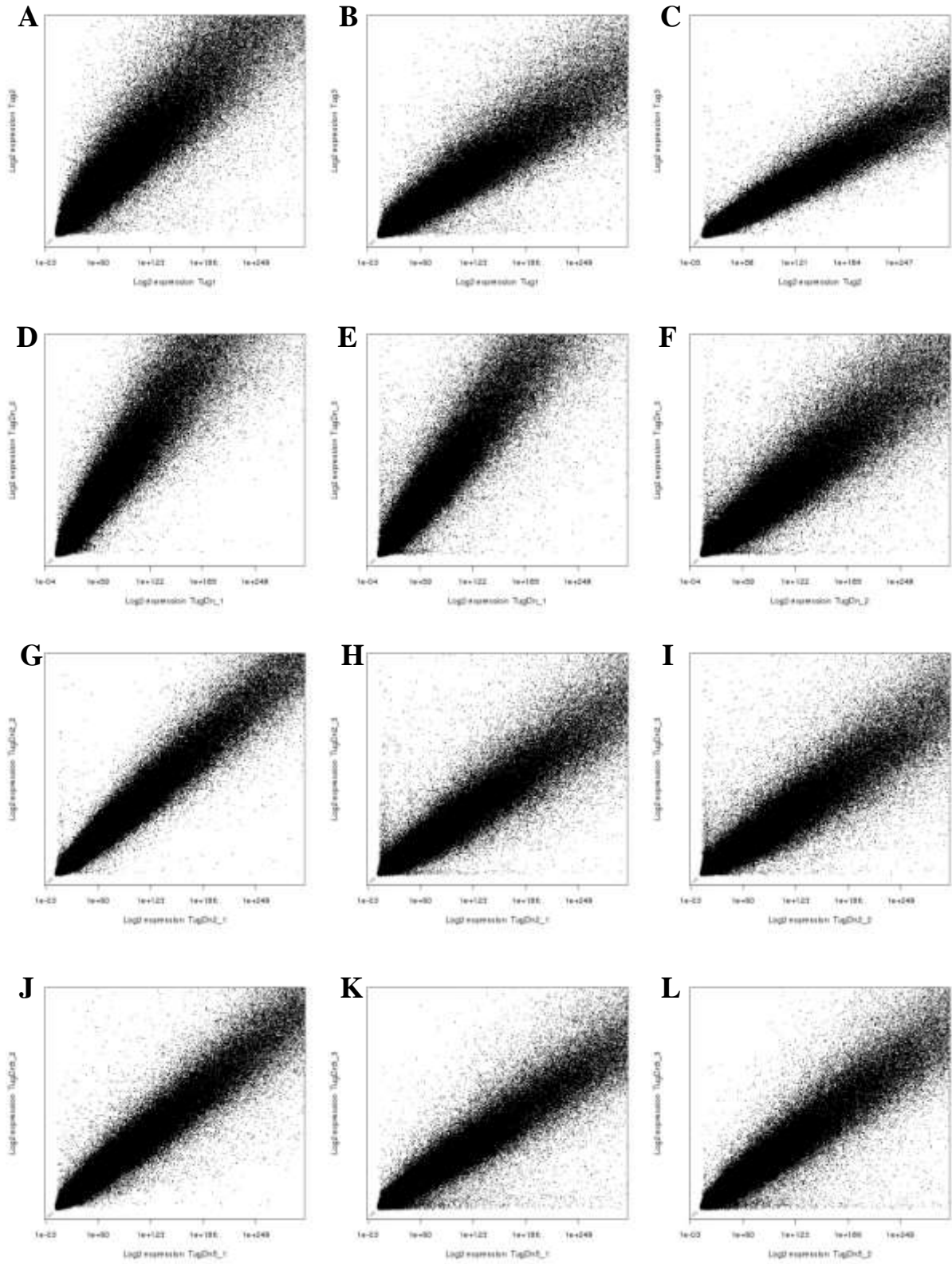




Figure Appx 5.7 - 12 Slides cont.

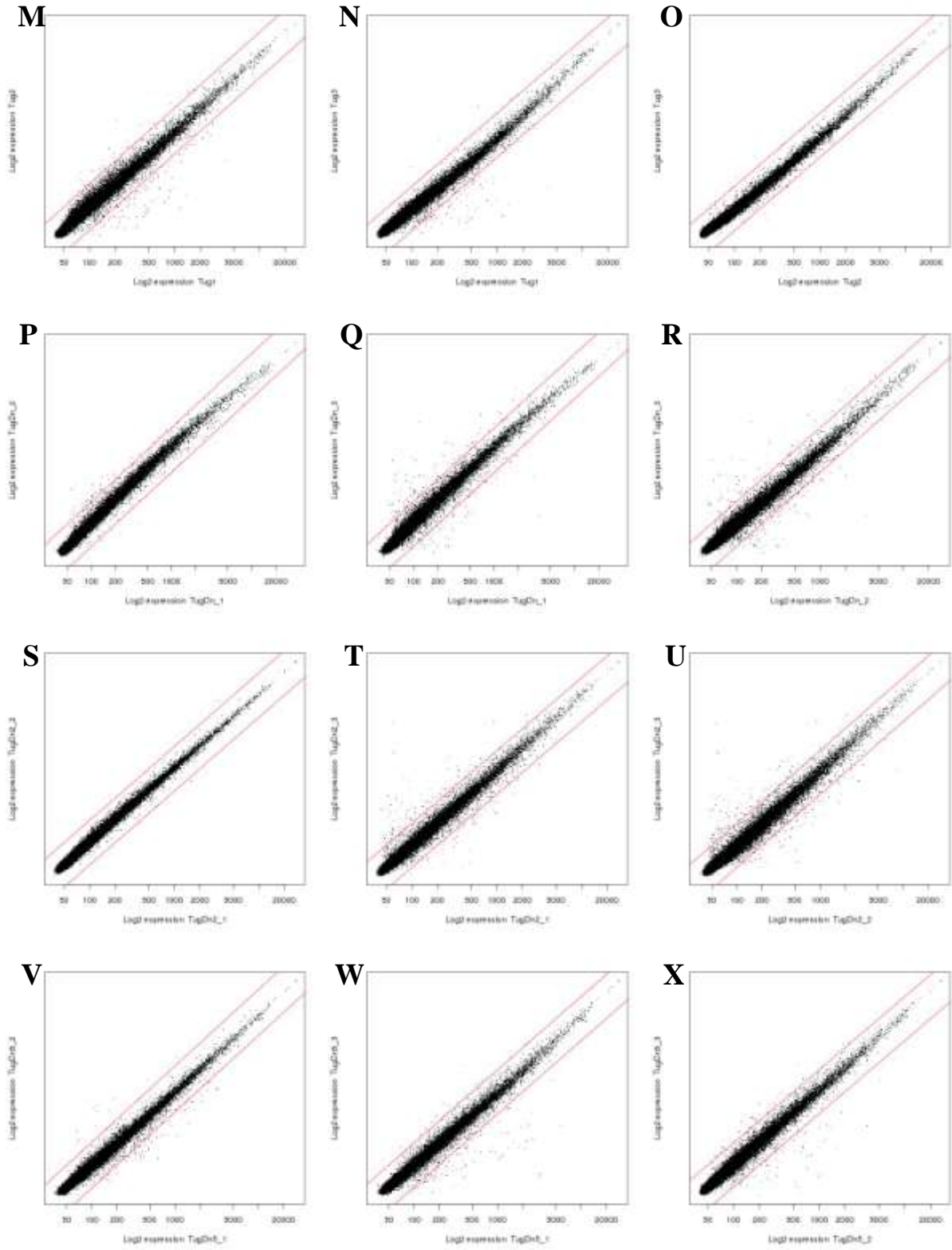


Figure Appx 5.7 - 12 Slides cont.

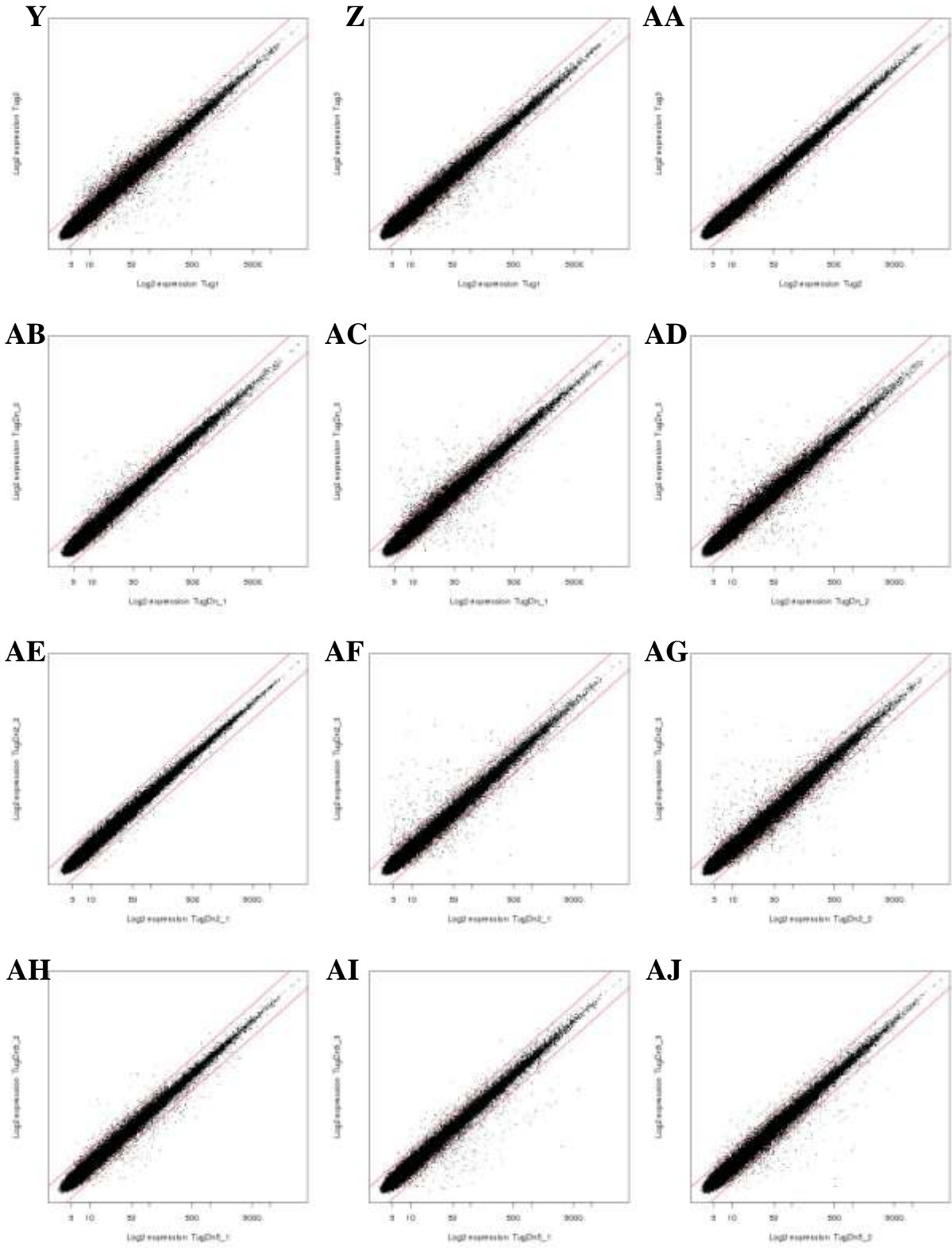
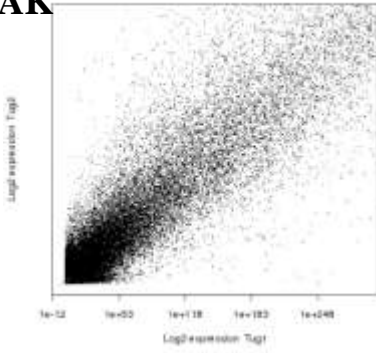
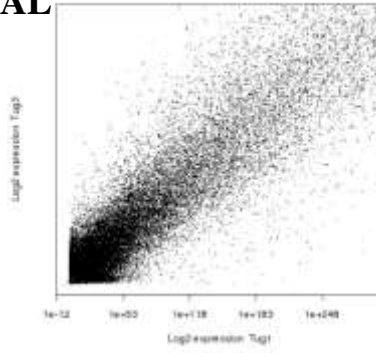


Figure Appx 5.7 - 12 Slides cont.

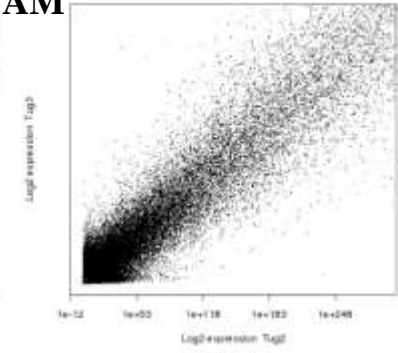
AK



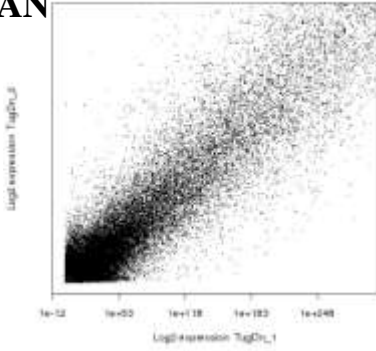
AL



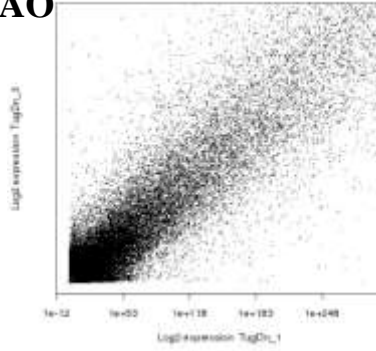
AM



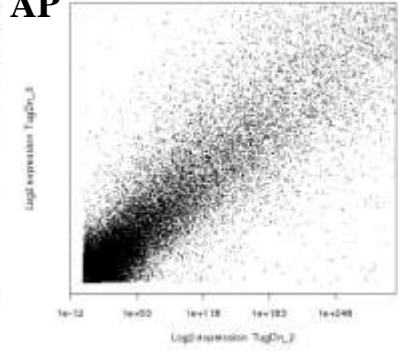
AN



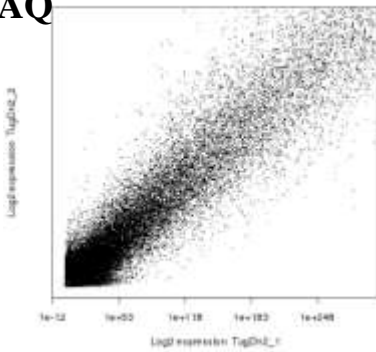
AO



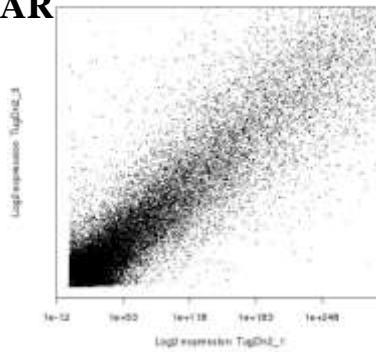
AP



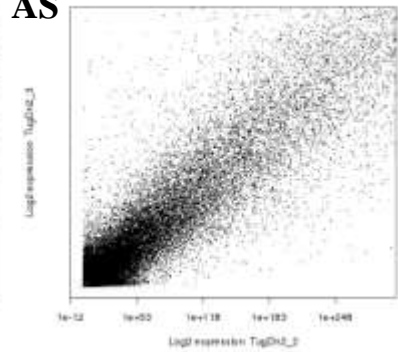
AQ



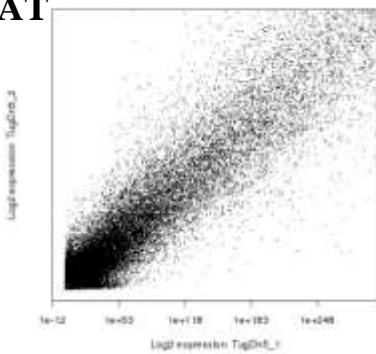
AR



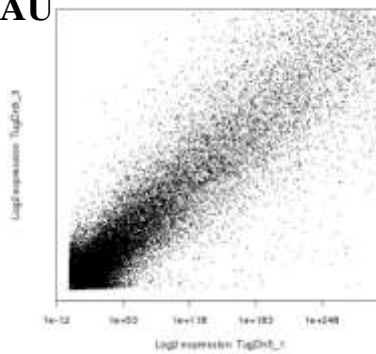
AS



AT



AU



AV

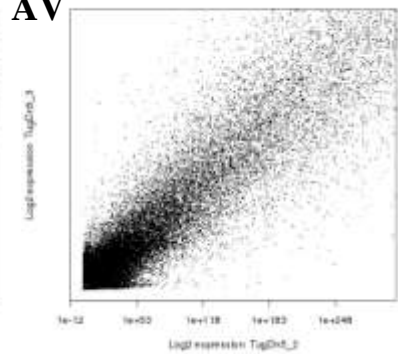
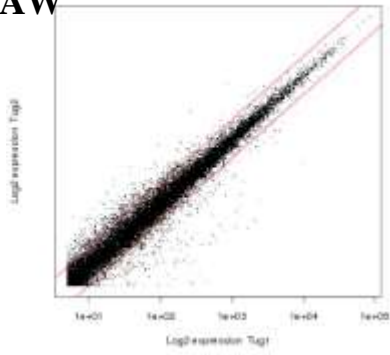
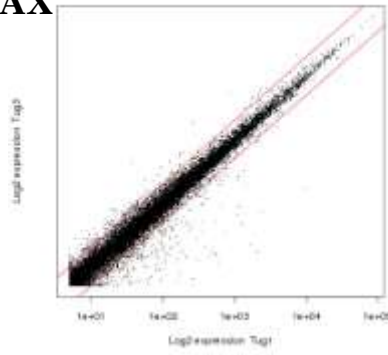


Figure Appx 5.7 - 12 Slides cont.

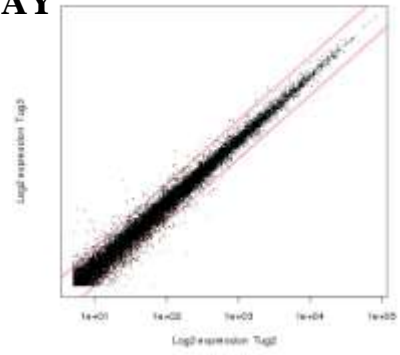
AW



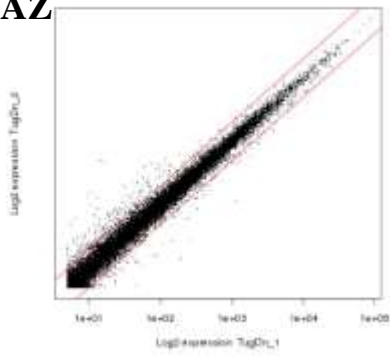
AX



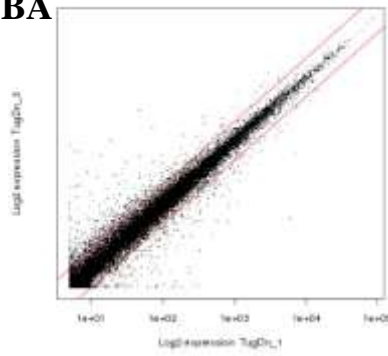
AY



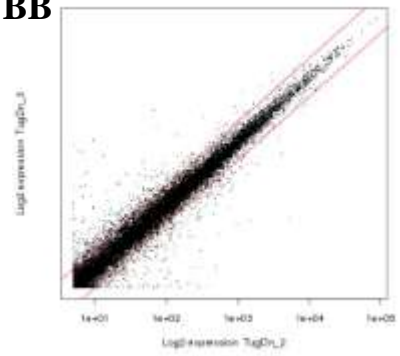
AZ



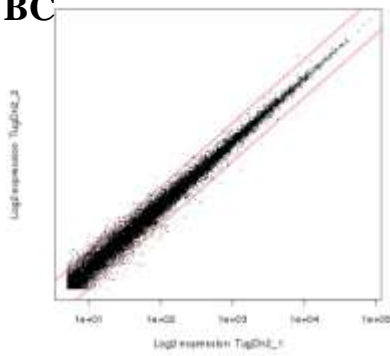
BA



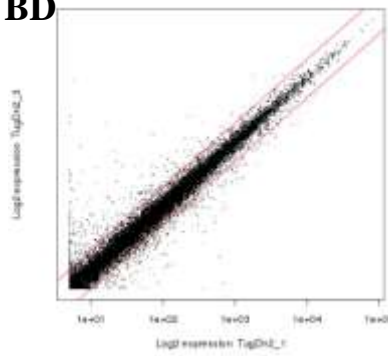
BB



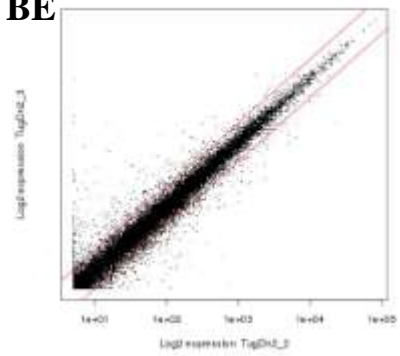
BC



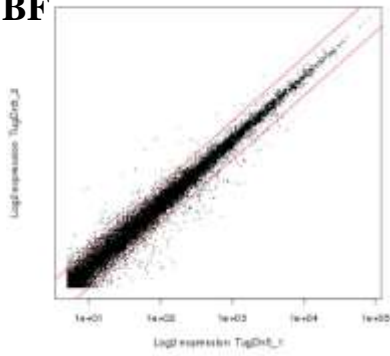
BD



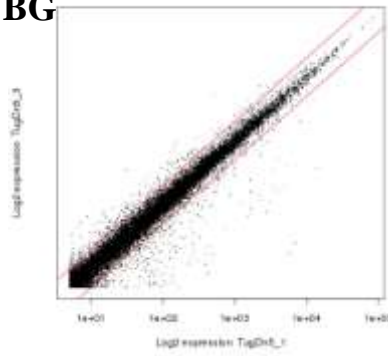
BE



BF



BG



BH

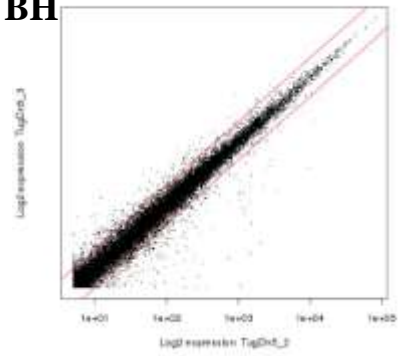
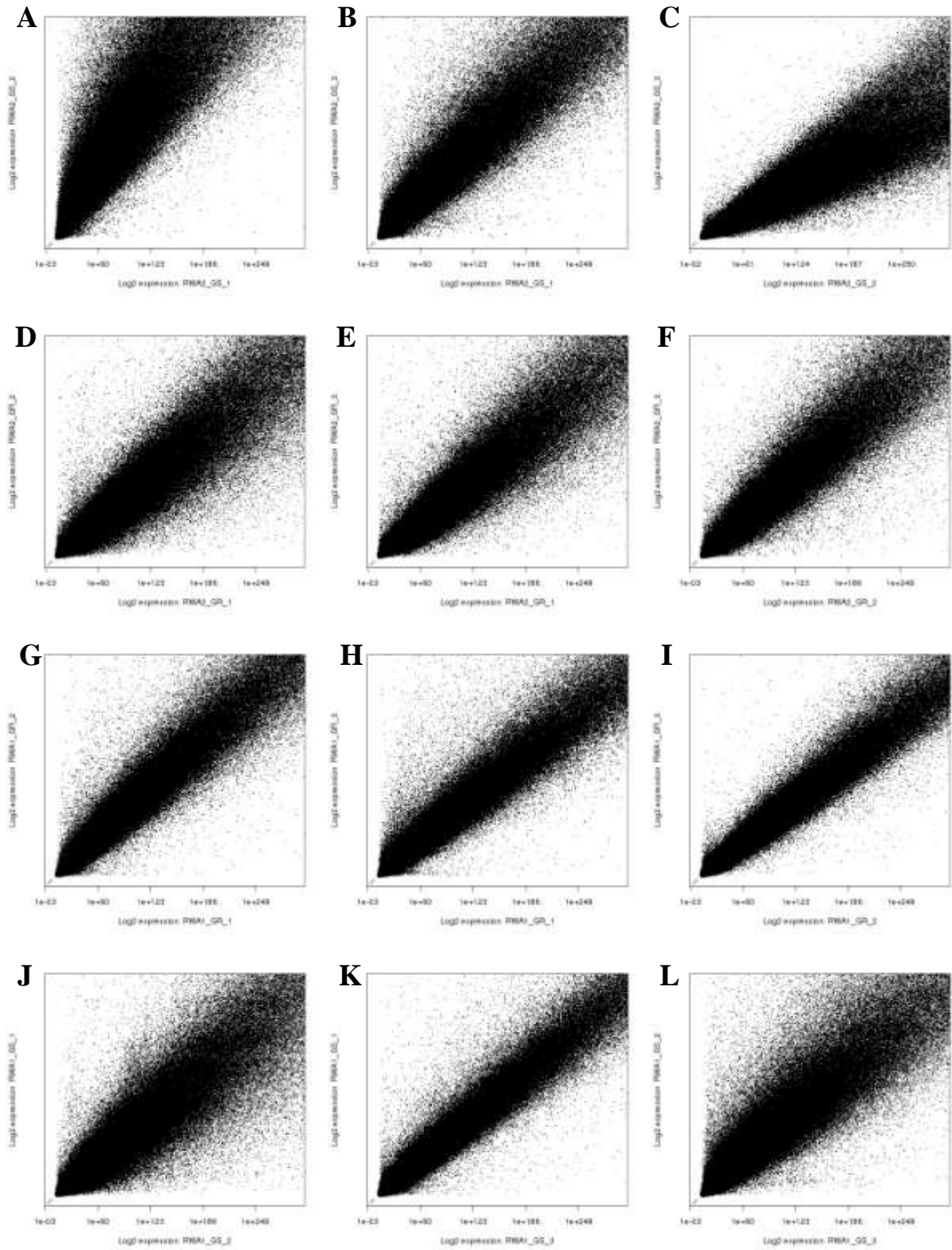




Figure Appx 5.7 - 18 Slides



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

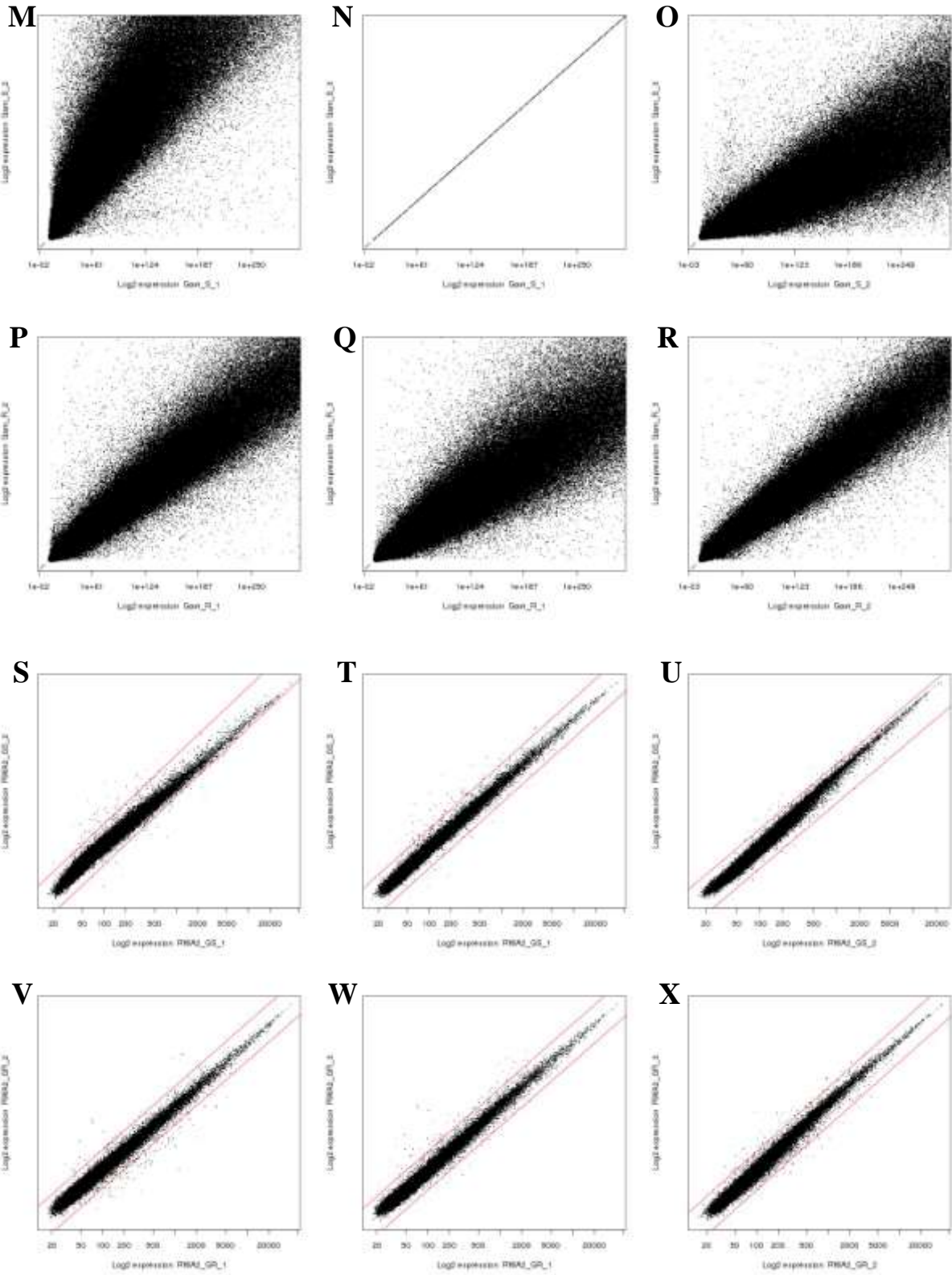


Figure Appx 5.7 - 18 Slides cont.

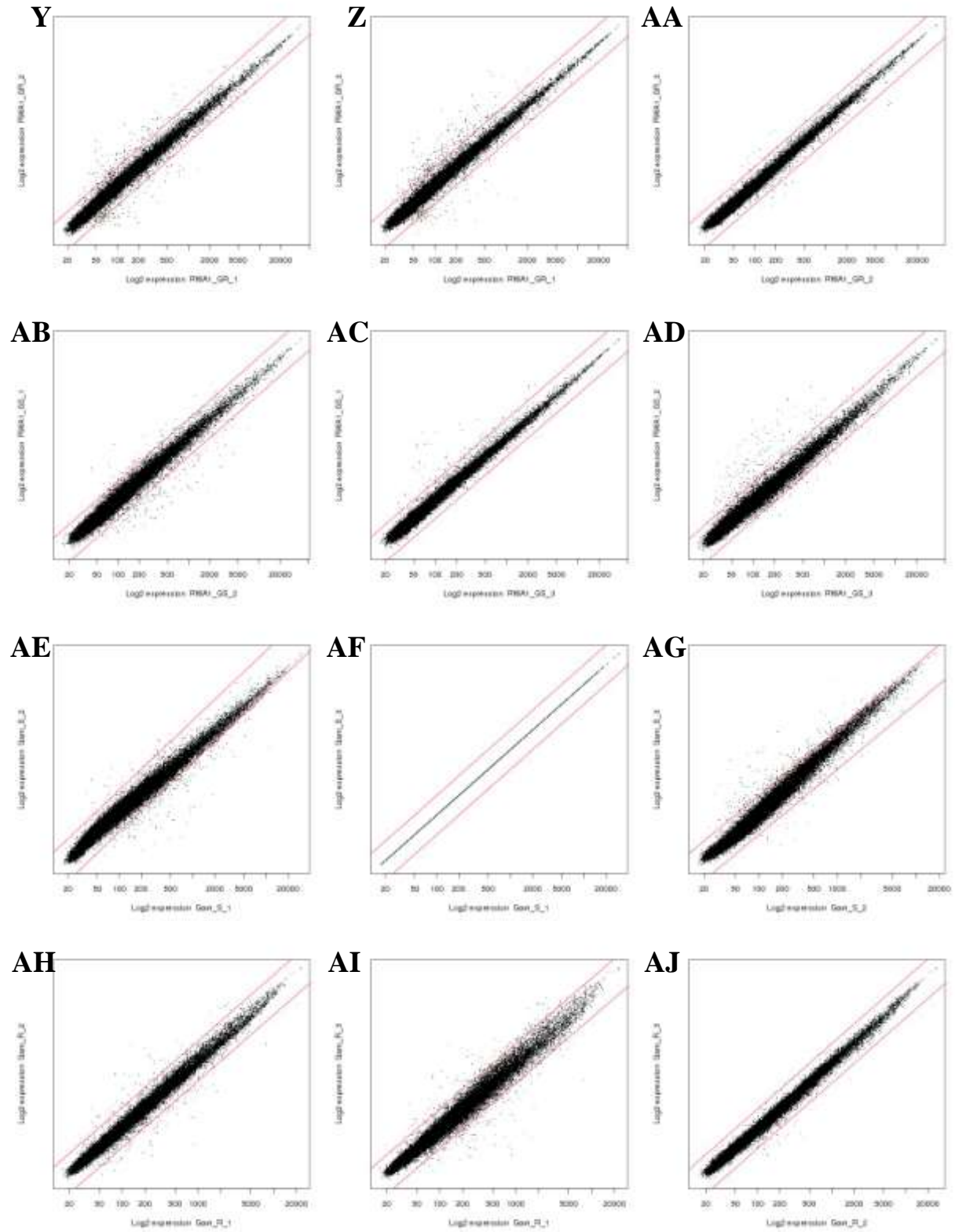
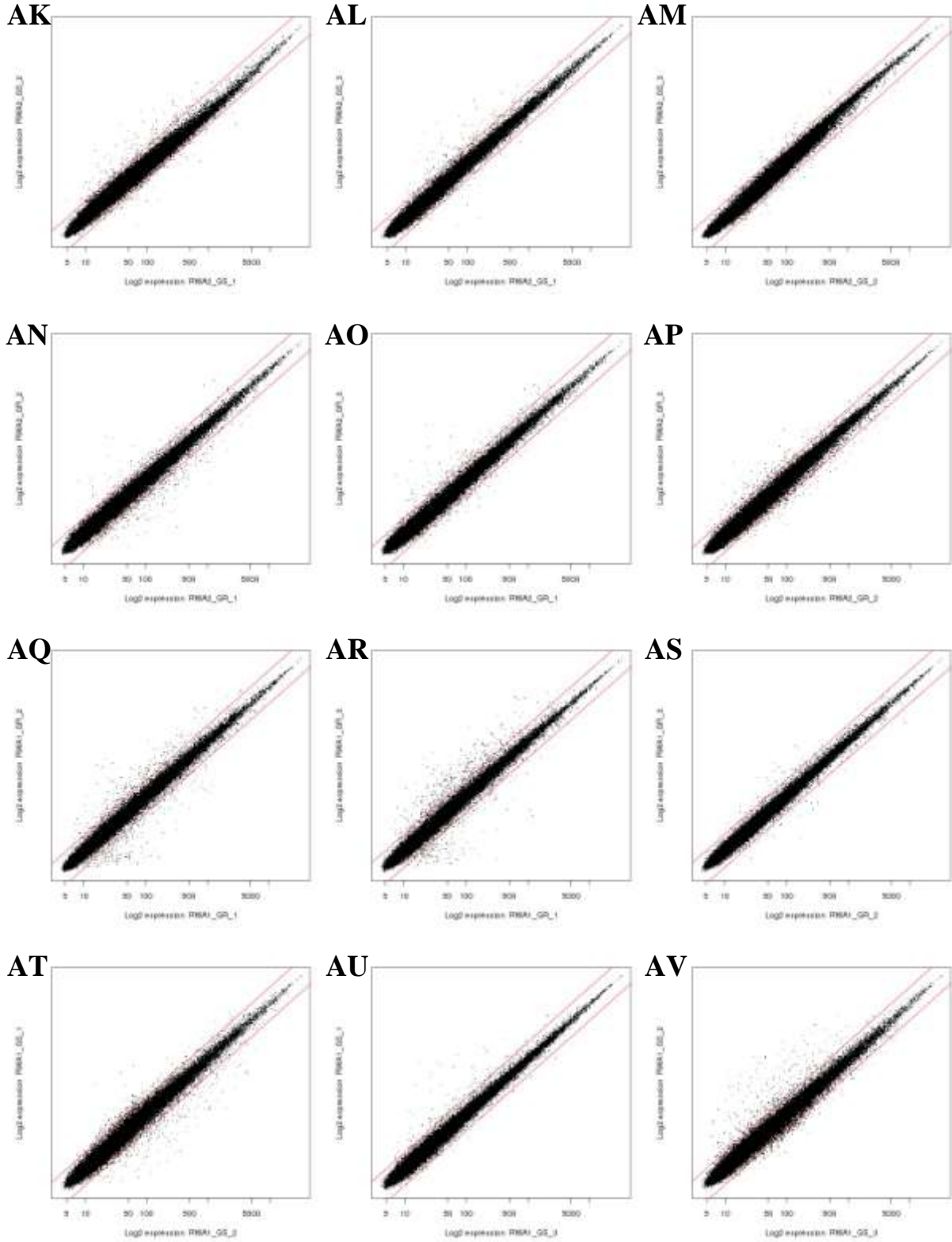


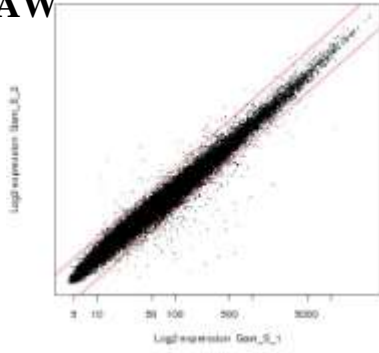
Figure Appx 5.7 - 18 Slides cont.



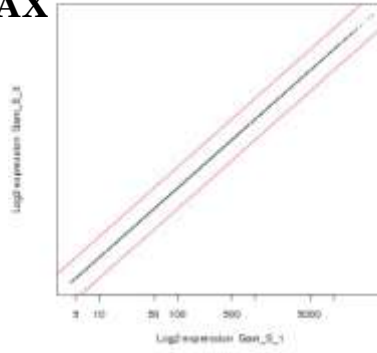


**Figure Appx 5.7 - 18 Slides cont.**

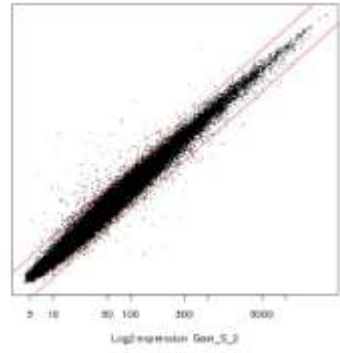
**AW**



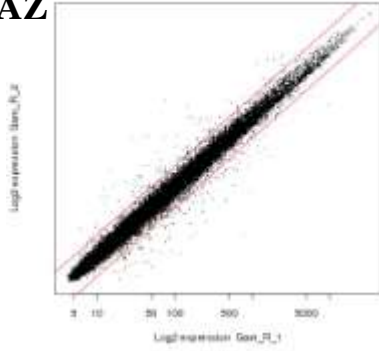
**AX**



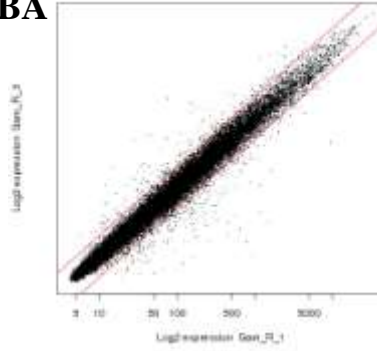
**AY**



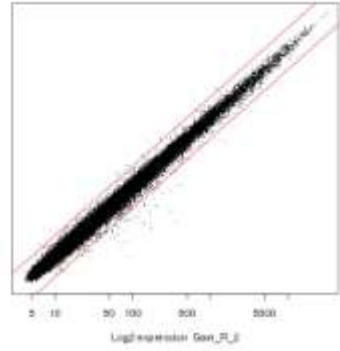
**AZ**



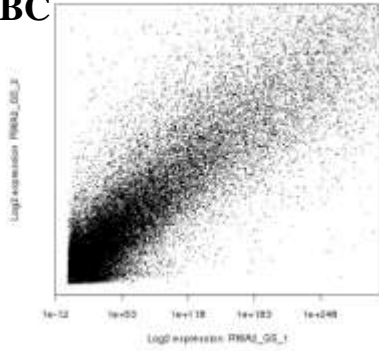
**BA**



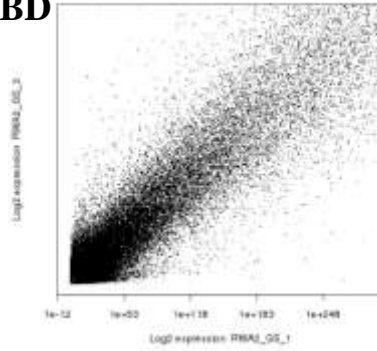
**BB**



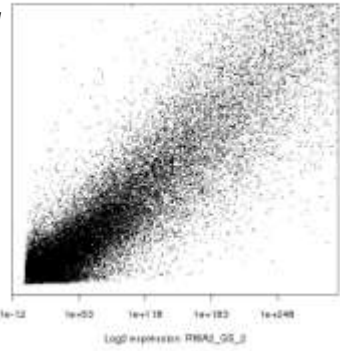
**BC**



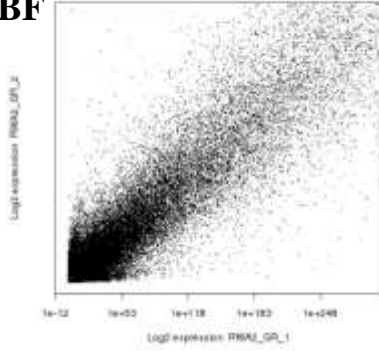
**BD**



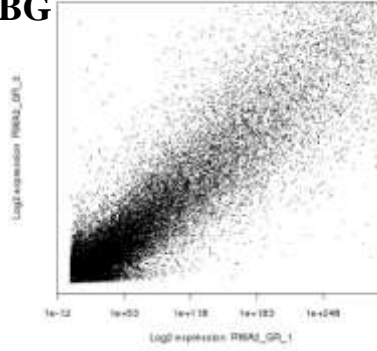
**BE**



**BF**



**BG**



**BH**

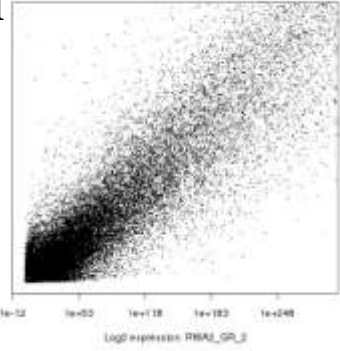


Figure Appx 5.7 - 18 Slides cont.

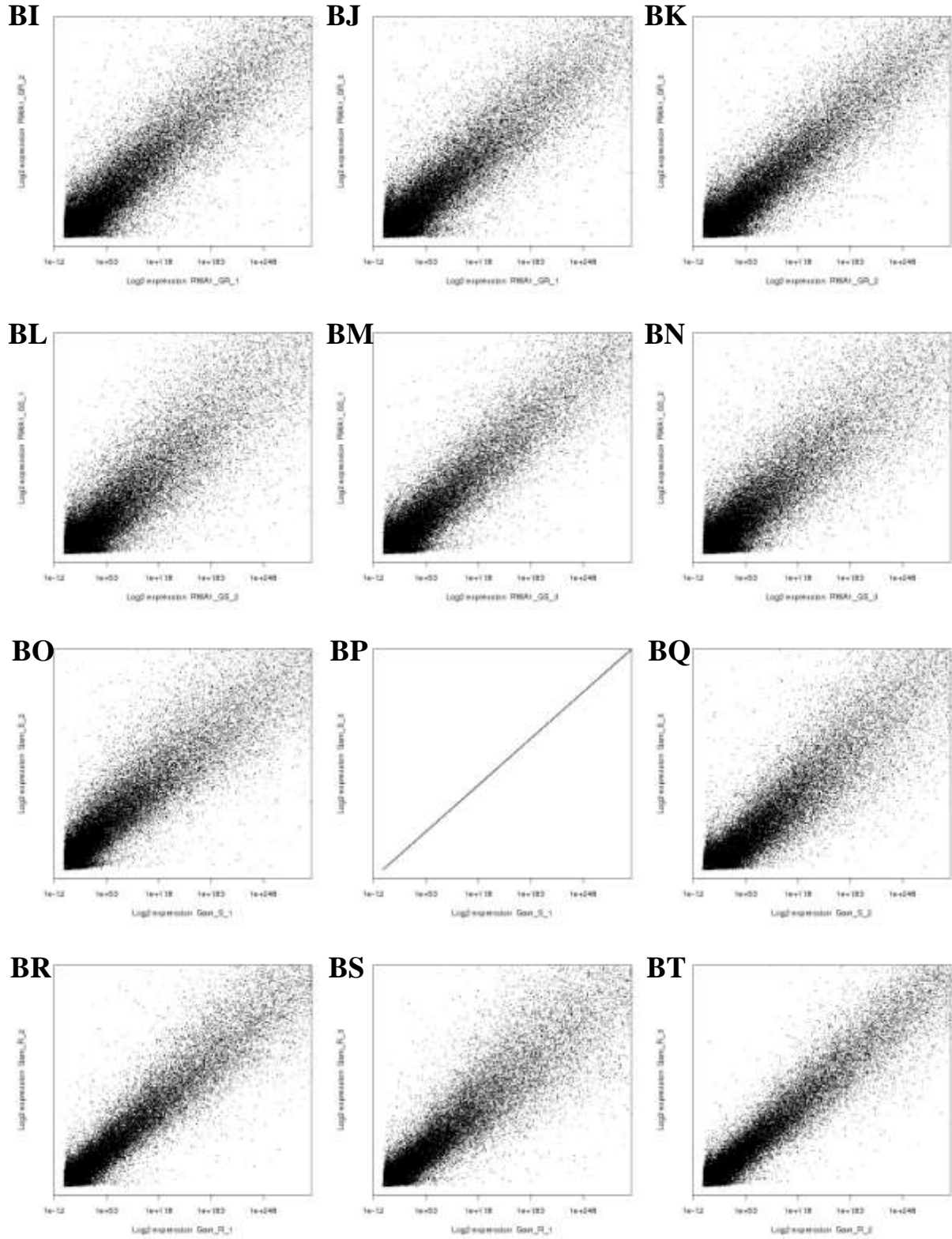
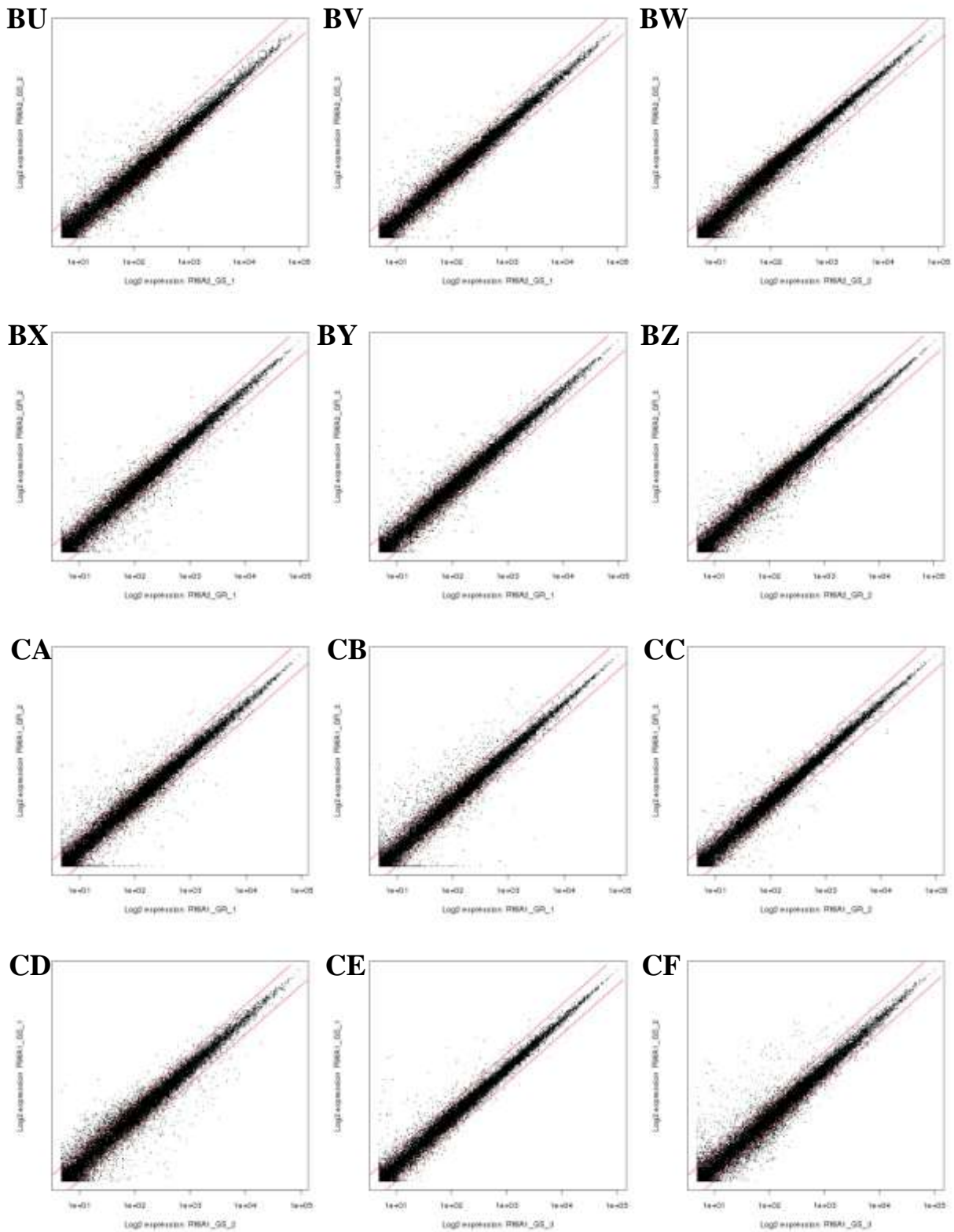
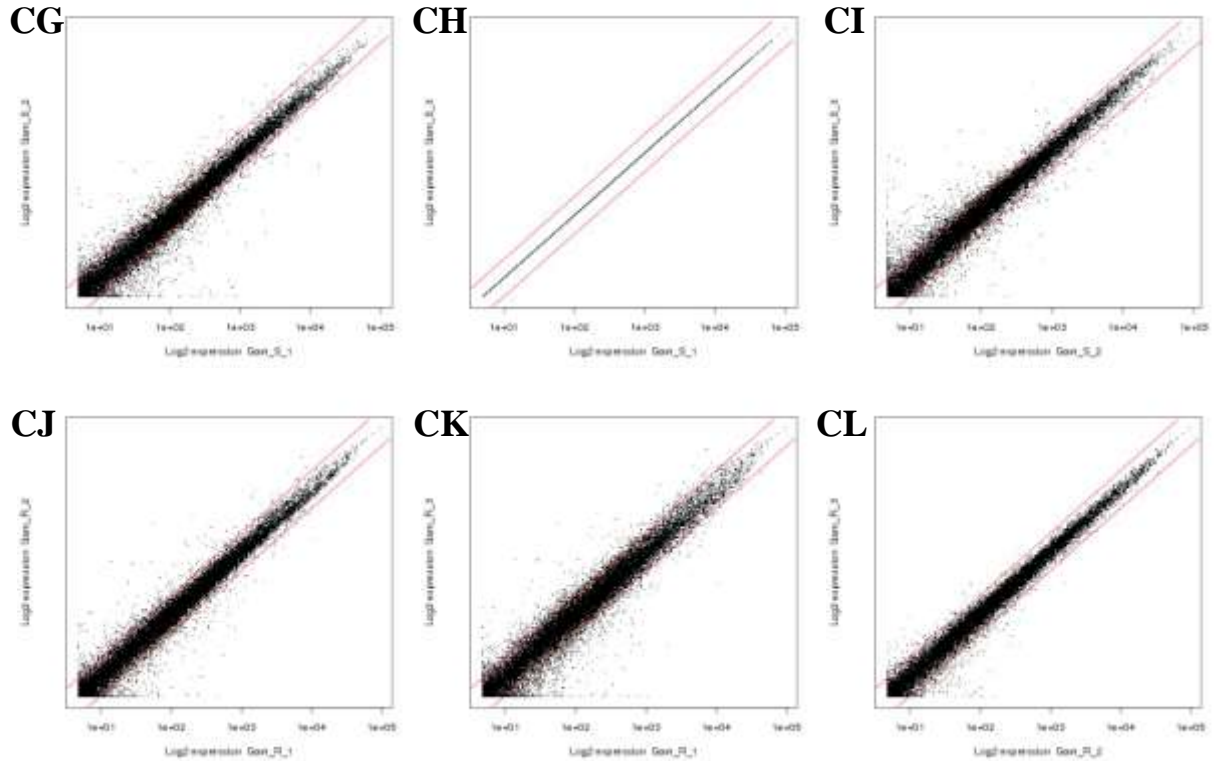


Figure Appx 5.7 - 18 Slides cont.



**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\_



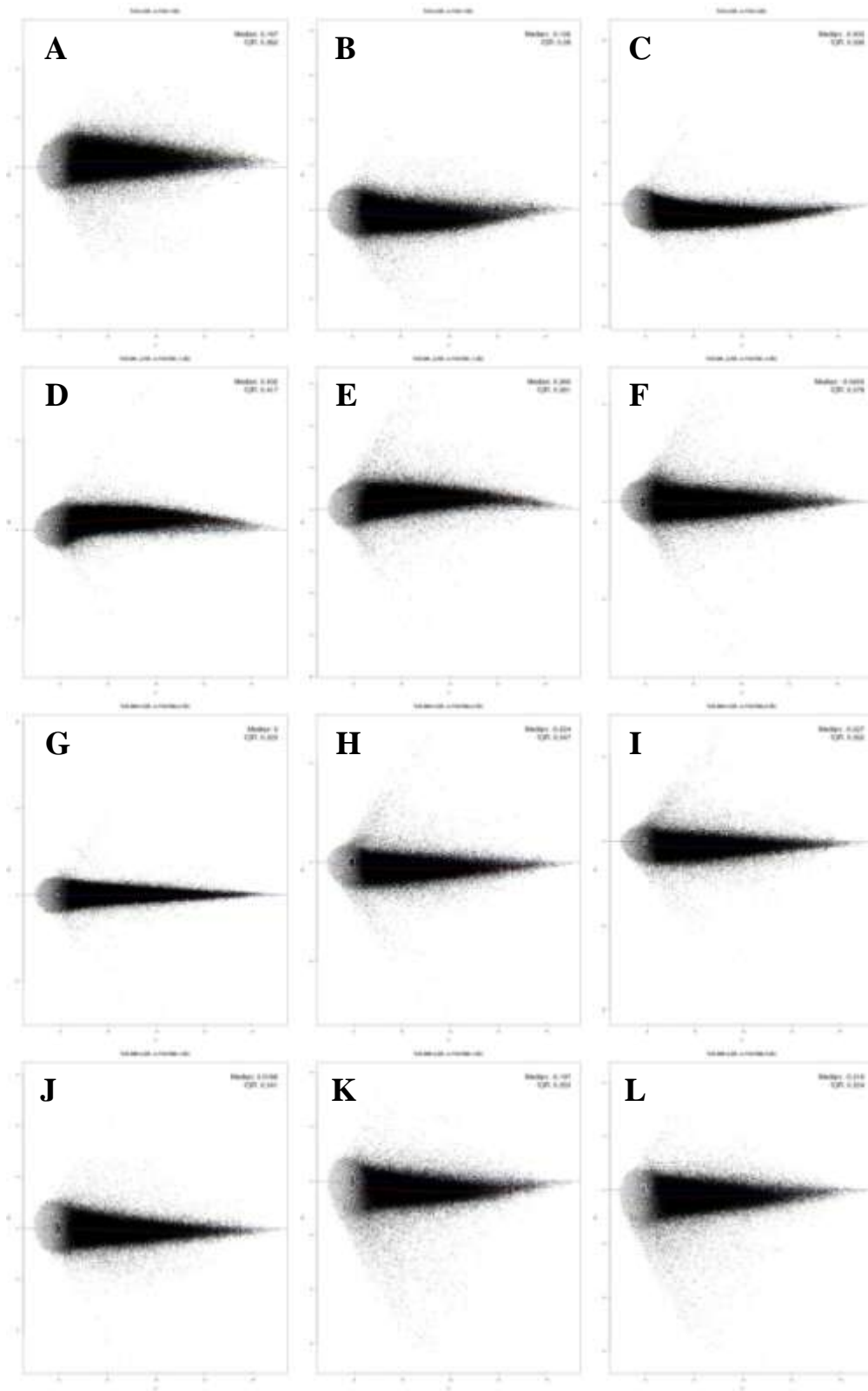
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The 18 Slides files are: (A) rawdata\_RWA2\_GS\_1\_vs\_RWA2\_GS\_2\_log2.jpg, (B) rawdata\_RWA2\_GS\_1\_vs\_RWA2\_GS\_3\_log2.jpg, (C) rawdata\_RWA2\_GS\_2\_vs\_RWA2\_GS\_3\_log2.jpg, (D) rawdata\_RWA2\_GR\_1\_vs\_RWA2\_GR\_2\_log2.jpg, (E) rawdata\_RWA2\_GR\_1\_vs\_RWA2\_GR\_3\_log2.jpg, (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.

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vs\_Gam\_S\_2\_log2.jpg, (BP) esetMAS\_Gam\_S\_1\_vs\_Gam\_S\_3\_log2.jpg, (BQ) esetMAS\_Gam\_-  
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Gam\_R\_1\_vs\_Gam\_R\_3\_log2.jpg, (BT) esetMAS\_Gam\_R\_2\_vs\_Gam\_R\_3\_log2.jpg, (BU) eset-  
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RWA2\_GR\_3\_log2.jpg, (BZ) esetGCRMA\_RWA2\_GR\_2\_vs\_RWA2\_GR\_3\_log2.jpg, (CA) eset-  
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Figure Appx 5.8 - 12 Slides





*Figure Appx 5.8 - 12 Slides cont.*

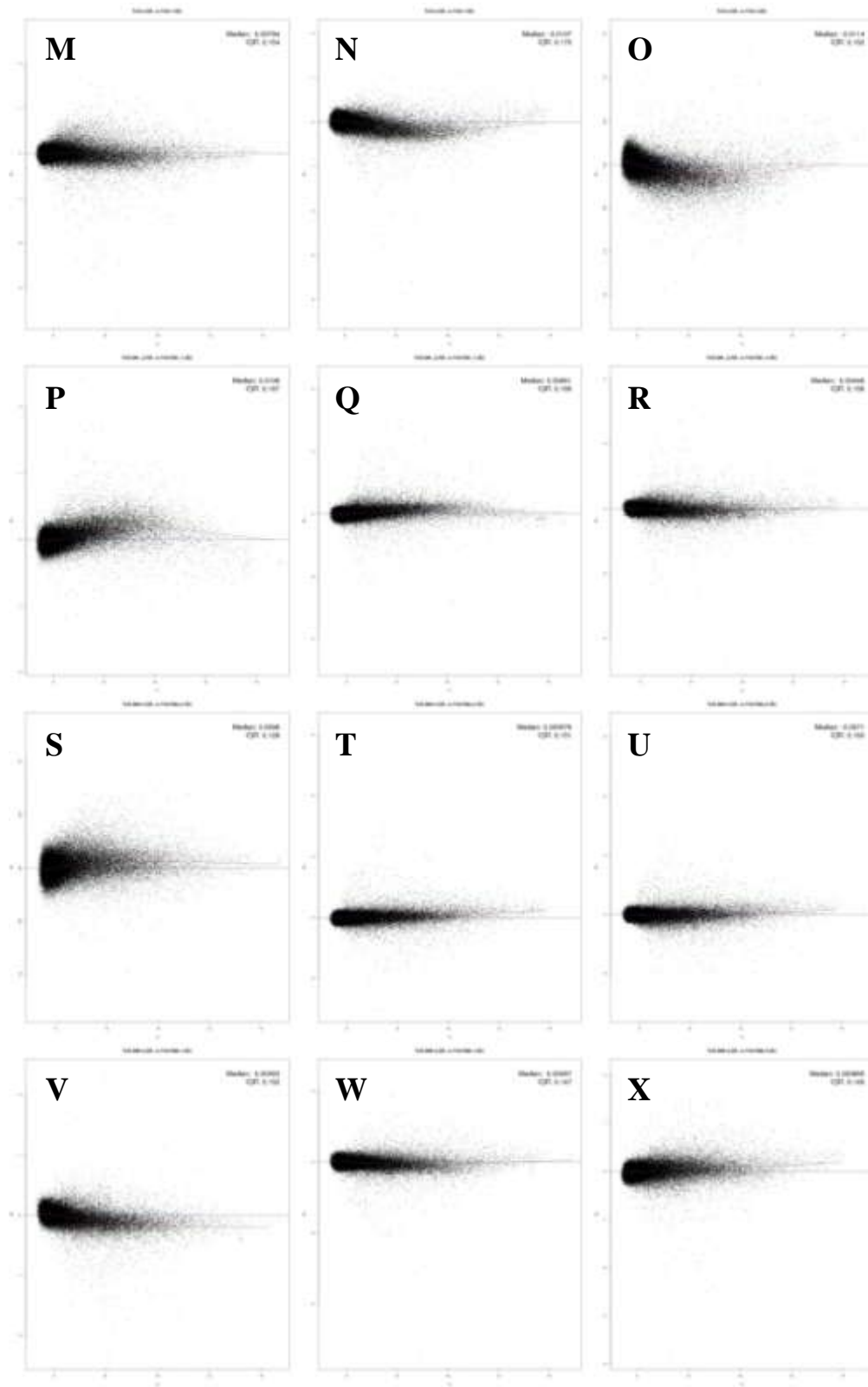


Figure Appx 5.8 - 12 Slides cont.

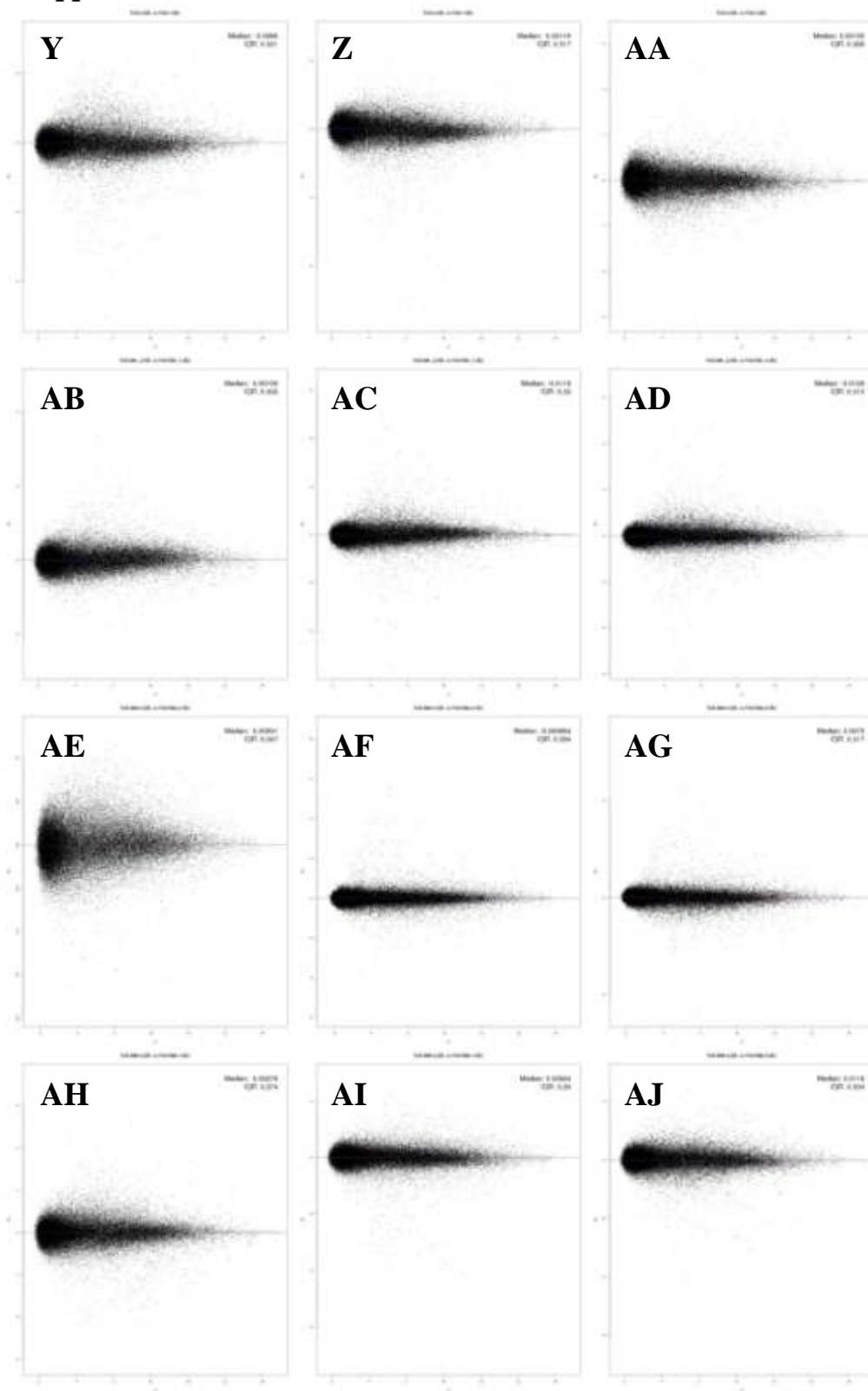
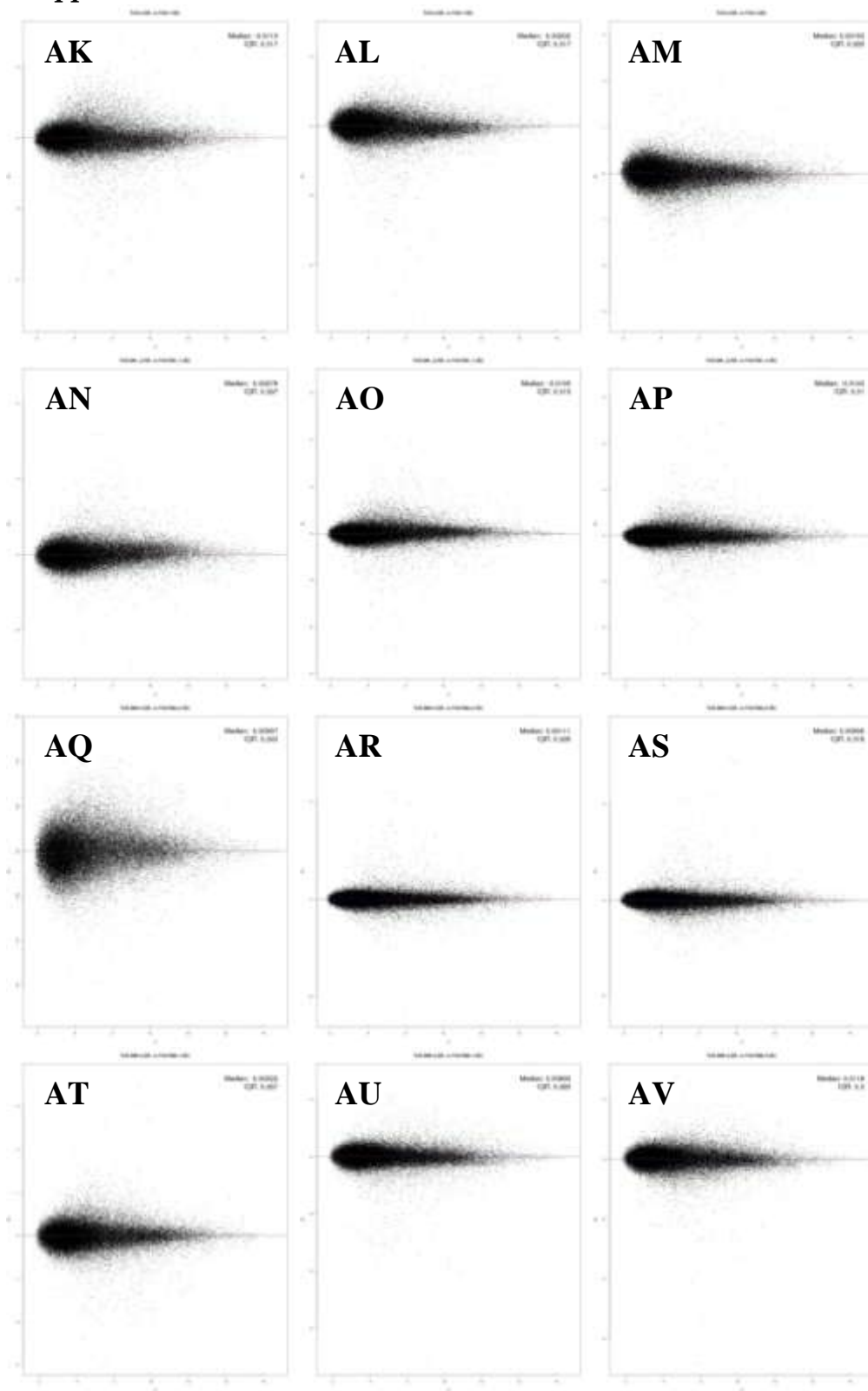


Figure Appx 5.8 - 12 Slides cont.



*Figure Appx 5.8 - 12 Slides cont.*

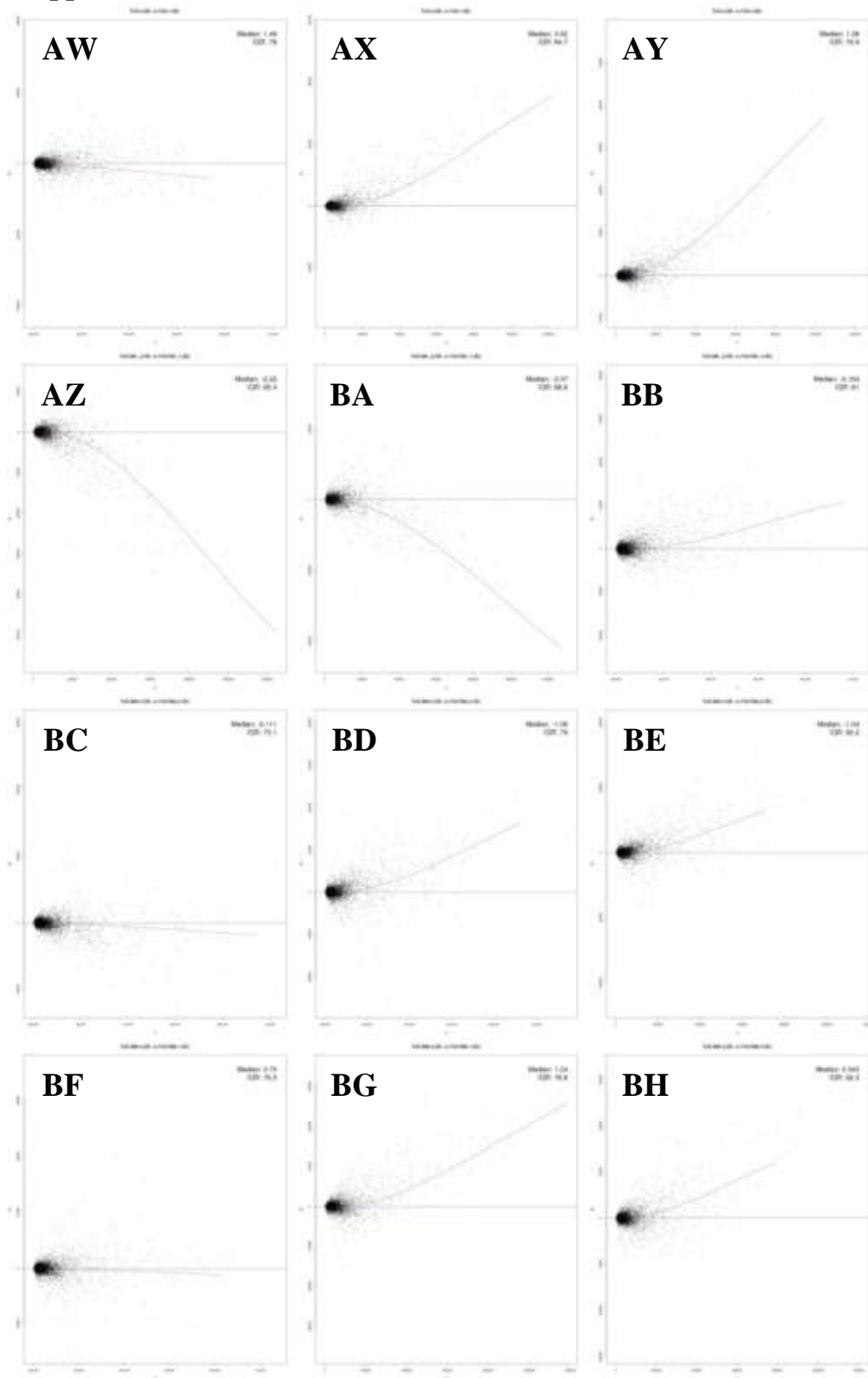


Figure Appx 5.8 - 12 Slides cont.

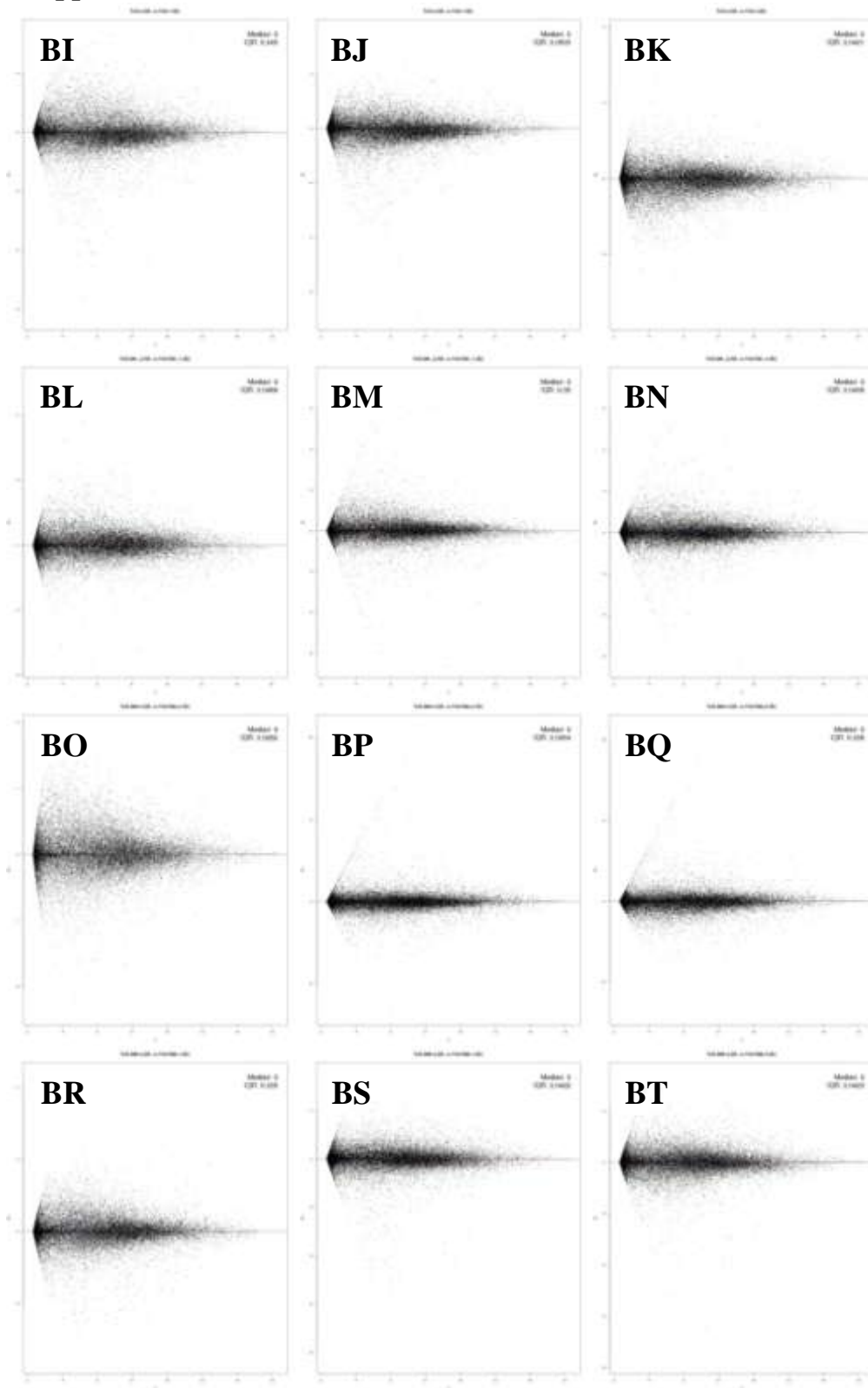


Figure Appx 5.8 - 18 Slides

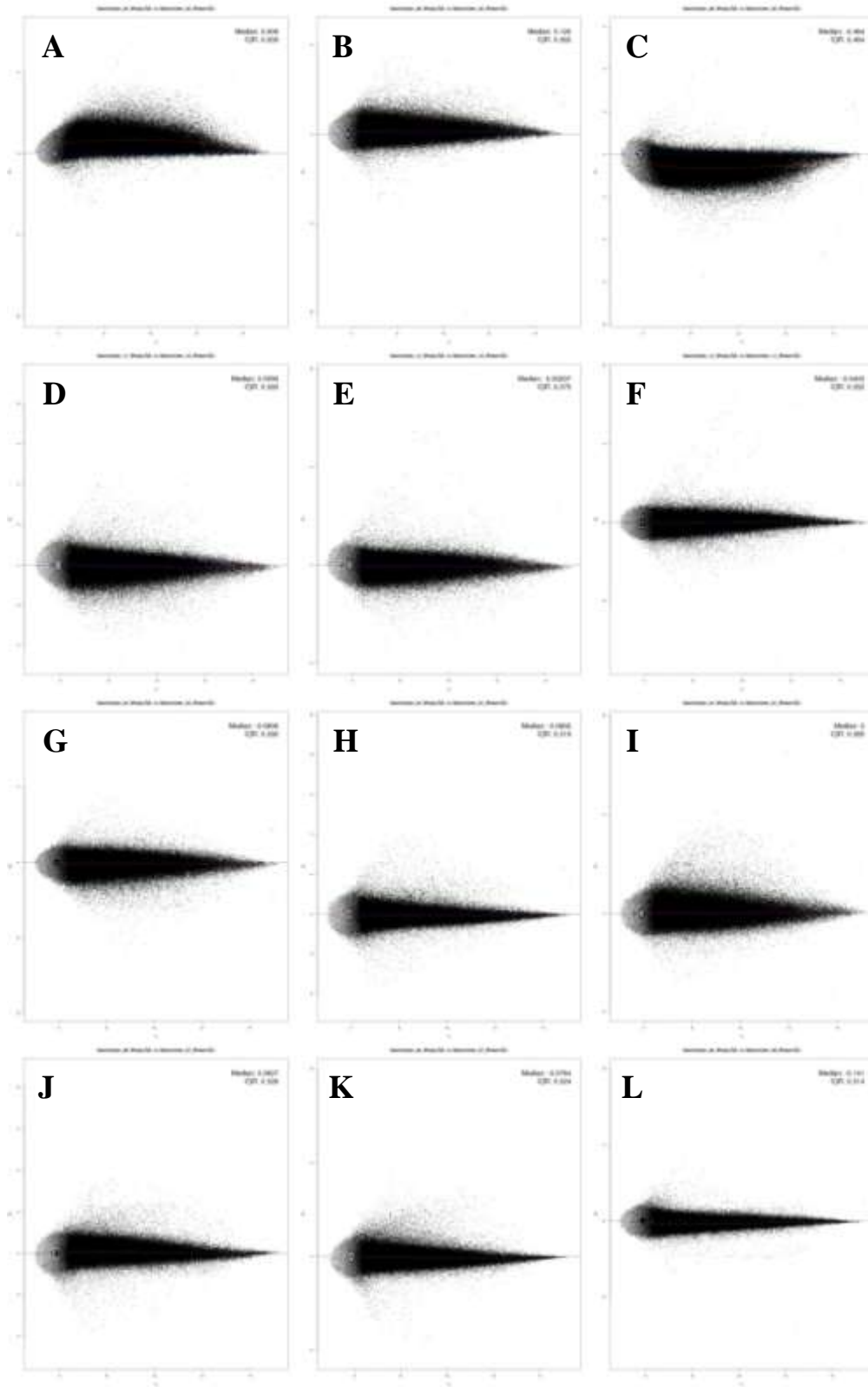


Figure Appx 5.8 - 18 Slides cont.

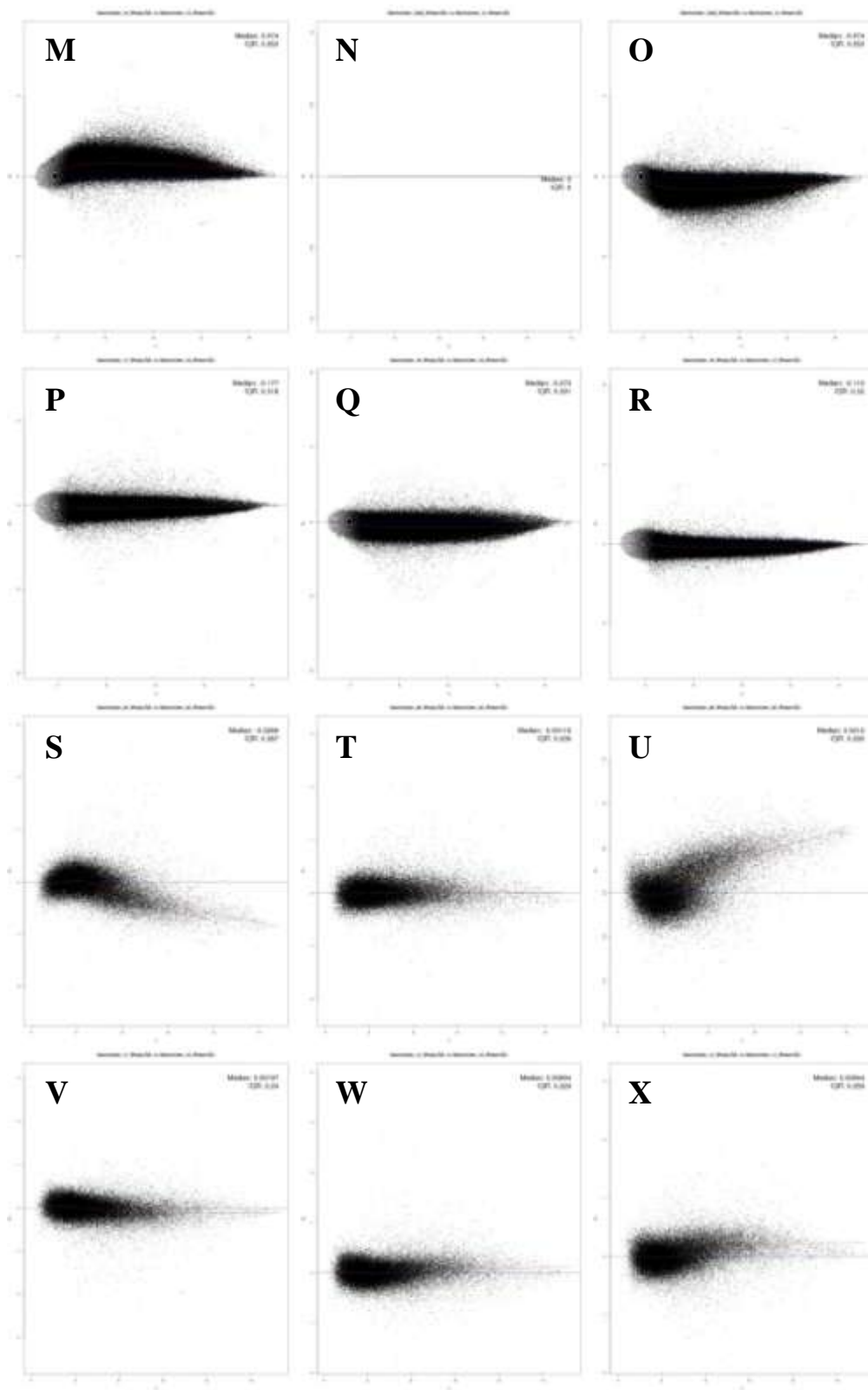


Figure Appx 5.8 - 18 Slides cont.

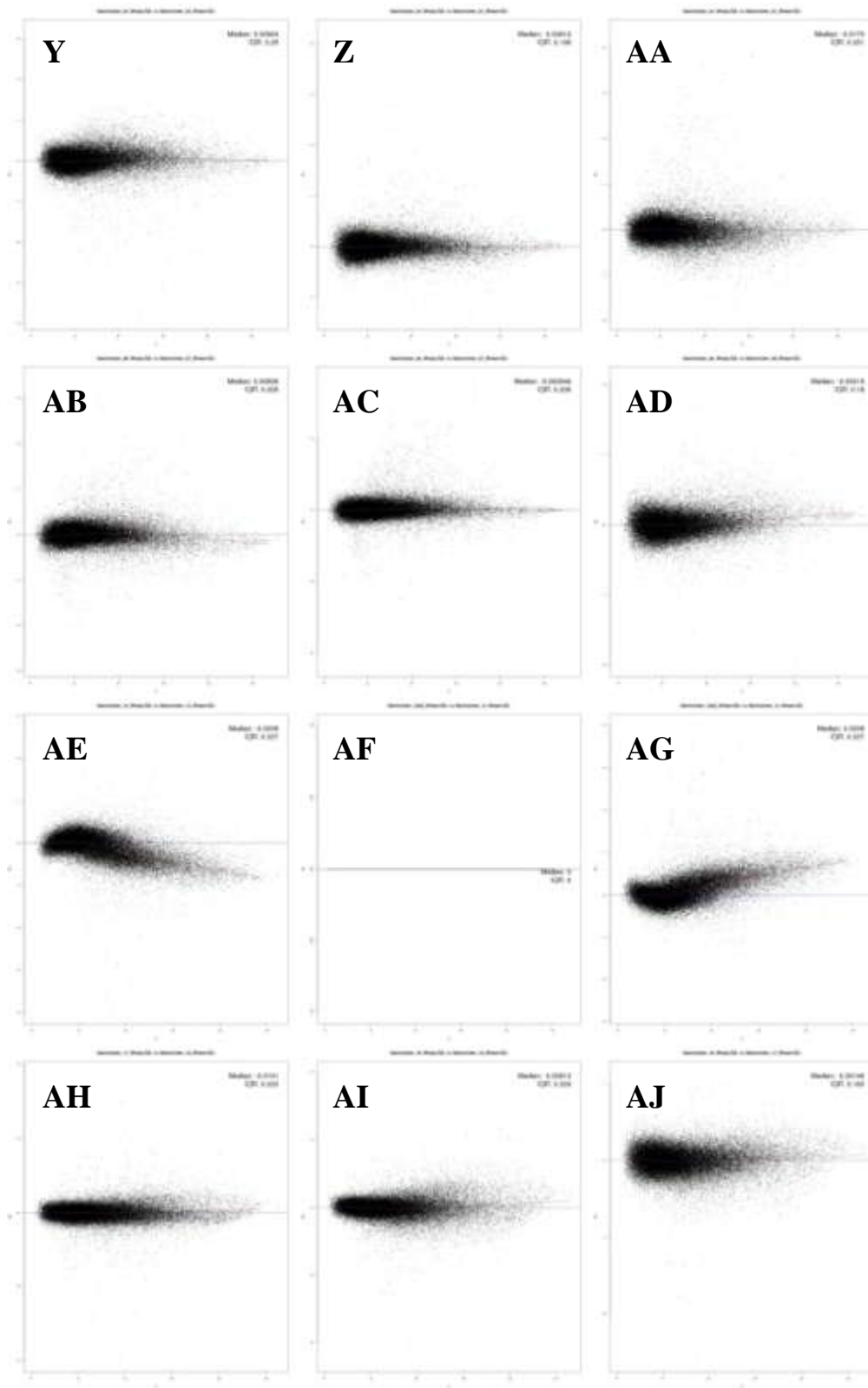




Figure Appx 5.8 - 18 Slides cont.

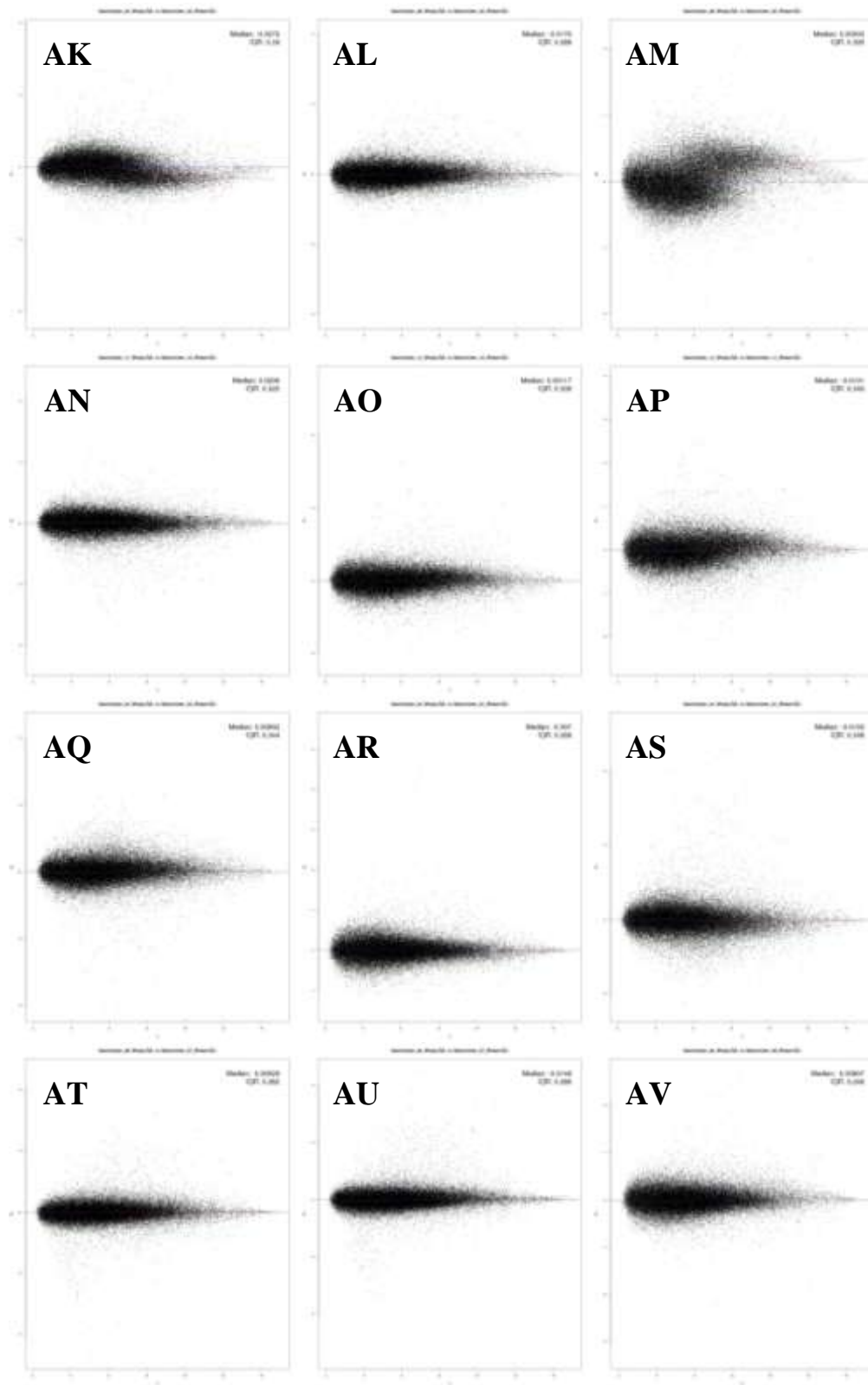


Figure Appx 5.8 - 18 Slides cont.

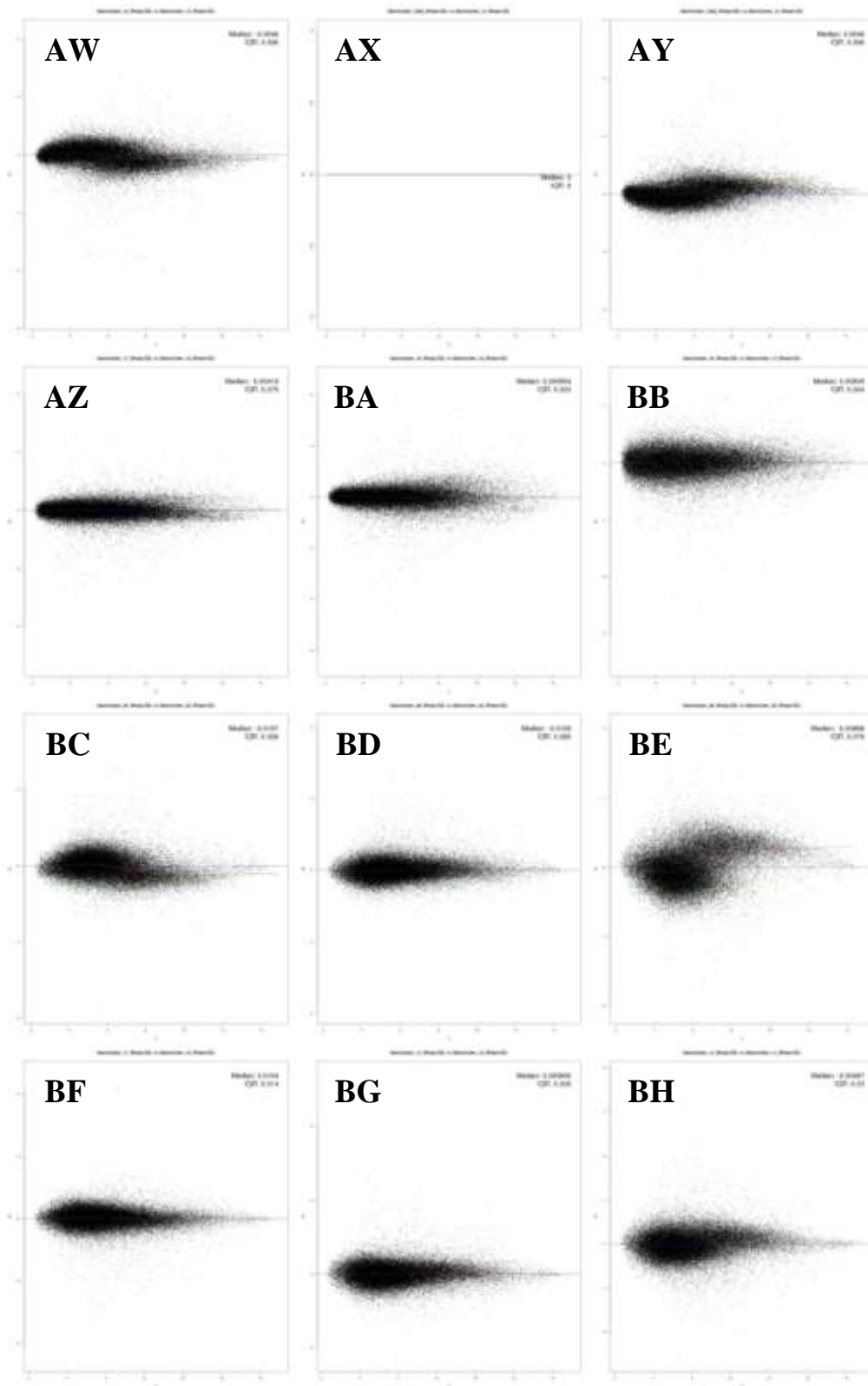


Figure Appx 5.8 - 18 Slides cont.

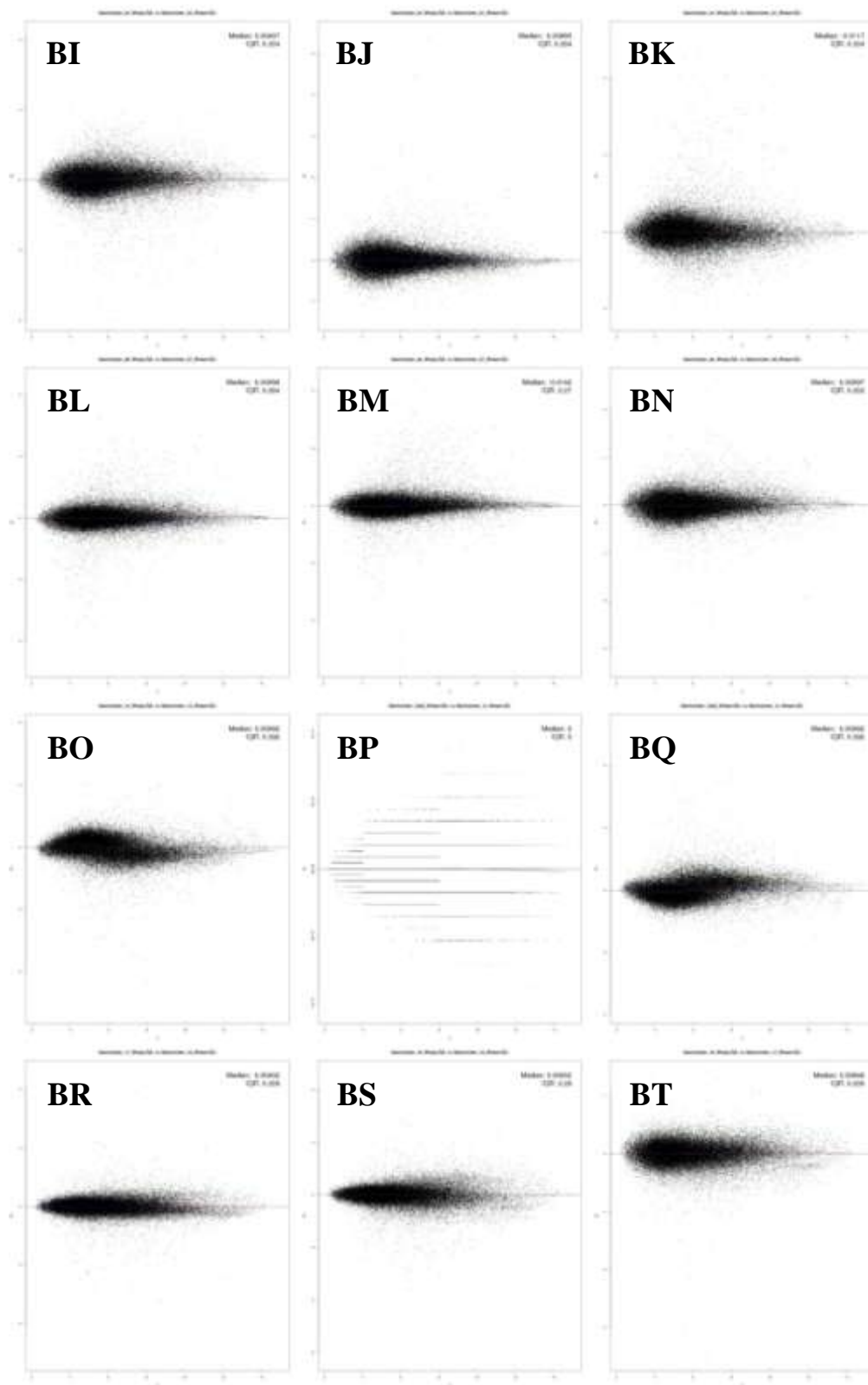


Figure Appx 5.8 - 18 Slides cont.

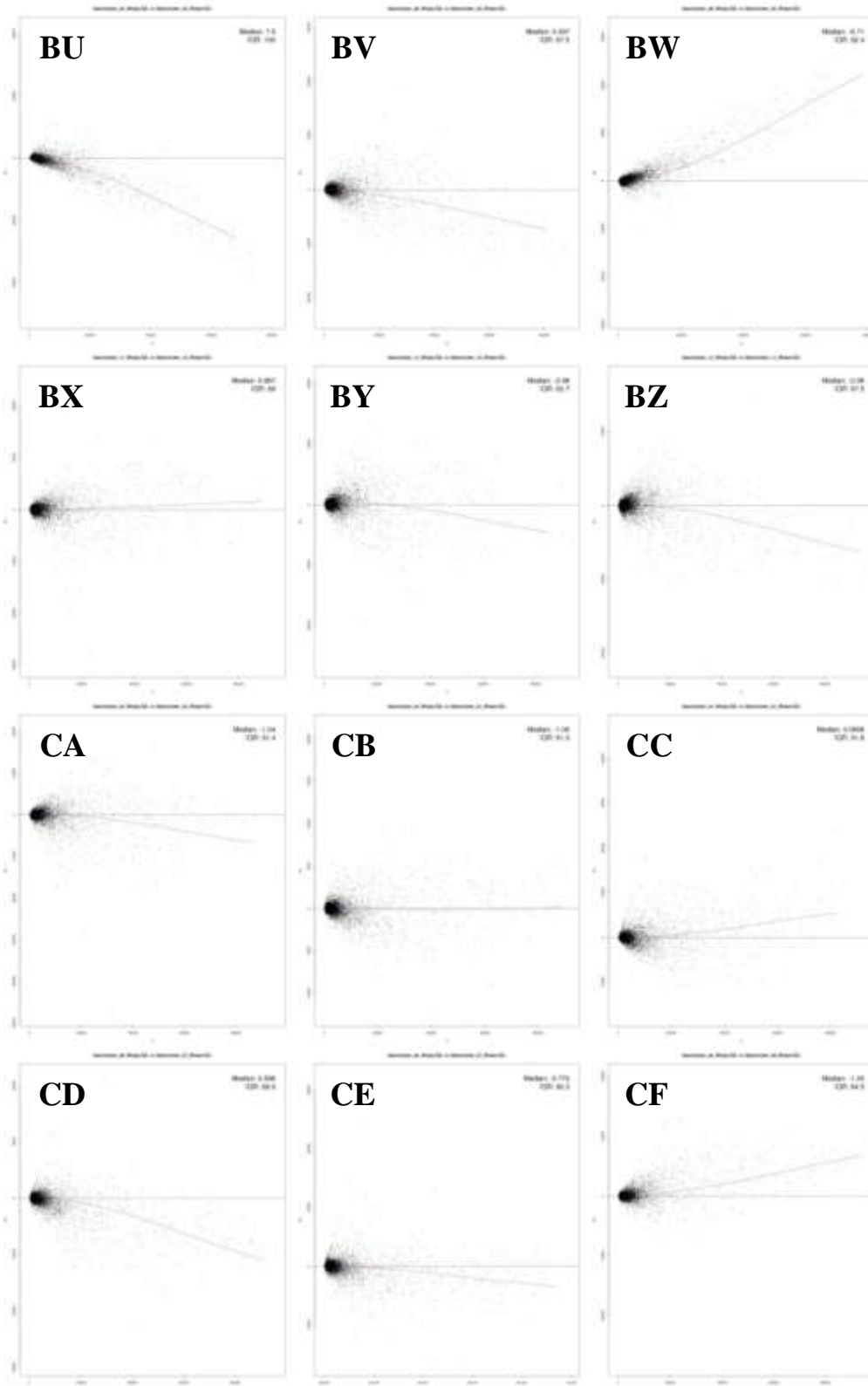
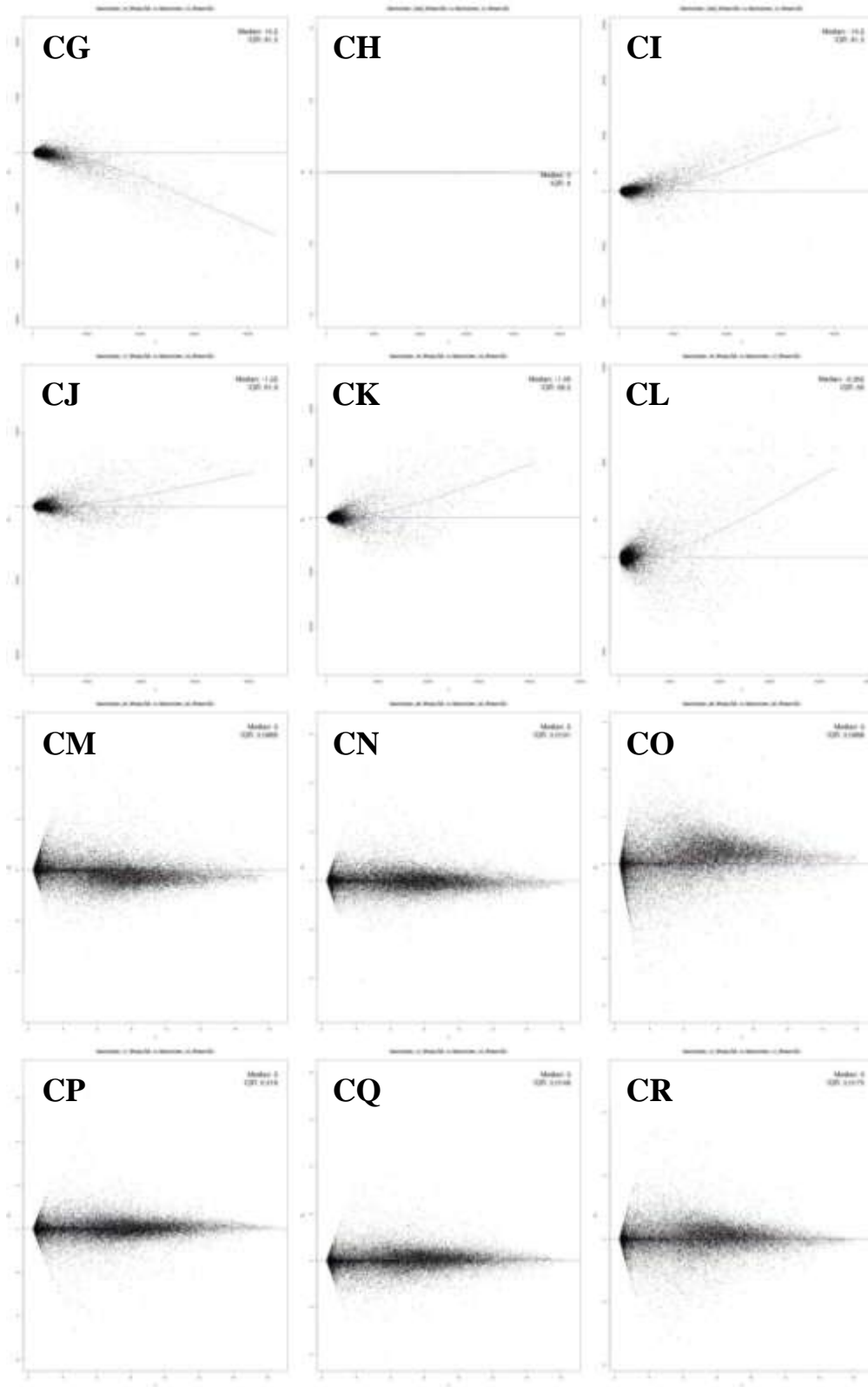
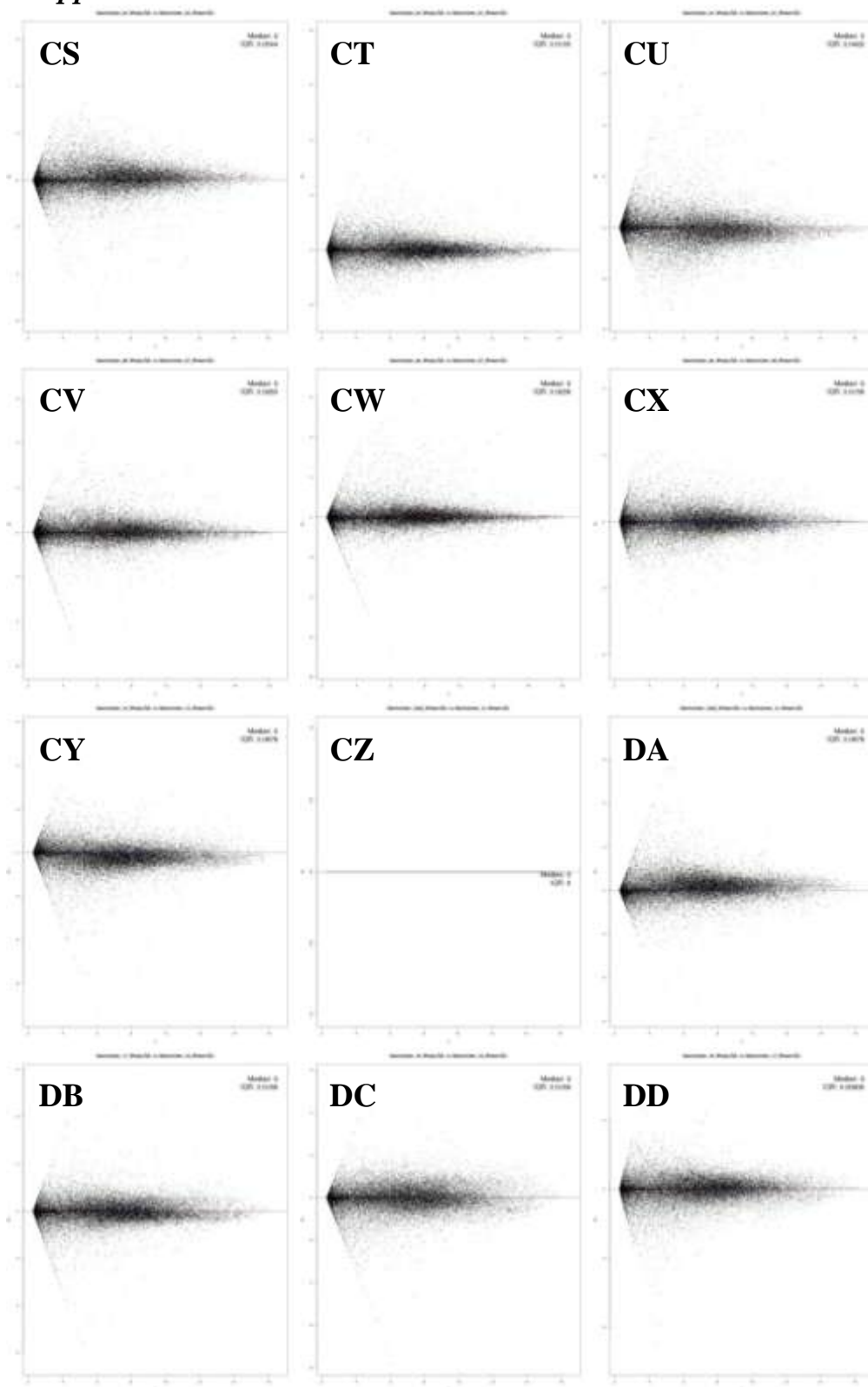


Figure Appx 5.8 - 18 Slides cont.



**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)



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3.jpg, (BH) esetPLM\_RWA2\_GR\_2\_vs\_RWA2\_GR\_3.jpg, (BI) esetPLM\_RWA1\_GR\_1\_vs\_-  
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GR\_2\_vs\_RWA1\_GR\_3.jpg, (BL) esetPLM\_RWA1\_GS\_2\_vs\_RWA1\_GS\_1.jpg, (BM) eset-  
PLM\_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) eset-  
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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) eset-  
MAS\_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) eset-  
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Gam\_R\_2\_vs\_Gam\_R\_3.jpg, (CM) esetGCRMA\_RWA2\_GS\_1\_vs\_RWA2\_GS\_2.jpg, (CN) eset-  
GCRMA\_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) eset-  
GCRMA\_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) eset-  
GCRMA\_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.

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

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

## CURRICULUM VITAE

Zacharias Hendrik Swanevelder was born June 1<sup>st</sup>, 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.