1. Data Preparation

Table 1. In this study, 22 patients are enrolled from which no disease, diabetes with complication, and diabetes without complication are 7, 8, and 7, respectively. Each subject has at least three replicates.

	No disease (N=7)	Diabet with complication (N=8)	Diabet without complication (N=7)
No treatment	21	26	25
Treated (with 30mM insulin)	21	26	25

Design matrix for differential expression analysis

- DwC: Disease with complication
- DwoC: Disease without complication
- NoD: No disease

Case_Number	Subject	Condition	Treatment
DwC_1026_30mM.1	1	disease_comp	insulin
DwC_1026_30mM.2	1	disease_comp	insulin
DwC_1026_30mM.3	1	disease_comp	insulin
DwC_1026_30mM.4	1	disease_comp	insulin
DwC_1026_30mM.5	1	disease_comp	insulin
DwC_1026_norm.1	2	disease_comp	control
DwC_1026_norm.2	2	disease_comp	control
DwC_1026_norm.3	2	disease_comp	control
DwC_1026_norm.4	2	disease_comp	control
DwC_1026_norm.5	2	disease_comp	control
DwC_6009_30mM.1	3	disease_comp	insulin
DwC_6009_30mM.2	3	disease_comp	insulin
DwC_6009_30mM.3	3	disease_comp	insulin
DwC_6009_norm.1	4	disease_comp	control
DwC_6009_norm.2	4	disease_comp	control
DwC_6009_norm.3	4	disease_comp	control
DwC_11167_30mM.1	5	disease_comp	insulin
DwC_11167_30mM.2	5	disease_comp	insulin
DwC_11167_30mM.3	5	disease_comp	insulin
DwC_11167_norm.1	6	disease_comp	control
DwC_11167_norm.2	6	disease_comp	control
DwC_11167_norm.3	6	disease_comp	control
DwC_13206_30mM.1	7	disease_comp	insulin
DwC_13206_30mM.2	7	disease_comp	insulin
DwC_13206_30mM.3	7	disease_comp	insulin

DwC_13206_norm.1	8	disease_comp	control
DwC_13206_norm.2	8	disease_comp	control
DwC_13206_norm.3	8	disease_comp	control
DwC_17075_30mM.1	9	disease_comp	insulin
DwC_17075_30mM.2	9	disease_comp	insulin
DwC_17075_30mM.3	9	disease_comp	insulin
DwC_17075_norm.1	10	disease_comp	control
DwC_17075_norm.2	10	disease_comp	control
DwC_17075_norm.3	10	disease_comp	control
DwC_21127_30mM.1	11	disease_comp	insulin
DwC_21127_30mM.2	11	disease_comp	insulin
DwC_21127_30mM.3	11	disease_comp	insulin
DwC_21127_norm.1	12	disease_comp	control
DwC_21127_norm.2	12	disease_comp	control
DwC_21127_norm.3	12	disease_comp	control
DwC_26008_30mM.1	13	disease_comp	insulin
DwC_26008_30mM.2	13	disease_comp	insulin
DwC_26008_30mM.3	13	disease_comp	insulin
DwC_26008_norm.1	14	disease_comp	control
DwC_26008_norm.2	14	disease_comp	control
DwC_26008_norm.3	14	disease_comp	control
DwC_27141_30mM.1	15	disease_comp	insulin
DwC_27141_30mM.2	15	disease_comp	insulin
DwC_27141_30mM.3	15	disease_comp	insulin
DwC_27141_norm.1	16	disease_comp	control
DwC_27141_norm.2	16	disease_comp	control
DwC_27141_norm.3	16	disease_comp	control
DwoC_2318_30mM.1	17	disease	insulin
DwoC_2318_30mM.2	17	disease	insulin
DwoC_2318_30mM.3	17	disease	insulin
DwoC_2318_30mM.4	17	disease	insulin
DwoC_2318_30mM.5	17	disease	insulin
DwoC_2318_norm.1	18	disease	control
DwoC_2318_norm.2	18	disease	control
DwoC_2318_norm.3	18	disease	control
DwoC_2318_norm.4	18	disease	control
DwoC_2318_norm.5	18	disease	control
DwoC_3395_30mM.1	19	disease	insulin
DwoC_3395_30mM.2	19	disease	insulin
DwoC_3395_30mM.3	19	disease	insulin
DwoC_3395_norm.1	20	disease	control
DwoC_3395_norm.2	20	disease	control
DwoC_3395_norm.3	20	disease	control
DwoC_6154_30mM.1	21	disease	insulin

DwoC_6154_30mM.2	21	disease	insulin
DwoC_6154_30mM.3	21	disease	insulin
DwoC_6154_norm.1	22	disease	control
DwoC_6154_norm.2	22	disease	control
DwoC_6154_norm.3	22	disease	control
DwoC_16362_30mM.1	23	disease	insulin
DwoC_16362_30mM.2	23	disease	insulin
DwoC_16362_30mM.3	23	disease	insulin
DwoC_16362_norm.1	24	disease	control
DwoC_16362_norm.2	24	disease	control
DwoC_16362_norm.3	24	disease	control
DwoC_18296_30mM.1	25	disease	insulin
DwoC_18296_30mM.2	25	disease	insulin
DwoC_18296_30mM.3	25	disease	insulin
DwoC_18296_norm.1	26	disease	control
DwoC_18296_norm.2	26	disease	control
DwoC_18296_norm.3	26	disease	control
DwoC_21183_30mM.1	27	disease	insulin
DwoC_21183_30mM.2	27	disease	insulin
DwoC_21183_30mM.3	27	disease	insulin
DwoC_21183_norm.1	28	disease	control
DwoC_21183_norm.2	28	disease	control
DwoC_21183_norm.3	28	disease	control
DwoC_25224_30mM.1	29	disease	insulin
DwoC_25224_30mM.2	29	disease	insulin
DwoC_25224_30mM.3	29	disease	insulin
DwoC_25224_30mM.4	29	disease	insulin
DwoC_25224_30mM.5	29	disease	insulin
DwoC_25224_norm.1	30	disease	control
DwoC_25224_norm.2	30	disease	control
DwoC_25224_norm.3	30	disease	control
DwoC_25224_norm.4	30	disease	control
DwoC_25224_norm.5	30	disease	control
NoD_7012_30mM.1	31	normal	insulin
NoD_7012_30mM.2	31	normal	insulin
NoD_7012_30mM.3	31	normal	insulin
NoD_7012_norm.1	32	normal	control
NoD_7012_norm.2	32	normal	control
NoD_7012_norm.3	32	normal	control
NoD_7344_30mM.1	33	normal	insulin
NoD_7344_30mM.2	33	normal	insulin
NoD_7344_30mM.3	33	normal	insulin
NoD_7344_norm.1	34	normal	control
NoD_7344_norm.2	34	normal	control

NoD_7344_norm.3	34	normal	control
NoD_11985_30mM.1	35	normal	insulin
NoD_11985_30mM.2	35	normal	insulin
NoD_11985_30mM.3	35	normal	insulin
NoD_11985_norm.1	36	normal	control
NoD_11985_norm.2	36	normal	control
NoD_11985_norm.3	36	normal	control
NoD_14381_30mM.1	37	normal	insulin
NoD_14381_30mM.2	37	normal	insulin
NoD_14381_30mM.3	37	normal	insulin
NoD_14381_norm.1	38	normal	control
NoD_14381_norm.2	38	normal	control
NoD_14381_norm.3	38	normal	control
NoD_14520_30mM.1	39	normal	insulin
NoD_14520_30mM.2	39	normal	insulin
NoD_14520_30mM.3	39	normal	insulin
NoD_14520_norm.1	40	normal	control
NoD_14520_norm.2	40	normal	control
NoD_14520_norm.3	40	normal	control
NoD_14569_30mM.1	41	normal	insulin
NoD_14569_30mM.2	41	normal	insulin
NoD_14569_30mM.3	41	normal	insulin
NoD_14569_norm.1	42	normal	control
NoD_14569_norm.2	42	normal	control
NoD_14569_norm.3	42	normal	control
NoD_14581_30mM.1	43	normal	insulin
NoD_14581_30mM.2	43	normal	insulin
NoD_14581_30mM.3	43	normal	insulin
NoD_14581_norm.1	44	normal	control
NoD_14581_norm.2	44	normal	control
NoD_14581_norm.3	44	normal	control

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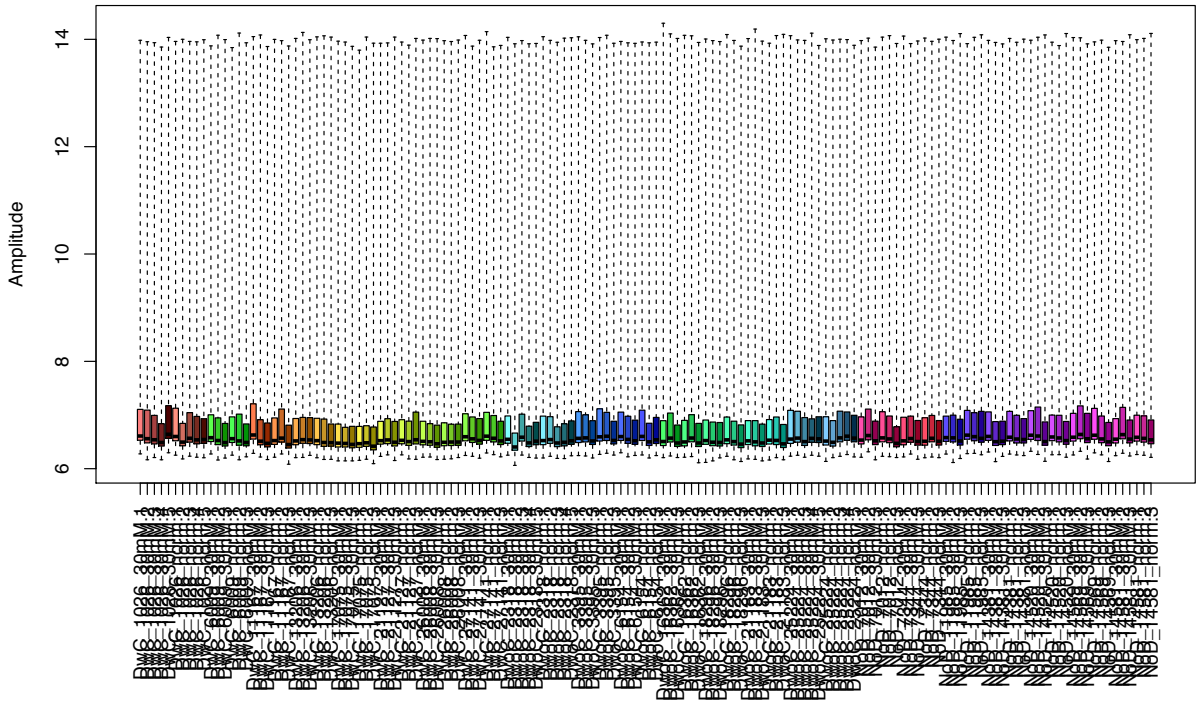
2. Data Preprocessing

Data matrix: 47282 (probes) x 144 (samples).

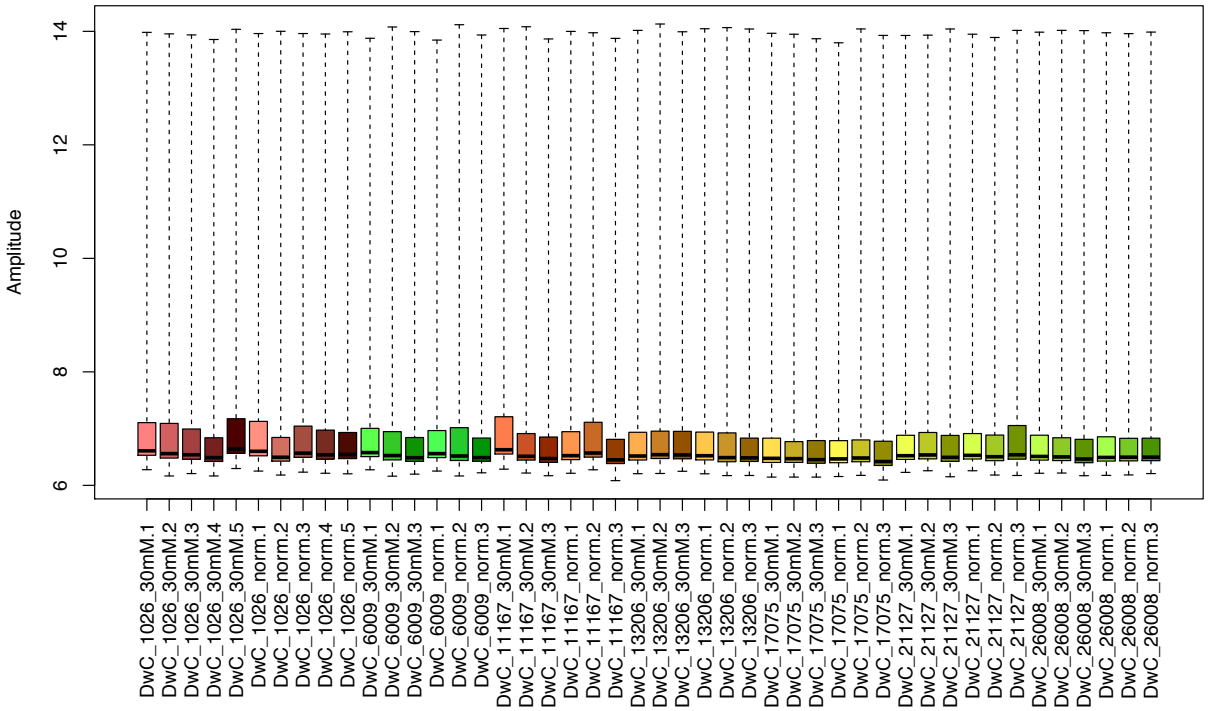
Note that 47282 is the common probes among the four different batches.

A. Raw data (box plot)

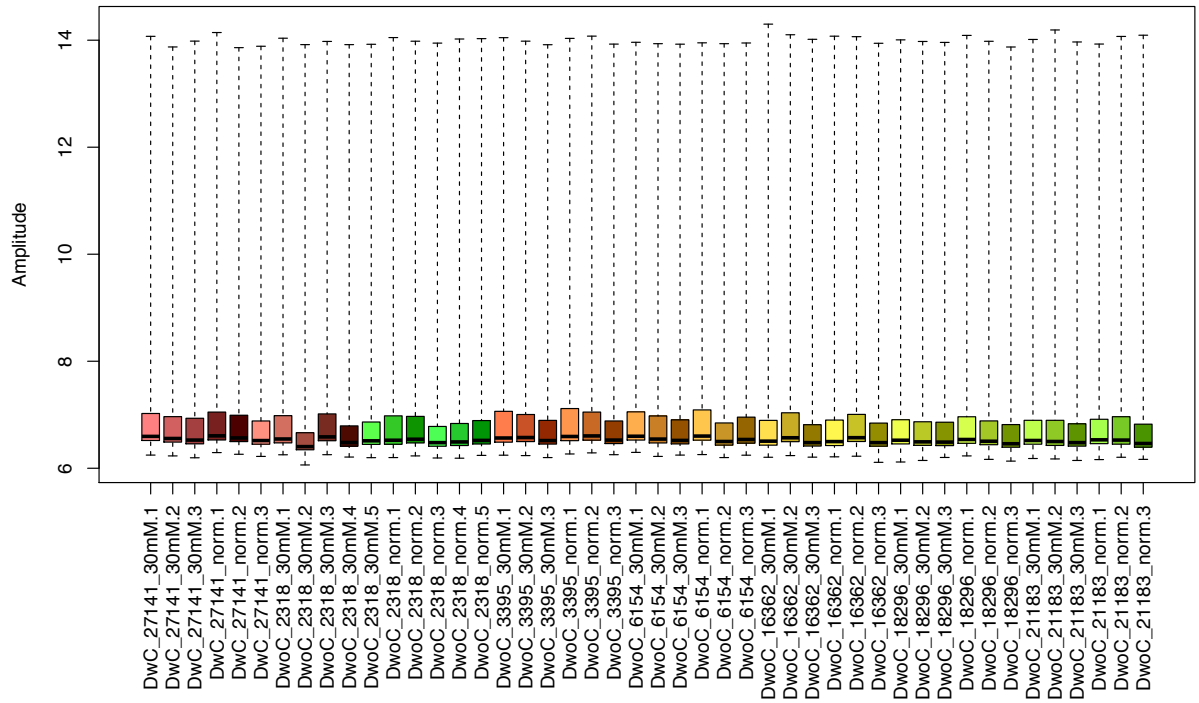
a. 1-144 samples all together



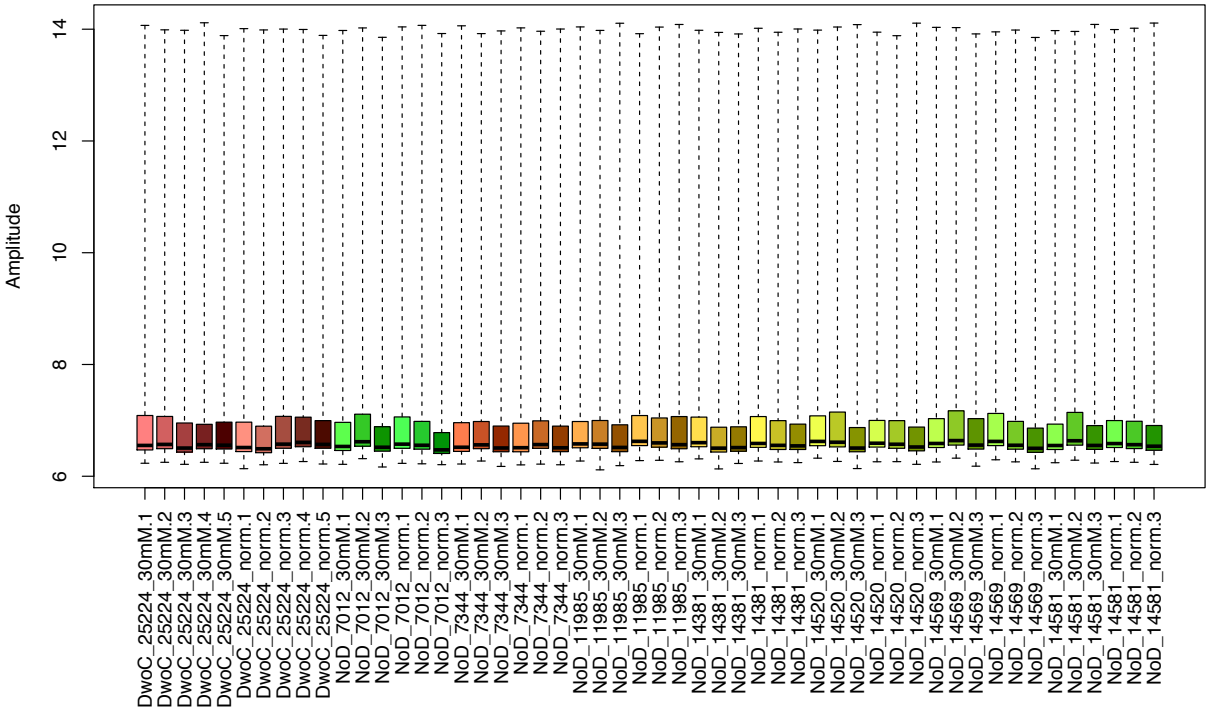
b. 1-46 samples only



c. 47-92 samples only

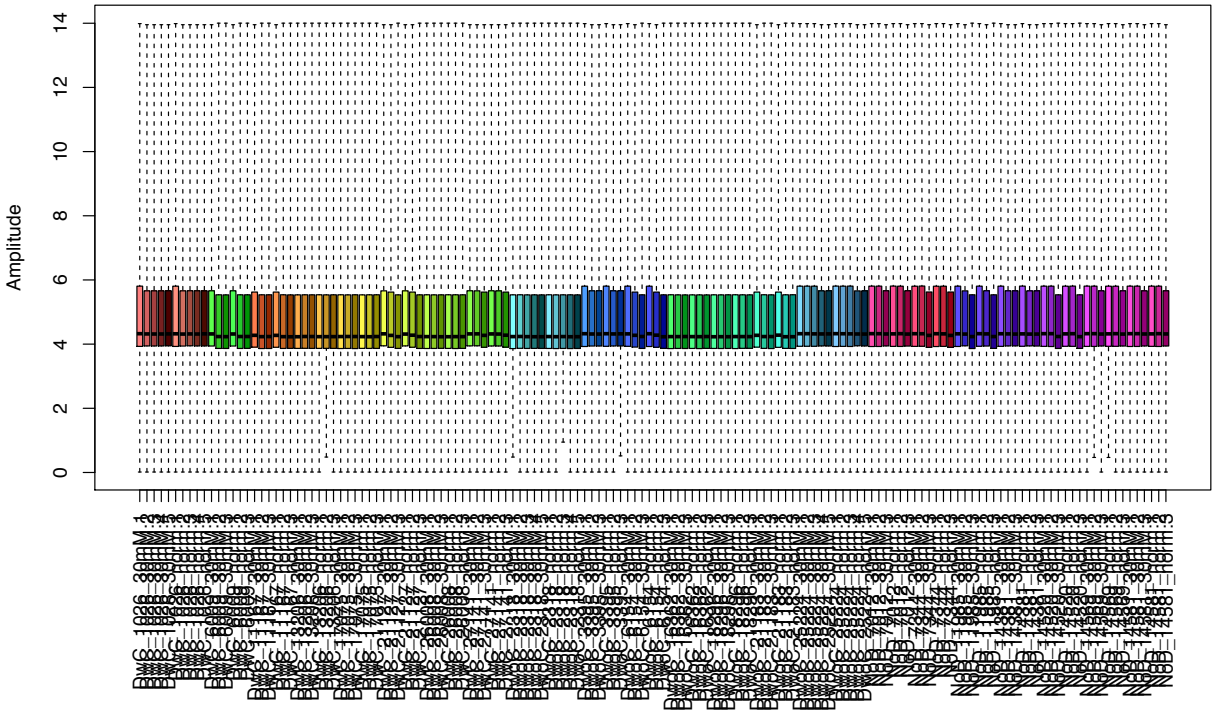


d. 93-144 samples only

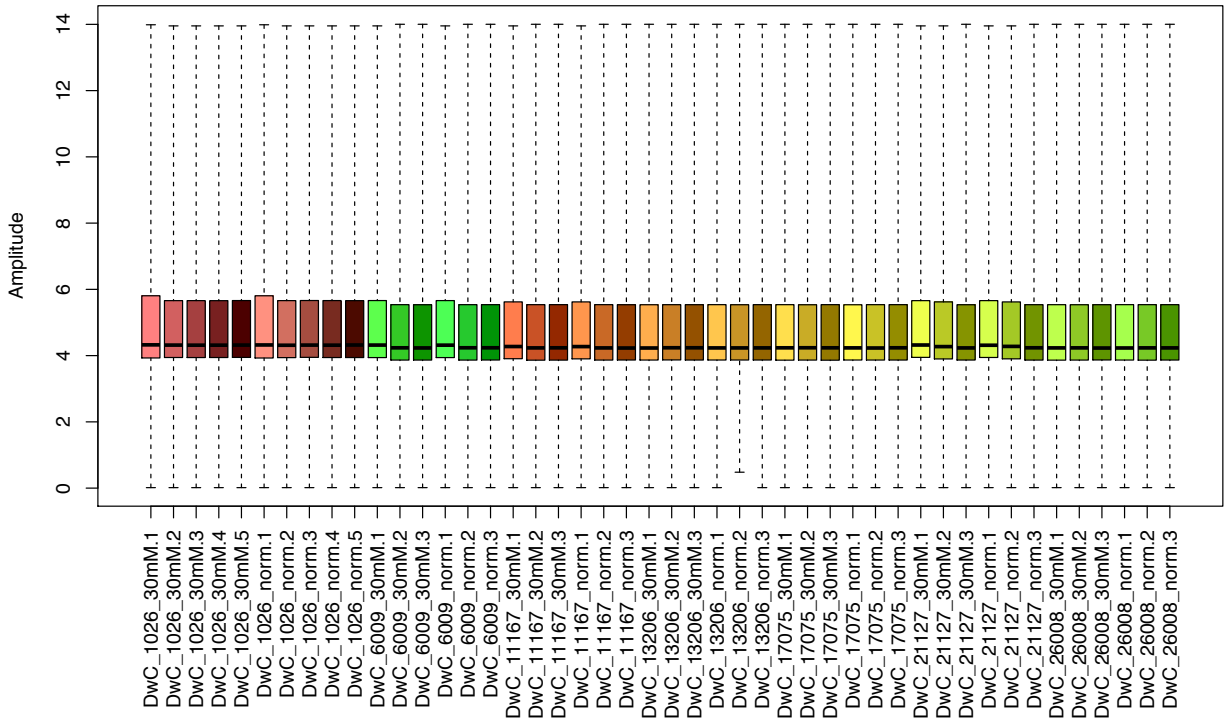


B. Normalized data (box plot)

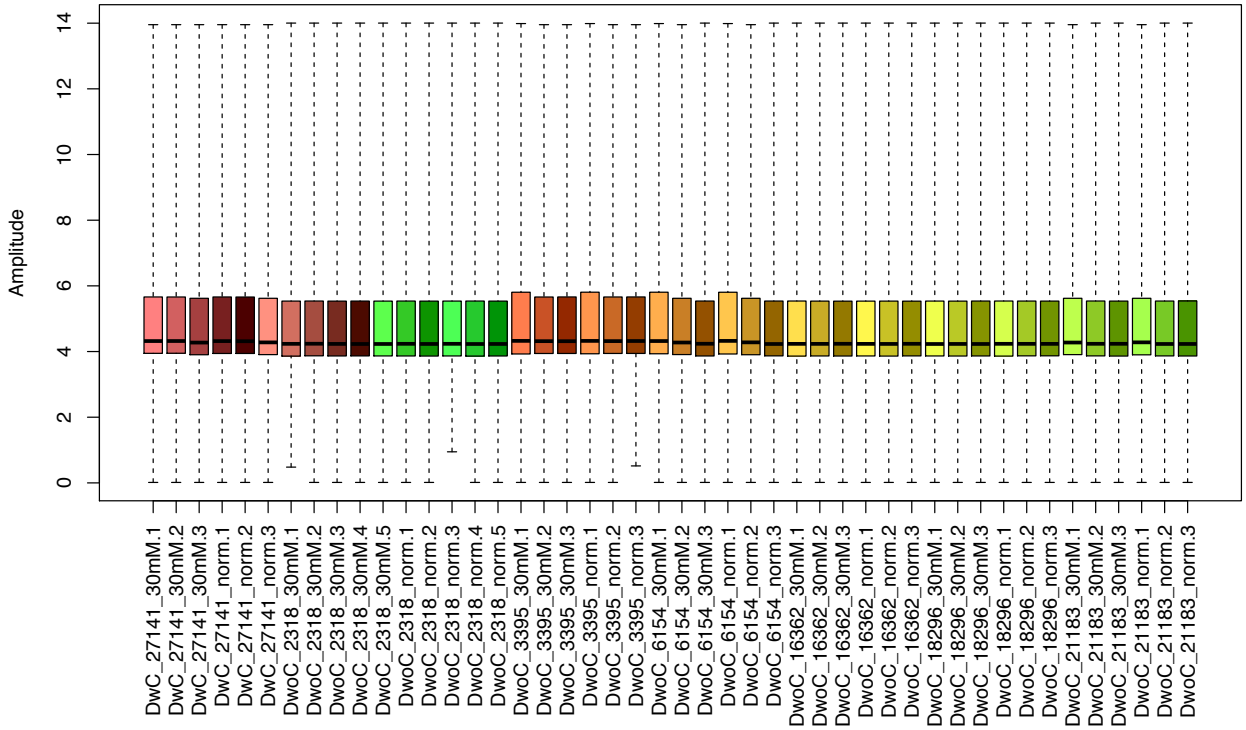
a. 1-144 samples all together



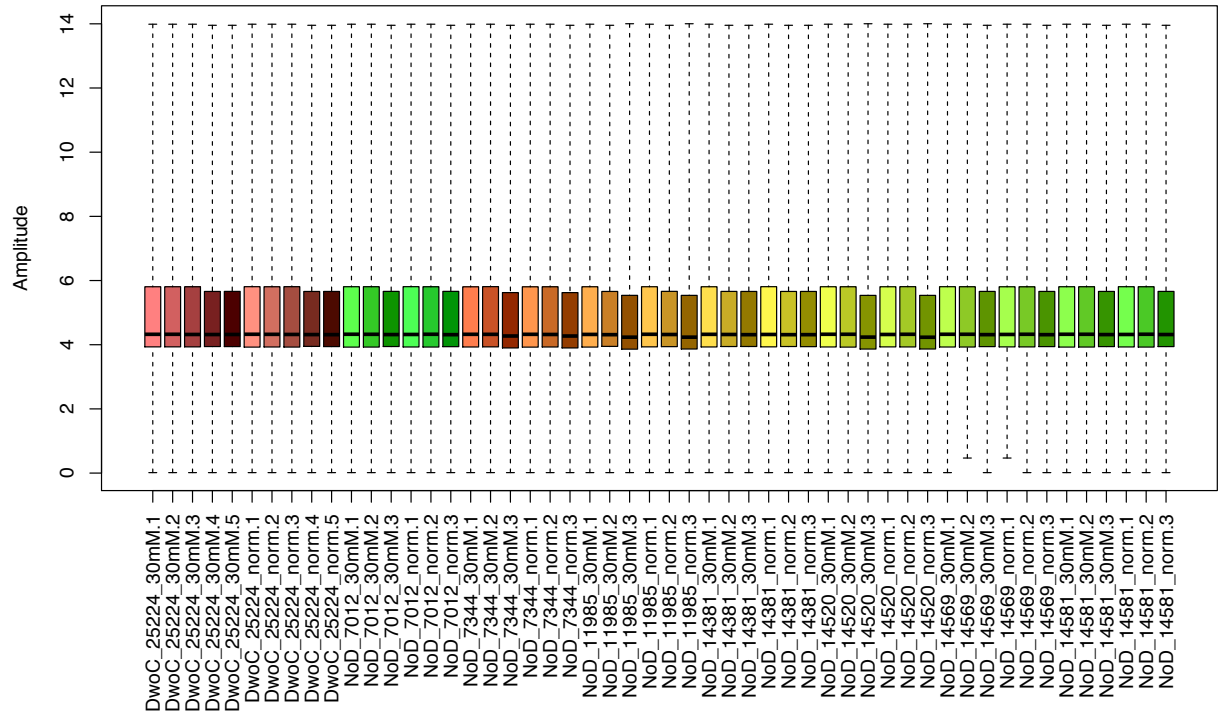
b. 1-46 samples only



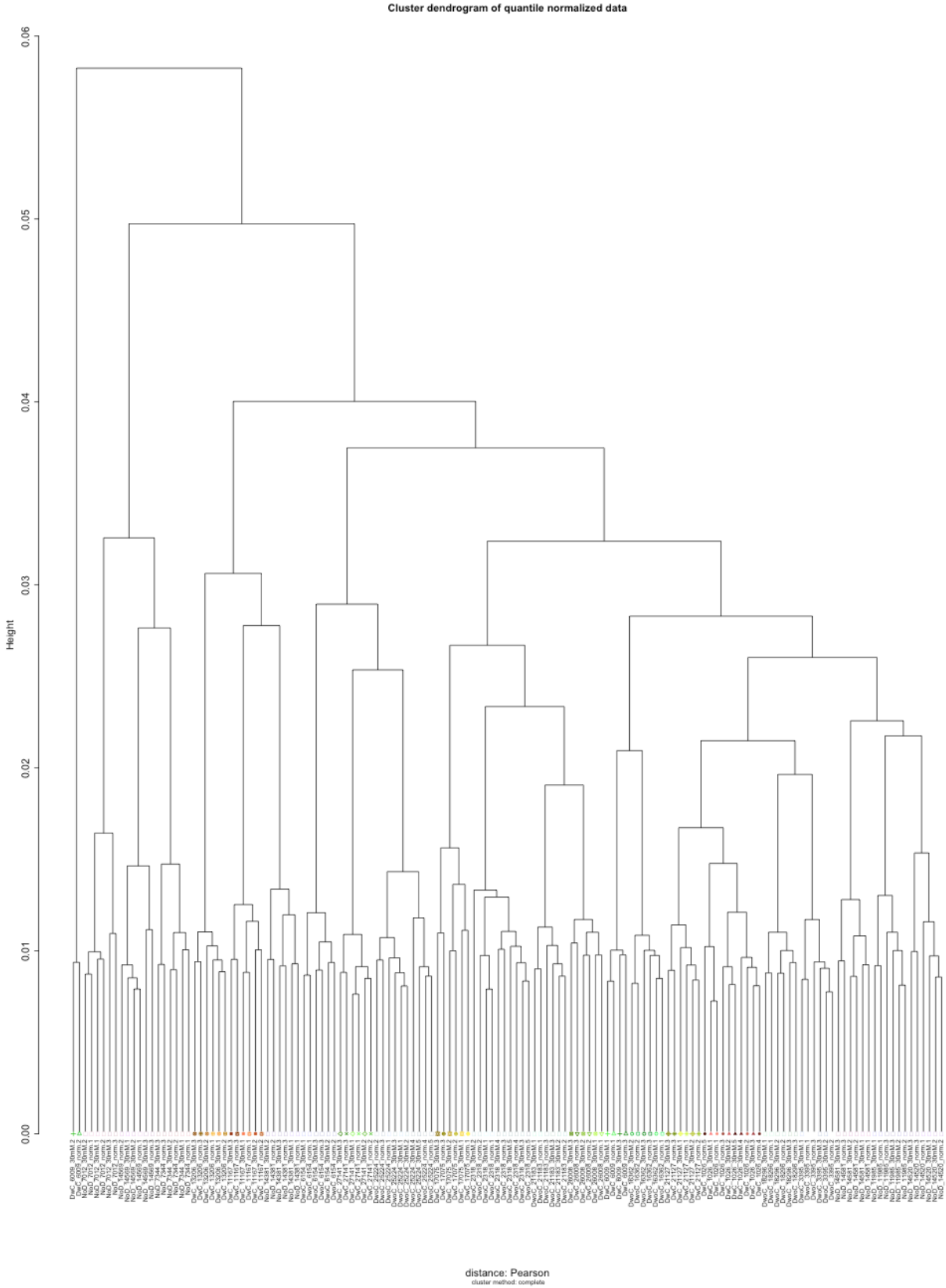
c. 47-92 samples only



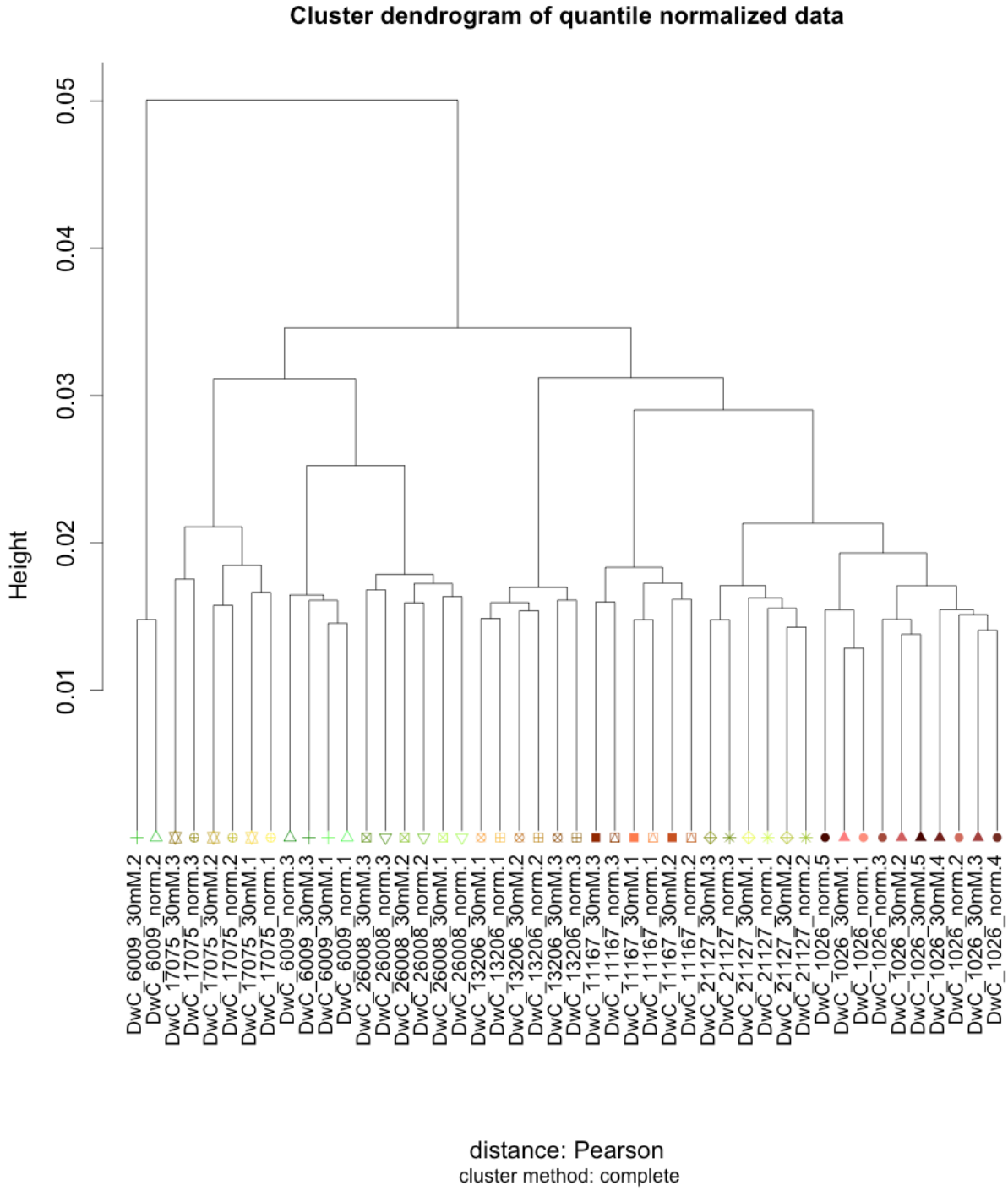
d. 93-144 samples only



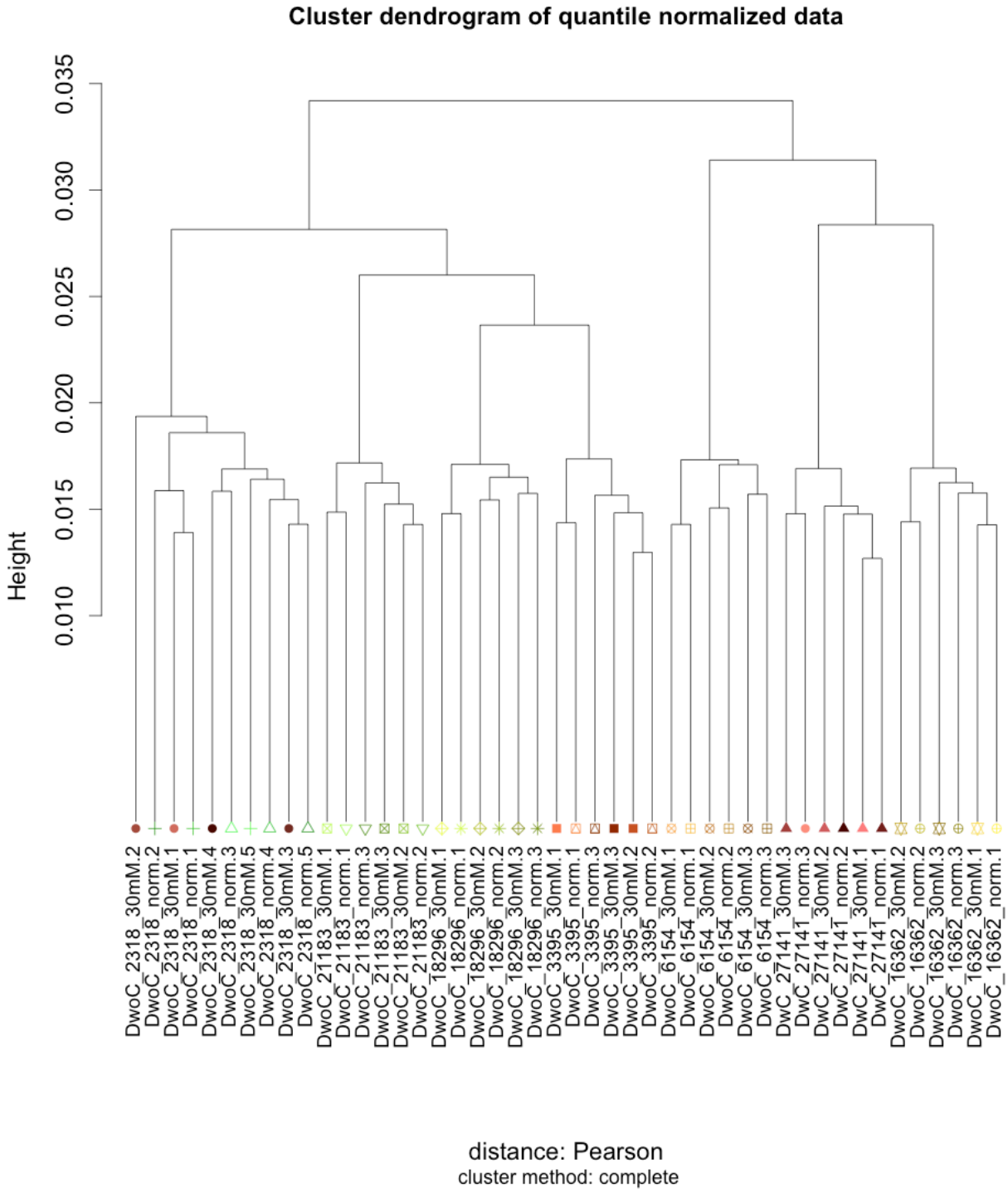
Data clustering (normalized data only: 144 samples all together)



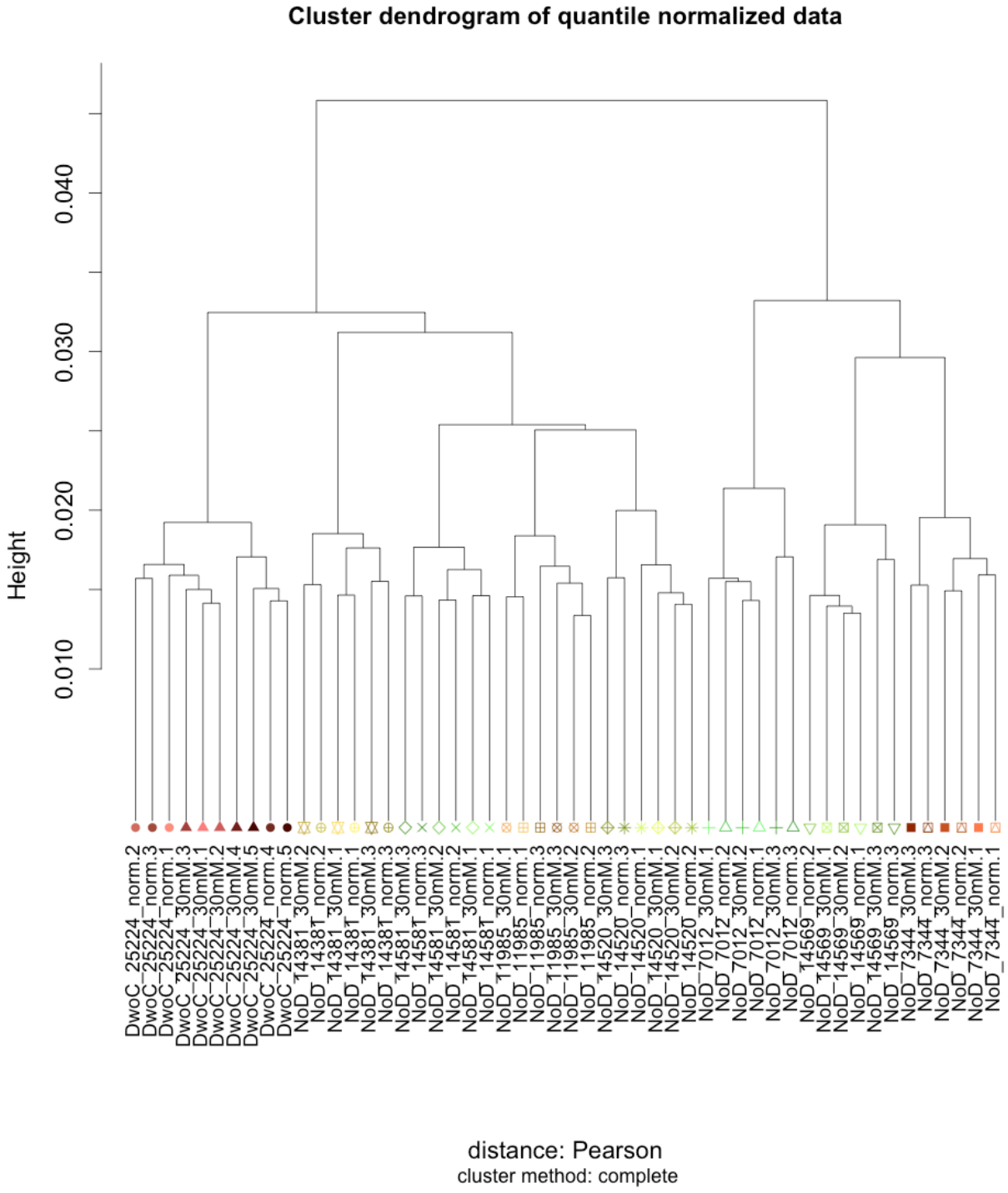
a. 1-46 samples only



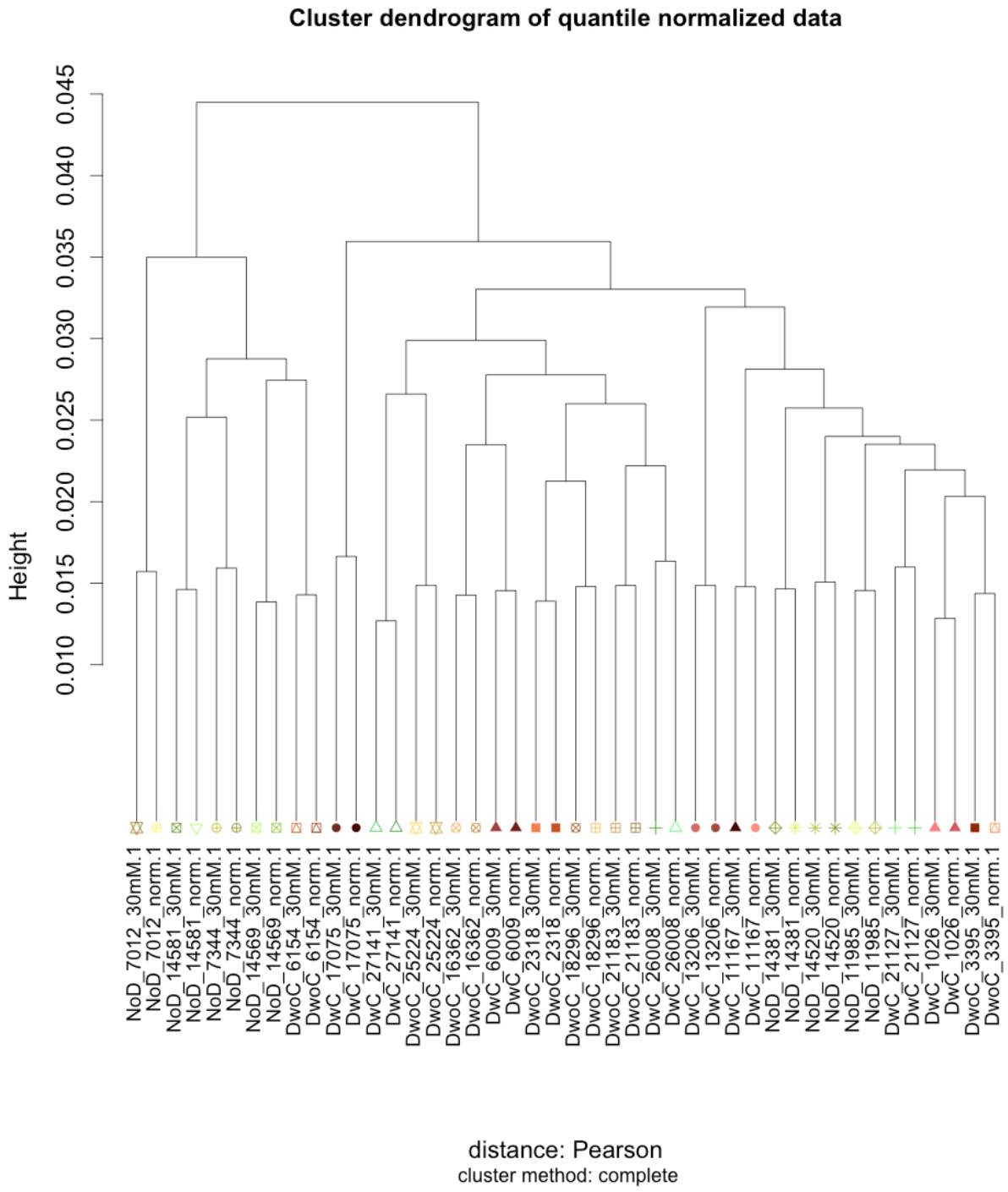
b. 47-92 samples only



c. 93-144 samples only



d. 44 samples without replicates



3. Data filtering

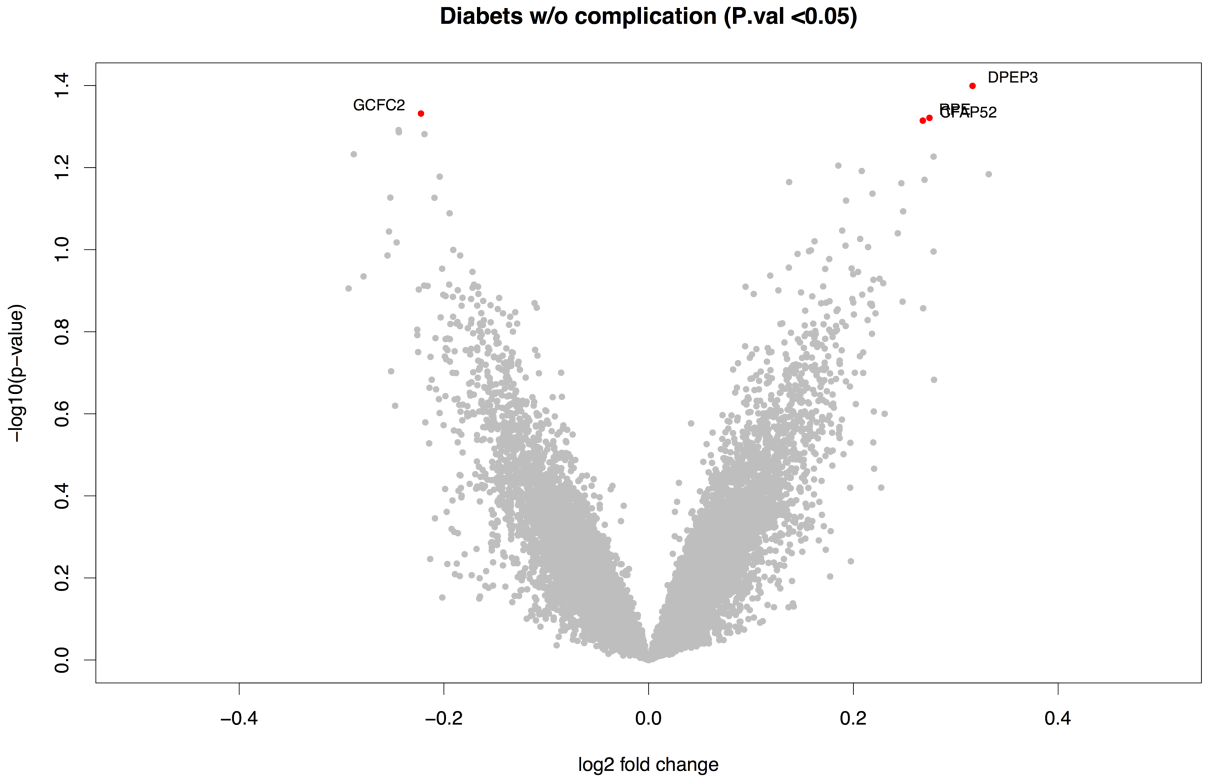
a. 16436 unexpressed probes are removed that will reduce false positives (matrix size: 30846 x 144)
b. 8871 probes that do not have annotated genes are removed (matrix size: 21975 x 144)

4. Differential expression analysis (using limma)

Input data matrix: 21975 probes x 144 samples

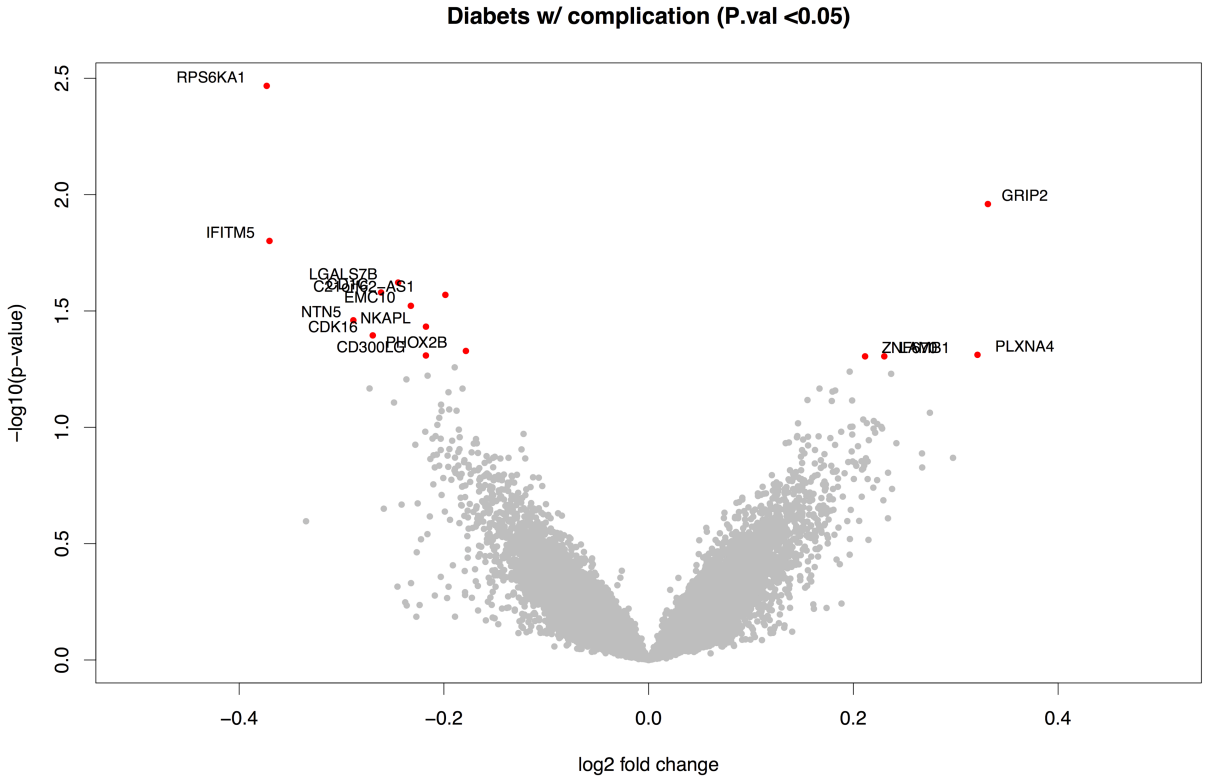
Comparison design (cutoff: p.val <0.05) – note that no significant q-value is achieved

a. disease without complication insulin vs control



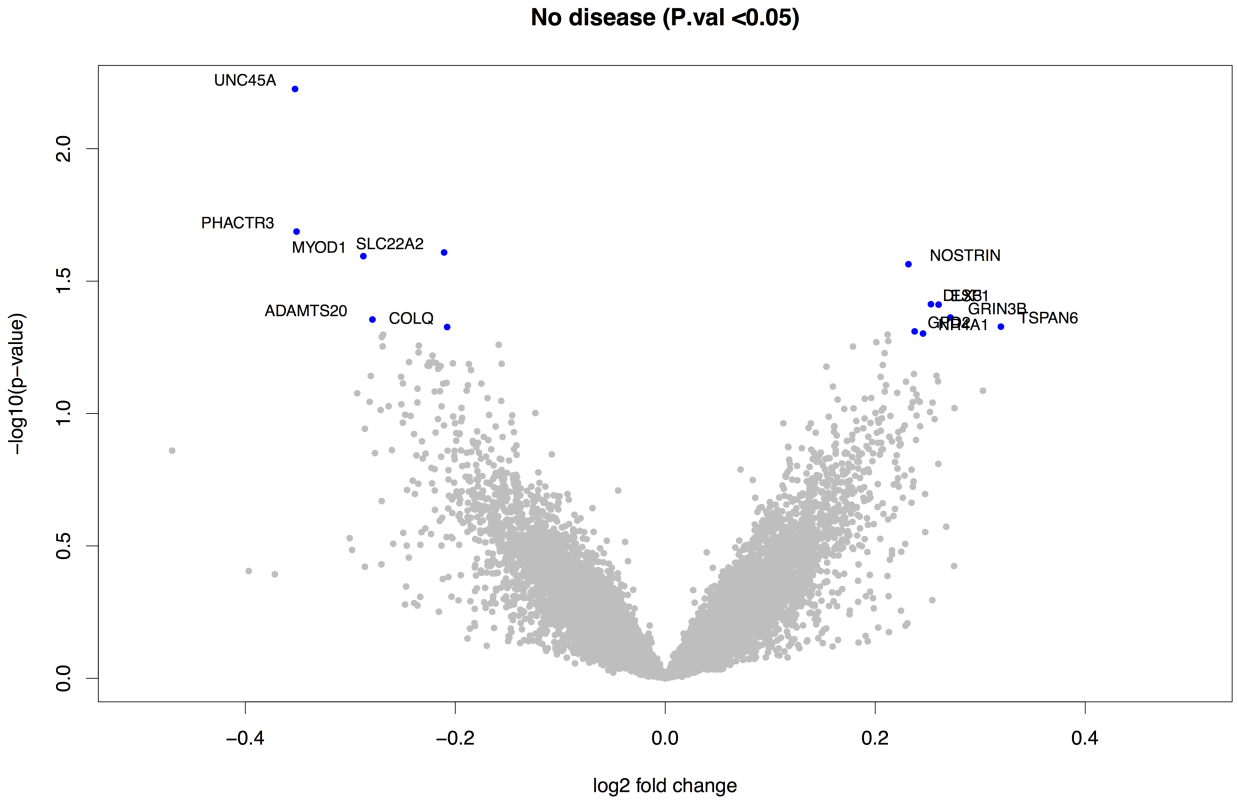
Gene Symbol	Gene Name	log2 FC	Ave Expr	P.Value
DPEP3	dipeptidase 3	0.316	3.767	0.040
GCFC2	GC-rich sequence DNA-binding factor 2	-0.222	4.325	0.047
RPE	ribulose-5-phosphate-3-epimerase	0.274	3.754	0.048
CFAP52	cilia and flagella associated protein 52	0.268	3.864	0.048

b. disease with complication insulin vs control



Gene Symbol	geneName	Log2 FC	Ave Expr	P.Value
RPS6KA1	ribosomal protein S6 kinase, 90kDa, polypeptide 1	-0.373	4.194	0.003
GRIP2	glutamate receptor interacting protein 2	0.331	4.230	0.011
IFITM5	interferon induced transmembrane protein 5	-0.370	3.617	0.016
LGALS7B	lectin, galactoside-binding, soluble, 7B	-0.245	3.897	0.024
CD1C	CD1c molecule	-0.261	3.892	0.026
C21orf62-AS1	C21orf62 antisense RNA 1	-0.199	4.477	0.027
EMC10	ER membrane protein complex subunit 10	-0.232	4.140	0.030
NTN5	netrin 5	-0.288	3.932	0.035
NKAPL	NFKB activating protein-like	-0.218	4.108	0.037
CDK16	cyclin-dependent kinase 16	-0.269	3.937	0.040
PHOX2B	paired-like homeobox 2b	-0.179	4.186	0.047
PLXNA4	plexin A4	0.321	3.747	0.049
CD300LG	CD300 molecule-like family member g	-0.218	4.103	0.049
LAMB1	laminin, beta 1	0.230	4.260	0.050
ZNF670	zinc finger protein 670	0.211	4.453	0.050

c. no disease insulin vs control



Gene Symbol	Gene Name	log2 FC	Ave Expr	P.Value
UNC45A	unc-45 myosin chaperone A	-0.353	4.097	0.006
PHACTR3	phosphatase and actin regulator 3	-0.351	3.956	0.021
SLC22A2	solute carrier family 22 (organic cation transporter), member 2	-0.211	4.329	0.025
MYOD1	myogenic differentiation 1	-0.288	4.002	0.025
NOSTRIN	nitric oxide synthase trafficking	0.232	4.121	0.027
DLX3	distal-less homeobox 3	0.253	4.198	0.039
ESF1	ESF1 nucleolar pre-rRNA processing protein homolog	0.260	3.925	0.039
GRIN3B	glutamate receptor, ionotropic, N-methyl-D-aspartate 3B	0.272	3.980	0.043
ADAMTS20	ADAM metallopeptidase with thrombospondin type 1 motif, 20	-0.279	3.858	0.044
TSPAN6	tetraspanin 6	0.320	3.878	0.047
COLQ	collagen-like tail subunit (single strand of homotrimer) of asymmetric acetylcholinesterase	-0.208	4.408	0.047
GPD2	glycerol-3-phosphate dehydrogenase 2 (mitochondrial)	0.238	4.114	0.049
NR4A1	nuclear receptor subfamily 4, group A, member 1	0.246	4.000	0.050