Microarray data analysis

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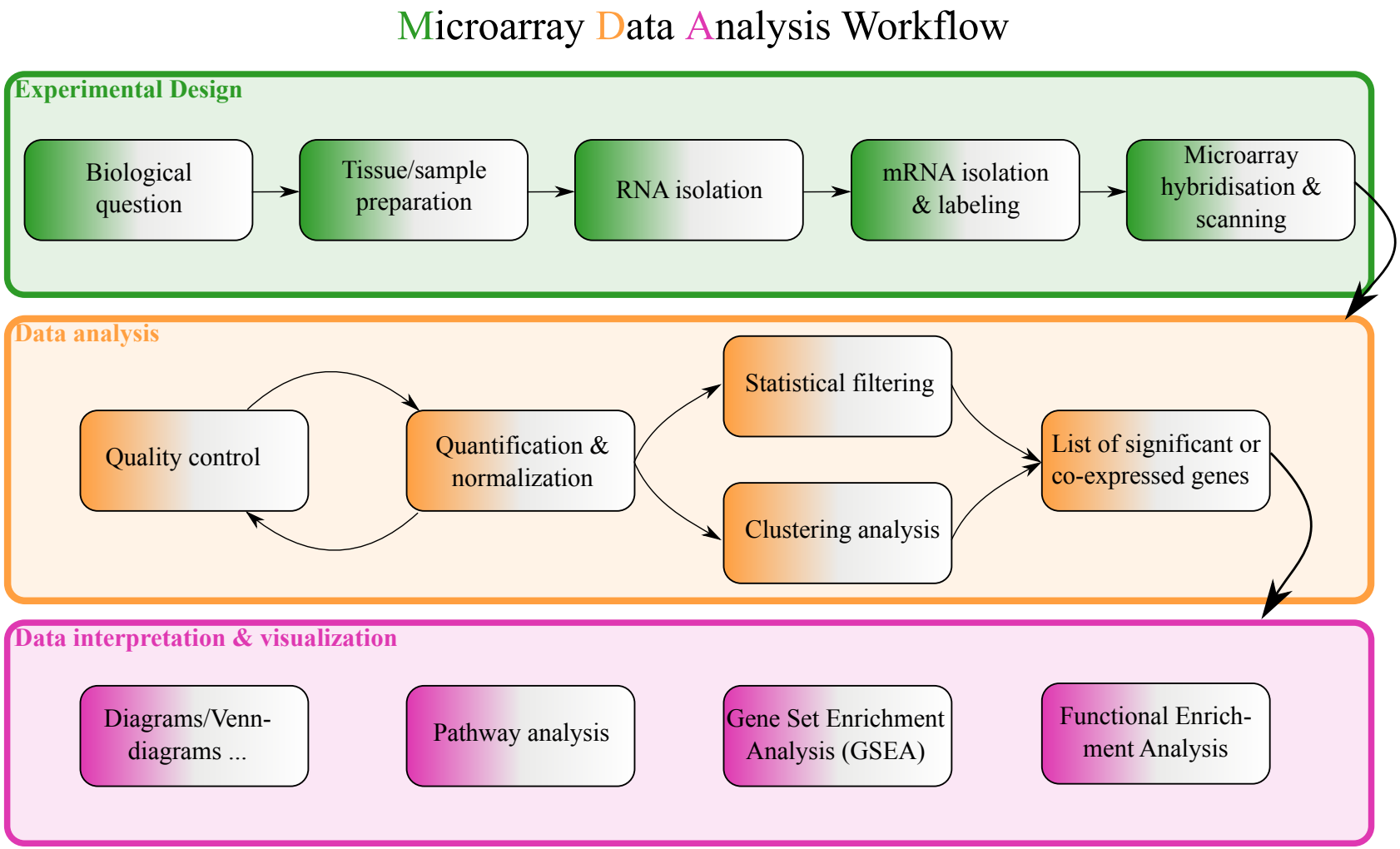
Workflow

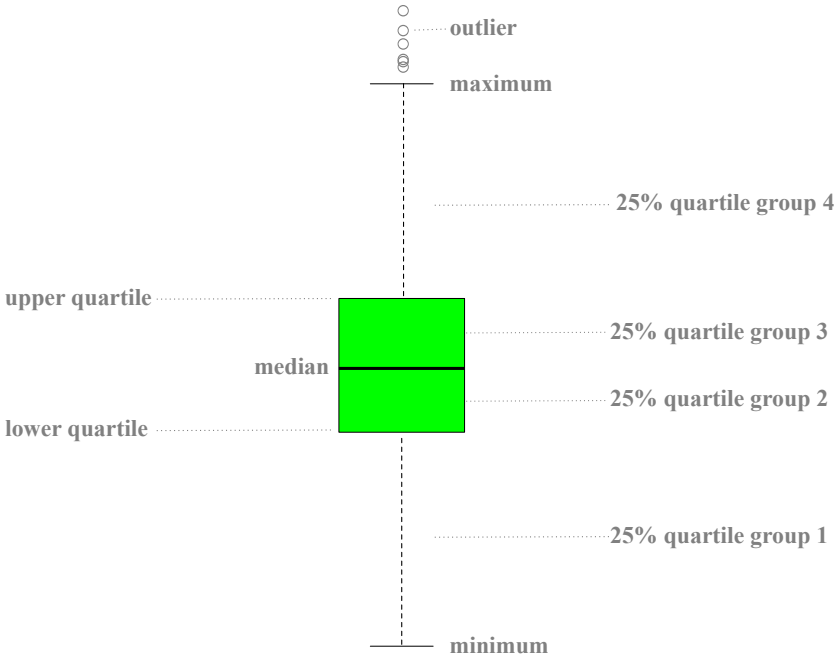

Microarray Data Analysis Workflow
Experimental Design
Biological question
Tissue/sample preparation
RNA isolation
mRNA isolation & labeling
Microarray hybridisation & scanning
Data analysis
Quality control
Quantification & normalization
Statistical filtering
Clustering analysis
List of significant or co-expressed genes
Data interpretation & visualization
Diagrams/Venn-diagrams ...
Pathway analysis
Gene Set Enrichment Analysis (GSEA)
Functional Enrich-ment Analysis

Quality control (QC)

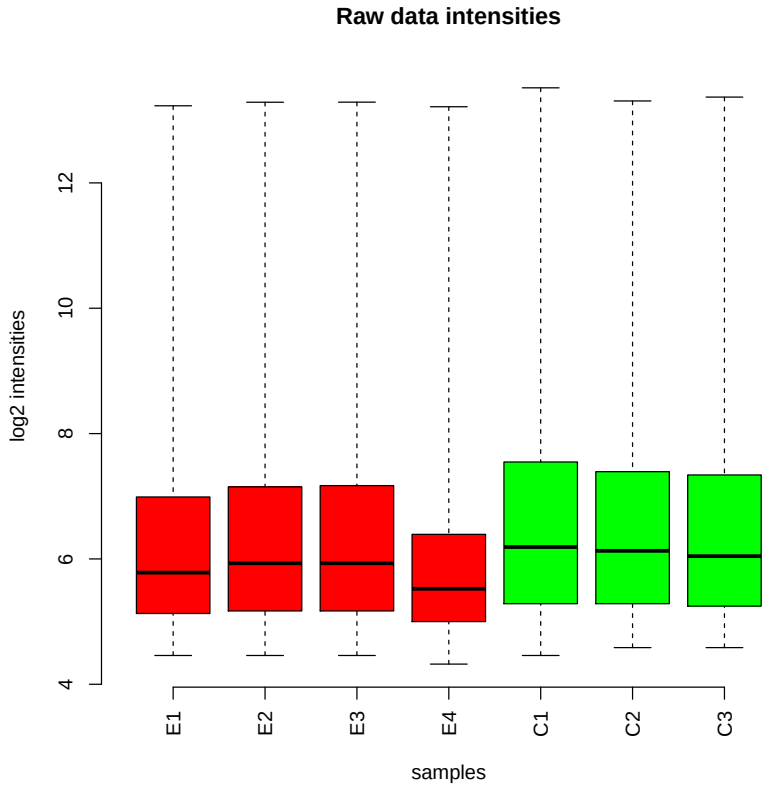
- is crucial to the reliability of the findings concluded from the analysis
- exclude samples of low quality
- several QC methods focussing on different aspects of the data
  - signal distribution $\rightarrow$ histogram, boxplots
  - array comparisons $\rightarrow$ PCA plots, array-array intensity correlation, hierarchical clustering

Intensity distribution: boxplots

- is a graphical representation of statistical measures

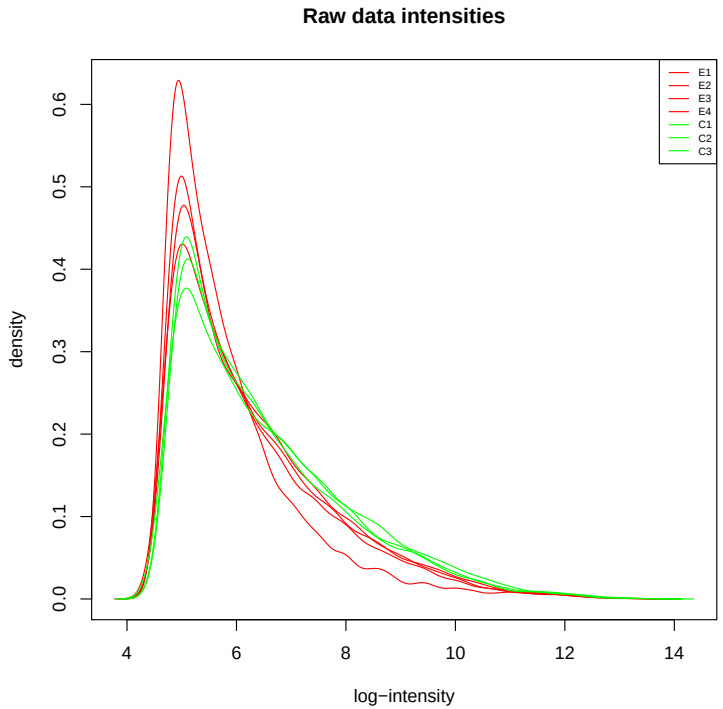


- used to detect major variations between the arrays



Density plot

- a smoothed histogram $\rightarrow$ shows intensity distribution of each array
- identifies arrays that need to be carefully examined before using in further analysis



Hierarchical clustering

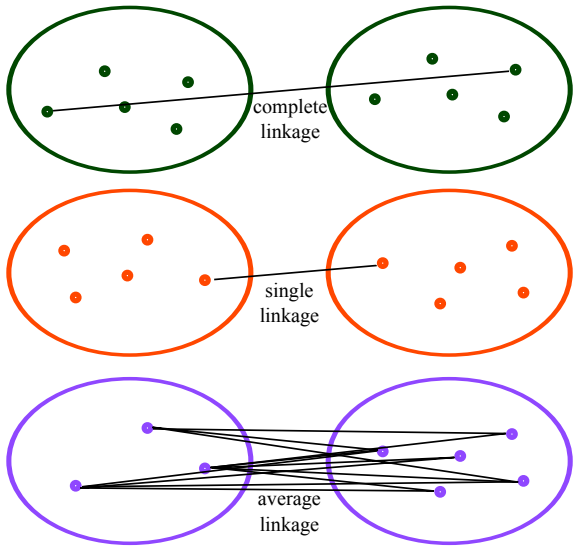
- widely used data analysis tool to combine or identify objects that are close or similar to each other into clusters
- cluster $\rightarrow$ a collection of data objects similar to one another within the same cluster & disimmilar to the objects in the other clusters
- idea $\rightarrow$ build a binary tree (dendrogram) of the data that successively merges similar groups of points
- requires measures of similarity/distances between individual points and groups of data points

Distance metrics

- distances are a numerical description of how far apart objects are
- choosing the right distance measure is a critical step in clustering
- examples:
  - Euclidean distance
  - Manhattan distance
  - $1 -$ correlation

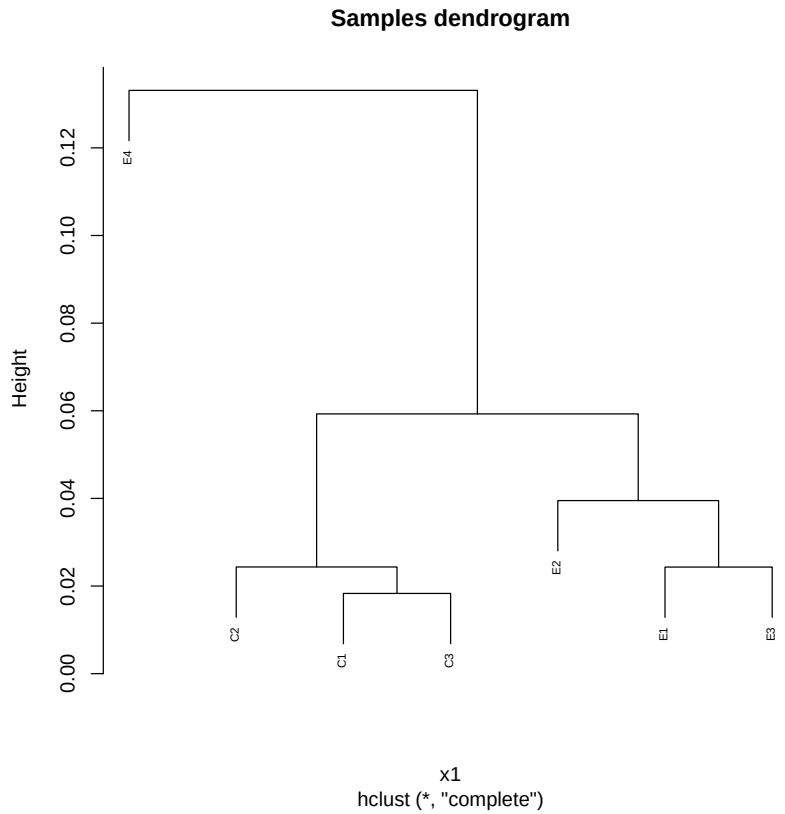
Group similarity

- is a measure of strength of relationship between two objects
- several methods to define intergroup similarity:
  1. single-linkage → is the minimum distance between any 2 objects, one from each cluster
  2. complete-linkage → is the maximum distance between 2 objects, one from each cluster
  3. average-linkage → is the average of all pairwise distances between the members of both clusters



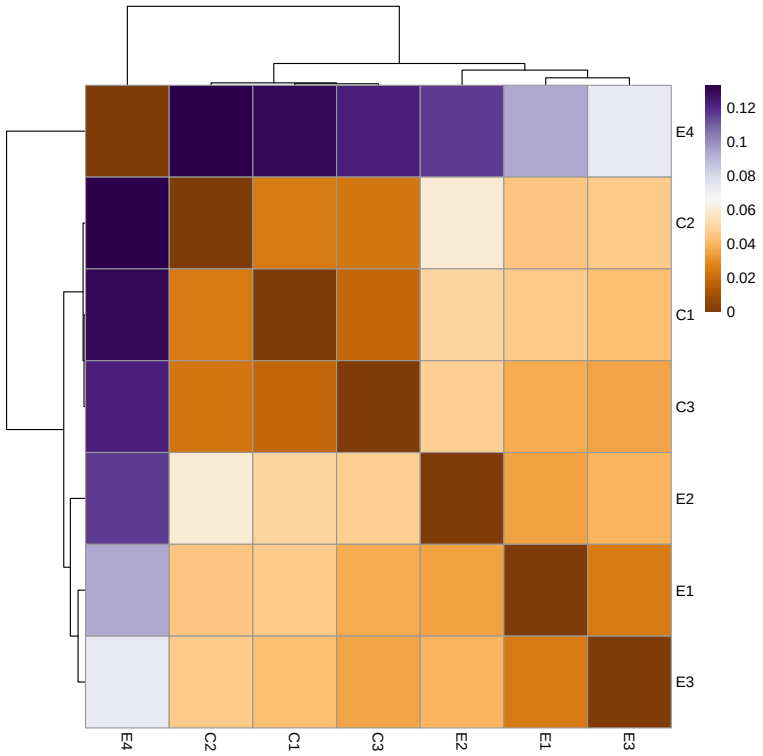
Dendrogram

- a tree that defines the relationships between objects and the distance between clusters



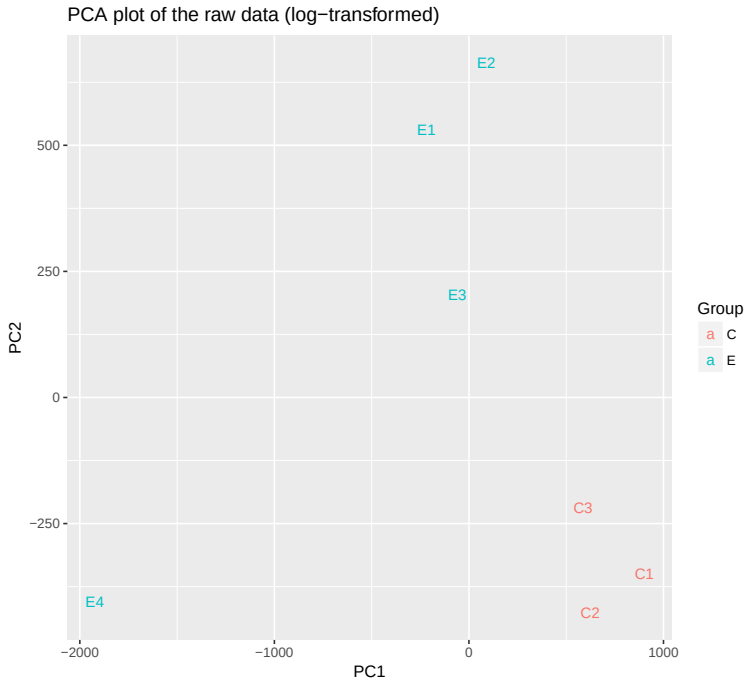
Array-array intensity correlation

- displays the correlation of all pairwise samples using heatmaps



Principle component analysis

- transforms the data from a high-dimensional space into a 2 or 3-dimensional one without losing much of the variation in the original values
- dimenisonality reduction allows visual inspection of the data
- idea $\rightarrow$ samples with similar intensities should cluster together



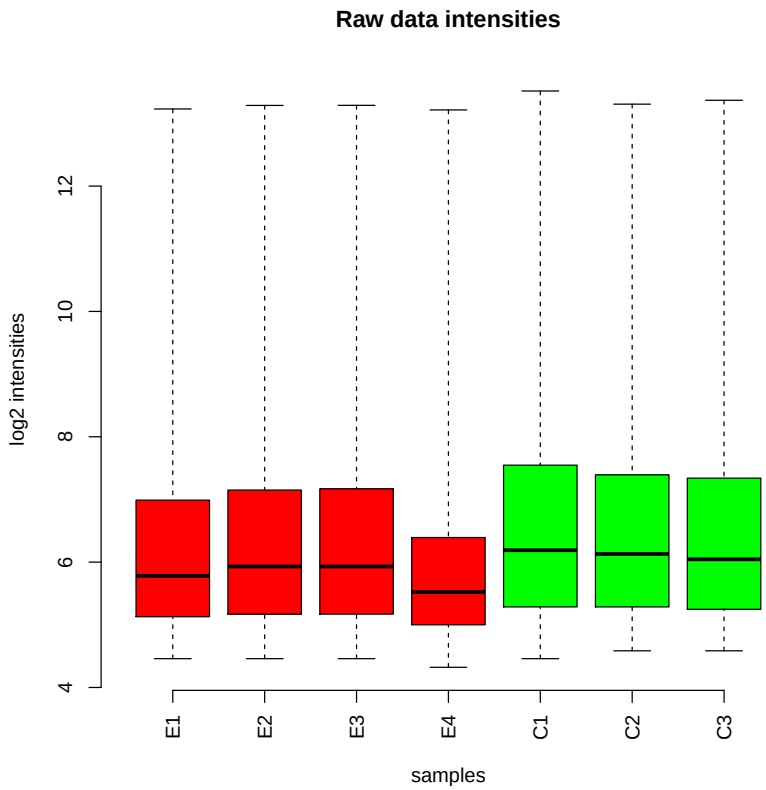
Normalisation

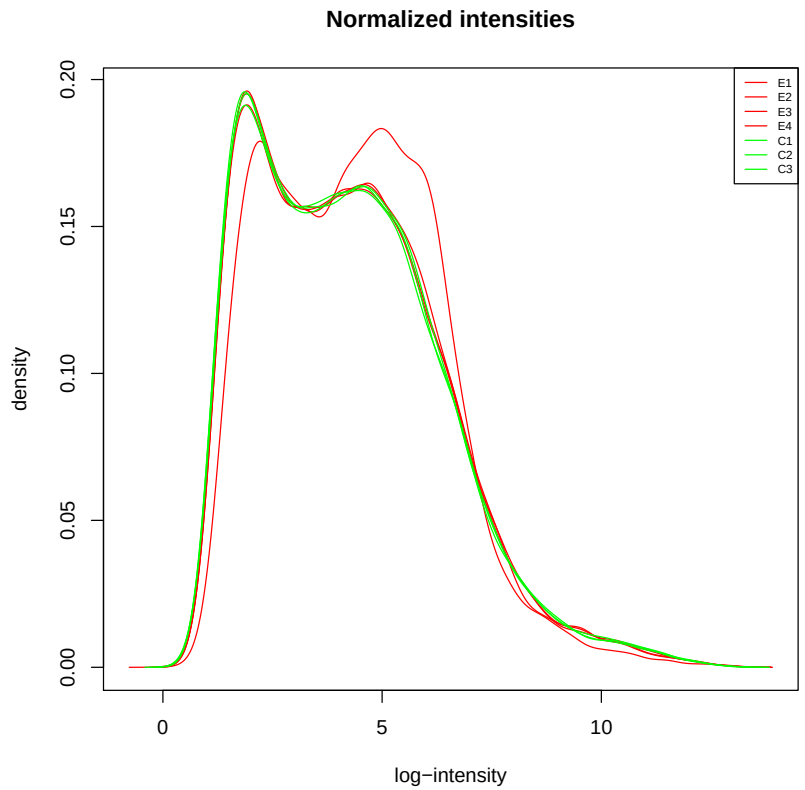
- compensates for systematic technical differences between chips
  - unequal quantities of starting RNA
  - different amounts of labelling
  - varied hybrdisation conditions across the physical extent of one array
  - different scanner settings
- normalisation techniques:
  - scale normalisation
  - lowess normalisation
  - MAS 5.0
  - RMA (quantile normalisation)

Robust multiarray average (RMA)

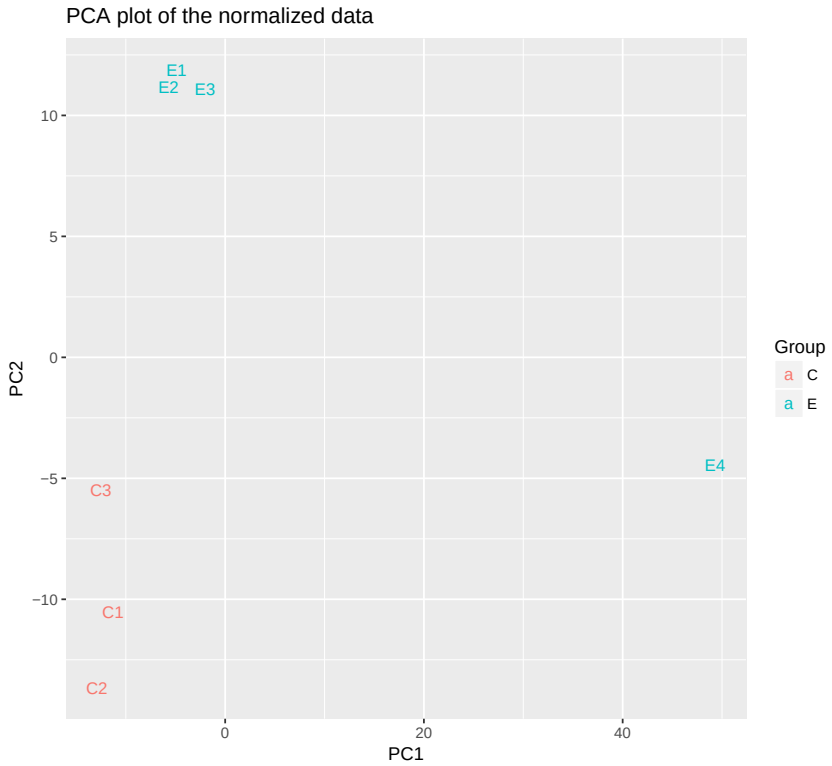
- one of the widest used normalisation methods for Affymetrix arrays
- uses only the PM probes on the chip
- normalizing at probe level avoids the loss of information
- 4 steps:
  - background correction
  - quantile normalisation
  - probe level intensity calculation
  - probe set summarization ('median polishing')

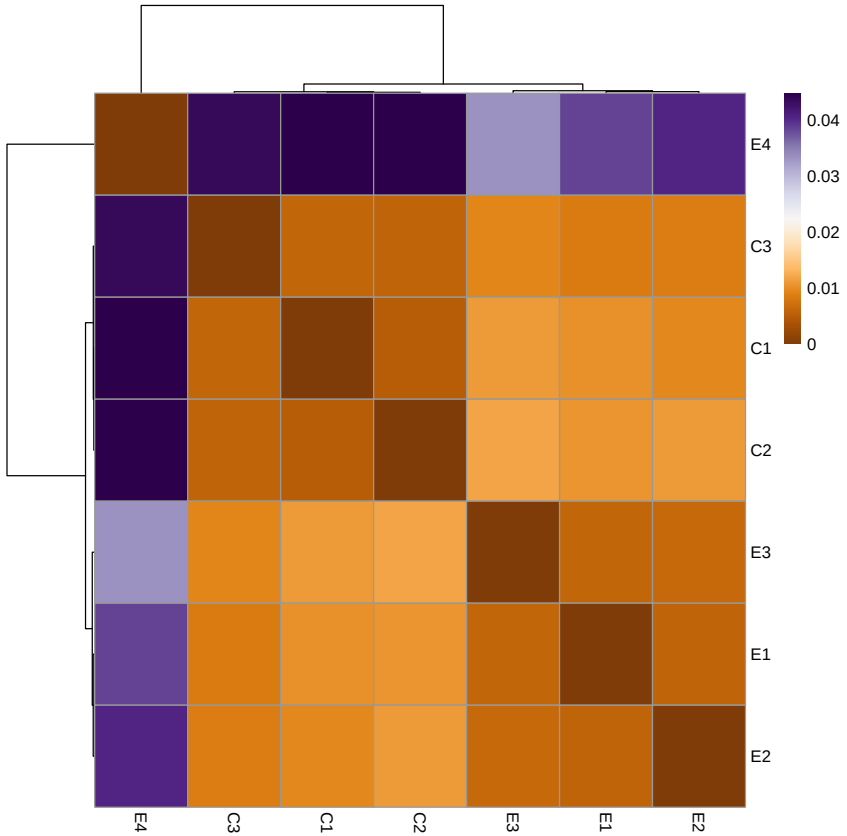
QC after normalisation (I)





QC after normalisation (II)





Identification of differentially expressed genes

- identify DE genes and apply statistical tests to assess the significance of the observed associations
- statistical tests:
  - comparison of two conditions: *Student's t-test* or *Wilcoxon rank sum test*
  - multiple/nested conditions: *ANOVA*
  - Linear models for microarray data (*LIMMA*)
- multiple testing $\rightarrow$ p-value adjustments (*FDR, FWER*)

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- multiple testing $\rightarrow$ p-value adjustments (*FDR, FWER*)
- statistical significance is not necessarily the same as biological significance

Linear models for microarray data (LIMMA)

- is an R package designed to analyse complex experiments involving comparisons between many samples simultaneously
- operates on a matrix of expression values
- it allows different levels of variability between genes and between samples

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- is an R package designed to analyse complex experiments involving comparisons between many samples simultaneously
- operates on a matrix of expression values
- it allows different levels of variability between genes and between samples
- simplified approach:
  - construct a linear model to describe the relation between observed values and experimental conditions
  - fit the linear model to each row of data to estimate the fold changes
  - apply empirical Bayes to calculate moderated t-statistics
  - output: moderated t-statistics

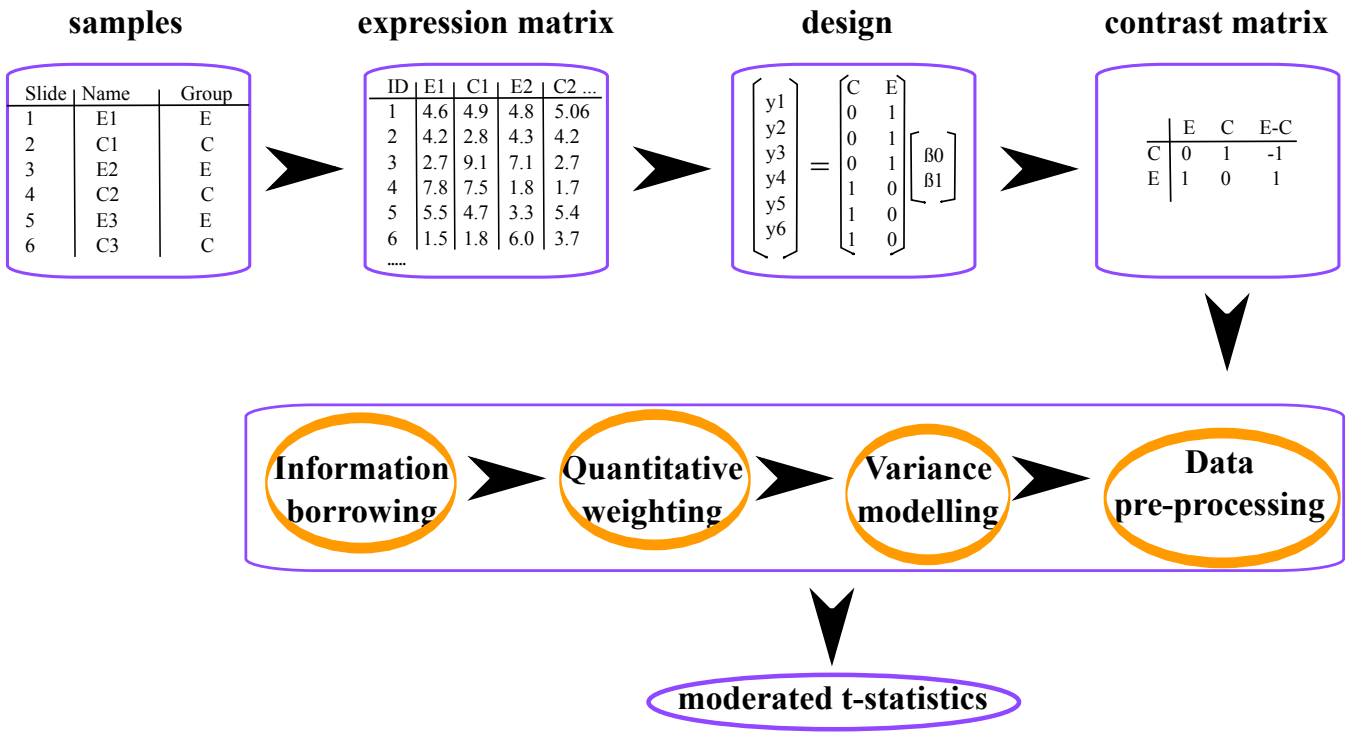
Linear models

- most used statistical methods
- can be used to compare 2 or more groups and for multifactorial designs
- requires a design matrix and a contrast matrix
  - design matrix $\rightarrow$ states which samples are allocated to which conditions
  - contrast matrix $\rightarrow$ describes which comparisons are of interest
- $y = X\beta + \epsilon$

y: vector of observed data, X: design matrix, β: vector of parameters to estimate

Example

- 2x3 factorial design: 2 conditions, each replicated three times
- goal: find differentially expressed genes between 2 conditions

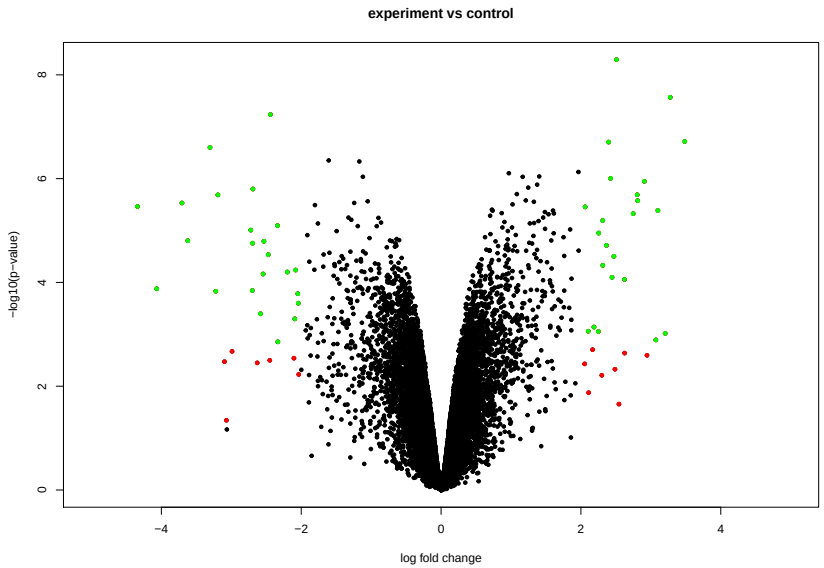


List of significantly differentially expressed genes

PROBEID	ENTREZID	SYMBOL	GENENAME	logFC	AveExpr	t	P.Value	adj.P.Val	B	E1	E2	E3	C1	C2	C3
TC01000017.hg.1	400728	FAM87B	family with sequence similarity 87 member B	-0.5108676	5.282430	-5.830297	7.381490e-04	0.0334267866	-0.057279721	5.082846	5.092984	4.905159	5.502273	5.673162	5.438157
TC01000113.hg.1	9563	H6PD	hexose-6-phosphate dehydrogenase (glucose 1-deh...	-0.4735258	7.655717	-5.053993	1.648450e-03	0.0467099019	-0.899082448	7.502889	7.309566	7.444406	7.936681	8.012458	7.728300
TC01000151.hg.1	5351	PLOD1	procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1	-0.5105864	6.892207	-5.476685	1.054191e-03	0.0386410376	-0.429817745	6.790599	6.673173	6.446968	7.162915	7.158692	7.120893
TC01000184.hg.1	23254	KAZN	kazrin, periplakin interacting protein	0.3506924	6.551819	5.353727	1.197689e-03	0.0409859801	-0.563584091	6.746468	6.774337	6.660690	6.430708	6.293886	6.404824
TC01000193.hg.1	6248	RSC1A1	regulatory solute carrier protein, family 1, member 1	-0.2682336	6.626843	-5.023315	1.704401e-03	0.0477554758	-0.934185613	6.500803	6.488452	6.488923	6.745079	6.733196	6.804605
TC01000371.hg.1	677774	SCARNA1	small Cajal body-specific RNA 1	-1.2962790	3.983974	-10.113995	2.556184e-05	0.0078580955	3.314046365	3.534069	3.035438	3.437998	4.641666	4.637176	4.617499
TC01000408.hg.1	347735	SERINC2	serine incorporator 2	-0.3554747	6.278629	-5.709592	8.321886e-04	0.0353095024	-0.182447479	6.115187	6.130025	6.057464	6.513044	6.481094	6.374961
TC01000424.hg.1	8668	EIF3I	eukaryotic translation initiation factor 3 subunit I	-0.3568492	8.093274	-5.177178	1.443535e-03	0.0445867614	-0.759565140	7.930683	7.993479	7.820386	8.322752	8.207641	8.284703
TC01000497.hg.1	64064	OXCT2	3-oxoacid CoA-transferase 2	-0.3896637	4.672660	-5.610016	9.199577e-04	0.0366517120	-0.287254955	4.544900	4.477226	4.411360	4.849566	4.794054	4.958857
TC01000536.hg.1	339539	LOC339539	uncharacterized LOC339539	0.6883077	5.218392	10.184796	2.445011e-05	0.0077400436	3.355994713	5.618360	5.462320	5.606956	4.822785	4.874720	4.925208
TC01000550.hg.1	6487	ST3GAL3	ST3 beta-galactoside alpha-2,3-sialyltransferase 3	0.3884467	5.964524	5.974156	6.413111e-04	0.0316322927	0.089253120	6.145311	6.078243	6.252688	5.746718	5.764427	5.799757
TC01000564.hg.1	6202	RPS8	ribosomal protein S8	-0.3657236	6.695883	-6.843642	2.879934e-04	0.0212561857	0.916921727	6.520853	6.478339	6.539872	6.870361	6.860791	6.905082
TC01000565.hg.1	94161	SNORD46	small nucleolar RNA, C/D box 46	-0.4804855	11.325241	-7.370833	1.840225e-04	0.0177412913	1.373998443	11.117220	11.003706	11.134068	11.518699	11.545139	11.632612
TC01000668.hg.1	26027	ACOT11	acyl-CoA thioesterase 11	0.4607009	5.485165	8.153243	9.907859e-05	0.0139638874	1.996975995	5.691272	5.727595	5.727680	5.266671	5.300785	5.196988
TC01000730.hg.1	3953	LEPR	leptin receptor	-0.2800242	5.489537	-5.210109	1.393693e-03	0.0439250668	-0.722656530	5.326151	5.383075	5.339350	5.631077	5.657286	5.600285
TC01000745.hg.1	1647	GADD45A	growth arrest and DNA damage inducible alpha	0.3319442	6.718726	5.824295	7.425322e-04	0.0334267866	-0.063455075	6.903872	6.926172	6.824051	6.572825	6.524426	6.561011
TC01000781.hg.1	256435	ST6GALNAC3	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-...	-0.7128701	3.674617	-6.640652	3.446840e-04	0.0231384560	0.732236807	3.521368	3.242929	3.190250	4.139107	3.906683	4.047368
TC01000874.hg.1	729970	LOC729970	hCG2028352-like	-0.5080706	5.747263	-5.653954	8.800092e-04	0.0358800205	-0.240834250	5.326493	5.645737	5.507452	5.951125	6.046520	6.006250
TC01000883.hg.1	58155	PTBP2	polypyrimidine tract binding protein 2	0.7157137	5.906043	9.355395	4.191649e-05	0.0101177096	2.841605787	6.163183	6.383313	6.245203	5.617368	5.540461	5.486728
TC01000892.hg.1	54873	PALMD	palmdelphin	-1.5961253	7.425419	-10.037111	2.683487e-05	0.0081723454	3.268093656	6.877048	6.704516	6.300505	8.343710	8.292006	8.034728
TC01000909.hg.1	1901	S1PR1	sphingosine-1-phosphate receptor 1	-0.7190823	6.329539	-5.878584	7.039221e-04	0.0326016120	-0.007776798	5.983407	5.941898	5.984688	6.452707	6.664954	6.949578
TC01001004.hg.1	56944	OLFML3	olfactomedin like 3	-0.3748706	6.466112	-5.435179	1.100358e-03	0.0393837151	-0.474724388	6.322097	6.200433	6.313499	6.570661	6.732215	6.657765
TC01001026.hg.1	8458	TTF2	transcription termination factor, RNA polymerase II	0.3761420	5.344338	5.353874	1.197504e-03	0.0409859801	-0.563422243	5.473276	5.511736	5.612214	5.220922	5.062530	5.185349
TC01001030.hg.1	54855	FAM46C	family with sequence similarity 46 member C	0.8025345	5.933439	7.559013	1.578103e-04	0.0167272092	1.529641277	6.425485	6.324733	6.253900	5.664675	5.312671	5.619168
TC01001053.hg.1	647121	EMBP1	embigin pseudogene 1	-0.9099513	4.783384	-5.778300	7.771126e-04	0.0343129955	-0.110949190	4.425107	4.577769	3.982349	5.118014	5.375836	5.221229
TC01001201.hg.1	10962	MLLT11	myeloid/lymphoid or mixed-lineage leukemia; transl...	-0.9376877	4.805903	-5.914541	6.795930e-04	0.0324783243	0.028875887	4.440566	4.448112	4.122501	4.956562	5.372954	5.494726
TC01001262.hg.1	4881	NPR1	natriuretic peptide receptor 1	0.4207164	6.176434	5.328641	1.229578e-03	0.0414553795	-0.591148043	6.452776	6.303448	6.404152	6.060845	5.996651	5.840730
TC01001274.hg.1	57198	ATP8B2	ATPase phospholipid transporting 8B2	0.4062779	6.365881	5.245222	1.342654e-03	0.0433614839	-0.683480409	6.688421	6.477253	6.541387	6.219719	6.191483	6.077025
TC01001348.hg.1	3428	IFI16	interferon, gamma-inducible protein 16	-0.7519609	6.766634	-7.434972	1.745707e-04	0.0173431723	1.427477250	6.290559	6.514843	6.366559	6.986561	7.133814	7.307469
TC01001377.hg.1	4817	NIT1	nitrilase 1	0.5312612	6.647760	6.077412	5.806069e-04	0.0302714482	0.192685120	6.760562	6.950099	7.029510	6.387677	6.298021	6.460690
TC01001422.hg.1	6675	UAP1	UDP-N-acetylglucosamine pyrophosphorylase 1	-0.4832461	6.574137	-5.148068	1.489258e-03	0.0451432083	-0.792327520	6.440064	6.268701	6.288776	6.908747	6.641255	6.897278
TC01001423.hg.1	4921	DDR2	discoidin domain receptor tyrosine kinase 2	-0.8498982	8.758723	-7.295497	1.958727e-04	0.0181157270	1.310608299	8.573590	8.318348	8.109385	9.167569	9.153386	9.230063
TC01001425.hg.1	4921	DDR2	discoidin domain receptor tyrosine kinase 2	-0.8240146	8.148635	-6.678313	3.332765e-04	0.0227676052	0.766880315	7.761718	7.793431	7.654735	8.530624	8.334582	8.816721
TC01001464.hg.1	100313835	MIR1255B2	microRNA 1255b-2	0.7645595	3.698043	7.833916	1.267770e-04	0.0155307630	1.750284059	3.980791	4.022401	4.237778	3.443932	3.189162	3.314197
TC01001484.hg.1	5396	PRRX1	paired related homeobox 1	0.9293720	5.456657	10.533458	1.971960e-05	0.0072212701	3.557543997	5.864014	5.815694	6.084322	4.915966	4.989748	5.070200

$\rightarrow$ easy to sort and filter out significantly differentially expressed genes: adj.P.Val < 0.05 and logFC $> \pm 2$

Volcano plot



p-value < 0.05 & logFC $> \pm 2$
adj.P.Val < 0.05 & logFC $> \pm 2$

- widely used visualisation technique to inspect the result of the statistical analysis
- large difference in expression $\rightarrow$ the more extreme the points will lie on the x-axis
- significant difference $\rightarrow$ the smaller the p-value & the higher the -$\log_{10}$(p-value)

Software - data analysis

- R & Bioconductor
  - *agilp* (Agilent Expression Arrays)
  - *oligo, exonmap* (Gene/Exon ST Arrays)
  - *affy* (3'biased Arrays)
  - *lumi, beadarray* (Illumina Expression Arrays)
  - *limma*
- Affymetrix Transcriptome Analysis Console (TAC)
- GeneSpring GX (Agilent, Affymetrix, Illumina arrays)

Thank you for your attention!