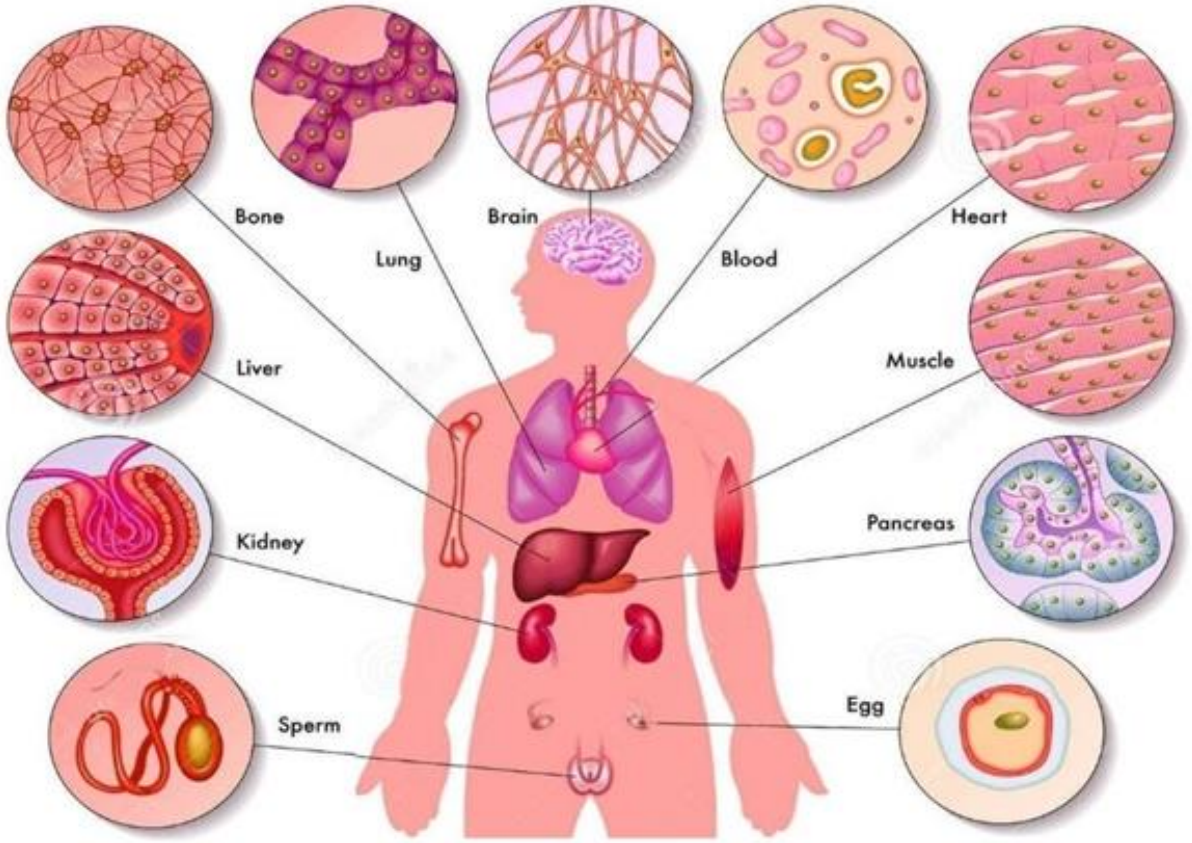
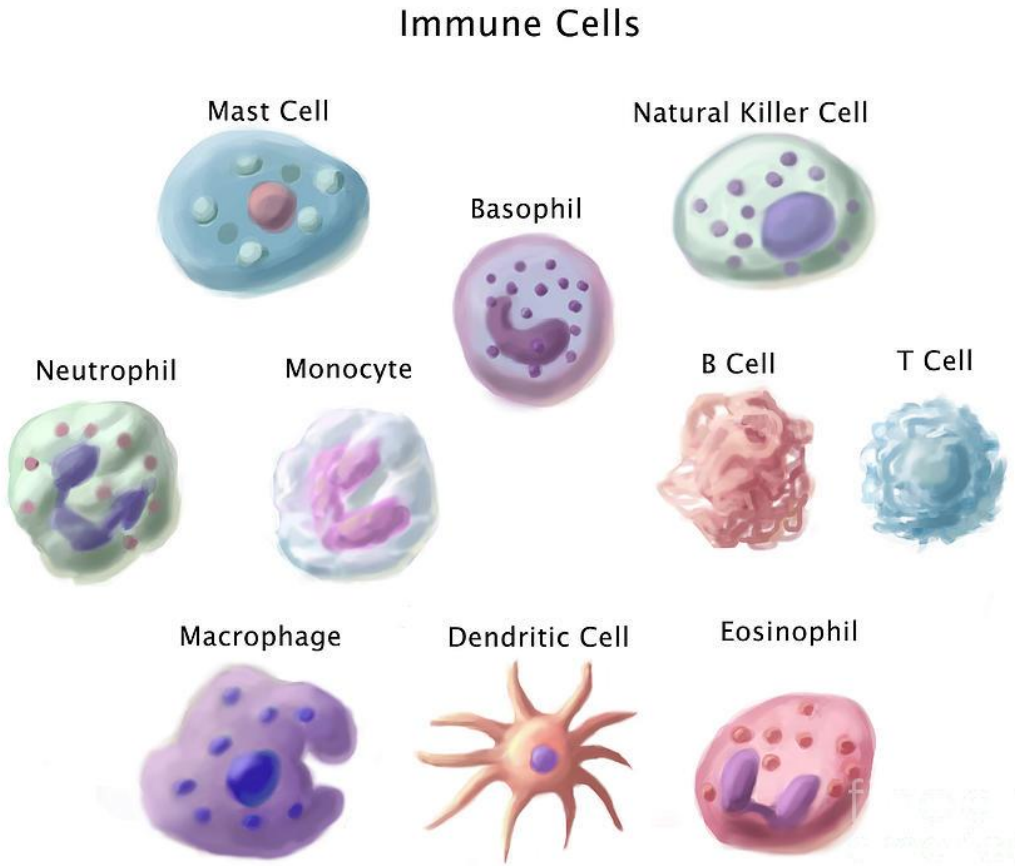
network-based analyses of transcriptomics data: new approaches to define cell signatures from tissue data

Prof Tom Freeman
Systems Immunology Group,
The Roslin Institute,
University of Edinburgh

KAJEKA
CLARITY FROM COMPLEXITY



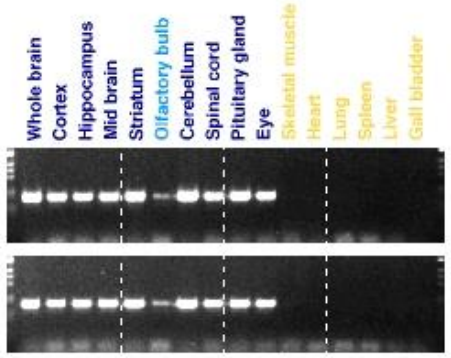
tissues



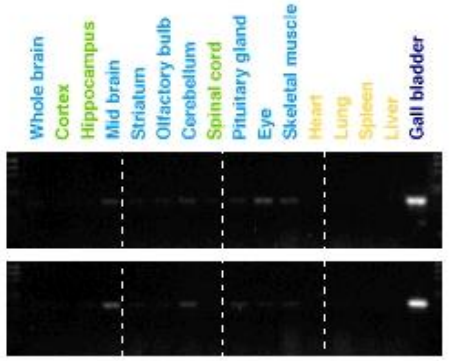
cells

- What does the expression pattern of a gene tell you about its likely function?
- What is the difference between the expression profile of tissues and cells, and how does this reflect their different functions?
- How is their expression profile changed during disease? – disease classification, patient stratification, drug targeting

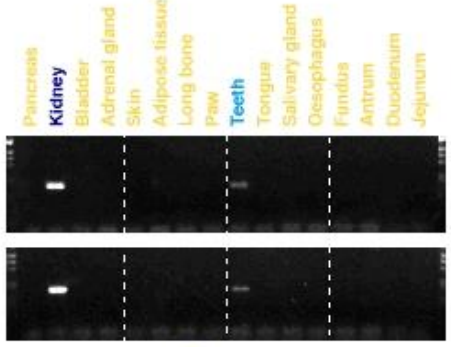
mouse expression atlas

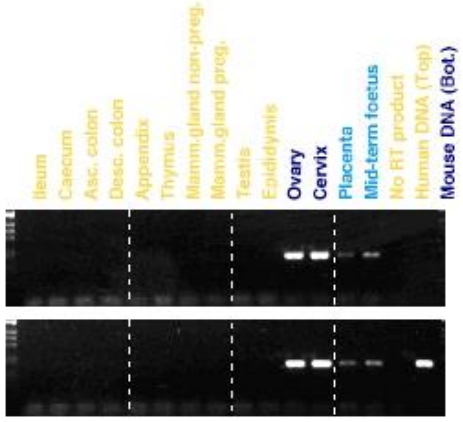


Gene/EST: Calbindin 28K
Symbol: *Calb1*
EMBL Ac. No.: M23663
Primers: 95/42
PCR;
Date: 8/2/96
$MgCl_2$: 3.5 mM
Temp: 55°C
Cycles: x40ex



Gene/EST: Villin
Symbol: *Vil*
EMBL Ac. No.: M98454
Primers: 95/67
PCR;
Date: 11/1/96
$MgCl_2$: 3.5 mM
Temp: 55°C
Cycles: x40ex





Amplific
signal:

Strong
Moderat
Trace
None
+ve Disc

Gene eXpression Database

Gene Expression Data

Reference **J:46439**
Freeman TC, et al., Expression Mapping of Mouse Genes. MGI Direct Data Submission. 1998;

Filter expression by: Anatomical System | Assay Type | Detected? | Theiler Stage | Wild type?

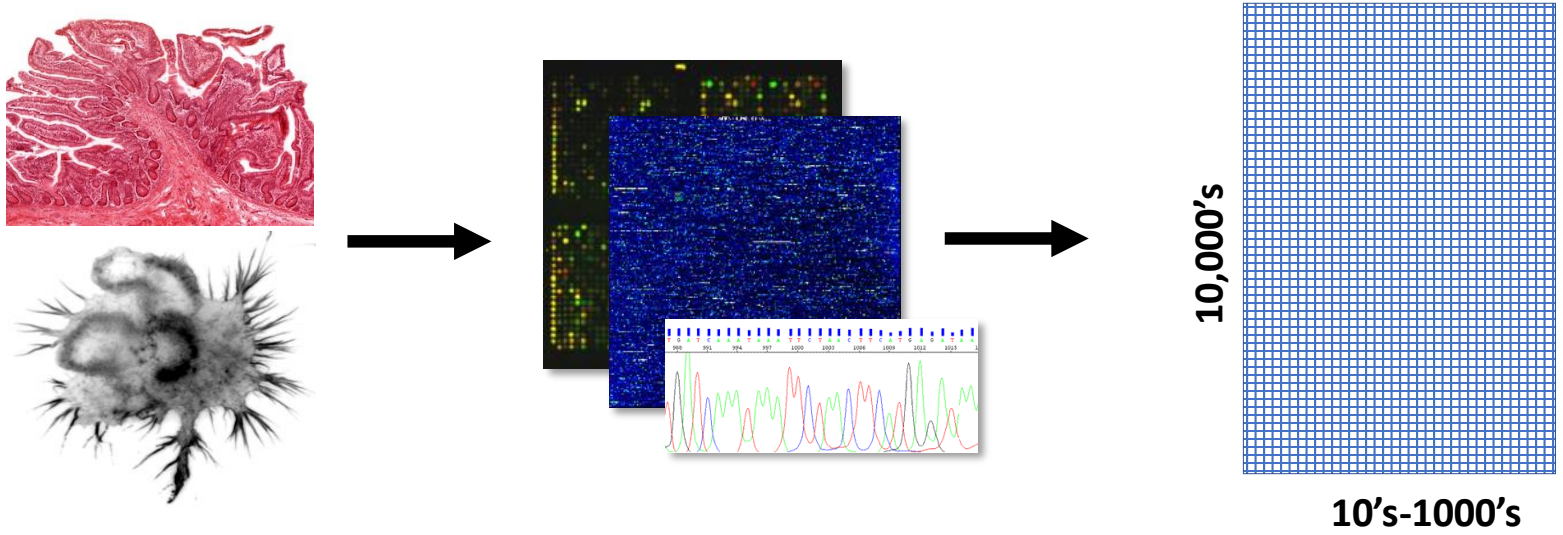
<< first < prev 1 2 3 next > last >> 100

Showing results(s) 1 - 100 of 706

Genes (517) | Assays (706) | Assay results(32476) | Images (706) | Tissue x Stage Matrix | Tissue x Gene Matrix

Gene	Assay Details	Assay Type	Reference
Abcb1a	data (MGI:1204240)	RT-PCR	J:46439 Freeman TC, et al., MGI Direct Data Submission. 1998;
Abcg1	data (MGI:1205113)	RT-PCR	J:46439 Freeman TC, et al., MGI Direct Data Submission. 1998;
Abi1	data (MGI:1204995)	RT-PCR	J:46439 Freeman TC, et al., MGI Direct Data Submission. 1998;
Abi1	data (MGI:1204997)	RT-PCR	J:46439 Freeman TC, et al., MGI Direct Data Submission. 1998;
Ace	data (MGI:1204117)	RT-PCR	J:46439 Freeman TC, et al., MGI Direct Data Submission. 1998;
Ache	data (MGI:1205463)	RT-PCR	J:46439 Freeman TC, et al., MGI Direct Data Submission. 1998;
Acrv1	data (MGI:1205074)	RT-PCR	J:46439 Freeman TC, et al., MGI Direct Data Submission. 1998;
Acta1	data (MGI:1204035)	RT-PCR	J:46439 Freeman TC, et al., MGI Direct Data Submission. 1998;
Acta1	data (MGI:1204037)	RT-PCR	J:46439 Freeman TC, et al., MGI Direct Data Submission. 1998;
Acta1	data (MGI:1204039)	RT-PCR	J:46439 Freeman TC, et al., MGI Direct Data Submission. 1998;

transcriptomics analyses



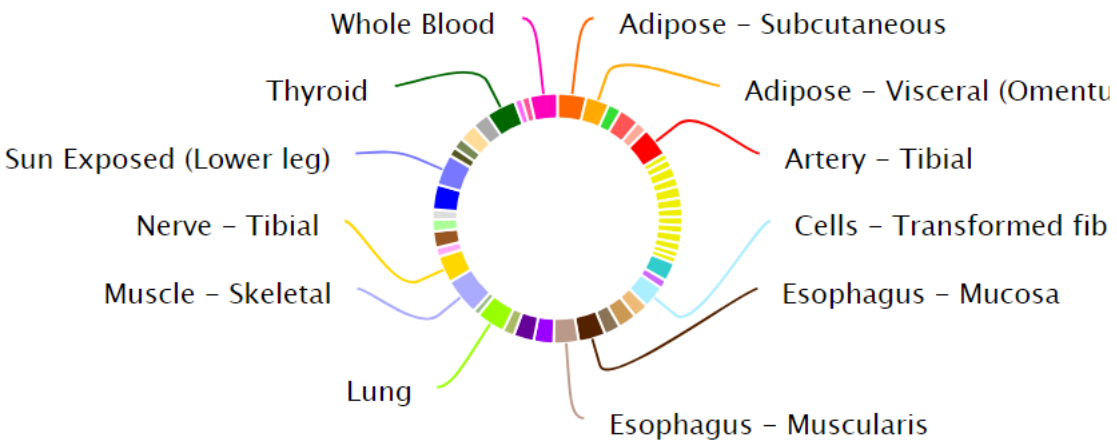
ArrayExpress

Gene Expression Omnibus

resources of gene expression data

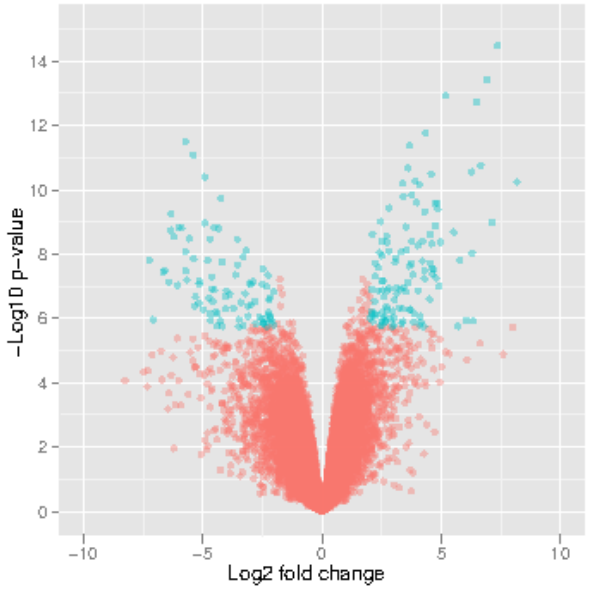
GTEx Portal

Total samples in all eQTL tissues: 10294

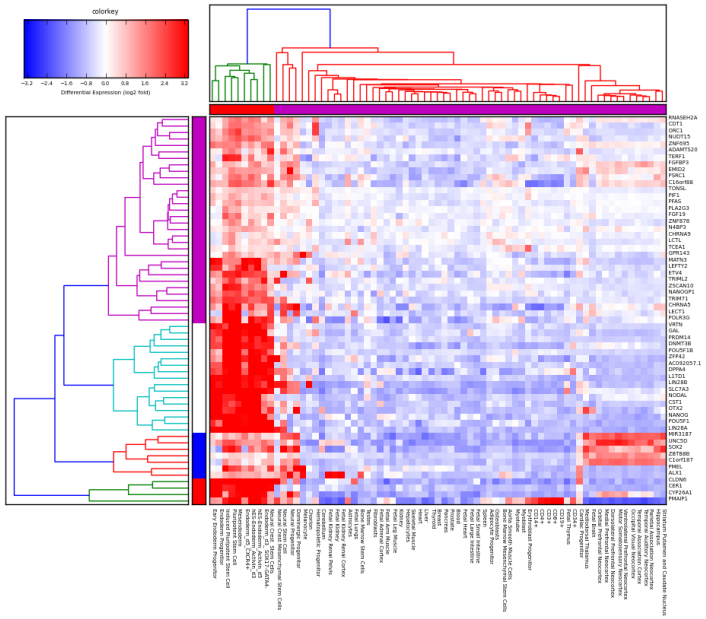


HUMAN CELL ATLAS

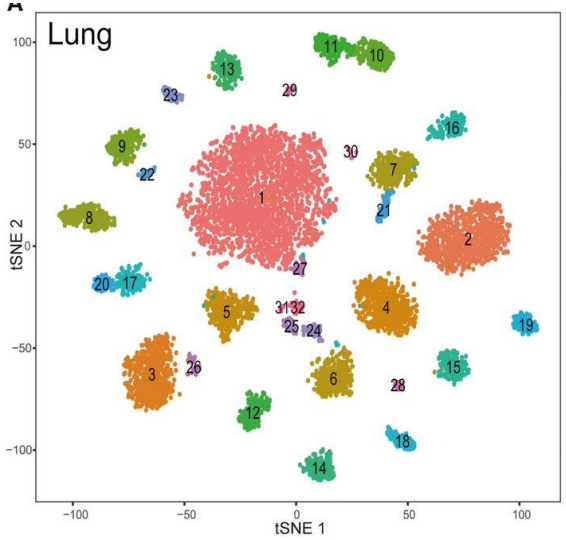
NIH THE CANCER GENOME ATLAS
National Cancer Institute
National Human Genome Research Institute



what is different?



what is similar?



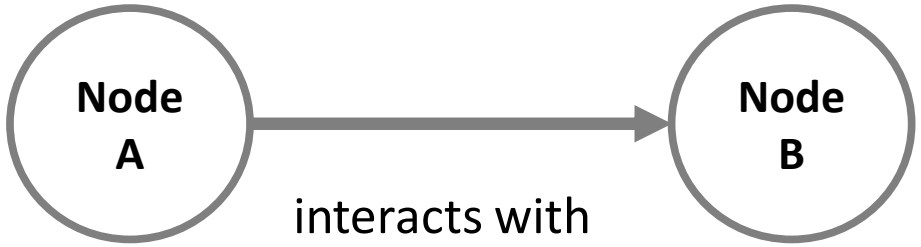
what groups?

what does it mean?

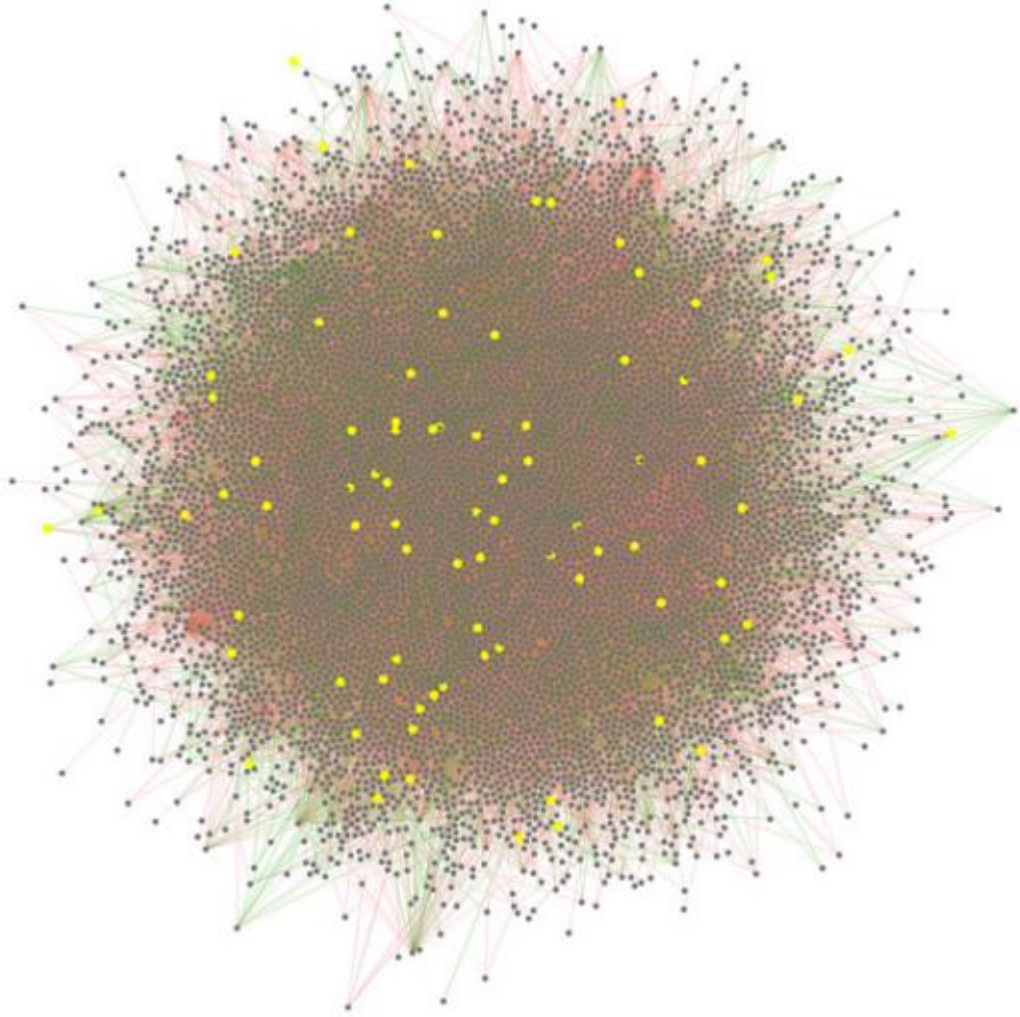
super computer

super visualisations

biological networks



interacts with
binds to
is regulated by
is correlated with



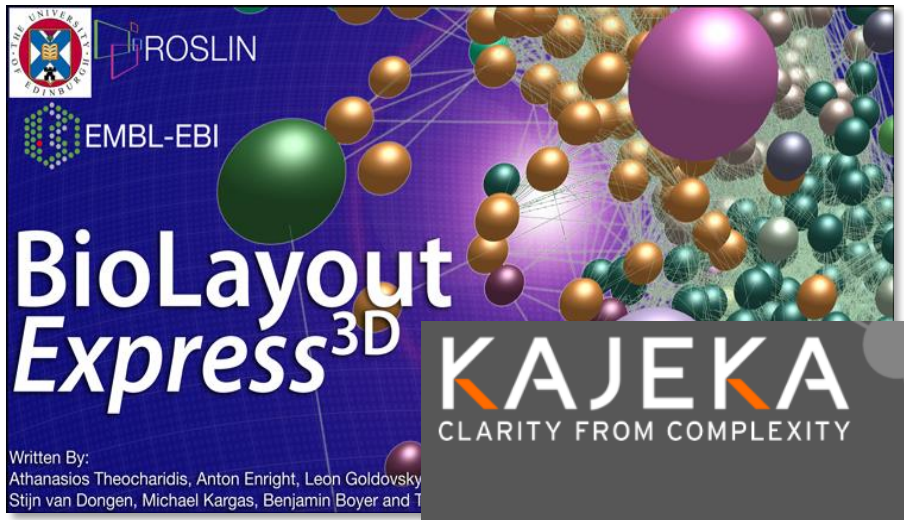
Prioritizing cancer-related genes with aberrant methylation based on a weighted protein-protein interaction network.

Liu H, Su J, Li J, Liu H, Lv J, Li B, Qiao H, Zhang Y - BMC Syst Biol (2011)

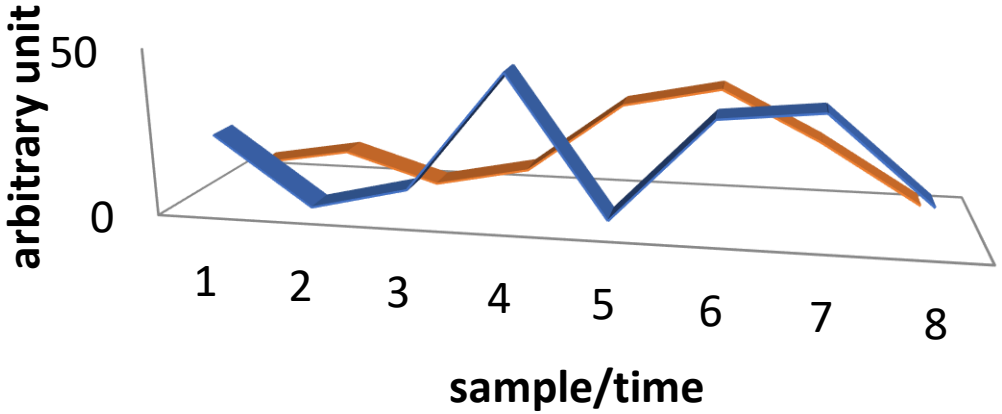
Cytoscape

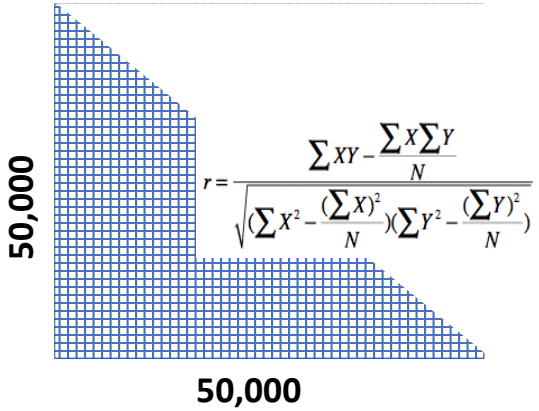
network analysis tools

developed over 15 year period with by scientists in Cambridge and Edinburgh, now by Kajeka Ltd.

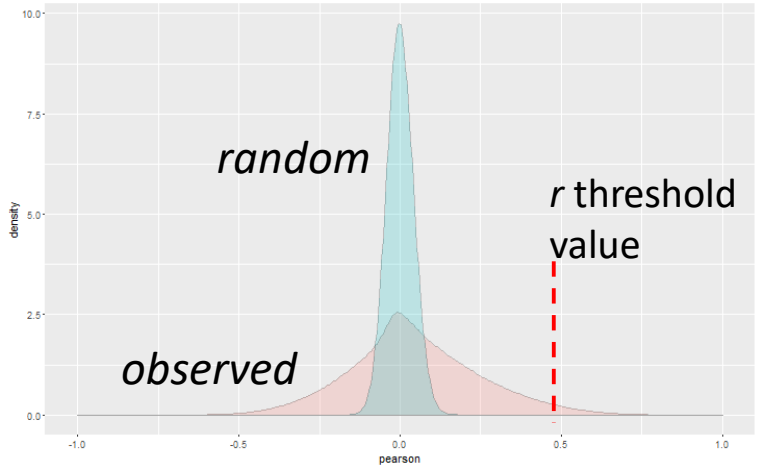


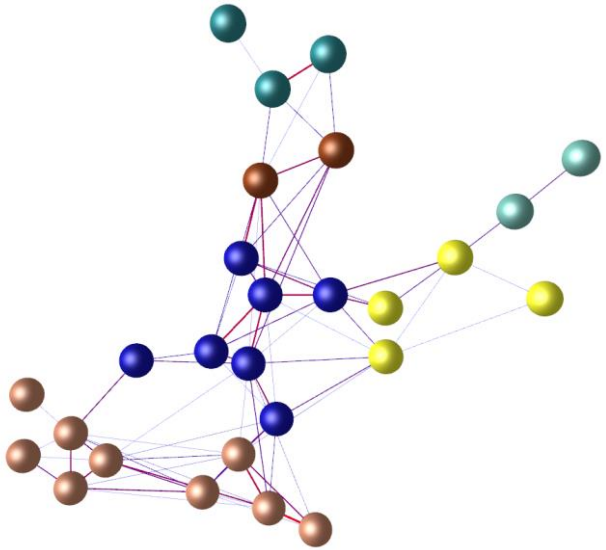
plotting the statistically improbable: principles of correlation networks





1.25 billion calculations





weighted, non-directional graph

Symbol	0-cont	24-con	0 h	1 h	2 h	4 h	6 h	8 h
OR4F17	14.1	13.7	13.4	13.4	12.8	12.9	12.8	13.1
OR4F16	21.8	21.7	20.7	22.2	20.8	22.5	20.9	19.7
LOC64383	58.1	41.1	37.8	52.7	69.8	112.2	90.3	95.8
SAMD11	159.3	140.5	129.4	103.5	123.3	127.0	134.6	139.6
KLHL17	151.2	132.2	119.9	113.0	92.9	105.5	112.3	117.2
PLEKHN1	95.5	84.4	74.2	69.9	58.4	68.6	71.0	71.7
ISG15	114.0	111.7	97.1	78.2	74.0	85.6	75.8	75.1
AGRN	212.4	201.6	209.8	226.9	175.5	162.0	136.0	143.1
MIR200B	81.3	104.6	85.7	84.9	66.6	65.9	74.0	77.6
MIR200A	16.3	15.4	15.3	15.4	14.8	16.4	15.9	18.0
MIR429	39.2	37.5	42.5					
TTLL10	136.0	131.6	123.0					
B3GALT6	197.0	181.7	173.7					

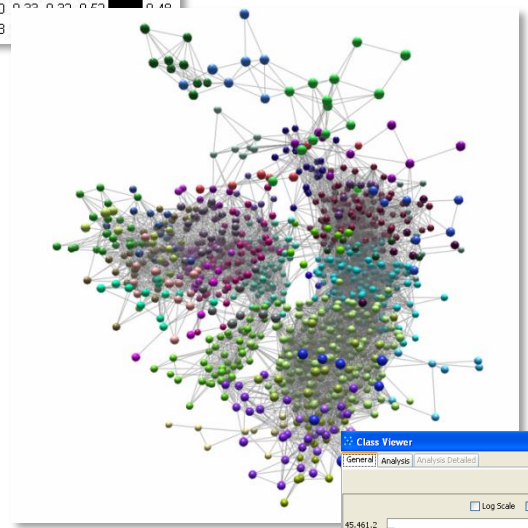
high dimensional data
100's columns, thousands rows

	0.97	0.85	0.57	0.56	0.52	0.52	0.48	0.51	0.56	0.49	0.50	0.50	0.42	0.42	0.09	0.11	0.07
0.97		0.91	0.62	0.58	0.55	0.50	0.47	0.50	0.55	0.48	0.50	0.49	0.41	0.41	0.09	0.11	0.05
0.85	0.91		0.58	0.56	0.52	0.48	0.43	0.48	0.54	0.47	0.48	0.48	0.42	0.38	0.14	0.16	0.07
0.57	0.62	0.58		0.53	0.53	0.42	0.45	0.41	0.41	0.42	0.42	0.39	0.37	0.35	0.17	0.22	0.17
0.56	0.58	0.56	0.53		0.56	0.42	0.42	0.44	0.43	0.43	0.44	0.39	0.38	0.38	0.17	0.25	0.17
0.52	0.55	0.52	0.53	0.56		0.33	0.35	0.32	0.34	0.34	0.34	0.29	0.30	0.28	0.17	0.22	0.15
0.52	0.50	0.48	0.42	0.42	0.33		0.92	0.94	0.89	0.87	0.88	0.92	0.78	0.86	0.26	0.30	0.24
0.48	0.47	0.43	0.45	0.42	0.35	0.92		0.86	0.80	0.80	0.82	0.84	0.73	0.78	0.26	0.30	0.26
0.51	0.50	0.48	0.41	0.44	0.32	0.94	0.86		0.89	0.88	0.89	0.92	0.78	0.84	0.28	0.32	0.25
0.56	0.55	0.54	0.41	0.43	0.34	0.89	0.80	0.89		0.83	0.84	0.86	0.75	0.77	0.26	0.29	0.21
0.49	0.48	0.47	0.42	0.43	0.34	0.87	0.80	0.88	0.83		0.84	0.83	0.75	0.77	0.27	0.32	0.26
0.50	0.50	0.48	0.42	0.44	0.34	0.88	0.82	0.89	0.84	0.84		0.85	0.74	0.78	0.24	0.29	0.23
0.50	0.49	0.48	0.39	0.39	0.29	0.92	0.84	0.92	0.86	0.83	0.85		0.75	0.82	0.27	0.30	0.23
0.42	0.41	0.42	0.37	0.38	0.30	0.78	0.73	0.78	0.75	0.75	0.74	0.75		0.75	0.29	0.33	0.27
0.42	0.41	0.38	0.35	0.38	0.28	0.86	0.78	0.84	0.77	0.77	0.78	0.82	0.75		0.29	0.32	0.29
0.09	0.09	0.14	0.17	0.17	0.17	0.26	0.26	0.28	0.26	0.27	0.24	0.27	0.29	0.29		0.52	0.49
0.11	0.11	0.16	0.22	0.25	0.22	0.30	0.30	0.32	0.29	0.32	0.29	0.30					
0.07	0.05	0.07	0.17	0.17	0.15	0.24	0.26	0.25	0.21	0.26	0.23	0.23					

calculate
correlation matrix

Graphia

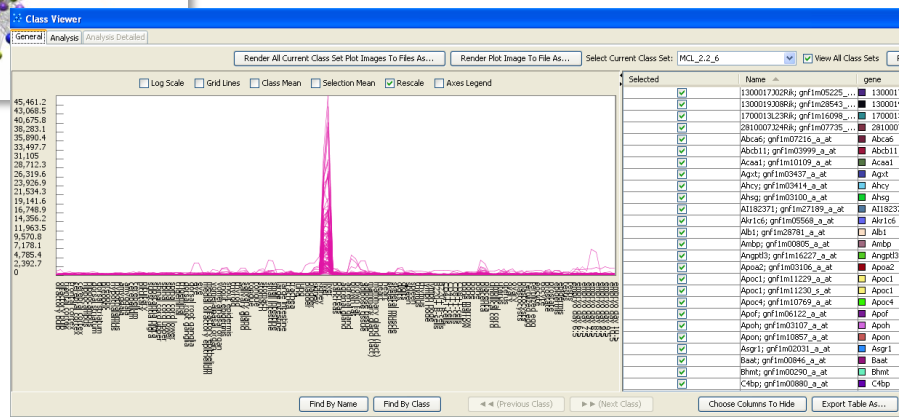
data analysis pipeline

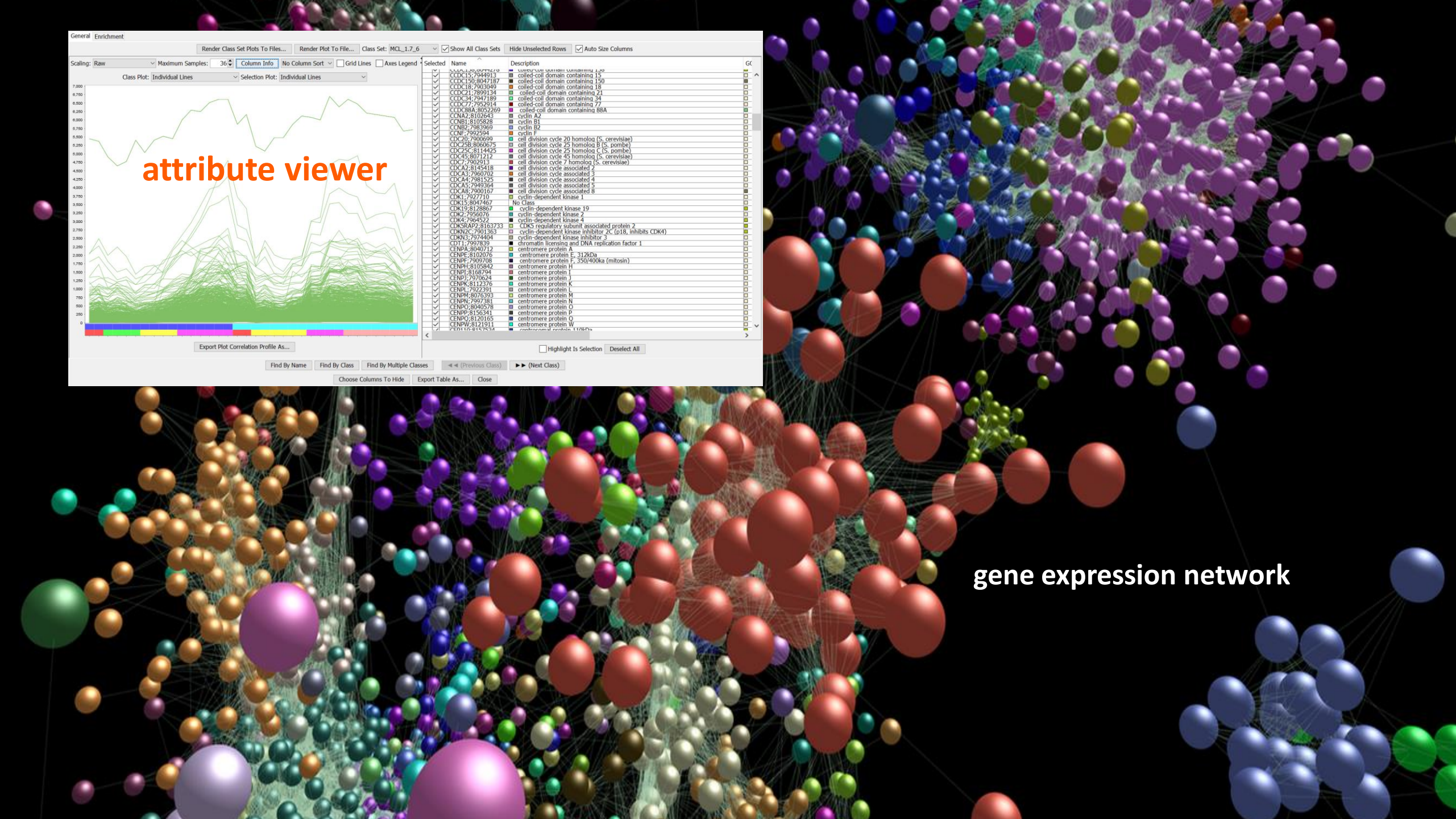


layout, dynamic visualisation and clustering of graphs (10's thousands nodes, millions of edges) in 2D or 3D

minutes

data integration and exploration




attribute viewer
gene expression network

Human Conjunctival Transcriptome Analysis Reveals the Prominence of Innate Defense in *Chlamydia trachomatis* Infection

Angels Natividad,[1]‡ Tom C. Freeman,[2] David Jeffries,[1,3] Matthew J. Burton,[1] David C. W. Mabey,[1] Robin L. Bailey,[1] and Martin J. Holland[1,3]*

Department of Clinical Research, London School of Hygiene and Tropical Medicine, University of London, London WC1E 7HT, United Kingdom[1]; The Roslin Institute, R(D)SVS, University of Edinburgh, Roslin Biocentre, Midlothian EH25 9PS, United Kingdom[2]; and Medical Research Council Laboratories, Viral Diseases Programme, Fajara, Banjul, the Gambia[3]

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Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

Functional clustering and lineage markers: Insights into cellular differentiation and gene function from large-scale microarray studies of purified primary cell populations

David A. Hume *, Kim M. Summers, Sobia Raza, J. Kenneth Baillie, Thomas C. Freeman

The Roslin Institute, Roslin Biocentre, Roslin EH25 9PS, Midlothian, UK
Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Roslin Biocentre, Roslin EH25 9PS, Midlothian, UK

Derivation of marker gene signatures from human skin and their use in the interpretation of the transcriptional changes associated with dermatological disorders

Barbara B Shih[1], Ajit J Nirmal[1], Denis J Headon[1], Arne N Akbar[2], Neil A Mabbott[1]* and Tom C Freeman[1]*

Journal of Pathology
J Pathol 2017; **241**: 600–613

Freeman *et al. BMC Biology* 2012, **10**:90
http://www.biomedcentral.com/1741-7007/10/90

BMC Biology incorporating Journal of Biology

RESEARCH ARTICLE Open Access

A gene expression atlas of the domestic pig

Tom C Freeman[1*], Alasdair Ivens[2,6], J Kenneth Baillie[1], Dario Beraldi[1,7], Mark W Barnett[1], David Dorward[1], Alison Downing[1], Lynsey Fairbairn[1], Ronan Kapetanovic[1], Sobia Raza[1], Andru Tomoiu[1], Ramiro Alberio[3], Chunlei Wu[4], Andrew I Su[4], Kim M Summers[1], Christopher K Tuggle[5], Alan L Archibald[1*] and David A Hume[1*]

RESEARCH ARTICLE

PLOS | GENETICS

A high resolution atlas of gene expression in the domestic sheep (*Ovis aries*)

Emily L. Clark[1☯*], Stephen J. Bush[1☯], Mary E. B. McCulloch[1], Iseabail L. Farquhar[1¤], Rachel Young[1], Lucas Lefevre[1], Clare Pridans[1], Hiu G. Tsang[1], Chunlei Wu[2], Cyrus Afrasiabi[2], Mick Watson[1], C. Bruce Whitelaw[1], Tom C. Freeman[1], Kim M. Summers[1,3], Alan L. Archibald[1*], David A. Hume[1,3*]

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.de/imbio

Meta-analysis of lineage-specific gene expression signatures in mouse leukocyte populations

Neil A. Mabbott*, J. Kenneth Baillie, David A. Hume*,[1], Tom C. Freeman [1]

The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Roslin Biocentre, Roslin EH25 9PS, Midlothian, UK

Mabbott *et al. BMC Genomics* 2013, **14**:632
http://www.biomedcentral.com/1471-2164/14/632

BMC Genomics

RESEARCH ARTICLE Open Access

An expression atlas of human primary cells: inference of gene function from coexpression networks

Neil A Mabbott*†, J Kenneth Baillie, Helen Brown, Tom C Freeman*† and David A Hume*†

Doig *et al. BMC Genomics* 2013, **14**:469
http://www.biomedcentral.com/1471-2164/14/469

BMC Genomics

RESEARCH ARTICLE Open Access

Coexpression analysis of large cancer datasets provides insight into the cellular phenotypes of the tumour microenvironment

Tamasin N Doig[1,3], David A Hume[3], Thanasis Theocharidis[3], John R Goodlad[2], Christopher D Gregory[1] and Tom C Freeman[3*]

ARTICLE

doi:10.1038/nature13182

A promoter-level mammalian expression atlas

The FANTOM Consortium and the RIKEN PMI and CLST (DGT)*

nature

over 150 publications using our software

advantages of network approaches

- data agnostic
- fast
- scalable – big data sets
- visualisation of 1,000,000s of data points
- powerful pattern finding – ideal for complex data
- visually engaging
- hypothesis free - explorative

Mabbott *et al. BMC Genomics* 2013, **14**:632
http://www.biomedcentral.com/1471-2164/14/632

BMC Genomics

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An expression atlas of human primary cells: inference of gene function from coexpression networks

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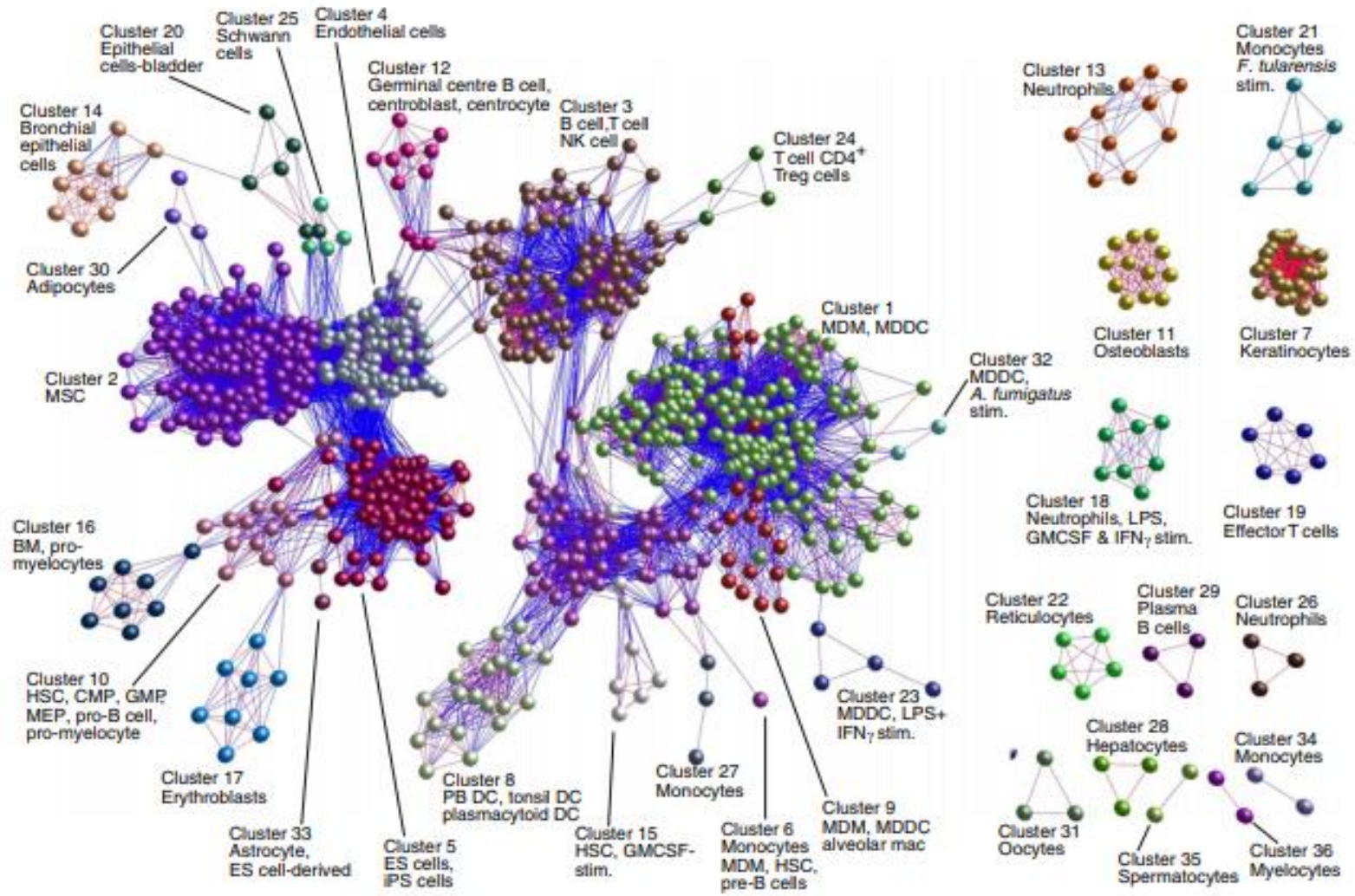


Figure 2 Clustering of samples based upon their gene global expression profiles. A Pearson correlation matrix was prepared comparing

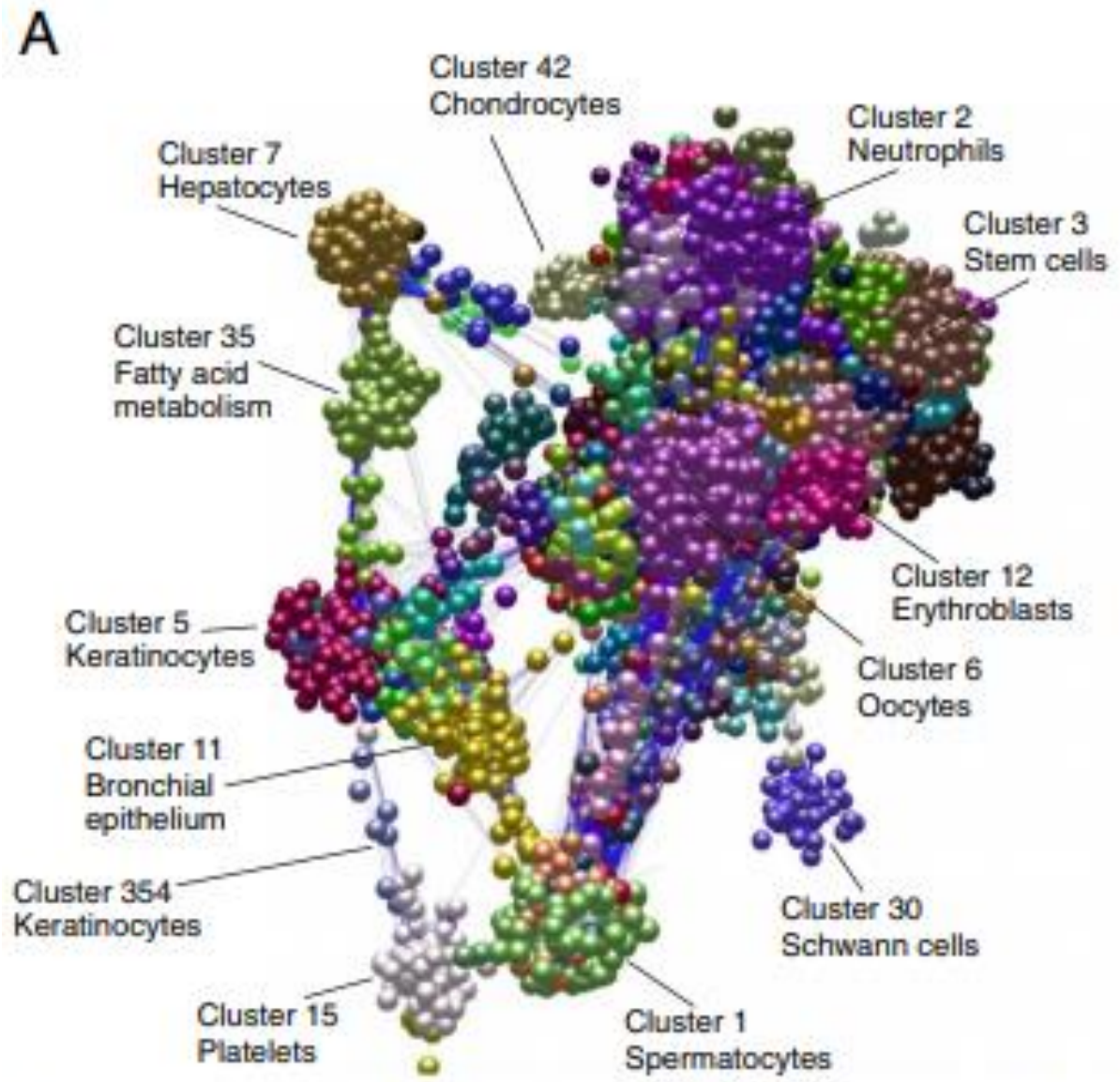
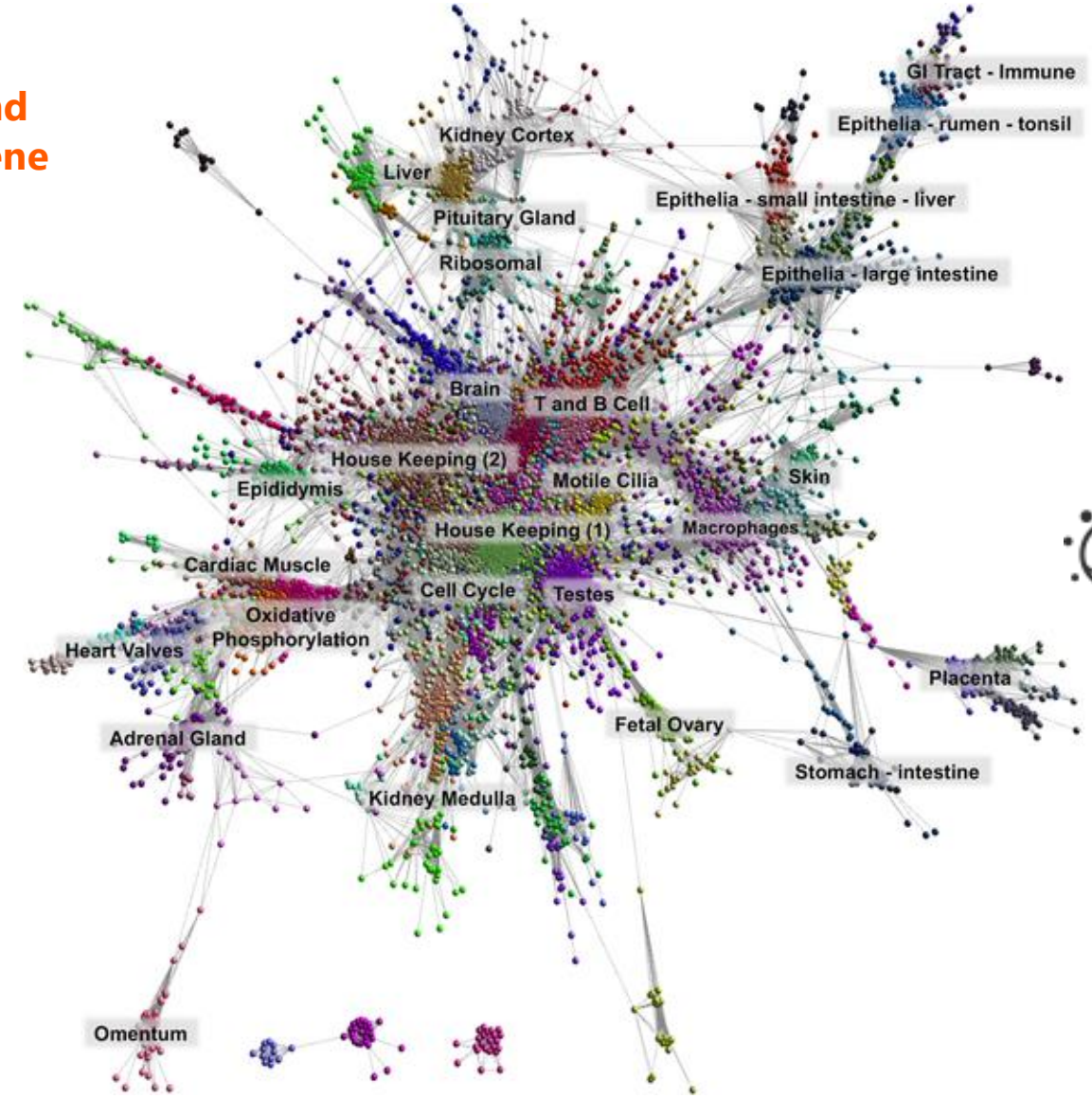


Figure 3 Network analysis of human primary cell transcriptomics data. (A

Network visualisation and clustering of the sheep gene expression atlas



PLOS | GENETICS

Clark EL et al. (2017) A high resolution atlas of gene expression in the domestic sheep (Ovis aries). *PLOS Genetics* **13**(9): e1006997. https://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1006997

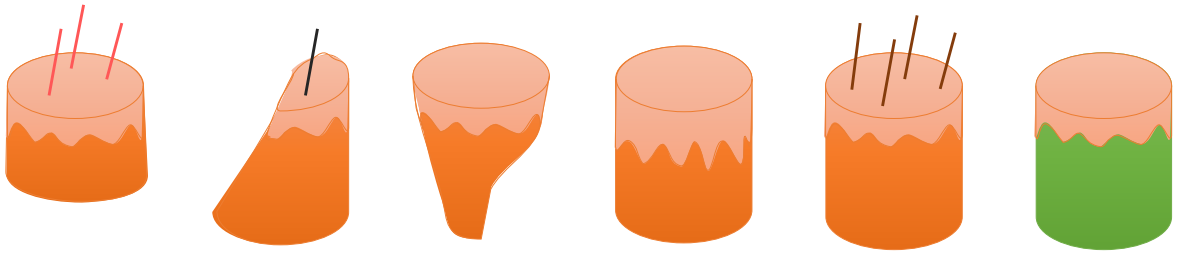
data deconvolution

computational approaches that can identify specific signals within heterogeneous data signals

in our case identify transcriptional **signatures** of cells and pathways from mixed cell populations, i.e. tissue samples

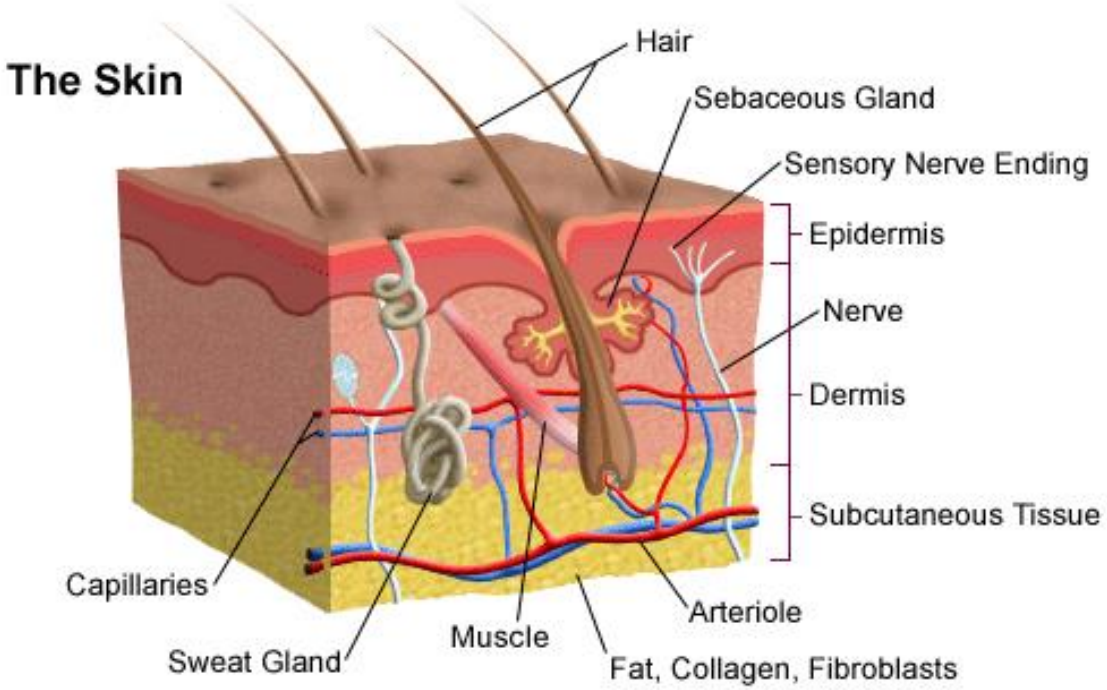
where a **signature** is a module (cluster) of genes that are robustly coexpressed because they are specifically transcribed by a given cell type or coregulated as they encode proteins that are part of the same pathway or process

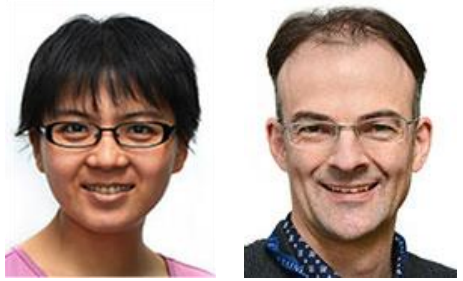
variation in clinical samples



every sample is a little different

an expression atlas of human skin





MRC Medical Research Council

Journal of Pathology
J Pathol 2017; **241**: 600–613
Published online 24 February 2017 in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/path.4864

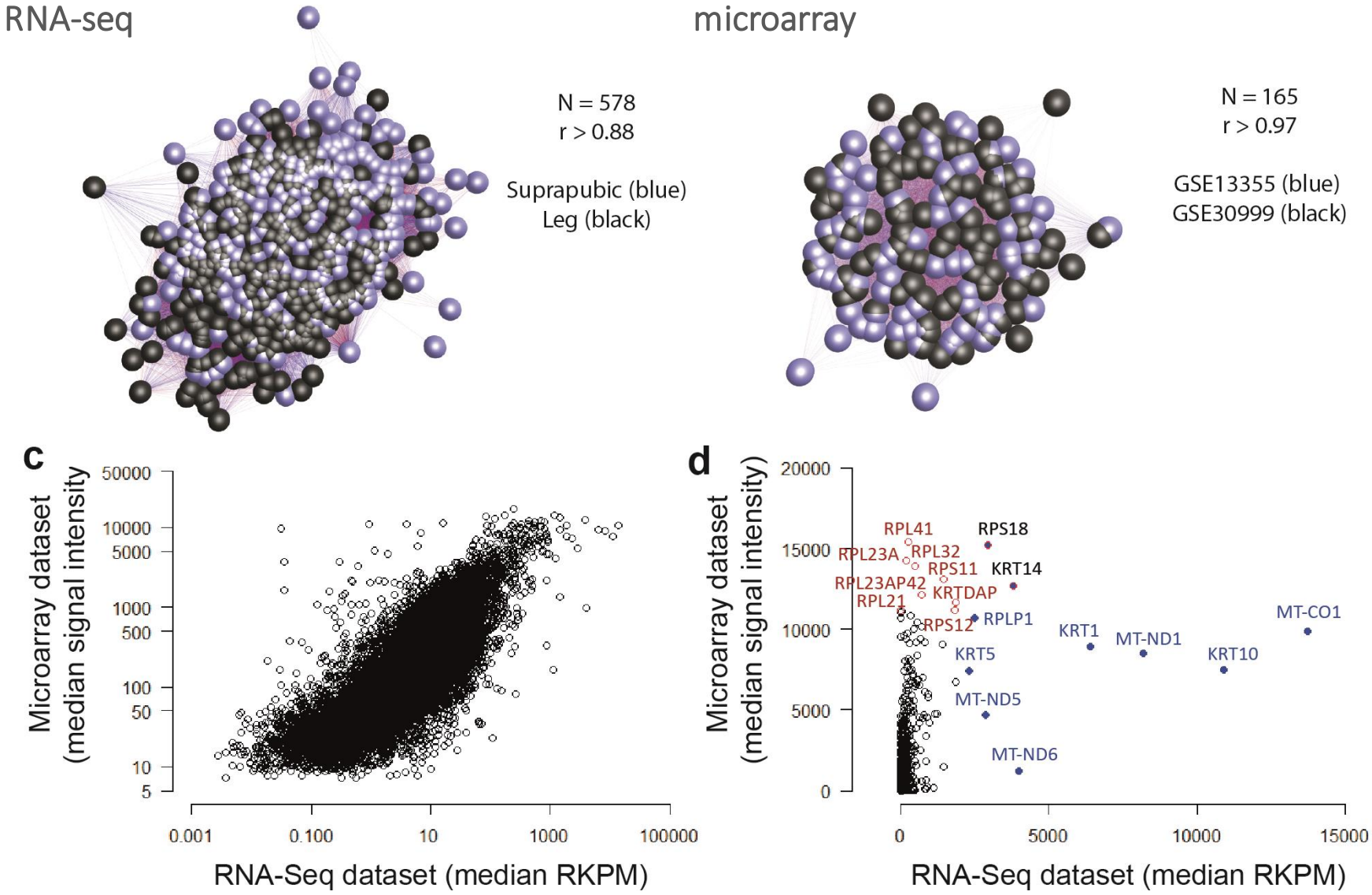
ORIGINAL PAPER

Derivation of marker gene signatures from human skin and their use in the interpretation of the transcriptional changes associated with dermatological disorders

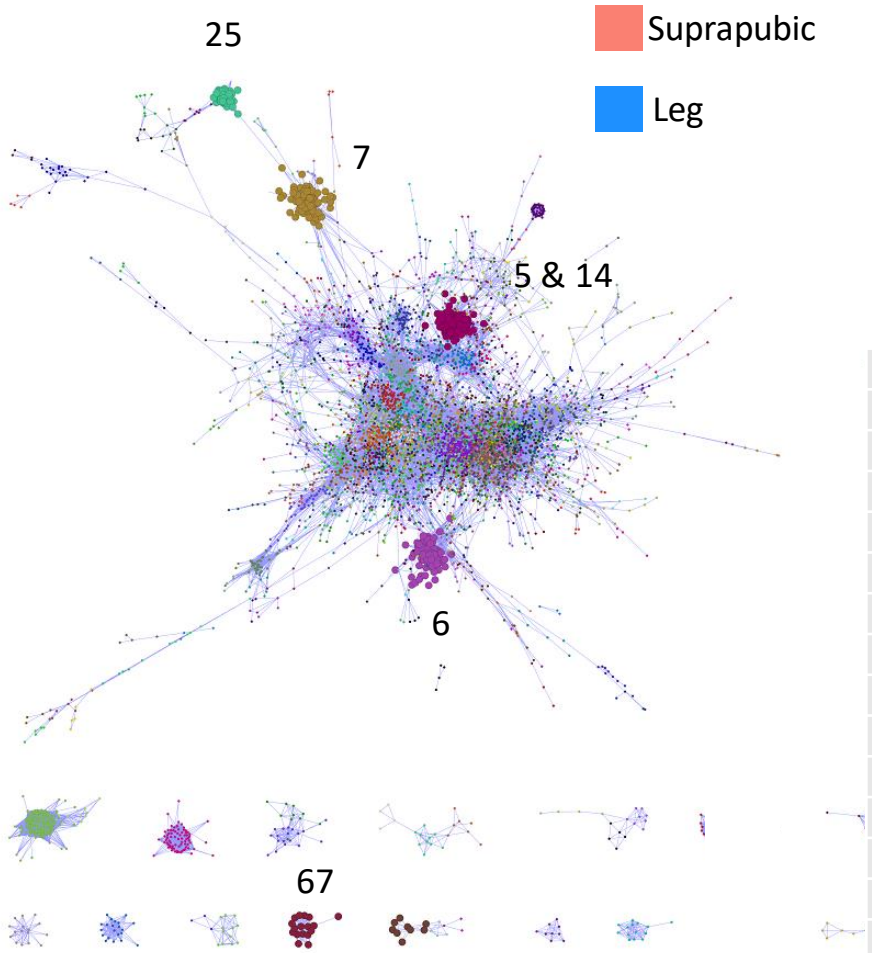
Barbara B Shih[1], Ajit J Nirmal[1], Denis J Headon[1], Arne N Akbar[2], Neil A Mabbott[1]* and Tom C Freeman[1]*

[1] *The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, Edinburgh, EH25 9RG, UK*
[2] *Division of Infection and Immunity, University College London, 90 Gower Street, London, WC1E 6BT, UK*

RNA-seq and microarray sample clustering and data comparison

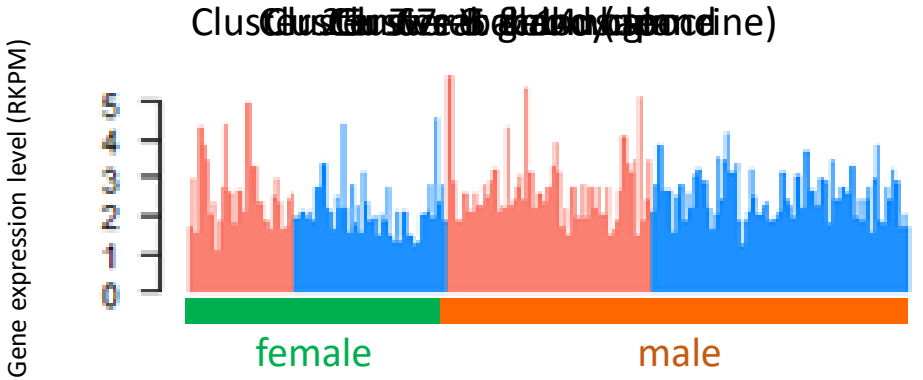


GTEx (RNA-seq) dataset



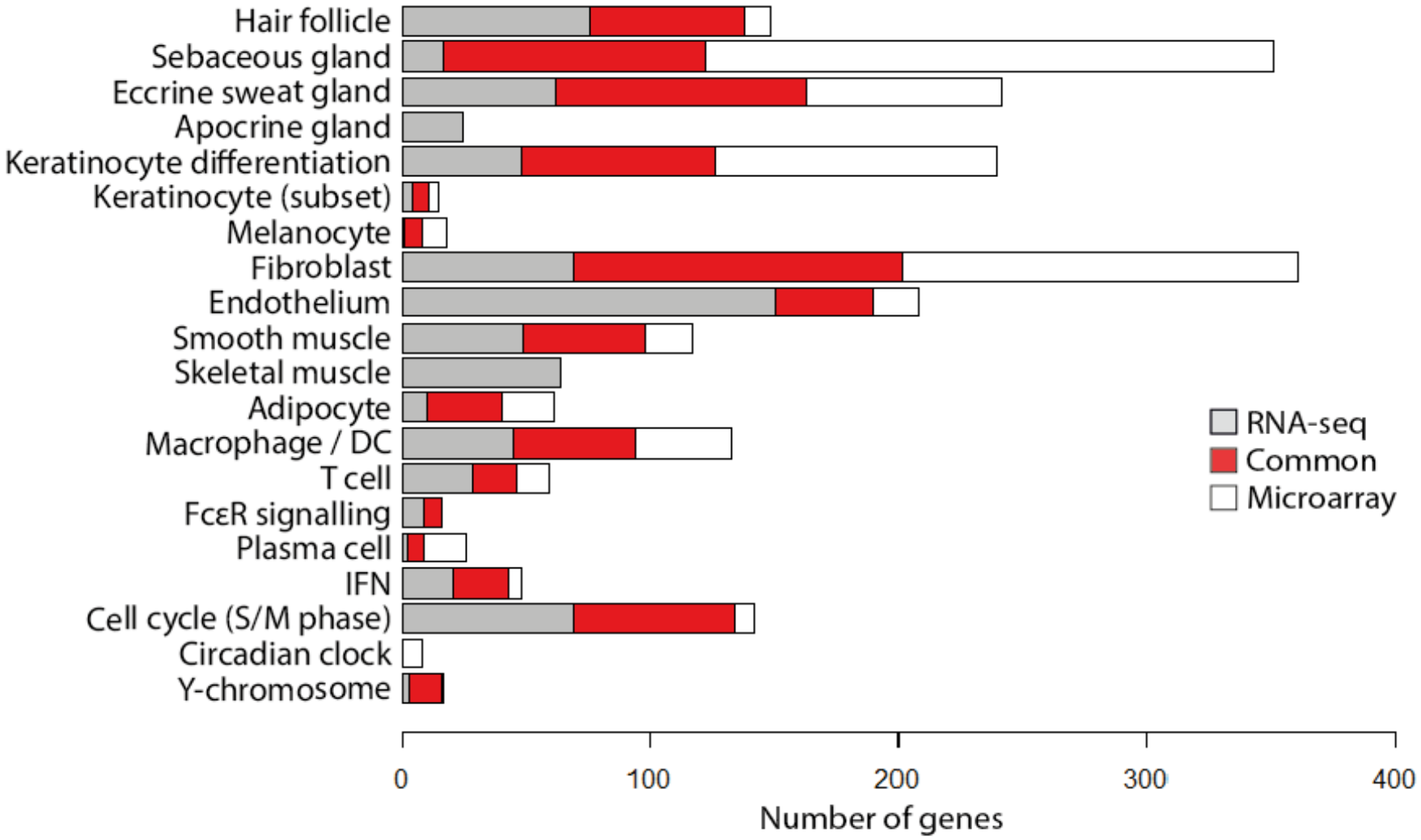
Pearson threshold: 0.73
MCLi: 2.2

Nodes: 8,317
Edges: 113,618

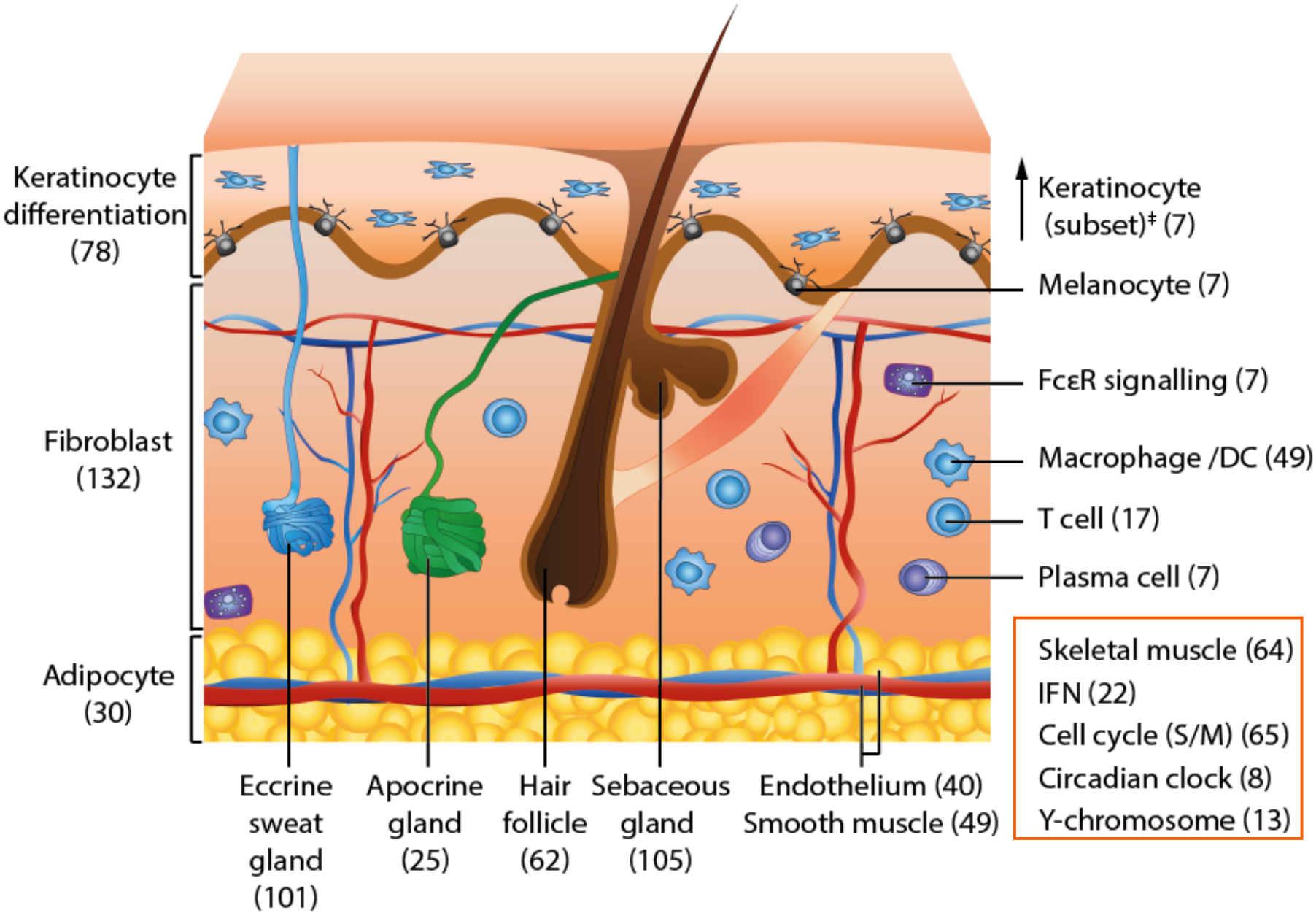


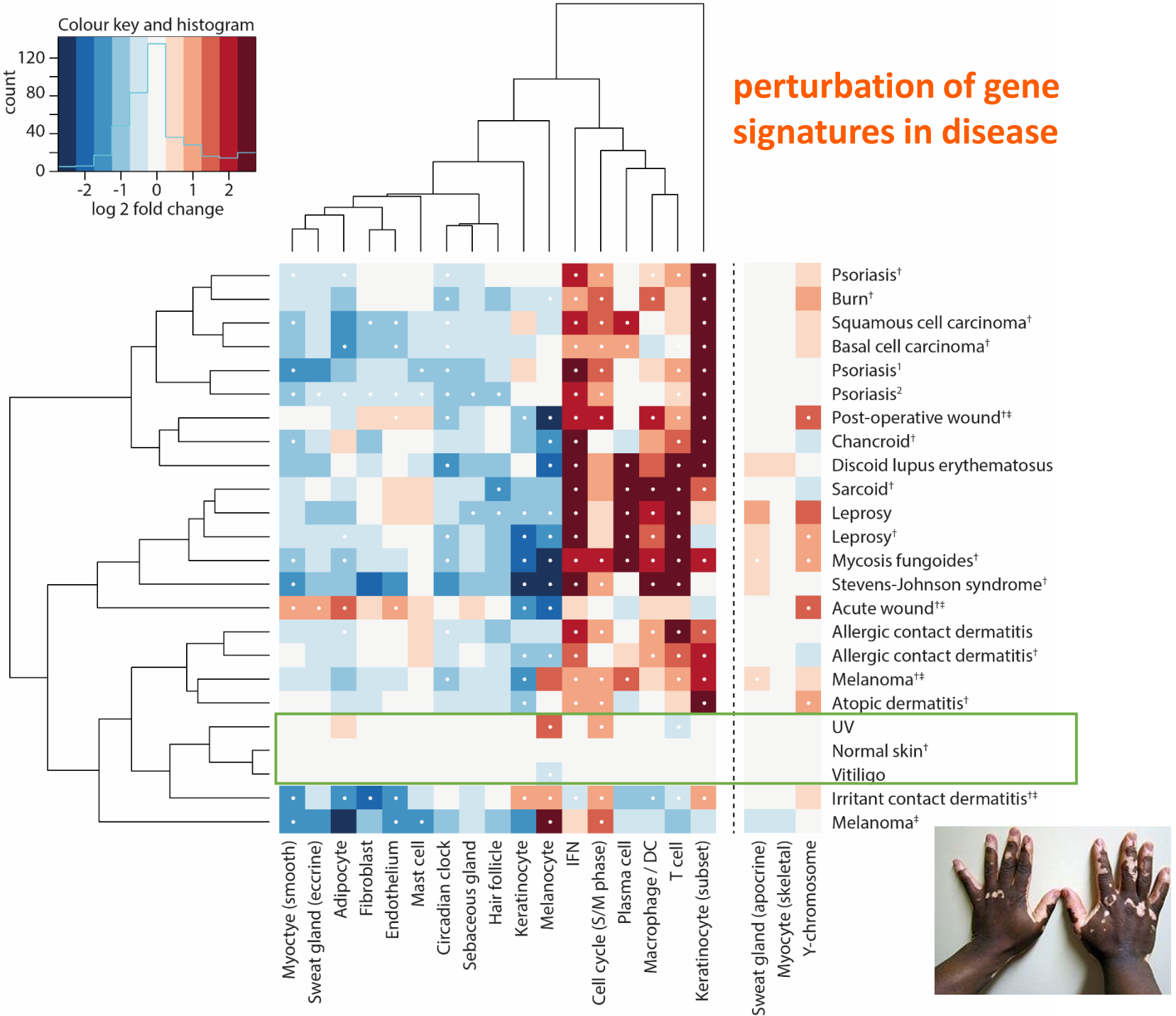
ANLN	CENPW	MCM2
ASPM	CEP55	MELK
AURKA	CKS2	MKI67
AURKB	DEPDC1	NCAPG
BUB1	DLGAP5	NDC80
BUB1B	DTL	NEK2
CASC5	E2F7	NUF2
CCNA2	ECT2	NUSAP1
CCNB1	GINS1	OIP5
CCNB2	GINS2	PBK
CDC20	HELLS	PCNA
CDCA3	HMMR	PRC1
CDCA5	KIAA0101	PTTG1
CDCA8	KIF11	RACGAP1
CDK1	KIF14	RAD51AP1
CDKN3	KIF18B	RRM2
CENPA	KIF20A	SPAG5
CENPE	KIF23	TK1
CENPF	KIF2C	TOP2A
CENPK	KIF4A	TPX2

overlap between datasets



SkinSig


Keratinocyte differentiation (78)
Fibroblast (132)
Adipocyte (30)
Keratinocyte (subset)‡ (7)
Melanocyte (7)
FcεR signalling (7)
Macrophage /DC (49)
T cell (17)
Plasma cell (7)
Skeletal muscle (64)
IFN (22)
Cell cycle (S/M) (65)
Circadian clock (8)
Y-chromosome (13)
Eccrine sweat gland (101)
Apocrine gland (25)
Hair follicle (62)
Sebaceous gland (105)
Endothelium (40)
Smooth muscle (49)

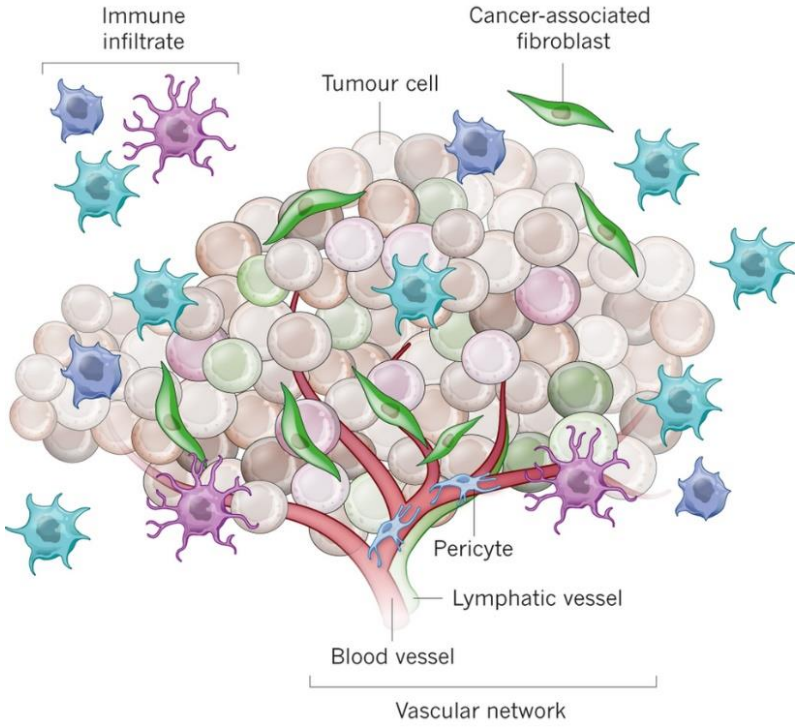

Colour key and histogram
count
120
80
40
0
-2
-1
0
1
2
log 2 fold change
perturbation of gene signatures in disease
Psoriasis†
Burn†
Squamous cell carcinoma†
Basal cell carcinoma†
Psoriasis¹
Psoriasis²
Post-operative wound†‡
Chancroid†
Discoid lupus erythematosus
Sarcoid†
Leprosy
Leprosy†
Mycosis fungoides†
Stevens-Johnson syndrome†
Acute wound†‡
Allergic contact dermatitis
Allergic contact dermatitis†
Melanoma†‡
Atopic dermatitis†
UV
Normal skin†
Vitiligo
Irritant contact dermatitis†‡
Melanoma‡
Myoctye (smooth)
Sweat gland (eccrine)
Adipocyte
Fibroblast
Endothelium
Mast cell
Circadian clock
Sebaceous gland
Hair follicle
Keratinocyte
Melanocyte
IFN
Cell cycle (S/M phase)
Plasma cell
Macrophage / DC
T cell
Keratinocyte (subset)
Sweat gland (apocrine)
Myocyte (skeletal)
Y-chromosome

immune microenvironment and cancer

growing interest in targeting the immune system in the treatment of cancer – the so called field of ***immuno-oncology***

numerous immune targets/strategies being explored as therapeutic targets, e.g. PD1, CTLA4, TLRs, CCRs etc

how do we analyse the immune microenvironment of tumours and relationship to outcome or treatment?



Melissa et.al. *Nature* 501, 346–354

Research Article

Cancer Immunology Research

Immune Cell Gene Signatures for Profiling the Microenvironment of Solid Tumors

Ajit J. Nirmal[1], Tim Regan[1], Barbara B. Shih[1], David A. Hume[1,3], Andrew H. Sims[2], and Tom C. Freeman[1]

Aim

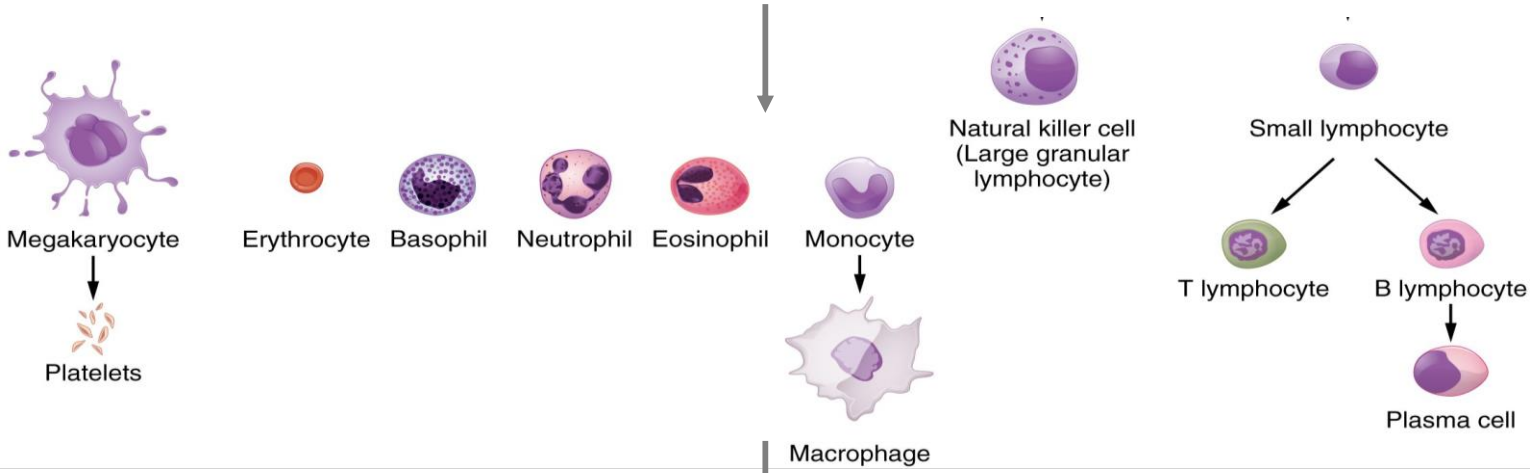
to derive robust immune gene signatures, that can be used to identify the presence and abundance of immune cells in a tissue (tumour) micro-environment from transcriptomics data

Ajit Johnson Nirmal

Cancer Immunol Res. 2018 Nov;6(11):1388-1400. doi: 10.1158/2326-6066.CIR-18-0342

derivation of markers

sort cells (usually from blood), sometimes cultured,
then analyse expression

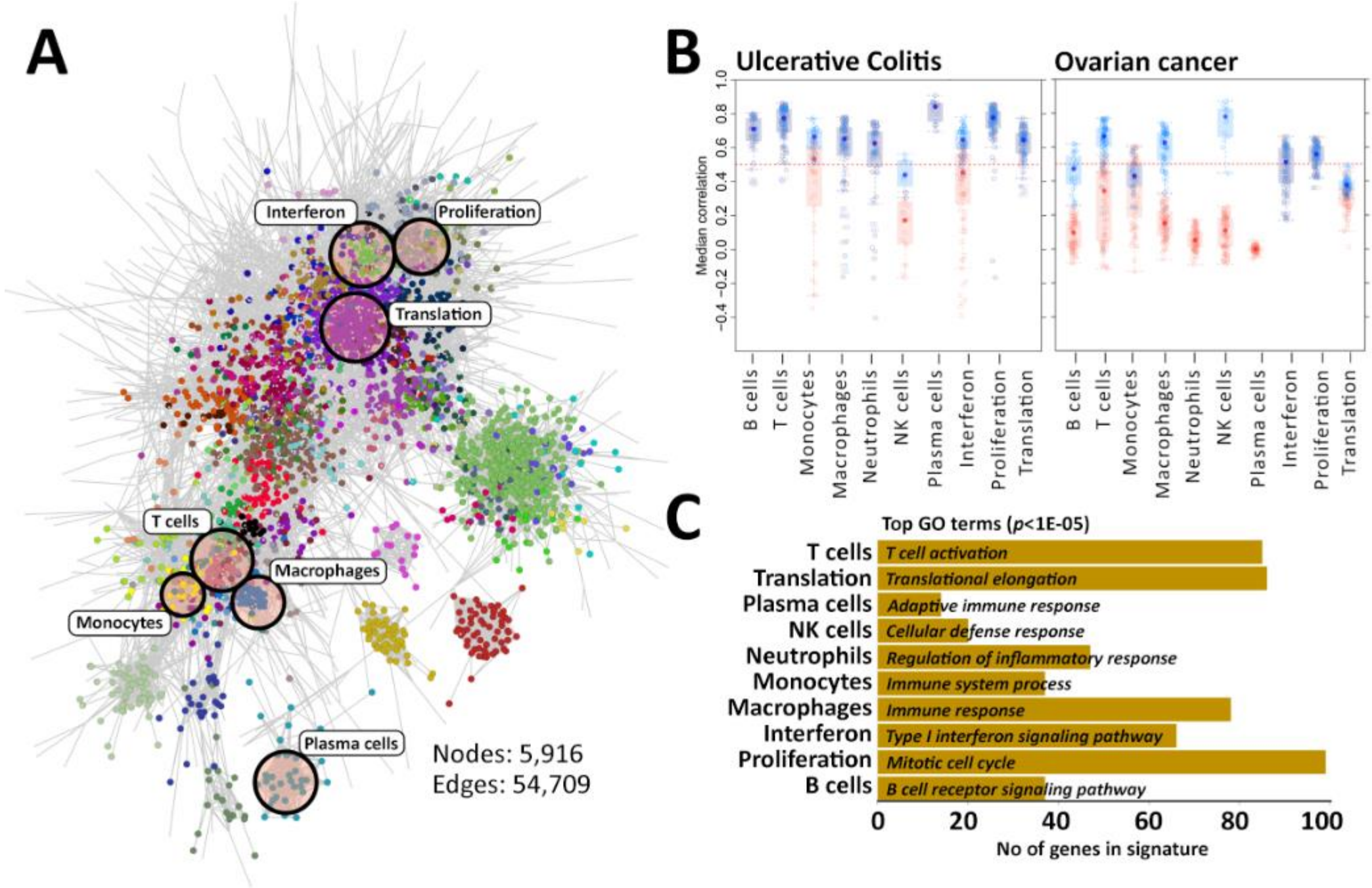


compare and derive cell type ‘specific’ markers

plugin into algorithm

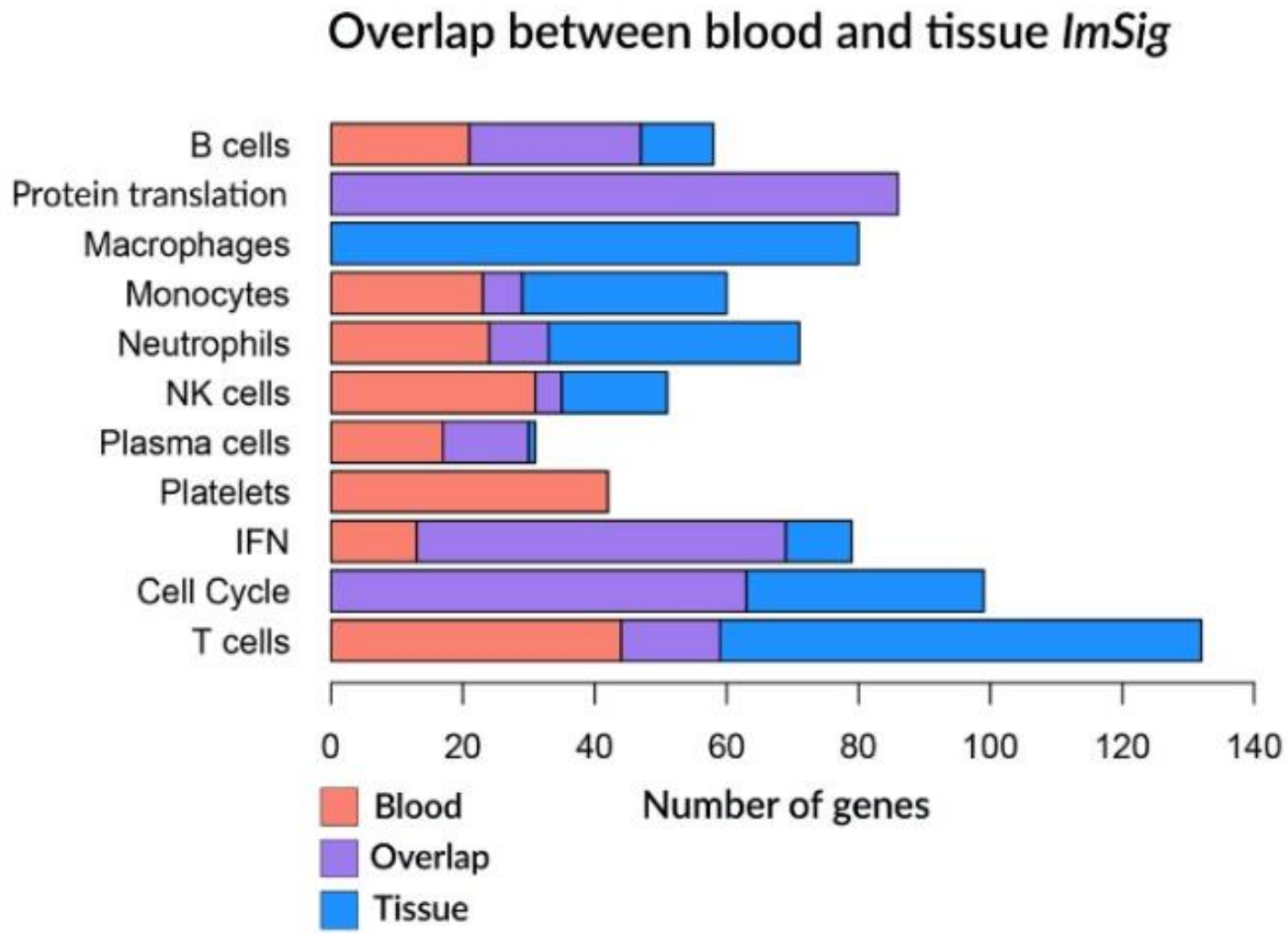
immune cell types and subtypes defined by published gene signatures

Study	Author, year (PMID)	Cell gene signatures (defined subtypes)	Unique genes
1	Becht *et al.* (27765066)	T (3), B, NK, Mono, DC's, Neutrophils, Other (2)	321
2	Angelova *et al.* (25853550)	T (14), B (3), DC's (4), NK (4), Eosinophil, Macrophages, Mast cells, Mono, Neutrophils, MDSC	812
3	Abbas *et al.* 2005 (19568420)	T (2), B (2), Plasma cells, NK (2),Monocytes (2), DC's (2), Neutrophils	100
4	Watkins *et al.* (19228925)	T (2), Mono, B, NK, Neutrophils, Erythroblast, Megakaryocyte	1,847
5	Bindea *et al.* (24138885)	B (34), T (11), NK (3), DC's (3), Eosinophils, Macrophages, Mast cells, Neutrophils, Others (4)	582
6	Abbas *et al.* 2009 (15789058)	B, DC's, Monocyte, Neutrophil, NK, T, Other(3)	1,757
7	Newman *et al.* (25822800)	B (2), Plasma cells, T (7), NK (2), Monocytes, Macrophages (3), DC's (2), Mast cells (2), Eosinophils, Neutrophils	547

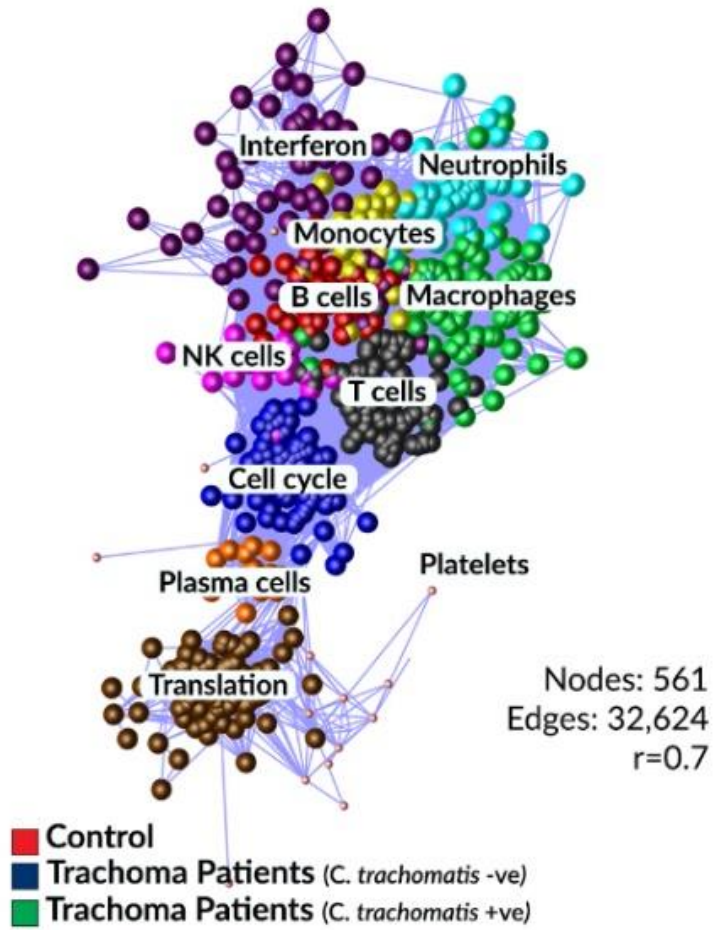

A
Interferon
Proliferation
Translation
T cells
Macrophages
Monocytes
Plasma cells
Nodes: 5,916
Edges: 54,709
B
Ulcerative Colitis
Ovarian cancer
Median correlation
1.0
0.8
0.6
0.4
0.2
0.0
−0.2
−0.4
B cells
T cells
Monocytes
Macrophages
Neutrophils
NK cells
Plasma cells
Interferon
Proliferation
Translation
C
Top GO terms (p<1E-05)
T cells
T cell activation
Translation
Translational elongation
Plasma cells
Adaptive immune response
NK cells
Cellular defense response
Neutrophils
Regulation of inflammatory response
Monocytes
Immune system process
Macrophages
Immune response
Interferon
Type I interferon signaling pathway
Proliferation
Mitotic cell cycle
B cells
B cell receptor signaling pathway
0
20
40
60
80
100
No of genes in signature

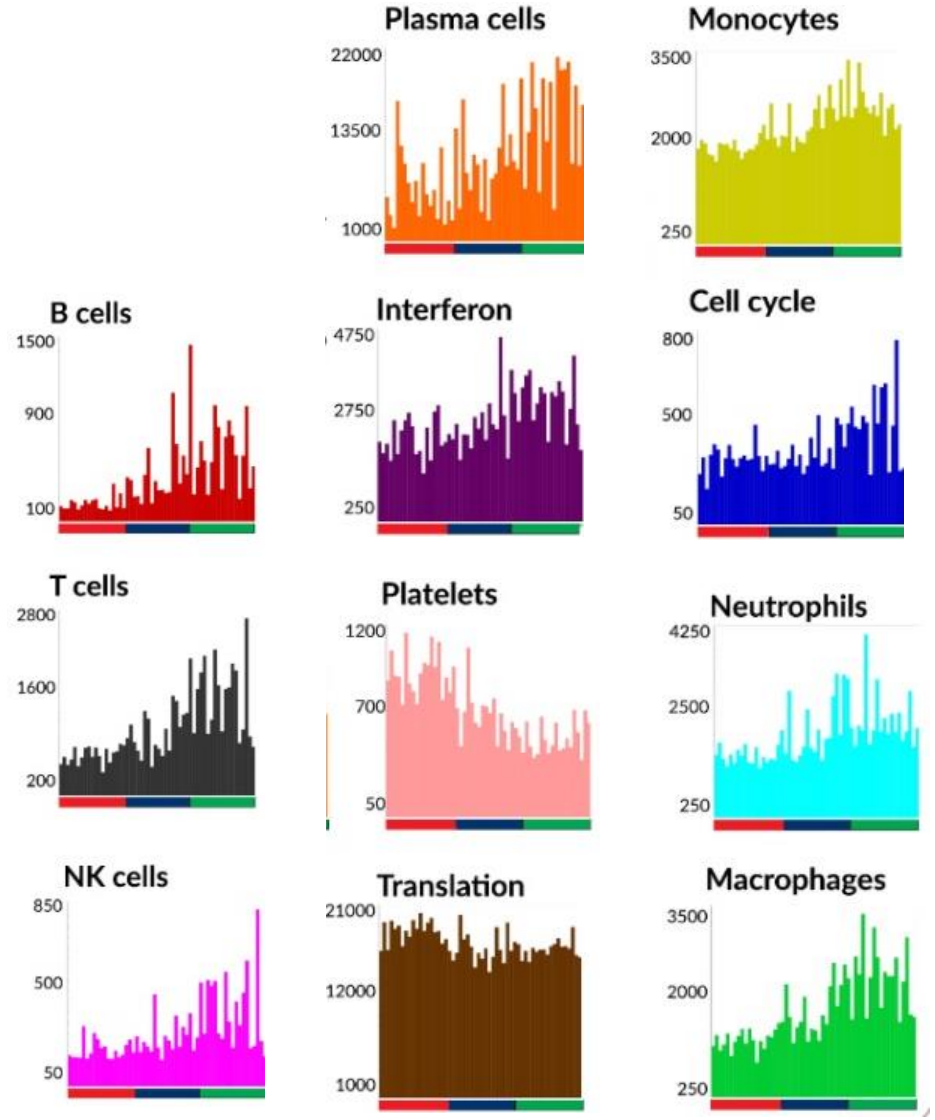
separate signatures for different applications

***ImSig*$_{blood}$ and *ImSig*$_{tissue}$**



$ImSig_{tissue}$



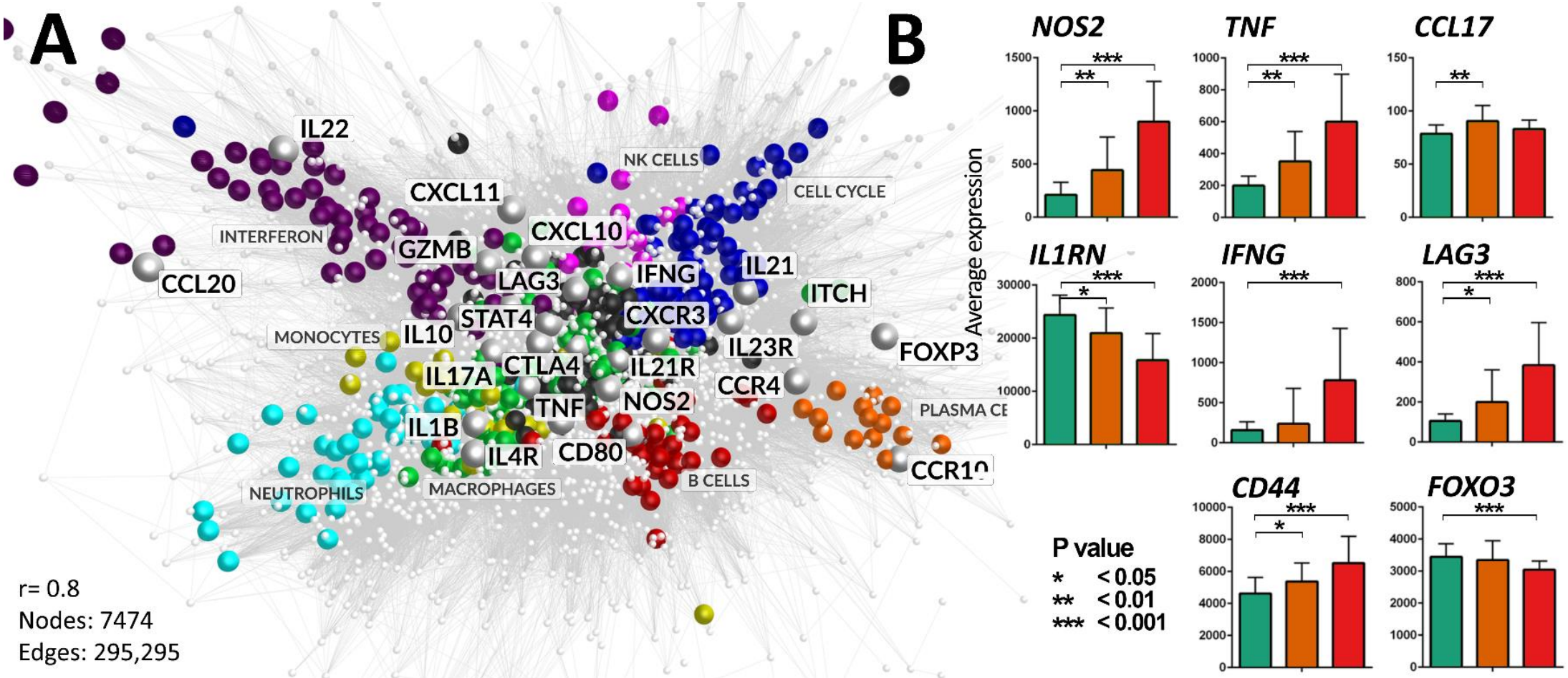


INFECTION AND IMMUNITY, Nov. 2010, p. 4895–4911
0019-9567/10/$12.00 doi:10.1128/IAI.00844-10
Copyright © 2010, American Society for Microbiology. All Rights Reserved.
Vol. 78, No. 11

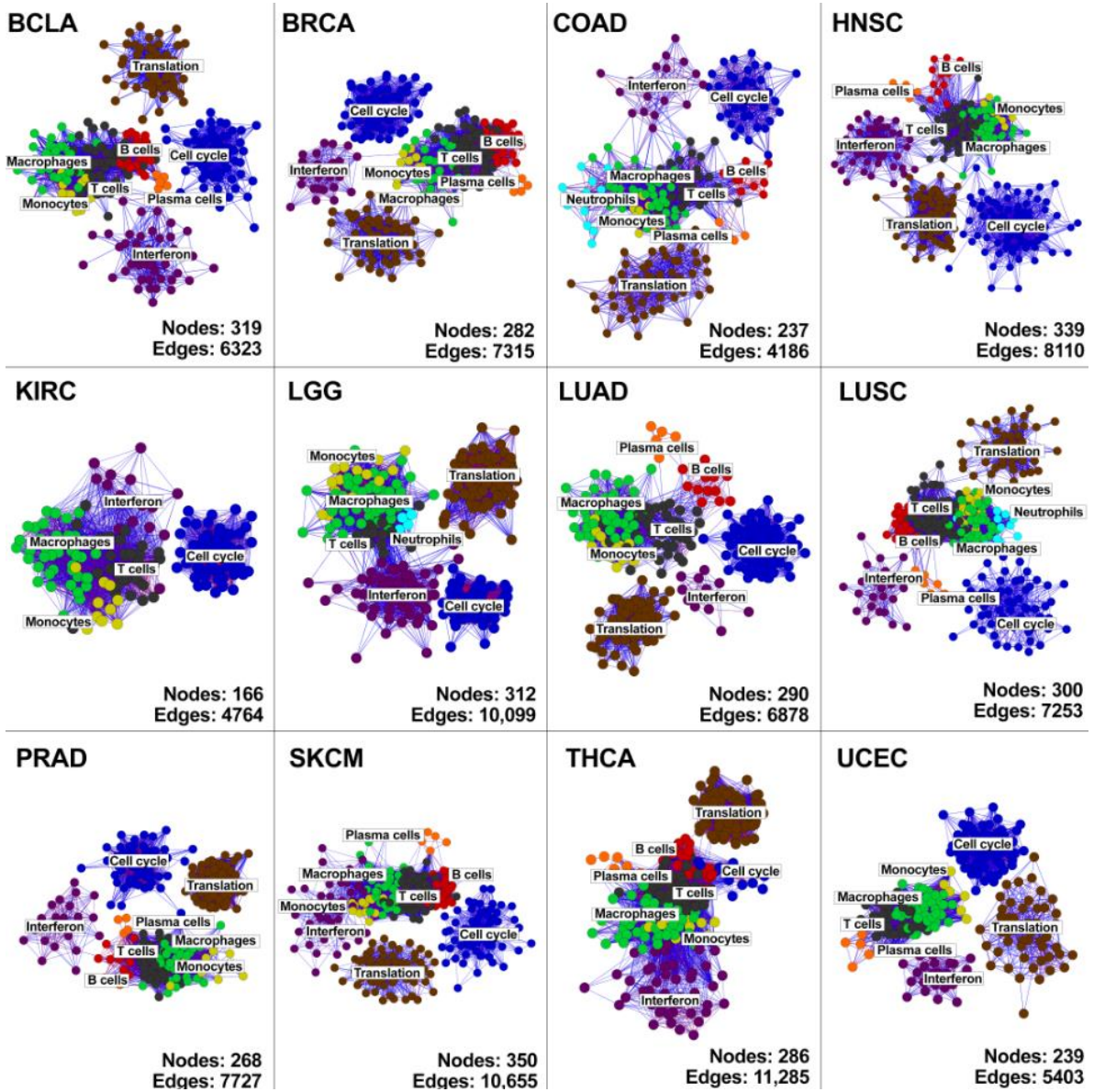
Human Conjunctival Transcriptome Analysis Reveals the Prominence of Innate Defense in *Chlamydia trachomatis* Infection▽†

Angels Natividad,[1]‡ Tom C. Freeman,[2] David Jeffries,[1,3] Matthew J. Burton,[1] David C. W. Mabey,[1] Robin L. Bailey,[1] and Martin J. Holland[1,3]*

what genes are coexpressed with signatures (subtype analysis)



deconvolution of TCGA cancer datasets

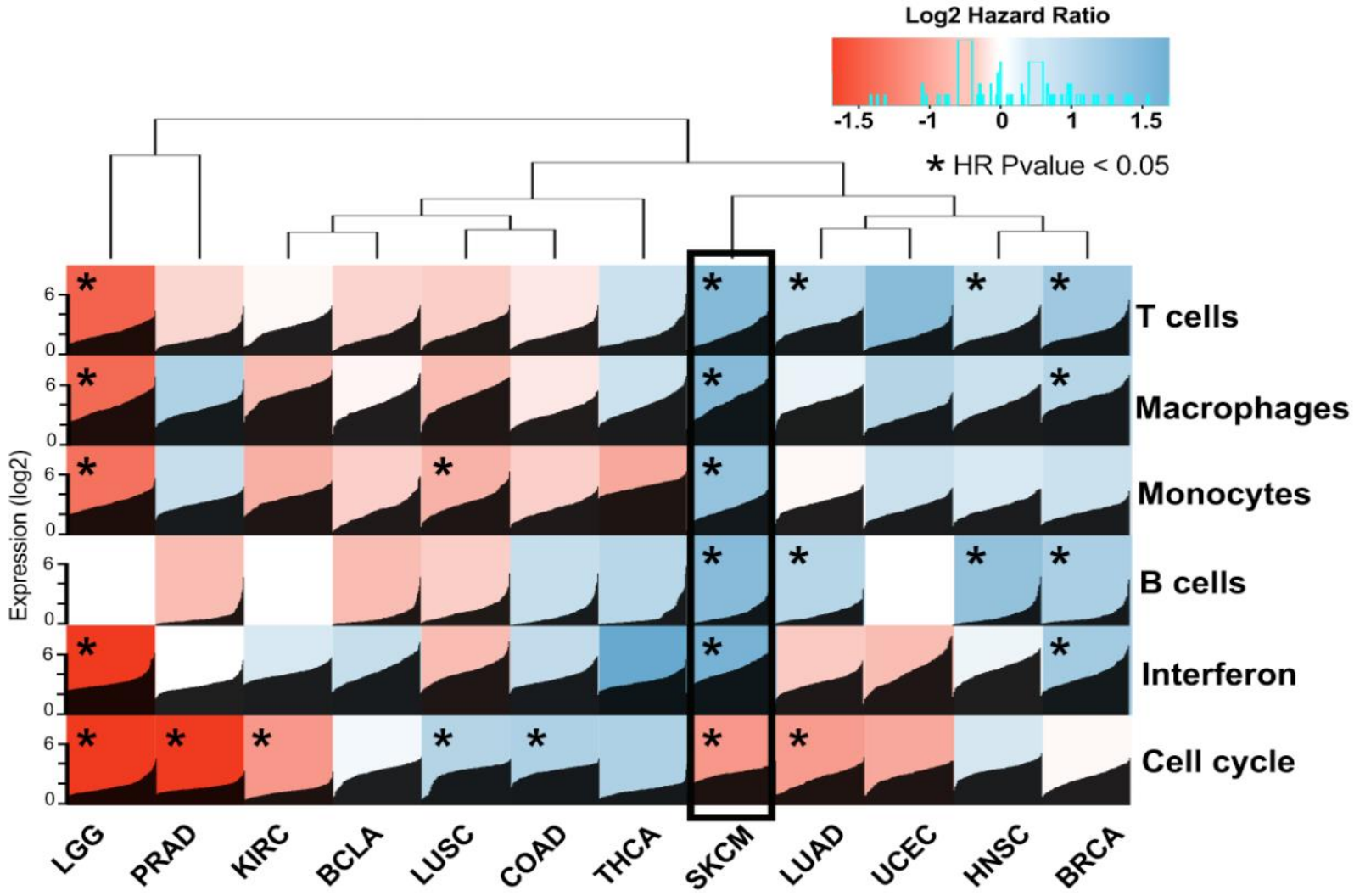


Cancer types (sample number)

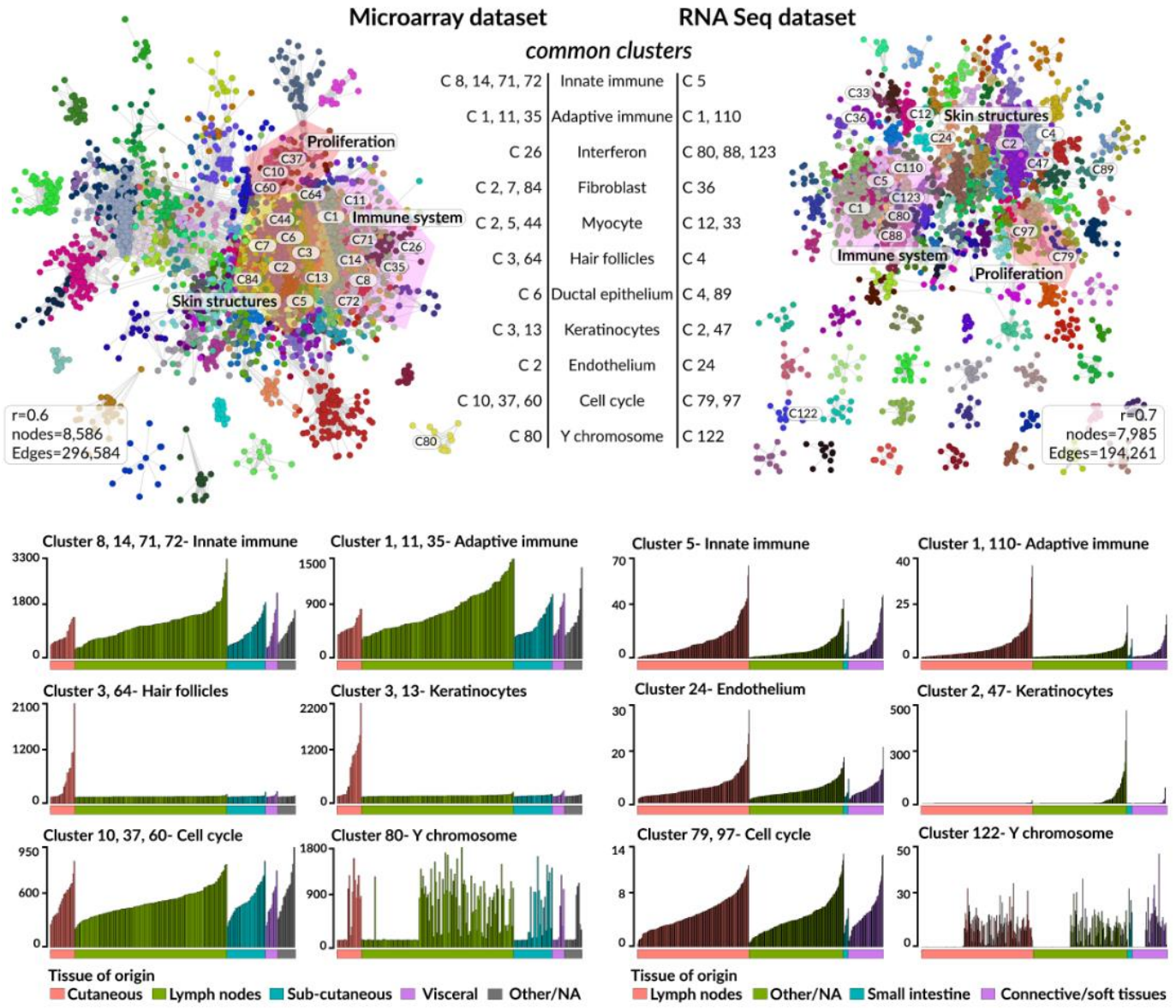
BCLA- Bladder Urothelial Carcinoma (408)
BRCA- Breast invasive carcinoma (1092)
COAD- Colon adenocarcinoma (456)
HNSC- Head and Neck squamous cell carcinoma (501)
KIRC- Kidney renal clear cell carcinoma (530)
LGG- Brain Lower Grade Glioma (512)
LUAD- Lung adenocarcinoma (515)
LUSC- Lung squamous cell carcinoma (501)
PRAD- Prostate adenocarcinoma (496)
SKCM- Skin Cutaneous Melanoma (468)
THCA- Thyroid carcinoma (502)
UCEC- Uterine Corpus Endometrial Carcinoma (555)

***ImSig*$_{\text{tissue}}$**

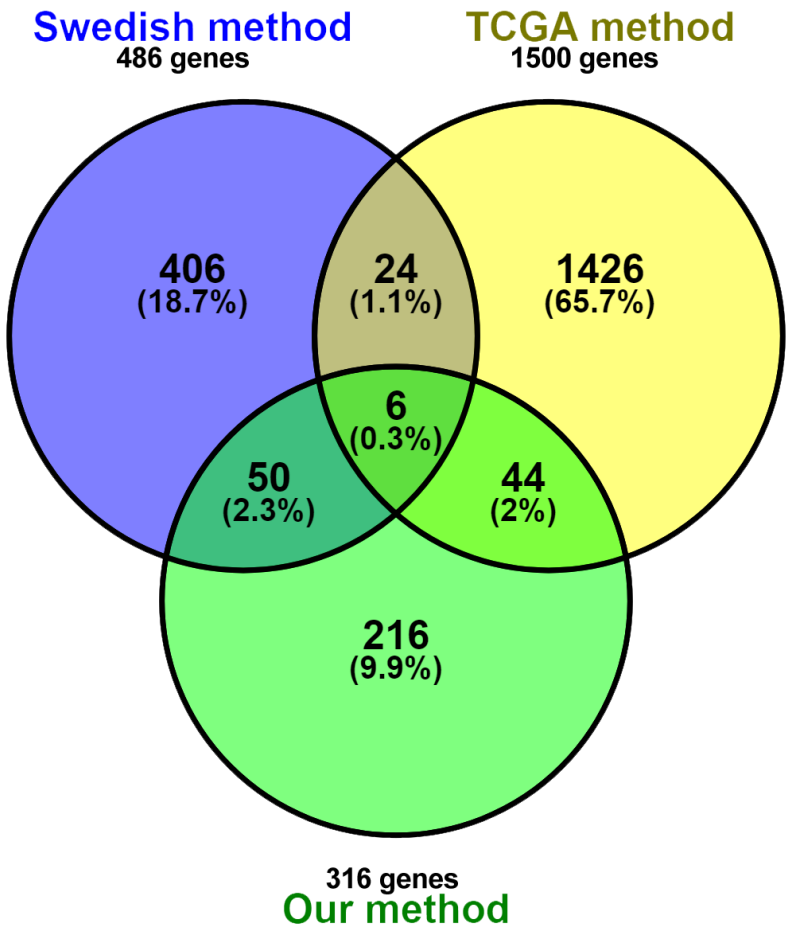
pan-cancer hazard ratio analysis

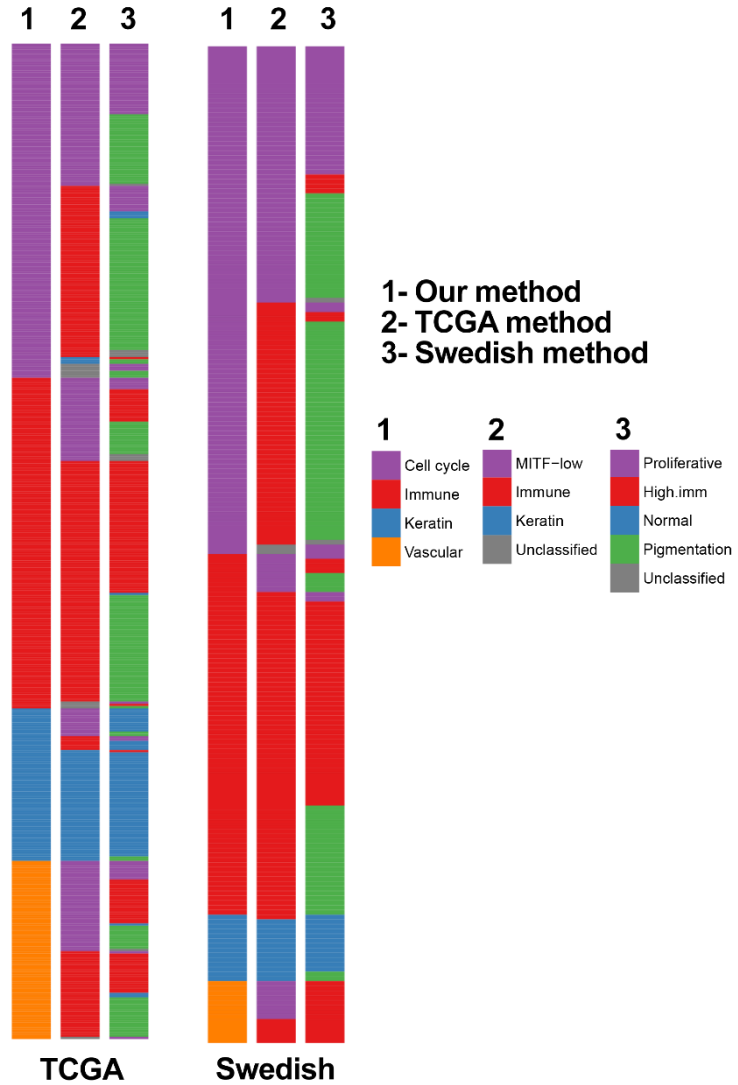


network analysis of melanoma datasets

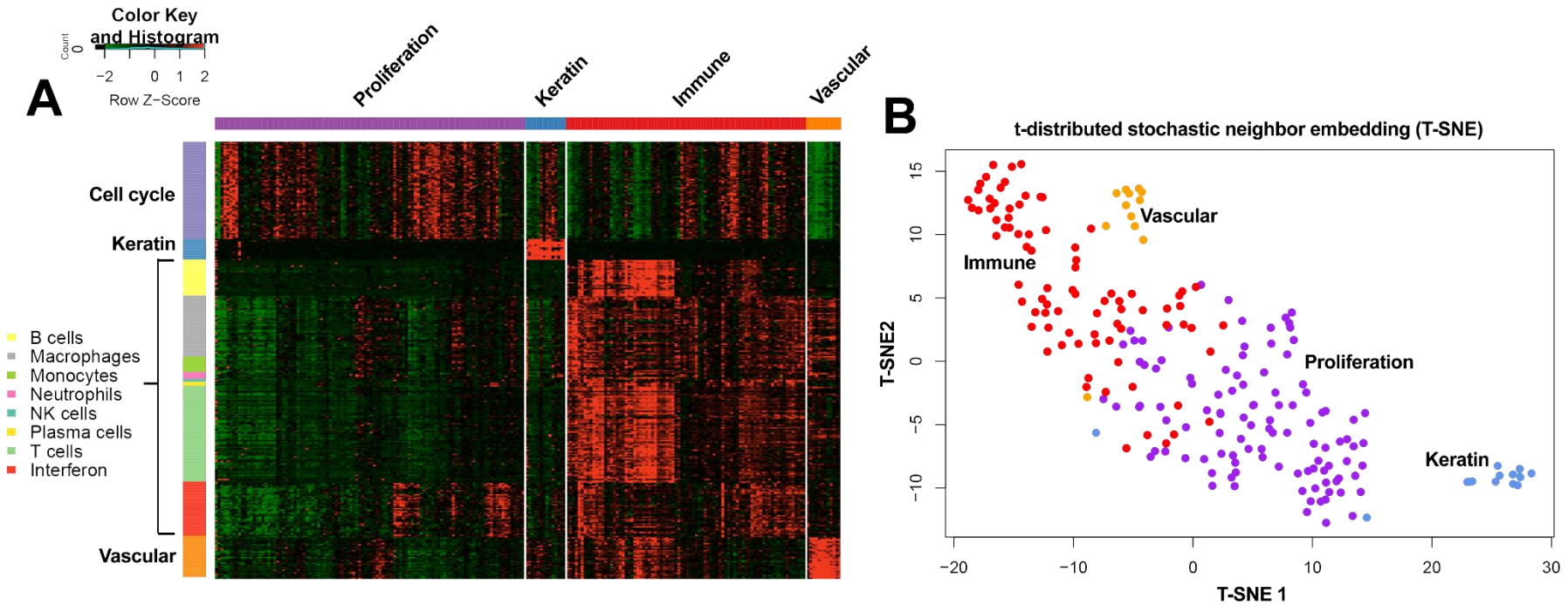


comparison of melanoma groupings

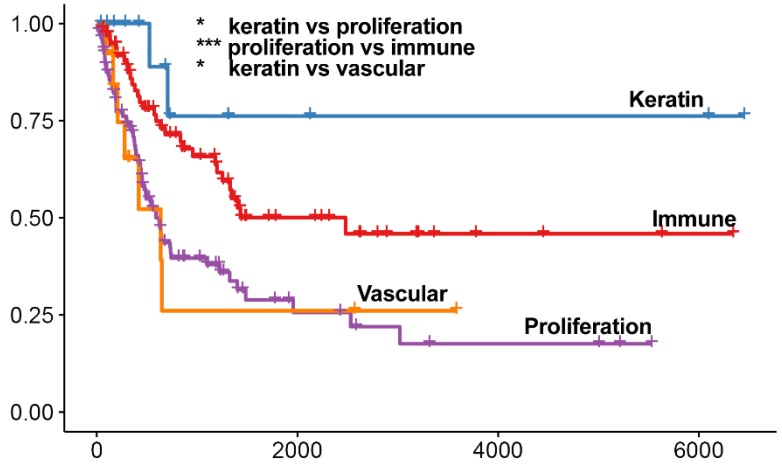




grouping of melanoma patients



Survival analysis



summary

- gene correlation network analysis approach supports exploration of tissue data allowing the derivation of cell and pathway-specific gene signatures
- knowing what constitutes normal, tells you a lot about disease
- still poor understanding of how immune microenvironment varies between cancers – what does a ‘good’ microenvironment look like?
- what immune profile is most likely to respond to immunotherapy? Can we predict responders to a given treatment?

ROSLIN

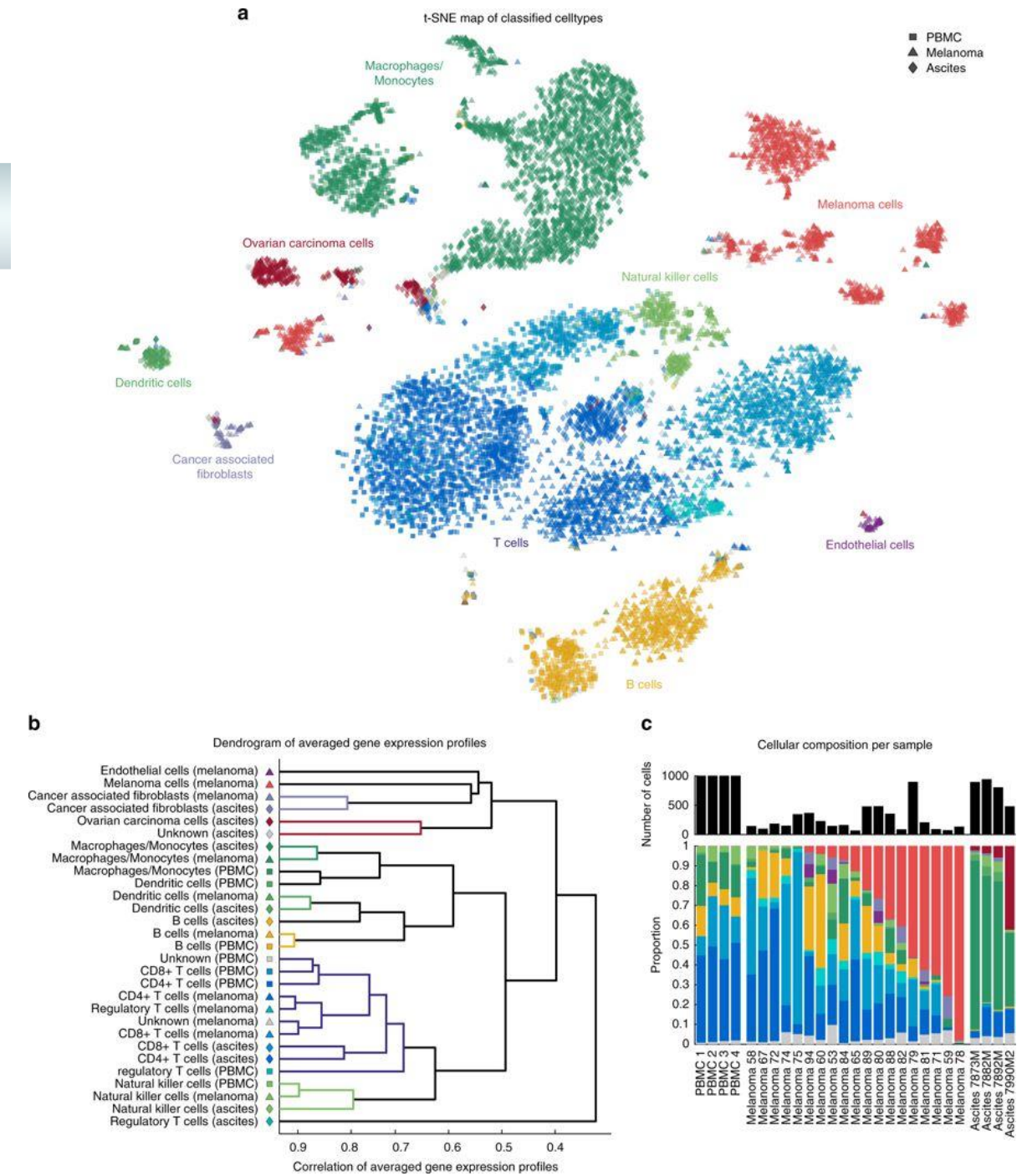
nature COMMUNICATIONS

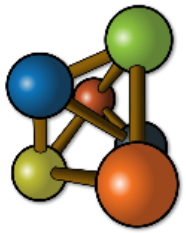
ARTICLE

DOI: 10.1038/s41467-017-02289-3 OPEN

Estimation of immune cell content in tumour tissue using single-cell RNA-seq data

Max Schelker[1,2], Sonia Feau[1], Jinyan Du[1], Nav Ranu[1], Edda Klipp[2], Gavin MacBeath[1], Birgit Schoeberl[1] & Andreas Raue[1]





GRAPHIA ENTERPRISE

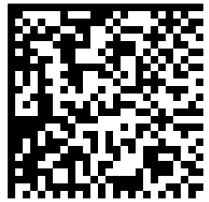
- designed and built from scratch
- user friendly
- bigger graphs, better rendering
- dynamic layout, input and filtering
- improved graph analytics, attribute handling, visualisation
- customisable for specific data types (plugin enabled)
- potential for direct connection to cloud resources

KAJEKA
CLARITY FROM COMPLEXITY

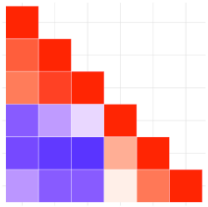
GRAPHIA ENTERPRISE

Genomics
Metagenomics
Transcriptomics
Single cell
Proteomics
Metabolomics
CHiP-seq
HiC
Multiparametric FACS
Image analysis

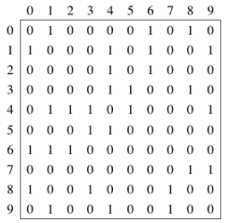
Interaction networks
DNA assembly networks
Pathways
Phylogenetic trees



data matrix

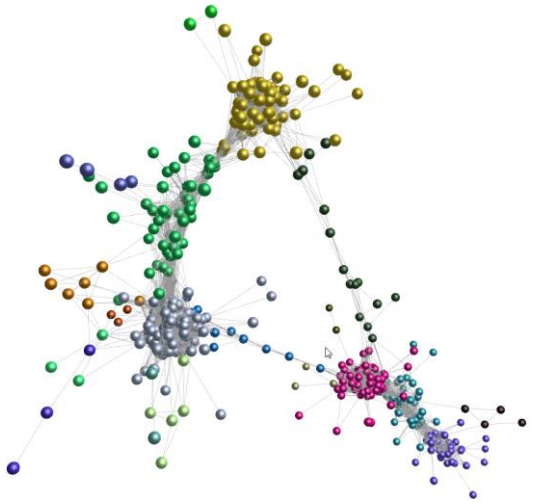


r matrix



	0	1	2	3	4	5	6	7	8	9
0	0	1	0	0	0	0	1	0	1	0
1	1	0	0	0	1	0	1	0	0	1
2	0	0	0	0	1	0	1	0	0	0
3	0	0	0	0	1	1	0	0	1	0
4	0	1	1	1	0	1	0	0	0	1
5	0	0	0	1	1	0	0	0	0	0
6	1	1	1	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	1	1
8	1	0	0	1	0	0	0	1	0	0
9	0	1	0	0	1	0	0	1	0	0

graph matrix



advanced network analysis solutions for the biological sciences

Acknowledgements

Tamasin Doig
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Barbara Shih
Anirudh Patir
Tim Regan

BBSRC bioscience for the future

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Chris Gregory

Andrew Sims

David Hume