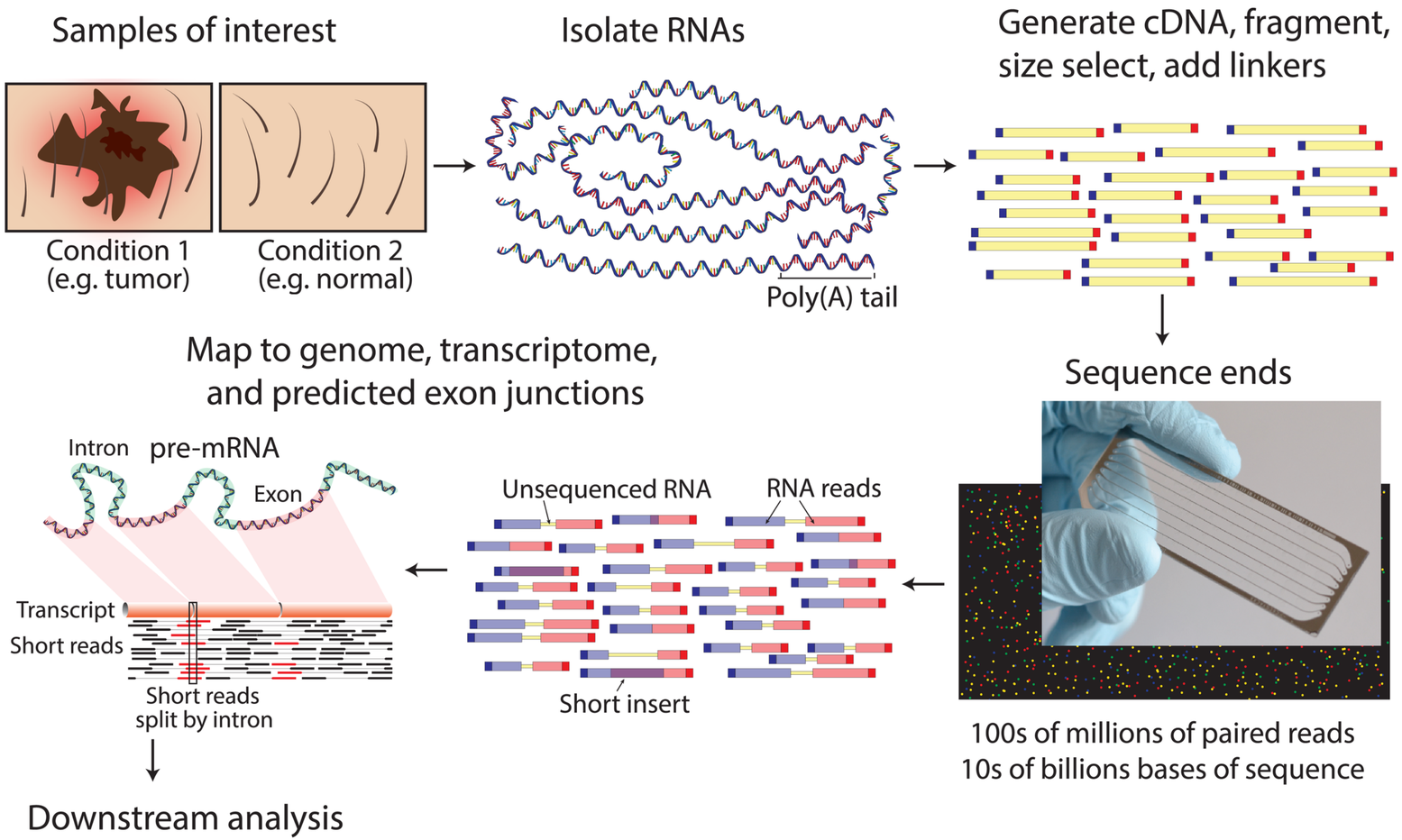
Common analyses with RNA-seq data

Matthew Sutcliffe
2020-06-05

https://github.com/JanesLab/shared-lab/tree/master/Matt-RNA-seq-analysis-tutorial

RNA-sequencing



Additional resources

- A brief introduction to RNA-seq at UVA (Shambhavi Singh)
  - smb://sammas.storage.virginia.edu/JanesLab/Matthew Sutcliffe/Resources/2019_06_05_RNAseqoverview.pptx

- RNA-seq alignment tutorial in UNIX
  - https://github.com/JanesLab/rna-seq-rivanna

- Video tutorials:
  - Part 1: https://youtu.be/jsB3fXnSgRQ
  - Part 2: https://youtu.be/2QHseQ422l4

- Intro to Git (Shambhavi Singh) - highly recommended version control system
  - smb://sammas.storage.virginia.edu/JanesLab/Matthew Sutcliffe/Resources/Sham intro to Git.pdf

- HarvardX Biomedical Data Science course
  - http://rafalab.github.io/pages/harvardx.html

Output of RNA-seq alignments

rsem_opcBulk

Search Sheet

Home | Insert | Page Layout | Formulas | Data | Review | View | Share

J1 | opcBulk_12dpi_CKO_21724_tdTpositive

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	ensgene	entrez	symbol	chr	start	end	strand	biotype	description	opcBulk_12c	opcBulk_12c	opcBulk_12c	opcBulk_12c
2	ENSMUSG00000000001	14679	Gnai3	3	108107280	108146146	-1	protein_codi	guanine nucl	1036	4271	435	760
3	ENSMUSG00000000003	54192	Pbsn	X	77837901	77853623	-1	protein_codi	probasin [So	0	0	0	0
4	ENSMUSG00000000028	12544	Cdc45	16	18780447	18811987	-1	protein_codi	cell division	23	26	6	8
5	ENSMUSG00000000037	107815	Scml2	X	161117193	161258213	1	protein_codi	sex comb on	47	96	6	18
6	ENSMUSG00000000049	11818	Apoh	11	108343354	108414396	1	protein_codi	apolipoprote	2	9	0	1
7	ENSMUSG00000000056	67608	Narf	11	121237253	121255856	1	protein_codi	nuclear prela	309	1237	98	195
8	ENSMUSG00000000058	12390	Cav2	6	17281185	17289115	1	protein_codi	caveolin 2 [S	172	304	29	240
9	ENSMUSG00000000078	23849	Klf6	13	5861482	5870394	1	protein_codi	Kruppel-like	331.01	471	53	307.35
10	ENSMUSG00000000085	29871	Scmh1	4	120405281	120530186	1	protein_codi	sex comb on	648.99	2645.99	216.99	436
11	ENSMUSG00000000088	12858	Cox5a	9	57521274	57532426	1	protein_codi	cytochrome	1481	4013	299.99	997
12	ENSMUSG00000000093	21385	Tbx2	11	85832551	85841948	1	protein_codi	T-box 2 [Sou	5	18	3	5
13	ENSMUSG00000000094	21387	Tbx4	11	85886422	85916097	1	protein_codi	T-box 4 [Sou	4	20.07	1	1
14	ENSMUSG00000000103	22768	Zfy2	Y	2106015	2170409	-1	protein_codi	zinc finger pı	0	0	0	0
15	ENSMUSG00000000120	18053	Ngfr	11	95568818	95587735	-1	protein_codi	nerve growtl	13.07	16.83	13.07	5.65
16	ENSMUSG00000000125	22415	Wnt3	11	103774150	103817957	1	protein_codi	wingless-typ	26	79	5	10
17	ENSMUSG00000000126	216795	Wnt9a	11	59306928	59333552	1	protein_codi	wingless-typ	22	27	6	20
18	ENSMUSG00000000127	14158	Fer	17	63896018	64139494	1	protein_codi	fer (fms/fps	151.75	666.13	63.51	71.36
19	ENSMUSG00000000131	74204	Xpo6	7	126101715	126200501	-1	protein_codi	exportin 6 [S	785.84	2887.91	213.8	564.09
20	ENSMUSG00000000134	209446	Tfe3	X	7762560	7775202	1	protein_codi	transcriptior	345.99	784.99	62.01	280
21	ENSMUSG00000000142	12006	Axin2	11	108920349	108950783	1	protein_codi	axin 2 [Sourc	277	670	85	165
22	ENSMUSG00000000148	231841	Brat1	5	140705011	140719379	1	protein_codi	BRCA1-assoc	72	182	15	24
23	ENSMUSG00000000149	14673	Gna12	5	140758408	140830431	-1	protein_codi	guanine nucl	547.05	708	81.46	317
24	ENSMUSG00000000154	18400	Slc22a18	7	143473736	143499334	1	protein_codi	solute carrie	3	12	0	1
25	ENSMUSG00000000157	16415	Itgb2l	16	96422288	96443619	-1	protein_codi	integrin beta	0	0	0	0
26	ENSMUSG00000000159	72058	Igsf5	16	96361668	96525580	1	protein_codi	immunoglob	8.32	3.6	0	0
27	ENSMUSG00000000167	72614	Rib1d2	9	50617321	50625000	1	protein_codi	RIH1 domair	5	28	4	14

rsem_opcBulk

Ready

140%

Samples

~34,000 rows (genes)

ENSMUSG00000000001

- Ensembl (annotation source)
- Mus musculus (mouse)
- Gene
- Unique ID

(One of the many reasons) why we don't open these files in Excel

rsem_opcBulk

Search Sheet

Home Insert Page Layout Formulas Data Review View Share

C87 2020-09-01

	A	B	C	D	E	F	G	H	I	J	K	L	M
83	ENSMUSG00000000435	17877	Myf5	10	107482908	107486134	-1	protein_codi	myogenic fac	0	1.42	0	
84	ENSMUSG00000000439	67027	Mkrn2	6	115601902	115622624	1	protein_codi	makorin, ring	451.37	1187.05	94.99	28
85	ENSMUSG00000000440	19016	Pparg	6	115360951	115490399	1	protein_codi	peroxisome p	36	297	13	
86	ENSMUSG00000000441	110157	Raf1	6	115618067	115676635	-1	protein_codi	v-raf-leukem	584.88	2341.91	166.77	31
87	ENSMUSG00000000486	54204	01-Sep	7	127214447	127233130	-1	protein_codi	septin 1 [Sou	24	92	3	
88	ENSMUSG00000000489	18591	Pdgfb	15	79995900	80014808	-1	protein_codi	platelet deriv	71.98	163.72	18.84	
89	ENSMUSG00000000530	11482	Acvrl1	15	101128522	101145336	1	protein_codi	activin A rec	15	24	7	
90	ENSMUSG00000000531	56149	Grasp	15	101224207	101232755	1	protein_codi	GRP1 (gener	100.8	446.2	39.06	
91	ENSMUSG00000000532	11479	Acvr1b	15	101174067	101213679	1	protein_codi	activin A rec	874	2864.01	205	
92	ENSMUSG00000000538	216810	Tom1l2	11	60226714	60352905	-1	protein_codi	target of my	1433	6185.02	453	
93	ENSMUSG00000000544	59290	Gpa33	1	166130238	166166510	1	protein_codi	glycoprotein	0	0	0	

rsem_opcBulk

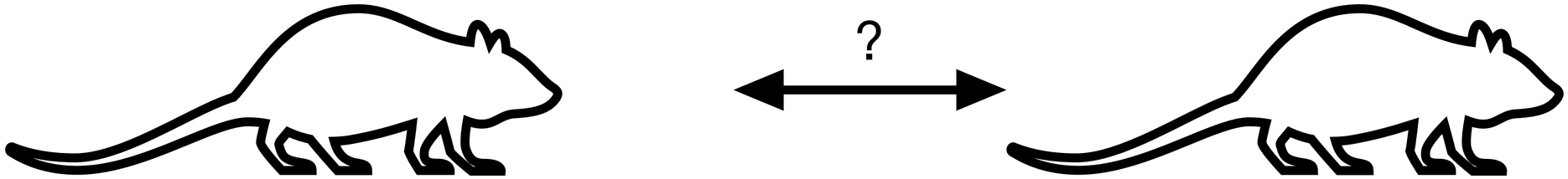
Ready 140%

rsem_opcBulk.csv

```
9,1011.87,901.45,818.41,1233.59,894.2700
.17,818.8599999999999,826.2099999999999,
79.9099999999999
ENSMUSG00000000486,54204,Sept1,7,1272144
coding,septin 1 [Source:MGI
Symbol;Acc:MGI:1858916],24,92,3,6,19,23,
23,14,17,44,10,5,19,15,10,4,2,11,43,24,1
53.01,8,7,10,4,7,0,18,13.999999999999998
17,9,5,11,87,64,16,32,37,22,4,0,0,16,33,
ENSMUSG00000000489,18591,Pdgfb,15,799959
```

Differential expression analysis with DESeq2

*Just pretend these rats are mice



wild-type

Trp53$^{-/-}$*Nf1*$^{-/-}$

Inputs to DESeq2

Counts matrix

RStudio Source Editor

rsem_counts

Filter

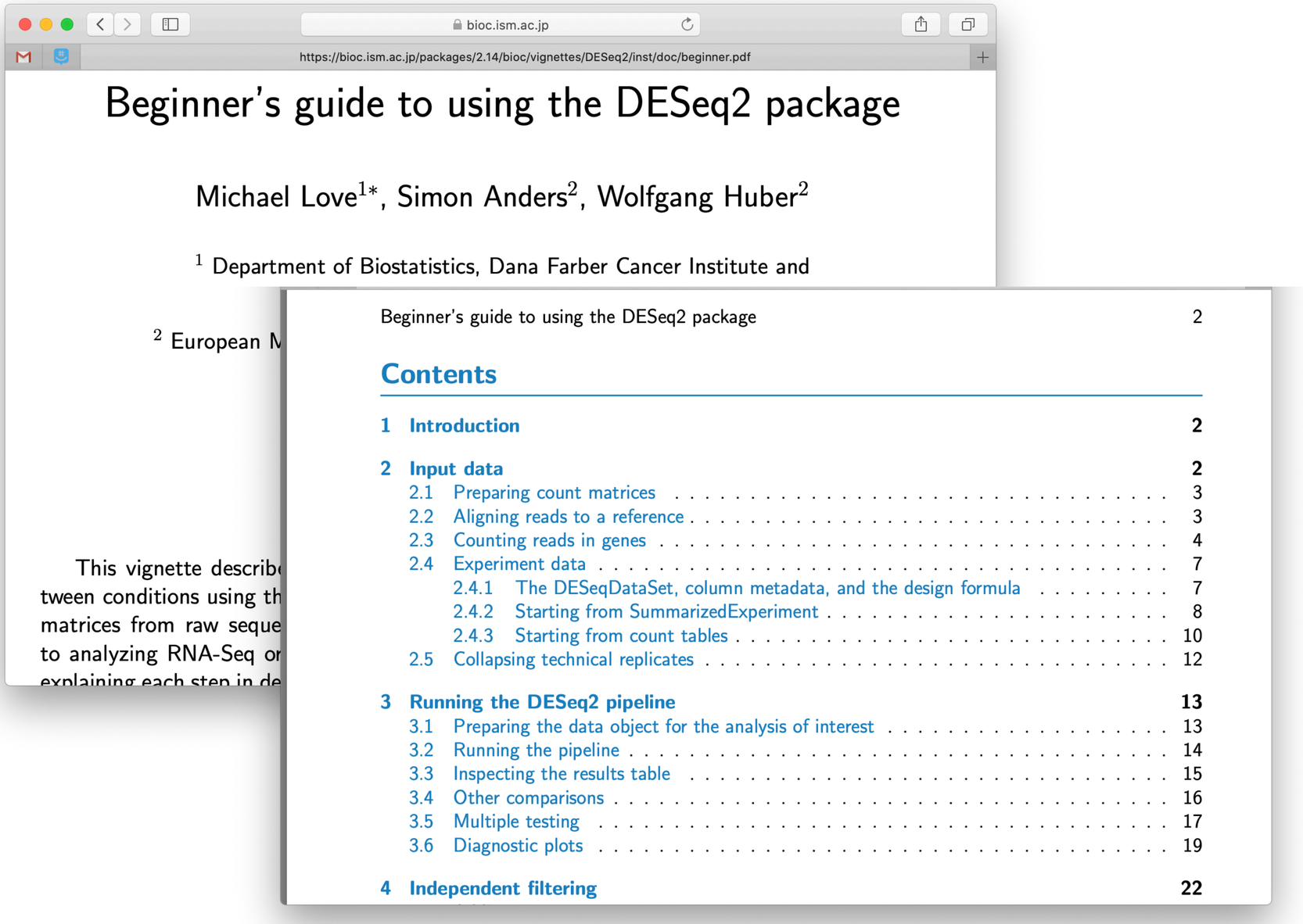
	opcBulk_150dpi_WT_21590_tdTpositive	opcBulk_150dpi_WT_21591_tdTpositive	opcBulk_150dpi_WT_21592_tdTpositive	opcBulk_150dpi_WT_21593_tdTpositive
Gnai3	624	571	306	1286
Pbsn	0	0	0	0
Cdc45	10	5	0	15
Scml2	43	60	79	64
Apoh	1	0	0	1
Narf	128	121	51	458
Cav2	160	433	201	295
Klf6	146	166	51	412
Scmh1	242	255	135	1004
Cox5a	842	372	285	1274
Tbx2	4	1	5	27
Tbx4	1	4	0	2
Zfy2	0	0	0	0
Ngfr	7	3	0	30
Wnt3	14	6	0	15
Wnt9a	4	2	1	9

Showing 1 to 16 of 34,623 entries, 13 total columns

Sample description

	genotype
opcBulk_150dpi_WT_21590_tdTpositive	WT
opcBulk_150dpi_WT_21591_tdTpositive	WT
opcBulk_150dpi_WT_21592_tdTpositive	WT
opcBulk_150dpi_WT_21593_tdTpositive	WT
opcBulk_150dpi_WT_22062_tdTpositive	WT
opcBulk_150dpi_WT_22064_tdTpositive	WT
opcBulk_150dpi_Tumor_16713_tdTpositive	Tumor
opcBulk_150dpi_Tumor_16839_tdTpositive	Tumor
opcBulk_150dpi_Tumor_16840_tdTpositive	Tumor
opcBulk_150dpi_Tumor_16844_tdTpositive	Tumor
opcBulk_150dpi_Tumor_21256_tdTpositive	Tumor
opcBulk_150dpi_Tumor_21349_tdTpositive	Tumor
opcBulk_150dpi_Tumor_21354_tdTpositive	Tumor

Resources from the creators of DESeq2



https://bioc.ism.ac.jp/packages/2.14/bioc/vignettes/DESeq2/inst/doc/beginner.pdf

In case you get stuck somewhere…

Bioconductor
OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

ASK QUESTION | LATEST 200 | NEWS 9 | JOBS | TUTORIALS | T

Question: Rescaling predictors in DESeq changes outcomes?

1

When I'm running a GLM I will sometimes need to rescale predictors (e.g. take z-scores) to make the coefficients of different predictors easier to compare to each other. My understanding is that this doesn't and shouldn't change anything about the model fit except the scale of the coefficient. When I do this in DESeq2, I find that it changes not only the log2FC estimates, but also the p-values (before adjustment). I don't understand how or why that would be happening, or what to do about it.

Does anyone understand this? Is it expected behaviour? What should I do about it?

Thank you!

5 days ago by
jc.szamosi • 10

deseq2 glm rescale • 49 views

ADD COMMENT • link • Not following modified 5 days ago by Michael Love ♦ 28k • written 5 days ago by jc.szamosi • 10

Answer: Rescaling predictors in DESeq changes outcomes?

0

Having predictors with very different scales can make the fitting numerically unstable, which is known in linear modeling, and it's typically recommended to not use a design matrix with very different scale of predictors.

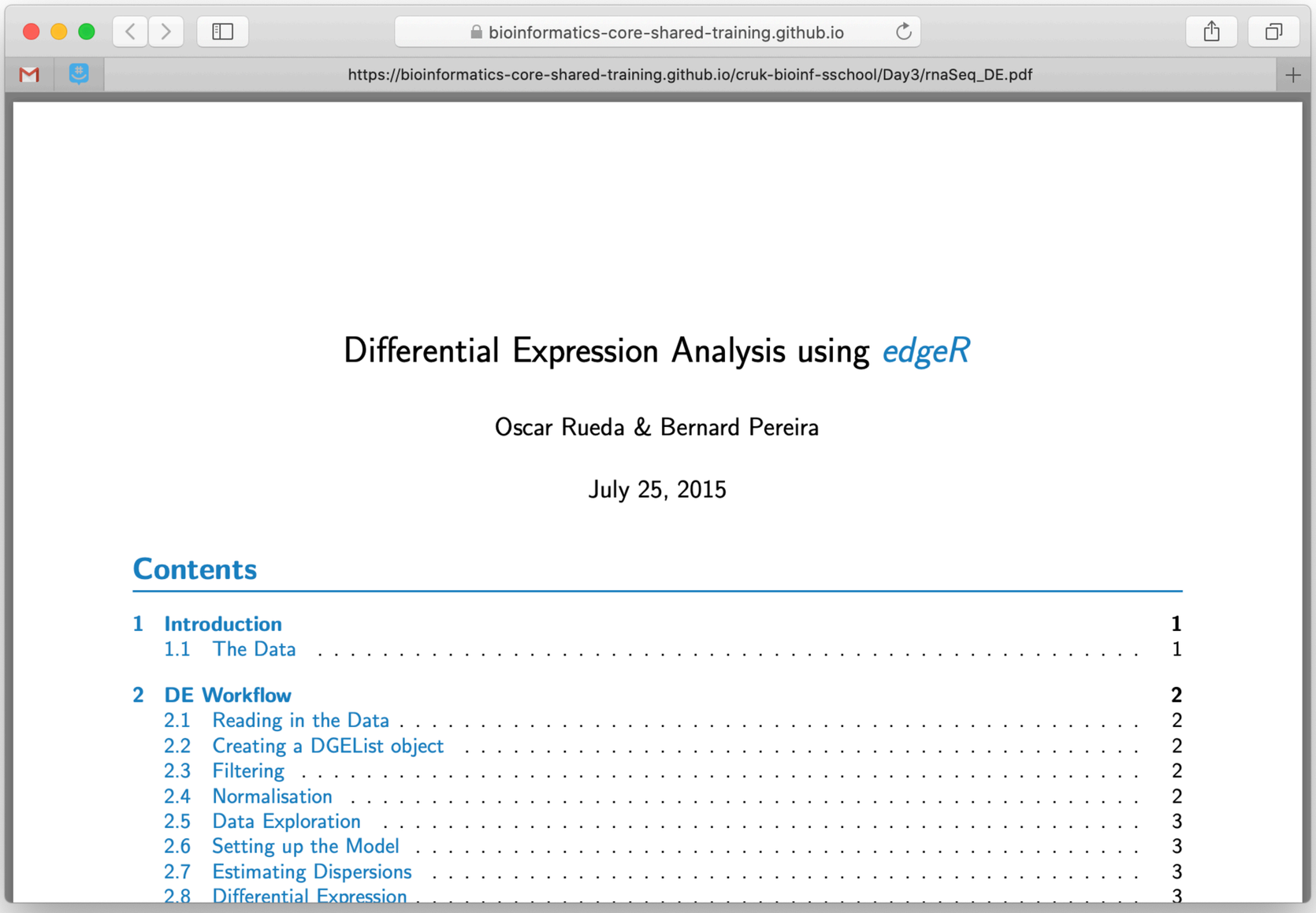
Because I noticed some users putting in very small and very large values in the design, DESeq2 gives a message to scale the predictors first since v1.26 (so current and previous release give this message).

ADD COMMENT • link

written 5 days ago by Michael Love ♦ 28k

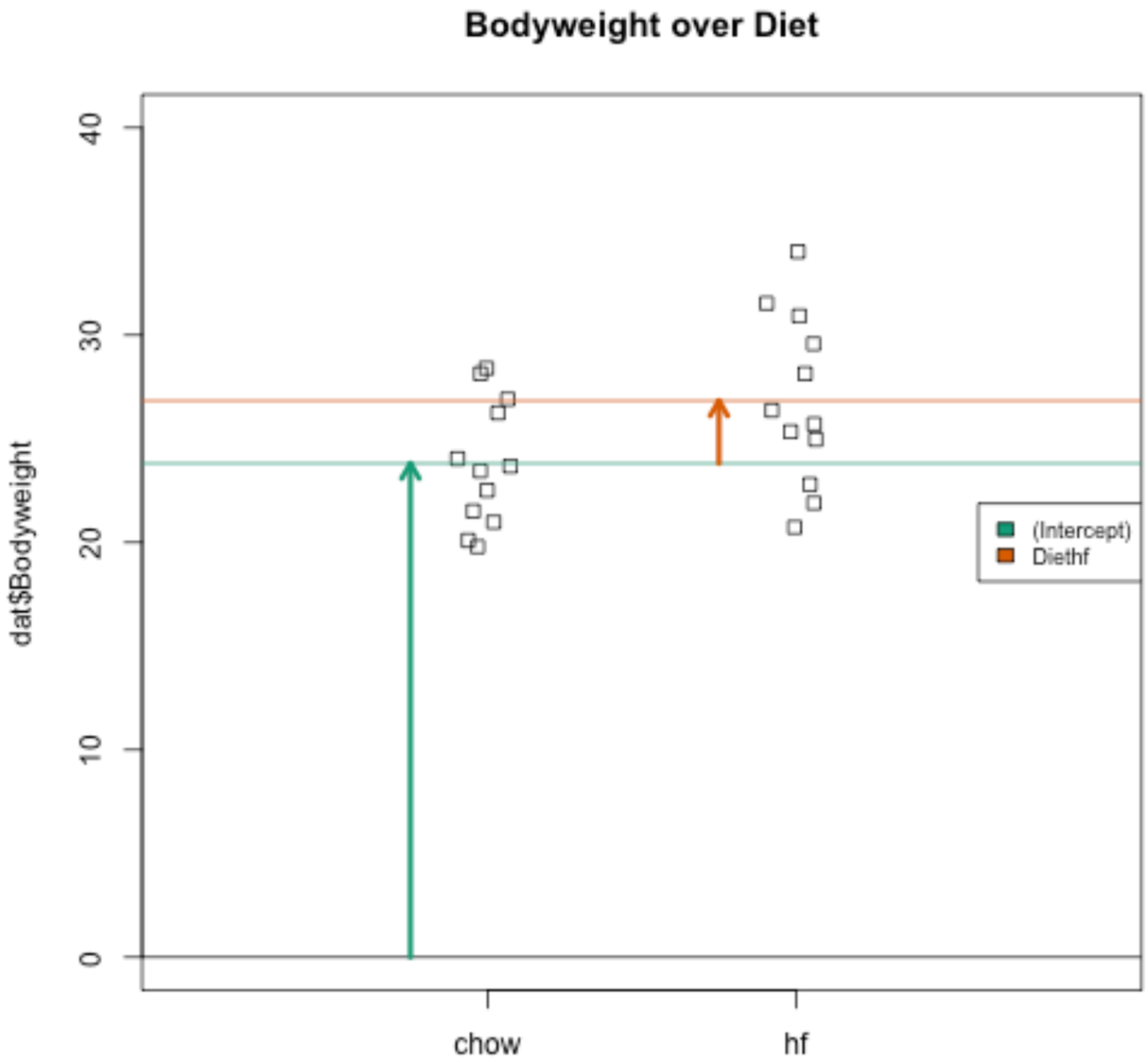
5 days ago by
Michael Love ♦ 28k
United States

Alternative to DESeq2: edgeR



https://www.bioconductor.org/packages/release/bioc/vignettes/edgeR/inst/doc/edgeRUsersGuide.pdf

Creating a study design matrix for edgeR



	(Intercept)	dataset_info$genotypeWT
1	1	1
2	1	1
3	1	1
4	1	1
5	1	1
6	1	1
7	1	0
8	1	0
9	1	0
10	1	0
11	1	0
12	1	0
13	1	0

http://genomicsclass.github.io/book/pages/linear_models_in_practice.html

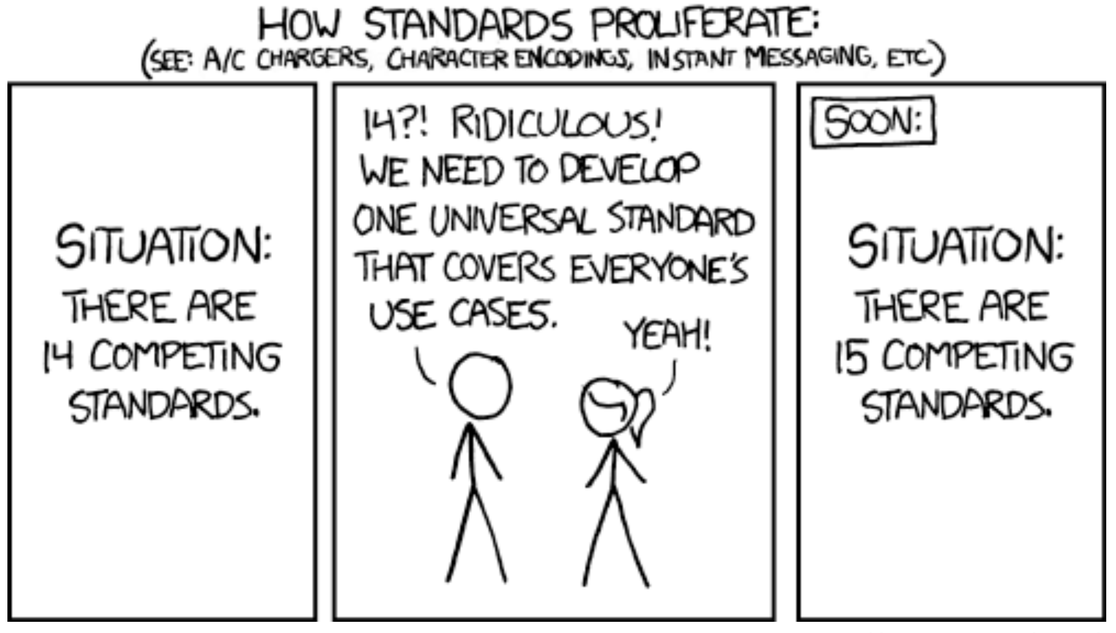
DESeq2 and edgeR produce similar results

Number of differentially expressed genes

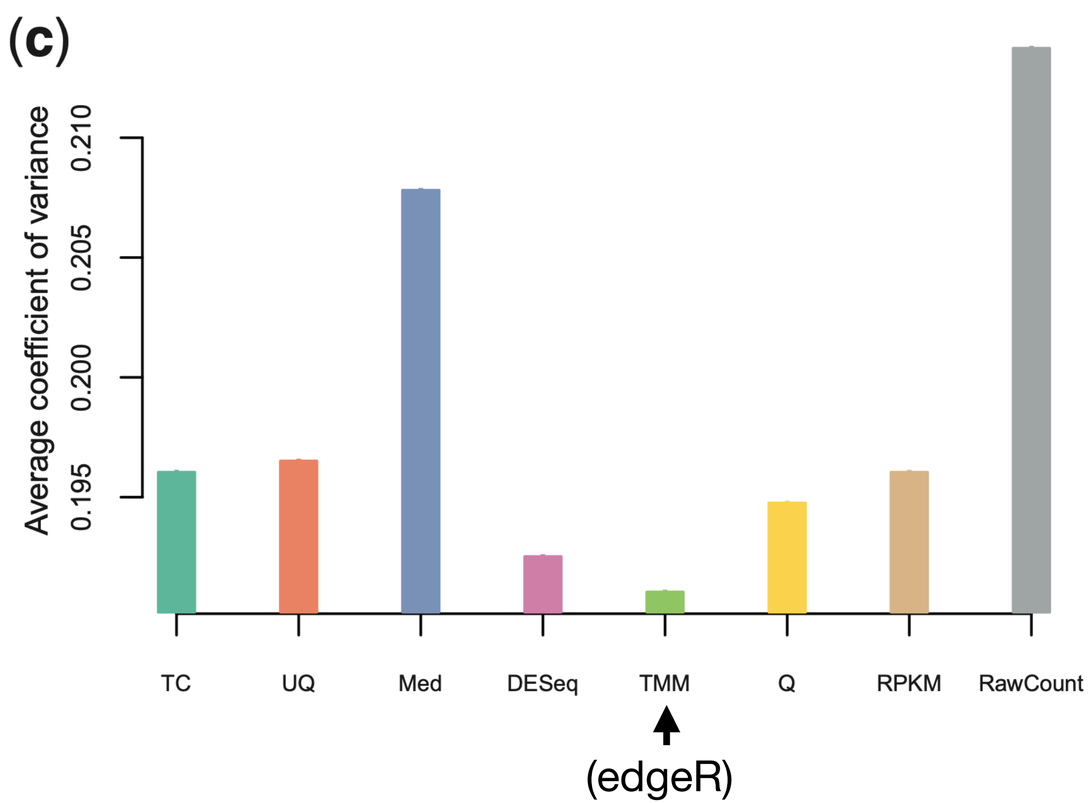
DESeq2 vs. edgeR vs. other: which should I use?

- Either (or both)
  - Using the intersection of multiple methods could produce a higher confidence DE gene list

- There is some evidence that edgeR's normalization method is superior to that of DESeq*

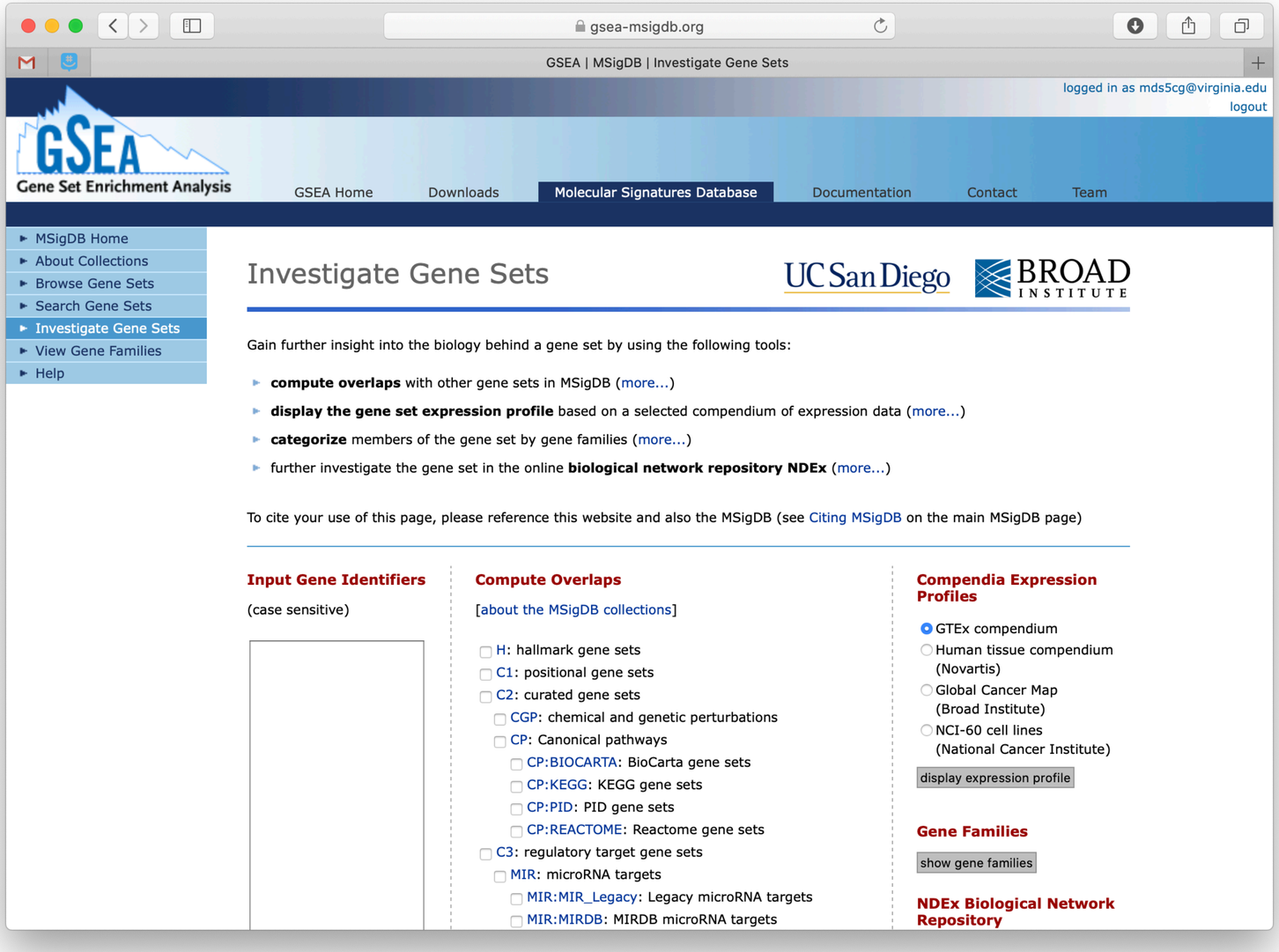


https://xkcd.com/927/



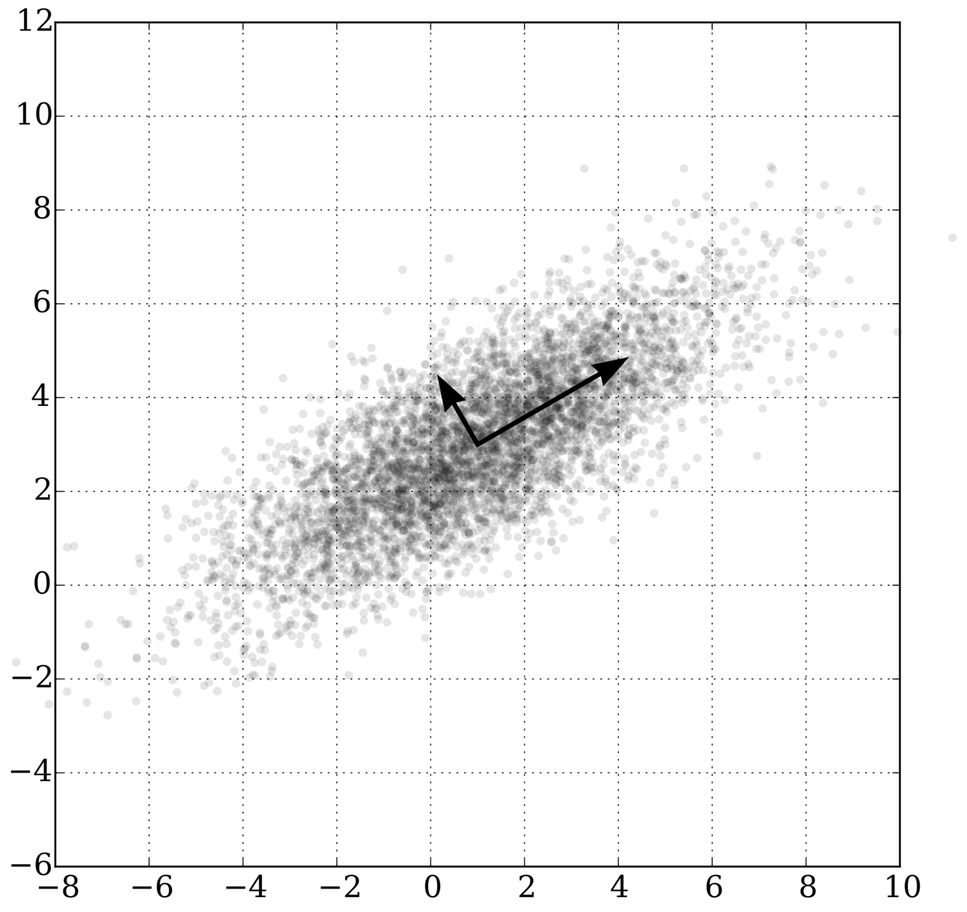
- DESeq: median ratio of geometric mean of all genes
- edgeR: "ratio of RNA production uses a weighted trimmed mean of the log expression ratios (trimmed mean of M values (TMM))"

Gene set enrichment analysis (GSEA)

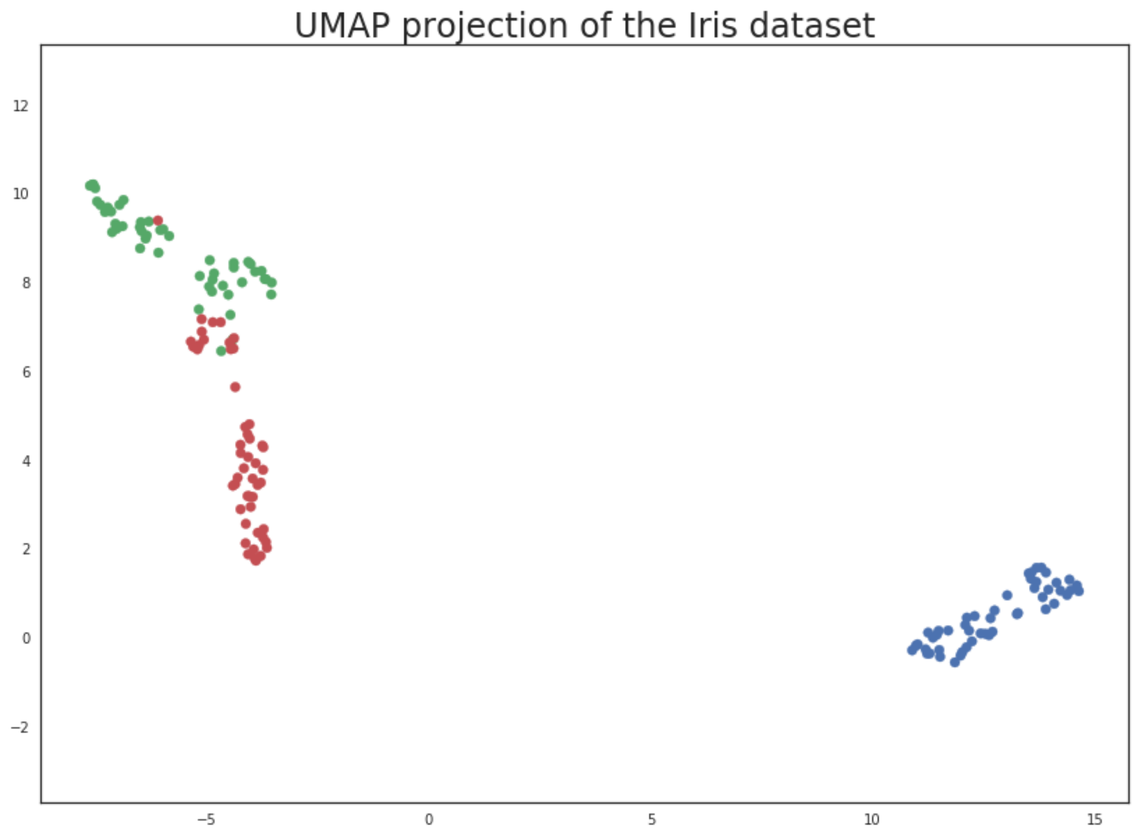


PCA and UMAP

PCA: linear dimensionality reduction



UMAP: nonlinear dimensionality reduction



When googling problems in R:

- Websites where you will commonly find answers
  - Stack Overflow
  - Bioconductor forums
  - R-bloggers