

SPOROS pipeline analysis of seed composition data using multinomial mixed models

1 Read in Output F from GitHub

```
> ## read in raw data from GitHub
>
> f.down <- "https://raw.githubusercontent.com/ebartom/SPOROS/main/Figure3.SPOROSpaper/differential/ADnTPDvsCtrl/pvalue/sRNA/F_seedExpand.Delta.sRNA.dn.txt"
> f.up <- "https://raw.githubusercontent.com/ebartom/SPOROS/main/Figure3.SPOROSpaper/differential/ADnTPDvsCtrl/pvalue/sRNA/F_seedExpand.Delta.sRNA.up.txt"
>
> f.up <- "G:\\PeterM_XXX\\Analysis\\Data\\MethodsPaper\\Files for Figure 3\\F_seedExpand.Delta.sRNA.up.txt"
> f.down <- "G:\\PeterM_XXX\\Analysis\\Data\\MethodsPaper\\Files for Figure 3\\F_seedExpand.Delta.sRNA.dn.txt"
>
>
> down <- read.table(f.down, header=TRUE, sep="\t")
> up <- read.table(f.up, header=TRUE, sep="\t")
```

There are 998 seeds in Up data.

There are 1000 seeds in Down data.

There are 1998 seeds in the combined data set.

Example of each data set:

```
> # look at each file to check that data were read and combined correctly
> up %>% head
```

	Seed	Sample	SeedID	Pos	Base
1	AAGGUU	Up	Up.Delta.1	1	A
2	AAGGUU	Up	Up.Delta.1	2	A
3	AAGGUU	Up	Up.Delta.1	3	G
4	AAGGUU	Up	Up.Delta.1	4	G
5	AAGGUU	Up	Up.Delta.1	5	U
6	AAGGUU	Up	Up.Delta.1	6	U

```
> down %>% head
```

	Seed	Sample	SeedID	Pos	Base
1	AAACAG	Down	Down.Delta.1	1	A
2	AAACAG	Down	Down.Delta.1	2	A
3	AAACAG	Down	Down.Delta.1	3	A
4	AAACAG	Down	Down.Delta.1	4	C
5	AAACAG	Down	Down.Delta.1	5	A
6	AAACAG	Down	Down.Delta.1	6	G

```
> dataCombined %>% head
```

	Seed	Sample	SeedID	Pos	Base
1	AAGGUU	Up	Up.Delta.1	1	A
2	AAGGUU	Up	Up.Delta.1	2	A
3	AAGGUU	Up	Up.Delta.1	3	G
4	AAGGUU	Up	Up.Delta.1	4	G
5	AAGGUU	Up	Up.Delta.1	5	U
6	AAGGUU	Up	Up.Delta.1	6	U

```
> dataCombined %>% tail
```

	Seed	Sample	SeedID	Pos	Base
11983	UUUUCC	Down	Down.Delta.1000	1	U
11984	UUUUCC	Down	Down.Delta.1000	2	U
11985	UUUUCC	Down	Down.Delta.1000	3	U
11986	UUUUCC	Down	Down.Delta.1000	4	U
11987	UUUUCC	Down	Down.Delta.1000	5	C
11988	UUUUCC	Down	Down.Delta.1000	6	C

Do the counts match?

```
> # check total seeds/id's
> (nrow(up)/6 + nrow(down)/6 ==
+   length(unique(dataCombined$SeedID)))
```

```
[1] TRUE
```

2 Save the data

Data saved for SAS analyses into:

```
[1] "G:\\PeterM_XXX\\Analysis\\Data\\MethodsPaper\\data.sas.Fig3.2021-08-12.
csv"
```

3 SAS Analysis using PROC GLIMMIX

```
> * update the path in the INFILE statement to match datapath above;
+ * note that sas uses single backslash in paths, e.g. "C:\\sasdata";
+
+ data seedlong;
+ informat seed $6. sample $20. seedid $30.;
+ infile "G:\\PeterM_XXX\\Analysis\\Data\\MethodsPaper\\data.sas.Fig3.2021-08-1
2.csv"
+   dlm="," firstobs=2;
+ input Seed $ sample $ seedid $ pos base $ ;
+ run;
+
+
+ * note: if the model has convergence issues, different estimation can be used
;
+ *       "method=laplace" was used in Fig. 3 data analysis;
+ *       "method = rmpl" was used in Fig. 2 data analysis;
+ proc glimmix data=seedlong outdesign = xx method=laplace;
+ class seed seedid sample(ref="Down") pos base(ref="A");
+ model base = sample pos sample*pos /dist=multinomial link=glogit s or(label)
ddfm=bw;
+ random intercept/ subject = seedid group=base type=chol;
+ store gmxres;
+ run;
```

Model Information	
Data Set	WORK.SEEDLONG
Response Variable	base
Response Distribution	Multinomial (nominal)
Link Function	Generalized Logit
Variance Function	Default
Variance Matrix Blocked By	seedid
Estimation Technique	Maximum Likelihood
Likelihood Approximation	Laplace
Degrees of Freedom Method	Between-Within

Class Level Information		
Class	Levels	Values
seed	88	AAACAG AAACCG AAGCGG AAGGUU AAUUGU AAUUUU ACGUGU ACUUCG AGACUA AGAGUG AGAUUA AGAUUC AGCUUC AGGAUC AGGCUC AGGGUC AGGUCC AGGUUC AGGUUG AGUACA AGUAGU AGUUUC AUCUUG AUGGAG AUUAGC AUUGGC AUUGUC CAAUAU CAGAUU CAGUAA CAUAAG CCAAGA CCAGAA CCCGAC CCCGGG CCGGUA CGGAGC CGGCCU CGGUAA CUA AAC CUCCAG CUCGGU CUGGAC GAACUG GAAGUG GAAUCG GAAUUG GAGGUU GAGUGU GAUUAU GAUUCG GAUUCU GAUUGU GGAAUG GGAGCG GGCAGU GGCCCC GGCGGA GGCUGU GGGAGC GGGAUU GGGUGU GGUAAA GGUAUU GGUUCC GGUUGU GUCAGU GUCGAU GUGUUA UAACUA UAUUGC UCACGG UCCAGA UCCCCG UCGACU UCGUGG UCUCUG UCUCUU UGACUG UGACUU UGAUUC UGCAUU UGGGGC UGUAUG UGUCUG UUGUGG UUUCUG UUUUCC
seedid	1998	Down.Delta.1 Down.Delta.10 Down.Delta.100 Down.Delta.1000 Down.Delta.101 Down.Delta.102 Down.Delta.103 Down.Delta.104 Down.Delta.105 Down.Delta.106 Down.Delta.107 Down.Delta.108 Down.Delta.109 Down.Delta.11 Down.Delta.110 Down.Delta.111 Down.Delta.112 Down.Delta.113 Down.Delta.114 Down.Delta.115 Down.Delta.116 Down.Delta.117 Down.Delta.118 Down.Delta.119 Down.Delta.12 Down.Delta.120 Down.Delta.121 Down.Delta.122 Down.Delta.123 Down.Delta.124

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 Up.Delta.980 Up.Delta.981 Up.Delta.982 Up.Delta.983 Up.Delta.984 Up.Delta.985
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 Up.Delta.991 Up.Delta.992 Up.Delta.993 Up.Delta.994 Up.Delta.995 Up.Delta.996
 Up.Delta.997 Up.Delta.998

sample	2	Up Down
pos	6	1 2 3 4 5 6
base	4	A C G U

Number of Observations Read	11988
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Number of Observations Used	11988
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Response Profile

Ordered	Total
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Value	base	Frequency
1	A	2327
2	C	1453
3	G	3692
4	U	4516

In modeling category probabilities,
base='A' serves as the reference
category.

Dimensions

G-side Cov. Parameters	3
Columns in X	63
Columns in Z per Subject	3
Subjects (Blocks in V)	1998
Max Obs per Subject	6

Optimization Information

Optimization Technique	Dual Quasi-Newton
Parameters in Optimization	39
Lower Boundaries	3
Upper Boundaries	0
Fixed Effects	Not Profiled
Starting From	GLM estimates

Iteration History					
Iteration	Restarts	Evaluations	Objective Function	Change	Max Gradient
0	0	4	21783.176573	.	1102.086
1	0	2	21123.365173	659.81139944	423.161
2	0	3	21122.306221	1.05895253	417.6768
3	0	4	21014.275125	108.03109540	260.8271
4	0	2	20968.685652	45.58947316	227.9763
5	0	2	20954.785929	13.89972266	69.56161
6	0	2	20951.020615	3.76531481	24.43601
7	0	3	20950.103258	0.91735640	38.05331
8	0	3	20949.498121	0.60513755	12.10819
9	0	3	20949.156716	0.34140464	8.945594
10	0	3	20949.056246	0.10047026	8.130956
11	0	3	20948.99494	0.06130605	4.947842
12	0	2	20948.974477	0.02046313	9.782639
13	0	4	20948.918067	0.05640952	2.822133
14	0	4	20948.784316	0.13375057	3.830968
15	0	2	20948.702941	0.08137551	5.603241
16	0	2	20948.623834	0.07910672	2.422144
17	0	3	20948.594599	0.02923494	1.8497
18	0	2	20948.556795	0.03780390	2.236341
19	0	2	20948.50031	0.05648498	1.753916

20	0	3	20948.466767	0.03354318	2.413428
21	0	2	20948.411885	0.05488224	1.338206
22	0	3	20948.399861	0.01202395	1.224934
23	0	2	20948.386503	0.01335831	2.024217
24	0	2	20948.371293	0.01520938	1.858681
25	0	2	20948.34717	0.02412377	1.107685
26	0	3	20948.330785	0.01638486	1.248665
27	0	2	20948.325155	0.00562993	2.218039
28	0	4	20948.306201	0.01895357	0.815453
29	0	2	20948.28558	0.02062088	0.655605
30	0	3	20948.282629	0.00295102	0.505051
31	0	4	20948.267217	0.01541252	1.222814
32	0	2	20948.245674	0.02154276	1.066225
33	0	3	20948.236967	0.00870724	0.805149
34	0	3	20948.233631	0.00333622	0.665553
35	0	4	20948.220206	0.01342478	0.421357
36	0	3	20948.218906	0.00129997	0.373942
37	0	4	20948.210212	0.00869384	0.400355
38	0	3	20948.209409	0.00080295	0.44697
39	0	4	20948.205	0.00440918	0.467956
40	0	2	20948.199508	0.00549158	0.630752
41	0	3	20948.198253	0.00125532	0.465919
42	0	4	20948.193008	0.00524492	0.355395

43	0	3	20948.190766	0.00224201	0.212836
44	0	3	20948.190054	0.00071246	0.301902
45	0	4	20948.183961	0.00609257	0.646081
46	0	3	20948.181942	0.00201847	0.29453
47	0	2	20948.18027	0.00167221	0.454104
48	0	2	20948.17795	0.00232074	0.320849
49	0	4	20948.16649	0.01145993	0.363537
50	0	3	20948.164308	0.00218162	0.436886
51	0	4	20948.15881	0.00549836	0.327065
52	0	3	20948.157981	0.00082838	0.331947
53	0	4	20948.154048	0.00393307	0.541978
54	0	2	20948.152457	0.00159141	0.648352
55	0	2	20948.150189	0.00226768	0.245159
56	0	3	20948.149004	0.00118487	0.332197
57	0	2	20948.148032	0.00097214	0.349204
58	0	2	20948.146727	0.00130519	0.198074
59	0	2	20948.145236	0.00149053	0.298491
60	0	3	20948.144638	0.00059810	0.24206
61	0	4	20948.134922	0.00971593	0.318445
62	0	3	20948.133071	0.00185107	0.490788
63	0	2	20948.131935	0.00113636	0.514495
64	0	4	20948.129102	0.00283298	0.430228
65	0	4	20948.114718	0.01438411	0.19267

66	0	3	20948.114436	0.00028205	0.210218
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Convergence criterion (GCONV=1E-8) satisfied.

Estimated G matrix is not positive definite.

Fit Statistics

-2 Log Likelihood	20948.11
AIC (smaller is better)	21020.11
AICC (smaller is better)	21020.34
BIC (smaller is better)	21221.71
CAIC (smaller is better)	21257.71
HQIC (smaller is better)	21094.14

Fit Statistics for Conditional Distribution

-2 log L(base r. effects)	20948.11
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Covariance Parameter Estimates

Cov Parm	Subject	Group	Estimate	Standard Error
CHOL(1,1)	seedid	base C	0	.
CHOL(1,1)	seedid	base G	0	.
CHOL(1,1)	seedid	base U	0	.

Solutions for Fixed Effects								
Effect	base	sample	pos	Estimate	Standard Error	DF	t Value	Pr > t
Intercept	C			1.4478	0.3503	1977	4.13	<.0001
Intercept	G			3.9195	0.3183	1977	12.31	<.0001
Intercept	U			3.7778	0.3188	1977	11.85	<.0001
sample	C	Up		2.9104	0.4887	1977	5.96	<.0001
sample	G	Up		-1.2101	0.4729	1977	-2.56	0.0106
sample	U	Up		-0.7666	0.4711	1977	-1.63	0.1038
sample	C	Down		0
sample	G	Down		0
sample	U	Down		0
pos	C		1	-4.5954	0.4576	1977	-10.04	<.0001
pos	G		1	-3.1089	0.3263	1977	-9.53	<.0001
pos	U		1	-5.0575	0.3436	1977	-14.72	<.0001
pos	C		2	-5.7400	0.4619	1977	-12.43	<.0001
pos	G		2	-5.7511	0.3319	1977	-17.33	<.0001
pos	U		2	-6.7973	0.3576	1977	-19.01	<.0001
pos	C		3	-3.5142	0.3746	1977	-9.38	<.0001
pos	G		3	-7.5800	0.4246	1977	-17.85	<.0001
pos	U		3	-3.9667	0.3256	1977	-12.18	<.0001
pos	C		4	-3.4175	0.4985	1977	-6.86	<.0001
pos	G		4	-3.5390	0.3567	1977	-9.92	<.0001

pos	U		4	-1.2282	0.3438	1977	-3.57	0.0004
pos	C		5	-0.5309	0.4785	1977	-1.11	0.2674
pos	G		5	-0.5415	0.4241	1977	-1.28	0.2018
pos	U		5	-0.01360	0.4235	1977	-0.03	0.9744
pos	C		6	0
pos	G		6	0
pos	U		6	0
sample*pos	C	Up	1	-2.9586	0.6192	5979	-4.78	<.0001
sample*pos	G	Up	1	0.3264	0.4832	5979	0.68	0.4994
sample*pos	U	Up	1	0.8524	0.4982	5979	1.71	0.0871
sample*pos	C	Up	2	2.7748	0.6129	5979	4.53	<.0001
sample*pos	G	Up	2	6.3789	0.5204	5979	12.26	<.0001
sample*pos	U	Up	2	5.1038	0.5432	5979	9.40	<.0001
sample*pos	C	Up	3	0.09304	0.5432	5979	0.17	0.8640
sample*pos	G	Up	3	7.4060	0.5767	5979	12.84	<.0001
sample*pos	U	Up	3	3.3988	0.5064	5979	6.71	<.0001
sample*pos	C	Up	4	0.5769	0.6376	5979	0.90	0.3656
sample*pos	G	Up	4	2.1113	0.5417	5979	3.90	<.0001
sample*pos	U	Up	4	1.4391	0.5237	5979	2.75	0.0060
sample*pos	C	Up	5	-2.8778	0.5997	5979	-4.80	<.0001
sample*pos	G	Up	5	-2.4834	0.5717	5979	-4.34	<.0001
sample*pos	U	Up	5	-1.1916	0.5584	5979	-2.13	0.0329
sample*pos	C	Up	6	0

sample*pos	G	Up	6	0
sample*pos	U	Up	6	0
sample*pos	C	Down	1	0
sample*pos	G	Down	1	0
sample*pos	U	Down	1	0
sample*pos	C	Down	2	0
sample*pos	G	Down	2	0
sample*pos	U	Down	2	0
sample*pos	C	Down	3	0
sample*pos	G	Down	3	0
sample*pos	U	Down	3	0
sample*pos	C	Down	4	0
sample*pos	G	Down	4	0
sample*pos	U	Down	4	0
sample*pos	C	Down	5	0
sample*pos	G	Down	5	0
sample*pos	U	Down	5	0
sample*pos	C	Down	6	0
sample*pos	G	Down	6	0
sample*pos	U	Down	6	0

Odds Ratio Estimates

Comparison	Estimate	DF	95% Confidence Limits
-------------------	-----------------	-----------	------------------------------

C: sample Up vs Down	12.328	1977	9.096	16.707
G: sample Up vs Down	2.944	1977	2.304	3.762
U: sample Up vs Down	2.302	1977	1.828	2.899
C: pos 1 vs 6	0.002	1977	0.001	0.004
G: pos 1 vs 6	0.053	1977	0.033	0.084
U: pos 1 vs 6	0.010	1977	0.006	0.016
C: pos 2 vs 6	0.013	1977	0.007	0.023
G: pos 2 vs 6	0.077	1977	0.046	0.129
U: pos 2 vs 6	0.014	1977	0.008	0.024
C: pos 3 vs 6	0.031	1977	0.018	0.053
G: pos 3 vs 6	0.021	1977	0.012	0.036
U: pos 3 vs 6	0.104	1977	0.063	0.170
C: pos 4 vs 6	0.044	1977	0.023	0.082
G: pos 4 vs 6	0.083	1977	0.049	0.142
U: pos 4 vs 6	0.601	1977	0.360	1.005
C: pos 5 vs 6	0.139	1977	0.077	0.251
G: pos 5 vs 6	0.168	1977	0.096	0.294
U: pos 5 vs 6	0.544	1977	0.314	0.940

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
sample	3	1977	95.36	<.0001
pos	15	1977	179.83	<.0001

sample*pos

15

5979

93.07

<.0001

```

>
+ proc plm restore=gmxres noclprint plots=none;
+ lsmeans sample/ilink oddsratio adj=tukey cl e;
+ slice sample*pos/sliceby=pos diff oddsratio adj=tukey cl;
+ ods output slicediffs= sampleposdiffs diffs= samplediffs;
+ run;

```

```

> proc sort data=sampleposdiffs;
+ by sample slice base;
+
+ proc print data=sampleposdiffs;
+ var sample _sample slice base OddsRatio AdjLowerOR AdjUpperOR AdjP;
+ run;

```

Obs	sample	_sample	Slice	base	OddsRatio	AdjLowerOR	AdjUpperOR	AdjP
1	Up	Down	pos 1	C	0.953	0.452	2.008	0.8992
2	Up	Down	pos 1	G	0.413	0.340	0.502	<.0001
3	Up	Down	pos 1	U	1.090	0.793	1.496	0.5962
4	Up	Down	pos 2	C	294.486	142.625	608.046	<.0001
5	Up	Down	pos 2	G	175.698	114.791	268.922	<.0001
6	Up	Down	pos 2	U	76.492	45.017	129.976	<.0001
7	Up	Down	pos 3	C	20.156	12.663	32.083	<.0001
8	Up	Down	pos 3	G	490.730	256.967	937.149	<.0001
9	Up	Down	pos 3	U	13.903	9.659	20.012	<.0001
10	Up	Down	pos 4	C	32.699	14.652	72.970	<.0001
11	Up	Down	pos 4	G	2.462	1.467	4.133	0.0007
12	Up	Down	pos 4	U	1.959	1.251	3.067	0.0033

13	Up	Down	pos 5	C	1.033	0.523	2.042	0.9251
14	Up	Down	pos 5	G	0.025	0.013	0.047	<.0001
15	Up	Down	pos 5	U	0.141	0.078	0.254	<.0001
16	Up	Down	pos 6	C	18.365	7.045	47.872	<.0001
17	Up	Down	pos 6	G	0.298	0.118	0.753	0.0105
18	Up	Down	pos 6	U	0.465	0.184	1.170	0.1037

```

> * update the path in the OUTFILE statement below to match datapath;
+ * note that sas uses single backslash for paths, e.g. "C:\sasdata";
+
+ proc export data=work.sampleposdiffs
+   outfile="G:\PeterM_XXX\Analysis\Data\MethodsPaper\F_OREstimates_Fig3_081221
+   .xlsx"
+   dbms=excel replace;
+   sheet="OR Position";
+ run;

```

4 Up vs. Down

Up vs. Down

