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.dicer <- "https://raw.githubusercontent.com/ebartom/SPOROS/main/Figure2.SPO
ROSpaper/totalCounts/sRNA/F_seedExpand.DicerKO.avg.sRNA.Figure2.txt"
> f.drosha <- "https://raw.githubusercontent.com/ebartom/SPOROS/main/Figure2.SP
OROSpaper/totalCounts/sRNA/F_seedExpand.DroshaKO.avg.sRNA.Figure2.txt"
> f.wildtype <- "https://raw.githubusercontent.com/ebartom/SPOROS/main/Figure2.
SPOROSpaper/totalCounts/sRNA/F_seedExpand.Wildtype.avg.sRNA.Figure2.txt"
>
> dicer <- read.table(f.dicer, header=TRUE, sep="\t")
> drosha <- read.table(f.drosha, header=TRUE, sep="\t")
> wildtype <- read.table(f.wildtype, header=TRUE, sep="\t")
```

There are 913 seeds in DicerKO data.

There are 889 seeds in DroshaKO data.

There are 951 seeds in Wildtype data.

There are 2753 seeds in the combined data set.

Example of each data set:

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

```
     Seed  Sample    SeedID Pos Base
  1 AAAGUG DicerKO DicerKO.1   1    A
  2 AAAGUG DicerKO DicerKO.1   2    A
  3 AAAGUG DicerKO DicerKO.1   3    A
  4 AAAGUG DicerKO DicerKO.1   4    G
  5 AAAGUG DicerKO DicerKO.1   5    U
  6 AAAGUG DicerKO DicerKO.1   6    G
```

```
> drosha %>% head
```

```
      Seed   Sample     SeedID Pos Base
  1 AAAAGC DroshaKO DroshaKO.1   1    A
  2 AAAAGC DroshaKO DroshaKO.1   2    A
  3 AAAAGC DroshaKO DroshaKO.1   3    A
  4 AAAAGC DroshaKO DroshaKO.1   4    A
  5 AAAAGC DroshaKO DroshaKO.1   5    G
  6 AAAAGC DroshaKO DroshaKO.1   6    C
```

```
> wildtype %>% head
```

```
      Seed   Sample     SeedID Pos Base
  1 AAAGAA Wildtype Wildtype.1   1    A
  2 AAAGAA Wildtype Wildtype.1   2    A
  3 AAAGAA Wildtype Wildtype.1   3    A
  4 AAAGAA Wildtype Wildtype.1   4    G
  5 AAAGAA Wildtype Wildtype.1   5    A
  6 AAAGAA Wildtype Wildtype.1   6    A
```

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

```
      Seed  Sample    SeedID Pos Base
  1 AAAGUG DicerKO DicerKO.1   1    A
  2 AAAGUG DicerKO DicerKO.1   2    A
  3 AAAGUG DicerKO DicerKO.1   3    A
  4 AAAGUG DicerKO DicerKO.1   4    G
  5 AAAGUG DicerKO DicerKO.1   5    U
  6 AAAGUG DicerKO DicerKO.1   6    G
```

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

```
          Seed   Sample       SeedID Pos Base
   16513 UUGGCA Wildtype Wildtype.951   1    U
   16514 UUGGCA Wildtype Wildtype.951   2    U
   16515 UUGGCA Wildtype Wildtype.951   3    G
   16516 UUGGCA Wildtype Wildtype.951   4    G
   16517 UUGGCA Wildtype Wildtype.951   5    C
   16518 UUGGCA Wildtype Wildtype.951   6    A
```

Do the counts match?

```
> # check total seeds/id's
> (nrow(dicer)/6 + nrow(drosha)/6 + nrow(wildtype)/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.seedLong.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.seedLong.2021-08-1
2.csv"
+   dlm="," firstobs=2;
+ input Seed $ sample $ seedid $ pos base $ ;
+ run;
+
+ proc glimmix data=seedlong outdesign = xx method=rmpl;
+ class seed seedid sample(ref="Wildtype") 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	Residual MPL
Degrees of Freedom Method	Between-Within

Class Level Information

Class	Levels	Values

seed	240	AAAAGC AAAGAA AAAGCA AAAGCU AAAGUG AACACC AACACU AACCGG AACGGA AAGCUG AAGGUG AAUACU AAUCCC ACAGUA ACAUUC ACCCCA ACCCGG ACCCGU ACCCUG ACCGAG ACCGCC ACCGGG ACCUCC ACCUCG ACCUGG ACGCCU ACGCGA ACUCUG ACUGCU ACUGGC AGCACC AGCAGC AGCCGU AGCCUG AGCGAG AGCUGC AGCUGG AGCUUA AGGAGC AGGUAG AGUACG AGUGCA AGUUUC AUAUAU AUCCAG AUCCCA AUCCCC AUCCGG AUCGGG AUGACA AUGCCU AUGGCA AUGGCG AUGGCU AUUAUU AUUGCA CAAAAC CAAAGU CAAGAG CACAGU CACCAG CACCGC CACCGG CACCUG CACUGC CAGGCU CAGUGC CAUAGC CCACCC CCACGG CCAGCU CCAGGA CCCACA CCCACC CCCACU CCCAUA CCCCAC CCCCAG CCCCCA CCCCGA CCCCGC CCCCGG CCCCGU CCCCUG CCCGCA CCCGCG CCCGGA CCCGGC CCCGGG CCCUGA CCCUGC CCCUGG CCCUGU CCGAGU CCGCCG CCGCGG CCGGAG CCGGCG CCGGCU CCGGGC CCGGGU CCUCAC CCUCGU CCUCUU CCUGCC CCUGCU CCUGGC CCUGGU CGAAAC CGAAUC CGACCG CGAGGA CGAUUC CGCACU CGCCGC CGCCUG CGCGAC CGCGGG CGGAGG CGGCGG CGGGCG CGGGUC CGGGUG CGUACC CGUACG CGUAUC CUACAU CUAUGC CUCACC CUCAUG CUCGCU CUCGGC CUCGGU CUCUCG CUGACU CUGAUU CUGCAG CUGCCC CUGGAC CUGGUC CUGGUU CUUUGG GAACAG GAGCCA GAGGGG GAGGGU GAGGUA GCACCA GCAGAG GCAGCA GCAUCC GCCCGC GCCGCG GCCGUG GCCUGG GCGAGG GCGCGU GCGGGC GCGGGG GCGGGU GCUACA GCUAUG GCUCAG GCUCGA GCUCGG GCUGGU GCUUAU GGAAGA GGAGAG GGCAAG GGCAGU GGCCGA GGCGGC GGCUCA GGCUGG GGGCUG GGGGCC GGGUCG GGUACG GGUGCG GUAAAC GUACCG GUAGCG GUAGUG GUGCAA GUGCGC GUUGUA GUUUCU UAAGCC UAAUAC UACAGU UAGACU UAGAGG UAGCAC UAGCGA UAGCUU UAUAAU UAUACG UAUCAG UCAAGU UCACAG UCACAU UCACCA UCACCU UCAGGA UCAUGG UCCAGC UCCAGU UCCCAC UCCCCG UCCCCU UCCCGG UCCCUC UCCCUG UCCGAG UCCGGC UCCGGG UCCUGC UCGCUU UCGGCG UCUAAA UCUCAC UCUCGC UCUCGG UCUUUG UGCAUA UGCAUU UGCCGC UGCGCA UGGUCC UGUACG UGUCUG UGUGCG UUCCCG UUCCGG UUCGAU UUCUCA UUGCAC UUGGCA UUUCCG
seedid	2753	not printed
sample	3	DicerKO DroshaKO Wildtype
pos	6	1 2 3 4 5 6
base	4	A C G U

Number of Observations Read	16518

Number of Observations Used	16518

Response Profile

Ordered Value	base	Total Frequency
1	A	3204
2	C	6355
3	G	4285
4	U	2674

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

Dimensions	
G-side Cov. Parameters	4
Columns in X	84
Columns in Z per Subject	4
Subjects (Blocks in V)	2753
Max Obs per Subject	6

Optimization Information	
Optimization Technique	Dual Quasi-Newton
Parameters in Optimization	4
Equality Constraints	1

Lower Boundaries	4
Upper Boundaries	1
Fixed Effects	Profiled
Starting From	Data

Iteration History

Iteration	Restarts	Subiterations	Objective Function	Change	Max Gradient
0	**0**	**7**	188693.55234	2.00000000	0.037381
1	**0**	**3**	189090.22873	2.00000000	0.000023
2	**0**	**2**	191080.04613	2.00000000	0.009061
3	**0**	**2**	191279.13083	0.23401438	3.222E-8
4	**0**	**2**	191283.77184	0.00009077	3.624E-8
5	**0**	**0**	191283.77528	0.00000000	3.09E-6

Convergence criterion (PCONV=1.11022E-8) satisfied.

Estimated G matrix is not positive definite.

Fit Statistics

-2 Res Log Pseudo-Likelihood	191283.8

Covariance Parameter Estimates

Cov Parm	Subject	Group	Estimate	Standard Error
CHOL(1,1)	seedid	base A	0.5000	.
CHOL(1,1)	seedid	base C	0.4217	0.03410
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			-1.3128	0.09827	2729	-13.36	<.0001
Intercept	G			-1.1292	0.09066	2729	-12.46	<.0001
Intercept	U			-1.1480	0.09131	2729	-12.57	<.0001
sample	C	DicerKO		1.7684	0.1341	2729	13.19	<.0001
sample	G	DicerKO		1.4992	0.1290	2729	11.63	<.0001
sample	U	DicerKO		0.4891	0.1514	2729	3.23	0.0013
sample	C	DroshaKO		2.9584	0.2031	2729	14.57	<.0001
sample	G	DroshaKO		3.5761	0.1919	2729	18.64	<.0001
sample	U	DroshaKO		2.8811	0.1983	2729	14.53	<.0001
sample	C	Wildtype		0	.	.	.	.
sample	G	Wildtype		0	.	.	.	.
sample	U	Wildtype		0	.	.	.	.
pos	C		1	-0.9188	0.1731	2729	-5.31	<.0001
pos	G		1	0.6124	0.1164	2729	5.26	<.0001

pos	**U**		**1**	-0.5293	0.1448	2729	-3.66	0.0003
pos	**C**		**2**	1.3850	0.1341	2729	10.33	<.0001
pos	**G**		**2**	1.6704	0.1232	2729	13.55	<.0001
pos	**U**		**2**	0.2229	0.1544	2729	1.44	0.1491
pos	**C**		**3**	2.2700	0.1315	2729	17.26	<.0001
pos	**G**		**3**	0.9229	0.1443	2729	6.40	<.0001
pos	**U**		**3**	1.1018	0.1411	2729	7.81	<.0001
pos	**C**		**4**	0.3252	0.1415	2729	2.30	0.0216
pos	**G**		**4**	0.6586	0.1252	2729	5.26	<.0001
pos	**U**		**4**	0.8303	0.1230	2729	6.75	<.0001
pos	**C**		**5**	1.9927	0.1392	2729	14.31	<.0001
pos	**G**		**5**	1.0889	0.1471	2729	7.40	<.0001
pos	**U**		**5**	1.9906	0.1332	2729	14.94	<.0001
pos	**C**		**6**	0	.	.	.	.
pos	**G**		**6**	0	.	.	.	.
pos	**U**		**6**	0	.	.	.	.
sample*pos	**C**	**DicerKO**	**1**	1.1915	0.2115	13735	5.63	<.0001
sample*pos	**G**	**DicerKO**	**1**	-2.9601	0.2422	13735	-12.22	<.0001
sample*pos	**U**	**DicerKO**	**1**	1.0444	0.2125	13735	4.91	<.0001
sample*pos	**C**	**DicerKO**	**2**	1.5772	0.2703	13735	5.84	<.0001
sample*pos	**G**	**DicerKO**	**2**	0.004345	0.2737	13735	0.02	0.9873
sample*pos	**U**	**DicerKO**	**2**	1.2570	0.3223	13735	3.90	<.0001
sample*pos	**C**	**DicerKO**	**3**	0.3778	0.2389	13735	1.58	0.1139

sample*pos	**G**	**DicerKO**	**3**	-0.2250	0.2645	13735	-0.85	0.3948
sample*pos	**U**	**DicerKO**	**3**	-0.04753	0.2919	13735	-0.16	0.8707
sample*pos	**C**	**DicerKO**	**4**	0.8536	0.2047	13735	4.17	<.0001
sample*pos	**G**	**DicerKO**	**4**	-0.2423	0.2020	13735	-1.20	0.2305
sample*pos	**U**	**DicerKO**	**4**	0.6045	0.2157	13735	2.80	0.0051
sample*pos	**C**	**DicerKO**	**5**	-3.2896	0.2056	13735	-16.00	<.0001
sample*pos	**G**	**DicerKO**	**5**	-0.8736	0.1923	13735	-4.54	<.0001
sample*pos	**U**	**DicerKO**	**5**	-1.5167	0.2053	13735	-7.39	<.0001
sample*pos	**C**	**DicerKO**	**6**	0	.	.	.	.
sample*pos	**G**	**DicerKO**	**6**	0	.	.	.	.
sample*pos	**U**	**DicerKO**	**6**	0	.	.	.	.
sample*pos	**C**	**DroshaKO**	**1**	0.2040	0.2696	13735	0.76	0.4492
sample*pos	**G**	**DroshaKO**	**1**	-2.2260	0.2319	13735	-9.60	<.0001
sample*pos	**U**	**DroshaKO**	**1**	-0.8743	0.2567	13735	-3.41	0.0007
sample*pos	**C**	**DroshaKO**	**2**	-1.1516	0.2454	13735	-4.69	<.0001
sample*pos	**G**	**DroshaKO**	**2**	-4.8489	0.2699	13735	-17.97	<.0001
sample*pos	**U**	**DroshaKO**	**2**	-2.9818	0.3009	13735	-9.91	<.0001
sample*pos	**C**	**DroshaKO**	**3**	-2.1477	0.2460	13735	-8.73	<.0001
sample*pos	**G**	**DroshaKO**	**3**	-2.8291	0.2557	13735	-11.06	<.0001
sample*pos	**U**	**DroshaKO**	**3**	-3.7410	0.2932	13735	-12.76	<.0001
sample*pos	**C**	**DroshaKO**	**4**	0.06387	0.2823	13735	0.23	0.8210
sample*pos	**G**	**DroshaKO**	**4**	-1.2034	0.2702	13735	-4.45	<.0001
sample*pos	**U**	**DroshaKO**	**4**	-0.6354	0.2734	13735	-2.32	0.0201

sample*pos	**C**	**DroshaKO**	**5**	-2.8822	0.2559	13735	-11.26	<.0001
sample*pos	**G**	**DroshaKO**	**5**	-1.8644	0.2492	13735	-7.48	<.0001
sample*pos	**U**	**DroshaKO**	**5**	-4.5446	0.2854	13735	-15.92	<.0001
sample*pos	**C**	**DroshaKO**	**6**	0	.	.	.	.
sample*pos	**G**	**DroshaKO**	**6**	0	.	.	.	.
sample*pos	**U**	**DroshaKO**	**6**	0	.	.	.	.
sample*pos	**C**	**Wildtype**	**1**	0	.	.	.	.
sample*pos	**G**	**Wildtype**	**1**	0	.	.	.	.
sample*pos	**U**	**Wildtype**	**1**	0	.	.	.	.
sample*pos	**C**	**Wildtype**	**2**	0	.	.	.	.
sample*pos	**G**	**Wildtype**	**2**	0	.	.	.	.
sample*pos	**U**	**Wildtype**	**2**	0	.	.	.	.
sample*pos	**C**	**Wildtype**	**3**	0	.	.	.	.
sample*pos	**G**	**Wildtype**	**3**	0	.	.	.	.
sample*pos	**U**	**Wildtype**	**3**	0	.	.	.	.
sample*pos	**C**	**Wildtype**	**4**	0	.	.	.	.
sample*pos	**G**	**Wildtype**	**4**	0	.	.	.	.
sample*pos	**U**	**Wildtype**	**4**	0	.	.	.	.
sample*pos	**C**	**Wildtype**	**5**	0	.	.	.	.
sample*pos	**G**	**Wildtype**	**5**	0	.	.	.	.
sample*pos	**U**	**Wildtype**	**5**	0	.	.	.	.
sample*pos	**C**	**Wildtype**	**6**	0	.	.	.	.
sample*pos	**G**	**Wildtype**	**6**	0	.	.	.	.

sample*pos	**U**	**Wildtype**	**6**	0	.	.	.	.

Odds Ratio Estimates

Comparison	Estimate	DF	95% Confidence Limits	
C: sample DicerKO vs Wildtype	6.598	2729	5.696	7.643
G: sample DicerKO vs Wildtype	2.188	2729	1.881	2.546
U: sample DicerKO vs Wildtype	2.040	2729	1.743	2.387
C: sample DroshaKO vs Wildtype	7.191	2729	6.238	8.289
G: sample DroshaKO vs Wildtype	4.113	2729	3.581	4.724
U: sample DroshaKO vs Wildtype	2.120	2729	1.806	2.490
C: pos 1 vs 6	0.635	2729	0.524	0.771
G: pos 1 vs 6	0.327	2729	0.267	0.402
U: pos 1 vs 6	0.623	2729	0.512	0.759
C: pos 2 vs 6	4.604	2729	3.687	5.748
G: pos 2 vs 6	1.057	2729	0.833	1.341
U: pos 2 vs 6	0.703	2729	0.537	0.921
C: pos 3 vs 6	5.366	2729	4.362	6.600
G: pos 3 vs 6	0.909	2729	0.729	1.134
U: pos 3 vs 6	0.851	2729	0.660	1.098
C: pos 4 vs 6	1.880	2729	1.526	2.315
G: pos 4 vs 6	1.193	2729	0.972	1.464
U: pos 4 vs 6	2.270	2729	1.835	2.809
C: pos 5 vs 6	0.937	2729	0.772	1.138

G: pos 5 vs 6	1.193	2729	0.994	1.431
U: pos 5 vs 6	0.971	2729	0.785	1.201

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
sample	6	2729	189.90	<.0001
pos	15	2729	138.39	<.0001
sample*pos	30	13735	56.61	<.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;
+ where _sample = "Wildtype";
+ var sample _sample slice base OddsRatio AdjLowerOR AdjUpperOR Adjp;
+ run;
```

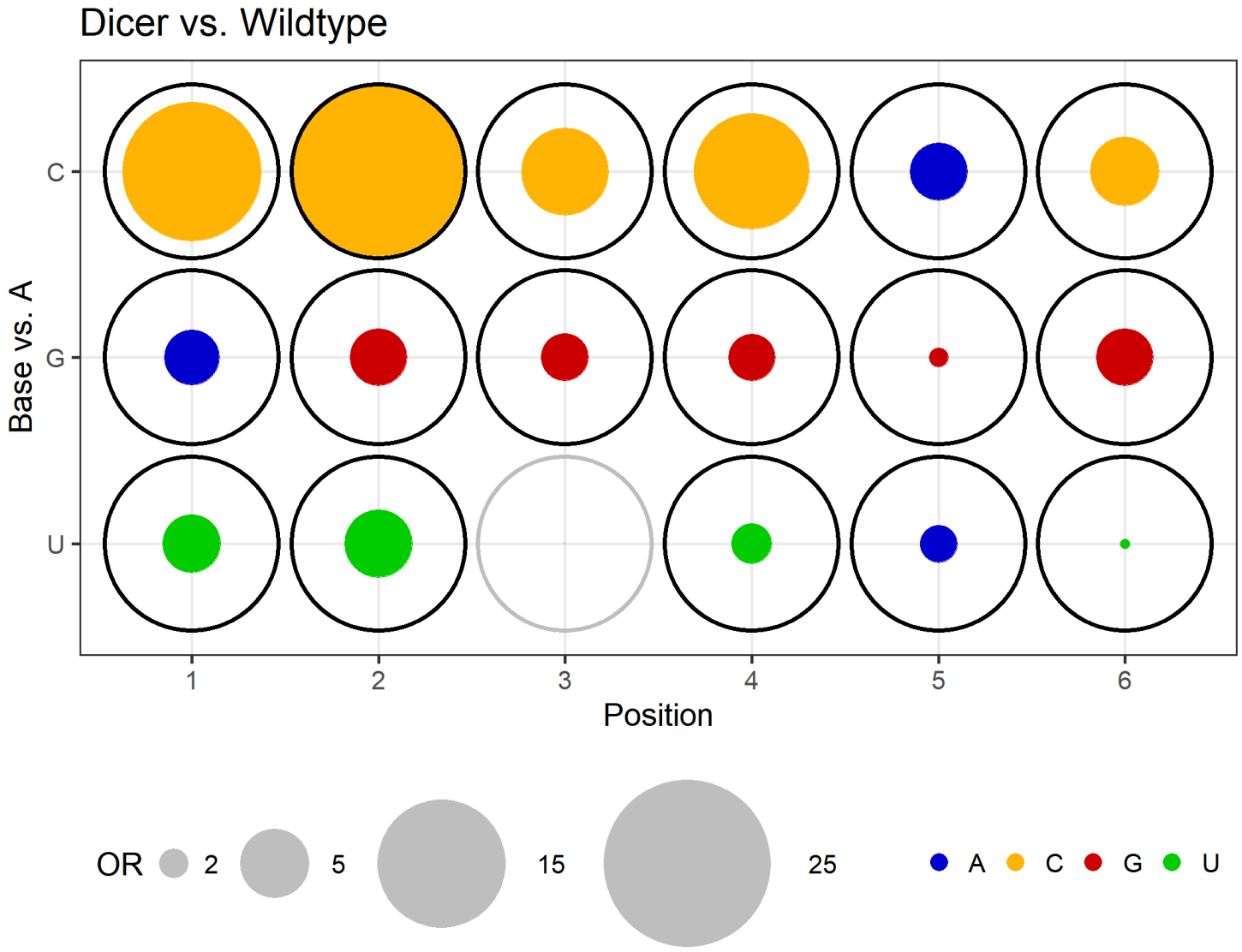
Obs	sample	_sample	Slice	base	OddsRatio	AdjLowerOR	AdjUpperOR	Adjp
2	DicerKO	Wildtype	pos 1	C	19.295	13.080	28.463	<.0001
4	DicerKO	Wildtype	pos 1	G	0.232	0.143	0.375	<.0001
6	DicerKO	Wildtype	pos 1	U	4.634	3.267	6.574	<.0001
8	DicerKO	Wildtype	pos 2	C	28.375	16.308	49.370	<.0001

10	DicerKO	Wildtype	pos 2	G	4.498	2.554	7.921	<.0001
12	DicerKO	Wildtype	pos 2	U	5.732	2.942	11.169	<.0001
14	DicerKO	Wildtype	pos 3	C	8.552	5.355	13.655	<.0001
16	DicerKO	Wildtype	pos 3	G	3.576	2.081	6.143	<.0001
18	DicerKO	Wildtype	pos 3	U	1.555	0.866	2.791	0.1800
20	DicerKO	Wildtype	pos 4	C	13.763	9.523	19.889	<.0001
22	DicerKO	Wildtype	pos 4	G	3.515	2.441	5.060	<.0001
24	DicerKO	Wildtype	pos 4	U	2.985	2.083	4.278	<.0001
26	DicerKO	Wildtype	pos 5	C	0.218	0.151	0.317	<.0001
28	DicerKO	Wildtype	pos 5	G	1.869	1.338	2.612	<.0001
30	DicerKO	Wildtype	pos 5	U	0.358	0.259	0.495	<.0001
32	DicerKO	Wildtype	pos 6	C	5.861	4.280	8.026	<.0001
34	DicerKO	Wildtype	pos 6	G	4.478	3.310	6.059	<.0001
36	DicerKO	Wildtype	pos 6	U	1.631	1.144	2.326	0.0036
37	DroshaKO	Wildtype	pos 1	C	23.628	15.512	35.989	<.0001
38	DroshaKO	Wildtype	pos 1	G	3.858	2.842	5.236	<.0001
39	DroshaKO	Wildtype	pos 1	U	7.439	5.076	10.904	<.0001
40	DroshaKO	Wildtype	pos 2	C	6.091	4.381	8.468	<.0001
41	DroshaKO	Wildtype	pos 2	G	0.280	0.179	0.437	<.0001
42	DroshaKO	Wildtype	pos 2	U	0.904	0.532	1.537	0.8967
43	DroshaKO	Wildtype	pos 3	C	2.249	1.614	3.134	<.0001
44	DroshaKO	Wildtype	pos 3	G	2.111	1.420	3.137	<.0001
45	DroshaKO	Wildtype	pos 3	U	0.423	0.255	0.702	0.0002

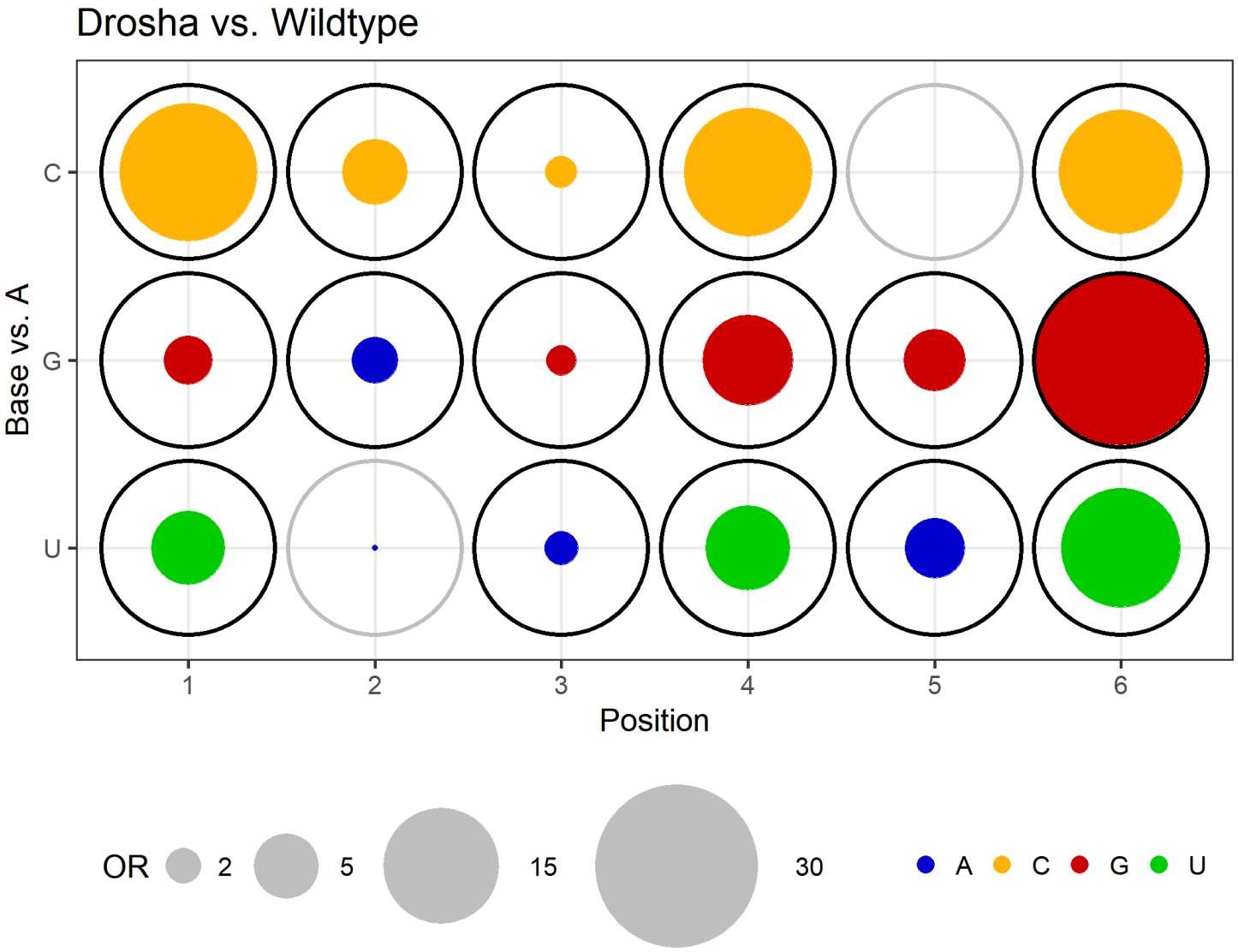
46	DroshaKO	Wildtype	pos 4	C	20.537	12.911	32.668	<.0001
47	DroshaKO	Wildtype	pos 4	G	10.726	6.868	16.753	<.0001
48	DroshaKO	Wildtype	pos 4	U	9.446	6.076	14.686	<.0001
49	DroshaKO	Wildtype	pos 5	C	1.079	0.745	1.563	0.8798
50	DroshaKO	Wildtype	pos 5	G	5.539	3.815	8.042	<.0001
51	DroshaKO	Wildtype	pos 5	U	0.189	0.117	0.307	<.0001
52	DroshaKO	Wildtype	pos 6	C	19.267	11.969	31.014	<.0001
53	DroshaKO	Wildtype	pos 6	G	35.734	22.791	56.027	<.0001
54	DroshaKO	Wildtype	pos 6	U	17.833	11.205	28.382	<.0001

```
> * 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_Fig2_081221
.xlsx"
+   dbms=excel replace;
+   sheet="OR Position";
+ run;
```

4 Dicer vs. Wildtype



5 Drosha vs. Wildtype


Drosha vs. Wildtype
Base vs. A
C
G
U
1
2
3
4
5
6
Position
OR
2
5
15
30
A
C
G
U