

# Lecture 9: Building Functions

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November 14, 2016

# R Functions are objects

R is a functional programming language. This means that functions are "objects", just like data frames, vectors, and other things that are assigned to variables and passed to other functions.

# A rose by any other name

The name of a function is actually the name of a variable that contains the function, in the same way that the

```
log
```

```
## function (x, base = exp(1)) .Primitive("log")
```

This means that we can create a copy of a function by assigning its value to a new variable.

```
myLogFunction <- log  
myLogFunction
```

```
## function (x, base = exp(1)) .Primitive("log")
```

# Functions are a kind of data and have a class

```
myNumber <- 7  
class(myNumber)
```

```
## [1] "numeric"
```

```
class(log)
```

```
## [1] "function"
```

# Creating a function

We can create a new function using the word "function" followed by the functions arguments and one or more R statements.

```
myDumbFunction <- function() 42  
myDumbFunction()
```

```
## [1] 42
```

# Creating a multi-statement function

If there is more than one statement in a function, they should be enclosed in curly brackets:

```
doubleIt <- function(x) {  
  myResult <- x * 2  
  myResult  
}  
doubleIt(5)
```

```
## [1] 10
```

The last statement within the curly brackets will be the value returned by the function.

# Functions live in their own little world

Inside a function, variables that existed in your environment can be used and even changed. However, any changes made, including changing data stored in variables and creating new variables, happens solely within the function. Your environment stays the same.

```
exists("myResult")
```

```
## [1] FALSE
```

```
myResult <- 1000
```

```
doubleItOutput <- doubleIt(2)
```

```
myResult
```

```
## [1] 1000
```

# Example Data Set

The data set used in today's lecture comes from an siRNA screen that we published a few years ago. The screen looked for genes that influence parkin translocation.

**High-content genome-wide RNAi screens identify regulators of parkin upstream of mitophagy.** Hasson SA, Kane LA, Yamano K, Huang CH, Sliter DA, Buehler E, Wang C, Heman-Ackah SM, Hessa T, Guha R, Martin SE, Youle RJ. Nature. 2013.

The data set will be available for download from the lectures portion of the class web page.



# Preview of the Data

Gene Symbol		Median Negative Control on Plate	Median Positive Control on Plate	PPT Sample as Percentage of Negative Control	PPT MAD Log Z-Score	Median PPT Log Mad Z-Score for all siRNAs having the same seed	Number of siRNAs having the same seed	Cell Count on Plate	Median Negative Control on Plate	Median Positive Control on Plate	Sample Cell Count MAD Z-Score Normalized to Negative	Median Negative Control on Plate	Median Positive Control on Plate	Sample Mitophagy MAD Z-Score Normalized to Negative	HUGO Gene Entrez	Row Number	Column Number	Ambion siRNA ID		
4	UAMN1	9.151514	38.61376	96.735081	-2.37885583	-2.41729712	-4.82722975	-2.09615864	128	128	1030.5	1.08517777	8.942457	10.462181	2.323055	0.04662106	1	4	818218	
6	MDI05	7.91536	33.146945	96.652828	23.7361874	-2.40057854	-4.77841378	-1.42101836	152	1203	1310.5	1.05015	2.24078157	16.791355	28.017045	49.016735	0.88787185	15	2	123866
7	PFN2	12.26818	33.166188	97.067871	25.4035876	-2.35813265	-5.53930527	-1.42101836	152	1177	1240	9.785	0.39941611	11.299915	29.03928	50.76025	-1.7000132	14	19	10379
8	KIAA1191	7.06218	33.765187	96.889565	25.6017581	-2.55312282	-5.51642745	-1.38836379	28	1060	1345.5	1.0615	1.02741248	11.132075	55.536245	27.7216	0.428081078	18	11	122099
9	CIBOR1	8.87857	34.335125	96.121174	25.8930085	-2.34678463	-4.46880947	-0.48514708	18	1277	1294.5	985	1.16824827	12.893802	12.099825	0.94152895	1	12	122581	
10	LOC50689	9.59596	36.724075	91.666073	26.1298885	-2.33993117	-4.54202707	-1.38836379	28	1093	1375.5	1.0615	0.56574784	14.181152	10.37487	1.8885533	2.08854154	1	2	123748
11	UAMN1	7.04537	26.493405	95.491585	26.4482474	-2.32679673	-4.39629723	-1.42101836	152	1209	1019.5	0.73184759	5.128205	8.7637465	20.23281	0.934840893	1	4	8219	
12	FAM73A	10.74058	38.900205	96.750781	26.94172518	-2.31930462	-4.366096	-0.762075318	28	854	1202	1059	0.21184759	23.633397	26.59421	44.646865	0.24361276	16	15	1545
13	CITR177	11.32515	40.214365	96.925455	28.181867	-2.28844919	-4.23547931	-0.17848188	75	1203	1355.5	1.025	0.33126082	17.049181	25.9359045	41.648632	0.65931957	17	17	1048
14	SAMD1	12.39604	43.454325	96.020801	28.282032	-2.77916385	-4.19737272	-0.35800881	18	1136	1208	1.005	0.46105972	9.154599	33.7626	1.06135717	SAMD1	1	14	122484
15	LOC42441	11.426256	38.900205	96.750781	28.5726185	-2.27826069	-4.15771176	-0.10011668	8	1126	1242	1059	0.02140758	34.63388	26.59421	44.646865	1.84802138	1	4	8208
16	WDR24	11.59552	38.900205	96.938275	28.85226575	-2.26704753	-4.16401364	-0.00417018	62	869	1345.5	1.0415	0.28469718	10.126582	27.585454	1.78558059	WDR24	1	18	138663
17	CARF7	9.84816	34.335125	96.121174	28.97445692	-2.267854123	-4.15158176	-1.60215688	18	1206	1294.5	985	0.344255071	10.414376	12.099825	0.12489771	1	6	49983	
18	LOC713855	10.84614	37.1028935	96.6317825	29.1279411	-2.26130825	-4.25445434	-0.41561139	50	1231	1355	1037.5	0.14738309	27.518622	27.637025	42.84036	0.67531389	1	13	161437
19	MGC16121	12.146893	41.1336935	96.96704	29.5327547	-2.253770524	-4.095566791	-0.00417018	62	1012	1333.5	1.025	0.17828563	26.482214	25.36282	40.9147165	0.84745085	1	1	339508
20	DEFB129	12.22562	41.674112	96.520455	30.04829953	-2.24664658	-4.0443201	-0.619984101	32	1091	1200	1.080	0.65846236	8.890925	10.76316	22.06597	0.05415426	16	5	125567
21	ATXN1L	11.61615	37.9373795	96.845925	30.05192033	-2.22617923	-3.988088623	-1.46110951	31	1154	1346	1019	0.35238956	26.918189	29.042115	0.277407092	ATXN1L	1	18	148186
22	CHP	12.38908	33.4170975	97.58857	30.638986	-2.2567747	-3.98954167	-0.712711886	30	854	1061	809.5	0.87093993	16.93442	13.868683	23.87019	1.37446038	1	1	339508
23	FLJ01215	12.38245	42.14365	96.524545	30.79110183	-2.21823206	-3.97234541	-1.971182144	78	1207	1355.5	1.025	0.23617941	24.109362	25.9359045	41.648632	0.399464678	1	16	145036
24	VPS13C	4.555059	14.602162	97.703815	30.9521131	-2.21842746	-3.959511391	-1.470715036	17	1224	1371	1088	0.007055429	21.977123	24.113465	37.634345	0.77891145	1	12	125443
25	AMOTL2	10.32165	33.146945	96.652828	30.95220613	-2.21740958	-3.959390665	-1.42101836	152	1232	1303.5	1.050	0.390613948	12.755519	28.0817045	49.016735	-1.44708005	1	7	1278109
26	TMC1	12.21442	39.331158	97.046655	31.06497146	-2.21513704	-3.94715939	-1.75059607	15	1198	1257.5	1.057	0.58183904	8.597663	13.744405	31.707255	0.781387307	1	1	124168
27	SOD3B	10.448464	33.603115	97.397395	31.1168715	-2.21356819	-3.94112778	-1.73716651	22	750	972.5	823	1.20215703	1.6	15.161405	24.5215945	-2.88626506	1	14	134777
28	LOC28423	10.766721	34.335125	96.121174	31.3577451	-2.20766512	-3.918557383	-1.82891671	37	1151	1294.5	985	0.221578634	7.211212	12.099825	-0.88878205	1	6	49924	
29	FAM747	10.759194	34.17813	94.94933	31.5174636	-2.2041356	-3.90358313	-1.87174786	16	1212	1311	1.005	0.30467197	17.19868	5.983825	31.66597	0.34400326	1	15	146102
30	NCL_1445727	10.32389	31.07602	95.879448	32.18321598	-2.18654936	-3.84119084	-1.76028254	16	1170	1355	1016	0.38117033	6.466647	10.476745	1.74687246	1	1	123632	
31	RASL1	10.23117	31.579675	97.040435	32.3984204	-2.17960151	-3.78968416	-0.94841356	43	1281	1373.5	1.078	0.63117114	8.430913	14.54753	25.32889	0.25325658	1	13	138853
32	LOC730187	10.32368	38.915025	96.71463	32.3984204	-2.17422673	-3.797746169	-0.46695723	29	1117	1238	1067	0.08616468	24.082363	28.46302	46.04568	0.07532204	1	13	106037
33	PRK5	12.43529	37.9373795	96.845925	32.78615406	-2.17233843	-3.79174041	-0.60513382	34	1305	1346	1019	0.74757371	32.413792	29.042115	45.512695	1.14259842	1	1	148186
34	SEMA6D	5.34974	16.330773	81.414805	32.75897595	-2.15960145	-3.75920446	-0.94841356	43	1281	1373.5	1.078	0.63117114	8.430913	14.54753	25.32889	0.25325658	1	13	138853
35	FAM708	10.29508	31.20667	95.909923	33.0009862	-2.16582798	-3.797988278	-1.23264529	34	1291	1328	1013.5	0.77388861	43.77723	14.492465	21.11532	1.745486812	1	18	123635
36	LOC728516	12.39008	37.94737	96.172192	33.1705254	-2.16141893	-3.752482408	-1.27594317	40	1202	1259	1015	0.60194531	11.813643	15.780395	22.121078	0.75330912	1	14	123616
37	LOC730176	13.31421	38.915025	96.71463	33.390604	-2.15960145	-3.75920446	-0.94841356	43	1281	1373.5	1.078	0.63117114	8.430913	14.54753	25.32889	0.25325658	1	13	138853
38	LOC729747	10.43478	38.987595	96.808715	33.4554733	-2.15431199	-3.72766608	-0.47700527	20	1166	1261	1022.5	0.31475863	11.053985	10.666215	24.43183	0.82569498	1	1	860101
39	LOC728511	11.44312	40.16214	96.488995	33.47220031	-2.15388601	-3.72451011	-0.76677254	62	1089	1325.5	1.072	0.70700914	21.30349	25.790215	42.022265	0.04945972	1	22	125849
40	LCOR	5.467801	16.330773	81.414805	33.4815106	-2.15279504	-3.72530737	-1.75059607	15	1430	1373.5	1.078	0.63117114	8.430913	14.54753	25.32889	0.25325658	1	4	49924
41	IL3	13.78189	41.1336935	96.96704	33.5048667	-2.15360422	-3.72331757	-1.89533346	13	1231	1233.5	1.025	0.230969879	21.750664	25.36282	40.9147165	0.11994521	1	9	124621
42	AGR2	13.86963	41.055187	96.555149	33.7348078	-2.14602159	-3.68989072	-1.971182144	78	1097	1312	1.062	0.48719706	11.668186	16.747305	20.578175	-0.404427157	1	6	49924
43	STC2	10.55555	31.20667	95.909923	33.8247919	-2.14595847	-3.69531221	-1.2424621	6	1031	1238	1.013	0.15157684	5.237633	14.492465	21.11532	-1.44126872	1	12	12591
44	TCF11	9.80099	29.06668	97.201075	34.0624856	-2.13896212	-3.67461123	-1.25342587	28	851	1028.5	802	0.64581251	11.054829	19.45772	34.010118	-1.008308	1	8	137905
45	LOC729054	11.846154	40.642891	96.750781	34.0672662	-2.13889599	-3.67421523	-1.75059607	15	1136	1249.5	1.020	0.51404137	9.950571	11.610969	25.99139	0.60126346	1	20	127727
46	INR151	13.75780	42.14365	96.524545	34.15641201	-2.13655124	-3.66854452	-0.46695723	29	1063	1353.5	1.025	0.39890761	18.72002	25.9359045	41.648632	0.047551865	1	6	49924
47	UKL1	13.757949	37.268825	96.49448	34.189998	-2.13592827	-3.66442013	-1.45604417	65	975	1253	1029	1.13480782	14.461538	23.895603	0.88828119	1	1	124168	
48	C10orf53	14.196078	41.255145	96.411655	34.1863882	-2.13579121	-3.64935452	-1.470715036	4	1223	1316	1089	0.352272194	14.478209	27.06845	25.934545	0.03807862	1	1	123632
49	FLJ36032	14.25012	41.674112	96.520455	34.1963183	-2.13554007	-3.663099273	-0.69739234	49	1177	1208	1080	-0.01799016	7.221175	10.76316	22.06597	-0.55899124	1	1	339508
50	KCN16	13.071896	38.712395	97.036425	34.2433771	-2.13427942	-3.65887406	-1.01916283	44	874	1328.5	835.5	0.429745018	13.501144	25.734465	37.937986	0.88828119	1	1	124168
51	LOC1	14.221232	41.674112	96.520455	34.2497088	-2.13425661	-3.64973913	-1.470715036	49	1177	1208	1080	-0.38085329	6.438939	14.76216	22.96597	0.77245059	1	9	124168
52	ZN787	12.23741	38.900205	96.938275	34.2664272	-2.13736055	-3.65706183	-0.84696966	6	1320	1173	961	2.28793658	15.515512	8.431358	29.063166	0.2781366	1	1	339508
53	PGBD1	12.112932	35.24025	96.21465	34.3688037	-2.13120251	-3.64438956	-2.35697735	28											

# Import the data

```
ambion <- read.csv("nature.parkin.gw.ambion.csv", stringsAsFactors = FALSE)
str(ambion)
```

```
## 'data.frame':    65196 obs. of  25 variables:
## $ Vendor.Supplied.Gene.Symbol      : chr
## $ Sample                          : num
## $ Median.Negative.Control.on.Plate : num
## $ Median.Positive.Control.on.Plate : num
## $ PPT.Sample.as.Percentage.of.Negative.Control : num
## $ PPT.MAD.Z.Score                  : num
## $ PPT.MAD.Log.MAD.Z.Score          : num
## $ Median.PPT.Log.Mad.Z.Score.for.all.siRNAs.having.the.same.seed.sequence: num
## $ Number.of.siRNAs.having.the.same.seed.sequence : int
## $ Cell.Count..Sample                : int
## $ Median.Negative.Control.Cell.Count.on.Plate    : num
## $ Median.Positive.Control.Cell.Count.on.Plate    : num
## $ Sample.Cell.Count..MAD.Z.Score.Normalized.to.Negative.Contol : num
## $ Sample.1                                : num
## $ Median.Negative.Control.Mitophagy.on.Plate     : num
## $ Median.Positive.Control.Mitophagy.on.Plate     : num
```

# Check for missing data

```
apply(ambion, 2, function(x) sum(is.na(x)))
```

```
##                               Vendor.Supplied.Gene.Symbol
##                                                                0
##                                                                Sample
##                                                                441
##                               Median.Negative.Control.on.Plate
##                                                                441
##                               Median.Positive.Control.on.Plate
##                                                                441
##                               PPT.Sample.as.Percentage.of.Negative.Control
##                                                                441
##                               PPT.MAD.Z.Score
##                                                                441
##                               PPT.MAD.Log.MAD.Z.Score
##                                                                441
## Median.PPT.Log.Mad.Z.Score.for.all.siRNAs.having.the.same.seed.sequence
##                                                                441
##                               Number.of.siRNAs.having.the.same.seed.sequence
##                                                                441
```

# Investigate Missing Data

```
ambion[is.na(ambion[,1]),][1,]
```

```
##      Vendor.Supplied.Gene.Symbol Sample Median.Negative.Control.on.Plate
## NA                                     <NA>      NA                        NA
##      Median.Positive.Control.on.Plate
## NA                                     NA
##      PPT.Sample.as.Percentage.of.Negative.Control PPT.MAD.Z.Score
## NA                                             NA                        NA
##      PPT.MAD.Log.MAD.Z.Score
## NA                                     NA
##      Median.PPT.Log.Mad.Z.Score.for.all.siRNAs.having.the.same.seed.sequence
## NA                                             NA
##      Number.of.siRNAs.having.the.same.seed.sequence Cell.Count..Sample
## NA                                             NA                        NA
##      Median.Negative.Control.Cell.Count.on.Plate
## NA                                             NA
##      Median.Positive.Control.Cell.Count.on.Plate
## NA                                             NA
##      Sample.Cell.Count..MAD.Z.Score.Normalized.to.Negative.Contol Sample.1
## NA                                             NA                        NA
```

# Eliminate Missing Data

```
ambion <- ambion[! is.na(ambion[,2]), ]  
apply(ambion, 2, function(x) sum(is.na(x)))
```

```
##                               Vendor.Supplied.Gene.Symbol  
##                               0  
##                               Sample  
##                               0  
##                               Median.Negative.Control.on.Plate  
##                               0  
##                               Median.Positive.Control.on.Plate  
##                               0  
##                               PPT.Sample.as.Percentage.of.Negative.Control  
##                               0  
##                               PPT.MAD.Z.Score  
##                               0  
##                               PPT.MAD.Log.MAD.Z.Score  
##                               0  
## Median.PPT.Log.Mad.Z.Score.for.all.siRNAs.having.the.same.seed.sequence  
##                               0  
##                               Number.of.siRNAs.having.the.same.seed.sequence
```

# Simplify the Data

Often it will be helpful to create a new data frame with only the data we wish to analyze.

```
ambion.simple <- ambion[,c(19,25,1,21,7,13,17)]  
ambion.simple[1,]
```

```
##      Entrez.GeneID Ambion.siRNA.ID Vendor.Supplied.Gene.Symbol  
## 1           3998           s8218                LMAN1  
##              DESCRIPTION PPT.MAD.Log.MAD.Z.Score  
## 1 lectin, mannose-binding, 1                -4.82223  
##      Sample.Cell.Count..MAD.Z.Score.Normalized.to.Negative.Contol  
## 1                                           1.108518  
##      Sample.Mitophagy..MAD.Z.Score.Normalized.to.Negative.Control  
## 1                                           0.04662911
```

# Simplify our Column Names

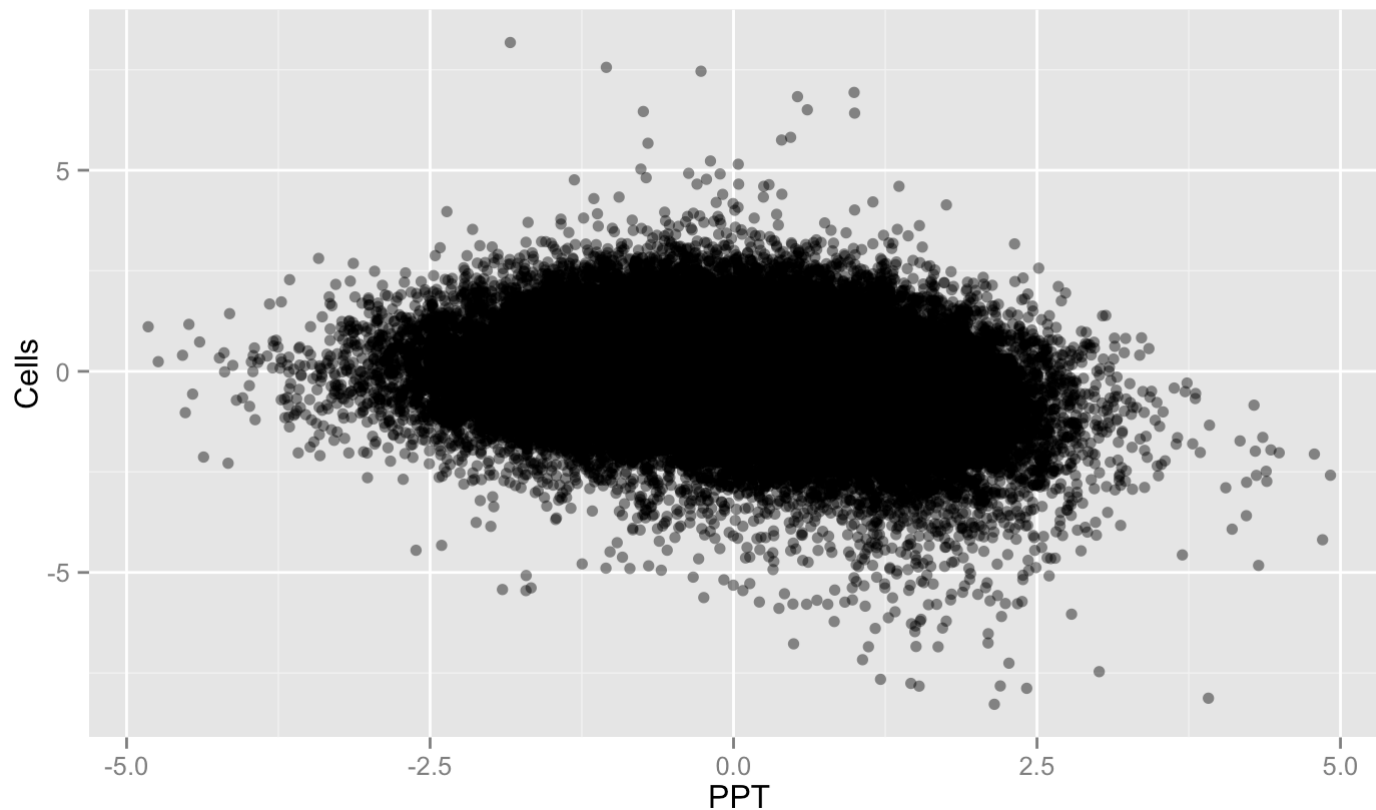
```
library(knitr, quietly = TRUE)
colnames(ambion.simple) <- c("GeneID", "siRNA", "Symbol", "Description",
                             "PPT", "Cells", "Mitophagy")
kable(head(ambion.simple, n=4), format = "markdown")
```

GeneID	siRNA	Symbol	Description	PPT	Cells	Mitophagy
3998	s8218	LMAN1	lectin, mannose-binding, 1	-4.822230	1.1085178	0.0466291
51586	s28366	MED15	mediator complex subunit 15	-4.739415	0.2405872	-0.8887362
5217	s10379	PFN2	profilin 2	-4.539309	0.3994161	-1.7000123
57179	s226909	KIAA1191	KIAA1191	-4.516443	-1.0274124	-0.4280631

---

# Evaluate how our variables interact

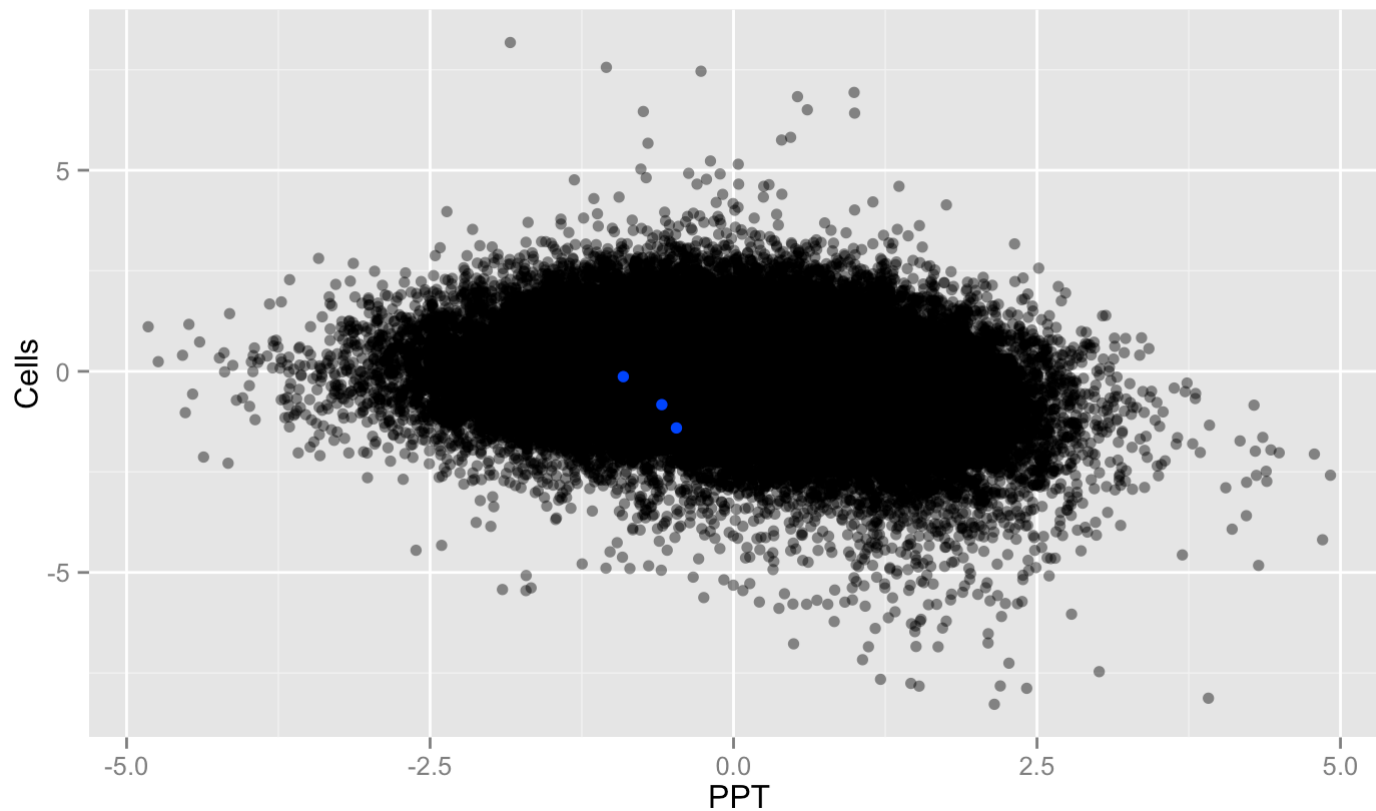
```
library(ggplot2, quietly = TRUE)  
ggplot(ambion.simple, aes(x=PPT, y=Cells)) + geom_point(alpha=0.5)
```





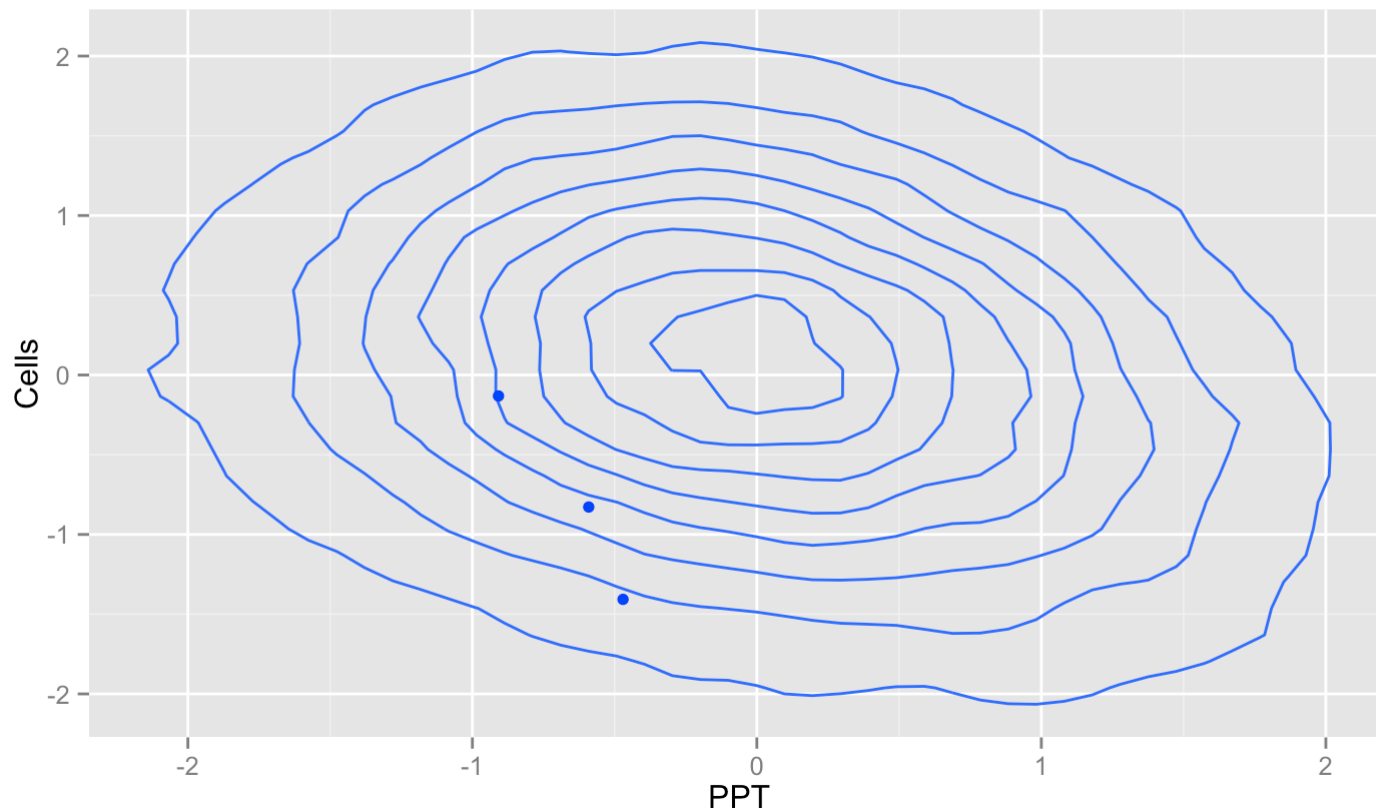
# Evaluate how our variables interact

```
ggplot(ambion.simple, aes(x=PPT, y=Cells)) + geom_point(alpha=0.5) +  
  geom_point(data = ambion.simple[ambion.simple$Symbol == "PARK2", ],  
            color="blue")
```



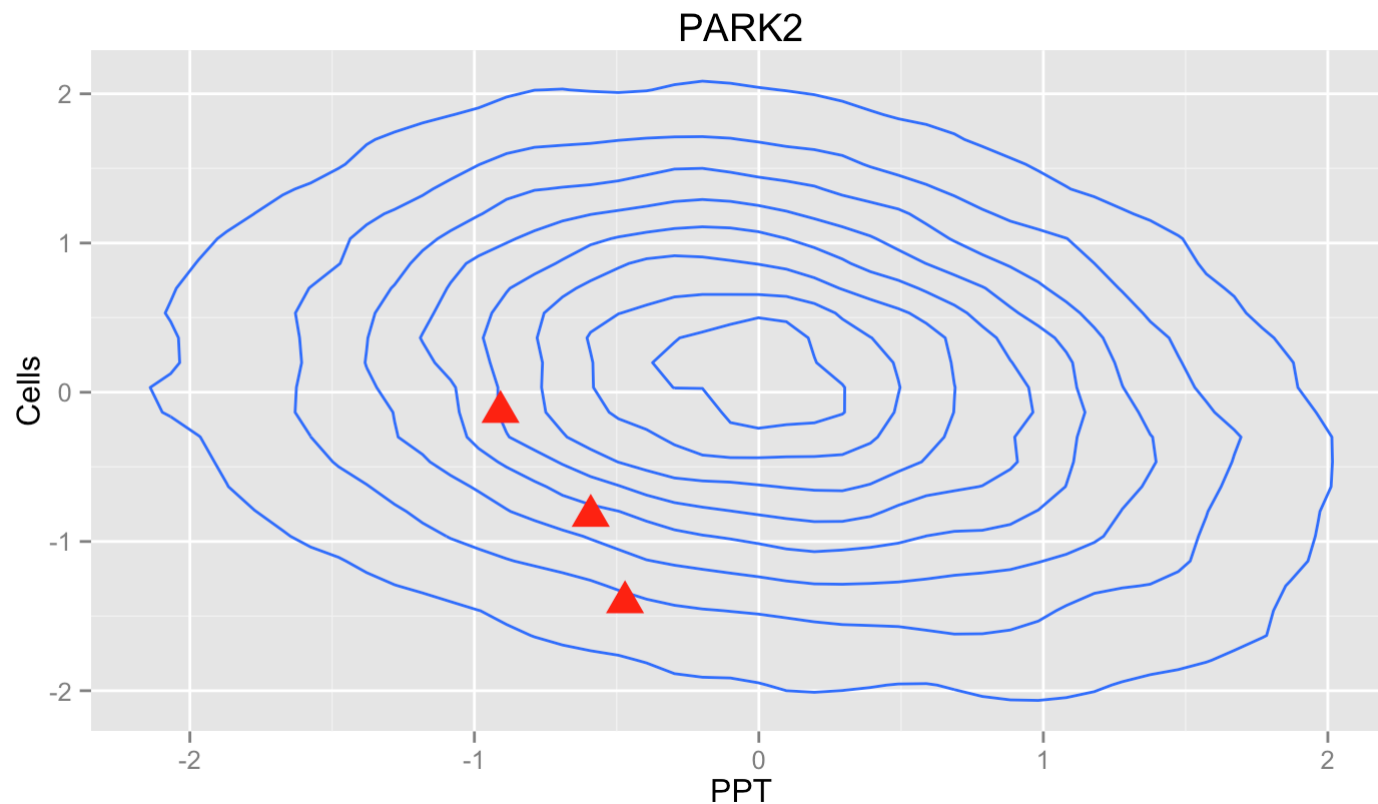
# Refine the plot

```
ggplot(ambion.simple, aes(x=PPT, y=Cells)) + geom_density2d() +  
  geom_point(data = ambion.simple[ambion.simple$Symbol == "PARK2", ],  
            color="blue")
```



# Further refine the plot

```
ggplot(ambion.simple, aes(x=PPT, y=Cells)) + geom_density2d() +  
  geom_point(data = ambion.simple[ambion.simple$Symbol == "PARK2",],  
            color="red", shape=17, size =5) +  
  ggtitle("PARK2")
```



# Adding gene description

```
description <- ambion.simple$Description[ambion.simple$Symbol == "PARK2"][1]  
description
```

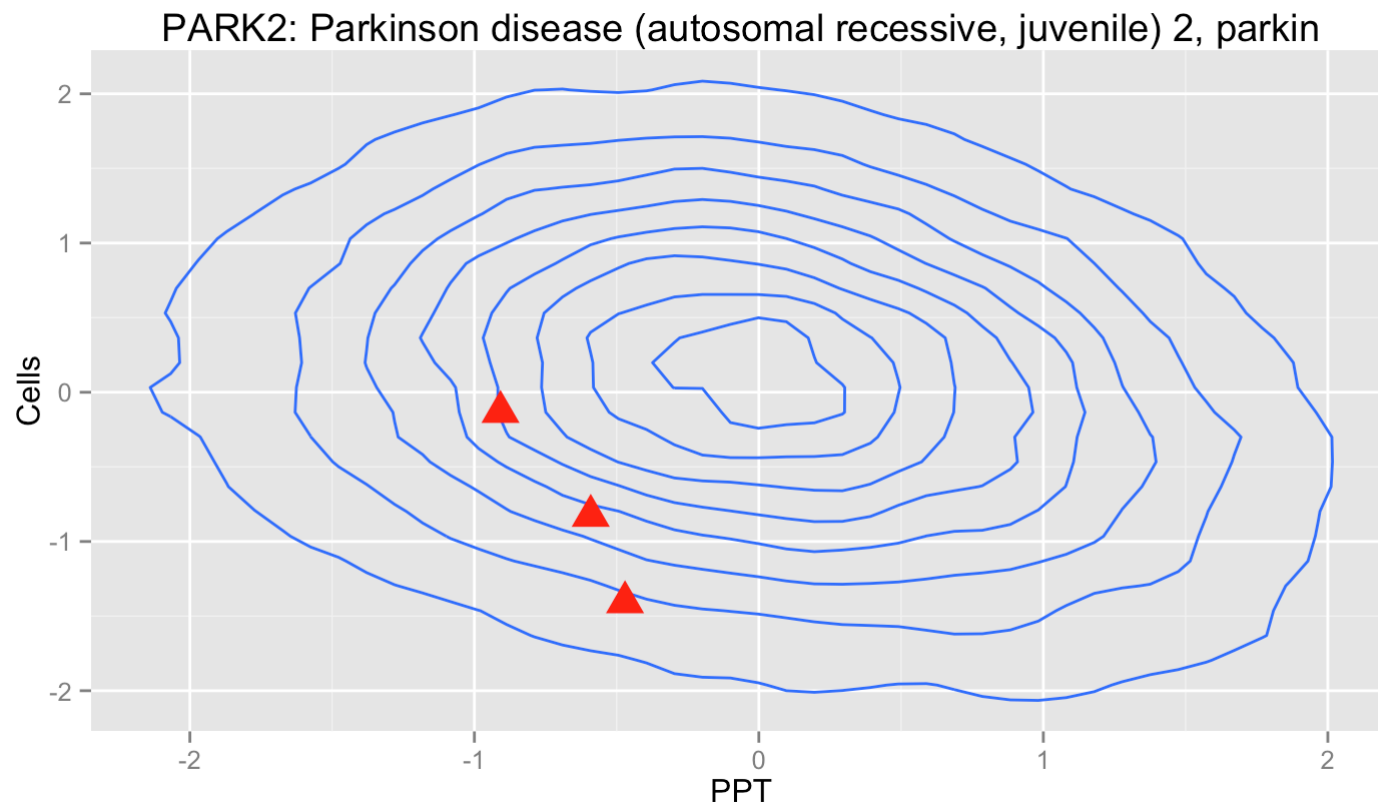
```
## [1] "Parkinson disease (autosomal recessive, juvenile) 2, parkin"
```

```
myTitle <- paste("PARK2",description,sep=": ")  
myTitle
```

```
## [1] "PARK2: Parkinson disease (autosomal recessive, juvenile) 2, parkin"
```

# Final version of plot

```
ggplot(ambion.simple, aes(x=PPT, y=Cells)) + geom_density2d() +  
  geom_point(data = ambion.simple[ambion.simple$Symbol == "PARK2", ],  
            color="red", shape=17, size =5) +  
  ggtitle(myTitle)
```



# Making the refined plot into a function

Now that we have our custom plot looking right, we would like to be able to do the same for other genes but without so much typing. First, make a new R Script in RStudio:

# Constructing a new function from your history

Frequently, making a function will simply be a function of selecting the right parts of your history and hitting the "to source" button.

# Function with PARK2 hard coded

```
graphGene <- function(gene) {  
  description <- ambion.simple$Description[ambion.simple$Symbol == "PARK2"][1]  
  myTitle <- paste("PARK2",description,sep=": ")  
  ggplot(ambion.simple, aes(x=PPT, y=Cells)) + geom_density2d() +  
    geom_point(data = ambion.simple[ambion.simple$Symbol == "PARK2",],  
              color="red", shape=17, size =5) +  
    ggtitle(myTitle)  
}
```

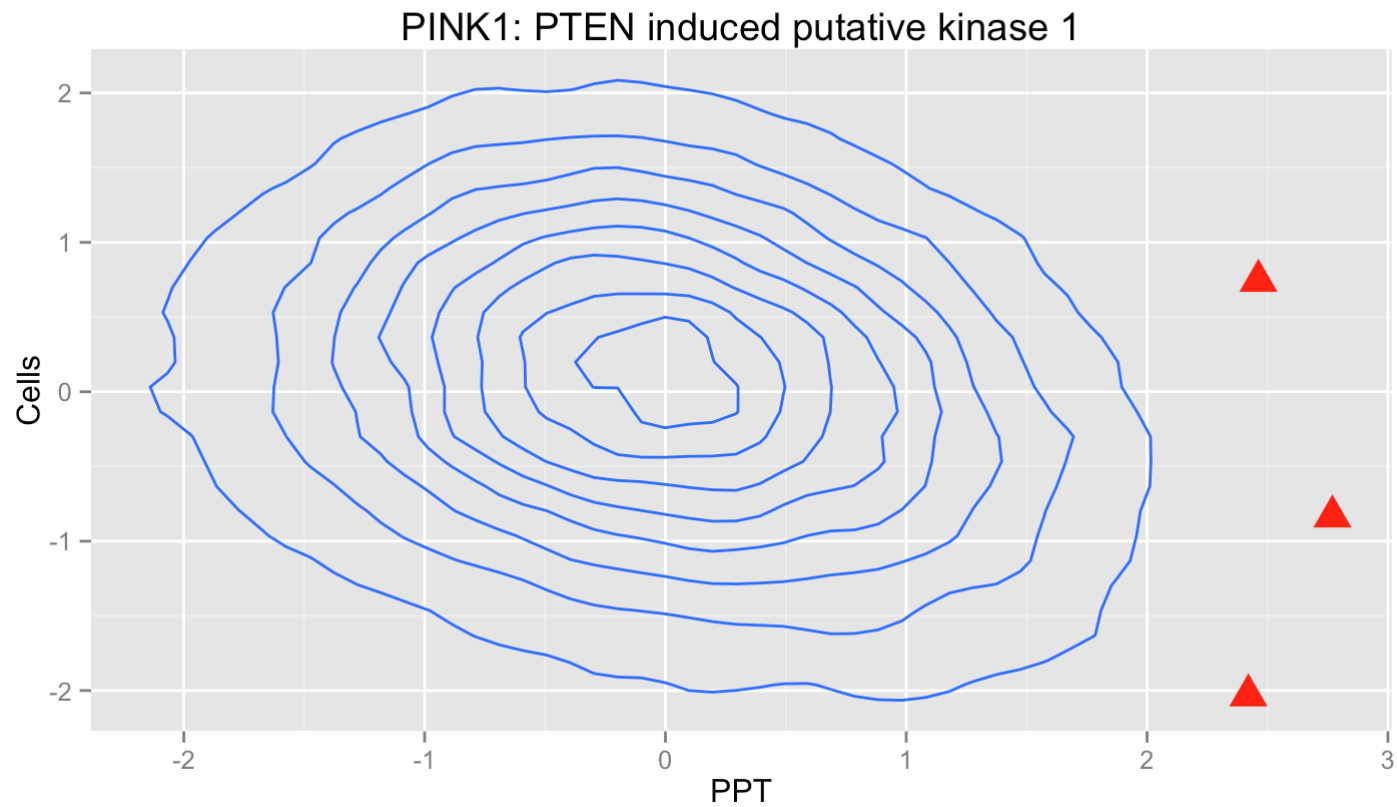


# Function made generic

```
graphGene <- function(gene) {  
  description <- ambion.simple$Description[ambion.simple$Symbol == gene][1]  
  myTitle <- paste(gene,description,sep=": ")  
  ggplot(ambion.simple, aes(x=PPT, y=Cells)) + geom_density2d() +  
    geom_point(data = ambion.simple[ambion.simple$Symbol == gene,],  
              color="red", shape=17, size =5) +  
    ggtitle(myTitle)  
}
```

# Our function in action

```
graphGene( "PINK1" )
```



# Default values for function arguments

```
pdfGene <- function(gene, file=paste(gene, ".pdf", sep="")) {  
  pdf(file, width=5, height=5)  
  graphGene(gene)  
  dev.off()  
}
```

# Passing on extra arguments to our function

We can use the ellipse notation (...) to indicate that extra arguments to our function should be passed on to a function that is inside our function (in this case pdf).

```
pdfGene <- function(gene, file=paste(gene, ".pdf", sep=""), ...) {  
  pdf(file, ...)  
  graphGene(gene)  
  dev.off()  
}  
pdfGene("PINK1", width=10, height=10)
```

```
## quartz_off_screen  
## 2
```

# Control of Flow: If/Else

We can decide whether something happens in our function using "if" and "if/else".

```
sillyFunction <- function(x) {  
  if (x < 5) {  
    returnValue <- x  
  }  
  else {  
    returnValue <- x / 2  
  }  
  returnValue  
}  
sillyFunction(12)
```

```
## [1] 6
```

# Control of Flow: For

```
pdfGenes <- function(genes, file=paste(gene, ".pdf", sep=""), ...) {  
  pdf(file, ...)  
  for (gene in genes) {  
    graphGene(gene)  
  }  
  dev.off()  
}  
pdfGenes(c("PLK1", "PINK1", "BRCA1"))
```