Analysis of C2C12 cell miRNA and mRNA expression data using the **miRNAmRNA** package

Maarten van Iterson

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1 minimal example

Download target predictions manually from PITA, TargetScan and microCosm.
Optionally construct parser for other prediction tools see ?addTable.

Construct the database and inspect its content.

```
> addTable(filePITA, tableName="pita", path=reultsDir, dbName=dbName, Org="Mm")
> addTable(fileMicrocosm, tableName="microcosm", path=resultsDir, dbName=dbName, Org="Mm")
> addTable(fileTargetScan, tableName="targetscan", path=resultsDir, dbName=dbName, Org="Mm")
> dbInfo(resultsDir, dbName)
> dbHeadTable(resultsDir, dbName, "pita")
> dbHeadTable(resultsDir, dbName, "targetscan")
> dbHeadTable(resultsDir, dbName, "microcosm", n=10)
```

Download microRNA and mRNA expression data and perform some preprocessing steps such as identifier mapping.

```
> ##extract and process data
> library(GEOquery)
> miRNA <- getGEO(filename=file.path(dataDir, "expression_data", "GSE9449_series_matrix.txt.
> miFeature <- pData(featureData(miRNA))
> miExprs <- exprs(miRNA)
> colnames(miExprs) <- c("Prol", "Conf", "+1d", "+2d", "+4d")
> strwhite <- function(x)
+   {
+     x <- sub("^[[:blank:]]*", "", x, perl=TRUE) ##leading
+     x <- sub("[[:blank:]]*$", "", x, perl=TRUE) ##trailing
+     x
+   }
> extractID <- function(x)
+   {
+     y <- unlist(strsplit(x, ","))
```

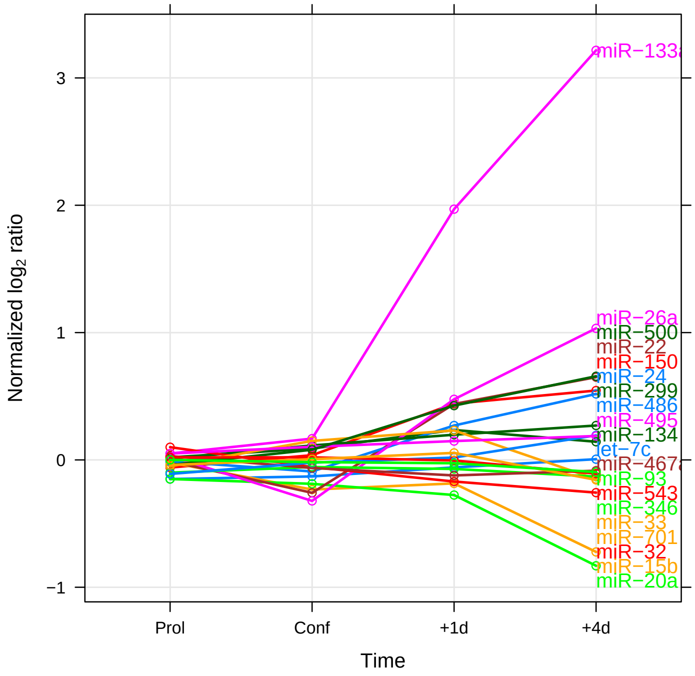
```
+     idx <- sapply(y, grepl, pattern="mmu")
+     if(any(idx))
+       return(strwhite(y[idx]))
+     NA
+   }
> mirID <- sapply(as.character(miFeature$SPOT_ID), extractID, USE.NAMES=FALSE)
> rownames(miExprs) <- mirID
> miExprs <- miExprs[,-4]
> miExprs <- miExprs[!is.na(rownames(miExprs)), ]
> miExprs <- miExprs[!duplicated(rownames(miExprs)),]
> ##some manual edits
> rownames(miExprs)[rownames(miExprs) == "mmu-miR-291a5p291b5p"] <- "mmu-miR-291a5p"
> rownames(miExprs)[rownames(miExprs) == "mmu-miR-133a133b"] <- "mmu-miR-133a"
> save(miExprs, file=file.path(resultsDir, "miExprs.RData"))
```

```
> mRNA <- getGEO(filename=file.path(dataDir, "expression_data", "GSE19968_series_matrix.txt.
> mFeature <- pData(featureData(mRNA))
> mExprs <- exprs(mRNA)
> mExprs <- cbind(rowMeans(mExprs[,1:3]), rowMeans(mExprs[,4:6]), rowMeans(mExprs[,7:9]), ro
> colnames(mExprs) <- c("Myoblast", "T0", "T24", "Myotube")
> mExprs <- mExprs[!duplicated(mFeature$GENE),,drop=FALSE] #extra calculation: remove all th
> rownames(mExprs) <- mFeature$GENE[!duplicated(mFeature$GENE)]
> save(mExprs, file=file.path(resultsDir, "mExprs.RData"))
```

Run the integrated analysis.

```
> library(miRNAmRNA)
> dbName <- "mir.Mm.db"
> resultsDir <- "/home/mviterson/Documents/packages/miRNAmRNA"
> load(file.path(resultsDir, "mExprs.RData"))
> load(file.path(resultsDir, "miExprs.RData"))
> results <- rungt(mirs=rownames(miExprs), X=mExprs, Y=miExprs, path=resultsDir, dbName=dbNa
> save(results, file=file.path(resultsDir, "C2C12pairs.RData"))
```

```
> library(lattice)
> library(directlabels)
> resultsDir <- "/home/mviterson/Documents/packages/miRNAmRNA"
> load(file.path(resultsDir, "miExprs.RData"))
> load(file=file.path(resultsDir, "C2C12pairs.RData"))
> topMirs <- head(results$mirs, n=20)
> X <- miExprs[rownames(miExprs) %in% rownames(topMirs), ]
> data <- data.frame(miExpr = as.vector(X),
+                    Time =  rep(factor(colnames(X), levels = colnames(X), ordered=TRUE), ea
+                    miRNA = gsub("mmu-", "", rep(rownames(X), ncol(X))))
> print(direct.label(xyplot(miExpr~Time, groups=miRNA, data, type=c("b", "g"), lwd=2, ylab=e
+              scales = list(x = list(labels = colnames(X))))))
```



```
> library(xtable)
> resultsDir <- "/home/mviterson/Documents/packages/miRNAmRNA"
> load(file=file.path(resultsDir, "C2C12pairs.RData"))
> topMirs <- head(results$mirs, n=20)
> topMiExprs <- miExprs[rownames(miExprs) %in% rownames(topMirs),c(1,4)]
> mirExprs <- topMiExprs[,1] < topMiExprs[,2]
> mirExprs[mirExprs==TRUE] <- "up"
> mirExprs[mirExprs==FALSE] <- "down"
> topMirs <- merge(topMirs, mirExprs, by="row.names")
> colnames(topMirs) <-  c("miRNA", "P-value", "\\# targets", "Regulation")
> xtable(topMirs[order(topMirs$'P-value'), ],
+        display=c("d", "s", "f", "d", "s"), digits= c(0, 0, 5, 0, 0),
+        caption="Overview of significant miRNA target sets with strict overlap between the
```

```
> print(xtable(mir22[,-c(4,5)],
+        display=c("d", "s", "f", "s"), digits=c(0,0,5,0),
+        caption="Overview of microRNA mmu-miR-22 targets with strict overlap between the th
+        tabular.environment="longtable", floating=FALSE)
```

	Symbol	Pvalue	Association

83767	Wasf1	0.00415	neg.
233902	Fbxl19	0.07967	neg.
76932	Arfip2	0.12357	neg.
19159	Cyth3	0.13766	neg.
67771	Arpc5	0.18739	neg.
14815	Nr3c1	0.31383	neg.
17536	Meis2	0.32297	neg.
22329	Vcam1	0.34188	neg.
21345	Tagln	0.45586	neg.
16852	Lgals1	0.47281	neg.
67074	Mon2	0.51303	neg.
230596	Prpf38a	0.54665	neg.
108112	Eif4ebp3	0.55761	neg.
67877	Naa20	0.57213	neg.
231887	Pdap1	0.66938	neg.
219094	Khnyn	0.76520	neg.
75770	Brsk2	0.90336	neg.
12226	Btg1	0.97314	neg.
12391	Cav3	0.03830	pos.
67092	Gatm	0.05633	pos.
232087	Mat2a	0.06313	pos.
56323	Dnajb5	0.12344	pos.
30948	Bin1	0.14616	pos.
104263	Kdm3a	0.21728	pos.
66240	Kcne1l	0.36748	pos.
107271	Yars	0.36959	pos.
216850	Kdm6b	0.52230	pos.
27281	Hrasls	0.54816	pos.
70315	Hdac8	0.54844	pos.
380916	Lrch1	0.55348	pos.
234964	Ccdc67	0.57421	pos.
229700	Rbm15	0.58935	pos.
13831	Epc1	0.60787	pos.
17257	Mecp2	0.69782	pos.
276952	Rasl10b	0.75741	pos.
16918	Mycl1	0.78415	pos.
18285	Odf1	0.78714	pos.
245469	Pdzd4	0.82422	pos.
239318	Plcxd3	0.87587	pos.
56349	Net1	0.96627	pos.
12978	Csf1r	0.97297	pos.

Table 2: Overview of microRNA mmu-miR-22 targets with strict overlap between the three databases TargetScan, Microcosm and PITA.

	miRNA	P-value	\# targets	Regulation
3	mmu-miR-134	0.00366	5	up
6	mmu-miR-20a	0.00677	134	down
17	mmu-miR-500	0.01090	23	up
2	mmu-miR-133a	0.01230	49	up
1	mmu-let-7c	0.01614	86	up
8	mmu-miR-24	0.02212	47	up
13	mmu-miR-346	0.02681	4	down
20	mmu-miR-93	0.03190	124	down
19	mmu-miR-701	0.03250	1	down
10	mmu-miR-299	0.03305	8	up
5	mmu-miR-15b	0.03308	100	down
15	mmu-miR-486	0.03409	13	up
11	mmu-miR-32	0.03616	120	down
9	mmu-miR-26a	0.03693	76	up
16	mmu-miR-495	0.05060	48	up
14	mmu-miR-467a	0.05228	34	down
18	mmu-miR-543	0.05705	55	down
12	mmu-miR-33	0.06556	20	down
4	mmu-miR-150	0.06703	17	up
7	mmu-miR-22	0.07198	41	up

Table 1: Overview of significant miRNA target sets with strict overlap between the three prediction tools TargetScan, MicroCosm and PITA.

```
> print(xtable(mir133a[,-c(4,5)],
+        display=c("d", "s", "f", "s"), digits=c(0,0,5,0),
+        caption="Overview of microRNA mmu-miR-133a targets with strict overlap between the
+        tabular.environment="longtable", floating=FALSE)
```

	Symbol	Pvalue	Association
108013	Celf4	0.00224	neg.
17300	Foxc1	0.00895	neg.
13017	Ctbp2	0.00962	neg.
29813	Zfp385a	0.01417	neg.
56195	Ptbp2	0.02202	neg.
76932	Arfip2	0.02642	neg.
56526	Sept6	0.03019	neg.
23873	Faim	0.03089	neg.
19671	Rce1	0.04030	neg.
13345	Twist2	0.04046	neg.
19052	Ppp2ca	0.05338	neg.
13639	Efna4	0.05490	neg.
70122	Mllt3	0.07573	neg.

21873	Tjp2	0.08822	neg.
223870	Senp1	0.09540	neg.
13681	Eif4a1	0.14082	neg.
17925	Myo9b	0.14631	neg.
66940	Shisa5	0.14670	neg.
17886	Myh9	0.14858	neg.
105522	Ankrd28	0.18061	neg.
14573	Gdnf	0.18734	neg.
19053	Ppp2cb	0.19079	neg.
74442	Sgms2	0.21377	neg.
83675	Bicc1	0.22581	neg.
72587	Pan3	0.26695	neg.
12757	Clta	0.28853	neg.
66500	Slc30a7	0.39067	neg.
22218	Sumo1	0.40243	neg.
66588	Cmpk1	0.40671	neg.
217214	Nags	0.55364	neg.
69257	Elf2	0.63642	neg.
76787	Ppfia3	0.66704	neg.
242667	Dlgap3	0.80598	neg.
224530	Acat3	0.80903	neg.
16873	Lhx5	0.86312	neg.
56389	Stx5a	0.86889	neg.
19272	Ptprk	0.00593	pos.
19228	Pth1r	0.12300	pos.
216190	Appl2	0.35621	pos.
78593	Nrip3	0.47936	pos.
243312	Elfn1	0.48227	pos.
216549	Aftph	0.52637	pos.
14167	Fgf12	0.54326	pos.
21854	Timm17a	0.57684	pos.
108071	Grm5	0.82700	pos.
19277	Ptpro	0.87367	pos.
13803	Enc1	0.93127	pos.
226896	Tcfap2d	0.99817	pos.
99326	Garnl3	0.99828	pos.

Table 3: Overview of microRNA mmu-miR-133a targets with strict overlap between the three databases TargetScan, Microcosm and PITA.

```
> print(xtable(mir26a[,-c(4,5)],
+        display=c("d", "s", "f", "s"), digits=c(0,0,5,0),
+        caption="Overview of microRNA mmu-miR-26a targets with strict overlap between the t
+        tabular.environment="longtable", floating=FALSE)
```

	Symbol	Pvalue	Association
230753	Thrap3	0.00975	neg.
15402	Hoxa5	0.01171	neg.
14163	Fgd1	0.01818	neg.
18753	Prkcd	0.03200	neg.
59027	Nampt	0.03455	neg.
15234	Hgf	0.03738	neg.
233902	Fbxl19	0.04957	neg.
18578	Pde4b	0.05925	neg.
68732	Lrrc16a	0.06011	neg.
23873	Faim	0.07397	neg.
264064	Cdk8	0.08502	neg.
66197	Cks2	0.09182	neg.
15446	Hpgd	0.13005	neg.
72549	Reep4	0.18209	neg.
13836	Epha2	0.18782	neg.
66980	Zdhhc6	0.20504	neg.
52708	Zfp410	0.22480	neg.
14056	Ezh2	0.23016	neg.
16647	Kpna2	0.23713	neg.
242466	Zfp462	0.31819	neg.
233833	Tnrc6a	0.41264	neg.
12018	Bak1	0.44428	neg.
235441	Usp3	0.59153	neg.
217154	Stac2	0.60107	neg.
13445	Cdk2ap1	0.61613	neg.
66695	Aspn	0.69023	neg.
503610	Zdhhc18	0.73335	neg.
77128	A930001N09Rik	0.78505	neg.
17532	Mras	0.89128	neg.
100019	Mdn1	0.90387	neg.
232288	Frmd4b	0.01550	pos.
215814	Ccdc28a	0.02566	pos.
27402	Pdhx	0.04878	pos.
22057	Tob1	0.04902	pos.
60613	Kcnq4	0.05639	pos.
11535	Adm	0.05794	pos.
22652	Mkrn3	0.06351	pos.
320538	Ubn2	0.07083	pos.
18712	Pim1	0.07706	pos.
234875	Ttc13	0.09932	pos.
77044	Arid2	0.10003	pos.
22065	Trpc3	0.11484	pos.
30928	Zfp238	0.12068	pos.
231290	Slc10a4	0.14205	pos.

213753	Zfp598	0.16269	pos.
229488	Fam160a1	0.17340	pos.
320213	Senp5	0.17368	pos.
231051	Mll3	0.17701	pos.
74132	Rnf6	0.18469	pos.
16650	Kpna6	0.19984	pos.
231600	Chfr	0.22044	pos.
19212	Pter	0.23132	pos.
208177	Phldb2	0.24115	pos.
99167	Ssx2ip	0.25254	pos.
76608	Hectd3	0.31757	pos.
71795	Pitpnc1	0.32328	pos.
19893	Rpgr	0.39004	pos.
227446	2310035C23Rik	0.41007	pos.
228983	Osbpl2	0.49724	pos.
74159	Acbd5	0.49863	pos.
192285	Phf21a	0.51123	pos.
22241	Ulk1	0.51187	pos.
17125	Smad1	0.51643	pos.
225055	Fbxo11	0.52365	pos.
238130	Dock4	0.53668	pos.
106369	Ypel1	0.53955	pos.
58242	Nudt11	0.54688	pos.
71069	Stox2	0.56330	pos.
14479	Usp15	0.56787	pos.
67071	Rps6ka6	0.64564	pos.
407823	Baz2b	0.67234	pos.
19266	Ptprd	0.68593	pos.
71673	Rnf215	0.71344	pos.
13831	Epc1	0.76169	pos.
269275	Acvr1c	0.76807	pos.
15112	Hao1	0.95523	pos.

Table 4: Overview of microRNA mmu-miR-26a targets with strict overlap between the three databases TargetScan, Microcosm and PITA.