Gene Classification (adjusted from MG)

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Functions

```
classify_expressed <- function(row, names, cutoff){</pre>
  #Checks if gene is expressed greater than [cutoff] in at least 1 of the tissues included in names.
  #Returns boolean
  row <- row[which(names(row) %in% names)]</pre>
  for(i in seq(1, length(names))){
    if (as.numeric(row[i]) > cutoff){
      return(TRUE)
    }
  }
  return(FALSE)
}
classify_trace <- function(row, names, cutoff){</pre>
  #Checks if gene is expressed less than [cutoff] in ALL of the tissues included in names.
  #Returns boolean
  num_trace <- 0</pre>
  row <- row[which(names(row) %in% names)]</pre>
  for(i in seq(1, length(names))){
    if (as.numeric(row[i]) < cutoff){</pre>
      num_trace <- num_trace + 1</pre>
    }
  if (num_trace == length(names)){
    return(TRUE)
  }else{return(FALSE)}
}
#calculate tau measure of tissue specificity
{\it \#https://github.com/severinEvo/gene\_expression}
tau<-function(x){</pre>
  if(any(is.na(x))) stop('NA\'s need to be 0.')
  if(any(x<0)) stop('Negative input values not permitted.')</pre>
  t < -sum(1-x/max(x))/(length(x)-1)
}
```

Load data

melanogaster (ore)

```
mel <- read.table("~/Desktop/data/aim_3/dmel.transTPM.txt", header = T)</pre>
mel_FB <- read.table("~/Desktop/data/aim_3/GSE99574_HiSAT2_dmel.transcript_level_TPM.FB.txt", header = '
mel_ore_f_full <- mel %>% select(1, 3, starts_with("oreR_f"))
mel_ore_f <- mel_ore_f_full %>%
  mutate(mean_head = rowMeans(select(mel_ore_f_full, contains("_hd_"))),
        mean_thorax = rowMeans(select(mel_ore_f_full, contains("_tx_"))),
        mean_abdomen = rowMeans(select(mel_ore_f_full, contains("_ac_"))),
        mean_digestive = rowMeans(select(mel_ore_f_full, contains("_dg_"))),
        mean_gonad = rowMeans(select(mel_ore_f_full, contains("_go_"))),
        mean_reproductive = rowMeans(select(mel_ore_f_full, contains("_re_"))),
        mean_genitalia = rowMeans(select(mel_ore_f_full, contains("_ge_")))) %>%
  select(1, 2, starts_with("mean"))
mel_ore_m_full <- mel %>% select(1, 3, starts_with("oreR_m"))
mel_ore_m <- mel_ore_m_full %>%
  mutate(mean_head = rowMeans(select(mel_ore_m_full, contains("_hd_"))),
        mean_thorax = rowMeans(select(mel_ore_m_full, contains("_tx_"))),
        mean_abdomen = rowMeans(select(mel_ore_m_full, contains("_ac_"))),
        mean_digestive = rowMeans(select(mel_ore_m_full, contains("_dg_"))),
        mean_gonad = rowMeans(select(mel_ore_m_full, contains("_go_"))),
        mean reproductive = rowMeans(select(mel ore m full, contains(" re "))),
        mean_genitalia = rowMeans(select(mel_ore_m_full, contains("_ge_")))) %>%
  select(1, 2, starts_with("mean"))
```

melanogaster (w1118)

ananassae

```
ana <- read.table("~/Desktop/data/aim_3/dana.transTPM.txt", header = T, fill = T)
ana <- na.omit(ana)</pre>
ana_f_full <- ana %>% select(1, starts_with("dana_f"))
ana_f <- ana_f_full %>%
  mutate(mean_head = rowMeans(select(ana_f_full, contains("_hd_"))),
        mean_thorax = rowMeans(select(ana_f_full, contains("_tx_"))),
        mean_abdomen = rowMeans(select(ana_f_full, contains("_ac_"))),
        mean_digestive = rowMeans(select(ana_f_full, contains("_dg_"))),
        mean gonad = rowMeans(select(ana f full, contains(" go "))),
        mean_reproductive = rowMeans(select(ana_f_full, contains("_re_")))) %>%
  select(1, starts with("mean"))
ana_m_full <- ana %>% select(1, starts_with("dana_m"))
ana_m <- ana_m_full %>%
  mutate(mean_head = rowMeans(select(ana_m_full, contains("_hd_"))),
        mean_thorax = rowMeans(select(ana_m_full, contains("_tx_"))),
        mean_abdomen = rowMeans(select(ana_m_full, contains("_ac_"))),
        mean_digestive = rowMeans(select(ana_m_full, contains("_dg_"))),
        mean_gonad = rowMeans(select(ana_m_full, contains("_go_"))),
        mean_reproductive = rowMeans(select(ana_m_full, contains("_re_")))) %>%
  select(1, starts_with("mean"))
```

mojavensis

persimilis

```
per <- read.table("~/Desktop/data/aim_3/dper.transTPM.txt", header = T, fill = T)</pre>
per <- na.omit(per)</pre>
per_f_full <- per %>% select(1, starts_with("dper_f"))
per_f <- per_f_full %>%
  mutate(mean_head = rowMeans(select(per_f_full, contains("_hd_"))),
        mean_thorax = rowMeans(select(per_f_full, contains("_tx_"))),
        mean_abdomen = rowMeans(select(per_f_full, contains("_ac_"))),
        mean_digestive = rowMeans(select(per_f_full, contains("_dg_"))),
        mean_gonad = rowMeans(select(per_f_full, contains("_go_"))),
        mean reproductive = rowMeans(select(per f full, contains(" re ")))) %>%
  select(1, starts_with("mean"))
per m full <- per %>% select(1, starts with("dper m"))
per_m <- per_m_full %>%
  mutate(mean_head = rowMeans(select(per_m_full, contains("_hd_"))),
        mean_thorax = rowMeans(select(per_m_full, contains("_tx_"))),
        mean_abdomen = rowMeans(select(per_m_full, contains("_ac_"))),
        mean_digestive = rowMeans(select(per_m_full, contains("_dg_"))),
        mean_gonad = rowMeans(select(per_m_full, contains("_go_"))),
        mean_reproductive = rowMeans(select(per_m_full, contains("_re_")))) %>%
  select(1, starts_with("mean"))
```

pseudoobscura

```
pse <- read.table("~/Desktop/data/aim_3/dpse.transTPM.txt", header = T, fill = T)
pse <- na.omit(pse)

pse_f_full <- pse %>% select(1, starts_with("dpse_f"))
```

```
pse_f <- pse_f_full %>%
  mutate(mean_head = rowMeans(select(pse_f_full, contains("_hd_"))),
        mean_thorax = rowMeans(select(pse_f_full, contains("_tx_"))),
        mean_abdomen = rowMeans(select(pse_f_full, contains("_ac_"))),
        mean_digestive = rowMeans(select(pse_f_full, contains("_dg_"))),
        mean_gonad = rowMeans(select(pse_f_full, contains("_go_"))),
        mean_reproductive = rowMeans(select(pse_f_full, contains("_re_")))) %>%
  select(1, starts with("mean"))
pse_m_full <- pse %>% select(1, starts_with("dpse_m"))
pse_m <- pse_m_full %>%
  mutate(mean_head = rowMeans(select(pse_m_full, contains("_hd_"))),
        mean_thorax = rowMeans(select(pse_m_full, contains("_tx_"))),
        mean_abdomen = rowMeans(select(pse_m_full, contains("_ac_"))),
        mean_digestive = rowMeans(select(pse_m_full, contains("_dg_"))),
        mean_gonad = rowMeans(select(pse_m_full, contains("_go_"))),
        mean_reproductive = rowMeans(select(pse_m_full, contains("_re_")))) %>%
  select(1, starts with("mean"))
```

virilis

```
vir <- read.table("~/Desktop/data/aim 3/dvir.transTPM.txt", header = T, fill = T)</pre>
vir <- na.omit(vir)</pre>
vir_f_full <- vir %>% select(1, starts_with("dvir_f"))
vir f <- vir f full %>%
  mutate(mean_head = rowMeans(select(vir_f_full, contains("_hd_"))),
        mean_thorax = rowMeans(select(vir_f_full, contains("_tx_"))),
        mean_abdomen = rowMeans(select(vir_f_full, contains("_ac_"))),
        mean_digestive = rowMeans(select(vir_f_full, contains("_dg_"))),
        mean_gonad = rowMeans(select(vir_f_full, contains("_go_"))),
        mean_reproductive = rowMeans(select(vir_f_full, contains("_re_")))) %>%
  select(1, starts_with("mean"))
vir_m_full <- vir %>% select(1, starts_with("dvir_m"))
vir m <- vir m full %>%
  mutate(mean_head = rowMeans(select(vir_m_full, contains("_hd_"))),
        mean_thorax = rowMeans(select(vir_m_full, contains("_tx_"))),
        mean_abdomen = rowMeans(select(vir_m_full, contains("_ac_"))),
        mean_digestive = rowMeans(select(vir_m_full, contains("_dg_"))),
        mean gonad = rowMeans(select(vir m full, contains(" go "))),
        mean_reproductive = rowMeans(select(vir_m_full, contains("_re_")))) %>%
  select(1, starts_with("mean"))
```

willistoni

```
wil <- read.table("~/Desktop/data/aim 3/dwil.transTPM.txt", header = T, fill = T)</pre>
wil <- na.omit(wil)</pre>
wil_f_full <- wil %>% select(1, starts_with("dwil_f"))
wil_f <- wil_f_full %>%
  mutate(mean_head = rowMeans(select(wil_f_full, contains("_hd_"))),
        mean_thorax = rowMeans(select(wil_f_full, contains("_tx_"))),
        mean_abdomen = rowMeans(select(wil_f_full, contains("_ac_"))),
        mean_digestive = rowMeans(select(wil_f_full, contains("_dg_"))),
        mean_gonad = rowMeans(select(wil_f_full, contains("_go_"))),
        mean_reproductive = rowMeans(select(wil_f_full, contains("_re_")))) %>%
  select(1, starts_with("mean"))
wil_m_full <- wil %>% select(1, starts_with("dwil_m"))
wil m <- wil m full %>%
  mutate(mean_head = rowMeans(select(wil_m_full, contains("_hd_"))),
        mean_thorax = rowMeans(select(wil_m_full, contains("_tx_"))),
        mean_abdomen = rowMeans(select(wil_m_full, contains("_ac_"))),
        mean_digestive = rowMeans(select(wil_m_full, contains("_dg_"))),
        mean_gonad = rowMeans(select(wil_m_full, contains("_go_"))),
        mean_reproductive = rowMeans(select(wil_m_full, contains("_re_")))) %>%
  select(1, starts_with("mean"))
```

yakuba

```
yak <- read.table("~/Desktop/data/aim_3/dyak.transTPM.txt", header = T, fill = T)</pre>
yak <- na.omit(yak)</pre>
yak f full <- yak %>% select(1, starts with("dyak f"))
yak f <- yak f full %>%
  mutate(mean_head = rowMeans(select(yak_f_full, contains("_hd_"))),
        mean_thorax = rowMeans(select(yak_f_full, contains("_tx_"))),
        mean_abdomen = rowMeans(select(yak_f_full, contains("_ac_"))),
        mean digestive = rowMeans(select(yak f full, contains(" dg "))),
        mean_gonad = rowMeans(select(yak_f_full, contains("_go_"))),
        mean_reproductive = rowMeans(select(yak_f_full, contains("_re_")))) %>%
  select(1, starts_with("mean"))
yak_m_full <- yak %>% select(1, starts_with("dyak_m"))
yak_m <- yak_m_full %>%
  mutate(mean_head = rowMeans(select(yak_m_full, contains("_hd_"))),
        mean_thorax = rowMeans(select(yak_m_full, contains("_tx_"))),
        mean abdomen = rowMeans(select(yak m full, contains(" ac "))),
        mean_digestive = rowMeans(select(yak_m_full, contains("_dg_"))),
```

```
mean_gonad = rowMeans(select(yak_m_full, contains("_go_"))),
    mean_reproductive = rowMeans(select(yak_m_full, contains("_re_")))) %>%
select(1, starts_with("mean"))
```

grimshawi

```
haw <- read.table("~/Desktop/data/aim_3/haw.transTPM.txt", header = T, fill = T)
haw <- na.omit(haw)</pre>
gri_f_full <- haw %>% select(1, starts_with("dgriG1_f"))
gri_f <- gri_f_full %>%
 mutate(mean head = rowMeans(select(gri f full, contains(" hd "))),
        mean_thorax = rowMeans(select(gri_f_full, contains("_tx_"))),
        mean_abdomen = rowMeans(select(gri_f_full, contains("_ac_"))),
        mean_digestive = rowMeans(select(gri_f_full, contains("_dg_"))),
        mean_gonad = rowMeans(select(gri_f_full, contains("_go_"))),
        mean_reproductive = rowMeans(select(gri_f_full, contains("_re_"))),
        mean_genitalia = rowMeans(select(gri_f_full, contains("_ge_")))) %>%
  select(1, starts_with("mean"))
gri_m_full <- haw %>% select(1, starts_with("dgriG1_m"))
gri_m <- gri_m_full %>%
  mutate(mean_head = rowMeans(select(gri_m_full, contains("_hd_"))),
        mean_thorax = rowMeans(select(gri_m_full, contains("_tx_"))),
        mean_abdomen = rowMeans(select(gri_m_full, contains("_ac_"))),
        mean_digestive = rowMeans(select(gri_m_full, contains("_dg_"))),
        mean gonad = rowMeans(select(gri m full, contains(" go "))),
        mean_reproductive = rowMeans(select(gri_m_full, contains("_re_"))),
        mean_genitalia = rowMeans(select(gri_m_full, contains("_ge_")))) %>%
  select(1, starts_with("mean"))
```

Calculate Tau

mel (ore) female

```
mel_ore_f_NA <- replace(mel_ore_f, mel_ore_f < 1, NA)
mel_ore_f_NA_log <- log2(mel_ore_f_NA[,3:9])
mel_ore_f_log <- replace(mel_ore_f_NA_log, is.na(mel_ore_f_NA_log), 0)

mel_ore_f_log$tau <- apply(mel_ore_f_log, 1, tau)

mel_ore_f_tau <- cbind(mel_ore_f[,1:2], mel_ore_f_log)

#filter rows where reproductive tissues have highest gene expression
mel_ore_f_RP <- mel_ore_f_tau %>%
    filter(mean_gonad > mean_head & mean_gonad > mean_thorax & mean_gonad > mean_abdomen & mean_gonad > mean_gonad > mean_abdomen & mean_gonad > mean_abdomen & mean_gonad > mean_gonad > mean_gonad > mean_abdomen & mean_a
```

```
mean_reproductive > mean_head & mean_reproductive > mean_thorax & mean_reproductive > mean_a
mean_genitalia > mean_head & mean_genitalia > mean_thorax & mean_genitalia > mean_abdomen & mean_senitalia > mean_abdomen & mean_genitalia > mean_genitalia > mean_abdomen & mean_genitalia > mean_abdomen & mean_genitalia > mean_genitalia > mean_abdomen & mean_abdomen & mean_genitalia > mean_abdomen & mean_abdom
```

mel (ore) male

```
mel_ore_m_NA <- replace(mel_ore_m, mel_ore_m < 1, NA)
mel_ore_m_NA_log <- log2(mel_ore_m_NA[,3:9])
mel_ore_m_log <- replace(mel_ore_m_NA_log, is.na(mel_ore_m_NA_log), 0)

mel_ore_m_log$tau <- apply(mel_ore_m_log, 1, tau)

mel_ore_m_tau <- cbind(mel_ore_m[,1:2], mel_ore_m_log)

#filter rows where reproductive tissues have highest gene expression
mel_ore_m_RP <- mel_ore_m_tau %>%
    filter(mean_gonad > mean_head & mean_gonad > mean_thorax & mean_abdomen & mean_gonad > mean_reproductive > mean_head & mean_reproductive > mean_thorax & mean_genitalia > mean_genitalia > mean_abdomen & mean_genitalia > mean_abdomen & mean_genitalia > mean_abdomen & mean_abdomen & mean_genitalia > mean_lore_m_RP %>% filter(tau > 0.8)
```

RP, SM, & GR

Classify

Overview of what I'm doing to classify genes:

For each species... 1. Identify SomExpressed genes (> 2 TPM in at least one somatic tissue; TRUE/FALSE) 2. Identify RepExpressed genes (> 2 TPM in at least one reproductive tissue; TRUE/FALSE) 3. Identify somatic trace expression genes (< 0.5 TPM in all som tissues; TRUE/FALSE) 4. Identify reproductive trace expression genes (< 0.5 TPM in all rep tissues; TRUE/FALSE)

- 5. Classify GR genes as those which are SomExpressed AND RepExpressed AND not expressed at trace levels in either (TRUE/FALSE)
- 6. Classify SM genes as those which are SomExpressed AND trace expressed in reproductive tissues AND not in the GR list (TRUE/FALSE)
- 7. Classify RP genes as those which are RepExpressed AND trace expressed in somatic tissues AND not in the GR list (TRUE/FALSE)

MELANOGASTER

```
upperCutoff <- 2
lowerCutoff <- 0.5
```

mel (ore) female

mel (ore) male

mel (w1118) female

mel (w1118) male

```
SomTrace = apply(mel_w1118_m, 1, classify_trace, names = c("mean_head", "mean_thomography (mel_w1118_m, 1, classify_trace, names = c("mean_gonad", "mean_remutate(GR = SomExpressed & RepExpressed & !SomTrace & !RepTrace,

SM = SomExpressed & RepTrace & !GR,

RP = RepExpressed & SomTrace & !GR)

mel_w1118_m_RP <- mel_w1118_m %>% filter(RP == TRUE)

mel_w1118_m_SM <- mel_w1118_m %>% filter(GR == TRUE)

mel_w1118_m_GR <- mel_w1118_m %>% filter(GR == TRUE)
```

ANANASSAE

ana female

ana male

MOJAVENSIS

moj female

moj male

PERSIMILIS

per female

per male

```
per_m <- per_m %>% mutate(SomExpressed = apply(per_m, 1, classify_expressed, names = c("mean_head", "mean_
RepExpressed = apply(per_m, 1, classify_expressed, names = c("mean_gonad", "mean_some = apply(per_m, 1, classify_trace, names = c("mean_head", "mean_thorax",
RepTrace = apply(per_m, 1, classify_trace, names = c("mean_gonad", "mean_reproduct")
```

PSEUDOOBSCURA

pse female

pse male

VIRILIS

vir female

vir male

WILLISTONI

wil female

wil male

```
RP = RepExpressed & SomTrace & !GR)
wil_m_RP <- wil_m %>% filter(RP == TRUE)
wil_m_SM <- wil_m %>% filter(SM == TRUE)
wil_m_GR <- wil_m %>% filter(GR == TRUE)
```

YAKUBA

yak female

yak male

GRIMSHAWI

gri female

```
RP = RepExpressed & SomTrace & !GR)

gri_f_RP <- gri_f %>% filter(RP == TRUE)
gri_f_SM <- gri_f %>% filter(SM == TRUE)
gri_f_GR <- gri_f %>% filter(GR == TRUE)
```

gri male