Frontloading_RMD

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Call modules representing higher expression by naive clams throughout the subsequent exposures

• 'Naive modules' == all mods of interest

- 'NaiveResponse_genes_data' call all genes that occured THREE times in 'Naive_modules'
- Why? These genes are represent those with persistant high expression relative to the pre-exposed (primed) clams!
- there are 315 total genes in this category

Calculate the contorl ratio (Y axis) and the foldchangie ratio (x axis)

```
# Day 7 gene expression
Day_WGCNA_genes <- Day7_exp_data %>%
  dplyr::filter(Gene %in% NaiveResponse_genes)
Day7_WGCNA_genes_melted <- Day_WGCNA_genes %>%
  reshape2::melt(id.var = 'Gene') %>%
  dplyr::rename(Sample.Name = variable)
```

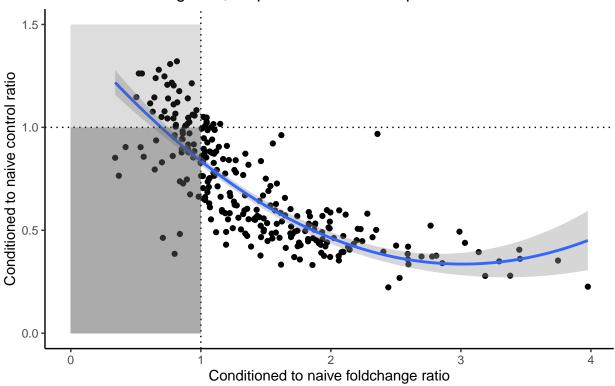
```
Day7_WGCNA_genes_Merge <- merge(Day7_WGCNA_genes_melted, Day7_Master.Exp.Metadata, by = 'Sample.Name')
  dplyr::group_by(Gene, All_Treatment) %>%
  dplyr::select(!'Sample.Name') %>%
  dplyr::summarise(meanExp = mean(value))
## 'summarise()' has grouped output by 'Gene'. You can override using the '.groups' argument.
Day7_READY <- dcast(Day7_WGCNA_genes_Merge, Gene ~ All_Treatment)</pre>
## Using meanExp as value column: use value.var to override.
for (i in 1:nrow(Day7_READY)) {
          # Moderate - higher expression AM > AA
        if (Day7_READY$AM[i] > Day7_READY$AA[i]) {
          Day7_READY$wgcna.xall_mod[i] <- ( (Day7_READY$MM[i] / Day7_READY$MA[i]) / (Day7_READY$AM[i] /
          Day7_READY$wgcna.yall_mod[i] <- (Day7_READY$MA[i] / Day7_READY$AA[i]) # Y Axis - this is simp
              } else {
                Day7_READY$wgcna.xall_mod[i] <- NA # X axis - call NA
                Day7_READY$wgcna.yall_mod[i] <- NA # Y Axis - call NA
            # Severe - higher expression AS > AA
          if (Day7 READY$AS[i] > Day7 READY$AA[i]) {
            Day7_READY$wgcna.xall_sev[i] <- ( (Day7_READY$MS[i] / Day7_READY$MA[i]) / (Day7_READY$AS[i]</pre>
            Day7_READY$wgcna.yall_sev[i] <- (Day7_READY$MA[i] / Day7_READY$AA[i])</pre>
                                                                                              # Y Axis -
                } else {
                  Day7_READY$wgcna.xall_sev[i] <- NA # X axis - call NA
                  Day7_READY$wgcna.yall_sev[i] <- NA # Y Axis - call NA
# Day7_READY
# Day 14 gene expression
Day_WGCNA_genes <- Day14_exp_data %>%
  dplyr::filter(Gene %in% NaiveResponse_genes)
Day14_WGCNA_genes_melted
                             <- Day_WGCNA_genes %>%
  reshape2::melt(id.var = 'Gene') %>%
  dplyr::rename(Sample.Name = variable)
Day14_WGCNA_genes_Merge <- merge(Day14_WGCNA_genes_melted, Day14_Master.Exp.Metadata, by = 'Sample.Name
  dplyr::group_by(Gene, All_Treatment) %>%
  dplyr::select(!'Sample.Name') %>%
  dplyr::summarise(meanExp = mean(value))
## 'summarise()' has grouped output by 'Gene'. You can override using the '.groups' argument.
Day14_READY <- dcast(Day14_WGCNA_genes_Merge, Gene ~ All_Treatment)</pre>
```

Using meanExp as value column: use value.var to override.

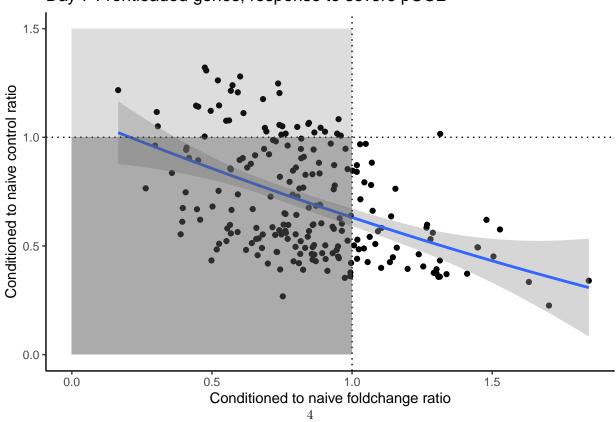
```
for (i in 1:nrow(Day14_READY)) {
          # Moderate - higher expression AM > AA
        if (Day14_READY$AM[i] > Day14_READY$AA[i]) {
          Day14_READY$wgcna.xall_mod[i] <- ( (Day14_READY$MM[i] / Day14_READY$MA[i]) / (Day14_READY$AM[
          Day14_READY$wgcna.yall_mod[i] <- (Day14_READY$MA[i] / Day14_READY$AA[i]) # Y Axis - this is s
                Day14_READY$wgcna.xall_mod[i] <- NA # X axis - call NA
                Day14 READY$wgcna.yall mod[i] <- NA # Y Axis - call NA
              }
            # Severe - higher expression AS > AA
          if (Day14_READY$AS[i] > Day14_READY$AA[i]) {
            Day14_READY$wgcna.xall_sev[i] <- ( (Day14_READY$MS[i] / Day14_READY$MA[i]) / (Day14_READY$A
            Day14_READY$wgcna.yall_sev[i] <- (Day14_READY$MA[i] / Day14_READY$AA[i])
                                                                                                 # Y Axi
                } else {
                  Day14_READY$wgcna.xall_sev[i] <- NA # X axis - call NA
                  Day14_READY$wgcna.yall_sev[i] <- NA # Y Axis - call NA
# Day14_READY
# Day 21 gene expression
Day_WGCNA_genes <- Day21_exp_data %>%
  dplyr::filter(Gene %in% NaiveResponse_genes)
Day21 WGCNA genes melted
                            <- Day WGCNA genes %>%
  reshape2::melt(id.var = 'Gene') %>%
  dplyr::rename(Sample.Name = variable)
Day21_WGCNA_genes_Merge <- merge(Day21_WGCNA_genes_melted, Day21_Master.Exp.Metadata, by = 'Sample.Name
  dplyr::group_by(Gene, All_Treatment) %>%
  dplyr::select(!'Sample.Name') %>%
  dplyr::summarise(meanExp = mean(value))
## 'summarise()' has grouped output by 'Gene'. You can override using the '.groups' argument.
Day21_READY <- dcast(Day21_WGCNA_genes_Merge, Gene ~ All_Treatment)</pre>
## Using meanExp as value column: use value.var to override.
for (i in 1:nrow(Day21 READY)) {
          # Moderate - higher expression AM > AA
        if (Day21 READY$AAM[i] > Day21 READY$AAA[i]) {
          Day21_READY$wgcna.xall_mod[i] <- ( (Day21_READY$MAM[i] / Day21_READY$MAA[i]) / (Day21_READY$A
          Day21_READY$wgcna.yall_mod[i] <- (Day21_READY$MAA[i] / Day21_READY$AAA[i]) # Y Axis - this is
              } else {
                Day21_READY$wgcna.xall_mod[i] <- NA # X axis - call NA
                Day21_READY$wgcna.yall_mod[i] <- NA # Y Axis - call NA
              }
# Day21_READY
```

Day 7 - Frontloaded genes (Plot)

Day 7 Frontloaded genes; response to moderate pCO2



Day 7 Frontloaded genes; response to severe pCO2

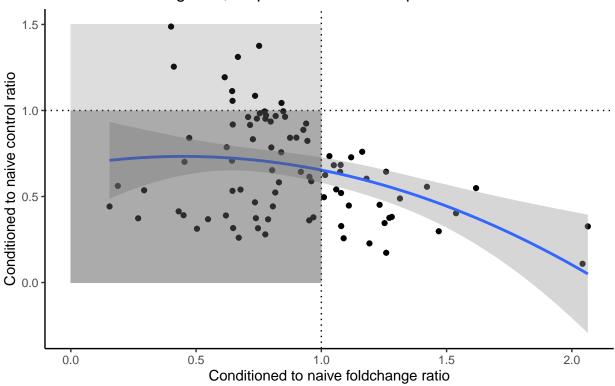


Day 7 - Frontloaded genes (Table)

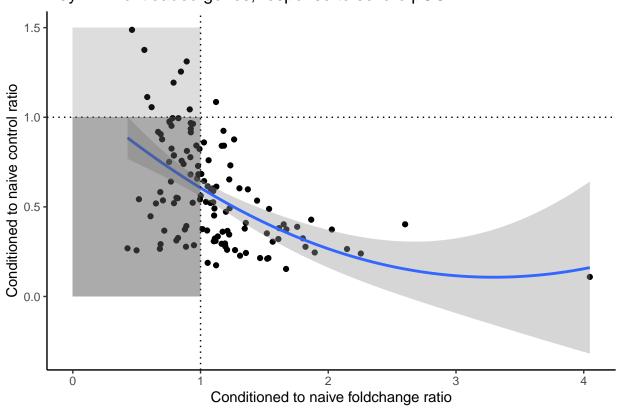
geneSymbol	Genes
PGEN00g026480	Ras-related protein Rab-32B
PGEN00g027620	V-type proton ATPase subunit D (V-ATPase subunit D) (Vacuolar proton pump subunit D)
PGEN00g048930	Alcohol dehydrogenase class-3 (EC 1.1.1.1) (Alcohol dehydrogenase class-III) (Glutathione-dependent
PGEN00g073190	Apolipophorins [Cleaved into: Apolipophorin-2 (Apolipophorin II) (apoLp-2); Apolipophorin-1 (Apolipophorin-2)
PGEN00g082240	Alpha-galactosidase A (EC 3.2.1.22) (Alpha-D-galactosidase A) (Alpha-D-galactoside galactohydrola
PGEN00g093570	NA
PGEN00g111730	V-type proton ATPase catalytic subunit A (V-ATPase subunit A) (EC 7.1.2.2) (V-ATPase 69 kDa s
PGEN00g116940	Glutathione peroxidase (PcGPx) (Se-PcGPx) (EC 1.11.1.9)
PGEN00g127120	3-hydroxyisobutyryl-CoA hydrolase, mitochondrial (EC 3.1.2.4) (3-hydroxyisobutyryl-coenzyme A hydroxyisobutyryl-coenzyme A hydroxyis
PGEN00g127760	Long-chain specific acyl-CoA dehydrogenase, mitochondrial (LCAD) (EC 1.3.8.8)
PGEN00g130790	All-trans-retinol 13,14-reductase (EC 1.3.99.23) (All-trans-13,14-dihydroretinol saturase A) (RetSat
PGEN00g153080	Hermansky-Pudlak syndrome 5 protein (Alpha-integrin-binding protein 63) (Ruby-eye protein 2 hon
PGEN00g186500	D-aspartate oxidase (DASOX) (DDO) (EC 1.4.3.1)
PGEN00g199980	NA NA
PGEN00g201290	Putative D-cysteine desulfhydrase 1, mitochondrial (EC 4.4.1.15) (OsD-CDes1) (D-CDes1)
PGEN00g203570	$15- hydroxyprostaglandin \ dehydrogenase \ [NAD(+)] \ (15-PGDH) \ (EC\ 1.1.1.141) \ (Prostaglandin \ dehydrogenase) \ (Prostaglandin \ dehyd$
PGEN00g204750	TBC1 domain family member 22A
PGEN00g213350	Carnitine O-palmitoyltransferase 2, mitochondrial (EC 2.3.1.21) (Carnitine palmitoyltransferase II)
PGEN00g213740	Soma ferritin (EC 1.16.3.1)
PGEN00g236660	NA
PGEN00g245510	NA
PGEN00g261210	Cytochrome P450 4Z1 (EC 1.14.14.1) (CYPIVZ1)
PGEN00g273190	Putative phosphoenolpyruvate synthase (Putative PEP synthase) (Pyruvate, water dikinase)
PGEN00g285980	Dimethylaniline monooxygenase [N-oxide-forming] 5 (EC 1.14.13.8) (Dimethylaniline oxidase 5) (Hep
PGEN00g298200	Cathepsin B (EC 3.4.22.1) (Cathepsin B1) [Cleaved into: Cathepsin B light chain; Cathepsin B heaven
PGEN00g298410	Cathepsin B (EC 3.4.22.1) (Cathepsin B1) [Cleaved into: Cathepsin B light chain; Cathepsin B heaven
PGEN00g300030	Peroxisomal acyl-coenzyme A oxidase 1 (AOX) (EC 1.3.3.6) (Palmitoyl-CoA oxidase) [Cleaved into:
PGEN00g305330	Poly(U)-specific endoribonuclease (EC 3.1) (Placental protein 11-related protein) (PP11-related protein)
PGEN00g306890	Adenylyl cyclase-associated protein 1 (CAP 1)
PGEN00g308530	Protein hunchback (Fragment)
PGEN00g312490	Tetratricopeptide repeat protein 39C (TPR repeat protein 39C)
PGEN00g313730	WASH complex subunit 2A
PGEN00g317830	Solute carrier family 12 member 9
PGEN00g334080	NA
PGEN00g345680	Cathepsin L1 (EC 3.4.22.15) (Cathepsin L) [Cleaved into: Cathepsin L1 heavy chain; Cathepsin L1

Genes
Ras-related protein Rab-32B
V-type proton ATPase subunit D (V-ATPase subunit D) (Vacuolar proton pump subunit D)
NA NA
Ethanolamine-phosphate cytidylyltransferase (EC 2.7.7.14) (CTP:phosphoethanolamine cytidylyltra
Alcohol dehydrogenase class-3 (EC 1.1.1.1) (Alcohol dehydrogenase class-III) (Glutathione-depender
Catalase (EC 1.11.1.6)
Apolipophorins [Cleaved into: Apolipophorin-2 (Apolipophorin II) (apoLp-2); Apolipophorin-1 (Apolipophorin-2)
Alpha-galactosidase A (EC 3.2.1.22) (Alpha-D-galactosidase A) (Alpha-D-galactoside galactohydrola
NA
V-type proton ATPase catalytic subunit A (V-ATPase subunit A) (EC 7.1.2.2) (V-ATPase 69 kDa s
Glutathione peroxidase (PcGPx) (Se-PcGPx) (EC 1.11.1.9)
3-hydroxyisobutyryl-CoA hydrolase, mitochondrial (EC 3.1.2.4) (3-hydroxyisobutyryl-coenzyme A hydroxyisobutyryl-coenzyme A hydroxyis
All-trans-retinol 13,14-reductase (EC 1.3.99.23) (All-trans-13,14-dihydroretinol saturase A) (RetSat
Vacuolar protein sorting-associated protein 29 (Vesicle protein sorting 29)
Hermansky-Pudlak syndrome 5 protein (Alpha-integrin-binding protein 63) (Ruby-eye protein 2 hon
NA
Putative D-cysteine desulfhydrase 1, mitochondrial (EC 4.4.1.15) (OsD-CDes1) (D-CDes1)
15-hydroxyprostaglandin dehydrogenase [NAD(+)] (15-PGDH) (EC 1.1.1.141) (Prostaglandin dehyd
TBC1 domain family member 22A
Carnitine O-palmitoyltransferase 2, mitochondrial (EC 2.3.1.21) (Carnitine palmitoyltransferase II)
Soma ferritin (EC 1.16.3.1)
NA
NA
Cytochrome P450 4Z1 (EC 1.14.14.1) (CYPIVZ1)
Putative phosphoenolpyruvate synthase (Putative PEP synthase) (Pyruvate, water dikinase)
FK506-binding protein 15 (FKBP-15) (133 kDa FK506-binding protein) (133 kDa FKBP) (FKBP-1
Dimethylaniline monooxygenase [N-oxide-forming] 5 (EC 1.14.13.8) (Dimethylaniline oxidase 5) (Her
Cathepsin B (EC 3.4.22.1) (Cathepsin B1) [Cleaved into: Cathepsin B light chain; Cathepsin B heav
Cathepsin B (EC 3.4.22.1) (Cathepsin B1) [Cleaved into: Cathepsin B light chain; Cathepsin B heav
Peroxisomal acyl-coenzyme A oxidase 1 (AOX) (EC 1.3.3.6) (Palmitoyl-CoA oxidase) [Cleaved into:
Poly(U)-specific endoribonuclease (EC 3.1) (Placental protein 11-related protein) (PP11-related p
Protein hunchback (Fragment)
Tetratricopeptide repeat protein 39C (TPR repeat protein 39C)
WASH complex subunit 2A
Solute carrier family 12 member 9
NA
Cathepsin L1 (EC 3.4.22.15) (Cathepsin L) [Cleaved into: Cathepsin L1 heavy chain; Cathepsin L1

Day 14 Frontloaded genes; response to moderate pCO2



Day 14 Frontloaded genes; response to severe pCO2



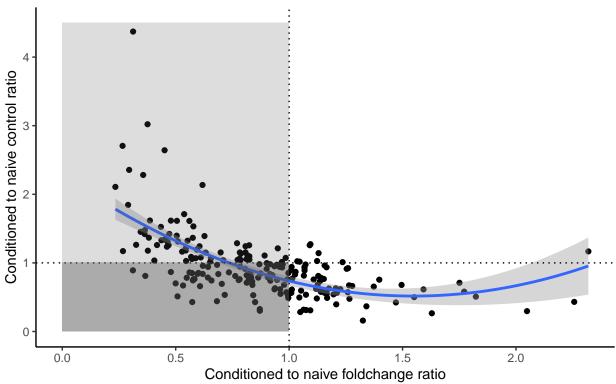
Day 14 - Frontloaded genes (Table)

geneSymbol	Genes
PGEN00g034480	ATP-binding cassette sub-family A member 5
PGEN00g047170	RUN and FYVE domain-containing protein 2
PGEN00g113630	Calponin-3 (Calponin, acidic isoform) (Calponin, non-muscle isoform)
PGEN00g228160	Collagen alpha-2(IV) chain
PGEN00g241240	NA
PGEN00g247890	NA
PGEN00g252190	Myosin regulatory light chain, smooth muscle
PGEN00g279950	Ribosome-recycling factor, mitochondrial (RRF) (Ribosome-releasing factor, mitochondrial)
PGEN00g334420	NA

geneSymbol	Genes
PGEN00g034480	ATP-binding cassette sub-family A member 5
PGEN00g047170	RUN and FYVE domain-containing protein 2
PGEN00g113630	Calponin-3 (Calponin, acidic isoform) (Calponin, non-muscle isoform)
PGEN00g228160	Collagen alpha-2(IV) chain
PGEN00g247890	NA
PGEN00g252190	Myosin regulatory light chain, smooth muscle
PGEN00g279950	Ribosome-recycling factor, mitochondrial (RRF) (Ribosome-releasing factor, mitochondrial)
PGEN00g334420	NA

Day 21 - Frontloaded genes (Plot)

Day 21 Frontloaded genes; response to moderate pCO2



Day 21 - Frontloaded genes (Table)

man a Cr 1 1	Compa
geneSymbol PGEN00g003940	Genes Regucalcin (RC) (Gluconolactonase) (GNL) (EC 3.1.1.17) (Senescence marker protein 30) (SMP-30)
PGEN00g003940 PGEN00g006490	Beta, beta-carotene 15,15'-dioxygenase (EC 1.13.11.63) (Beta-carotene dioxygenase 1) (Beta-carotene
PGEN00g008880	Hydroxysteroid dehydrogenase-like protein 2 (EC 1)
PGEN00g014860	Molybdenum cofactor biosynthesis protein 1 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 1 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 1 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 1 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 1 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 1 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 1 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 1 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 1 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 1 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 1 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 1 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 1 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 1 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 1 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 1 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 1 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 1 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 2 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 2 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 2 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 2 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 2 [Includes: GTP 3',8-cyclase (EC 4.1.99.22) (Molybdenum cofactor biosynthesis protein 2 [I
PGEN00g018570	Mammalian ependymin-related protein 1 (MERP-1)
PGEN00g024970	NA
PGEN00g027970	NA
PGEN00g030010	Homeobox protein prospero
PGEN00g031490	NA
PGEN00g035820	Prosaposin (Proactivator polypeptide) [Cleaved into: Saposin-A (Protein A); Saposin-B (Cerebroside)
PGEN00g039520	NA
PGEN00g042910	Sulfotransferase 1C4 (ST1C4) (EC 2.8.2) (Sulfotransferase 1C2) (SULT1C#2)
PGEN00g046630	L-rhamnonate dehydratase (RhamD) (EC 4.2.1.90)
PGEN00g047200	Trypsin beta (EC 3.4.21.4)
PGEN00g048930	Alcohol dehydrogenase class-3 (EC 1.1.1.1) (Alcohol dehydrogenase class-III) (Glutathione-dependent Maprin, A subunit alpha (EC 3.4.24.18) (Endoportidese 2) (Endoportidese 24.18 subunit alpha) (E.
PGEN00g049840 PGEN00g064390	Meprin A subunit alpha (EC 3.4.24.18) (Endopeptidase-2) (Endopeptidase-24.18 subunit alpha) (E-Putative defense protein 1 (DFP-1)
PGEN00g064390 PGEN00g070040	Apolipophorins [Cleaved into: Apolipophorin-2 (Apolipophorin II) (apoLp-2); Apolipophorin-1 (Apolipophorin-1)
PGEN00g070040 PGEN00g077860	Sorting nexin-12
PGEN00g084890	Protocadherin-11 X-linked (Protocadherin-11) (Protocadherin on the X chromosome)
PGEN00g085780	Transforming growth factor-beta-induced protein ig-h3 (Beta ig-h3) (Kerato-epithelin) (RGD-contain
PGEN00g095390	Transforming growth factor-beta-induced protein ig-h3 (Beta ig-h3)
PGEN00g106710	NA
PGEN00g114200	Caprin-2 (C1q domain-containing protein 1) (Cytoplasmic activation/proliferation-associated protein
PGEN00g114370	NA
PGEN00g119160	Perlucin
PGEN00g121940	Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic (GPD-C) (GPDH-C) (EC 1.1.1.8)
PGEN00g122110	Poly(rC)-binding protein 3 (Alpha-CP3) (PCBP3-overlapping transcript) (PCBP3-overlapping trans
PGEN00g122510	NA
PGEN00g123000	NA
PGEN00g130020	Fatty acid-binding protein 1
PGEN00g130760	NA NA
PGEN00g133710	NA Description of a series of A series 2 (EC 1 2 2 C) (Described sheir and Ca A series) (DDCAC).
PGEN00g135600	Peroxisomal acyl-coenzyme A oxidase 3 (EC 1.3.3.6) (Branched-chain acyl-CoA oxidase) (BRCACox
PGEN00g150640 PGEN00g155300	Oxysterol-binding protein 2 (Oxysterol-binding protein-related protein 4) (ORP-4) (OSBP-related p
PGEN00g155300 PGEN .00g155710	RUN domain-containing protein 1
PGEN00g155710 PGEN .00g156260	Prostaglandin reductase-3 (PTGR-3) (EC 1.3.1.48) (15-oxoprostaglandin 13-reductase) (Zinc-binding
PGEN00g169020	NA
PGEN00g187320	Interferon-induced very large GTPase 1 (Very large-inducible GTPase-1) (VLIG-1)
PGEN00g199190	NA
PGEN00g204750	TBC1 domain family member 22A
PGEN00g214740	Adiponectin receptor protein
PGEN00g228160	Collagen alpha-2(IV) chain
PGEN00g237580	Pancreatic triacylglycerol lipase (PL) (PTL) (Pancreatic lipase) (EC 3.1.1.3) (Fragment)
PGEN00g240360	NA
PGEN00g248130	NA
PGEN00g251840	Copper homeostasis protein cutC homolog
PGEN00g258220	NA
PGEN00g265000	NA
PGEN00g273190	Putative phosphoenolpyruvate synthase (Putative PEP synthase) (Pyruvate, water dikinase)
PGEN00g281080	NA DDILE
PGEN00g281550	DBH-like monooxygenase protein 1 (EC 1.14.17) (DBH-related protein) (Monooxygenase X)
PGEN00g285980	Dimethylaniline monooxygenase [N-oxide-forming] 5 (EC 1.14.13.8) (Dimethylaniline oxidase 5) (Hej
PGEN00g286040	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial (MCAD) (EC 1.3.8.7) Perlucin-like protein
PGEN00g293270 PGEN00g306160	NA
1 GEN00g300100	1VA