**Supplementary Material 3. Script for juveniles analysis (Figure 7).**

**(A) Linux commands**

#0 Analysis environment

Ubuntu 18.04.6 LTS

#1. Making directories

workDir=/mnt/d/takahashi/Juveniles

mkdir $workDir

scDir=/mnt/d/takahashi/Juveniles/00\_reference

mkdir $scDir&& cd $\_

#Copying sample information file to 00\_reference

cp /mnt/d/takahashi/sample.csv $\_

#Making directories from the information of the sample file

cat sample.csv | awk -F "," '{print $2}'  > filename.txt

cat sample.csv | awk -F "," '{print $3}'  > workDir.txt

paste -d "/" workDir.txt filename.txt > FilenameDir.txt

cat FilenameDir.txt | {

while read dir1

  do

      mkdir -p $dir1 && cd $\_

      mkdir 01\_raw-seq 02\_fastqc 03\_valid-seq 04\_abundant-check 05\_mapping 06\_count 07\_clustering 08\_DESeq2

  done

}

cd $workDir

tree

#2. Making index for HISAT2

cd $scDir

wget ftp://ftp.ensembl.org/pub/release-108/fasta/nothobranchius\_furzeri/dna/Nothobranchius\_furzeri.Nfu\_20140520.dna.toplevel.fa.gz

gzip -d Nothobranchius\_furzeri.Nfu\_20140520.dna.toplevel.fa.gz

hisat2-build Nothobranchius\_furzeri.Nfu\_20140520.dna.toplevel.fa Nothobranchius\_furzeri.Nfu\_20140520.dna.toplevel

cd $scDir

hisat2-build nfu.abundant.fa nfu.abundant

#3-1. Download of Genome Data from Ensembl

cd $scDir

wget ftp://ftp.ensembl.org/pub/release-108/gtf/nothobranchius\_furzeri/Nothobranchius\_furzeri.Nfu\_20140520.108.gtf.gz

gzip -d Nothobranchius\_furzeri.Nfu\_20140520.108.gtf.gz

#3-2. Making homologues list

#See Supplementary Figure 8(A) Linux commands #3-2. Making homologues list (pages 2-4)

#4. check of raw data in 01\_rawdata

workDir=/mnt/d/takahashi/Juveniles

scDir=/mnt/d/takahashi/Juveniles/00\_reference

cd $scDir

cat FilenameDir.txt | {

while read dir1

  do

    cd $dir1/01\_raw-seq

    for d in `find ./ -type d`;

        do echo $dir1,`ls "$d" | wc -l`;

    done

    md5sum Lib\*gz > sum.txt

  done

}

#5. RUN FastQC

workDir=/mnt/d/takahashi/Juveniles

scDir=/mnt/d/takahashi/Juveniles/00\_reference

cd $scDir

cat FilenameDir.txt | {

  while read dir1

  do

      fastqc -t 8 --nogroup $dir1/01\_raw-seq/Lib\*.gz -o $dir1/02\_fastqc

      multiqc $dir1/02\_fastqc

      mv ./multiqc\_report\*.html $dir1/02\_fastqc/

  done

}

#6. Processing sequence data with Trim galore

workDir=/mnt/d/takahashi/Juveniles

scDir=/mnt/d/takahashi/Juveniles/00\_reference

cd $scDir

cat FilenameDir.txt | {

  while read dir1

  do

  Max=$((`find $dir1/01\_raw-seq -type f | wc -l`/2))

  for i in `seq -f "%02g" $Max`

  do

  trim\_galore -q 30 --length 35 \

  --paired $dir1/01\_raw-seq/Lib${i}\*R1\_001.fastq.gz $dir1/01\_raw-seq/Lib${i}\*R2\_001.fastq.gz \

  -o $dir1/03\_valid-seq \

  &> $dir1/03\_valid-seq/fastq${i}.trim-galore.log;

  done

  done

}

#7 genome mapping with HISAT2

workDir=/mnt/d/takahashi/Juveniles

scDir=/mnt/d/takahashi/Juveniles/00\_reference

cd $scDir

cat FilenameDir.txt | {

  while read dir1

  do

  Max=$((`find $dir1/01\_raw-seq -type f | wc -l`/2))

  for i in `seq -f "%02g" $Max`

  do

    hisat2 -p 8 -x $scDir/nfu.abundant \

      -1 $dir1/03\_valid-seq/Lib${i}\*val\_1.fq.gz \

      -2 $dir1/03\_valid-seq/Lib${i}\*val\_2.fq.gz \

      -S $dir1/04\_abundant-check/Lib${i}.abundant.sam \

      --no-unal --no-hd \

      2>$dir1/04\_abundant-check/Lib${i}.abundant.log;

  done

  done

}

workDir=/mnt/d/takahashi/Juveniles

scDir=/mnt/d/takahashi/Juveniles/00\_reference

cd $scDir

cat FilenameDir.txt | {

  while read dir1

  do

  echo -e "library\tchrM\teGFP\tphiX174\trRNA\tmRNA" > $dir1/04\_abundant-check/abundant.summary.log;

  Max=$((`find $dir1/01\_raw-seq -type f | wc -l`/2))

  echo $Max;

  done

}

cat FilenameDir.txt | {

  while read dir1

  do

  Max=$((`find $dir1/01\_raw-seq -type f | wc -l`/2))

  for i in `seq -f "%02g" $Max`

  do

  paste <(echo $dir1/04\_abundant-check/Lib${i}) \

  <(grep -c chrM    $dir1/04\_abundant-check/Lib${i}.abundant.sam) \

  <(grep -c eGFP    $dir1/04\_abundant-check/Lib${i}.abundant.sam) \

  <(grep -c phiX174 $dir1/04\_abundant-check/Lib${i}.abundant.sam) \

  <(grep -c EU780557    $dir1/04\_abundant-check/Lib${i}.abundant.sam) \

  <(sed -n 3P $dir1/04\_abundant-check/Lib${i}.abundant.log | cut -f 5 -d ' ') \

  >> $dir1/04\_abundant-check/abundant.summary.log;

  done

  done

}

cat FilenameDir.txt | {

  while read dir1

  do

  R --no-save --args $dir1/04\_abundant-check/abundant.summary.log $dir1/04\_abundant-check/abundant < $scDir/logPlot.R #H-1 script

  done

}

workDir=/mnt/d/takahashi/Juveniles

scDir=/mnt/d/takahashi/Juveniles/00\_reference

cd $scDir

cat FilenameDir.txt | {

  while read dir1

  do

  Max=$((`find $dir1/01\_raw-seq -type f | wc -l`/2))

  for i in `seq -f "%02g" $Max`

  do

  hisat2 -p 8 -x $scDir/Nothobranchius\_furzeri.Nfu\_20140520.dna.toplevel --rna-strandness RF \

  -1 $dir1/03\_valid-seq/Lib${i}\*val\_1.fq.gz -2 $dir1/03\_valid-seq/Lib${i}\*val\_2.fq.gz \

  --dta \

  -S $dir1/05\_mapping/Lib${i}.sam 2> $dir1/05\_mapping/Lib${i}.mapping.log;

  done

  done

}

workDir=/mnt/d/takahashi/Juveniles

scDir=/mnt/d/takahashi/Juveniles/00\_reference

cd $scDir

cat FilenameDir.txt | {

  while read dir1

  do

  Max=$((`find $dir1/01\_raw-seq -type f | wc -l`/2))

  for i in `seq -f "%02g" $Max`

  do

  samtools sort -@ 8 $dir1/05\_mapping/Lib${i}.sam > $dir1/05\_mapping/Lib${i}.bam;

  samtools index -@ 8 $dir1/05\_mapping/Lib${i}.bam;

  done

  done

}

workDir=/mnt/d/takahashi/Juveniles

scDir=/mnt/d/takahashi/Juveniles/00\_reference

cd $scDir

cat FilenameDir.txt | {

  while read dir1

  do

    echo -e "library\tunmapped\tmulti-mapped\tuniq-mapped" > $dir1/05\_mapping/mapping.summary.log;

    Max=$((`find $dir1/01\_raw-seq -type f | wc -l`/2))

    for i in `seq -f "%02g" $Max`

    do

        paste <(echo $dir1/05\_mapping/Lib${i}) \

        <(sed -n 3P $dir1/05\_mapping/Lib${i}.mapping.log | cut -f 5 -d ' ') \

        <(sed -n 5P $dir1/05\_mapping/Lib${i}.mapping.log | cut -f 5 -d ' ') \

        <(sed -n 4P $dir1/05\_mapping/Lib${i}.mapping.log | cut -f 5 -d ' ') \

        >> $dir1/05\_mapping/mapping.summary.log;

    done

    R --no-save --args $dir1/05\_mapping/mapping.summary.log $dir1/05\_mapping/mapping <  $scDir/logPlot.R #H-1 script

  done

}

#9.Counting with fetureCounts

workDir=/mnt/d/takahashi/Juveniles

scDir=/mnt/d/takahashi/Juveniles/00\_reference

cd $scDir

cat FilenameDir.txt | {

    while read dir1

    do

    featureCounts -T 8 -p -B -C -t exon -g gene\_id -a $scDir/Nothobranchius\_furzeri.Nfu\_20140520.108.gtf -o $dir1/06\_count/all.featurecounts.txt $dir1/05\_mapping/Lib\*.bam;

  done

}

#Manually delete the first line of the text file "all.featurecounts.txt",  and rename it to "all.featurecounts2.txt".

workDir=/mnt/d/takahashi/Juveniles

scDir=/mnt/d/takahashi/Juveniles/00\_reference

cd $scDir

cat FilenameDir.txt | {

    while read dir1

    do

    R --no-save --args $dir1/06\_count/all.featurecounts2.txt $dir1/06\_count/ $dir1/06\_count/colnames.txt <  $scDir/counts\_to\_tpm2.R #H-2 script

    R --no-save --args $dir1/06\_count/TPMfeaturecounts.txt $dir1/06\_count $dir1/06\_count/colnames.txt<  $scDir/TH.R #H-3 script

    R --no-save --args /mnt/d/takahashi/blast/ncbi-blast-2.13.0+-src/c++/nfu\_zeb\_medaka.txt $dir1/06\_count/TPMfeaturecounts.txt $dir1/06\_count/ <  /mnt/d/takahashi/blast/ncbi-blast-2.13.0+-src/c++/blast3.R #H-11 script

    R --no-save --args $dir1/06\_count/TPMfeaturecounts\_max10.txt $dir1/06\_count  <  $dir1/06\_count/Fig7A.R #H-4 script

  done

}

#10. PCA(Fig.7B) & DEseq2 (Supplementary Fig.4-5)

workDir=/mnt/d/takahashi/Juveniles

scDir=/mnt/d/takahashi/Juveniles/00\_reference

cd $workDir

cd $scDir

cat FilenameDir.txt | {

  while read dir1

  do

  wdir=$dir1/08\_DESeq2

  data=$dir1/06\_count/all.featurecounts2.txt

  INDEX=$dir1/08\_DESeq2/samplenames.txt

  GTF=$scDir/Nothobranchius\_furzeri.Nfu\_20140520.108.gtf

  default=default

  TPMdata=$dir1/06\_count/TPMfeaturecounts\_max10.txt

  newTPMdata=$dir1/06\_count/newTPMfeaturecounts2.txt

  THtpm=10

  ID=/mnt/d/takahashi/blast/ncbi-blast-2.13.0+-src/c++/nfu\_zeb\_medaka.txt

  R --no-save --args $wdir $data $INDEX $GTF defalt $TPMdata $newTPMdata $THtpm $ID < $dir1/08\_DESeq2/DESeq2.R #H-5 script

  done

  done

}

#11. Heatmap and clustering of DEGs between stage1/2 and stage6 (Fig.7C)

workDir=/mnt/d/takahashi/Juveniles

scDir=/mnt/d/takahashi/Juveniles/00\_reference

cd $workDir

cd $scDir

cat FilenameDir.txt | {

    while read dir1

    do

      R --no-save --args $dir1/07\_clustering/commonDEGs.csv commonDEGs-TPM.txt $dir1/06\_count/newTPMfeaturecounts2.txt $dir1/07\_clustering <  $dir1/07\_clustering/ID-TPMv1.R #H-6 script

      R --no-save --args $dir1/07\_clustering/commonDEGs-TPM.txt $dir1/07\_clustering  <  $dir1/07\_clustering/Fig7C.R #H-7 script

    done

}

#12. graphs of the expression change of cell scenesence-related genes (Fig.7D, Supplementary Fig.6)

workDir=/mnt/d/takahashi/Juveniles

scDir=/mnt/d/takahashi/Juveniles/00\_reference

cd $workDir

cd $scDir

cat FilenameDir.txt | {

    while read dir1

    do

    R --no-save --args $dir1/06\_count/graph.txt graph-TPM.txt $dir1/06\_count/newTPMfeaturecounts2.txt $dir1/06\_count <  $dir1/07\_clustering/ID-TPMv1.R #H-6 script

    R --no-save --args $dir1/06\_count/graph-TPM.txt $dir1/06\_count graph-TPM# < $dir1/06\_count/beeswarm2.R #H-8 script

    done

}

#13. DEGs number (Fig.7E)

workDir=/mnt/d/takahashi/Juveniles

scDir=/mnt/d/takahashi/Juveniles/00\_reference

cd $scDir

cat FilenameDir.txt | {

  while read dir1

  do

  wdir=$dir1/08\_DESeq2

  data=$dir1/06\_count/all.featurecounts2.txt

  INDEX=$dir1/08\_DESeq2/samplenames.txt

  GTF=$scDir/Nothobranchius\_furzeri.Nfu\_20140520.108.gtf

  default=default

  TPMdata=$dir1/06\_count/TPMfeaturecounts\_max50.txt

  newTPMdata=$dir1/06\_count/newTPMfeaturecounts2.txt

  THtpm=50

  R --no-save --args $wdir $data $INDEX $GTF defalt $TPMdata $newTPMdata $THtpm < $dir1/08\_DESeq2/DESeq2v4-2.R #H-9 script

  done

}

#14. Heatmap for Fig7F

workDir=/mnt/d/takahashi/Juveniles

scDir=/mnt/d/takahashi/Juveniles/00\_reference

cd $workDir

cd $scDir

cat FilenameDir.txt | {

    while read dir1

    do

      wdir=$dir1/08\_DESeq2

      data=$dir1/08\_DESeq2/DESeq2\_result\_DEGall\_FC4\_fdr0.01\_TPMmax50.txt

      INDEX=$dir1/08\_DESeq2/samplenames.txt

      newTPMdata=$dir1/06\_count/newTPMfeaturecounts2.txt

      name="allDEGsFC4\_fdr0.01\_TPMmax50\_TPMcorrelation\_All.txt"

      R --no-save --args $wdir $data $INDEX $newTPMdata $name < $scDir/correlation3.R #H-10 script

      R --no-save --args $dir1/08\_DESeq2/allDEGsFC4\_fdr0.01\_TPMmax50\_TPMcorrelation\_All.txt $dir1/08\_DESeq2 <  $dir1/08\_DESeq2/Fig7F.R #H-11 script

    done

}

#Supplementary Fig.4-5

workDir=/mnt/d/takahashi/Juveniles

scDir=/mnt/d/takahashi/Juveniles/00\_reference

cd $workDir

cd $scDir

cat FilenameDir.txt | {

    while read dir1

    do

    R --no-save --args $dir1/06\_count/up.csv up-TPM.txt $dir1/06\_count/newTPMfeaturecounts2.txt ./ <  .$dir1/06\_count/ID-TPMv1.R #H-6 script

    R --no-save --args $dir1/06\_count/down.csv down-TPM.txt $dir1/06\_count/newTPMfeaturecounts2.txt ./ <  $dir1/06\_count/ID-TPMv1.R #H-6 script

    done

}

#DEGS (S2-4 vs G1-4)\_Supplementary Fig.7-8

workDir=/mnt/d/takahashi/Juveniles

scDir=/mnt/d/takahashi/Juveniles/00\_reference

cd $scDir

cat FilenameDir.txt | {

  while read dir1

  do

  wdir=$dir1/08\_DESeq2

  data=$dir1/06\_count/all.featurecounts2.txt

  INDEX=$dir1/08\_DESeq2/samplenames2.txt

  GTF=$scDir/Nothobranchius\_furzeri.Nfu\_20140520.108.gtf

  default=default

  TPMdata=$dir1/06\_count/TPMfeaturecounts\_max10.txt

  newTPMdata=$dir1/06\_count/newTPMfeaturecounts2.txt

  THtpm=10

  ID=/mnt/d/takahashi/blast/ncbi-blast-2.13.0+-src/c++/nfu\_zeb\_medaka.txt

  R --no-save --args $wdir $data $INDEX $GTF defalt $TPMdata $newTPMdata $THtpm $ID $INDEX2< $dir1/08\_DESeq2/DESeq2v6.R #H-12 script

  done

}

**(B) sample.csv**

1,male,/mnt/d/takahashi/Juveniles,

2,female,/mnt/d/takahashi/Juveniles,

**(C) nfu.abundant.fa**

See Supplementary Figure 8(C) nfu.abundant.fa (pages 11-17)

**(D) colnames.txt**

geneID,GeneLength,G9d\_1,G9d\_2,G9d\_3,G9d\_4,G13d\_1,G13d\_2,G13d\_3,G13d\_4,G17d\_1,G17d\_2,G17d\_3,G17d\_4,G21d\_1,G21d\_2,G21d\_3,G21d\_4,G28d\_1,G28d\_2,G28d\_3,G28d\_4,G35d\_1,G35d\_2,G35d\_3,G35d\_4,S9d\_1,S9d\_2,S9d\_3,S9d\_4,S10d\_1,S10d\_2,S10d\_3,S10d\_4,S11d\_1,S11d\_2,S11d\_3,S11d\_4,S17d\_1,S17d\_2,S17d\_3,S17d\_4,S21d\_1,S21d\_2,S21d\_3,S21d\_4

**(E) samplenames.txt**

(E-1) samplenames.txt

G9d,G9d,G9d,G9d,G13d,G13d,G13d,G13d,G17d,G17d,G17d,G17d,G21d,G21d,G21d,G21d,G28d,G28d,G28d,G28d,G35d,G35d,G35d,G35d,S9d,S9d,S9d,S9d,S10d,S10d,S10d,S10d,S11d,S11d,S11d,S11d,S17d,S17d,S17d,S17d,S21d,S21d,S21d,S21d

(E-2) samplenames2.txt

Gj,Gj,Gj,Gj,Gj,Gj,Gj,Gj,Gj,Gj,Gj,Gj,Gj,Gj,Gj,Gj,G28d,G28d,G28d,G28d,G35d,G35d,G35d,G35d,Sj,Sj,Sj,Sj,Sj,Sj,Sj,Sj,Sj,Sj,Sj,Sj,S17d,S17d,S17d,S17d,S21d,S21d,S21d,S21d

**(F)** **commonDEGs.csv**

**(F-1) male >** **07\_clustering > commonDEGs.csv**

ENSNFUG00015013763

ENSNFUG00015016999

ENSNFUG00015004461

ENSNFUG00015011555

ENSNFUG00015007621

ENSNFUG00015016896

ENSNFUG00015020193

ENSNFUG00015017486

ENSNFUG00015006758

ENSNFUG00015018841

ENSNFUG00015010429

ENSNFUG00015013536

ENSNFUG00015009758

ENSNFUG00015010595

ENSNFUG00015006779

ENSNFUG00015017910

ENSNFUG00015010711

ENSNFUG00015025289

ENSNFUG00015013330

ENSNFUG00015012483

ENSNFUG00015006914

ENSNFUG00015003233

ENSNFUG00015001595

ENSNFUG00015013426

ENSNFUG00015013464

ENSNFUG00015003476

ENSNFUG00015016057

ENSNFUG00015012290

ENSNFUG00015019695

ENSNFUG00015003981

ENSNFUG00015003776

ENSNFUG00015025307

ENSNFUG00015000894

ENSNFUG00015008466

ENSNFUG00015023790

ENSNFUG00015016486

ENSNFUG00015014629

ENSNFUG00015007153

ENSNFUG00015021139

ENSNFUG00015008878

ENSNFUG00015004991

ENSNFUG00015014299

ENSNFUG00015020269

ENSNFUG00015020573

ENSNFUG00015001304

ENSNFUG00015009909

ENSNFUG00015021422

ENSNFUG00015005810

ENSNFUG00015004739

ENSNFUG00015002339

ENSNFUG00015018028

ENSNFUG00015006628

ENSNFUG00015021545

ENSNFUG00015008767

ENSNFUG00015010722

ENSNFUG00015015054

ENSNFUG00015020032

ENSNFUG00015018114

ENSNFUG00015008089

ENSNFUG00015010862

ENSNFUG00015000800

ENSNFUG00015007052

ENSNFUG00015007574

ENSNFUG00015023555

ENSNFUG00015007657

ENSNFUG00015018780

ENSNFUG00015010818

ENSNFUG00015007832

ENSNFUG00015024586

ENSNFUG00015025049

ENSNFUG00015015333

ENSNFUG00015011388

ENSNFUG00015014135

ENSNFUG00015005872

ENSNFUG00015002229

ENSNFUG00015022509

ENSNFUG00015022812

ENSNFUG00015021366

ENSNFUG00015006905

ENSNFUG00015007443

ENSNFUG00015017825

ENSNFUG00015021543

ENSNFUG00015017867

ENSNFUG00015021546

ENSNFUG00015025279

ENSNFUG00015018989

ENSNFUG00015007370

ENSNFUG00015022400

ENSNFUG00015015479

ENSNFUG00015023021

ENSNFUG00015004017

ENSNFUG00015007595

ENSNFUG00015016689

ENSNFUG00015015449

ENSNFUG00015024742

ENSNFUG00015000547

ENSNFUG00015016621

ENSNFUG00015023557

ENSNFUG00015018687

ENSNFUG00015024735

ENSNFUG00015010772

ENSNFUG00015021921

ENSNFUG00015020816

ENSNFUG00015010844

ENSNFUG00015020525

ENSNFUG00015012293

ENSNFUG00015003894

ENSNFUG00015000041

ENSNFUG00015022100

ENSNFUG00015014569

ENSNFUG00015020545

ENSNFUG00015007841

ENSNFUG00015010245

ENSNFUG00015003143

ENSNFUG00015018291

ENSNFUG00015022168

ENSNFUG00015012345

ENSNFUG00015024441

ENSNFUG00015003973

ENSNFUG00015007919

ENSNFUG00015021264

ENSNFUG00015003036

ENSNFUG00015000814

ENSNFUG00015013595

ENSNFUG00015002566

ENSNFUG00015007656

ENSNFUG00015020984

ENSNFUG00015007630

ENSNFUG00015009959

ENSNFUG00015018650

ENSNFUG00015016492

ENSNFUG00015024320

ENSNFUG00015007611

ENSNFUG00015020333

ENSNFUG00015018786

ENSNFUG00015018686

ENSNFUG00015001018

ENSNFUG00015009627

ENSNFUG00015014701

ENSNFUG00015002319

ENSNFUG00015021358

ENSNFUG00015007590

ENSNFUG00015015003

ENSNFUG00015006859

ENSNFUG00015020348

ENSNFUG00015003964

ENSNFUG00015003976

ENSNFUG00015025432

ENSNFUG00015020809

ENSNFUG00015010160

ENSNFUG00015018651

ENSNFUG00015002083

ENSNFUG00015006413

ENSNFUG00015020256

ENSNFUG00015022933

ENSNFUG00015007213

ENSNFUG00015015838

ENSNFUG00015018722

ENSNFUG00015002697

ENSNFUG00015006801

ENSNFUG00015017802

ENSNFUG00015007588

ENSNFUG00015011925

ENSNFUG00015018234

ENSNFUG00015009255

ENSNFUG00015010960

ENSNFUG00015011241

ENSNFUG00015006757

ENSNFUG00015010085

ENSNFUG00015017471

ENSNFUG00015007564

ENSNFUG00015007691

ENSNFUG00015017780

ENSNFUG00015019128

ENSNFUG00015009349

ENSNFUG00015003574

ENSNFUG00015014768

ENSNFUG00015018188

ENSNFUG00015019234

ENSNFUG00015015059

ENSNFUG00015004588

ENSNFUG00015023064

ENSNFUG00015021686

ENSNFUG00015017985

ENSNFUG00015014159

ENSNFUG00015016044

ENSNFUG00015007720

ENSNFUG00015021094

ENSNFUG00015022729

ENSNFUG00015015495

ENSNFUG00015013469

ENSNFUG00015010294

ENSNFUG00015014325

ENSNFUG00015010908

ENSNFUG00015021289

ENSNFUG00015006246

ENSNFUG00015020047

ENSNFUG00015009729

ENSNFUG00015024043

ENSNFUG00015021222

ENSNFUG00015004893

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ENSNFUG00015013132

ENSNFUG00015021002

ENSNFUG00015008032

ENSNFUG00015024552

ENSNFUG00015025346

ENSNFUG00015016281

ENSNFUG00015013219

ENSNFUG00015023343

ENSNFUG00015022759

ENSNFUG00015006322

ENSNFUG00015008655

ENSNFUG00015009448

ENSNFUG00015002603

ENSNFUG00015007755

ENSNFUG00015004974

ENSNFUG00015008015

ENSNFUG00015006867

ENSNFUG00015003723

ENSNFUG00015019537

ENSNFUG00015017001

ENSNFUG00015002715

ENSNFUG00015024014

ENSNFUG00015009731

ENSNFUG00015018960

ENSNFUG00015020695

ENSNFUG00015010424

ENSNFUG00015003607

ENSNFUG00015024482

ENSNFUG00015021678

ENSNFUG00015014049

ENSNFUG00015009802

ENSNFUG00015007606

ENSNFUG00015003870

ENSNFUG00015022858

ENSNFUG00015011347

ENSNFUG00015017681

ENSNFUG00015024506

ENSNFUG00015001496

ENSNFUG00015001503

ENSNFUG00015012931

ENSNFUG00015013610

ENSNFUG00015018243

ENSNFUG00015020601

ENSNFUG00015017623

ENSNFUG00015015117

ENSNFUG00015024760

ENSNFUG00015008429

ENSNFUG00015019309

ENSNFUG00015013054

ENSNFUG00015016124

ENSNFUG00015018171

ENSNFUG00015002862

ENSNFUG00015008657

ENSNFUG00015001340

ENSNFUG00015007224

ENSNFUG00015020115

ENSNFUG00015022784

ENSNFUG00015000553

ENSNFUG00015007184

ENSNFUG00015009087

ENSNFUG00015003351

ENSNFUG00015024126

ENSNFUG00015007174

ENSNFUG00015021039

ENSNFUG00015018774

ENSNFUG00015022162

ENSNFUG00015021268

ENSNFUG00015012600

ENSNFUG00015022548

ENSNFUG00015023943

ENSNFUG00015000591

ENSNFUG00015002138

ENSNFUG00015003917

ENSNFUG00015000476

ENSNFUG00015003140

ENSNFUG00015008440

ENSNFUG00015018700

ENSNFUG00015005718

ENSNFUG00015007736

ENSNFUG00015023746

ENSNFUG00015007598

ENSNFUG00015001718

ENSNFUG00015016626

ENSNFUG00015006385

ENSNFUG00015014155

ENSNFUG00015021622

ENSNFUG00015010015

ENSNFUG00015017938

ENSNFUG00015017520

ENSNFUG00015012669

ENSNFUG00015005268

ENSNFUG00015010155

ENSNFUG00015024469

ENSNFUG00015019852

ENSNFUG00015012582

ENSNFUG00015003901

ENSNFUG00015022487

ENSNFUG00015004919

ENSNFUG00015010290

ENSNFUG00015017198

ENSNFUG00015015699

ENSNFUG00015017012

ENSNFUG00015022246

ENSNFUG00015022208

ENSNFUG00015024322

ENSNFUG00015002469

ENSNFUG00015012566

ENSNFUG00015023026

ENSNFUG00015010600

ENSNFUG00015017019

ENSNFUG00015022293

ENSNFUG00015002592

ENSNFUG00015005672

ENSNFUG00015010888

ENSNFUG00015014179

ENSNFUG00015000477

ENSNFUG00015023365

ENSNFUG00015002002

ENSNFUG00015012267

ENSNFUG00015003372

ENSNFUG00015012496

ENSNFUG00015012588

ENSNFUG00015001575

ENSNFUG00015018903

ENSNFUG00015001130

ENSNFUG00015012215

ENSNFUG00015019268

ENSNFUG00015005758

ENSNFUG00015016922

ENSNFUG00015004344

ENSNFUG00015012611

ENSNFUG00015008908

ENSNFUG00015004714

ENSNFUG00015019792

ENSNFUG00015024316

ENSNFUG00015011825

ENSNFUG00015009097

ENSNFUG00015003845

ENSNFUG00015007566

ENSNFUG00015020938

ENSNFUG00015008615

ENSNFUG00015001071

ENSNFUG00015019830

ENSNFUG00015015249

ENSNFUG00015003267

ENSNFUG00015005939

ENSNFUG00015023018

ENSNFUG00015011022

ENSNFUG00015003379

ENSNFUG00015013998

ENSNFUG00015007457

ENSNFUG00015013485

ENSNFUG00015017257

ENSNFUG00015010230

ENSNFUG00015015926

ENSNFUG00015025264

ENSNFUG00015010284

ENSNFUG00015004574

ENSNFUG00015007237

ENSNFUG00015009598

ENSNFUG00015012644

ENSNFUG00015021272

ENSNFUG00015003461

ENSNFUG00015000043

ENSNFUG00015009557

ENSNFUG00015018125

ENSNFUG00015013454

ENSNFUG00015010961

ENSNFUG00015003306

ENSNFUG00015018355

ENSNFUG00015010644

ENSNFUG00015004973

ENSNFUG00015018689

ENSNFUG00015000143

ENSNFUG00015017084

ENSNFUG00015003384

ENSNFUG00015013643

ENSNFUG00015021065

ENSNFUG00015016301

ENSNFUG00015022721

ENSNFUG00015012094

ENSNFUG00015019950

ENSNFUG00015011056

ENSNFUG00015013926

ENSNFUG00015020740

ENSNFUG00015023269

ENSNFUG00015020451

ENSNFUG00015004958

ENSNFUG00015000269

ENSNFUG00015011622

ENSNFUG00015023325

ENSNFUG00015013605

ENSNFUG00015015485

ENSNFUG00015017565

ENSNFUG00015003847

ENSNFUG00015006015

ENSNFUG00015024297

ENSNFUG00015018751

ENSNFUG00015018055

ENSNFUG00015018952

ENSNFUG00015009050

ENSNFUG00015007277

ENSNFUG00015009233

ENSNFUG00015005936

ENSNFUG00015001594

ENSNFUG00015015740

ENSNFUG00015022701

ENSNFUG00015019915

ENSNFUG00015017241

ENSNFUG00015007783

ENSNFUG00015010007

ENSNFUG00015005532

ENSNFUG00015013589

ENSNFUG00015019996

ENSNFUG00015020168

ENSNFUG00015009099

ENSNFUG00015011852

ENSNFUG00015009511

ENSNFUG00015009085

ENSNFUG00015016273

ENSNFUG00015014223

ENSNFUG00015007037

ENSNFUG00015022908

ENSNFUG00015012597

ENSNFUG00015010498

ENSNFUG00015015642

ENSNFUG00015018946

ENSNFUG00015016756

ENSNFUG00015018908

ENSNFUG00015020617

ENSNFUG00015014679

ENSNFUG00015018409

ENSNFUG00015014396

ENSNFUG00015025172

ENSNFUG00015008971

ENSNFUG00015001558

ENSNFUG00015001480

ENSNFUG00015011184

ENSNFUG00015011034

ENSNFUG00015003382

ENSNFUG00015024877

ENSNFUG00015000240

ENSNFUG00015003177

ENSNFUG00015020583

ENSNFUG00015023747

ENSNFUG00015016849

ENSNFUG00015013259

ENSNFUG00015005736

ENSNFUG00015004106

ENSNFUG00015005248

ENSNFUG00015014798

ENSNFUG00015013245

ENSNFUG00015002984

ENSNFUG00015012949

ENSNFUG00015012559

ENSNFUG00015001391

ENSNFUG00015016260

ENSNFUG00015023009

ENSNFUG00015021994

ENSNFUG00015004407

ENSNFUG00015008299

ENSNFUG00015004258

ENSNFUG00015017627

ENSNFUG00015018748

ENSNFUG00015004277

ENSNFUG00015023976

ENSNFUG00015000234

ENSNFUG00015023275

ENSNFUG00015012757

ENSNFUG00015005433

ENSNFUG00015007157

ENSNFUG00015016401

ENSNFUG00015011644

ENSNFUG00015025036

ENSNFUG00015022793

ENSNFUG00015015870

ENSNFUG00015014856

ENSNFUG00015012298

ENSNFUG00015010583

ENSNFUG00015001721

ENSNFUG00015018292

ENSNFUG00015013325

ENSNFUG00015024882

ENSNFUG00015020063

ENSNFUG00015001487

ENSNFUG00015020824

ENSNFUG00015022354

ENSNFUG00015004935

ENSNFUG00015020771

ENSNFUG00015002376

ENSNFUG00015012103

ENSNFUG00015009842

ENSNFUG00015011727

ENSNFUG00015022029

ENSNFUG00015009007

ENSNFUG00015005016

ENSNFUG00015022086

ENSNFUG00015006281

ENSNFUG00015001604

ENSNFUG00015023344

ENSNFUG00015008636

ENSNFUG00015020679

ENSNFUG00015022868

ENSNFUG00015010982

ENSNFUG00015023887

ENSNFUG00015021231

ENSNFUG00015008081

ENSNFUG00015010677

ENSNFUG00015017561

ENSNFUG00015004493

ENSNFUG00015015179

ENSNFUG00015018285

ENSNFUG00015004413

ENSNFUG00015017907

ENSNFUG00015018041

ENSNFUG00015021000

ENSNFUG00015022838

ENSNFUG00015020698

ENSNFUG00015009781

ENSNFUG00015005351

**(G)** **graph.txt**

**(G-1) male >** **06\_count > graph.txt**

ENSNFUG00015017902

ENSNFUG00015003177

ENSNFUG00015002592

ENSNFUG00015014290

ENSNFUG00015010294

ENSNFUG00015019695

ENSNFUG00015010245

ENSNFUG00015024488

ENSNFUG00015015851

ENSNFUG00015017155

ENSNFUG00015021982

**(G-2) female >** **06\_count > graph.txt**

ENSNFUG00015017902

ENSNFUG00015003177

ENSNFUG00015002592

ENSNFUG00015014290

ENSNFUG00015010294

ENSNFUG00015019695

ENSNFUG00015010245

ENSNFUG00015022764

ENSNFUG00015021077

ENSNFUG00015017155

ENSNFUG00015019719

**(H) R scripts**

**(H-1) logPlot.R**

See Supplementary Figure 8(H-1) logPlot.R (pages 18-19)

**(H-2) counts\_to\_tpm2.R**

See Supplementary Figure 8(H-1) counts\_to\_tpm2.R (page 19)

**(H-3) TH.R**

See Supplementary Figure 8(H-1) counts\_to\_tpm2.R (pages 19-20)

**(H-4) Fig7A.R**

#Heatmap

#Correlation coefficients between samples in the gene sets with TPM > 10 in at least one sample.

library(lattice)

library(latticeExtra)

library(gplots)

library(matrixStats)

library(genefilter)

library(dplyr)

my.col1 <- colorRampPalette(c("blue","white","magenta"))

comm <- commandArgs(trailingOnly = T)

head (comm)

infile1 <- comm[1]

outdir <- comm[2]

indata <- read.delim(infile1, header=T, sep="\t")

x<-indata[,3:46]

x<-x+1

x <- as.matrix(x)

log\_x<-log(x,base=2)

cor<-cor(log\_x)

cor2<-round(cor, digits = 2)

correlationPlot <- function(x) {

  cor<-cor(log\_x);

  fig<-levelplot(cor, col.regions=colorRampPalette(c("yellow", "red"), space = "rgb")(120), scales=list(x=list(rot=55)), main="TPMlibirary\_Correlation plot")

  fig

}

outfile <- paste(outdir, "Fig7A.pdf", sep="/")

pdf(file=outfile,onefile=FALSE,paper="special",height=15,width=15,family="Helvetica",pointsize=15)

correlationPlot(log\_x)

dev.off()

**(H-5) DESeq2.R**

#PCA(VST)&DESeq2

options(warn=1)

options(scipen=100)

options( java.parameters = "-Xmx64g" )

library( DESeq2 )

packageVersion("DESeq2")

library("dplyr")

library(xlsx)

library("genefilter")

library("gplots")

my.col1 <- colorRampPalette(c("blue","white","magenta"))

args <- commandArgs(T)

workDir   <- args[1]

data      <- args[2]

head(data)

name    <- args[3]

gtfFile   <- args[4]

gene\_type <- args[5]

subName   <- ifelse(gene\_type=="default","",paste0(".",gene\_type))

markers   <- c()

TPMdata <- args[6]

#nzm<-args[7]

newTPMdata<-args[7]

THtpm <- args[8]

infile3 <- args[9];#zebraID�ϊ��\

outdir <- workDir

outDir    <- file.path(workDir)

outDir

outFile   <- file.path(outDir, paste0("TPM",".xlsx"))

outPCA    <- file.path(outDir, paste0("PCA",subName,".xlsx"))

outBAR    <- file.path(outDir, paste0("barplot",subName,".pdf"))

saveData  <- file.path(outDir, paste0("DESeq2\_HISAT2",subName,".Rdata"))

name1 <- read.delim(name,header=F, sep=",")

name1<-as.vector(name1)

Group <- data.frame(con = factor(name1))

data<-read.table(data,header=T,row.names=1,sep="\t")

head(data)

#data TPM>10

TPMdata<-read.delim(TPMdata,header=T,sep="\t")

TPMdata1<-as.matrix(TPMdata)

TPMmax10<-TPMdata1[,1]

print(head(TPMdata1))

print(head(TPMmax10))

L1 <- length(TPMmax10)

L1

#TPM

t5 <- c()

t4 <- subset (data, row.names(data) == TPMmax10[1])

t4 <- t4

for (i in 2:L1) {

  t5 <- subset (data, row.names(data) ==  TPMmax10[i], )

  t4 <- rbind (t4, t5)

}

head(t4)

data<-t4

data1<-row.names(data)

data2<-data[6:ncol(data)]

data3<-cbind(data1, data2)

#DESeq2

dds <- DESeqDataSetFromMatrix(countData=data2, colData=Group, design=~ con)

genelength <- matrix( unlist(data [5]), ncol = 1)

mcols(dds)$basepairs <- as.numeric(genelength)

dds <- estimateSizeFactors(dds)

dds <- estimateDispersions(dds)

dds <- nbinomWaldTest(dds)

#vst\_value\_PCA\_plot(Fig.7B)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

vst <- vst(dds, blind=FALSE)

head(assay(vst), 3)

vst1<-assay(vst)

colnames(vst1) <- c("G9d\_1", "G9d\_2", "G9d\_3", "G9d\_4", "G13d\_1", "G13d\_2", "G13d\_3", "G13d\_4","G17d\_1", "G17d\_2", "G17d\_3", "G17d\_4", "G21d\_1", "G21d\_2", "G21d\_3", "G21d\_4", "G28d\_1", "G28d\_2", "G28d\_3", "G28d\_4", "G35d\_1", "G35d\_2", "G35d\_3", "G35d\_4", "S9d\_1", "S9d\_2", "S9d\_3", "S9d\_4",  "S10d\_1", "S10d\_2", "S10d\_3", "S10d\_4", "S11d\_1", "S11d\_2", "S11d\_3", "S11d\_4", "S17d\_1", "S17d\_2", "S17d\_3", "S17d\_4",  "S21d\_1", "S21d\_2", "S21d\_3", "S21d\_4")

outfile <- paste(outdir, "VST\_value.txt", sep="/")

write.table(vst1, file=outfile, sep="\t")

res <- prcomp(t(assay(vst)), scale=T)

v <- res$sdev^2

v <- v / sum(v)

v[1]<-round(v[1]\*100,1)

v[2]<-round(v[2]\*100,1)

v[3]<-round(v[3]\*100,1)

v[4]<-round(v[4]\*100,1)

v[5]<-round(v[5]\*100,1)

v[6]<-round(v[6]\*100,1)

v[7]<-round(v[7]\*100,1)

v[8]<-round(v[8]\*100,1)

v[9]<-round(v[9]\*100,1)

v[1]

v[2]

v[3]

v[4]

v[5]

PC1 <- res$x[, 1]

PC2 <- res$x[, 2]

PC3 <- res$x[, 3]

PC4 <- res$x[, 4]

PC5 <- res$x[, 5]

PC6 <- res$x[, 6]

PC7 <- res$x[, 7]

PC8 <- res$x[, 8]

PC9 <- res$x[, 9]

PC1\_9<- res$x[,1:9]

outfile <- paste(outdir, "Score\_VST\_PCA\_prcomp.txt", sep="/")

write.table(PC1\_9, file=outfile, sep="\t")

col <- c("skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","skyblue3","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1","palevioletred1")

pch <- c(0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1)

label3 <- c("1", "1", "1", "1", "2", "2", "2", "2","3", "3", "3", "3", "4", "4", "4", "4", "5", "5", "5", "5", "6", "6", "6", "6", "2", "2", "2", "2", "3", "3", "3", "3", "4", "4", "4", "4",  "5", "5", "5", "5",  "6", "6", "6", "6")

outfile <- paste(outdir, "VST\_PCA\_prcomp\_PC1-2\_#2.pdf", sep="/");

pdf(file=outfile,onefile=FALSE,paper="special",height=15,width=15,family="Times",pointsize=20)

xname <- paste("PC1\_", v[1], "%", sep="");

yname <- paste("PC2\_", v[2], "%", sep="");

plot(PC1, PC2, pch=1, cex=6, col = col,xlab=xname, ylab=yname, main="TPMlibirary\_PCA\_prcomp\_PC1-2")

text(PC1, PC2, label3, col = col, cex=2.5)

dev.off();

#loading factors

fc.l2<-sweep(res$rotation, MARGIN=2, res$sdev, FUN="\*")

newTPMdata<-read.delim(newTPMdata,header=T,sep="\t")

colname <- newTPMdata[,1];

colname <- as.matrix(colname)

nfu\_G <- data3[,1]

head(nfu\_G)

nfu\_zeb<-read.delim(infile3, sep="\t",header=F);

nfu\_zeb <- as.matrix(nfu\_zeb)

head(nfu\_zeb)

L1 <- length(nfu\_G)

L2 <- L1+1

L2

t5 <- c()

t4 <- subset (nfu\_zeb, nfu\_zeb[,1] == nfu\_G[1], 4)

t4 <- t4[1]

for (i in 2:L1) {

  t5 <- subset (nfu\_zeb, nfu\_zeb[,1] ==  nfu\_G[i], 4)

  t6 <- t5[1]

  t4 <- c (t4, t6)

}

zebraID <- t4

zebraID <- as.matrix(zebraID)

colnames(zebraID)<-c("zebra\_geneID")

length(zebraID)

head(zebraID)

t5 <- c()

t4 <- subset (nfu\_zeb, nfu\_zeb[,1] == nfu\_G[1], 5)

t4 <- t4[1]

for (i in 2:L1) {

  t5 <- subset (nfu\_zeb, nfu\_zeb[,1] ==  nfu\_G[i], 5)

  t6 <- t5[1]

  t4 <- c (t4, t6)

}

zebraGName <- t4

zebraGName <- as.matrix(zebraGName)

colnames(zebraGName)<-c("zebra\_geneName")

t5 <- c()

t4 <- subset (nfu\_zeb, nfu\_zeb[,1] == nfu\_G[1], 6)

t4 <- t4[1]

for (i in 2:L1) {

  t5 <- subset (nfu\_zeb, nfu\_zeb[,1] ==  nfu\_G[i], 6)

  t6 <- t5[1]

  t4 <- c (t4, t6)

}

zebraGene <- t4

zebraGene <- as.matrix(zebraGene)

colnames(zebraGene)<-c("zebra\_gene")

fc.l<-cbind(data3,zebraID,zebraGName,zebraGene,fc.l2)

outfile <- paste(outdir, "FactorLoadings\_VST\_PCA\_prcomp.txt", sep="/")

write.table(fc.l, file=outfile, sep="\t")

#\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

#DEGs(For Supplementary Fig.5-6)

norm.dt   <- data.frame(counts(dds, normalized=T), check.names=F)

fpkm.dt   <- data.frame(fpkm(dds, robust=F), check.names=F)

tpm.dt    <- data.frame(sweep(fpkm.dt\*1e6, 2, colSums(fpkm.dt),"/"), check.names=F)

cpm.dt    <- data.frame(fpm(dds, robust=F), check.names=F)

outfile <- paste(outDir, "DESeq2\_results.Rdata", sep="/")

#TPM

wb <- createWorkbook(type="xlsx")

sheet  <- createSheet(wb, sheet="TPM")

row.names(tpm.dt)<-row.names(data)

colnames(tpm.dt)<-name1

addDataFrame(tpm.dt, sheet, row.names=T)

saveWorkbook(wb, file=outFile)

outfile <- paste(outDir, "TPM.txt", sep="/")

write.table(tpm.dt, file=outfile, row.names=T)

dds <- DESeqDataSetFromMatrix(countData=data2, colData=Group, design=~ con)

head(dds)

genelength <- matrix( unlist(data [5]), ncol = 1)

mcols(dds)$basepairs <- as.numeric(genelength)

dds <- DESeq(dds)

head(dds)

res<-results(dds)

head(res)

terms<-c("S21d","S17d","S11d","S10d","S9d","G35d","G28d","G21d","G17d","G13d","G9d")

terms2<-combn(terms,2)

terms2

n<-ncol(terms2)

newTPMdata<-as.matrix(newTPMdata)

L1 <- length(TPMmax10)

L1

#TPM

t5 <- c()

t4 <- subset (newTPMdata, newTPMdata[,1] == TPMmax10[1])

t4 <- t4

for (i in 2:L1) {

  t5 <- subset (newTPMdata, newTPMdata[,1] ==  TPMmax10[i], )

  t4 <- rbind (t4, t5)

}

head(t4)

newTPMdata<-t4

head(newTPMdata)

FC<-log2(3)

print(FC)

for (j in 1:n) {

  terms3<-terms2[,j]

  terms4<-append("con",terms3)

  print(terms4)

  res<-results(dds, contrast=terms4)

  res$TPM <-newTPMdata

  tpm<-THtpm

  print(tpm)

  data2 <- as.data.frame(res)

  outfilename <- paste0("DESeq2\_result\_", terms3[1],"vs",terms3[2],"\_TPMmax10\_All.txt")

  out\_f <- paste(outDir, outfilename, sep="/");

  write.table(data2, out\_f, sep="\t", append=F, quote=F, row.names=T, col.names=T)

  data2upAll <- subset(data2, (data2$pad<=0.01 & (data2$log2FoldChange>=FC )))

  outfilename <- paste0("DESeq2\_result\_", terms3[1],"vs",terms3[2],"\_TPMmax10\_3.0upAll.txt")

  out\_f <- paste(outDir, outfilename, sep="/");

  write.table(data2upAll, out\_f, sep="\t", append=F, quote=F, row.names=T, col.names=T)

  data2downAll <- subset(data2, (data2$pad<=0.01 & (data2$log2FoldChange<=-FC )))

  outfilename <- paste0("DESeq2\_result\_", terms3[1],"vs",terms3[2],"\_TPMmax10\_3.0downAll.txt")

  out\_f <- paste(outDir, outfilename, sep="/");

  write.table(data2downAll, out\_f, sep="\t", append=F, quote=F, row.names=T, col.names=T)

}

**(H-6) ID-TPMv1.R**

#ID->TPM

comm <- commandArgs(trailingOnly = T)

infile1 <- comm[1]

infile2 <- comm[2]

infile3 <- comm[3]

outdir <- comm[4]

TPM <- read.delim(infile3, sep="\t");

TPM <- as.matrix(TPM)

downcomID <- read.delim(infile1,header=F,sep=",")

downcomID <-as.matrix(downcomID)

downcomID

L1 <- length(downcomID)

L1

#TPM

t5 <- c()

t4 <- subset (TPM, TPM[,1] == downcomID[1])

t4 <- t4

for (i in 2:L1) {

   t5 <- subset (TPM, TPM[,1] ==  downcomID[i], )

   t4 <- rbind (t4, t5)

}

head(t4)

outfile <- paste(outdir, infile2, sep="/")

write.table(t4, file=outfile, sep="\t")

**(H-7) Fig7C.R**

library(lattice)

library(latticeExtra)

library(gplots)

library(matrixStats)

library(genefilter)

library(dplyr)

my.col1 <- colorRampPalette(c("blue","white","magenta"))

comm <- commandArgs(trailingOnly = T);

head (comm)

infile1 <- comm[1];

outdir <- comm[2];

data <- read.delim(infile1, header=T, sep="\t");

x<-data[,3:46]

x<-x+1

x <- as.matrix(x)

m1<-rowMeans(x[,1:4])

m2<-rowMeans(x[,5:8])

m3<-rowMeans(x[,9:12])

m4<-rowMeans(x[,13:16])

m5<-rowMeans(x[,17:21])

m6<-rowMeans(x[,22:24])

m7<-rowMeans(x[,25:28])

m8<-rowMeans(x[,29:32])

m9<-rowMeans(x[,33:36])

m10<-rowMeans(x[,37:40])

m11<-rowMeans(x[,41:44])

mx<-cbind(m1,m7,m2,m8,m3,m9,m4,m10,m5,m11,m6)

mx\_a<-mx/rowMeans(mx)

log\_mx\_a<-log(mx\_a, base=2)

outfile <- paste(outdir, "Fig7C\_heatmap.pdf", sep="/");

pdf(file=outfile,onefile=FALSE,paper="special",height=15,width=15,family="Helvetica",pointsize=20)

heatmap.2(as.matrix(log\_mx\_a),col=my.col1(269),Colv=NA, scale="none",key=TRUE, symm=F,symkey=F,symbreaks=T, breaks=c(seq(-2,2,length=270)), trace="none",distfun = function(x) {dist(x, method="euclidean")},hclustfun = function(x) {hclust(x, method="ward.D2")})

dev.off();

outfile <- paste(outdir, "Fig7C\_clustering.pdf", sep="/");

pdf(file=outfile,onefile=FALSE,paper="special",height=15,width=15,family="Helvetica",pointsize=20)

heatmap.2(as.matrix(log\_mx\_a),col=my.col1(269),scale="none",key=TRUE, symm=F,symkey=F,symbreaks=T, breaks=c(seq(-2,2,length=270)), trace="none",distfun = function(x) {dist(x, method="euclidean")},hclustfun = function(x) {hclust(x, method="ward.D2")})

dev.off();

**(H-8) beeswarm2.R**

args <- commandArgs(T)

TPMdata <- args[1]

outDir <- args[2]

name <- args[3]

TPMdata <- read.delim(TPMdata, header=T, sep="\t", row.names=1)

TPMdata <- as.matrix(TPMdata)

N<-matrix(NA,nrow(TPMdata),4)

head(N)

print(nrow(TPMdata))

print(nrow(N))

head(TPMdata[,3:26])

head(TPMdata[,27:46])

dx <- cbind(TPMdata[,3:26],N,TPMdata[,27:46])

head(dx)

label<-c(1,1,1,1,1,1,1,1,2,2,2,2,2,2,2,2,3,3,3,3,3,3,3,3,4,4,4,4,4,4,4,4,5,5,5,5,5,5,5,5,6,6,6,6,6,6,6,6)

label2<-c(1,1,1,1,2,2,2,2,2,2,2,2,3,3,3,3,3,3,3,3,4,4,4,4,4,4,4,4,5,5,5,5,6,6,6,6,6,6,6,6,7,7,7,7,7,7,7,7)

X = ceiling(nrow(dx)/30)

X

print(X)

i <- 1

for(i in 1:X){

  print(i)

  filename <- paste0(name,"bw",i,".pdf")

  outfile <- paste(outDir, filename, sep="/");

  pdf(file=outfile,onefile=FALSE,paper="special",height=15,width=15,family="Helvetica",pointsize=8)

  H <- ceiling(nrow(dx)/2)

  H

  par(mfrow=c(5,6))

  a<-30\*(i-1)+1

  if(i==X){

    b<-nrow(dx)

  }else{

    b<-30\*i

  }

  for(j in a:b){

    y1<-c(as.numeric(dx[j,1:4]), as.numeric(dx[j,25:28]))

    y2<-c(as.numeric(dx[j,5:8]), as.numeric(dx[j,29:32]))

    y3<-c(as.numeric(dx[j,9:12]),as.numeric(dx[j,33:36]))

    y4<-c(as.numeric(dx[j,13:16]),as.numeric(dx[j,37:40]))

    y5<-c(as.numeric(dx[j,17:20]),as.numeric(dx[j,41:44]))

    y6<-c(as.numeric(dx[j,21:24]),as.numeric(dx[j,45:48]))

    col<-c("#00A0E9","#00A0E9","#00A0E9","#00A0E9","#FFB2A4", "#FFB2A4", "#FFB2A4", "#FFB2A4")

    v1<-cbind.data.frame(y1,col)

    colnames(v1)<-c("TPM","col")

    v2<-cbind.data.frame(y2,col)

    colnames(v2)<-c("TPM","col")

    v3<-cbind.data.frame(y3,col)

    colnames(v3)<-c("TPM","col")

    v4<-cbind.data.frame(y4,col)

    colnames(v4)<-c("TPM","col")

    v5<-cbind.data.frame(y5,col)

    colnames(v5)<-c("TPM","col")

    v6<-cbind.data.frame(y6,col)

    colnames(v6)<-c("TPM","col")

    y<-rbind.data.frame(v1,v2,v3,v4,v5,v6)

    dx2<-cbind.data.frame(label,y)

    title=TPMdata[j,55:56]

    max=max(as.numeric(dx[j,c(1:24,29:48)]))+10

    print(max)

    if(!is.na(max)){

      beeswarm(TPM~label, data=dx2,pch=16,cex=1.5, yaxs="i", las = 1, xlab = "", cex.lab  = 1.4, cex.axis=1.5, cex.names=1.5, las = 2, main=title,pwcol=c("#00A0E9","#00A0E9","#00A0E9", "#00A0E9","#FFB2A4", "#FFB2A4", "#FFB2A4","#FFB2A4","#00A0E9","#00A0E9","#00A0E9", "#00A0E9","#FFB2A4", "#FFB2A4", "#FFB2A4","#FFB2A4","#00A0E9","#00A0E9","#00A0E9", "#00A0E9","#FFB2A4", "#FFB2A4", "#FFB2A4","#FFB2A4","#00A0E9","#00A0E9","#00A0E9", "#00A0E9","#FFB2A4", "#FFB2A4", "#FFB2A4","#FFB2A4","#00A0E9","#00A0E9","#00A0E9", "#00A0E9","#FFB2A4", "#FFB2A4", "#FFB2A4","#FFB2A4","#00A0E9","#00A0E9","#00A0E9", "#00A0E9","#FFB2A4", "#FFB2A4", "#FFB2A4","#FFB2A4"),labels = c("G9", "G13S9", "G17S10", "G21S11","G28S17","G35S21"),ylim=c(0,max))

      t1<-as.numeric(dx[j,1:4])

      t2<-as.numeric(dx[j,25:28])

      t3<-as.numeric(dx[j,5:8])

      t4<-as.numeric(dx[j,29:32])

      t5<-as.numeric(dx[j,9:12])

      t6<-as.numeric(dx[j,33:36])

      t7<-as.numeric(dx[j,13:16])

      t8<-as.numeric(dx[j,37:40])

      t9<-as.numeric(dx[j,17:20])

      t10<-as.numeric(dx[j,41:44])

      t11<-as.numeric(dx[j,21:24])

      t12<-as.numeric(dx[j,45:48])

      dx3<-cbind(t1,t2,t3,t4,t5,t6,t7,t8,t9,t10,t11,t12)

      m <- apply(dx3, 2, mean, na.rm = TRUE)

      s <- apply(dx3, 2, sd, na.rm = TRUE)

      b <- c(1,1,2,2,3,3,4,4,5,5,6,6)

      arrows (b, m,

              b, m + s,

              length = 0.08,

              angle = 90,

              col=c("#00A0E9","#FFB2A4","#00A0E9","#FFB2A4", "#00A0E9","#FFB2A4",  "#00A0E9", "#FFB2A4"))

      arrows (b, m,

              b, m - s,

              length = 0.08,

              angle = 90,

              col=c("#00A0E9","#FFB2A4","#00A0E9","#FFB2A4", "#00A0E9","#FFB2A4",  "#00A0E9", "#FFB2A4"))

      arrows (b, m,

              b+0.2, m,

              length = 0,

              angle = 90,

              col=c("#00A0E9","#FFB2A4","#00A0E9","#FFB2A4", "#00A0E9","#FFB2A4",  "#00A0E9", "#FFB2A4"))

      arrows (b, m,

              b-0.2, m,

              length = 0,

              angle = 90,

              col=c("#00A0E9","#FFB2A4","#00A0E9","#FFB2A4", "#00A0E9","#FFB2A4",  "#00A0E9", "#FFB2A4"))

    }else{

    }

  }

  dev.off();

}

**(H-9) DESeq2v4-2.R**

#DEGs TPM50, FC4 (FDR0.01)

options(warn=1)

options(scipen=100)

options( java.parameters = "-Xmx64g" )

library( DESeq2 )

packageVersion("DESeq2")

library("dplyr")

library(xlsx)

library("genefilter")

library("gplots")

my.col1 <- colorRampPalette(c("blue","white","magenta"))

args <- commandArgs(T)

workDir   <- args[1]

data      <- args[2]

head(data)

name    <- args[3]

gtfFile   <- args[4]

gene\_type <- args[5]

subName   <- ifelse(gene\_type=="default","",paste0(".",gene\_type))

markers   <- c()

TPMdata <- args[6]

#nzm<-args[7]

newTPMdata<-args[7]

THtpm <- args[8]

outDir    <- file.path(workDir)

outDir

outFile   <- file.path(outDir, paste0("TPM",".xlsx"))

outPCA    <- file.path(outDir, paste0("PCA",subName,".xlsx"))

outBAR    <- file.path(outDir, paste0("barplot",subName,".pdf"))

saveData  <- file.path(outDir, paste0("DESeq2\_HISAT2",subName,".Rdata"))

name1 <- read.delim(name,header=F, sep=",")

name1<-as.vector(name1)

Group <- data.frame(con = factor(name1))

data<-read.table(data,header=T,row.names=1,sep="\t")

TPMdata<-read.delim(TPMdata,header=T,sep="\t")

TPMdata1<-as.matrix(TPMdata)

TPMmax50<-TPMdata1[,1]

print(head(TPMdata1))

print(head(TPMmax50))

L1 <- length(TPMmax50)

L1

#TPM

t5 <- c()

t4 <- subset (data, row.names(data) == TPMmax50[1])

t4 <- t4

for (i in 2:L1) {

  t5 <- subset (data, row.names(data) ==  TPMmax50[i], )

  t4 <- rbind (t4, t5)

}

head(t4)

data<-t4

data1<-row.names(data)

data2<-data[6:ncol(data)]

data3<-cbind(data1, data2)

#DEseq2

dds <- DESeqDataSetFromMatrix(countData=data2, colData=Group, design=~ con)

genelength <- matrix( unlist(data [5]), ncol = 1)

mcols(dds)$basepairs <- as.numeric(genelength)

dds <- estimateSizeFactors(dds)

dds <- estimateDispersions(dds)

dds <- nbinomWaldTest(dds)

norm.dt   <- data.frame(counts(dds, normalized=T), check.names=F)

fpkm.dt   <- data.frame(fpkm(dds, robust=F), check.names=F)

tpm.dt    <- data.frame(sweep(fpkm.dt\*1e6, 2, colSums(fpkm.dt),"/"), check.names=F)

cpm.dt    <- data.frame(fpm(dds, robust=F), check.names=F)

outfile <- paste(outDir, "DESeq2\_results\_FC2\_0.01.Rdata", sep="/")

#TPM

wb <- createWorkbook(type="xlsx")

sheet  <- createSheet(wb, sheet="TPM")

row.names(tpm.dt)<-row.names(data)

colnames(tpm.dt)<-name1

addDataFrame(tpm.dt, sheet, row.names=T)

saveWorkbook(wb, file=outFile)

outfile <- paste(outDir, "TPM\_FC2\_0.01.txt", sep="/")

write.table(tpm.dt, file=outfile, row.names=T)

dds <- DESeqDataSetFromMatrix(countData=data2, colData=Group, design=~ con)

head(dds)

genelength <- matrix( unlist(data [5]), ncol = 1)

mcols(dds)$basepairs <- as.numeric(genelength)

dds <- DESeq(dds)

res<-results(dds)

terms<-c("S21d","S17d","S11d","S10d","S9d","G35d","G28d","G21d","G17d","G13d","G9d")

terms2<-combn(terms,2)

terms2

n<-ncol(terms2)

newTPMdata<-read.delim(newTPMdata,header=T,sep="\t")

newTPMdata<-as.matrix(newTPMdata)

L1 <- length(TPMmax50)

L1

#TPM

t5 <- c()

t4 <- subset (newTPMdata, newTPMdata[,1] == TPMmax50[1])

t4 <- t4

for (i in 2:L1) {

  t5 <- subset (newTPMdata, newTPMdata[,1] ==  TPMmax50[i], )

  t4 <- rbind (t4, t5)

}

newTPMdata<-t4

DEGall\_FC4\_fdr0.01<-c()

for (j in 1:n) {

  terms3<-terms2[,j]

  terms4<-append("con",terms3)

  print(terms4)

  res<-results(dds, contrast=terms4)

  res$TPM <-newTPMdata

  tpm<-THtpm

  print(tpm)

  data2 <- as.data.frame(res)

  data2upAll <- subset(data2, (data2$pad<=0.01 & (data2$log2FoldChange>=2 )))

  DEG<-rownames(data2upAll)

  DEGall\_FC4\_fdr0.01<-unique(c(DEG, DEGall\_FC4\_fdr0.01))

  outfilename <- paste0("DESeq2\_result\_", terms3[1],"vs",terms3[2],"\_TPMmax50\_upAll\_FC4\_0.01.txt")

  out\_f <- paste(outDir, outfilename, sep="/");

  write.table(data2upAll, out\_f, sep="\t", append=F, quote=F, row.names=T, col.names=T)

  data2downAll <- subset(data2, (data2$pad<=0.01 & (data2$log2FoldChange<=-2 )))

  DEG<-rownames(data2downAll)

  DEGall\_FC4\_fdr0.01<-unique(c(DEG, DEGall\_FC4\_fdr0.01))

  outfilename <- paste0("DESeq2\_result\_", terms3[1],"vs",terms3[2],"\_TPMmax50\_downAll\_FC4\_0.01.txt")

  out\_f <- paste(outDir, outfilename, sep="/");

  write.table(data2downAll, out\_f, sep="\t", append=F, quote=F, row.names=T, col.names=T)

}

#Add TPM data

head(DEGall\_FC4\_fdr0.01)

data <- args[2]

data<-read.delim(data,header=T,row.names=1,sep="\t")

data1<-row.names(data)

data2<-data[6:ncol(data)]

data3<-cbind(data1, data2)

newTPMdata<-args[7]

newTPMdata<-read.delim(newTPMdata,header=T,row.names=1,sep="\t")

newTPMdata1<-row.names(newTPMdata)

newTPMdata3<-cbind(newTPMdata1, newTPMdata)

L1<-length(DEGall\_FC4\_fdr0.01)

L1

DEGallTPM1<-matrix()

DEGallTPM2<-matrix()

DEGallcount1<-matrix()

DEGallcount2<-matrix()

DEGallTPM1<-subset(newTPMdata, newTPMdata3[,1]==DEGall\_FC4\_fdr0.01[1])

DEGallTPM1

DEGallcount1<-subset(data, data3[,1]==DEGall\_FC4\_fdr0.01[1])

DEGallcount1

for (i in 2:L1){

  DEGallTPM2<-subset(newTPMdata, newTPMdata3[,1]==DEGall\_FC4\_fdr0.01[i])

  DEGallTPM1<-rbind(DEGallTPM1,DEGallTPM2)

  DEGallcount2<-subset(data, data3[,1]==DEGall\_FC4\_fdr0.01[i])

  DEGallcount1<-rbind(DEGallcount1,DEGallcount2)

}

outfilename <- paste0("DESeq2\_result\_DEGall\_FC4\_fdr0.01","\_TPMmax50.txt")

out\_f <- paste(outDir, outfilename, sep="/");

write.table(DEGallTPM1, out\_f, sep="\t", append=F, quote=F, row.names=T, col.names=T)

outfilename <- paste0("DESeq2\_result\_DEGall\_FC4\_fdr0.01","\_countRAWmax50.txt")

out\_f <- paste(outDir, outfilename, sep="/");

write.table(DEGallcount1, out\_f, sep="\t", append=F, quote=F, row.names=T, col.names=T)

**(H-10)** **correlation3.R**

#Fig.7C

options(warn=1)

options(scipen=100)

options( java.parameters = "-Xmx64g" )

library(xlsx)

args <- commandArgs(T)

workDir   <- args[1]

data      <- args[2]

name    <- args[3]

newTPMdata <- args[4]

newTPMdata<-read.delim(newTPMdata,header=T,row.names=1,sep="\t")

outname <- args[5]

outDir    <- file.path(workDir)

name1 <- read.delim(name,header=F, sep=",")

name1<-as.vector(name1)

data<-read.delim(data,header=T,row.names=1,sep="\t")

data1<-row.names(data)

data2<-data[2:45]

data3<-cbind(data1, data2)

data3<-as.data.frame(data3)

colnames(data2)<-name1

data2<-as.data.frame(data2)

terms<-name1

n<-ncol(terms)

n2<-n/4

k<-0

Average<-c()

for (j in 1:n2) {

  print(j)

  m<-1+(4\*k)

  i<-m+3

  Mean<-rowMeans(data2[,m:i])

  Mean<-as.matrix(Mean)

  print(head(Mean))

  print(ncol(Mean))

  print(length(data1))

  print(nrow(Mean))

  A<-Mean[,1]

  Average<-cbind(Average,A)

  k<-k+1

}

log<-log(Average+1, base=2)

cor1<-c()

n3<-length(data1)

for (j in 1:n3) {

  cor2<-cor(log[j,1:5],log[j,6:10])

  cor1<-rbind(cor1,cor2)

}

data3$cor<-cor1

Alldata<-cbind(data1,data3$cor,log,data)

outfile <- paste(workDir, outname, sep="/")

write.table(Alldata, file=outfile, sep="\t", append=F, quote=F, row.names=T, col.names=T)

**(H-10)** **Fig7F.R**

#Figure7F, correlation heatmap

library(lattice)

library(latticeExtra)

library(gplots)

library(matrixStats)

library(genefilter)

library(dplyr)

my.col1 <- colorRampPalette(c("blue","white","magenta"))

comm <- commandArgs(trailingOnly = T);

infile1 <- comm[1];

outdir <- comm[2];

data<-read.delim(infile1, header=T, sep="\t")

x<-data[,15:58]

x<-x+1

x <- as.matrix(x)

m1<-rowMeans(x[,1:4])

m2<-rowMeans(x[,5:8])

m3<-rowMeans(x[,9:12])

m4<-rowMeans(x[,13:16])

m5<-rowMeans(x[,17:21])

m6<-rowMeans(x[,22:24])

m7<-rowMeans(x[,25:28])

m8<-rowMeans(x[,29:32])

m9<-rowMeans(x[,33:36])

m10<-rowMeans(x[,37:40])

m11<-rowMeans(x[,41:44])

mx<-cbind(m1,m2,m3,m4,m5,m6,m7,m8,m9,m10,m11)

log\_mx<-log(mx, base=2)

cor<-cor(log\_mx);

cor2<-round(cor, digits = 2)

correlationPlot <- function(x) {

  cor<-cor(log\_mx);

  fig<-levelplot(cor, col.regions=colorRampPalette(c("white","yellow", "red"), space = "rgb")(120), scales=list(x=list(rot=55)), main="Correlation Plot")

  fig+layer(panel.text(x,y,labels=cor2))

}

outfile <- paste(outdir, "Fig7F.pdf", sep="/");

pdf(file=outfile,onefile=FALSE,paper="special",height=15,width=15,family="Helvetica",pointsize=20)

correlationPlot(log\_mx);

dev.off();

**(H-11) DESeq2v6.R**

options(warn=1)

options(scipen=100)

options( java.parameters = "-Xmx64g" )

library( DESeq2 )

packageVersion("DESeq2")

#1.28.1

library("dplyr")

library(xlsx)

library("genefilter")

library("gplots")

my.col1 <- colorRampPalette(c("blue","white","magenta"))

args <- commandArgs(T)

workDir   <- args[1]

data      <- args[2]

head(data)

name    <- args[3]

gtfFile   <- args[4]

gene\_type <- args[5]

subName   <- ifelse(gene\_type=="default","",paste0(".",gene\_type))

markers   <- c()

TPMdata <- args[6]

newTPMdata<-args[7]

THtpm <- args[8]

infile3 <- args[9]

outdir <- workDir

outDir    <- file.path(workDir)

outDir

outFile   <- file.path(outDir, paste0("TPM",".xlsx"))

outPCA    <- file.path(outDir, paste0("PCA",subName,".xlsx"))

outBAR    <- file.path(outDir, paste0("barplot",subName,".pdf"))

saveData  <- file.path(outDir, paste0("DESeq2\_HISAT2",subName,".Rdata"))

name1 <- read.delim(name,header=F, sep=",")

name1<-as.vector(name1)

Group <- data.frame(con = factor(name1))

data<-read.table(data,header=T,row.names=1,sep="\t")

#data TPM>10

TPMdata<-read.delim(TPMdata,header=T,sep="\t")

TPMdata1<-as.matrix(TPMdata)

TPMmax10<-TPMdata1[,1]

print(head(TPMdata1))

print(head(TPMmax10))

L1 <- length(TPMmax10)

L1

#TPM

t5 <- c()

t4 <- subset (data, row.names(data) == TPMmax10[1])

t4 <- t4

for (i in 2:L1) {

  t5 <- subset (data, row.names(data) ==  TPMmax10[i], )

  t4 <- rbind (t4, t5)

}

data<-t4

data1<-row.names(data)

data2<-data[6:ncol(data)]

data3<-cbind(data1, data2)

#DEseq2

dds <- DESeqDataSetFromMatrix(countData=data2, colData=Group, design=~ con)

genelength <- matrix( unlist(data [5]), ncol = 1)

mcols(dds)$basepairs <- as.numeric(genelength)

dds <- estimateSizeFactors(dds)

dds <- estimateDispersions(dds)

dds <- nbinomWaldTest(dds)

norm.dt   <- data.frame(counts(dds, normalized=T), check.names=F)

fpkm.dt   <- data.frame(fpkm(dds, robust=F), check.names=F)

tpm.dt    <- data.frame(sweep(fpkm.dt\*1e6, 2, colSums(fpkm.dt),"/"), check.names=F)

cpm.dt    <- data.frame(fpm(dds, robust=F), check.names=F)

outfile <- paste(outDir, "DESeq2\_results.Rdata", sep="/")

#TPM

wb <- createWorkbook(type="xlsx")

sheet  <- createSheet(wb, sheet="TPM")

row.names(tpm.dt)<-row.names(data)

colnames(tpm.dt)<-name1

addDataFrame(tpm.dt, sheet, row.names=T)

saveWorkbook(wb, file=outFile)

outfile <- paste(outDir, "TPM.txt", sep="/")

write.table(tpm.dt, file=outfile, row.names=T)

dds <- DESeqDataSetFromMatrix(countData=data2, colData=Group, design=~ con)

genelength <- matrix( unlist(data [5]), ncol = 1)

mcols(dds)$basepairs <- as.numeric(genelength)

dds <- DESeq(dds)

res<-results(dds)

terms<-c("S21d","S17d","Sj","G35d","G28d","Gj")

terms2<-combn(terms,2)

terms2

n<-ncol(terms2)

print(resultsNames(dds))

#newTPMdata TPM>10

newTPMdata<-read.delim(newTPMdata,header=T,sep="\t")

newTPMdata<-as.matrix(newTPMdata)

L1 <- length(TPMmax10)

L1

#TPM

t5 <- c()

t4 <- subset (newTPMdata, newTPMdata[,1] == TPMmax10[1])

t4 <- t4

for (i in 2:L1) {

  t5 <- subset (newTPMdata, newTPMdata[,1] ==  TPMmax10[i], )

  t4 <- rbind (t4, t5)

}

head(t4)

newTPMdata<-t4

head(newTPMdata)

FC<-log2(1.5)

for (j in 1:n) {

  terms3<-terms2[,j]

  terms4<-append("con",terms3)

  print(terms4)

  res<-results(dds, contrast=terms4)

  res$TPM <-newTPMdata

  tpm<-THtpm

  data2 <- as.data.frame(res)

  data2upAll <- subset(data2, (data2$pad<=0.05 & (data2$log2FoldChange>=FC )))

  print(head(data2upAll))

  outfilename <- paste0("DESeq2\_result\_", terms3[1],"vs",terms3[2],"\_TPMmax10\_upAll\_1.5.txt")

  out\_f <- paste(outDir, outfilename, sep="/");

  write.table(data2upAll, out\_f, sep="\t", append=F, quote=F, row.names=T, col.names=T)

  data2downAll <- subset(data2, (data2$pad<=0.05 & (data2$log2FoldChange<=-FC )))

  outfilename <- paste0("DESeq2\_result\_", terms3[1],"vs",terms3[2],"\_TPMmax10\_downAll\_1.5.txt")

  out\_f <- paste(outDir, outfilename, sep="/");

  write.table(data2downAll, out\_f, sep="\t", append=F, quote=F, row.names=T, col.names=T)

}