**Supplementary Material 2. Script for livers analysis (Figure 6).**

**(A) Linux commands**

#0 Analysis environment

Ubuntu 18.04.6 LTS

#1. Making directories

workDir=/mnt/d/takahashi/Livers

mkdir $workDir

scDir=/mnt/d/takahashi/Livers/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/Livers

scDir=/mnt/d/takahashi/Livers/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/Livers

scDir=/mnt/d/takahashi/Livers/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/Livers

scDir=/mnt/d/takahashi/Livers/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/Livers

scDir=/mnt/d/takahashi/Livers/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/Livers

scDir=/mnt/d/takahashi/Livers/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

  done

}

workDir=/mnt/d/takahashi/Livers

scDir=/mnt/d/takahashi/Livers/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/Livers

scDir=/mnt/d/takahashi/Livers/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/Livers

scDir=/mnt/d/takahashi/Livers/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

    done

}

#9.Counting with fetureCounts

workDir=/mnt/d/takahashi/Livers

scDir=/mnt/d/takahashi/Livers/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/Livers

scDir=/mnt/d/takahashi/Livers/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-9 script

    R --no-save --args $dir1/06\_count/TPMfeaturecounts\_max10.txt $dir1/06\_count  <  $dir1/06\_count/Fig6B.R #H-4 script

  done

}

#10. DEseq2

#Manually delete ' of the text file "newTPMfeaturecounts.txt" in Excel (microsoft),  and rename it to "newTPMfeaturecounts2.txt".

workDir=/mnt/d/takahashi/Livers/male

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

wdir=$workDir/08\_DESeq2/

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

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

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

default=default

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

newTPMdata=$workDir/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< $workDir/08\_DESeq2/DESeq2.R #H-5 script

#11. Clustering of 1588\_age\_related\_genes

workDir=/mnt/d/takahashi/Livers/male/06\_count

cd $workDir

R --no-save --args ./1588\_age\_related\_genes.csv 1588\_age\_related\_genes-TPM.txt ./newTPMfeaturecounts2.txt ./ <  ./ID-TPMv1.R #H-6 script

workDir=/mnt/d/takahashi/Livers/male

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

cd $workDir/07\_clustering

R --no-save --args $workDir/07\_clustering/1588\_age\_related\_genes-TPM.txt $workDir/07\_clustering <  $workDir/07\_clustering/Fig6C.R #H-7 script

#12. graphs of the expression change of cell scenesence-related genes

workDir=/mnt/d/takahashi/Livers/male/06\_count

cd $workDir

R --no-save --args ./liver\_graph.txt liver\_graph-TPM.txt ./newTPMfeaturecounts2.txt ./ <  ./ID-TPMv1.R  #H-6 script

R --no-save --args liver\_graph-TPM.txt ./ liver\_graph-TPM# < ./Fig6D.R #H-8 script

**(B) sample.csv**

1,male,/mnt/d/takahashi/Livers,

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

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

**(D) colnames.txt**

geneID,GeneLength,G7w\_1,G7w\_2,G7w\_3,G7w\_4,G7w\_5,G7w\_6,G7w\_7,G7w\_8,G7w\_9,G7w\_10,S7w\_1,S7w\_2,S7w\_3,S7w\_4,S7w\_5,S7w\_6,S7w\_7,S7w\_8,S7w\_9,S7w\_10,G14w\_2,G14w\_3,G14w\_4,G14w\_5,G14w\_6,G14w\_7,G14w\_8,G14w\_9,G14w\_10,S14w\_1,S14w\_2,S14w\_3,S14w\_4,S14w\_5,S14w\_6,S14w\_7,S14w\_8,S14w\_9,S14w\_10

**(E) samplenames.txt**

G7w,G7w,G7w,G7w,G7w,G7w,G7w,G7w,G7w,G7w,S7w,S7w,S7w,S7w,S7w,S7w,S7w,S7w,S7w,S7w,G14w,G14w,G14w,G14w,G14w,G14w,G14w,G14w,G14w,S14w,S14w,S14w,S14w,S14w,S14w,S14w,S14w,S14w,S14w

**(F)** **1588\_age\_related\_genes.csv**

ENSNFUG00015000772

ENSNFUG00015000843

ENSNFUG00015000960

ENSNFUG00015001123

ENSNFUG00015001228

ENSNFUG00015001233

ENSNFUG00015001437

ENSNFUG00015001617

ENSNFUG00015001635

ENSNFUG00015001689

ENSNFUG00015001762

ENSNFUG00015002061

ENSNFUG00015002471

ENSNFUG00015003275

ENSNFUG00015003337

ENSNFUG00015003758

ENSNFUG00015003956

ENSNFUG00015004128

ENSNFUG00015004196

ENSNFUG00015004389

ENSNFUG00015004396

ENSNFUG00015004485

ENSNFUG00015004651

ENSNFUG00015004961

ENSNFUG00015005562

ENSNFUG00015005723

ENSNFUG00015006102

ENSNFUG00015007473

ENSNFUG00015008635

ENSNFUG00015010592

ENSNFUG00015011950

ENSNFUG00015012230

ENSNFUG00015013022

ENSNFUG00015013833

ENSNFUG00015013978

ENSNFUG00015019376

ENSNFUG00015023111

ENSNFUG00015023259

ENSNFUG00015000248

ENSNFUG00015000262

ENSNFUG00015000765

ENSNFUG00015000830

ENSNFUG00015001104

ENSNFUG00015001111

ENSNFUG00015001224

ENSNFUG00015001247

ENSNFUG00015001349

ENSNFUG00015001395

ENSNFUG00015001485

ENSNFUG00015001681

ENSNFUG00015001750

ENSNFUG00015002179

ENSNFUG00015002333

ENSNFUG00015002672

ENSNFUG00015002701

ENSNFUG00015002840

ENSNFUG00015003271

ENSNFUG00015003351

ENSNFUG00015003622

ENSNFUG00015003795

ENSNFUG00015004047

ENSNFUG00015004349

ENSNFUG00015004753

ENSNFUG00015005988

ENSNFUG00015006460

ENSNFUG00015006536

ENSNFUG00015007397

ENSNFUG00015007556

ENSNFUG00015007712

ENSNFUG00015011445

ENSNFUG00015011619

ENSNFUG00015012163

ENSNFUG00015012229

ENSNFUG00015012910

ENSNFUG00015013813

ENSNFUG00015014427

ENSNFUG00015017855

ENSNFUG00015018683

ENSNFUG00015019083

ENSNFUG00015020606

ENSNFUG00015020686

ENSNFUG00015020954

ENSNFUG00015021050

ENSNFUG00015025184

ENSNFUG00015000548

ENSNFUG00015000838

ENSNFUG00015001388

ENSNFUG00015001893

ENSNFUG00015002145

ENSNFUG00015002387

ENSNFUG00015002425

ENSNFUG00015002710

ENSNFUG00015002854

ENSNFUG00015002962

ENSNFUG00015003024

ENSNFUG00015003360

ENSNFUG00015003378

ENSNFUG00015003768

ENSNFUG00015003902

ENSNFUG00015003922

ENSNFUG00015004149

ENSNFUG00015004262

ENSNFUG00015005770

ENSNFUG00015006652

ENSNFUG00015007234

ENSNFUG00015012586

ENSNFUG00015012711

ENSNFUG00015012739

ENSNFUG00015012877

ENSNFUG00015015976

ENSNFUG00015017390

ENSNFUG00015018533

ENSNFUG00015023972

ENSNFUG00015024451

ENSNFUG00015025159

ENSNFUG00015025241

ENSNFUG00015000474

ENSNFUG00015000520

ENSNFUG00015000665

ENSNFUG00015000932

ENSNFUG00015001155

ENSNFUG00015001402

ENSNFUG00015001408

ENSNFUG00015001464

ENSNFUG00015001534

ENSNFUG00015001790

ENSNFUG00015002674

ENSNFUG00015003305

ENSNFUG00015003313

ENSNFUG00015003441

ENSNFUG00015003479

ENSNFUG00015003488

ENSNFUG00015003491

ENSNFUG00015003820

ENSNFUG00015004861

ENSNFUG00015005612

ENSNFUG00015009049

ENSNFUG00015009113

ENSNFUG00015009973

ENSNFUG00015010069

ENSNFUG00015010496

ENSNFUG00015011900

ENSNFUG00015012290

ENSNFUG00015012293

ENSNFUG00015012295

ENSNFUG00015012799

ENSNFUG00015013734

ENSNFUG00015013807

ENSNFUG00015016298

ENSNFUG00015016407

ENSNFUG00015019132

ENSNFUG00015023040

ENSNFUG00015023520

ENSNFUG00015024370

ENSNFUG00015024416

ENSNFUG00015000103

ENSNFUG00015000317

ENSNFUG00015000425

ENSNFUG00015000499

ENSNFUG00015000710

ENSNFUG00015000882

ENSNFUG00015001521

ENSNFUG00015001827

ENSNFUG00015002121

ENSNFUG00015002131

ENSNFUG00015002305

ENSNFUG00015002327

ENSNFUG00015002638

ENSNFUG00015002713

ENSNFUG00015002917

ENSNFUG00015002933

ENSNFUG00015002976

ENSNFUG00015003037

ENSNFUG00015003414

ENSNFUG00015003681

ENSNFUG00015004091

ENSNFUG00015004142

ENSNFUG00015004144

ENSNFUG00015004278

ENSNFUG00015004300

ENSNFUG00015004309

ENSNFUG00015004569

ENSNFUG00015005364

ENSNFUG00015006147

ENSNFUG00015006209

ENSNFUG00015006755

ENSNFUG00015007563

ENSNFUG00015007620

ENSNFUG00015008204

ENSNFUG00015008271

ENSNFUG00015008597

ENSNFUG00015008618

ENSNFUG00015008674

ENSNFUG00015010534

ENSNFUG00015011707

ENSNFUG00015011800

ENSNFUG00015014789

ENSNFUG00015015424

ENSNFUG00015017287

ENSNFUG00015020230

ENSNFUG00015022132

ENSNFUG00015000125

ENSNFUG00015000523

ENSNFUG00015000581

ENSNFUG00015000624

ENSNFUG00015000651

ENSNFUG00015000835

ENSNFUG00015001183

ENSNFUG00015001572

ENSNFUG00015002204

ENSNFUG00015002610

ENSNFUG00015002614

ENSNFUG00015002816

ENSNFUG00015003371

ENSNFUG00015003372

ENSNFUG00015003375

ENSNFUG00015003489

ENSNFUG00015004027

ENSNFUG00015004164

ENSNFUG00015004570

ENSNFUG00015004688

ENSNFUG00015006585

ENSNFUG00015006589

ENSNFUG00015007706

ENSNFUG00015010233

ENSNFUG00015010942

ENSNFUG00015010958

ENSNFUG00015010964

ENSNFUG00015010981

ENSNFUG00015013943

ENSNFUG00015015054

ENSNFUG00015015059

ENSNFUG00015015209

ENSNFUG00015016532

ENSNFUG00015017980

ENSNFUG00015019674

ENSNFUG00015020192

ENSNFUG00015020297

ENSNFUG00015020426

ENSNFUG00015020430

ENSNFUG00015022769

ENSNFUG00015024501

ENSNFUG00015024660

ENSNFUG00015024733

ENSNFUG00015001364

ENSNFUG00015003798

ENSNFUG00015003878

ENSNFUG00015004394

ENSNFUG00015004647

ENSNFUG00015005613

ENSNFUG00015006258

ENSNFUG00015007012

ENSNFUG00015007298

ENSNFUG00015007690

ENSNFUG00015008874

ENSNFUG00015010218

ENSNFUG00015011622

ENSNFUG00015017484

ENSNFUG00015017826

ENSNFUG00015018792

ENSNFUG00015019247

ENSNFUG00015020743

ENSNFUG00015023046

ENSNFUG00015004398

ENSNFUG00015004822

ENSNFUG00015005910

ENSNFUG00015006411

ENSNFUG00015006602

ENSNFUG00015006938

ENSNFUG00015007711

ENSNFUG00015009546

ENSNFUG00015010404

ENSNFUG00015011169

ENSNFUG00015011184

ENSNFUG00015011190

ENSNFUG00015011873

ENSNFUG00015012375

ENSNFUG00015012928

ENSNFUG00015012936

ENSNFUG00015012947

ENSNFUG00015013329

ENSNFUG00015014351

ENSNFUG00015015123

ENSNFUG00015015726

ENSNFUG00015019164

ENSNFUG00015019551

ENSNFUG00015020026

ENSNFUG00015020755

ENSNFUG00015021708

ENSNFUG00015022366

ENSNFUG00015022783

ENSNFUG00015023162

ENSNFUG00015023357

ENSNFUG00015023858

ENSNFUG00015024126

ENSNFUG00015005017

ENSNFUG00015006015

ENSNFUG00015006124

ENSNFUG00015007373

ENSNFUG00015008323

ENSNFUG00015008558

ENSNFUG00015008595

ENSNFUG00015009386

ENSNFUG00015011464

ENSNFUG00015012362

ENSNFUG00015013021

ENSNFUG00015013378

ENSNFUG00015013669

ENSNFUG00015013981

ENSNFUG00015016258

ENSNFUG00015018822

ENSNFUG00015020742

ENSNFUG00015020812

ENSNFUG00015021324

ENSNFUG00015021373

ENSNFUG00015022116

ENSNFUG00015022818

ENSNFUG00015022911

ENSNFUG00015023235

ENSNFUG00015023248

ENSNFUG00015025232

ENSNFUG00015004698

ENSNFUG00015005064

ENSNFUG00015005986

ENSNFUG00015006013

ENSNFUG00015006050

ENSNFUG00015006067

ENSNFUG00015006550

ENSNFUG00015006835

ENSNFUG00015007704

ENSNFUG00015007925

ENSNFUG00015007950

ENSNFUG00015008369

ENSNFUG00015008591

ENSNFUG00015009233

ENSNFUG00015010162

ENSNFUG00015010583

ENSNFUG00015011206

ENSNFUG00015012929

ENSNFUG00015013578

ENSNFUG00015013878

ENSNFUG00015014553

ENSNFUG00015014582

ENSNFUG00015015382

ENSNFUG00015016424

ENSNFUG00015017956

ENSNFUG00015018071

ENSNFUG00015018825

ENSNFUG00015018942

ENSNFUG00015018959

ENSNFUG00015019091

ENSNFUG00015019155

ENSNFUG00015019437

ENSNFUG00015019633

ENSNFUG00015019939

ENSNFUG00015020163

ENSNFUG00015020541

ENSNFUG00015020617

ENSNFUG00015020684

ENSNFUG00015020810

ENSNFUG00015020832

ENSNFUG00015021082

ENSNFUG00015021189

ENSNFUG00015022111

ENSNFUG00015022530

ENSNFUG00015022746

ENSNFUG00015022888

ENSNFUG00015023595

ENSNFUG00015023968

ENSNFUG00015023987

ENSNFUG00015025135

ENSNFUG00015004999

ENSNFUG00015005105

ENSNFUG00015005444

ENSNFUG00015005456

ENSNFUG00015005506

ENSNFUG00015005757

ENSNFUG00015005871

ENSNFUG00015006235

ENSNFUG00015006284

ENSNFUG00015006391

ENSNFUG00015006407

ENSNFUG00015006445

ENSNFUG00015006450

ENSNFUG00015006466

ENSNFUG00015006559

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ENSNFUG00015000023

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**(G)** **liver\_graph.txt**

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**(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) Fig6B.R**

#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[,3:41]

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("white","yellow", "red"), space = "rgb")(120), scales=list(x=list(rot=55)), main="TPMlibirary\_Correlation plot")

   fig

}

outfile <- paste(outdir, "Fig6B.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**

#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]

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))

head(Group)

typeof(Group)

mode(Group)

class(Group)

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

head(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

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

head(data)

data1<-row.names(data)

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

head(data2)

data3<-cbind(data1, data2)

#DEseq2�ŉ��

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)

head(norm.dt)

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("S14w","S7w","G14w","G7w")

terms2<-combn(terms,2)

terms2

n<-ncol(terms2)

DEGall\_FC1\_fdr0.05<-c()

DEGall\_FC1\_fdr0.01<-c()

DEGall\_FC2\_fdr0.05<-c()

DEGall\_FC2\_fdr0.01<-c()

DEGall\_FC3\_fdr0.05<-c()

DEGall\_FC3\_fdr0.01<-c()

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)

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)

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

  outfilename <- paste0("DESeq2\_result\_", terms3[1],"vs",terms3[2],"\_TPMmax10\_1.5upAll.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\_1.5downAll.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) Fig6C.R**

#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]

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

x<-data[,3:41]

x<-x+1

x <- as.matrix(x)

x\_a<-x/rowMeans(x)

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

outfile <- paste(outdir, "Fig6C.pdf", sep="/")

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

heatmap.2(as.matrix(log\_x\_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) Fig6D.R**

library(beeswarm)

args <- commandArgs(T)

TPMdata <- args[1]

outDir <- args[2]

name <- args[3]

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

TPMdata <- as.matrix(TPMdata)

head(TPMdata)

N<-matrix(NA,nrow(TPMdata),1)

head(N)

print(nrow(TPMdata))

print(nrow(N))

head(TPMdata[,3:22])

head(TPMdata[,23:41])

dx <- cbind(TPMdata[,3:22],N,TPMdata[,23:41])

dx<-as.matrix(dx)

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

X = ceiling(nrow(dx)/30)

X

print(X)

i <- 1

for(i in 1:X){

  print(i)

  filename <- paste0(name,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,1:10]))

    y2<-c(as.numeric(dx[j,11:20]))

    y3<-c(as.numeric(dx[j,21:30]))

    y4<-c(as.numeric(dx[j,31:40]))

    col1<-c("#00A0E9","#00A0E9","#00A0E9","#00A0E9","#00A0E9","#00A0E9","#00A0E9","#00A0E9","#00A0E9","#00A0E9")

    col2<-c("#FFB2A4", "#FFB2A4", "#FFB2A4", "#FFB2A4","#FFB2A4", "#FFB2A4", "#FFB2A4", "#FFB2A4", "#FFB2A4", "#FFB2A4")

    v1<-cbind.data.frame(y1,col1)

    colnames(v1)<-c("TPM","col")

    v2<-cbind.data.frame(y2,col2)

    colnames(v2)<-c("TPM","col")

    v3<-cbind.data.frame(y3,col1)

    colnames(v3)<-c("TPM","col")

    v4<-cbind.data.frame(y4,col2)

    colnames(v4)<-c("TPM","col")

    y<-rbind.data.frame(v1,v2,v3,v4)

    dx2<-cbind.data.frame(label,y)

    title=TPMdata[j,42]

    print(title)

    max=max(as.numeric(TPMdata[j,c(3:41)]))+10

    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, main=title,pwcol=c("#00A0E9","#00A0E9","#00A0E9","#00A0E9","#00A0E9","#00A0E9","#00A0E9","#00A0E9","#00A0E9","#00A0E9","#FFB2A4", "#FFB2A4", "#FFB2A4", "#FFB2A4","#FFB2A4", "#FFB2A4", "#FFB2A4", "#FFB2A4", "#FFB2A4", "#FFB2A4","#00A0E9","#00A0E9","#00A0E9","#00A0E9","#00A0E9","#00A0E9","#00A0E9","#00A0E9","#00A0E9","#00A0E9","#FFB2A4", "#FFB2A4", "#FFB2A4", "#FFB2A4","#FFB2A4", "#FFB2A4", "#FFB2A4", "#FFB2A4", "#FFB2A4", "#FFB2A4"),labels = c("G7","S7","G14","S14"),ylim=c(0,max))

      t1<-as.numeric(dx[j,1:10])

      t2<-as.numeric(dx[j,11:20])

      t3<-as.numeric(dx[j,21:30])

      t4<-as.numeric(dx[j,31:40])

      dx3<-cbind(t1,t2,t3,t4)

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

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

      b <- c(1,2,3,4)

      b2 <- c(1,2,2,3,3,4,4,5,6,6,7,7)

      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();

}