2022年度 PAGS・DDBJ合同 初級者情報解析講習会

2022/10/20

RNA-seq 解析

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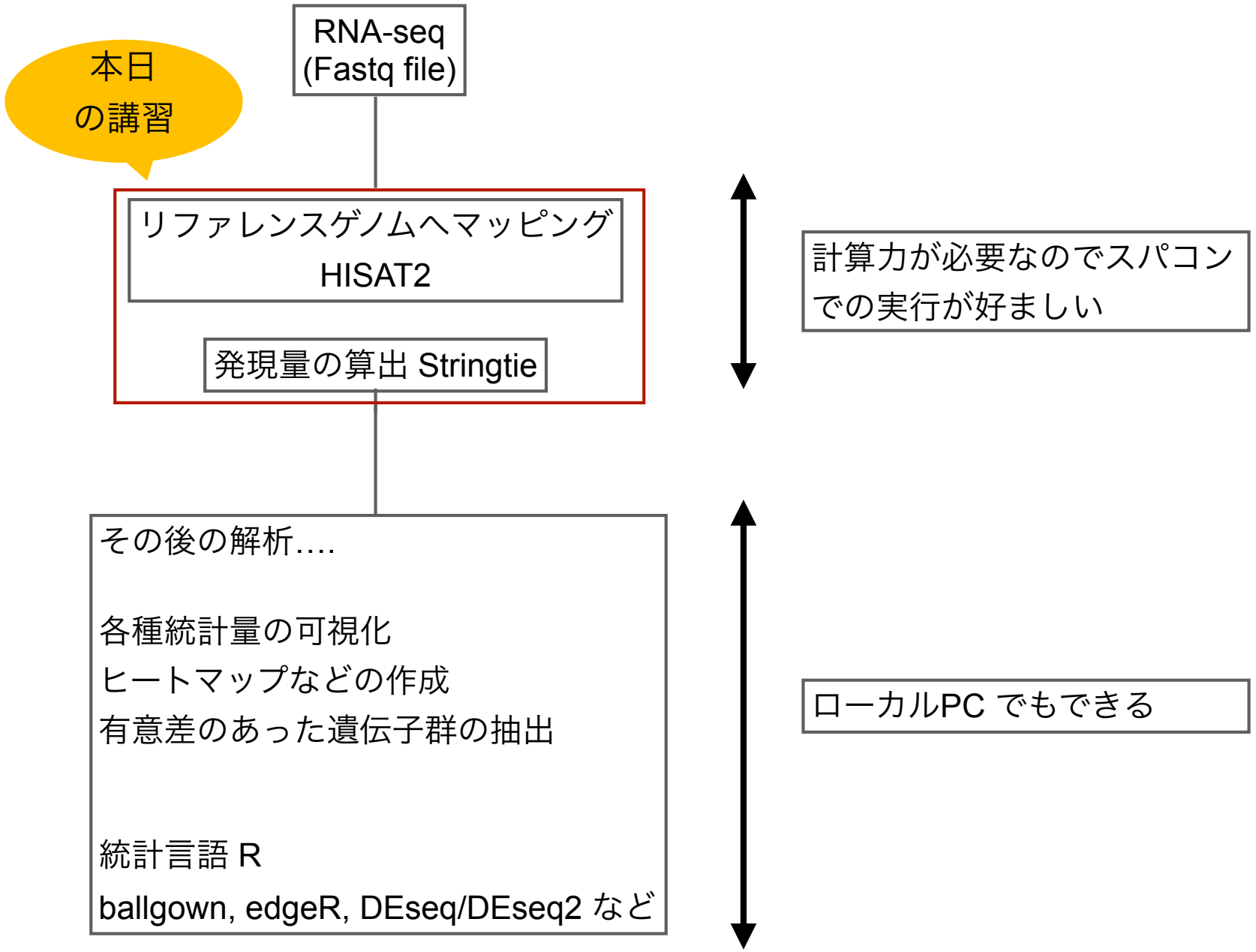
本日の講習の流れ RNA-seq 発現量解析



配布データのコピー

ホームディレクトリ に移動

```
$ cd
```

ホームディレクトリ に20221020 というファイル/ディレクトリがないかを確認。

```
$ ls 20221020
ls: 20221021 にアクセスできません: そのようなファイルやディレクトリはありません
```

あれば、講習時間だけファイル/ディレクトリ 名前を一時的に変更してください。

```
$ mv 20221020 20221020_tmp
```

講習データをコピー

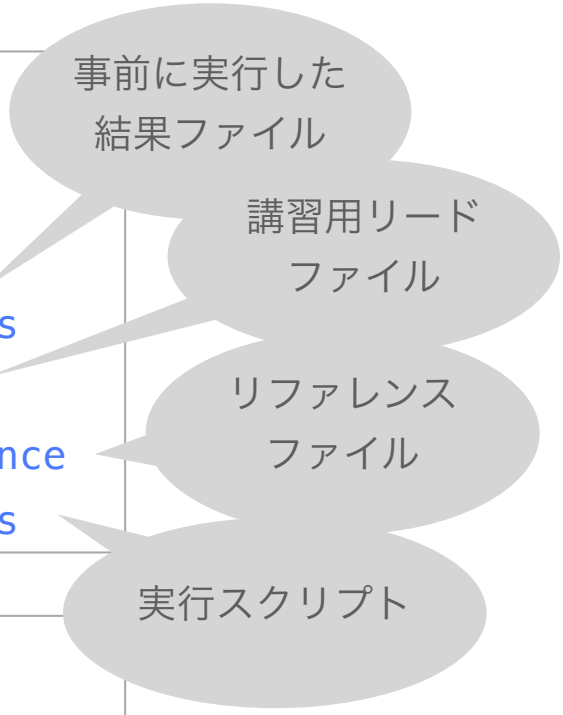
```
$ cp -r /home/ddbjshare/public/lecture/20221020 .
```

本日の講習はこちらのディレクトリで

```
$ cd 20221020
```

配布データの確認 (1)

```
$ ls -al
合計 24
drwxr-xr-x 6 koshu3 koshu 4096 10月  9 14:41 .
drwxr-x--- 9 koshu3 koshu 4096 10月  9 14:41 ..
drwxr-xr-x 2 koshu3 koshu 4096 10月  9 14:41 outputs
drwxr-xr-x 2 koshu3 koshu 4096 10月  9 12:23 reads
drwxr-xr-x 2 koshu3 koshu 4096 10月  9 14:25 reference
drwxr-xr-x 2 koshu3 koshu 4096 10月  9 14:35 scripts
```



それぞれのディレクトリ の中身は

```
$ ls outputs
$ ls outputs/hisat2_index
$ ls outputs/hisat2
$ ls outputs/stringtie
$ ls reads
$ ls reference
$ ls scripts
```

配布データの確認 (2)

配布データの構成

```
20201020
  |____ outputs  # 解析結果
              |___hisat2
              |___hisat2_index
              |___stringtie
  |----- reads      # リードファイル格納用
              |___SRR453566_1.fastq.gz
              |___SRR453566_2.fastq.gz
              |___SRR453569_1.fastq.gz
              |___SRR453569_2.fastq.gz
  |----- reference   # リファレンスファイル
              |___s288c.fa
              |___s288c.gff
  |----- scripts     # スクリプト
              |___hisat2.sh        # condaにて実装
              |___hisat2_index.sh  # condaにて実装
              |___stringtie.sh     # condaにて実装
              |___hisat2_singularity.sh        # singularityにて実装
              |___hisat2_index_singularity.sh  # singularityにて実装
              |___stringtie_singularity.sh       # singularityにて実装
```

Conda activate

```
$ conda activate pags_rnaseq
```

講習用 RNA-seq データ

JOURNAL ARTICLE

A comprehensive comparison of RNA-Seq-based transcriptome analysis from reads to differential gene expression and cross-comparison with microarrays: a case study in *Saccharomyces cerevisiae*

Intawat Nookaew, Marta Papini, Natapol Pornputtapong, Gionata Scalcinati, Linn Fagerberg, Matthias Uhlén, Jens Nielsen ✉ Author Notes

Nucleic Acids Research, Volume 40, Issue 20, 1 November 2012, Pages 10084–10097, https://doi.org/10.1093/nar/gks804

Published: 08 September 2012 **Article history** ▾

Saccharomyces cerevisiae CEN.PK113-7D

バッチ培養	ケモスタット培養
SRR453566	SRR453569
SRR453567	SRR453570
SRR453568	SRR453571

Biological replicates 3回ずつ

```
$ ls reads/
SRR453566_1.fastq.gz  SRR453566_2.fastq.gz  SRR453569_1.fastq.gz  SRR453569_2.fastq.gz
```

Paired-end データ

Paired-end データ

FASTQ フォーマット

4行で1配列の情報を表す。

```
$ zcat reads/SRR453566_1.fastq.gz | more
@SRR453566.1 HWI-ST167:4:1101:1597:1986/1
NAAAACTTTGGATGACTTCAACAACTATTCTTCTGAAATCAACAAAATATCACCAACTTCCGCCAACACAAAGTCTTACAGTGCAACAACAAGTGATGTTG
+
#4=DFFFFHHHHHJJJJJJJJJJJJJJJJIJJJJJJJJJJJJJJJJJJJJJJJJJJIIJJJIJJJJJJJHHHEFFFFFFFEECEECCC@ABDACCC@CCCCD
```

1行目: @ の後ろにその配列のID
2行目: 配列
3行目: + を記載する。(配列のID を記載してもしなくてもよい)
4行目: その配列のクオリティ値

クオリティ値はアスキーコードで表示
アスキー値 - 33 が クオリティ値

```
  SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS.....................................................
  ..........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX.....................
  ..............................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII.....................
  ..............................JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ.....................
  LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLL.....................................................
  PPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPP
  !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
  |                         |    |        |                              |                   |
 33                        59   64       73                            104                 126
  0.........................26...31.......40
                           -5....0........9.............................40
                                0........9.............................40
                                   3.....9.............................41
  0.2.......................26...31........41
  0..................20........30........40........50.....................................93
```

```
S - Sanger        Phred+33,  raw reads typically (0, 40)
X - Solexa        Solexa+64, raw reads typically (-5, 40)
I - Illumina 1.3+ Phred+64,  raw reads typically (0, 40)
J - Illumina 1.5+ Phred+64,  raw reads typically (3, 41)
    with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
    (Note: See discussion above).
L - Illumina 1.8+ Phred+33,  raw reads typically (0, 41)
P - PacBio        Phred+33,  HiFi reads typically (0, 93)
```

<- SRA

クオリティ値 @ の場合

64 - 33 = **31**

https://en.wikipedia.org/wiki/FASTQ_format

講習用 リファレンスファイル　ゲノム配列 fasta

```
$ ls reference/
s288c.fa  s288c.gff
```

ゲノム配列

ファイルの中身を確認

```
$ more reference/s288c.fa
>NC_001133.9 Saccharomyces cerevisiae S288C chromosome I, complete sequence
CCACACCACACCCACACACCCACACACCACACCACACACCACACCACACCCACACACACACATCCTAACA
CTACCCTAACACAGCCCTAATCTAACCCTGGCCAACCTGTCTCTCAACTTACCCTCCATTACCCTGCCTC
CACTCGTTACCCTGTCCCATTCAACCATACCACTCCGAACCACCATCCATCCCTCTACTTACTACCACTC
ACCCACCGTTACCCTCCAATTACCCATATCCAACCCACTGCCACTTACCCTACCATTACCCT
```

染色体16本分の配列

FASTA ヘッダの出力

```
$ grep ">" reference/s288c.fa
>NC_001133.9 Saccharomyces cerevisiae S288C chromosome I, complete sequence
>NC_001134.8 Saccharomyces cerevisiae S288C chromosome II, complete sequence
>NC_001135.5 Saccharomyces cerevisiae S288C chromosome III, complete sequence
>NC_001136.10 Saccharomyces cerevisiae S288C chromosome IV, complete sequence
>NC_001137.3 Saccharomyces cerevisiae S288C chromosome V, complete sequence
>NC_001138.5 Saccharomyces cerevisiae S288C chromosome VI, complete sequence
>NC_001139.9 Saccharomyces cerevisiae S288C chromosome VII, complete sequence
>NC_001140.6 Saccharomyces cerevisiae S288C chromosome VIII, complete sequence
>NC_001141.2 Saccharomyces cerevisiae S288C chromosome IX, complete sequence
>NC_001142.9 Saccharomyces cerevisiae S288C chromosome X, complete sequence
>NC_001143.9 Saccharomyces cerevisiae S288C chromosome XI, complete sequence
>NC_001144.5 Saccharomyces cerevisiae S288C chromosome XII, complete sequence
>NC_001145.3 Saccharomyces cerevisiae S288C chromosome XIII, complete sequence
>NC_001146.8 Saccharomyces cerevisiae S288C chromosome XIV, complete sequence
>NC_001147.6 Saccharomyces cerevisiae S288C chromosome XV, complete sequence
>NC_001148.4 Saccharomyces cerevisiae S288C chromosome XVI, complete sequence
```

講習用 リファレンスファイル　アノテーションファイル

```
$ ls reference/
s288c.fa  s288c.gff
```

アノテーションファイル

ファイルの中身を確認

```
$ more reference/s288c.gff
##gff-version 3
#!gff-spec-version 1.21
#!processor NCBI annotwriter
#!genome-build R64
#!genome-build-accession NCBI_Assembly:GCF_000146045.2
#!annotation-source SGD R64-3-1
##sequence-region NC_001133.9 1 230218
##species https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=559292
NC_001133.9  RefSeq  region  1  230218  .  +  .  ID=NC_001133.9:1..230218;Dbxref=taxor
NC_001133.9  RefSeq  telomere  1  801  .  -  .  ID=id-NC_001133.9:1..801;Dbxref=SGD:S0
NC_001133.9  RefSeq  origin_of_replication  707  776  .  +  .  ID=id-NC_001133.9:707..
NC_001133.9  RefSeq  gene  1807  2169  .  -  .  ID=gene-YAL068C;Dbxref=GeneID:851229;N
NC_001133.9  RefSeq  mRNA  1807  2169  .  -  .  ID=rna-NM_001180043.1;Parent=gene-YAL0
NC_001133.9  RefSeq  exon  1807  2169  .  -  .  ID=exon-NM_001180043.1-1;Parent=rna-NM
NC_001133.9  RefSeq  CDS  1807  2169  .  -  0  ID=cds-NP_009332.1;Parent=rna-NM_001180
```

GFF フォーマット

遺伝子アノテーションのフォーマット

```
##gff-version 3
#!gff-spec-version 1.21
#!processor NCBI annotwriter
#!genome-build R64
#!genome-build-accession NCBI_Assembly:GCF_000146045.2
#!annotation-source SGD R64-2-1
##sequence-region NC_001133.9 1 230218
##species https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=559292
NC_001133.9  RefSeq gene   1807  2169  .  -  .  ID=gene0;Dbxref=GeneID:851229;Name=PAU8;end_range=2169,.;gbkey=Gene;gene=PAU8;
NC_001133.9  RefSeq mRNA   1807  2169  .  -  .  ID=rna0;Parent=gene0;Dbxref=GeneID:851229,Genbank:NM_001180043.1;Name=NM_0011
NC_001133.9  RefSeq exon   1807  2169  .  -  .  ID=id3;Parent=rna0;Dbxref=GeneID:851229,Genbank:NM_001180043.1;end_range=2169,.;gb
NC_001133.9  RefSeq CDS    1807  2169  .  -  0  ID=cds0;Parent=rna0;Dbxref=SGD:S000002142,GeneID:851229,Genbank:NP_009332.1;Nam
NC_001133.9  RefSeq gene   2480  2707  .  +  .  ID=gene1;Dbxref=GeneID:1466426;Name=YAL067W-A;end_range=2707,.;gbkey=Gene;gene
NC_001133.9  RefSeq mRNA   2480  2707  .  +  .  ID=rna1;Parent=gene1;Dbxref=GeneID:1466426,Genbank:NM_001184582.1;Name=NM_001
NC_001133.9  RefSeq exon   2480  2707  .  +  .  ID=id4;Parent=rna1;Dbxref=GeneID:1466426,Genbank:NM_001184582.1;end_range=2707,.;
NC_001133.9  RefSeq CDS    2480  2707  .  +  0  ID=cds1;Parent=rna1;Dbxref=SGD:S000028593,GeneID:1466426,Genbank:NP_878038.1;Nam
NC_001133.9  RefSeq gene   7235  9016  .  -  .  ID=gene2;Dbxref=GeneID:851230;Name=SEO1;end_range=9016,.;gbkey=Gene;gene=SEO1;
NC_001133.9  RefSeq mRNA   7235  9016  .  -  .  ID=rna2;Parent=gene2;Dbxref=GeneID:851230,Genbank:NM_001178208.1;Name=NM_0011
NC_001133.9  RefSeq exon   7235  9016  .  -  .  ID=id5;Parent=rna2;Dbxref=GeneID:851230,Genbank:NM_001178208.1;end_range=9016,.;gb
NC_001133.9  RefSeq CDS    7235  9016  .  -  0  ID=cds2;Parent=rna2;Dbxref=SGD:S000000062,GeneID:851230,Genbank:NP_009333.1;Nam
```

タブ区切りフォーマット。値がない場合は、”.” が設定される。

1. seqname：染色体 or スキャフォールドの名前
2. source：アノテーションを生成したプログラムまたはデータソースの名前
3. feature：フィーチャータイプ（mRNA, gene, exon, CDS ….）
4. start：スタートポジション (1bp ～)
5. end：エンドポジション (1bp ～)
6. score：スコア
7. strand：+(forward)、-(reverse)または '.'
8. frame：翻訳フレーム（0, 1, 2）
9. attribute：追加情報。セミコロンで区切られたタグと値のペアのリスト。

リファレンスゲノムへリードをマッピング

ステップ

1. リファレンスゲノムのインデックスを作成

   hisat2_index.sh

2. リードをリファレンスゲノムへマッピング

   hisat2.sh　　2サンプル分をアレイジョブで同時実行

スクリプト

```
$ ls scripts/hisat2*
scripts/hisat2.sh  scripts/hisat2_index.sh
scripts/hisat2_index_singularity.sh  scripts/hisat2_singularity.sh
```

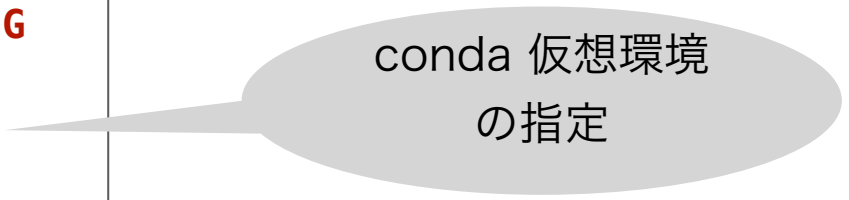
マッピング用インデックスの作成　　スクリプトの確認

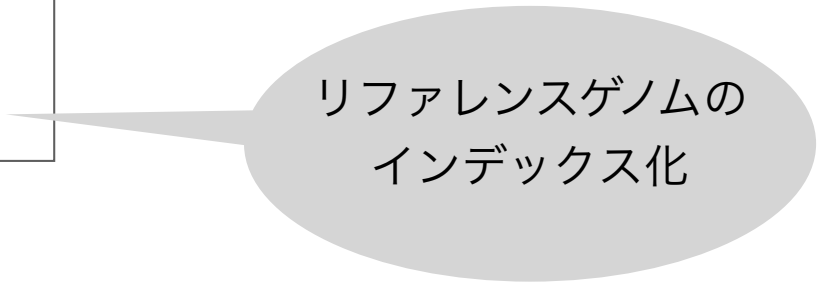
```
$ more scripts/hisat2_index.sh
#$ -S /bin/bash
#$ -pe def_slot 2
#$ -cwd
#$ -l mem_req=10G,s_vmem=10G


conda activate pags_rnaseq

GENOME=./reference/s288c.fa
INDEX=./reference/s288c

hisat2-build $GENOME $INDEX
```





qsub コマンドのオプション

-S 使用するインタプリタのパス

-pe def_slot 1 ジョブスロット数

-cwd　ホームディレクトリではなく、qsubコマンド実行時のディレクトリでジョブ を実行。
　　標準出力 / 標準エラー出力ファイルは、qsubコマンド実行時のディレクトリに出力。

-l 主にキューの選択、メモリ利用上限の変更に使う

mem_req: 使用するメモリの量を宣言する。(ジョブ管理システムUGEのジョブリソース管理に対する宣言)

s_vmem: ジョブが使用可能な仮想メモリの上限値。 (OS に対する宣言)

キューの指定: Thin ノードへの投入は、キューの指定は不要。

マッピング用インデックスの作成　　オプションの確認

```
$ hisat2-build -h
HISAT2 version 2.2.1 by Daehwan Kim (infphilo@gmail.com, http://www.ccb.jhu.edu/people/infphilo)
Usage: hisat2-build [options]* <reference_in> <ht2_index_base>
    reference_in            comma-separated list of files with ref sequences
    hisat2_index_base       write ht2 data to files with this dir/basename
Options:
    -c                      reference sequences given on cmd line (as
                            <reference_in>)
    --large-index           force generated index to be 'large', even if ref
                            has fewer than 4 billion nucleotides
    -a/--noauto             disable automatic -p/--bmax/--dcv memory-fitting
    -p <int>                number of threads
    --bmax <int>            max bucket sz for blockwise suffix-array builder
    --bmaxdivn <int>        max bucket sz as divisor of ref len (default: 4)
    --dcv <int>             diff-cover period for blockwise (default: 1024)
    --nodc                  disable diff-cover (algorithm becomes quadratic)
    -r/--noref              don't build .3/.4.ht2 (packed reference) portion
    -3/--justref            just build .3/.4.ht2 (packed reference) portion
    -o/--offrate <int>      SA is sampled every 2^offRate BWT chars (default: 5)
    -t/--ftabchars <int>    # of chars consumed in initial lookup (default: 10)
    --localoffrate <int>    SA (local) is sampled every 2^offRate BWT chars (default: 3)
    --localftabchars <int>  # of chars consumed in initial lookup in a local index (default: 6)
    --snp <path>            SNP file name
    --haplotype <path>      haplotype file name
    --ss <path>             Splice site file name
    --exon <path>           Exon file name
    --repeat-ref <path>     Repeat reference file name
    --repeat-info <path>    Repeat information file name
    --repeat-snp <path>     Repeat snp file name
    --repeat-haplotype <path>   Repeat haplotype file name
    --seed <int>            seed for random number generator
    -q/--quiet              disable verbose output (for debugging)
    -h/--help               print detailed description of tool and its options
    --usage                 print this usage message
    --version               print version information and quit
```

マッピング用インデックスの作成　　実行

実行

```
$ qsub scripts/hisat2_index.sh
Your job 16626048 ("hisat2_index.sh") has been submitted
```

ステータスの確認

```
$ qstat
job-ID     prior   name       user    state submit/start at     queue          jclass slots ja-task-ID
-----------------------------------------------------------------------------------------------------
  16624661 0.25493 QLOGIN     koshu3  r     10/09/2022 17:58:41 login.q@at137             1
  16626048 0.00000 hisat2_ind koshu3  qw    10/10/2022 10:57:31                           2
```

実行待機

```
$ qstat
job-ID     prior   name       user    state submit/start at     queue          jclass slots ja-task-ID
-----------------------------------------------------------------------------------------------------
  16624661 0.25493 QLOGIN     koshu3  r     10/09/2022 17:58:41 login.q@at137             1
  16626048 0.25028 hisat2_ind koshu3  r     10/10/2022 10:57:39 epyc.q@at153              2
```

実行中

```
$ qstat
job-ID     prior   name    user    state submit/start at     queue          jclass slots ja-task-ID
-----------------------------------------------------------------------------------------------
  16624661 0.25493 QLOGIN  koshu3  r     10/09/2022 17:58:41 login.q@at137             1
```

ジョブが終了すると該当ジョブID が表示されなくなる。

マッピング用インデックスの作成　　実行結果の確認

```
$ ls -al
合計 32
drwxr-xr-x  6 koshu3 koshu 4096 10月 10 10:57 .
drwxr-x--- 10 koshu3 koshu 4096 10月 10 10:51 ..
-rw-r--r--  1 koshu3 koshu 2262 10月 10 10:57 hisat2_index.sh.e16626048
-rw-r--r--  1 koshu3 koshu 3919 10月 10 10:57 hisat2_index.sh.o16626048
-rw-r--r--  1 koshu3 koshu    0 10月 10 10:57 hisat2_index.sh.pe16626048
-rw-r--r--  1 koshu3 koshu    0 10月 10 10:57 hisat2_index.sh.po16626048
drwxr-xr-x  2 koshu3 koshu 4096 10月  9 14:41 outputs
drwxr-xr-x  2 koshu3 koshu 4096 10月  9 12:23 reads
drwxr-xr-x  2 koshu3 koshu 4096 10月 10 10:57 reference
drwxr-xr-x  2 koshu3 koshu 4096 10月 10 10:51 scripts
```

ログの確認

```
$ more hisat2_index.sh.e16626048
Settings:
  Output files: "./reference/s288c.fa.*.ht2"
  Line rate: 6 (line is 64 bytes)
  Lines per side: 1 (side is 64 bytes)
  Offset rate: 4 (one in 16)
  FTable chars: 10
    .
    .
    .
Total time for call to driver() for forward index: 00:0
```

```
$ more hisat2_index.sh.o16626048
Building DifferenceCoverSample
  Building sPrime
  Building sPrimeOrder
  V-Sorting samples
      .
      .
      .
Returning block of 1908813 for bucket 7
```

インデックスが作成された

```
$  ls -al reference/
合計 46228
drwxr-xr-x 2 koshu3 koshu     4096 10月 10 10:57 .
drwxr-xr-x 6 koshu3 koshu     4096 10月 10 10:57 ..
-rw-r--r-- 1 koshu3 koshu 12245035 10月  9 15:25 s288c.fa
-rw-r--r-- 1 koshu3 koshu  8219756 10月 10 10:57 s288c.fa.1.ht2
-rw-r--r-- 1 koshu3 koshu  3017836 10月 10 10:57 s288c.fa.2.ht2
-rw-r--r-- 1 koshu3 koshu      152 10月 10 10:57 s288c.fa.3.ht2
-rw-r--r-- 1 koshu3 koshu  3017832 10月 10 10:57 s288c.fa.4.ht2
-rw-r--r-- 1 koshu3 koshu  5357645 10月 10 10:57 s288c.fa.5.ht2
-rw-r--r-- 1 koshu3 koshu  3071004 10月 10 10:57 s288c.fa.6.ht2
-rw-r--r-- 1 koshu3 koshu       12 10月 10 10:57 s288c.fa.7.ht2
-rw-r--r-- 1 koshu3 koshu        8 10月 10 10:57 s288c.fa.8.ht2
-rw-r--r-- 1 koshu3 koshu 12377219 10月 10 12:28 s288c.gff
```

ジョブが終わらなかった場合、事前実行した結果を確認

```
$ ls outputs/hisat2_index/
$ more outputs/hisat2_index/hisat2_index.sh.e16626048
$ more outputs/hisat2_index/hisat2_index.sh.o16626048
```

マッピング　スクリプトの確認

```
$ more scripts/hisat2.sh
#$ -S /bin/bash
#$ -pe def_slot 4
#$ -cwd
#$ -t 1-2:1
#$ -l mem_req=8G,s_vmem=8G

conda activate pags_rnaseq

# Batch culture: SRR453566
# chemostat: SRR453569
ACESSIONS=(453566 453569)
no=`expr ${SGE_TASK_ID} - 1`

NUM=${ACESSIONS[${no}]}
PREFIX=SRR${NUM}

# read file
DIR=./reads/
QUERY1_1=${DIR}${PREFIX}"_1.fastq.gz"
QUERY1_2=${DIR}${PREFIX}"_2.fastq.gz"


hisat2 -p ${NSLOTS} -x reference/s288c.fa --dta \
       -1 ${QUERY1_1} -2 ${QUERY1_2} \
       -S ${PREFIX}.sam

# convert sam to bam
# sort by position
samtools sort -@ ${NSLOTS} ${PREFIX}.sam -o ${PREFIX}.sorted.bam
```

アレイジョブを指定

アレイジョブのタスクID

—dta: reports alignments tailored for transcript assemblers

HISAT2 オプションの確認

```
$ hisat2 --help
HISAT2 version 2.2.1 by Daehwan Kim (infphilo@gmail.com, www.ccb.jhu.edu/people/infphilo)
Usage:
  hisat2 [options]* -x <ht2-idx> {-1 <m1> -2 <m2> | -U <r>} [-S <sam>]

  <ht2-idx>  Index filename prefix (minus trailing .X.ht2).
  <m1>       Files with #1 mates, paired with files in <m2>.
             Could be gzip'ed (extension: .gz) or bzip2'ed (extension: .bz2).
  <m2>       Files with #2 mates, paired with files in <m1>.
             Could be gzip'ed (extension: .gz) or bzip2'ed (extension: .bz2).
  <r>        Files with unpaired reads.
             Could be gzip'ed (extension: .gz) or bzip2'ed (extension: .bz2).
  <sam>      File for SAM output (default: stdout)

  <m1>, <m2>, <r> can be comma-separated lists (no whitespace) and can be
  specified many times.  E.g. '-U file1.fq,file2.fq -U file3.fq'.

Options (defaults in parentheses):

 Input:
  -q                 query input files are FASTQ .fq/.fastq (default)
  --qseq             query input files are in Illumina's qseq format
  -f                 query input files are (multi-)FASTA .fa/.mfa
  -r                 query input files are raw one-sequence-per-line
  -c                 <m1>, <m2>, <r> are sequences themselves, not files
  -s/--skip <int>    skip the first <int> reads/pairs in the input (none)
  -u/--upto <int>    stop after first <int> reads/pairs (no limit)
  -5/--trim5 <int>   trim <int> bases from 5'/left end of reads (0)
  -3/--trim3 <int>   trim <int> bases from 3'/right end of reads (0)
  --phred33          qualities are Phred+33 (default)
  --phred64          qualities are Phred+64
  --int-quals        qualities encoded as space-delimited integers

 Presets:                 Same as:
   --fast                 --no-repeat-index
   --sensitive            --bowtie2-dp 1 -k 30 --score-min L,0,-0.5
   --very-sensitive       --bowtie2-dp 2 -k 50 --score-min L,0,-1

 Alignment:
  --bowtie2-dp <int> use Bowtie2's dynamic programming alignment algorithm (0) - 0: no dynamic programming, 1: conditional
dynamic programming, and 2: unconditional dynamic programming (slowest)
  --n-ceil <func>    func for max # non-A/C/G/Ts permitted in aln (L,0,0.15)
  --ignore-quals     treat all quality values as 30 on Phred scale (off)
  --nofw             do not align forward (original) version of read (off)
  --norc             do not align reverse-complement version of read (off)
  --no-repeat-index  do not use repeat index
```

マッピング　　samtools sort オプションの確認

```
$ samtools sort --help
sort: unrecognized option '--help'
Usage: samtools sort [options...] [in.bam]
Options:
  -l INT     Set compression level, from 0 (uncompressed) to 9 (best)
  -u         Output uncompressed data (equivalent to -l 0)
  -m INT     Set maximum memory per thread; suffix K/M/G recognized [768M]
  -M         Use minimiser for clustering unaligned/unplaced reads
  -K INT     Kmer size to use for minimiser [20]
  -n         Sort by read name (not compatible with samtools index command)
  -t TAG     Sort by value of TAG. Uses position as secondary index (or read name if -n is set)
  -o FILE    Write final output to FILE rather than standard output
  -T PREFIX  Write temporary files to PREFIX.nnnn.bam
      --no-PG
               Do not add a PG line
      --template-coordinate
               Sort by template-coordinate
      --input-fmt-option OPT[=VAL]
               Specify a single input file format option in the form
               of OPTION or OPTION=VALUE
  -O, --output-fmt FORMAT[,OPT[=VAL]]...
               Specify output format (SAM, BAM, CRAM)
      --output-fmt-option OPT[=VAL]
               Specify a single output file format option in the form
               of OPTION or OPTION=VALUE
      --reference FILE
               Reference sequence FASTA FILE [null]
  -@, --threads INT
              Number of additional threads to use [0]
      --write-index
               Automatically index the output files [off]
      --verbosity INT
               Set level of verbosity
```

SAM フォーマット/ BAM フォーマット

```
@HD    VN:1.0 SO:unsorted
@SQ    SN:NC_001133.9      LN:230218
@SQ    SN:NC_001134.8      LN:813184
@SQ    SN:NC_001135.5      LN:316620
@SQ    SN:NC_001136.10     LN:1531933
@SQ    SN:NC_001137.3      LN:576874
@SQ    SN:NC_001138.5      LN:270161
@SQ    SN:NC_001139.9      LN:1090940
@SQ    SN:NC_001140.6      LN:562643
@SQ    SN:NC_001141.2      LN:439888
@SQ    SN:NC_001142.9      LN:745751
@SQ    SN:NC_001143.9      LN:666816
@SQ    SN:NC_001144.5      LN:1078177
@SQ    SN:NC_001145.3      LN:924431
@SQ    SN:NC_001146.8      LN:784333
@SQ    SN:NC_001147.6      LN:1091291
@SQ    SN:NC_001148.4      LN:948066
@SQ    SN:NC_001224.1      LN:85779
@PG ID:hisat2   PN:hisat2   VN:2.2.1    CL:"/home/koshu3/miniconda3/envs/pags_rnaseq/bin/hisat2-align-s --wrapper basic-0 -p 4 -x referen
SRR453566.24 163    NC_001139.9  727518 60     69M    =      727620 203    TTAATCAAG….  =DFFFFHHHH…. AS:i:0 XN:i:0 XM:i:0 XO:i:0 XG:i:0 NM:i:0 MD
SRR453566.22 99     NC_001142.9  509705 60     101M   =      509740 136    CAAAGCGTA….  CCCFFFFFHG…. AS:i:-6       XN:i:0 XM:i:1 XO:i:0 XG:i:0 NM
SRR453566.22 147    NC_001142.9  509740 60     101M   =      509705 -136   GGTATATTT….  @DA@:>>>@C…. AS:i:-4       ZS:i:-7       XN:i:0 XM:i:1 XO
SRR453566.23 99     NC_001134.8  674240 60     101M   =      674286 131    TTTTCTTCA….  @BCFFFFFHH…. AS:i:-6       XN:i:0 XM:i:1 XO:i:0 XG:i:0 NM
SRR453566.23 147    NC_001134.8  674286 60     85M    =      674240 -131   AACAAAAGC….  ??>C>@5(@>…. AS:i:-4       ZS:i:-10      XN:i:0 XM:i:1 XO
```

QNAME FLG RNAME POS MAPQ CIGAR RNEXT PNEXT TLEN SEQ QUAL optional fields

@ヘッダ行

HD: ヘッダ行 SAMフォーマットのバージョンなど

SQ: リファレンスの情報

PG ツールの実行情報

QNAME	リード名
FLG	アラインメント情報。参考 https://broadinstitute.github.io/picard/explain-flags.html
RNAME	マップされたリファレンス名
POS	マップポジション
MAPQ	マッピングスコア
CIGAR	マッピングの状況 ex) M アライメントマッチ I リファレンスにインサーションあり など
RNEXT	ペアエンドの場合、ペアのリード名（=: QNAME）。
PNEXT	ペアエンドの場合、ペアのマップされた開始位置。
TLEN	ペアエンドのリード間の距離。
SEQ	FASTQ の塩基配列データ
QUAL	FASTQ のクオリティデータ。

BAM は SAM をバイナリ形式にしたファイル

マッピング　実行

```
$ qsub scripts/hisat2.sh
Your job-array 16626062.1-2:1 ("hisat2.sh") has been submitted

$ qstat
job-ID     prior   name       user    state submit/start at     queue          jclass slots ja-task-ID
-----------------------------------------------------------------------------------------------------
  16624661 0.25505 OLOGIN     koshu3  r     10/09/2022 17:58:41 login.q@at137              1
  16626062 0.25087 hisat2.sh  koshu3  r     10/10/2022 11:21:53 epyc.q@at144               4 1
  16626062 0.25087 hisat2.sh  koshu3  r     10/10/2022 11:21:53 epyc.q@at162               4 2
```

```
(pags_rnaseq) ls -al
合計 8310037
drwxr-xr-x  6 koshu3 koshu       4096 10月 10 11:23 .
drwxr-x--- 10 koshu3 koshu       4096 10月 10 11:21 ..
-rw-r--r--  1 koshu3 koshu 4173908387 10月 10 11:23 SRR453566.sam
-rw-r--r--  1 koshu3 koshu  821523867 10月 10 11:23 SRR453566.sorted.bam
-rw-r--r--  1 koshu3 koshu 2922686958 10月 10 11:22 SRR453569.sam
-rw-r--r--  1 koshu3 koshu  591294985 10月 10 11:22 SRR453569.sorted.bam
-rw-r--r--  1 koshu3 koshu        693 10月 10 11:23 hisat2.sh.e16626062.1
-rw-r--r--  1 koshu3 koshu        688 10月 10 11:22 hisat2.sh.e16626062.2
-rw-r--r--  1 koshu3 koshu          0 10月 10 11:21 hisat2.sh.o16626062.1
-rw-r--r--  1 koshu3 koshu          0 10月 10 11:21 hisat2.sh.o16626062.2
-rw-r--r--  1 koshu3 koshu          0 10月 10 11:21 hisat2.sh.pe16626062.1
-rw-r--r--  1 koshu3 koshu          0 10月 10 11:21 hisat2.sh.pe16626062.2
-rw-r--r--  1 koshu3 koshu          0 10月 10 11:21 hisat2.sh.po16626062.1
-rw-r--r--  1 koshu3 koshu          0 10月 10 11:21 hisat2.sh.po16626062.2
-rw-r--r--  1 koshu3 koshu       2262 10月 10 10:57 hisat2_index.sh.e16626048
-rw-r--r--  1 koshu3 koshu       3919 10月 10 10:57 hisat2_index.sh.o16626048
-rw-r--r--  1 koshu3 koshu          0 10月 10 10:57 hisat2_index.sh.pe16626048
-rw-r--r--  1 koshu3 koshu          0 10月 10 10:57 hisat2_index.sh.po16626048
drwxr-xr-x  2 koshu3 koshu       4096 10月  9 14:41 outputs
drwxr-xr-x  2 koshu3 koshu       4096 10月  9 12:23 reads
drwxr-xr-x  2 koshu3 koshu       4096 10月 10 10:57 reference
drwxr-xr-x  2 koshu3 koshu       4096 10月 10 11:21 scripts
```

ジョブが終わらなかった場合

$ ls -al outputs/hisat2/

マッピング　実行結果　ログの確認

```
$ more hisat2.sh.e16626062.1
5725730 reads; of these:
  5725730 (100.00%) were paired; of these:
    1222756 (21.36%) aligned concordantly 0 times
    4258780 (74.38%) aligned concordantly exactly 1 time
    244194 (4.26%) aligned concordantly >1 times
    ----
    1222756 pairs aligned concordantly 0 times; of these:
      128491 (10.51%) aligned discordantly 1 time
    ----
    1094265 pairs aligned 0 times concordantly or discordantly; of these:
      2188530 mates make up the pairs; of these:
        1470694 (67.20%) aligned 0 times
        662896 (30.29%) aligned exactly 1 time
        54940 (2.51%) aligned >1 times
87.16% overall alignment rate
[bam_sort_core] merging from 4 files and 4 in-memory blocks…
```

ジョブが終わらなかった場合

$ more outputs/hisat2/hisat2.sh.e16626062.1

```
$ more hisat2.sh.e16626062.2
4032514 reads; of these:
  4032514 (100.00%) were paired; of these:
    975045 (24.18%) aligned concordantly 0 times
    2882289 (71.48%) aligned concordantly exactly 1 time
    175180 (4.34%) aligned concordantly >1 times
    ----
    975045 pairs aligned concordantly 0 times; of these:
      89479 (9.18%) aligned discordantly 1 time
    ----
    885566 pairs aligned 0 times concordantly or discordantly; of these:
      1771132 mates make up the pairs; of these:
        1274482 (71.96%) aligned 0 times
        459285 (25.93%) aligned exactly 1 time
        37365 (2.11%) aligned >1 times
84.20% overall alignment rate
[bam_sort_core] merging from 0 files and 4 in-memory blocks..
```

ジョブが終わらなかった場合

$ more outputs/hisat2/hisat2.sh.e16626062.2

マッピング　実行結果　アライメントファイル (sam)の確認

```
$ more SRR453566.sam
@HD VN:1.0 SO:unsorted
@SQ SN:NC_001133.9 LN:230218
@SQ SN:NC_001134.8 LN:813184
@SQ SN:NC_001135.5 LN:316620
@SQ SN:NC_001136.10   LN:1531933
@SQ SN:NC_001137.3 LN:576874
@SQ SN:NC_001138.5 LN:270161
@SQ SN:NC_001139.9 LN:1090940
@SQ SN:NC_001140.6 LN:562643
@SQ SN:NC_001141.2 LN:439888
@SQ SN:NC_001142.9 LN:745751
@SQ SN:NC_001143.9 LN:666816
@SQ SN:NC_001144.5 LN:1078177
@SQ SN:NC_001145.3 LN:924431
@SQ SN:NC_001146.8 LN:784333
@SQ SN:NC_001147.6 LN:1091291
@SQ SN:NC_001148.4 LN:948066
@PG ID:hisat2  PN:hisat2  VN:2.2.1   CL:"/home/koshu3/miniconda3/envs/pags_rnaseq/bin/hisat2-align-s --wrapper basic-0 -p 4 -x re
SRR453566.3    73  NC_001142.9    76325   60  7S88M6S=763250NATGGCTGCTCAAAAGTCTTTCAGAATCAAGCAAAAAATGGCTAAGGCTAAGAAGCAAAACAGACCATTGC
SRR453566.3    133 NC_001142.9    76325   0   *=763250NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
SRR453566.1    73  NC_001145.3    887941  60  101M=8879410NAAAACTTTGGATGACTTCAACAACTATTCTTCTGAAATCAACAAAATATCACCAACTTCCGCCAACACAAAG
```

ジョブが終わらなかった場合

$ more outputs/hisat2/SRR453566.sam

```
$ more SRR453569.sam
@HD VN:1.0 SO:unsorted
@SQ SN:NC_001133.9 LN:230218
@SQ SN:NC_001134.8 LN:813184
@SQ SN:NC_001135.5 LN:316620
@SQ SN:NC_001136.10   LN:1531933
@SQ SN:NC_001137.3 LN:576874
@SQ SN:NC_001138.5 LN:270161
@SQ SN:NC_001139.9 LN:1090940
@SQ SN:NC_001140.6 LN:562643
@SQ SN:NC_001141.2 LN:439888
@SQ SN:NC_001142.9 LN:745751
@SQ SN:NC_001143.9 LN:666816
@SQ SN:NC_001144.5 LN:1078177
@SQ SN:NC_001145.3 LN:924431
@SQ SN:NC_001146.8 LN:784333
@SQ SN:NC_001147.6 LN:1091291
@SQ SN:NC_001148.4 LN:948066
@PG ID:hisat2  PN:hisat2  VN:2.2.1   CL:"/home/koshu3/miniconda3/envs/pags_rnaseq/bin/hisat2-align-s --wrapper basic-0 -p 4 -x ref
SRR453569.2    77  *   0   0   *   *   0   0   NTGGACTTTAGTGCGTTGAACGACATATTAAATGAACATGGTATTAGTATACTCCCAGCTAACGCATCACAATATGTCAAAAGATC
SRR453569.2    141 *   0   0   *   *   0   0   NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
SRR453569.1    89  NC_001136.10   761970  60  101M=7619700CGCATTGATGAACAATCAATAAATACGACCAGCAGTAACTCGACCACAACATCGAGCATGTTTACAGATGCACT
```

ジョブが終わらなかった場合

$ more outputs/hisat2/SRR453569.sam

発現量の算出

```
$ more stringtie.sh
#$ -S /bin/bash
#$ -pe def_slot 4
#$ -cwd
#$ -l mem_req=8G,s_vmem=8G

conda activate pags_rnaseq

ACESSIONS=(453566 453569)
for NUM in ${ACESSIONS[@]}
do
  PREFIX=SRR${NUM}
  BAM=${PREFIX}".sorted.bam"
  stringtie -e -B -p ${NSLOTS} \
         -G reference/s288c.gff \
         -o ballgown/$PREFIX/${PREFIX}.out.gtf \
         -A ${PREFIX}.gene_abund.tab \
         $BAM
done
```

発現量の算出

```
$ stringtie -h
StringTie v2.2.1 usage:

stringtie <in.bam ..> [-G <guide_gff>] [-l <prefix>] [-o <out.gtf>] [-p <cpus>]
 [-v] [-a <min_anchor_len>] [-m <min_len>] [-j <min_anchor_cov>] [-f <min_iso>]
 [-c <min_bundle_cov>] [-g <bdist>] [-u] [-L] [-e] [--viral] [-E <err_margin>]
 [--ptf <f_tab>] [-x <seqid,..>] [-A <gene_abund.out>] [-h] {-B|-b <dir_path>}
 [--mix] [--conservative] [--rf] [--fr]
Assemble RNA-Seq alignments into potential transcripts.
Options:
 --version : print just the version at stdout and exit
 --conservative : conservative transcript assembly, same as -t -c 1.5 -f 0.05
 --mix : both short and long read data alignments are provided
        (long read alignments must be the 2nd BAM/CRAM input file)
 --rf : assume stranded library fr-firststrand
 --fr : assume stranded library fr-secondstrand
 -G reference annotation to use for guiding the assembly process (GTF/GFF)
 --ptf : load point-features from a given 4 column feature file <f_tab>
 -o output path/file name for the assembled transcripts GTF (default: stdout)
 -l name prefix for output transcripts (default: STRG)
 -f minimum isoform fraction (default: 0.01)
 -L long reads processing; also enforces -s 1.5 -g 0 (default:false)
 -R if long reads are provided, just clean and collapse the reads but
    do not assemble
 -m minimum assembled transcript length (default: 200)
 -a minimum anchor length for junctions (default: 10)
 -j minimum junction coverage (default: 1)
 -t disable trimming of predicted transcripts based on coverage
    (default: coverage trimming is enabled)
 -c minimum reads per bp coverage to consider for multi-exon transcript
    (default: 1)
 -s minimum reads per bp coverage to consider for single-exon transcript
    (default: 4.75)
 -v verbose (log bundle processing details)
 -g maximum gap allowed between read mappings (default: 50)
 -M fraction of bundle allowed to be covered by multi-hit reads (default:1)
 -p number of threads (CPUs) to use (default: 1)
 -A gene abundance estimation output file
 -E define window around possibly erroneous splice sites from long reads to
    look out for correct splice sites (default: 25)
 -B enable output of Ballgown table files which will be created in the
    same directory as the output GTF (requires -G, -o recommended)
 -b enable output of Ballgown table files but these files will be
    created under the directory path given as <dir_path>
 -e only estimate the abundance of given reference transcripts (requires -G)
 --viral : only relevant for long reads from viral data where splice sites
```

発現量の算出

```
$ qsub scripts/stringtie.sh
Your job 16626115 ("stringtie.sh") has been submitted
```

```
$ ls -al
合計 8311004
drwxr-xr-x  7 koshu3 koshu       4096 10月 10 12:38 .
drwxr-x--- 10 koshu3 koshu       4096 10月 10 12:36 ..
-rw-r--r--  1 koshu3 koshu     490409 10月 10 12:37 SRR453566.gene_abund.tab
-rw-r--r--  1 koshu3 koshu 4173908387 10月 10 11:23 SRR453566.sam
-rw-r--r--  1 koshu3 koshu  821523867 10月 10 11:23 SRR453566.sorted.bam
-rw-r--r--  1 koshu3 koshu     491170 10月 10 12:38 SRR453569.gene_abund.tab
-rw-r--r--  1 koshu3 koshu 2922686958 10月 10 11:22 SRR453569.sam
-rw-r--r--  1 koshu3 koshu  591294985 10月 10 11:22 SRR453569.sorted.bam
drwxr-xr-x  4 koshu3 koshu       4096 10月 10 12:37 ballgown
-rw-r--r--  1 koshu3 koshu        693 10月 10 11:23 hisat2.sh.e16626062.1
-rw-r--r--  1 koshu3 koshu        688 10月 10 11:22 hisat2.sh.e16626062.2
-rw-r--r--  1 koshu3 koshu          0 10月 10 11:21 hisat2.sh.o16626062.1
-rw-r--r--  1 koshu3 koshu          0 10月 10 11:21 hisat2.sh.o16626062.2
-rw-r--r--  1 koshu3 koshu          0 10月 10 11:21 hisat2.sh.pe16626062.1
-rw-r--r--  1 koshu3 koshu          0 10月 10 11:21 hisat2.sh.pe16626062.2
-rw-r--r--  1 koshu3 koshu          0 10月 10 11:21 hisat2.sh.po16626062.1
-rw-r--r--  1 koshu3 koshu          0 10月 10 11:21 hisat2.sh.po16626062.2
-rw-r--r--  1 koshu3 koshu       2262 10月 10 10:57 hisat2_index.sh.e16626048
-rw-r--r--  1 koshu3 koshu       3919 10月 10 10:57 hisat2_index.sh.o16626048
-rw-r--r--  1 koshu3 koshu          0 10月 10 10:57 hisat2_index.sh.pe16626048
-rw-r--r--  1 koshu3 koshu          0 10月 10 10:57 hisat2_index.sh.po16626048
drwxr-xr-x  2 koshu3 koshu       4096 10月  9 14:41 outputs
drwxr-xr-x  2 koshu3 koshu       4096 10月  9 12:23 reads
drwxr-xr-x  2 koshu3 koshu       4096 10月 10 12:33 reference
drwxr-xr-x  2 koshu3 koshu       4096 10月 10 12:36 scripts
-rw-r--r--  1 koshu3 koshu          0 10月 10 12:37 stringtie.sh.e16626115
-rw-r--r--  1 koshu3 koshu          0 10月 10 12:37 stringtie.sh.o16626115
-rw-r--r--  1 koshu3 koshu          0 10月 10 12:37 stringtie.sh.pe16626115
-rw-r--r--  1 koshu3 koshu          0 10月 10 12:37 stringtie.sh.po16626115
```

ジョブが終わらなかった場合

$ ls outputs/stringtie

```
$ ls ballgown/*
ballgown/SRR453566:
SRR453566.out.gtf  e2t.ctab  e_data.ctab
i2t.ctab  i_data.ctab  t_data.ctab

ballgown/SRR453569:
SRR453569.out.gtf  e2t.ctab  e_data.ctab
i2t.ctab  i_data.ctab  t_data.ctab
```

次のステップとして、R などを用いることで可視化などができる。

ballgown を使う場合は、このballgown ディレクトリを
そのまま入力データとして使用できる。

ジョブが終わらなかった場合

$ ls outputs/stringtie/ballgown

結果ファイルの確認

SRR453566.gene_abund.tab ファイル

```
$ more SRR453566.gene_abund.tab
```

Gene ID	Gene Name	Reference	Strand	Start	End	Coverage	FPKM	TPM
gene-YAL068C	PAU8	NC_001133.9	-	1807	2169	1.011341	1.058091	1.143341
gene-YAL030W	SNC1	NC_001133.9	+	87286	87752	70.827682	74.101746	80.072098
gene-YAL029C	MYO4	NC_001133.9	-	87855	92270	35.674591	37.323677	40.330833
gene-YAL028W	FRT2	NC_001133.9	+	92900	94486	5.316950	5.562730	6.010918
gene-YAL027W	SAW1	NC_001133.9	+	94687	95472	24.430025	25.559322	27.618629
gene-YAL026C	DRS2	NC_001133.9	-	95630	99697	27.811796	29.097420	31.441788
gene-YNCA0001W	HRA1	NC_001133.9	+	99305	99868	3.410652	3.568312	3.855810
gene-YAL025C	MAK16	NC_001133.9	-	100225	101145	146.168289	152.925034	165.246155
gene-YAL067C	SEO1	NC_001133.9	-	7235	9016	0.000000	0.000000	0.000000
gene-YAL065C	-	NC_001133.9	-	11565	11951	0.130491	0.136523	0.147523
gene-YAL064W-B	-	NC_001133.9	+	12046	12426	1.191600	1.246683	1.347127
gene-YAL064C-A	TDA8	NC_001133.9	-	13363	13743	0.000000	0.000000	0.000000
gene-YAL064W	-	NC_001133.9	+	21566	21850	0.011696	0.012237	0.013223
gene-YAL063C-A	-	NC_001133.9	-	22395	22685	0.000000	0.000000	0.000000
gene-YAL063C	FLO9	NC_001133.9	-	24000	27968	1.093222	1.143757	1.235909
gene-YAL062W	GDH3	NC_001133.9	+	31567	32940	7.488355	7.834511	8.465734
gene-YAL061W	BDH2	NC_001133.9	+	33448	34701	14.996013	15.689216	16.953291

発現量のノーマライズ

FPKM: Fragments Per Kilobase of exon per Million reads mapped
TPM: Transcripts Per kilobase Milion

FPKMもTPMも以下の二つで補正するが、補正する順番が異なる。

(1) 総リード数での補正 (総リード数 100万)

(2) 遺伝子長での補正 (遺伝子長 1000b)

FPKM (1) -> (2)
TPM (2) -> (1)

Singularity を利用したスクリプト

```
$ more scripts/hisat2_singularity.sh
#$ -S /bin/bash
#$ -pe def_slot 4
#$ -cwd
#$ -t 1-2:1
#$ -l mem_req=8G,s_vmem=8G

conda activate pags_rnaseq

# Batch culture: SRR453566
# chemostat: SRR453569
ACESSIONS=(453566 453569)
no=`expr ${SGE_TASK_ID} - 1`

NUM=${ACESSIONS[${no}]}
PREFIX=SRR${NUM}

# read file
DIR=./reads/
QUERY1_1=${DIR}${PREFIX}"_1.fastq.gz"
QUERY1_2=${DIR}${PREFIX}"_2.fastq.gz"

singularity exec /usr/local/biotools/h/hisat2:2.2.1--h87f3376_4 \
 hisat2 -p ${NSLOTS} -x reference/s288c.fa --dta \
        -1 ${QUERY1_1} -2 ${QUERY1_2} \
        -S ${PREFIX}.sam

# convert sam to bam
# sort by position
singularity exec /usr/local/biotools/s/samtools:1.16.1--h6899075_0 \
 samtools sort -@ ${NSLOTS} ${PREFIX}.sam -o ${PREFIX}.sorted.bam
```

おわり