RNA-Seq分析流程V0.1.0版SOP

建立工作目录

根据批次号（batchID）建立整个项目的工作目录

e.g.：`projectID=RNASEQ_0001`

目前RNA-SEQ分析目录：

`/Bioinfo/ScientificResearchService/project`

配置文件

目前流程较为固定，暂无可选参数

makefile所需配置文件

- 运行配置文件（如下例子所示），`=` 中间**不能有空格**，空行是可以的。

```
#[software&scripts]
SAMTOOLS=/Bioinfo/ScientificResearchService/bin/samtools/samtools-
1.2/bin/samtools
HISAT2=/Bioinfo/ScientificResearchService/bin/hisat/hisat2-2.0.5/hisat2
HISAT2SPS=/Bioinfo/ScientificResearchService/bin/hisat/hisat2-
```

```
2.0.5/hisat2_extract_splice_sites.py
STRINGTIE=/Bioinfo/ScientificResearchService/bin/stringtie/stringtie-
1.3.3b.Linux_x86_64/stringtie
JAVA=/usr/bin/java
TRIMMOMATIC=/Bioinfo/ScientificResearchService/pylib/yiTiJi/pip/bin/trimmomat
ic-0.36.jar
FASTQC=/Bioinfo/ScientificResearchService/pylib/yiTiJi/pip/bin/FastQC/fastqc
FEATURECOUNTS=/Bioinfo/Software/subread-1.5.0-p1-Linux-
x86_64/bin/featureCounts
R=/Bioinfo/Software/R-3.3.2/bin/R
STAR_DIR=/Bioinfo/ScientificResearchService/bin/star/STAR-
2.5.2b/bin/Linux_x86_64_static/
GATK=/Bioinfo/Software/gatk-3.5.0/GenomeAnalysisTK.jar
PICARD=/Bioinfo/ScientificResearchService/bin/picard/picard-2.17.3/picard.jar
COSEQR=/Bioinfo/MDDRD2/PMO/chenyl/PROJECTS/RNA-seq/src/coseq.R
COR=/Bioinfo/Software/R-3.4.4/bin/Rscript
PLOTR=/Bioinfo/MDDRD2/PMO/chenyl/PROJECTS/RNA-seq/src/plot_figures.R

#[RNACocktail]
RNACOCKTAIL=/Bioinfo/MDDRD2/PMO/chenyl/venv_python/py_RNACocktail/bin/run_rna
cocktail.py

#[database]
REF=/Bioinfo/Database/reference/human/ucsc.hg19.fasta
REFHISAT2=/Bioinfo/Database/reference/human/ucsc.hg19.hisat2
GENEGTF=/Bioinfo/Test/rna-seq-test-by-cyl/gencode.v27lift37.annotation.gtf
DBSNP=/Bioinfo/MDDRD2/PMO/chenyl/test/yitiji_control_test/database/ucsc_dbsnp
_138.hg19.vcf
STRANDPOSBED=/Bioinfo/Test/rna-seq-test-by-cyl/database/GRCh37_strand_pos.bed
GENESPOSBED=/Bioinfo/Test/rna-seq-test-by-cyl/database/GRCh37_genes_pos.bed
TRUSEQPE=/Bioinfo/Database/Illumina/TruSeq3-PE.fa
```

inputlist配置

1. inputlist主要包括需要分析的样本信息
2. inputlist分为4列，以tab分割
3. 第一列到第四列输入的分别为 样本ID ， 路径/read1.fq.gz ， 路径/read2.fq.gz ， condition
4. 样本ID ：只能有[数字，下划线，字母]组成
5. fq.gz ：必须为绝对路径
6. condition ：表示样本为control或case，只能标记 control 或者 case （全小写）

e.g.

```
S-A /Bioinfo/ScientificResearchService/Rawdata/data_P101SC18010822-01-B1-
22/raw_data/S-A_H75N5DMXX_L1_1.fq.gz
/Bioinfo/ScientificResearchService/Rawdata/data_P101SC18010822-01-B1-
22/raw_data/S-A_H75N5DMXX_L1_2.fq.gz   case
S-D /Bioinfo/ScientificResearchService/Rawdata/data_P101SC18010822-01-B1-
22/raw_data/S-D_H75N5DMXX_L1_1.fq.gz
/Bioinfo/ScientificResearchService/Rawdata/data_P101SC18010822-01-B1-
22/raw_data/S-D_H75N5DMXX_L1_2.fq.gz   control
2S-A    /Bioinfo/ScientificResearchService/Rawdata/P101SC18011607-
01shajingjing/P101SC18011607-01-2018-03-05_data/2S-A_HK77MCCXY_L5_1.fq.gz
/Bioinfo/ScientificResearchService/Rawdata/P101SC18011607-
01shajingjing/P101SC18011607-01-2018-03-05_data/2S-A_HK77MCCXY_L5_2.fq.gz
case
2S-D    /Bioinfo/ScientificResearchService/Rawdata/P101SC18011607-
01shajingjing/P101SC18011607-01-2018-03-05_data/2S-D_HK77MCCXY_L5_1.fq.gz
/Bioinfo/ScientificResearchService/Rawdata/P101SC18011607-
01shajingjing/P101SC18011607-01-2018-03-05_data/2S-D_HK77MCCXY_L5_2.fq.gz
control
```

生成分析脚本

脚本路径

`/Bioinfo/MDDRD2/PMO/chenyl/PROJECTS/RNA-seq/generateMakefile.py`

运行方式

```
python3 {路径}/RNA-seq/generateMakefile.py \     # 本流程主程序
    -l {路径1}/input_list.txt \      #输入list
    -o {路径2}/RNA_RESULT/ \     #输出路径
    -m {路径3}/makefile \     # makefile的输出文件
    -p {项目名称} # 项目名称，可以是RNAseq-xxx
```

- `路径` ：RNA-seq主要流程脚本目录
- `路径1` ：配置文件路径
- `路径2` ：结果输出路径

- 路径3 ：运行脚本生成路径

结果 ：
在 路径3 下生成一个 makefile ，会分析 inputlist 中的样本，并将分析结果以 项目名称 的前缀输出到 路径2 中。

makefile例子：

```
include $(CONFIG)
export
PATH:=/Bioinfo/MDDRD2/PMO/chenyl/venv_python/py_RNACocktail/bin:${PATH}
mkdir1:
  mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-A/QC
  mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-A/ALIGN
  mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-
A/RECONSTRUCT
  mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-A/VARIANT
  mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-A/ANNO
  mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-A/WORKDIR
fastqc1:mkdir1
  $(FASTQC) /Bioinfo/ScientificResearchService/Rawdata/data_P101SC18010822-
01-B1-22/raw_data/S-A_H75N5DMXX_L1_1.fq.gz
/Bioinfo/ScientificResearchService/Rawdata/data_P101SC18010822-01-B1-
22/raw_data/S-A_H75N5DMXX_L1_2.fq.gz --outdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-A/QC --threads 1 -extract -q -f fastq -
-java $(JAVA)
  $(JAVA) -jar -XX:ParallelGCThreads=4 -Xmx10g $(TRIMMOMATIC) PE
/Bioinfo/ScientificResearchService/Rawdata/data_P101SC18010822-01-B1-
22/raw_data/S-A_H75N5DMXX_L1_1.fq.gz
/Bioinfo/ScientificResearchService/Rawdata/data_P101SC18010822-01-B1-
22/raw_data/S-A_H75N5DMXX_L1_2.fq.gz /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-A/QC/S-A.clean.R1.fastq.gz
/Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-A/QC/S-
A.cleanUp.R1.fastq.gz /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-A/QC/S-A.clean.R2.fastq.gz
/Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-A/QC/S-
A.cleanUp.R2.fastq.gz -trimlog /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-A/WORKDIR/fastqc.trim.log -threads 4 -
phred33 ILLUMINACLIP:$(TRUSEQPE):2:30:10 LEADING:3 TRAILING:3
SLIDINGWINDOW:4:15 MINLEN:36
align1:fastqc1
  $(RNACOCKTAIL) align --align_idx $(REFHISAT2) --outdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-A/ALIGN --workdir /Bioinfo/Test/RNA-
```

```makefile
Seq_chenyl/pipeline_test/AcellsTest_S-A/WORKDIR --ref_gtf $(GENEGTF) --1
/Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-A/QC/S-
A.clean.R1.fastq.gz --2 /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-A/QC/S-A.clean.R2.fastq.gz --hisat2
$(HISAT2) --hisat2_sps $(HISAT2SPS) --samtools $(SAMTOOLS)  --threads 10 --
sample S-A
reconstruct1:align1
    $(RNACOCKTAIL) reconstruct --alignment_bam /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-A/ALIGN/hisat2/S-
A/alignments.sorted.bam --outdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-A/RECONSTRUCT --workdir
/Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-A/WORKDIR --ref_gtf
$(GENEGTF) --stringtie $(STRINGTIE) --threads 10 --sample S-A
variant1:align1
    $(RNACOCKTAIL) variant --alignment /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-A/ALIGN/hisat2/S-
A/alignments.sorted.bam --CleanSam --outdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-A/VARIANT --workdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-A/WORKDIR --picard $(PICARD) --gatk
$(GATK) --threads 10 --sample S-A --ref_genome $(REF) --IndelRealignment --
knownsites $(DBSNP) --java $(JAVA)
S-A:mkdir1 fastqc1 align1 reconstruct1 variant1 mkdir2:
    mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-D/QC
    mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-D/ALIGN
    mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-
D/RECONSTRUCT
    mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-D/VARIANT
    mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-D/ANNO
    mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-D/WORKDIR
fastqc2:mkdir2
    $(FASTQC) /Bioinfo/ScientificResearchService/Rawdata/data_P101SC18010822-
01-B1-22/raw_data/S-D_H75N5DMXX_L1_1.fq.gz
/Bioinfo/ScientificResearchService/Rawdata/data_P101SC18010822-01-B1-
22/raw_data/S-D_H75N5DMXX_L1_2.fq.gz --outdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-D/QC --threads 1 -extract -q -f fastq -
-java $(JAVA)
    $(JAVA) -jar -XX:ParallelGCThreads=4 -Xmx10g $(TRIMMOMATIC) PE
/Bioinfo/ScientificResearchService/Rawdata/data_P101SC18010822-01-B1-
22/raw_data/S-D_H75N5DMXX_L1_1.fq.gz
/Bioinfo/ScientificResearchService/Rawdata/data_P101SC18010822-01-B1-
22/raw_data/S-D_H75N5DMXX_L1_2.fq.gz /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-D/QC/S-D.clean.R1.fastq.gz
/Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-D/QC/S-
```

```makefile
D.cleanUp.R1.fastq.gz /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-D/QC/S-D.clean.R2.fastq.gz
/Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-D/QC/S-
D.cleanUp.R2.fastq.gz -trimlog /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-D/WORKDIR/fastqc.trim.log -threads 4 -
phred33 ILLUMINACLIP:$(TRUSEQPE):2:30:10 LEADING:3 TRAILING:3
SLIDINGWINDOW:4:15 MINLEN:36
align2:fastqc2
	$(RNACOCKTAIL) align --align_idx $(REFHISAT2) --outdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-D/ALIGN --workdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-D/WORKDIR --ref_gtf $(GENEGTF) --1
/Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-D/QC/S-
D.clean.R1.fastq.gz --2 /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-D/QC/S-D.clean.R2.fastq.gz --hisat2
$(HISAT2) --hisat2_sps $(HISAT2SPS) --samtools $(SAMTOOLS)  --threads 10 --
sample S-D
reconstruct2:align2
	$(RNACOCKTAIL) reconstruct --alignment_bam /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-D/ALIGN/hisat2/S-
D/alignments.sorted.bam --outdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-D/RECONSTRUCT --workdir
/Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_S-D/WORKDIR --ref_gtf
$(GENEGTF) --stringtie $(STRINGTIE) --threads 10 --sample S-D
variant2:align2
	$(RNACOCKTAIL) variant --alignment /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-D/ALIGN/hisat2/S-
D/alignments.sorted.bam --CleanSam --outdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-D/VARIANT --workdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-D/WORKDIR --picard $(PICARD) --gatk
$(GATK) --threads 10 --sample S-D --ref_genome $(REF) --IndelRealignment --
knownsites $(DBSNP) --java $(JAVA)
S-D:mkdir2 fastqc2 align2 reconstruct2 variant2 mkdir3:
	mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-A/QC
	mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-A/ALIGN
	mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-
A/RECONSTRUCT
	mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-A/VARIANT
	mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-A/ANNO
	mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-A/WORKDIR
fastqc3:mkdir3
	$(FASTQC) /Bioinfo/ScientificResearchService/Rawdata/P101SC18011607-
01shajingjing/P101SC18011607-01-2018-03-05_data/2S-A_HK77MCCXY_L5_1.fq.gz
/Bioinfo/ScientificResearchService/Rawdata/P101SC18011607-
```

```
01shajingjing/P101SC18011607-01-2018-03-05_data/2S-A_HK77MCCXY_L5_2.fq.gz --
outdir /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-A/QC --
threads 1 -extract -q -f fastq --java $(JAVA)
  $(JAVA) -jar -XX:ParallelGCThreads=4 -Xmx10g $(TRIMMOMATIC) PE
/Bioinfo/ScientificResearchService/Rawdata/P101SC18011607-
01shajingjing/P101SC18011607-01-2018-03-05_data/2S-A_HK77MCCXY_L5_1.fq.gz
/Bioinfo/ScientificResearchService/Rawdata/P101SC18011607-
01shajingjing/P101SC18011607-01-2018-03-05_data/2S-A_HK77MCCXY_L5_2.fq.gz
/Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-A/QC/2S-
A.clean.R1.fastq.gz /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-
A/QC/2S-A.cleanUp.R1.fastq.gz /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-A/QC/2S-A.clean.R2.fastq.gz
/Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-A/QC/2S-
A.cleanUp.R2.fastq.gz -trimlog /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-A/WORKDIR/fastqc.trim.log -threads 4 -
phred33 ILLUMINACLIP:$(TRUSEQPE):2:30:10 LEADING:3 TRAILING:3
SLIDINGWINDOW:4:15 MINLEN:36
align3:fastqc3
  $(RNACOCKTAIL) align --align_idx $(REFHISAT2) --outdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-A/ALIGN --workdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-A/WORKDIR --ref_gtf $(GENEGTF) --1
/Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-A/QC/2S-
A.clean.R1.fastq.gz --2 /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-A/QC/2S-A.clean.R2.fastq.gz --hisat2
$(HISAT2) --hisat2_sps $(HISAT2SPS) --samtools $(SAMTOOLS)    --threads 10 --
sample 2S-A
reconstruct3:align3
  $(RNACOCKTAIL) reconstruct --alignment_bam /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-A/ALIGN/hisat2/2S-
A/alignments.sorted.bam --outdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-A/RECONSTRUCT --workdir
/Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-A/WORKDIR --ref_gtf
$(GENEGTF) --stringtie $(STRINGTIE) --threads 10 --sample 2S-A
variant3:align3
  $(RNACOCKTAIL) variant --alignment /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-A/ALIGN/hisat2/2S-
A/alignments.sorted.bam --CleanSam --outdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-A/VARIANT --workdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-A/WORKDIR --picard $(PICARD) --gatk
$(GATK) --threads 10 --sample 2S-A --ref_genome $(REF) --IndelRealignment --
knownsites $(DBSNP) --java $(JAVA)
2S-A:mkdir3 fastqc3 align3 reconstruct3 variant3 mkdir4:
  mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-D/QC
```

```
  mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-D/ALIGN
  mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-
D/RECONSTRUCT
  mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-D/VARIANT
  mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-D/ANNO
  mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-D/WORKDIR
fastqc4:mkdir4
  $(FASTQC) /Bioinfo/ScientificResearchService/Rawdata/P101SC18011607-
01shajingjing/P101SC18011607-01-2018-03-05_data/2S-D_HK77MCCXY_L5_1.fq.gz
/Bioinfo/ScientificResearchService/Rawdata/P101SC18011607-
01shajingjing/P101SC18011607-01-2018-03-05_data/2S-D_HK77MCCXY_L5_2.fq.gz --
outdir /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-D/QC --
threads 1 -extract -q -f fastq --java $(JAVA)
  $(JAVA) -jar -XX:ParallelGCThreads=4 -Xmx10g $(TRIMMOMATIC) PE
/Bioinfo/ScientificResearchService/Rawdata/P101SC18011607-
01shajingjing/P101SC18011607-01-2018-03-05_data/2S-D_HK77MCCXY_L5_1.fq.gz
/Bioinfo/ScientificResearchService/Rawdata/P101SC18011607-
01shajingjing/P101SC18011607-01-2018-03-05_data/2S-D_HK77MCCXY_L5_2.fq.gz
/Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-D/QC/2S-
D.clean.R1.fastq.gz /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-
D/QC/2S-D.cleanUp.R1.fastq.gz /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-D/QC/2S-D.clean.R2.fastq.gz
/Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-D/QC/2S-
D.cleanUp.R2.fastq.gz -trimlog /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-D/WORKDIR/fastqc.trim.log -threads 4 -
phred33 ILLUMINACLIP:$(TRUSEQPE):2:30:10 LEADING:3 TRAILING:3
SLIDINGWINDOW:4:15 MINLEN:36
align4:fastqc4
  $(RNACOCKTAIL) align --align_idx $(REFHISAT2) --outdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-D/ALIGN --workdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-D/WORKDIR --ref_gtf $(GENEGTF) --1
/Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-D/QC/2S-
D.clean.R1.fastq.gz --2 /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-D/QC/2S-D.clean.R2.fastq.gz --hisat2
$(HISAT2) --hisat2_sps $(HISAT2SPS) --samtools $(SAMTOOLS)    --threads 10 --
sample 2S-D
reconstruct4:align4
  $(RNACOCKTAIL) reconstruct --alignment_bam /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-D/ALIGN/hisat2/2S-
D/alignments.sorted.bam --outdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-D/RECONSTRUCT --workdir
/Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_2S-D/WORKDIR --ref_gtf
$(GENEGTF) --stringtie $(STRINGTIE) --threads 10 --sample 2S-D
```

```
variant4:align4
  $(RNACOCKTAIL) variant --alignment /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-D/ALIGN/hisat2/2S-
D/alignments.sorted.bam --CleanSam --outdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-D/VARIANT --workdir /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-D/WORKDIR --picard $(PICARD) --gatk
$(GATK) --threads 10 --sample 2S-D --ref_genome $(REF) --IndelRealignment --
knownsites $(DBSNP) --java $(JAVA)
2S-D:mkdir4 fastqc4 align4 reconstruct4 variant4 mkdir5:
  mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_DIFF
  mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_COEXP
  mkdir -p /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_WORKDIR
diff5:align1 align2 align3 align4 mkdir5
  $(RNACOCKTAIL) diff --alignments /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-D/ALIGN/hisat2/S-
D/alignments.sorted.bam,/Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-D/ALIGN/hisat2/2S-
D/alignments.sorted.bam /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_S-A/ALIGN/hisat2/S-
A/alignments.sorted.bam,/Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_2S-A/ALIGN/hisat2/2S-
A/alignments.sorted.bam --sample S-D,2S-D S-A,2S-A --ref_gtf $(GENEGTF) --
outdir /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_DIFF --workdir
/Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_WORKDIR --featureCounts
$(FEATURECOUNTS) --R $(R) --stringtie $(STRINGTIE)
  $(COR) $(PLOTR) /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_WORKDIR/deseq2/S-D,2S-D-S-A,2S-
A/deseq2.rda 0.1 /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_DIFF
coexp5:diff5
  $(COR) $(COSEQR) /Bioinfo/Test/RNA-
Seq_chenyl/pipeline_test/AcellsTest_WORKDIR/deseq2/S-D,2S-D-S-A,2S-
A/deseq2.rda /Bioinfo/Test/RNA-Seq_chenyl/pipeline_test/AcellsTest_COEXP 0.1
ALL:S-A S-D 2S-A 2S-D diff5 coexp5
```

运行分析脚本

```
make -f {路径}/makefile CONFIG={路径}/RNA-seq/rna_config -j8 ALL
```

参数 `-j8` 表示最大可执行并行数目为8

检查分析是否完成

输出无错误即可