# Convert a FASTQ file to an indexed BAM

## **Description**

This function takes in FASTQ files and performs quality control before aligning to a reference genome. It assumes paired-end samples are of the same file name with an underscore (\_) and a number to signify different reads of the same sample. Each sample's outputs will be stored in a separate directory.

## Usage

```
fastqProcession(
  file = list_files_with_exts(dir = dir, exts = "fastq"),
  dir = getwd(),
  genome,
  genomedir,
  paired = FALSE,
  threads = 4L,
  molecule = "RNA"
)
```

## **Arguments**

file	A list of paths to FASTQ files. If no paths are entered, defaults to all fastq files in dir.
dir	Output directory.
genome	A BSgenome object, GmapGenome object, or a character string indicating the genome name eg. "hg19".
genomedir	A directory containing the reference genome. Otherwise, it is the parent directory of the reference genome is genome is a character string or BSgenome object.
paired	Indicates whether the samples are from paired-end or single-end reads.
threads	The number of cores to use in the process.
molecule	A character string indicating either DNA or RNA samples.

#### Value

A list of file paths to created BAM files

# Analyse differential gene expression

## **Description**

This function analyses differentially expressed genes between sample groups. It assumes that sample BAM files are ordered in a directory structure such as "group/sample/" as fastqProcession() would order it. There should be more than one sample per group or else differential expression analysis won't work It outputs a CSV file showing differential expression (ordered by p-value). It works similarly to gene\_Counting(), but then further analyses those counts to obtain differential expression data.

#### **Usage**

```
geneExpression(
  dir = getwd(),
  groups,
  TxDb,
  orgDb,
  outputdir = getwd(),
  threads = 4L,
  paired = FALSE
)
```

#### **Arguments**

dir	The parent directory of the sample groups.
groups	Folder names of the sample groups. The default is all folders in dir.
TxDb	A TxDb object upon which regions of the genome are counted.

orgDb An orgDb object for annotating the CSV with gene symbols and Ensembl IDs.

outputdir Output directory of CSV file.
threads Number of cores to use.

paired Indicates whether the samples are from paired-end reads.

#### Value

A data frame list containing all of the differential expression comparison.

# Count genes

## **Description**

This function counts reads of gene regions between sample groups. It assumes that sample BAM files are ordered in a directory structure such as "group/sample/" as fastqProcession() would order it. It outputs a CSV file showing gene counts. Works similarly to geneExpression(), but outputs count data instead of differential expression data.

#### **Usage**

```
gene_Counting(
   dir = getwd(),
   groups,
   TxDb,
   orgDb,
   outputdir = getwd(),
   threads = 4L,
   paired = FALSE
)
```

#### **Arguments**

dir The parent directory of the sample groups.

groups Folder names of the sample groups. The default is all folders in dir.

TxDb A TxDb object upon which regions of the genome are counted.

orgDb An orgDb object for annotating the CSV with gene symbols and Ensembl IDs.

outputdir Output directory of CSV file.

threads Number of cores to use.

paired Indicates whether the samples are from paired-end reads.

#### **Value**

A data frame containing gene counts.

# Call single nucleotide polymorphism variants

## **Description**

This function calls SNP variants from BAM files. The results are formatted into a VCF file and the ID column is populated with dbSNP IDs.

#### **Usage**

callSNP(bam, genome, genomedir, SNPlocs, threads = 4L, outputdir = getwd())

#### **Arguments**

bam A list of paths to BAM files

genome A BSgenome object, a GmapGenome object, or a character string indicating the

reference genome eg. "hg19"

genomedir The directory containing the reference genome or, if genome is a character string, the

parent directory of the reference genome directory.

SNPlocs Object containing dbSNP IDs.

threads The number of cores to use.

outputdir The output directory for the VCF file.

#### **Value**

A list of file paths to the VCF files.

# Call insertion/deletion variants

## **Description**

This function calls indel variants from BAM files. The results are formatted into a VCF file and the ID column is populated with dbSNP IDs.

#### Usage

callIndel(bam, genome, genomedir, SNPlocs, threads, outputdir = getwd())

#### **Arguments**

bam A list of paths to BAM files

genome A BSgenome object, a GmapGenome object, or a character string indicating the

reference genome eg. "hg19"

genomedir The directory containing the reference genome or, if genome is a character string, the

parent directory of the reference genome directory.

SNPlocs An SNPlocs object containing dbSNP IDs. For indels, this may be an XtraSNPlocs object.

threads The number of cores to use. Cores should equal a factor of reference genome sequence

levels ie. chromosome contigs should be equally divisible between cores.

outputdir The output directory for the VCF file.

#### **Value**

A list of file paths to the VCF files.

# Call copy number variants

## **Description**

This function calls copy number variants from sample BAM files compared to control BAM files. It assumes that BAM files are stored in separate folders as is created by fastqProcession(). This function requires that control BAM files are provided. Once complete, it creates a CSV file containing copy number information.

#### **Usage**

```
callCNV(
  controldir,
  control = NULL,
  experimentdir,
  experiment = NULL,
  bed,
  outputdir = getwd()
)
```

#### **Arguments**

controldir The parent directory of the sample directories.

control The names of the folders in which control BAM files are. If NULL, all folders in

controldir will be checked for BAM files.

experimentdir The parent directory of sample on which to investigate copy numbers.

experiment The names of the folders in which sample BAM files are. If NULL, all folders in

experimentdir will be checked for BAM files.

bed A character string indicating BED file path or a TxDb object from which to extract a

BED file.

outputdir The directory in which to place copy number call.

#### **Value**

A data frame containing copy number calls.