

# Package ‘CODEX’

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**Type** Package

**Title** A Normalization and Copy Number Variation Detection Method for Whole Exome Sequencing

**Version** 1.41.0

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**Description** A normalization and copy number variation calling procedure for whole exome DNA sequencing data. CODEX relies on the availability of multiple samples processed using the same sequencing pipeline for normalization, and does not require matched controls. The normalization model in CODEX includes terms that specifically remove biases due to GC content, exon length and targeting and amplification efficiency, and latent systemic artifacts. CODEX also includes a Poisson likelihood-based recursive segmentation procedure that explicitly models the count-based exome sequencing data.

**License** GPL-2

**Depends** R (>= 3.2.3), Rsamtools, GenomeInfoDb, BSgenome.Hsapiens.UCSC.hg19, IRanges, Biostrings, S4Vectors

**Suggests** WES.1KG.WUGSC

**biocViews** ImmunoOncology, ExomeSeq, Normalization, QualityControl, CopyNumberVariation

**LazyData** yes

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|               |                                                                                              |
|---------------|----------------------------------------------------------------------------------------------|
| CODEX-package | <i>A Normalization and Copy Number Variation Detection Method for Whole Exome Sequencing</i> |
|---------------|----------------------------------------------------------------------------------------------|

---

**Description**

CODEX is a normalization and copy number variation calling procedure for whole exome DNA sequencing data. CODEX relies on the availability of multiple samples processed using the same sequencing pipeline for normalization, and does not require matched controls. The normalization model in CODEX includes terms that specifically remove biases due to GC content, exon length and targeting and amplification efficiency, and latent systemic artifacts. CODEX also includes a Poisson likelihood-based recursive segmentation procedure that explicitly models the count-based exome sequencing data.

**Details**

Package: CODEX  
Type: Package  
Version: 0.99.0  
Date: 2015-01-13  
License: GPL-2

CODEX takes as input the bam files/directories for whole exome sequencing datasets and bed files for exonic positions, returns raw and normalized coverage for each exon, and calls copy number variations with genotyping results.

**Author(s)**

Yuchao Jiang <yuchaoj@wharton.upenn.edu>, Nancy R. Zhang

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|               |                                            |
|---------------|--------------------------------------------|
| bambedObjDemo | <i>Demo data pre-stored for bambedObj.</i> |
|---------------|--------------------------------------------|

---

**Description**

Pre-stored bambedObj data for demonstration purposes.

**Usage**

```
data(bambedObjDemo)
```

**Details**

Pre-computed using whole exome sequencing data of 46 HapMap samples.

**Value**

bambedObj demo data (list) pre-computed.

**Author(s)**

Yuchao Jiang <yuchaoj@wharton.upenn.edu>

**Examples**

```
bamdir <- bambedObjDemo$bamdir
samname <- bambedObjDemo$samname
ref <- bambedObjDemo$ref
projectname <- bambedObjDemo$projectname
chr <- bambedObjDemo$chr
```

---

|           |                                                  |
|-----------|--------------------------------------------------|
| choiceofK | <i>Determine the number of latent factors K.</i> |
|-----------|--------------------------------------------------|

---

**Description**

Determines the number of latent variables K via AIC, BIC, and deviance reduction. A pdf file containing all three plots is generated.

**Usage**

```
choiceofK(AIC, BIC, RSS, K, filename)
```

**Arguments**

|          |                                                                  |
|----------|------------------------------------------------------------------|
| AIC      | vector of AIC for each K returned from <a href="#">normalize</a> |
| BIC      | vector of BIC for each K returned from <a href="#">normalize</a> |
| RSS      | vector of RSS for each K returned from <a href="#">normalize</a> |
| K        | vector of K returned from <a href="#">normalize</a>              |
| filename | Filename of the output plot of AIC and RSS                       |

**Details**

AIC: Akaike information criterion, used for model selection; BIC: Bayesian information criterion, used for model selection; RSS: residue sum of squares, used to plot scree plot and determine the 'elbow'.

**Value**

pdf file with three plots: AIC, BIC, and percentage of variance explained versus the number of latent factors.

**Author(s)**

Yuchao Jiang <yuchaoj@wharton.upenn.edu>

**See Also**

[normalize](#), [segment](#)

**Examples**

```
AIC <- normObjDemo$AIC
BIC <- normObjDemo$BIC
RSS <- normObjDemo$RSS
K <- normObjDemo$K
projectname <- bambedObjDemo$projectname
chr <- bambedObjDemo$chr
choiceofK(AIC, BIC, RSS, K, filename = paste(projectname, "_", chr,
  "_choiceofK", ".pdf", sep = ""))
```

---

coverageObjDemo

*Demo data pre-stored for coverageObj.*

---

**Description**

Pre-stored coverageObj data for demonstration purposes.

**Usage**

```
data(coverageObjDemo)
```

**Details**

Pre-computed using whole exome sequencing data of 46 HapMap samples.

**Value**

coverageObj demo data (list) pre-computed.

**Author(s)**

Yuchao Jiang <yuchaoj@wharton.upenn.edu>

**Examples**

```
Y <- coverageObjDemo$Y
readlength <- coverageObjDemo$readlength
```

gcDemo

*Demo data pre-stored for GC content.***Description**

Pre-stored GC content data for demonstration purposes.

**Usage**

```
data(gcDemo)
```

**Details**

Pre-computed using whole exome sequencing data of 46 HapMap samples.

**Value**

gc demo data (vector) pre-computed.

**Author(s)**

Yuchao Jiang <yuchaoj@wharton.upenn.edu>

**Examples**

```
head(round(gcDemo, 2))
```

getbambed

*Get bam file directories, sample names, and exonic positions***Description**

Gets bam file directories, sample names from .txt file, and exonic positions from .bed file.

**Usage**

```
getbambed(bamdir, bedFile, sampname, projectname, chr)
```

**Arguments**

|             |                                                                                                                                          |
|-------------|------------------------------------------------------------------------------------------------------------------------------------------|
| bamdir      | Column vector. Each line specifies directory of a bam file. Should be in same order as sample names in sampname.                         |
| bedFile     | Path to bed file specifying exonic targets. Is of type character.                                                                        |
| sampname    | Column vector. Each line specifies name of a sample corresponding to the bam file. Should be in same order as bam directories in bamdir. |
| projectname | String specifying the name of the project. Data will be saved using this as prefix.                                                      |
| chr         | Chromosome.                                                                                                                              |

**Value**

|             |                                            |
|-------------|--------------------------------------------|
| bamdir      | Bam directories                            |
| sampname    | Sample names                               |
| ref         | IRanges object specifying exonic positions |
| projectname | String specifying the name of the project. |
| chr         | Chromosome                                 |

**Author(s)**

Yuchao Jiang <yuchaoj@wharton.upenn.edu>

**References**

Lawrence M, Huber W, Pages H, Aboyoun P, Carlson M, Gentleman R, Morgan M and Carey V (2013). "Software for Computing and Annotating Genomic Ranges." PLoS Computational Biology, 9.

**See Also**

[getcoverage](#)

**Examples**

```
library(WES.1KG.WUGSC)
dirPath <- system.file("extdata", package = "WES.1KG.WUGSC")
bamFile <- list.files(dirPath, pattern = '*.bam$')
bamdir <- file.path(dirPath, bamFile)
sampsnameFile <- file.path(dirPath, "sampsname")
sampsname <- as.matrix(read.table(sampsnameFile))
chr <- 22
bambedObj <- getbambed(bamdir = bamdir, bedFile = file.path(dirPath,
  "chr22_400_to_500.bed"), sampsname = sampsname,
  projectname = "CODEX_demo", chr)
bamdir <- bambedObj$bamdir
sampsname <- bambedObj$sampsname
ref <- bambedObj$ref
projectname <- bambedObj$projectname
chr <- bambedObj$chr
```

---

getcoverage

*Get depth of coverage from whole exome sequencing*

---

**Description**

Gets depth of coverage for each exon across all samples from whole exome sequencing files.

**Usage**

```
getcoverage(bambedObj, mapqthres)
```

**Arguments**

|           |                                                |
|-----------|------------------------------------------------|
| bambedObj | Object returned from <a href="#">getbambed</a> |
| mapqthres | Mapping quality threshold hold of reads.       |

**Value**

|            |                                       |
|------------|---------------------------------------|
| Y          | Read depth matrix                     |
| readlength | Vector of read length for each sample |

**Author(s)**

Yuchao Jiang <yuchaoj@wharton.upenn.edu>

**See Also**

[getbambed](#)

**Examples**

```
library(WES.1KG.WUGSC)
dirPath <- system.file("extdata", package = "WES.1KG.WUGSC")
bamFile <- list.files(dirPath, pattern = '*.bam$')
bamdir <- file.path(dirPath, bamFile)
samnameFile <- file.path(dirPath, "samname")
samname <- as.matrix(read.table(samnameFile))
chr <- 22
bambedObj <- getbambed(bamdir = bamdir, bedFile = file.path(dirPath,
  "chr22_400_to_500.bed"), samname = samname,
  projectname = "CODEX_demo", chr)
bamdir <- bambedObj$bamdir
samname <- bambedObj$samname
ref <- bambedObj$ref
projectname <- bambedObj$projectname
chr <- bambedObj$chr
coverageObj <- getcoverage(bambedObj, mapqthres = 20)
Y <- coverageObj$Y
readlength <- coverageObj$readlength
```

---

getgc

*Get GC content for each exonic target*


---

**Description**

Computes GC content for each exon. Will be later used in QC procedure and normalization.

**Usage**

```
getgc(chr, ref)
```

**Arguments**

|     |                                                        |
|-----|--------------------------------------------------------|
| chr | Chromosome returned from <a href="#">getbambed</a>     |
| ref | IRanges object returned from <a href="#">getbambed</a> |

**Value**

Vector of GC content for each exon.

**Author(s)**

Yuchao Jiang <yuchaoj@wharton.upenn.edu>

**References**

Team TBD. BSgenome.Hsapiens.UCSC.hg19: Full genome sequences for Homo sapiens (UCSC version hg19). R package version 1.3.99.

**See Also**

[getbambed](#), [qc](#), [normalize](#)

**Examples**

```
ref <- IRanges(st = 51207851, end = 51207982)
gc <- getgc(chr = 22, ref)
```

---

getmapp

*Get mappability for each exonic target*

---

**Description**

Computes mappability for each exon. To save running time, take values from pre-computed results. Will be later used in QC procedure.

**Usage**

```
getmapp(chr, ref)
```

**Arguments**

|     |                                                        |
|-----|--------------------------------------------------------|
| chr | Chromosome returned from <a href="#">getbambed</a>     |
| ref | IRanges object returned from <a href="#">getbambed</a> |

**Details**

To calculate the exonic mappability, we first construct consecutive reads of length 90 that are one base pair apart along the exon. The sequences are taken from the hg19 reference. We then find possible positions across the genome that the reads can map to allowing for two mismatches. We compute the mean of the probabilities that the overlapped reads map to the target places where they are generated and use this as the mappability of the exon.

**Value**

Vector of mappability for each exon.

**Author(s)**

Yuchao Jiang <yuchaoj@wharton.upenn.edu>



**See Also**[getbambed](#), [qc](#)**Examples**

```
ref <- IRanges(st = 51207851, end = 51207982)
mapp <- getmapp(chr = 22, ref)
```

---

|             |                                   |
|-------------|-----------------------------------|
| mappability | <i>Pre-computed mappabilities</i> |
|-------------|-----------------------------------|

---

**Description**

The results of pre-computed mappabilities to save running time.

**Usage**

```
data(mappability)
```

**Details**

Pre-computed mappabilities. Method used is detailed in [getmapp](#).

**Value**

List of length 24 with pre-computed mappability of each chromosome.

**Author(s)**

Yuchao Jiang <yuchaoj@wharton.upenn.edu>

**See Also**[getmapp](#)**Examples**

```
# mappability of chromosome 1
head(round(mappability[[1]], 2))
```

---

|          |                                              |
|----------|----------------------------------------------|
| mappDemo | <i>Demo data pre-stored for mappability.</i> |
|----------|----------------------------------------------|

---

**Description**

Pre-stored mappability data for demonstration purposes.

**Usage**

```
data(mappDemo)
```

**Details**

Pre-computed using whole exome sequencing data of 46 HapMap samples.

**Value**

mapp demo data (vector) pre-computed.

**Author(s)**

Yuchao Jiang <yuchaoj@wharton.upenn.edu>

**Examples**

```
head(round(mappDemo, 2))
```

---

|          |                                                                 |
|----------|-----------------------------------------------------------------|
| mapp_ref | <i>Position reference for pre-computed mappability results.</i> |
|----------|-----------------------------------------------------------------|

---

**Description**

List consisting of IRanges objects specifying exonic positions whose mappabilities are pre-computed across the genome.

**Usage**

```
data(mapp_ref)
```

**Details**

Genomic positions for pre-computed mappabilities. Method used is detailed in [getmapp](#).

**Value**

List of length 24 with genomic positions of pre-computed mappability of each chromosome.

**Author(s)**

Yuchao Jiang <yuchaoj@wharton.upenn.edu>

**See Also**[getmapp](#)**Examples**

```
# mappability exon reference of chromosome 1
mapp_ref[[1]]
```

---

|           |                                                                |
|-----------|----------------------------------------------------------------|
| normalize | <i>Normalization of read depth from whole exome sequencing</i> |
|-----------|----------------------------------------------------------------|

---

**Description**

Fits a Poisson log-linear model that normalizes the read depth data from whole exome sequencing. Includes terms that specifically remove biases due to GC content, exon capture and amplification efficiency, and latent systemic artifacts.

**Usage**

```
normalize(Y_qc, gc_qc, K)
```

**Arguments**

|       |                                                                                                                                                                                 |
|-------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Y_qc  | Read depth matrix after quality control procedure returned from <a href="#">qc</a>                                                                                              |
| gc_qc | Vector of GC content for each exon after quality control procedure returned from <a href="#">qc</a>                                                                             |
| K     | Number of latent Poisson factors. Can be an integer if optimal solution has been chosen or a vector of integers so that AIC, BIC, and RSS are computed for choice of optimal k. |

**Value**

|      |                                  |
|------|----------------------------------|
| Yhat | Normalized read depth matrix     |
| AIC  | AIC for model selection          |
| BIC  | BIC for model selection          |
| RSS  | RSS for model selection          |
| K    | Number of latent Poisson factors |

**Author(s)**

Yuchao Jiang <yuchaoj@wharton.upenn.edu>

**See Also**[qc](#), [choiceofK](#)

**Examples**

```

Y_qc <- qcObjDemo$Y_qc
gc_qc <- qcObjDemo$gc_qc
normObj <- normalize(Y_qc, gc_qc, K = 1:5)
Yhat <- normObj$Yhat
AIC <- normObj$AIC
BIC <- normObj$BIC
RSS <- normObj$RSS
K <- normObj$K

```

normalize2

*Normalization of read depth from whole exome sequencing under the case-control setting*

**Description**

Fits a Poisson log-linear model that normalizes the read depth data from whole exome sequencing. Includes terms that specifically remove biases due to GC content, exon capture and amplification efficiency, and latent systemic artifacts. If the WES is designed under case-control setting, CODEX estimates the exon-wise Poisson latent factor using only the read depths in the control cohort, and then computes the sample-wise latent factor terms for the case samples by regression.

**Usage**

```
normalize2(Y_qc, gc_qc, K, normal_index)
```

**Arguments**

|              |                                                                                                                                                                                 |
|--------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Y_qc         | Read depth matrix after quality control procedure returned from <a href="#">qc</a>                                                                                              |
| gc_qc        | Vector of GC content for each exon after quality control procedure returned from <a href="#">qc</a>                                                                             |
| K            | Number of latent Poisson factors. Can be an integer if optimal solution has been chosen or a vector of integers so that AIC, BIC, and RSS are computed for choice of optimal k. |
| normal_index | Indices of control samples.                                                                                                                                                     |

**Value**

|      |                                  |
|------|----------------------------------|
| Yhat | Normalized read depth matrix     |
| AIC  | AIC for model selection          |
| BIC  | BIC for model selection          |
| RSS  | RSS for model selection          |
| K    | Number of latent Poisson factors |

**Author(s)**

Yuchao Jiang <yuchaoj@wharton.upenn.edu>

**See Also**

[qc](#), [choiceofK](#)

**Examples**

```

Y_qc <- qcObjDemo$Y_qc
gc_qc <- qcObjDemo$gc_qc
normObj <- normalize2(Y_qc, gc_qc, K = 1:5, normal_index = seq(1, 45, 2))
Yhat <- normObj$Yhat
AIC <- normObj$AIC
BIC <- normObj$BIC
RSS <- normObj$RSS
K <- normObj$K

```

normObjDemo

*Demo data pre-stored for normObj.***Description**

Pre-stored normObj data for demonstration purposes.

**Usage**

```
data(normObjDemo)
```

**Details**

Pre-computed using whole exome sequencing data of 46 HapMap samples.

**Value**

normObj demo data (list) pre-computed.

**Author(s)**

Yuchao Jiang <yuchaoj@wharton.upenn.edu>

**Examples**

```

Yhat <- normObjDemo$Yhat
AIC <- normObjDemo$AIC
BIC <- normObjDemo$BIC
RSS <- normObjDemo$RSS
K <- normObjDemo$K

```

---

|    |                                                        |
|----|--------------------------------------------------------|
| qc | <i>Quality control procedure for depth of coverage</i> |
|----|--------------------------------------------------------|

---

### Description

Applies a quality control procedure to the depth of coverage matrix both sample-wise and exon-wise before normalization.

### Usage

```
qc(Y, sampname, chr, ref, mapp, gc, cov_thresh, length_thresh, mapp_thresh,
    gc_thresh)
```

### Arguments

|               |                                                                                                              |
|---------------|--------------------------------------------------------------------------------------------------------------|
| Y             | Original read depth matrix returned from <a href="#">getcoverage</a>                                         |
| sampname      | Vector of sample names returned from <a href="#">getbambed</a>                                               |
| chr           | Chromosome.                                                                                                  |
| ref           | IRanges object specifying exonic positions returned from <a href="#">getbambed</a>                           |
| mapp          | Vector of mappability for each exon returned from <a href="#">getmapp</a>                                    |
| gc            | Vector of GC content for each exon returned from <a href="#">getgc</a>                                       |
| cov_thresh    | Vector specifying the upper and lower bound of exonic median coverage threshold for QC. 20-4000 recommended. |
| length_thresh | Vector specifying the upper and lower bound of exonic length threshold for QC. 20-2000 recommended.          |
| mapp_thresh   | Scalar variable specifying exonic mappability threshold for QC. 0.9 recommended.                             |
| gc_thresh     | Vector specifying the upper and lower bound of exonic GC content threshold for QC. 20-80 recommended.        |

### Details

It is suggested that analysis by CODEX be carried out in a batch-wise fashion if multiple batches exist. CODEX further filters out exons that: have extremely low coverage—median read depth across all samples less than 20 or greater than 4000; are extremely short—less than 20 bp; are extremely hard to map—mappability less than 0.9; have extreme GC content—less than 20 or greater than 80. The above filtering thresholds are recommended and can be user-defined to be adapted to different sequencing protocols.

### Value

|             |                                                      |
|-------------|------------------------------------------------------|
| Y_qc        | Updated Y after QC                                   |
| sampname_qc | Updated sampname after QC                            |
| gc_qc       | Updated gc after QC                                  |
| mapp_qc     | Updated mapp after QC                                |
| ref_qc      | Updated ref after QC                                 |
| qcmat       | Matrix specifying results of exon-wise QC procedures |

**Author(s)**

Yuchao Jiang <yuchaoj@wharton.upenn.edu>

**See Also**

[getbambed](#), [getgc](#), [getmapp](#)

**Examples**

```
Y <- coverageObjDemo$Y
sampname <- bambedObjDemo$sampname
chr <- bambedObjDemo$chr
ref <- bambedObjDemo$ref
gc <- gcDemo
mapp <- mappDemo
cov_thresh <- c(20, 4000)
length_thresh <- c(20, 2000)
mapp_thresh <- 0.9
gc_thresh <- c(20, 80)
qcObj <- qc(Y, sampname, chr, ref, mapp, gc, cov_thresh, length_thresh,
  mapp_thresh, gc_thresh)
Y_qc <- qcObj$Y_qc
sampname_qc <- qcObj$sampname_qc
gc_qc <- qcObj$gc_qc
mapp_qc <- qcObj$mapp_qc
ref_qc <- qcObj$ref_qc
qcmat <- qcObj$qcmat
```

---

qcObjDemo

*Demo data pre-stored for qcObj.*

---

**Description**

Pre-stored qcObj data for demonstration purposes.

**Usage**

```
data(qcObjDemo)
```

**Details**

Pre-computed using whole exome sequencing data of 46 HapMap samples.

**Value**

qcObj demo data (list) pre-computed.

**Author(s)**

Yuchao Jiang <yuchaoj@wharton.upenn.edu>

## Examples

```
Y_qc <- qcObjDemo$Y_qc
samprname_qc <- qcObjDemo$samprname_qc
gc_qc <- qcObjDemo$gc_qc
mapp_qc <- qcObjDemo$mapp_qc
ref_qc <- qcObjDemo$ref_qc
```

---

segment

Recursive segmentation algorithm for CNV detection and genotyping

---

## Description

Recursive segmentation algorithm for CNV detection and genotyping, using normalized read depth from whole exome sequencing.

## Usage

```
segment(Y_qc, Yhat, optK, K, samprname_qc, ref_qc, chr, lmax, mode)
```

## Arguments

|              |                                                                                                                                                                                 |
|--------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Y_qc         | Raw read depth matrix after quality control procedure returned from <a href="#">qc</a>                                                                                          |
| Yhat         | Normalized read depth matrix returned from <a href="#">normalize</a>                                                                                                            |
| optK         | Optimal value K returned from <a href="#">choiceofK</a>                                                                                                                         |
| K            | Number of latent Poisson factors. Can be an integer if optimal solution has been chosen or a vector of integers so that AIC, BIC, and RSS are computed for choice of optimal k. |
| samprname_qc | Vector of sample names after quality control procedure returned from <a href="#">qc</a>                                                                                         |
| ref_qc       | IRanges object of genomic positions of each exon after quality control procedure returned from <a href="#">qc</a>                                                               |
| chr          | Chromosome number returned from <a href="#">getbambed</a>                                                                                                                       |
| lmax         | Maximum CNV length in number of exons returned.                                                                                                                                 |
| mode         | Can be either "integer" or "fraction", which respectively correspond to format of the returned copy numbers.                                                                    |

## Value

Final callset of CNVs with genotyping results.

## Author(s)

Yuchao Jiang <yuchaoj@wharton.upenn.edu>

## See Also

[normalize](#), [choiceofK](#)



**Examples**

```
Y_qc <- qcObjDemo$Y_qc
Yhat <- normObjDemo$Yhat
BIC <- normObjDemo$BIC
K <- normObjDemo$K
samprname_qc <- qcObjDemo$samprname_qc
ref_qc <- qcObjDemo$ref_qc
chr <- bambedObjDemo$chr
finalcall <- segment(Y_qc, Yhat, optK = K[which.max(BIC)], K = K, samprname_qc,
  ref_qc, chr, lmax = 200, mode = "integer")
finalcall
```

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