

# Package ‘Rbowtie2’

April 25, 2025

**Type** Package

**Title** An R Wrapper for Bowtie2 and AdapterRemoval

**Version** 2.15.0

**Date** 2021-10-5

**Description** This package provides an R wrapper of the popular bowtie2 sequencing reads aligner and AdapterRemoval, a convenient tool for rapid adapter trimming, identification, and read merging. The package contains wrapper functions that allow for genome indexing and alignment to those indexes. The package also allows for the creation of .bam files via Rsamtools.

**License** GPL (>= 3)

**Depends** R (>= 4.1.0)

**Encoding** UTF-8

**Suggests** knitr, testthat (>= 3.0.0), rmarkdown

**Imports** magrittr, Rsamtools

**SystemRequirements** C++11, GNU make, samtools

**Archs** x64

**RoxygenNote** 7.1.1

**biocViews** Sequencing, Alignment, Preprocessing

**VignetteBuilder** knitr

**Config/testthat/edition** 3

**git\_url** <https://git.bioconductor.org/packages/Rbowtie2>

**git\_branch** devel

**git\_last\_commit** 175c396

**git\_last\_commit\_date** 2025-04-15

**Repository** Bioconductor 3.22

**Date/Publication** 2025-04-24

**Author** Zheng Wei [aut, cre],  
Wei Zhang [aut]

**Maintainer** Zheng Wei <wzweizheng@qq.com>

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|             |                                      |
|-------------|--------------------------------------|
| .callbinary | <i>Make system call for binaries</i> |
|-------------|--------------------------------------|

---

## Description

Function that makes a system call for the bowtie binaries. Note it is not intended to be used outside of the package.

## Usage

```
.callbinary(
  bin1,
  args1,
  op = NULL,
  bin2 = NULL,
  args2 = NULL,
  path = NULL,
  lang = NULL
)
```

## Arguments

|       |                                                                                                                                                |
|-------|------------------------------------------------------------------------------------------------------------------------------------------------|
| bin1  | Character. The binary used for the system call.                                                                                                |
| args1 | Character. The arguments to pass to the binary.                                                                                                |
| op    | Character. Optional: Generally used if needed to pipe to another binary.                                                                       |
| bin2  | Character. Optional: Another binary that can be used in the system call. Generally the output of the first binary is piped to the this binary. |
| args2 | Character Optional: The arguments to pass to the second binary.                                                                                |
| path  | Character Optional: If passed to function, returns the path. Needed for Rsamtools to convert from sam to bam.                                  |
| lang  | Character. The interpreter used to execute the binary                                                                                          |

**Value**

The output of the system call or the path provided.

**Author(s)**

Zheng Wei

---

adapterremoval\_usage    *Print available arguments for adapterremoval*

---

**Description**

Print available arguments for adapterremoval. Note that some arguments to the adapterremoval are invalid if they are already handled as explicit function arguments.

**Usage**

adapterremoval\_usage()

**Value**

AdapterRemoval available arguments and their usage.

**Author(s)**

Zheng Wei

**References**

Schubert, Lindgreen, and Orlando (2016). AdapterRemoval v2: rapid adapter trimming, identification, and read merging. BMC Research Notes, 12;9(1):88.

**Examples**

adapterremoval\_usage()

---

adapterremoval\_version    *Print version information of adapterremoval*

---

**Description**

Print version information of adapterremoval

**Usage**

adapterremoval\_version()

**Value**

An invisible Integer of call status. The value is 0 when there is not any mistakes

**Author(s)**

Zheng Wei

**References**

Schubert, Lindgreen, and Orlando (2016). AdapterRemoval v2: rapid adapter trimming, identification, and read merging. BMC Research Notes, 12;9(1):88.

**Examples**

```
adapterremoval_version()
```

---

bowtie2

---

*Interface to bowtie2 of bowtie2-2.2.3*


---

**Description**

This function can be use to call wrapped bowtie2 binary.

**Usage**

```
bowtie2(
  bt2Index,
  samOutput,
  seq1,
  ...,
  seq2 = NULL,
  interleaved = FALSE,
  overwrite = FALSE
)
```

**Arguments**

|             |                                                                                                                                                                                                                                                      |
|-------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| bt2Index    | Character scalar. bowtie2 index files prefix: 'dir/basename' (minus trailing '.*.bt2' of 'dir/basename.*.bt2').                                                                                                                                      |
| samOutput   | Character scalar. A path to a SAM file used for the alignment output.                                                                                                                                                                                |
| seq1        | Character vector. For single-end sequencing, it contains sequence file paths. For paired-end sequencing, it can be file paths with #1 mates paired with file paths in seq2. And it can also be interleaved file paths when argument interleaved=TRUE |
| ...         | Additional arguments to be passed on to the binaries. See below for details.                                                                                                                                                                         |
| seq2        | Character vector. It contains file paths with #2 mates paired with file paths in seq1. For single-end sequencing files and interleaved paired-end sequencing files(argument interleaved=TRUE), it must be NULL.                                      |
| interleaved | Logical. Set TRUE when files are interleaved paired-end sequencing data.                                                                                                                                                                             |
| overwrite   | Logical. Force overwriting of existing files if setting TRUE.                                                                                                                                                                                        |

## Details

All additional arguments in ... are interpreted as additional parameters to be passed on to bowtie2. All of them should be Character or Numeric scalar. You can put all additional arguments in one Character (e.g. "--threads 8 --no-mixed") with white space splited just like command line, or put them in different Character (e.g. "--threads", "8", "--no-mixed"). Note that some arguments ("-x", "--interleaved", "-U", "-1", "-2", "-S") to the bowtie2 are invalid if they are already handled as explicit function arguments. See the output of `bowtie2_usage()` for details about available parameters.

## Value

An invisible Integer of call status. The value is 0 when there is not any mistakes Otherwise the value is non-zero.

## Author(s)

Zheng Wei

## References

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature methods*, 9(4), 357-359.

## Examples

```
td <- tempdir()
## Building a bowtie2 index
refs <- dir(system.file(package="Rbowtie2", "extdata", "bt2", "refs"),
full=TRUE)
bowtie2_build(references=refs, bt2Index=file.path(td, "lambda_virus"),
"--threads 4 --quiet", overwrite=TRUE)
## Alignments
reads_1 <- system.file(package="Rbowtie2", "extdata", "bt2", "reads",
"reads_1.fastq")
reads_2 <- system.file(package="Rbowtie2", "extdata", "bt2", "reads",
"reads_2.fastq")
if(file.exists(file.path(td, "lambda_virus.1.bt2"))){
  cmdout<-bowtie2(bt2Index = file.path(td, "lambda_virus"),
    samOutput = file.path(td, "result.sam"),
    seq1=reads_1, seq2=reads_2, overwrite=TRUE, "--threads 3");cmdout
  head(readLines(file.path(td, "result.sam")))
}
```

---

bowtie2-build

*Interface to bowtie2-2.4.4 build function*

---

## Description

This function can be use to call the bowtie2-build wrapper which wraps the bowtie2-build-s and the bowtie2-build-l binaries.

**Usage**

```
bowtie2_build(references, bt2Index, ..., overwrite = FALSE)
```

**Arguments**

|            |                                                                                                                                                                                    |
|------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| references | Character vector. The path to the files containing the references for which to build a bowtie index.                                                                               |
| bt2Index   | Character scalar. The path where the bowtie2 index files should be created. Include the basename of the index file at the end of the path (i.e. path_to_index_dir/index_basename). |
| ...        | Additional arguments to be passed on to the binaries. See below for details.                                                                                                       |
| overwrite  | Logical. Force overwriting of existing files if setting TRUE.                                                                                                                      |

**Details**

All additional arguments in ... are interpreted as additional parameters to be passed on to bowtie2\_build wrapper. All of them should be Character or Numeric scalar. You can put all additional arguments in one Character (e.g. "--threads 8 --quiet") with white space separation, or put them in different Character (e.g. "--threads","8","--quiet"). See the output of bowtie2\_build\_usage() for details about available parameters.

**Value**

An invisible Integer of call status. The value is 0 when there is not any mistakes Otherwise the value is non-zero.

**Author(s)**

Zheng Wei

**References**

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. Nature methods, 9(4), 357-359.

**Examples**

```
td <- tempdir()

## Building a bowtie2 index

refs <- dir(system.file(package="Rbowtie2", "extdata", "bt2", "refs"),
full=TRUE)

bowtie2_build(references=refs, bt2Index=file.path(td, "lambda_virus"),
"--threads 4 --quiet",overwrite=TRUE)

## Use additional arguments in another way

bowtie2_build(references=refs, bt2Index=file.path(td, "lambda_virus"),
"--threads",4,"--quiet",overwrite=TRUE)

## The function will print the output during the process without "--quiet" argument.
bowtie2_build(references=refs, bt2Index=file.path(td, "lambda_virus"),
overwrite=TRUE)
```

---

|                     |                                                                        |
|---------------------|------------------------------------------------------------------------|
| bowtie2_build_usage | <i>Print available arguments that can be passed to bowtie2_build()</i> |
|---------------------|------------------------------------------------------------------------|

---

**Description**

Calling bowtie2\_build\_usage() prints the available arguments that can be passed to the ... argument of the bowtie2\_build() function of the package. Note that some arguments are invalid if they are already handled as explicit function arguments.

**Usage**

```
bowtie2_build_usage()
```

**Value**

Information about available arguments that can be passed to bowtie2\_build()

**Author(s)**

Zheng Wei

**References**

Langmead B, Salzberg S. Fast gapped-read alignment with Bowtie 2. Nature Methods. 2012, 9:357-359.

**Examples**

```
bowtie2_build_usage()
```

---

|                  |                                                  |
|------------------|--------------------------------------------------|
| bowtie2_samtools | <i>Interface to bowtie2-2.4.4 align function</i> |
|------------------|--------------------------------------------------|

---

**Description**

This function can be use to call the bowtie2 wrapper which wraps the bowtie2-align-s and the bowtie2-align-l binaries.

**Usage**

```
bowtie2_samtools(  
  bt2Index,  
  output,  
  outputType = "sam",  
  seq1 = NULL,  
  seq2 = NULL,  
  bamFile = NULL,  
  ...,  
  interleaved = FALSE,  
  overwrite = FALSE  
)
```

**Arguments**

|             |                                                                                                                                                                                                                                                        |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| bt2Index    | Character scalar. The path where the bowtie2 index files are located. Include the basename of the index files at the end of the path (i.e. path_to_index_dir/index_basename). Do not include the bowtie2 index file extension (.bt2 or .bt2l)          |
| output      | Character scalar. The path where the alignment output file should be created. Include the basename of the alignment file at the end of the path (i.e. path_to_output_dir/output_basename). Do not include the alignment file extension (.sam or .bam). |
| outputType  | Character scalar. Specify the output alignment file type. Default is set to "sam" but can also be changed to "bam".                                                                                                                                    |
| seq1        | Character vector. For single-end sequencing, it contains sequence file paths. For paired-end sequencing, it can be file paths with #1 mates paired with file paths in seq2. And it can also be interleaved file paths when argument interleaved=TRUE.  |
| seq2        | Character vector. It contains file paths with #2 mates paired with file paths in seq1. For single-end sequencing files and interleaved paired-end sequencing files (argument interleaved=TRUE), it must be NULL.                                       |
| bamFile     | Character vector. A path to a bam file that contains unaligned reads. If a bam file is provided then seq1 and seq2 must be set to NULL                                                                                                                 |
| ...         | Additional arguments to be passed on to the bowtie2 wrapper. See below for details.                                                                                                                                                                    |
| interleaved | Logical. Set TRUE when files are interleaved paired-end sequencing data.                                                                                                                                                                               |
| overwrite   | Logical. Force overwriting of existing files if setting TRUE.                                                                                                                                                                                          |

**Details**

All additional arguments in ... are interpreted as additional parameters to be passed to bowtie2 wrapper. All of them should be Character or Numeric scalar. You can put all additional arguments in one Character (e.g. "--threads 8 --no-mixed") with white space separation, or put them in different Character (e.g. "--threads","8","--no-mixed"). Note that some arguments ("x","--interleaved","-U","-1","-2","-b","-S") are invalid if they are already handled as explicit function arguments. See the output of bowtie2\_usage() for details about available parameters.

**Value**

An invisible Integer of call status. The value is 0 when there is not any mistakes Otherwise the value is non-zero.

**Author(s)**

Zheng Wei

**References**

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature methods*, 9(4), 357-359.

**Examples**

```

td <- tempdir()

## Building a bowtie2 index
refs <- dir(system.file(package="Rbowtie2", "extdata", "bt2","refs"), full=TRUE)
bowtie2_build(references=refs, bt2Index=file.path(td, "lambda_virus"),"--threads 4 --quiet",overwrite=TRUE)

## Alignments
reads_1 <- system.file(package="Rbowtie2", "extdata", "bt2", "reads", "reads_1.fastq")
reads_2 <- system.file(package="Rbowtie2", "extdata", "bt2", "reads", "reads_2.fastq")

## Sam file created
bowtie2_samtools(bt2Index = file.path(td,"lambda_virus"), output = file.path(td,"example"), seq1 = reads_1,
seq2 = reads_2, overwrite = TRUE)

## Bam file created
bowtie2_samtools(bt2Index = file.path(td,"lambda_virus"), output = file.path(td,"example"), outputType = "bam",
seq1 = reads_1, seq2 = reads_2, overwrite = TRUE)

```

---

bowtie2\_usage

---

*Print available arguments that can be passed to bowtie2()*


---

**Description**

Calling bowtie2\_usage() prints the available arguments that can be passed to the ... argument of the bowtie2() function of the package. Note that some arguments are invalid if they are already handled as explicit function arguments.

**Usage**

```
bowtie2_usage()
```

**Value**

Information about available arguments that can be passed to bowtie2().

**Author(s)**

Zheng Wei

**References**

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. Nature methods, 9(4), 357-359.

**Examples**

```
bowtie2_usage()
```

---

|                 |                                                   |
|-----------------|---------------------------------------------------|
| bowtie2_version | <i>Print version information of bowtie2-2.4.4</i> |
|-----------------|---------------------------------------------------|

---

**Description**

Calling bowtie2\_version() prints the version information of the bowtie package used.

**Usage**

```
bowtie2_version()
```

**Value**

An invisible Integer of call status. The value is 0 when there is not any mistakes Otherwise the value is non-zero.

**Author(s)**

Zheng Wei

**References**

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. Nature methods, 9(4), 357-359.

**Examples**

```
bowtie2_version()
```

---

|                     |                                   |
|---------------------|-----------------------------------|
| checkFileCreateable | <i>Check if file is creatable</i> |
|---------------------|-----------------------------------|

---

**Description**

This is an internal function that is not meant to be used outside of the package. It determines whether the file at the end of the path can be created.

**Usage**

```
checkFileCreateable(filePath, argname, overwrite)
```

**Arguments**

|           |                                                    |
|-----------|----------------------------------------------------|
| filePath  | Character scalar. Path to file to be created.      |
| argname   | Character scalar. Name of the argument passed.     |
| overwrite | Logical Will warn user of overwriting if set TRUE. |

**Value**

Indicates whether the file can be created at the path given.

**Author(s)**

Zheng Wei

---

|                |                             |
|----------------|-----------------------------|
| checkFileExist | <i>Check if file exists</i> |
|----------------|-----------------------------|

---

**Description**

This is an internal function that is not meant to be used outside of the package. It determines whether a specific file exists at the end of the path given to the function.

**Usage**

```
checkFileExist(filePath, argname)
```

**Arguments**

|          |                                                |
|----------|------------------------------------------------|
| filePath | Character scalar. Path to file of interest.    |
| argname  | Character scalar. Name of the argument passed. |

**Value**

Stops the function if the file does not exist.

**Author(s)**

Zheng Wei

---

|                |                                     |
|----------------|-------------------------------------|
| checkIndexType | <i>Determine bowtie2 index type</i> |
|----------------|-------------------------------------|

---

**Description**

This is an internal function that is not meant to be used outside of the package. It determines whether the given index library comprises of small indexes (.bt2) or large indexes (.bt2l).

**Usage**

```
checkIndexType(filePath)
```

**Arguments**

|          |                                                                                                                                                                                                                                            |
|----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| filePath | Character scalar. The path where bowtie2 index files are located. Include the basename of the index files at the end of the path (i.e. path_to_index_dir/index_basename). Do not include the bowtie2 index file extension (.bt2 or .bt2l). |
|----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**Details**

The function first tries to determine whether path\_to\_index\_dir/index\_basename.1.bt2 exists and if it doesn't exist then it tries to determine whether path\_to\_index\_dir/index\_basename.1.bt2l exists. If neither of those files exist then there is an issue with the index files that must be addressed.

**Value**

Character scalar. Returns either "SMALL" if the .bt2 file is found, "LARGE" if the .bt2l file is found, or "ERROR" if neither the .bt2 nor .bt2l file is found.

---

|                |                             |
|----------------|-----------------------------|
| checkPathExist | <i>Check if path exists</i> |
|----------------|-----------------------------|

---

**Description**

This is an internal function that is not meant to be used outside of the package. It determines whether the path passed to the function exists.

**Usage**

```
checkPathExist(filePath, argname)
```

**Arguments**

|          |                                                |
|----------|------------------------------------------------|
| filePath | Character scalar. Path of interest.            |
| argname  | Character scalar. Name of the argument passed. |

**Value**

Stops the function if the path does not exist.

**Author(s)**

Zheng Wei

---

|                     |                                               |
|---------------------|-----------------------------------------------|
| checkSamtoolsExists | <i>Check if samtools exists on the system</i> |
|---------------------|-----------------------------------------------|

---

**Description**

This is an internal function that is not meant to be used outside of the package. It checks whether samtools exists on the system.

**Usage**

```
checkSamtoolsExists()
```

**Value**

Returns TRUE if samtools exists on the system, else FALSE

---

|                   |                                               |
|-------------------|-----------------------------------------------|
| identify_adapters | <i>identify adapters for paired-end reads</i> |
|-------------------|-----------------------------------------------|

---

## Description

This function can be use to call wrapped AdapterRemoval binary for adapters identifying.

## Usage

```
identify_adapters(file1, file2, ..., basename = NULL, overwrite = FALSE)
```

## Arguments

|           |                                                                                                                                                                                              |
|-----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| file1     | Character vector. It can be file paths with #1 mates paired with file paths in file2 And it can also be interleaved file paths when argument interleaved=TRUE                                |
| file2     | Character vector. It contains file paths with #2 mates paired with file paths in file1. For interleaved paired-end sequencing files(argument interleaved=TRUE),it must to be setted to NULL. |
| ...       | Additional arguments to be passed on to the binaries. See below for details.                                                                                                                 |
| basename  | Character. The outputfile path prefix. Default: your_output                                                                                                                                  |
| overwrite | Logical. Force overwriting of existing files if setting TRUE.                                                                                                                                |

## Details

All additional arguments in ... are interpreted as additional parameters to be passed on to identify\_adapters. All of them should be Character or Numeric scalar. You can put all additional arguments in one Character(e.g. "--threads 8") with white space splited just like command line, or put them in different Character(e.g. "--threads","8"). Note that some arguments("--identify-adapters", "--file1", "--file2", "--basename") to the identify\_adapters are invalid if they are already handled as explicit function arguments. See the output of adapterremoval\_usage() for details about available parameters.

## Value

An invisible Character vector of adapters for each mate.

## Author(s)

Zheng Wei

## References

Schubert, Lindgreen, and Orlando (2016). AdapterRemoval v2: rapid adapter trimming, identification, and read merging. BMC Research Notes, 12;9(1):88.

## Examples

```
td <- tempdir()
reads_1 <- system.file(package="Rbowtie2", "extdata", "adrm", "reads_1.fq")
reads_2 <- system.file(package="Rbowtie2", "extdata", "adrm", "reads_2.fq")
adapters <- identify_adapters(file1=reads_1,file2=reads_2,
  basename = file.path(td,"reads")
  , "--threads 2", overwrite=TRUE)
adapters
```

---

|                 |                                                      |
|-----------------|------------------------------------------------------|
| remove_adapters | <i>Interface to bowtie2 of adapterremoval-2.2.1a</i> |
|-----------------|------------------------------------------------------|

---

## Description

This function can be use to call wrapped AdapterRemoval binary.

## Usage

```
remove_adapters(
  file1,
  ...,
  adapter1 = NULL,
  output1 = NULL,
  file2 = NULL,
  adapter2 = NULL,
  output2 = NULL,
  basename = NULL,
  interleaved = FALSE,
  overwrite = FALSE
)
```

## Arguments

|          |                                                                                                                                                                                                                                                      |
|----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| file1    | Character vector. For single-end sequencing, it contains sequence file paths. For paired-end sequencing, it can be file paths with #1 mates paired with file paths in file2 And it can also be interleaved file paths when argument interleaved=TRUE |
| ...      | Additional arguments to be passed on to the binaries. See below for details.                                                                                                                                                                         |
| adapter1 | Character. It is an adapter sequence for file1. Default: AGATCGGAAGAG-CACACGTCTGAACTCCAGTCACNNNNNNATCTCGTATGCCGTCTTCTGCTTG                                                                                                                           |
| output1  | Character. The trimmed mate1 reads output file path for file1. Default: base-name.pair1.truncated (paired-end), basename.truncated (single-end), or base-name.paired.truncated (interleaved)                                                         |
| file2    | Character vector. It contains file paths with #2 mates paired with file paths in file1. For single-end sequencing files and interleaved paired-end sequencing files(argument interleaved=TRUE), it must be NULL.                                     |
| adapter2 | Character. It is an adapter sequence for file2. Default: AGATCGGAAGAGCGTCGT-GTAGGGAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT                                                                                                                                  |

|             |                                                                                                                                                                                   |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| output2     | Character. The trimmed mate2 reads output file path for file2. Default: BASE-NAME.pair2.truncated (only used in PE mode, but not if <code>--interleaved-output</code> is enabled) |
| basename    | Character. The outputfile path prefix. Default: <code>your_output</code>                                                                                                          |
| interleaved | Logical. Set TRUE when files are interleaved paired-end sequencing data.                                                                                                          |
| overwrite   | Logical. Force overwriting of existing files if setting TRUE.                                                                                                                     |

### Details

All additional arguments in ... are interpreted as additional parameters to be passed on to `remove_adapters`. All of them should be Character or Numeric scalar. You can put all additional arguments in one Character (e.g. `"--threads 8"`) with white space splited just like command line, or put them in different Character (e.g. `"--threads", "8"`). Note that some arguments (`"-file1", "-file2", "-adapter1", "-adapter2", "-output1", "-output2", "-basename", "-interleaved"`) to the `identify_adapters` are invalid if they are already handled as explicit function arguments. See the output of `adapterremoval_usage()` for details about available parameters.

### Value

An invisible Integer of call status. The value is 0 when there is not any mistake. Otherwise the value is non-zero.

### Author(s)

Zheng Wei

### References

Schubert, Lindgreen, and Orlando (2016). AdapterRemoval v2: rapid adapter trimming, identification, and read merging. BMC Research Notes, 12;9(1):88.

### Examples

```
td <- tempdir()

# Identify adapters
reads_1 <- system.file(package="Rbowtie2", "extdata", "adrm", "reads_1.fq")
reads_2 <- system.file(package="Rbowtie2", "extdata", "adrm", "reads_2.fq")
adapters <- identify_adapters(file1=reads_1, file2=reads_2,
  basename=file.path(td, "reads"), "--threads 3", overwrite=TRUE)

# Remove adapters
cmdout <- remove_adapters(file1=reads_1, file2=reads_2, adapter1 = adapters[1],
  adapter2 = adapters[2],
  output1=file.path(td, "reads_1.trimmed.fq"),
  output2=file.path(td, "reads_2.trimmed.fq"),
  basename=file.path(td, "reads.base"), overwrite=TRUE, "--threads 3");cmdout
```

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