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# **InSilicoSeq Documentation**

***Release 1.1.0***

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InSilicoSeq is a sequencing simulator producing realistic Illumina reads. Primarily intended for simulating metagenomic samples, it can also be used to produce sequencing data from a single genome.

InSilicoSeq is written in python, and use kernel density estimators to model the read quality of real sequencing data.

InSilicoSeq support substitution, insertion and deletion errors. If you don't have the use for insertion and deletion error a basic error model is provided.



## 1.1 Installing InSilicoSeq

### 1.1.1 Using pip

To install InSilicoSeq, type the following in your terminal:

```
pip install InSilicoSeq
```

It will install InSilicoSeq as well as the following dependencies:

- biopython
- numpy
- pysam
- scipy

### Advanced options

- Upgrading InSilicoSeq to the latest version:

```
pip install --upgrade InSilicoSeq
```

- If you don't have administration rights on your machine:

```
pip install --user InSilicoSeq
```

- if you wish to install InSilicoSeq at a custom location (i.e with a module system):

```
prefix="/path/to/install/prefix"  
pip install --install-option="--prefix=$prefix" InSilicoSeq
```

then add `$prefix/bin` to your `PATH`, and `$prefix/lib/pythonX.X/site-packages` to your `PYTHONPATH` (replacing `pythonX.X` with your python version)

## 1.1.2 Using docker

If you wish to use InSilicoSeq using docker

```
docker pull hadrieng/insilicoseq:1.0.1
```

To use InSilicoSeq with docker, you need to provide a *volume* to the `docker run` command. Given with the `-v` option, the volume is your way to exchanging data (in this case, your input and output files) with the docker container.

```
docker run -v /Users/hadrien/data:/mnt/data -it --rm \
  hadrieng/insilicoseq:1.0.1 iss generate \
  --genomes /mnt/data/genomes.fasta -m miseq \
  -o /mnt/data/reads
```

The above command will mount the local folder `/Users/hadrien/data` onto `/mnt/data` on the docker side. The output reads will be located in `/Users/hadrien/data` when InSilicoSeq has finished running.

## 1.2 Generating reads

InSilicoSeq comes with a set of pre-computed error models to allow the user to easily generate reads from the most popular Illumina instruments:

- HiSeq
- MiSeq
- NovaSeq

Per example generate 1 million MiSeq reads from a set of input genomes:

```
iss generate --genomes genomes.fasta --model miseq --output miseq_reads
```

This will create 2 fastq files, *miseq\_reads\_R1.fastq* and *miseq\_reads\_R2.fastq* in your current directory, as well as *miseq\_reads\_abundance.txt*, a tab-delimited file containing the abundance of each genomes.

If you have created your custom model, change `--model miseq` to your custom model file:

```
iss generate --genomes genomes.fasta --model model.npz --output model_reads
```

### 1.2.1 Required input files

By default, InSilicoSeq only requires 1 file in order to start generating reads: 1 (multi-)fasta files containing your input genome(s).

If you don't want to use a multi-fasta file or don't have one at hand but are equipped with an Internet connection, you can download random genomes from the ncbi:

```
iss generate --ncbi bacteria -u 10 --model miseq --output miseq_ncbi
```

or for bacteria and viruses:



```
iss generate -k bacteria viruses -u 10 4 --model miseq --output miseq_ncbi
```

In addition to the 2 fastq files and the abundance file, the downloaded genomes will be saved in *miseq\_ncbi\_genomes.fasta* in your current directory.

*Note: If possible, I recommend using InSilicoSeq with a fasta file as input. The eutils utilities from the ncbi can be slow and quirky.*

## 1.2.2 Abundance distribution

With default settings, the abundance of the input genomes is drawn from a log-normal distribution.

Alternatively, you can use other distributions with the `--abundance` parameter: *uniform*, *halfnormal*, *exponential* or *zero-inflated-lognormal*

If you wish to fine-tune the distribution of your genomes, InSilicoSeq also accepts an abundance file:

```
iss generate -g genomes.fasta --abundance_file abundance.txt -m HiSeq -o HiSeq_reads
```

Example abundance file for a multi-fasta containing 2 genomes: *genome\_A* and *genome\_B*.

```
genome_A    0.2
genome_B    0.8
```

For the abundance to make sense, the total abundance in your abundance file must equal 1.

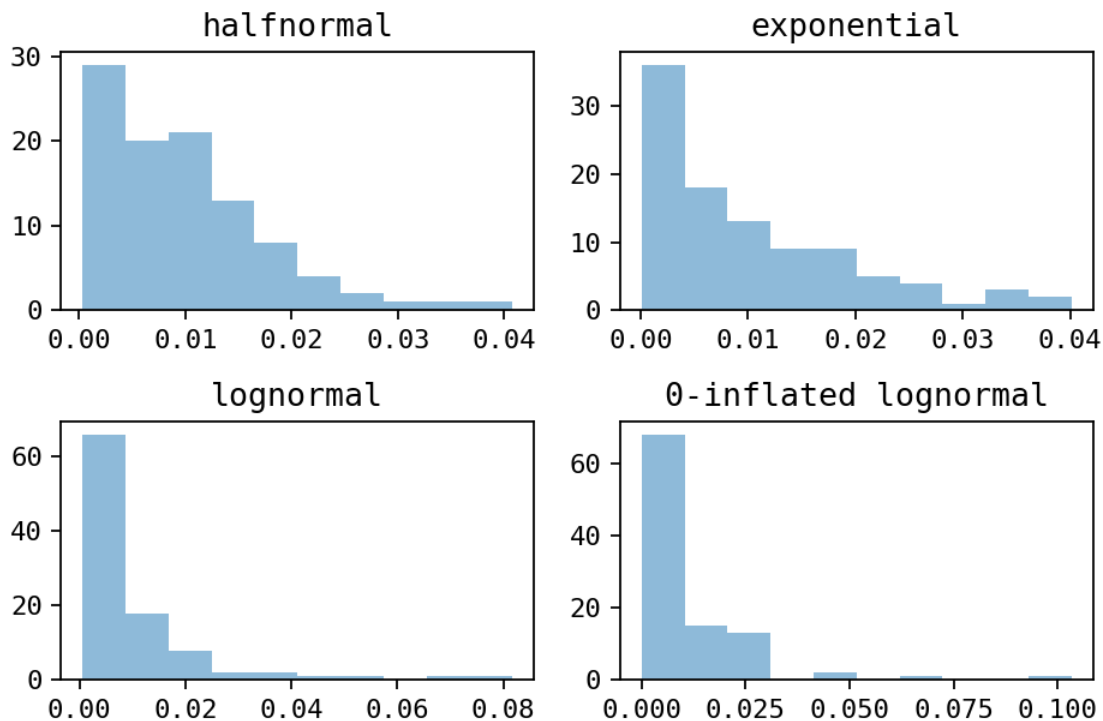


Fig. 1.1: Histograms of the different distribution (drawn with 100 samples)

### 1.2.3 Full list of options

#### **–genomes**

Input genome(s) from where the reads will originate

#### **–ncbi**

Download input genomes from RefSeq instead of using –genomes. Requires –n\_genomes option. Can be bacteria, viruses, archaea or a combination of the three (space-separated)

#### **–n\_genomes**

How many genomes will be downloaded from the ncbi. Required if –ncbi is set. If more than one kingdom is set with –ncbi, multiple values are necessary (space-separated).

#### **–abundance**

Abundance distribution (default: lognormal). Can be uniform, halfnormal, exponential, lognormal or zero\_inflated\_lognormal.

#### **–abundance\_file**

Abundance file for coverage calculations (default: None).

#### **–n\_reads**

Number of reads to generate (default: 1000000). Allows suffixes k, K, m, M, g and G (ex 0.5M for 500000).

#### **–mode**

Error model. If not specified, using kernel density estimation (default: kde). Can be ‘kde’ or ‘basic’

#### **–model**

Error model file. (default: None). Use HiSeq, NovaSeq or MiSeq for a pre-computed error model provided with the software, or a file generated with iss model. If you do not wish to use a model, use –mode basic. The name of the built-in models is case insensitive.

#### **–gc\_bias**

If set, may fail to sequence reads with abnormal GC content. Does not guarantee –n\_reads (default: False)

#### **–cpus**

Number of cpus to use. (default: 2).

**–quiet**

Disable info logging

**–debug**

Enable debug logging

**–output**

Output file prefix (Required)

## 1.3 Creating an Error Model

If you do not wish to use the pre-computed error models provided with InSilicoSeq, it is possible to create your own. InSilicoSeq creates error models from .bam files. The input bam file should be a set of reads aligned against a reference genome or metagenome.

Given you have two read files, *reads\_R1.fastq.gz* and ‘reads\_R2.fastq.gz’, and a reference metagenome *ref.fasta*:

### 1.3.1 Align you reads against the reference

```
bowtie2-build ref.fasta ref
bowtie2 -x ref -1 reads_R1.fastq.gz \
    -2 reads_R2.fastq.gz | samtools view -bS | samtools sort -o ref.bam
samtools index ref.bam
```

### 1.3.2 Build the model

```
iss model -b ref.bam -o my_model
```

which will create a *my\_model.npz* file containing your newly built model

### 1.3.3 Full list of options

**–bam**

aligned reads from which the model will be inferred (Required)

**–model**

Error model to build. If not specified, using kernel density estimation (default: kde). Can be ‘kde’ or ‘cdf’

**–output**

Output file prefix (Required)

**-quiet**

Disable info logging

**-debug**

Enable debug logging

## 1.4 iss package

### 1.4.1 Subpackages

#### iss.error\_models package

##### Submodules

##### iss.error\_models.basic module

**class** `iss.error_models.basic.BasicErrorModel`

Bases: `iss.error_models.ErrorModel`

Basic Error Model class

Basic error model. The phred scores are based on a normal distribution. Only substitutions errors occur. The substitution rate is assumed equal between all nucleotides.

**gen\_phred\_scores** (*mean\_quality, orientation*)

Generate a normal distribution, transform to phred scores

Generate a list of phred score according to a normal distribution centered around the ErrorModel quality

**Parameters** `mean_quality` (*int*) – mean phred score

**Returns** list of phred scores following a normal distribution

**Return type** list

**random\_insert\_size** ()

Fake random function returning the default insert size of the basic error model

**Returns** insert size

**Return type** int

##### iss.error\_models.cdf module

##### iss.error\_models.kde module

**class** `iss.error_models.kde.KDEErrorModel` (*npz\_path*)

Bases: `iss.error_models.ErrorModel`

KDEErrorModel class.

Error model based on an .npz files derived from read alignments. the npz file must contain:

- the length of the reads

- the mean insert size
- the size of mean sequence quality bins (for R1 and R2)
- **a cumulative distribution function of quality scores for each position** (for R1 and R2)
- the substitution for each nucleotide at each position (for R1 and R2)
- the insertion and deletion rates for each position (for R1 and R2)

**gen\_phred\_scores** (*cdfs, orientation*)

Generate a list of phred scores based on cdfs and mean bins

For each position, draw a phred score from the cdf and append to the phred score list

**Parameters**

- **cdfs** (*ndarray*) – array containing the cdfs
- **orientation** (*string*) – orientation of the read. Can be ‘forward’ or ‘reverse’

**Returns** a list of phred scores

**Return type** list

**random\_insert\_size** ()

Draw a random insert size from the insert size cdf

**Parameters** **i\_size\_cdf** – cumulative distribution function of the insert size

**Returns** an insert size

**Return type** int

## Module contents

**class** `iss.error_models.ErrorModel`

Bases: `object`

Main ErrorModel Class

This class is used to create inheriting classes and contains all the functions that are shared by all ErrorModel classes

**adjust\_seq\_length** (*mut\_seq, orientation, full\_sequence, bounds*)

Truncate or Extend reads to make them fit the read length

When insertions or deletions are introduced to the reads, their length will change. This function takes a (mutable) read and a reference sequence, and extend or truncate the read if it has had an insertion or a deletion

**Parameters**

- **mut\_seq** (*MutableSeq*) – a mutable sequence
- **orientation** (*string*) – orientation of the read. Can be ‘forward’ or ‘reverse’
- **full\_sequence** (*Seq*) – the reference sequence from which mut\_seq comes from
- **bounds** (*tuple*) – the position of the read in the full\_sequence

**Returns** a sequence fitting the ErrorModel

**Return type** Seq

**introduce\_error\_scores** (*record, orientation*)

Add phred scores to a SeqRecord according to the error\_model

**Parameters**

- **record** (*SeqRecord*) – a read record
- **orientation** (*string*) – orientation of the read. Can be ‘forward’ or ‘reverse’

**Returns** a read record with error scores

**Return type** SeqRecord

**introduce\_indels** (*record, orientation, full\_seq, bounds*)

Introduce insertions or deletions in a sequence

Introduce insertion and deletion errors according to the probabilities present in the indel choices list

**Parameters**

- **record** (*SeqRecord*) – a sequence record
- **orientation** (*string*) – orientation of the read. Can be ‘forward’ or ‘reverse’
- **full\_seq** (*Seq*) – the reference sequence from which mut\_seq comes from
- **bounds** (*tuple*) – the position of the read in the full\_sequence

**Returns** a sequence with (eventually) indels

**Return type** Seq

**load\_npz** (*npz\_path, model*)

load the error profile .npz file

**Parameters**

- **npz\_path** (*string*) – path to the npz file
- **model** (*string*) – type of model. Could be ‘cdf’ or ‘kde’. ‘cdf’ has been deprecated and is no longer available

**Returns**

**numpy object containg variables necessary** for error model construction

**Return type** ndarray

**logger**

**mut\_sequence** (*record, orientation*)

Introduce substitution errors to a sequence

If a random probability is higher than the probability of the basecall being correct, introduce a substitution error

**Parameters**

- **record** (*SeqRecord*) – a read record with error scores
- **orientation** (*string*) – orientation of the read. Can be ‘forward’ or ‘reverse’

**Returns** a sequence

**Return type** Seq

## 1.4.2 Submodules

### 1.4.3 iss.abundance module

`iss.abundance.exponential(record_list)`

**Generate scaled exponential abundance distribution from a number of records**

**Parameters** `record_list` (*list*) – a list of record.id

**Returns** a dictionary with records as keys, abundance as values

**Return type** dict

`iss.abundance.halfnormal(record_list)`

**Generate scaled halfnormal abundance distribution from a number of records**

**Parameters** `record_list` (*list*) – a list of record.id

**Returns** a dictionary with records as keys, abundance as values

**Return type** dict

`iss.abundance.lognormal(record_list)`

**Generate scaled lognormal abundance distribution from a number of records**

**Parameters** `record_list` (*list*) – a list of record.id

**Returns** a dictionary with records as keys, abundance as values

**Return type** dict

`iss.abundance.parse_abundance_file(abundance_file)`

Parse an abundance file

The abundance file is a flat file of the format “genome\_id<TAB>abundance”

**Parameters** `abundance_file` (*string*) – the path to the abundance file

**Returns** genome\_id as keys, abundance as values

**Return type** dict

`iss.abundance.to_coverage(total_n_reads, species_abundance, read_length, genome_size)`

Calculate the coverage of a genome in a metagenome given its size and abundance

**Parameters**

- **total\_n\_reads** (*int*) – total amount of reads in the dataset
- **species\_abundance** (*float*) – abundance of the species, between 0 and 1
- **read\_length** (*int*) – length of the reads in the dataset
- **genome\_size** (*int*) – size of the genome

**Returns** coverage of the genome

**Return type** float

`iss.abundance.to_file(abundance_dic, output)`

Write the abundance dictionary to a file

**Parameters**

- **abundance\_dic** (*dict*) – the abundance dictionary
- **output** (*str*) – the output file name

`iss.abundance.uniform(record_list)`

Generate uniform abundance distribution from a number of records

**Parameters** **record\_list** (*list*) – a list of record.id

**Returns** a dictionary with records as keys, abundance as values

**Return type** dict

`iss.abundance.zero_inflated_lognormal(record_list)`

Generate scaled zero-inflated lognormal abundance distribution from a number of records

**Parameters** **record\_list** (*list*) – a list of record.id

**Returns** a dictionary with records as keys, abundance as values

**Return type** dict

## 1.4.4 iss.app module

`iss.app.generate_reads(args)`

Main function for the *iss generate* submodule

**This submodule generates reads from an ErrorModel and write them to** `args.output + _R(1/2).fastq`

**Parameters** **args** (*object*) – the command-line arguments from argparse

`iss.app.main()`

`iss.app.model_from_bam(args)`

Main function for the *iss model* submodule

This submodule write all variables necessary for building an ErrorModel to `args.output + .npz`

**Parameters** **args** (*object*) – the command-line arguments from argparse

## 1.4.5 iss.bam module

`iss.bam.random()` → *x* in the interval [0, 1).

`iss.bam.read_bam(bam_file, n_reads=1000000)`

Bam file reader. Select random mapped reads from a bam file

**Parameters** **bam\_file** (*string*) – path to a bam file

**Yields** *read* – a pysam read object

`iss.bam.to_model(bam_path, output)`

from a bam file, write all variables needed for modelling reads in a .npz model file

**For a brief description of the variables that will be written to the** `output` file, see the `bam.write_to_file` function

**Parameters**



- **bam\_path** (*string*) – path to a bam file
- **output** (*string*) – prefix of the output file

`iss.bam.write_to_file(model, read_length, mean_f, mean_r, hist_f, hist_r, sub_f, sub_r, ins_f, ins_r, del_f, del_r, i_size, output)`

Write variables to a .npz file

#### Parameters

- **model** (*string*) – the type of error model
- **read\_length** (*int*) – read length of the dataset
- **mean\_f** (*list*) – list of mean bin sizes
- **mean\_r** (*list*) – list of mean bin sizes
- **hist\_f** (*list*) – list of cumulative distribution functions for the forward read quality
- **hist\_r** (*list*) – list of cumulative distribution functions for the reverse read quality
- **sub\_f** (*list*) – list of dictionaries representing the substitution probabilities for the forward reads
- **sub\_r** (*list*) – list of dictionaries representing the substitution probabilities for the reverse reads
- **ins\_f** (*list*) – list of dictionaries representing the insertion probabilities for the forward reads
- **ins\_r** (*list*) – list of dictionaries representing the insertion probabilities for the reverse reads
- **del\_f** (*list*) – list of dictionaries representing the deletion probabilities for the forward reads
- **del\_r** (*list*) – list of dictionaries representing the deletion probabilities for the reverse reads
- **i\_size** (*int*) – distribution of insert size for the aligned reads
- **output** (*string*) – prefix of the output file

### 1.4.6 iss.generator module

`iss.generator.cleanup(file_list)`

remove temporary files

**Parameters** **file\_list** (*list*) – a list of files to be removed

`iss.generator.concatenate(file_list, output)`

Concatenate fastq files together

Outputs two files: output\_R1.fastq and output\_R2.fastq

#### Parameters

- **file\_list** (*list*) – the list of input files prefix
- **output** (*string*) – the output files prefix

`iss.generator.reads(record, ErrorModel, n_pairs, cpu_number, output, gc_bias=False)`

Simulate reads from one genome (or sequence) according to an ErrorModel

This function makes use of the `simulate_read` function to simulate reads and save them in a fastq file

**Parameters**

- **record** (*SeqRecord*) – sequence or genome of reference
- **ErrorModel** (*ErrorModel*) – an ErrorModel
- **n\_pairs** (*int*) – the number of reads to generate
- **cpu\_number** (*int*) – an int identifying the cpu that is used by the function. Is used for naming the output file
- **output** (*str*) – the output file prefix
- **gc\_bias** (*bool*) – if set, the function may skip a read due to abnormal GC content

**Returns** the name of the output file

**Return type** *str*

`iss.generator.simulate_read(record, ErrorModel, i)`

From a read pair from one genome (or sequence) according to an ErrorModel

Each read is a SeqRecord object returns a tuple containing the forward and reverse read.

**Parameters**

- **record** (*SeqRecord*) – sequence or genome of reference
- **ErrorModel** (*ErrorModel*) – an ErrorModel class
- **i** (*int*) – a number identifying the read

**Returns** tuple containing a forward read and a reverse read

**Return type** *tuple*

`iss.generator.to_fastq(generator, output)`

Write reads to a fastq file

**Take a generator or a list containing read pairs (tuples) and write them** in two fastq files: `output_R1.fastq` and `output_R2.fastq`

**Parameters**

- **generator** (*generator*) – a read generator (or list)
- **output** (*string*) – the output files prefix

## 1.4.7 iss.modeller module

`iss.modeller.dispatch_indels(read)`

Return the x and y position of a insertion or deletion to be inserted in the indel matrix.

The substitution matrix is a 2D array of size 301 \* 9 The x axis (301) corresponds to the position in the read, while the y axis (9) represents the match or indel (see the dispatch dict in the function). Positions 0 is match or substitution, other positions in 'N1' are insertions, 'N2 are deletions'

The size of x axis is 301 because we haven't calculated the read length yet

**Parameters** **read** (*read*) – an aligned read object

**Yields** *tuple* – a tuple with the x, y position for dispatching the indel in the indel matrix

`iss.modeller.dispatch_subst (base, read, read_has_indels)`

Return the x and y position of a substitution to be inserted in the substitution matrix.

The substitution matrix is a 2D array of size 301 \* 16 The x axis (301) corresponds to the position in the read, while the y axis (16) represents the match or substitution (see the dispatch dict in the function). Positions 0, 4, 8 and 12 are matches, other positions are substitutions

The size of x axis is 301 because we haven't calculated the read length yet

#### Parameters

- **base** (*tuple*) – one base from an alignment object. According to the pysam documentation: an alignment is a list of tuples: aligned read (query) and reference positions. the parameter `with_seq` adds the ref sequence as the 3rd element of the tuples. substitutions are lower-case.
- **read** (*read*) – a read object, from which the alignment comes from
- **read\_has\_indels** (*bool*) – a boolean flag to keep track if the read has an indel or not

**Returns** x and y position for incrementing the substitution matrix and a third element: True if an indel has been detected, False otherwise

**Return type** *tuple*

`iss.modeller.divide_qualities_into_bins (qualities, n_bins=4)`

Divides the raw quality scores in bins according to the mean phred quality of the sequence they come from

#### Parameters

- **qualities** (*list*) – raw count of all the phred scores and mean sequence quality
- **n\_bins** (*int*) – number of bins to create (default: 4)

**Returns** a list of lists containing the binned quality scores

**Return type** *list*

`iss.modeller.indel_matrix_to_choices (indel_matrix, read_length)`

Transform an indel matrix into probabilities of indels for at every position

From the raw indel count at one position, returns a dictionary with probabilities of indel

#### Parameters

- **indel\_matrix** (*np.array*) – the substitution matrix is a 2D array of size `read_length * 16`. the x axis (`read_length`) corresponds to the position in the read, while the y axis (9) represents the match or indel. Positions 0 is match or substitution, other positions in 'N1' are insertions, 'N2' are deletions
- **read\_length** (*int*) – read length

**Returns** tuple containing two lists of dictionaries representing the insertion or deletion probabilities for a collection of reads

**Return type** *tuple*

`iss.modeller.insert_size (insert_size_distribution)`

Calculate cumulative distribution function from the raw insert size distributin. Uses 1D kernel density estimation.

#### Parameters

- **insert\_size\_distribution** (*list*) – list of insert sizes from aligned

- **pairs**(*read*) –

**Returns** a cumulative density function

**Return type** 1darray

`iss.modeller.quality_bins_to_histogram(bin_lists)`

Wrapper function to generate cdfs for each quality bins

Generate cumulative distribution functions for a number of mean quality bins

**Parameters**

- **bins\_lists**(*list*) – list of list containing raw count of all phred
- **scores** –

**Returns** a list of lists containg cumulative density functions

**Return type** list

`iss.modeller.raw_qualities_to_histogram(qualities)`

Approximate the distribution of base quality at each position in a read using a pseudo 2d kernel density estimation

Generate cumulative distribution functions

**Parameters** **qualities**(*list*) – raw count of all phred scores

**Returns**

**list of cumulative distribution functions. One cdf per base. The** list has the size of the read length

**Return type** list

`iss.modeller.subst_matrix_to_choices(substitution_matrix, read_length)`

Transform a substitution matrix into probabilities of substitutions for each base and at every position

From the raw mismatches at one position, returns a dictionary with probabilities of substitutions

**Parameters**

- **substitution\_matrix**(*np.array*) – the substitution matrix is a 2D array of size `read_length * 16`. the x axis (`read_length`) corresponds to the position in the read, while the y axis (16) represents the match or substitution. Positions 0, 4, 8 and 12 are matches, other positions are substitutions
- **read\_length**(*int*) – read length

**Returns**

**list of dictionaries representing** the substitution probabilities for a collection of reads

**Return type** list

### 1.4.8 iss.util module

`iss.util.convert_n_reads(unit)`

For strings representing a number of bases and ending with k, K, m, M, g, and G converts to a plain old number

**Parameters** **n**(*str*) – a string representing a number ending with a suffix

**Returns** a number of reads

**Return type** float

`iss.util.count_records(fasta_file)`  
 Count the number of records in a fasta file and return a list of records id

**Parameters** `fasta_file` (*string*) – the path to a fasta file

**Returns** a list of record ids

**Return type** list

`iss.util.genome_file_exists(filename)`  
 Checks if the output file from the `-ncbi` option already exists

**Parameters** `filename` (*str*) – a file name

`iss.util.nplog(type, flag)`

`iss.util.phred_to_prob(q)`  
 Convert a phred score (Sanger or modern Illumina) in probability

Given a phred score `q`, return the probability `p` of the call being right

**Parameters** `q` (*int*) – phred score

**Returns** probability of basecall being right

**Return type** float

`iss.util.prob_to_phred(p)`  
 Convert a probability into a phred score (Sanger or modern Illumina)

Given a probability `p` of the basecall being right, return the phred score `q`

**Parameters** `p` (*int*) – probability of basecall being right

**Returns** phred score

**Return type** int

`iss.util.reservoir(records, record_list, n=None)`  
 yield a number of records from a fasta file using reservoir sampling

**Parameters** `records` (*obj*) – fasta records from SeqIO.parse

**Yields** `record` (*obj*) – a fasta record

`iss.util.rev_comp(s)`  
 A simple reverse complement implementation working on strings

**Parameters** `s` (*string*) – a DNA sequence (IUPAC, can be ambiguous)

**Returns** reverse complement of the input sequence

**Return type** list

`iss.util.split_list(l, n_parts=1)`  
 Split a list in a number of parts

**Parameters**

- `l` (*list*) – a list
- `n_parts` (*in*) – the number of parts to split the list in

**Returns** a list of `n_parts` lists

**Return type** list

### 1.4.9 Module contents

## CHAPTER 2

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