

iAssembler

Introduction

iAssembler is a standalone package to assemble ESTs generated using Sanger and/or 454 pyrosequencing technologies into unigenes. The pipeline gives much higher accuracy in EST assembly by employing an iterative assembly strategy and automatic error corrections of mis-assemblies. iAssembler first performs iterative assemblies using [MIRA](#) and [CAP3](#) (default: four cycles of MIRA assembly followed by one CAP3 assembly) to correct assembly errors (mostly two similar sequences are not assembled) which occur in just one round of assembly. The program then performs post-assembly quality checking by 1) aligning each cDNA sequence to its corresponding unigene sequence to identify mis-assemblies; and 2) comparing unigene sequences against themselves to identify sequences from same genes that were not assembled together. The identified mis-assemblies are then corrected by the program automatically.

System requirement and dependencies

- Linux (required)
- Perl version 5.10.0 or higher (required). [Perl](#) was installed by default on most Linux systems
- BioPerl version 1.006 or higher (required). Please check <http://www.bioperl.org> and [wiki/Installing_BioPerl](#) for more details on installation of BioPerl.
- [NCBI BLAST package](#) (required). Provided in iAssembler.
- [MIRA assembly program](#) (required). Provided in iAssembler.
- [CAP3 assembly program](#) (required). Provided in iAssembler.

Installation

Installation of iAssembler is straightforward. Just [download](#) the appropriate version of iAssembler and uncompress the downloaded file.

```
shell$ tar -xzf iAssembler-1.0b.x32.tar.gz
```

This will generate a folder named "iAssembler-1.0b.x32" on a 32 bit machine or "iAssembler-1.0b.x64" on a 64 bit machine (we call this fold "iAssembler home folder"). iAssembler home folder includes two subfolders, a "bin" folder which contains all executables and a "doc" folder which contains the program documentation and the example configure file (see below). The home folder also contains a perl script, iAssembler.pl, which is the core script to run the whole iAssembler pipeline.

Run iAssembler

Quick Start

1. Put the EST sequence file in FASTA format (assuming the file name is **input_EST_seq**) into iAssembler home folder

2. Go to iAssembler home folder and run iAssembler with the following command

```
shell$ perl iAssembler.pl -i input_EST_seq
```

3. The program will generate an output folder named **input_EST_seq_output** which contains all the output files. See [below](#) for the description of the output files.

Parameters

(Note: Based on our experiences, the default settings of iAssembler program can achieve very high quality assemblies for most Sanger and/or 454 ESTs.)

Section 1: Input parameters

- i [String] Name of the input sequence file in FASTA format (*required*)
- z [String] Name of the parameter configuration file (default: none)
iAssembler allows users to put their preferred assembly parameters in a file so users do not need to type the same parameters every time they run the program. (see below for detail description of [iAssembler assembly parameters](#)). Here is how to create the parameter configuration file.
 1. Copy the example parameter configuration file (config.manually) from the "doc" folder to iAssembler home folder and change the parameters in the file accordingly.
 2. The command running iAssembler with the parameter configuration file will be:

```
shell$ perl iAssembler.pl -i input EST seq -z  
config.manually
```

Section 2: Assembly parameters

- b [String] BLAST program used for clustering and alignments of ESTs to their corresponding unigenes (megablast or blastn; default = megablast)
- a [Integer] number of CPUs used for blast program (default = 1)
- e [Integer] maximum length of end clips (0~100; default = 30)
- h [Integer] minimum overlap length (≥ 30 ; default = 30)
- x [Integer] minimum percent identity for sequence clustering (95~99; default = 97)
- p [Integer] minimum percent identify for sequence assembly (95~100; default = 95)

Section 3 : Output parameters

- u **[String]** prefix used for IDs of the assembled unigenes (default = UN)
iAssembler names the resulted unigenes with a prefix and trailing numbers, e.g., UN00001
- l **[Integer]** length of the trailing numbers in unigene IDs (\geq default; default = number characters of the maximum number assigned to unigenes)
For example, if the maximum trailing number assigned to the resulted unigenes is 5000, then the default of -l is 4 ('5000' has 4 characters). In this case users can set a number greater than or equal to 4.
- s **[Integer]** start number of unigene ID trailing number (≥ 1 ; default = 1)
- o **[String]** Name of the output directory (default = "input file name" + "_output")

Output files

iAssembler generates four files in the output directory.

1. unigene_seq

Unigene sequences (FASTA format) generated from the EST assembly process.

2. contig_member

A tab-delimited txt file containing unigenes and their corresponding EST members.

3. unigene_mp

A tab-delimited txt file containing the mapping details of EST members to their corresponding unigenes

EST ID	EST Length	Unigene ID	Unigene length	Query Start	Query End	Hit Start	Hit End	Strand	% Identity
EST0001	116	UN0001	1195	11	108	650	747	1	100.00

4. member_position_stat

A tab-delimited file containing the summary statistics of aligning ESTs to their corresponding unigenes.

Len/%ID	100-99	99-98	98-97	97-96	96-95	95-94	94-93	93-92	92-91	91-90	<90
000-100	0	0	0	0	0	0	0	0	0	0	0
100-200	614	123	68	13	2	1	1	0	0	0	0
200-300	1009	216	64	11	4	8	5	0	0	0	0
300-400	3546	493	134	17	10	2	1	0	0	0	0
400-500	2805	422	118	40	11	2	0	0	0	0	0
>500	222	26	6	3	3	0	0	0	0	0	0

Download

Current version of iAssembler is v1.0-beta. It's available for both 32 and 64 bit linux systems.

[iAssembler for 32-bit Linux systems](#) (v1.0-beta)

[iAssembler for 64-bit Linux systems](#) (v1.0-beta)

Contact

For questions and suggestions, please contact us at bioinfo@cornell.edu