



Tutorial: Prediction of Ribosomal RNA Genes Using RNAmmer Software



Introduction

3



Ribosomal RNA (rRNA) genes are known to be an integral part of ribosome synthesis machinery hence been studied extensively. Due to their repetitive nature, evolutionary conserveness, and ubiquitous distribution /omnipresence, these genes are playing a key role in varying functions and mechanisms including maintenance of genome integrity, control of cellular aging, and serving as standard phylogenetic markers in molecular taxonomic studies, etc. Considering their significance, several databases and computational tools have been developed to organize, annotate and analyze rRNA gene data. Of these, only RNAmmer accurately predicts the location of 5s/8s, 16s/18s, and 23s/28s rRNA in full genome sequences. It made predictions using Hidden Markov Model (HMM) and to design this tool training data sets from the 5S ribosomal RNA database and the European ribosomal RNA database project was used.

Tutorial

1. RNAmmer is freely available for academic users at the following link: [Download](#)
2. After filling out the form, you will receive an email with the download link.
3. Now put the ***.tar.gz** file in an appropriate folder. like **/home/user/tool/RNAMMER/**.
4. Run the following command to extract the files.

```
tar -xzf rnammer-1.2.src.tar.gz
```

5. RNAmmer dependencies:

It required the following three dependencies:

a. [Perl](#)

b. [Hmmer2](#)

c. [libxml-simple-perl](#)

6. After installation of the above dependencies, change **line 35** of **rnammer** script

Default line my \$INSTALL_PATH = "/usr/cbs/bio/src/rnammer-1.2"; *After changing* my \$INSTALL_PATH = "/home/user/tool/RNAMMER";

7. Now also change **line 50** of **rnammer** script:

Default line

```
$HMMSEARCH_BINARY = "/usr/cbs/bio/bin/linux64/hmmsearch";
```

After changing

```
$HMMSEARCH_BINARY = "/home/user/tool/HMMER/hmmsearch2";
```

1. Now **RNAmmer** can be used.
2. It requires the following arguments to predict rRNAs from the genome:

-S: This option is used to specify the kingdom of our genome whether it is bacteria, archaea, or eukaryotes. For bacteria, archaea, and eukaryotes we use “bac”, “arc” and “euk” respectively.

-m: Molecule type can be **tsu** for 5/8s rRNA, **ssu** for 16/18s rRNA, **lsu** for 23/28s rRNA, or any combination separated by a comma.

-multi: If you want to run it parallel then use the **-multi** argument.

-gff: Specifies a filename for output in GFF version 2 output.

-xml: Specifies a filename for output in XML output.

-h: hmmreport: is used to specify the filename for the output HMM report.

-f: Specifies filename for output FASTA sequence of predicted rRNA genes.

1. Now here are the commands to run **RNAmmer** for different kinds of organisms. Before that first export it in the **\$PATH** variable

```
export PATH="/home/user/tool/RNAMMER/:$PATH"
```

For Bacteria:

```
perl rnammer -S bac -m lsu,ssu,tsu -multi -gff ecoli.rrna.gff -xml ecoli.rrna.xr
```

For Archaea:

```
perl rnammer -S arc -m lsu,ssu,tsu -multi -gff A_pernix.rrna.gff -xml A_pernix.r
```

For Eukaryote:

```
perl rnammer -S euk -m lsu,ssu,tsu -multi -gff H_sapien.rrna.gff -xml H_sapien.r
```

1. After running this command, RNAmmer produces four files GFF file, XML file, HMM report file, and FASTA sequence.
 - a. GFF file (genomic feature format) containing the genomic coordinate information that describes the starting and end of the gene at which strand that gene is present and which subunit is coded by that gene. It also gives the alignment score for that particular gene.
 - b. FASTA file is containing the nucleotide sequence of the predicted rRNAs.

c. The HMM report file contains all statistical and detailed information of the search which is done by the HMMER program. It contains cutoff values, alignment detail information, E-value, and this kind of detail information.

d. In the XML file, the output is generated in the XML file format.

Critical Parameters/Troubleshooting

Problem 1

Can't open Perl script "rnammer": No such file or directory (or when you are not in the directory where rnammer is installed)

Reason:

Perl is not installed or you are not in the directory where RNAmmer is installed.

Solution:

Perl is not installed or you are not in the directory where RNAmmer is installed.

Problem 2

No output file generated

Reason:

Hmmer2 is not installed or Hmmer 3 is installed.

Solution:

RNAmmer use HMMER2, install HMMER2, and set the path accordingly in **rnammer** script.

Problem 3

```
Can't locate XML/Simple.pm in @INC (you may need to install the XML::Simple module)
BEGIN failed--compilation aborted at /home/user/Desktop/rnammer-1.2/xml2gff line
error converting xml into gff
```

Reason:

XML::Simple module is not installed

Solution:

Install the **XML::Simple** module

Problem 4

FATAL: Failed to open HMM file /home/user/Desktop/rnammer-1.2/lib/bac.tsu.rnammer.hmm

Reason:

lib folder is deleted.


Solution:


Download the RNAmmer package again and get the lib folder.

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11 weeks ago by [Dr.Animo](#) • 70

 This is great! But it looks like that link to RNAmmer is already broken. Is this software no longer available?
1

 Edit: Nevermind-I was able to get a new link from the creators. Note: There is a typo in the command for eukaryotes. It should be "-S euk" instead of "-S bac"

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 Thanks, I updated the post.

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[RNAmmer running](#) •

2.0 years ago by [kishorssf91](#) • 10

Dear, I am Kishor from Shanghai. Recently I have been trying to use RNAmmer. But yet to successfully run it. I have made two changes in the...

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I am using RNAmmer version 1.2, available after sign-up at <http://www.cbs.dtu.dk/services/RNAmmer/>, for download from an e-mailed link. I'...

[RNAmmer rna prediction](#) •

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High. I used the RNAmmer site to search for rRNA genes in a draft prokaryotic genome. RNAmmer found rRNA

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Hi, I am new to bioinformatics I used a SLURM script from a [website]
(<https://informatics.fas.harvard.edu/trinotate-workflow-example-...>

[Best way for rRNA prediction of Eukaryotic Genome\(~500mb\)](#) •
7.1 years ago by [mks002](#) ▲ 220

Dear all, I have used the online RNAmmer 1.2 Server for rRNA prediction which works for smaller genome. can anyone suggest me a other...

[how to chose one 16s rRNA from each genome](#) •
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I predicted all 16s rRNAs using rnammer. For each genome there is more than two 16s rRNAs.. So how to choose one 16s RNA for each genome fo...

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I have **raw paired-end reads** (not yet aligned) that may be bacteria/archaea but could also be eukaryote. I would like to determine their...

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4.3 years ago by [maricom](#) • 0

Hi all, I have a draft genome of bacterial gene and try to find out whether it has an operon for respective rRNA genes (5S, 23S, 16S). ...

[How to identify 16s sequences from binning data\(contigs\)?](#) •
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Hi everyone, I got some binning data and I want to retrieve 16s sequences from them. The binning data are supposed to be pure single genome...

[Best method to submit an annotated prokaryotic genome in NCBI](#) •
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What is the best method to submit prokaryotic genome assemblies along with annotations (mainly the predicted genes, tRNA and rRNA) into NCB...

[How To Annotate A Previously Unknown Bacterial Organism With Glimmer3?](#) •
updated 10.7 years ago by [Neilfws](#) ⚡ 49k • written 10.7 years ago by [hugh.winchester](#) • 0

Hi there! Firstly apologies it this seems really basic, but I've been set an assignment to annotate an previously unknown bacterial organ...

[Which Is An Acceptable Pipeline From Sequencing To Deep Annotation?](#) •
9.7 years ago by [Lluís R.](#) ★ 1.2k

I know there are several approach depending on which are your goals. But we can agree that all the genome projects have at least a sequenci...

[Installation issues with hmmer2](#) •
updated 20 months ago by [Ram](#) ⚡ 40k • written 9.0 years ago by [JV](#) ▲ 450

Hi everyone, I want to use rnammer with my favorite annotation pipeline prokka (as a supposedly more sensitive option than the default-t...

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Hey all, I'm working with a lot of data from NCBI and at the moment I'm kind of stuck. I have a ton of fasta files, either containing...

[How to get correct Taxonomy UID from NCBI Taxonomy using Entrez E-utilities efetch?](#) •
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**Basically the short form of my question is: how can I use Entrez esearch with STRICT search terms (so it gives me only the most appropri...

updated 11.1 years ago by [Pierre Lindenbaum](#) ⚡ 157k • written 11.1 years ago by [Burke](#) ▲ 290

I would like to analyze some metadata about a publication and I have a perl script that parses PubMed XML formatted files. However, I do no...

[How To Install BioPerl Without Root Privileges in Ubuntu/Linux](#) •

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[How To Extract Introns Positions And Domains From Entrez Gene Xml?](#) •

updated 11.6 years ago by [Joachim](#) ★ 2.9k • written 11.6 years ago by [Dror](#) ▲ 280

Entrez gene XML is very complicated, is there a way to extract all the genomic information (exon-introns positions) and the assigned protei...

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updated 11.2 years ago by [Matt Shirley](#) ⚡ 10k • written 11.2 years ago by [li_eunice2009](#) • 0

I am new to use Perl. My purpose is to convert one owl file into obo.step 1. I set up perl environment and I am sure that it can work now.s...

[perl orthoxml parser](#) •

6.8 years ago by [qqq2395](#) • 0

Hello I am looking for PERL parser for OMA orthoXML file. can XML parsers handle the orthoXML file? Thanks

[Prokka installation - compilation aborted](#) •

4 months ago by [eimanpharmacist](#) ▲ 20

Hi, I installed Prokka using this code posted at <https://github.com/tseemann/prokka> sudo apt-get install libdatetime-perl libxml...

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updated 10.1 years ago by [Biostar](#) ⚡ 20 • written 11.6 years ago by [Casual](#) ▲ 90

__Hi,guys~Need your help__. I got tens of thousands of protein seqs. Because they're from a rare organism and have too many seqs, DAVID and...

[question about MEME suite installation](#) •

updated 2.7 years ago by [Mensur Dlakic](#) ★ 25k • written 2.7 years ago by [13719756608](#) • 0

hello,everyone i was trying to install MEME suite,and when i do the "perl dependencies pl",the system response "Checking Required Perl Mod...

[Missing module error at Galaxy instance](#) •

19 months ago by [Ibrahim Tanyalcin](#) ★ 1.2k

I have a Galaxy installation at a cluster that makes use of default `python 2.7`. I can see that there are 2 symlinks called `python2` and ...

[How To Convert Xml Into A Decent Parseable Format?](#) •

updated 12.4 years ago by [Jrbeaman](#) ▲ 30 • written 12.4 years ago by [Lyco](#) ★ 2.3k

Please excuse the provocative title, I am well aware that for most of you out there XML is the best thing since sliced bread. However, I am...

[Problems with perl-xml-parser-2.44-4](#) •

updated 10 months ago by [Mensur Dlakic](#) ★ 25k • written 10 months ago by [Юлия](#) ▲ 10

Hello everyone! I tried to install this tool: <https://github.com/zhangrengang/SubPhaser> However, when I try to install needed packages fro...

[Biomart: Biomart Or Perl Api Or Xml Query](#) •

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Hi all, I have used biomaRt quite often in the past but mainly using a Perl API or R biomaRt package. I realize there is a shift to biomaR...

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