AnalysePrimer user manual

AnalysePrimer Version 1.0 user manual

Gerald Weber and Pâmella Miranda
gweberbh@gmail.com
Departamento de Física, Universidade Federal de Minas Gerais, Brazil

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1 Introduction

AnalysePrimer is a software package to evaluate DNA mismatches in primers and probes used in PCR- and LAMP-based methods and other techniques. It aligns the primer against the genomes of interest, calculates the melting temperature of perfect match and up to three consecutive mismatches and calculates their coverage [1]. The limit of three contiguous mismatches relates to the available melting temperature parameters [2], that is, alignments with four or more contiguous mismatches are considered as not aligned.

What will this software do for you? Given a set of primers/probes and genomes of interest, you will be able to align them and achieve all possible alignments in a perfect and mismatch way. Then, it will calculate the melting temperatures and the coverages for perfect match and up to three contiguous mismatches.

Evidently, this is work in progress. We hope to add new guidelines for mismatches in structural modifications of DNA/RNA and other considerations. Furthermore, there may be aspects of it which are not yet completely understood.

We would find it truly helpful indeed if you would let us know if this software is of any use to you. Showing a list of interested users to funding agencies often helps to secure the necessary resources to keeping such projects running. So, please, if you find this software useful let us know and if you use it for your scientific work please cite the appropriate papers which are listed at the end of this manual.

We wish you all the best in using AnalysePrimer

Gerald and Pâmella

Belo Horizonte, April 28, 2025

2 Installation

2.1 Download the software package

You can find the compiled binary files, for many Linux distributions, as well as source code, at this location:

https://bioinf.fisica.ufmg.br/software/

Browse to the desired version of the softwares and then download the appropriate packages for your Linux distribution. If your distribution is not covered then download the source code and compile manually, see below for instructions.

You need download and install the following software packages

1. `TfReg` (version 5.3 or higher)
2. `AlignPrimer` (version 1.0 or higher)
3. analyse_primers_lamp.tar.gz

The latter is a package of Bash and Perl scripts to carry AnalysePrimer out, examples of primer files and its documentation.

2.2 What will be installed?

Typically, there will be at least a binary executable file for TfReg and AlignPrimer

`/usr/bin/tfreg`

`/usr/bin/align_primer`

The documentation (which you are reading right now) is located at

http://bioinf.fisica.ufmg.br/software/analyse_primers_lamp.tar.gz

named analyseprimer-user-manual.pdf.

2.3 Specific instructions

All packages were fully tested for OpenSUSE 15.0 and 15.1.

TfReg and AlignPrimer Download the appropriate package for your system from

https://bioinf.fisica.ufmg.br/software/

for example if your system is 64bits, you may download the packages

`http://bioinf.fisica.ufmg.br/software/tfreg-6.0/openSUSE_Leap_42.3/TfReg-1.0-1.2.1.x86_64.rpm`

`http://bioinf.fisica.ufmg.br/software/AlignPrimer-1.0/openSUSE_Leap_42.3/AlignPrimer-1.0-4.1.x86_64.rpm`

note: version numbers may vary from this example. Then install

```
zypper install TfReg-1.0-1.2.1.x86_64.rpm
```

```
zypper install AlignPrimer-1.0-4.1.x86_64.rpm
```

analyse_primers_lamp Download the source package from webpage

http://bioinf.fisica.ufmg.br/software/analyse_primers_lamp.tar.gz

The package is called `analyse_primers_lamp.tar.gz`. You need to create a new folder or put the package in the folder of your choice. Unpacking the `tar` package

```
tar -xvf analyse_primers_lamp.tar.gz
```

The scripts and examples of primer files will be accessible.

2.4 If things go wrong

Most problems will come from missing library packages or from erroneous usage of your system. It is not possible for me to cover everything that may go wrong, so please feel free to contact me. Please include a detailed description of error messages, which system you are using and a step by step description of what you tried to do. Please understand that I will need as much information as possible. I can do nothing with messages saying simply "TfReg is not working on Ubuntu".

3 Genome and primer files

3.1 Download the genomes

Download the genomes of interest in FASTA format, which, usually, come in a single file. Split off the FASTA file into a single folder.

Some suggested databases are

- NCBI (https://www.ncbi.nlm.nih.gov/sars-cov-2/)
- GISAID (https://www.gisaid.org/)
- ENA (https://www.ebi.ac.uk/ena/browser/home)
- CNCB (https://bigd.big.ac.cn/ncov/release_genome).

3.1.1 Formatting the genome files

The FASTA files follow a pattern format (header + genome). However, each database constructs the header in a particular way. The NCBI and GISAID examples are shown below, respectively

```
>NC_045512.2 |Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome
ATTAAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGTAGATCT
GTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACT
CACGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATC
```

```
>hCoV-19/France/IDF-IPP03691/2021|EPI_ISL_1036400|2021-02-04
AGATCTGTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACTCACGCAGTATAATT
AATAACTAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATCTTCTGCAGGCTGCTTACGGTTTCGTCCGTGTTGC
AGCCGATCATCAGCACATCTAGGTTTTGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGA
```

where `NC_045512.2` and `EPI_ISL_1036400` are the accession code and are located in different positions. To carry AnalysePrimer out, the header of FASTA files should follow NCBI pattern: accession code, space and `|`. An example of GISAID file following our criterion

```
>EPI_ISL_1036400 |hCoV-19/France/IDF-IPP03691/2021|2021-02-04
AGATCTGTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACTCACGCAGTATAATT
AATAACTAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATCTTCTGCAGGCTGCTTACGGTTTCGTCCGTGTTGC
AGCCGATCATCAGCACATCTAGGTTTTGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGA
```

3.2 Formatting the primer files

The primer file should be in FASTA format and constructed as shown below

```
>N_Set1_F3
TGGCTACTACCGAAGAGCT
>N_Set1_B3
TGCAGCATTGTTAGCAGGAT
```

the FASTA identifiers will be used throughout the analysis. Examples of primer files in FASTA format can be found in the script package.

3.3 Formatting the accession code files

You will need a file with the list of accession codes. An example of how the file is constructed

```
LC528232.1
LC528233.1
MW079841.1
```

4 Carrying out the alignments

4.1 Algorithm basis

The primer is aligned against the genomes of interest using a Smith-Waterman algorithm [3]. Matching base pairs AT and CG is given score 2, mismatches score -1, and no gaps is considered. Alignments are carried out in two strand configurations. One for the genome sequences as obtained from the database and taking the primer sequence as complementary strand

$$5'-(\text{unmodified target genome sequence})-3'$$
$$3'-(\text{primer/probe})-5'$$

and the other by taking the complementary of the genome sequence

$$5'-(\text{complementary target genome sequence})-3'$$
$$3'-(\text{primer/probe})-5'$$

The alignments are carried out regardless if the primer/probe is identified as forward or reverse. In all cases the nominal directions of the primers are identified correctly.

4.2 Run the alignments

The alignments of primers against genomes are calculated by the software package *AlignPrimer*. To carry it out, you should use the helper script `align_primer.sh`. The command line to run it

```
./align_primer.sh genomes id-genomes.dat primer
```

where"genomes" is the folder in which are all genomes, "id-genomes.dat" is the file with genomes accession codes list and "primer" the name of primer file without the extension ".fasta". An example using the primer file `alves21.fasta`

```
./align_primer.sh ../genomes id-genomes.dat cdc
```

5 Evaluating the melting temperatures

5.1 Peyrard-Bishop Mesoscopic Model (PB)

This is the original Peyrard-Bishop model proposed in Ref. [4] which uses a Morse potential for modelling the hydrogen bonds

$$V_{\text{Morse}}(y_i) = D\left(e^{-y_i/\lambda} - 1\right)^2, \tag{5.1}$$

and the nearest-neighbour stacking interaction as a harmonic oscillator

$$w_{\text{harm.}}(y_i, y_{i-1}) = \frac{k}{2}\left(y_i - y_{i-1}\right)^2. \tag{5.2}$$

Because of the divergence of the partition function [5] we modified this to

$$w_{\text{harm.}}(y_i, y_{i-1}) = \frac{k}{2}\left(y_i^2 - 2y_i y_{i-1}\cos\theta + y_{i-1}^2\right), \tag{5.3}$$

Hamiltonian	model parameter	program parameter	units	type
$D\left(e^{-y_i/\lambda} - 1\right)^2$	D	`Morse.D`	eV	BP
	λ	`Morse.lambda`	Å	BP
$\frac{k}{2}\left(y_i^2 - 2y_i y_{i-1}\cos\theta + y_{i-1}^2\right)$	k	`harmonic.k`	eV/Å^2	NN
	θ	`harmonic.theta`	rad	NN

5.2 Calculating melting temperatures

Melting temperatures T_m are calculated from

$$T_m = a_0 + a_1\tau, \tag{5.4}$$

where τ is a statistical index calculated from the classical partition function of a model Hamiltonian, and a_0 and a_1 are regression coefficients. The index τ is calculated for each primer aligned against the genomes using the parameters reported in Ref. [2] for up to three consecutive mismatched base pairs. For a complete description of the melting temperature calculation and experimental conditions see Ref. [2, 6].

5.3 Run melting temperatures

To calculate the melting temperature of primers, you should run the script `calculate_primer_tm.pl`, which assumes perfect complementarity and is used as reference. An example

```
perl calculate_primer_tm.pl alves21
```

where the "alves21" is the primer file without the extension ".fasta" as used in the previous example.

After this, you should calculate the melting temperatures of all alignments, including those mismatched, using the script `calculate_alignments_tm.pl`

```
perl calculate_alignments_tm.pl id-genomes.dat alves21
```

where "alves21" is the primer file and "id-genomes.dat" is the same accession codes list used before.

6 Evaluating the coverage

6.1 Calculating the coverages

The coverage for a strictly non-mismatched alignment C_{strict} is calculated as

$$C_{\text{strict}} = \frac{N_G - N_{\text{n.a.}} - N_{\text{MM}}}{N_G} \tag{6.1}$$

where N_G is the total number of genomes, $N_{\text{n.a.}}$ the number of genomes for which no alignment was found, and N_{MM} the number of genomes for which a partial alignment with up to three consecutive mismatches was found. The coverage is calculated for genomes which are at least 25000 bp in size.

The difference between the melting temperature T_{MM} for each N_{MM} partial alignments taking into account the mismatches and the reference temperature $T_{\text{ref.}}$ is

$$\Delta T_{\text{MM}} = T_{\text{ref.}} - T_{\text{MM}} \tag{6.2}$$

The coverage for a partially mismatched alignment $C_{\text{part.}}$ is calculated as

$$C_{\text{part.}} = \frac{N_G - N_{\text{n.a.}} - N_{\text{low}}(\Delta T_{\text{lim.}})}{N_G} \tag{6.3}$$

where N_{low} is the number of primers satisfying

$$\Delta T_{\text{MM}} \leq \Delta T_{\text{lim.}} \tag{6.4}$$

For example, if you use $\Delta T_{\text{lim.}} = 5$ °C it will consider that mismatched primers with T_{MM} no more than 5 °C below the reference temperature $T_{\text{ref.}}$ are still acceptable.

6.2 Run coverages

To calculation the coverages, you should run the script `analyse_coverages.pl`

```
perl analyse_coverages.pl primer genomes tech temp
```

where “primer” is the primer file, “genomes” the folder containing genome files, “tech” is the technique analysed and “temp” the range of temperature in celsius ($\Delta T_{\text{lim.}}$). An example to run it

```
perl analyse_coverages.pl alves21 genomes-ncbi lamp 5
```

7 Resulting files

Alignments There will be four files resulting from alignments between primers and genomes from which three are non-empty. The first part of the filename is the identification of the genome FASTA files and the second part that of the primer file. An example using the primer file `alves21.fasta`

```
NC_045512.2-alves21.backtrace
NC_045512.2-alves21.dat
NC_045512.2-alves21.echo
NC_045512.2-alves21-tfreg.dat
```

where `NC_045512.2` is the genome accession code and `alves21` the primer filename.

The file `NC_045512.2-alves21.dat` shows all alignments found. An example

file of type genome-primer.dat

```
N_Set1_F3 NC_045512.2    19 29903 dir=D 1.00000 38 -nan 0.631579 0.631579 0.631579
dir=r
29212 (29213)

TTCTTCGGAATGTCGCGCA (genome 5'->3')
XX||XXXXXX|XXXXXX||
TCGAGAAGCCATCATCGGT (primer 3'->5')
```

where, in the first line, `N_Set1_F3` is the primer name, `NC_045512.2` is the genome accession code, `19` is the primer sequence lenght, `29903` is the genome sequence length and the rest of the line is the best alignment. In the example above, the second and third lines indicate the direction of alignment and the position in the genome. The direction code (`dir`) has the following convention

D genome is displayed 5′ to 3′ and primer on the next line is 3′ to 5′

r the complementary reverse of the genome is shown 5′ to 3′ and aligned to the primer shown 3′ to 5′

In the alignment, the `|` represents a perfect match and the `X` a mismatch. The file with extension `.echo` shows if the alignment was processed without errors. An example

file of type .echo

```
*** align_primer version=1120 system= builddate=2020-05-05T21:12:15-03:00
Used: -g=../../genomas-ncbi/NC_045512.2.fasta -o=alves21/NC_045512.2-alves21 -p=../primers-lamp/alves21.fasta
-tf=alves21
/NC_045512.2-alves21-tfreg.dat
  g = ../../genomas-ncbi/NC_045512.2.fasta (necessary and provided)
      genome fasta file name                                                    [string]
  o = alves21/NC_045512.2-alves21 (necessary and provided)
      output files basename                                                     [string]
  p = ../primers-lamp/alves21.fasta (necessary and provided)
      primer fasta file name                                                    [string]
 tf = alves21/NC_045512.2-alves21-tfreg.dat (optional and provided)
      output file for TfReg                                                     [string]
CPU user   : 2.995440s
CPU system : 0.43933s
Memory     : 17408KB (17MB)
```

In the example, it was processed without errors and shows the files used and created. The file ended in `-tfreg.dat` is a base file to the calculation of melting temperatures, see Chapter 5. An example

file of type tfreg.dat

```
temperatures #(1120) -g=../../genomas-ncbi/NC_045512.2.fasta -p=../primers-lamp/alves21.fasta
-o=alves21/NC_045512.2-alves21 -tf=alves21/NC_045512.2-alves21-tfreg.dat
TTCTTCGGAATGTCGCGCA TCGAGAAGCCATCATCGGT 60.0 50 0.5 #N_Set1_F3@NC_045512.2@29212 mm=14 lcm=6
algn=XX||XXXXXX|XXXXXX|| dir=r genome=5'->3' primer=3'->5'
AGCTCTTCGGTAGTAGCCA TCGAGAAGCCATCATCGGT 60.0 50 0.5 #N_Set1_F3@NC_045512.2@1360 mm=0 lcm=0
algn=|||||||||||||||||| dir=D complementary_genome=5'->3' primer=3'->5'
```

The first line shows informations about execution of the script (genome file, primer file, output name and file itself). The next lines show the alignment sequence, base values for melting temperature calculation, the primer name and the genome aligned, the total number of mismatches (`mm`) and maximum number of consecutive mismatches (`lcm`) and data from previous file (`NC_045512.2-alves21.dat`).

Melting temperature of primers An example of resulting file is `alves21-tm-primer.dat`, where "alves21" is the name of analysed primer

```
Main/Complementary alpha salt_concentration species_concentration temperature.measured temperature.adjusted
temperature.predicted enthalpy.measured enthalpy.adjusted enthalpy.predicted entropy.measured entropy.adjusted
entropy.predicted prediction_method thermal_equivalence thermal_index partition_function helmholtz_energy
TGGCTACTACCGAAGAGCT/ACCGATGATGGCTTCTCGA 4 50 0.5 60 60 61.6292 0 0 0 0 0 0 -1 12.908 3.59277 0 0 #N_Set1_F3
TGCAGCATTGTTAGCAGGAT/ACGTCGTAACAATCGTCCTA 4 50 0.5 60 60 62.5404 0 0 0 0 0 0 -1 13.165 3.62836 0 0 #N_Set1_B3
```

The first line is the header and next lines show the primer sequence, its complementary and the calculated melting temperature (`temperature.predicted`), in the example is `61.6292`.

Melting temperature of alignments The files of calculation of melting temperature to the alignments are similar to previous file. An example of file for strictly alignments is `alves21-res-can.dat` and for partially alignments `alves21-res-mm.dat`, where "alves21" is the name of analysed primer once more.

```
Main/Complementary alpha salt_concentration species_concentration temperature.measured temperature.adjusted
temperature.predicted enthalpy.measured enthalpy.adjusted enthalpy.predicted entropy.measured
entropy.adjusted entropy.predicted prediction_method thermal_equivalence thermal_index
partition_function helmholtz_energy
AGCTCTTCGGTAGTAGCCA/TCGAGAAGCCATCATCGGT 4 50 0.5 60 60 61.6292 0 0 0 0 0 0 -1 12.908 3.59277 0 0
#N_Set1_F3@LC528232.1@1356 mm=0 lcm=0 algn=|||||||||
||||||||| dir=D complementary_genome=5'->3' primer=3'->5'
ATCCTGCTAACAATGCTGCA/TAGGACGATTGTTACGACGT 4 50 0.5 60 60 62.5404 0 0 0 0 0 0 -1 13.165 3.62836 0 0
#N_Set1_B3@LC528232.1@28724 mm=0 lcm=0 algn=||||||
|||||||||||||| dir=r genome=5'->3' primer=3'->5'
```

The example above is to partially alignments (`res-mm`). For strictly alignments (`res-can`), the main difference is both `mm=` and `lcm=` will be always `0` (zero).

Coverages The resulting files for the calculation of coverages are three

```
alves21-genomes-ncbi-analysis.txt
alves21-genomes-ncbi-not-aligned.txt
alves21-genomes-ncbi-summary.txt
```

An example of the first one

file of type analysis.txt

```
primer=alves21 genomes=genomas-ncbi
total genome files=21665 files larger than 25000=21665
Lowest probe RdRp_F2 Tm=56.6261
E_Set1_B1c Tm=62.5157
genomes=21665 aligned=21537 no-alignment=128
(delta match) mm=0 genomes=21482
(delta match) perfect alignment (21482) =21482 coverage=99.1553196399723%
(delta Tm) Too low=55
(delta Tm) no-alignment (128) + too low (55) =183 coverage=99.1553196399723%
(delta probe) mm=0 genomes=21482
(delta probe) mm=1 genomes=1 MT966086.1(57.3613 -0.735199999999999 D 1)
(delta probe) Too low=54
(delta probe) no-alignment or too low=182 coverage=99.1599353796446%
```

where first line shows the primer and genome filenames, second one the number of files and third one is the primer with the lowest T_m. This resulting file lists each primer and shows how many genomes the primer aligns with. Carrying on, it shows the number of mismatches (`mm`), the total number of aligned genomes (`genomes`) and the coverage of them. For strictly alignments is `(delta match)`, for alignments with $\Delta T_{\text{lim.}}$ below of T_m of perfect alignment of the primer is `(delta Tm)` and alignments with $\Delta T_{\text{lim.}}$ below of T_m of the lowest probe is `(delta probe)`. When the genomes are listed

```
(delta Tm) mm=1 genomes=9 LR877996.1(67.1292 2.05670000000001 D 1),LR880588.1(66.6753 2.51060000000001 D 1),
LR880634.1(66.6753 2.51060000000001 D 1),LR882323.1(66.6753 2.51060000000001 D 1),
LR882471.1(66.6753 2.51060000000001 D 1),MT293160.1(67.1292 2.05670000000001 D 1),MT345857.1(67.1292 2.05670
000000001 D 1),MT558660.1(67.1292 2.05670000000001 D 1),MT873316.1(68.1775 1.00840000000001 D 1)
```

where `67.1292` is the T_m, `2.05670000000001` the difference between T_m of perfect match of primer and the alignment and `r` the direction of the alignment. The file `alves21-genomes-ncbi-not-aligned.txt` lists the genomes not aligned to the primer and with T_m too low. An example

file of type not-aligned.txt

```
E_Set1_B1c (no-alignment): LR814001.2,LR814006.2,LR814021.2,LR814034.2,LR814040.3,LR814043.2,LR814048.3,
LR814118.1,LR814122.1,LR814136.1,LR814137.1
E_Set1_B1c (delta Tm miss): LR877821.1(52.2201 10.2956 D 1),MT512443.1(52.2201 10.2956 D 1),
MT611451.1(50.2041 12.3116 D 1),MT627747.1(52.2201 10.2956 D 1)
E_Set1_B1c (delta probe miss): LR877821.1(52.2201 10.2956 D 1),MT512443.1(52.2201 10.2956 D 1),
MT611451.1(50.2041 12.3116 D 1),MT627747.1(52.2201 10.2956 D 1)
```

At the end of the file are listed the genomes not covered by any primer and genomes.

```
(delta Tm) LR822003.1 not covered by any primer
(delta probe) LR814006.2 not covered by any primer
(delta Tm) LC542809.1 96.875% primer coverage E_Set1_B1c,E_Set1_B2,E_Set1_B3,E_Set1_F1c,E_Set1_F2,E_Set1_LB,
E_Set1_LF,N_Set1_B1c,N_Set1_B2,N_Set1_B3,N_Set1_F1c,N_Set1_F2,N_Set1_F3,N_Set1_LB,N_Set1_LF,N_Set2_B1c,
N_Set2_B2,N_Set2_B3,N_Set2_F1c,N_Set2_F2,N_Set2_F3,N_Set2_LB,N_Set2_LF,
RdRp_B1c,RdRp_B2,RdRp_B3,RdRp_F1c,RdRp_F2,RdRp_F3,RdRp_LB,RdRp_LF
(delta probe) MW079834.1 96.875% primer coverage E_Set1_B1c,E_Set1_B2,E_Set1_B3,E_Set1_F1c,E_Set1_F2,E_Set1_F3,
E_Set1_LB,E_Set1_LF,N_Set1_B1c,N_Set1_B2,N_Set1_B3,N_Set1_F1c,N_Set1_F2,N_Set1_F3,N_Set1_LB,N_Set1_LF,N_Set2_B1c,
N_Set2_B2,N_Set2_B3,N_Set2_F1c,N_Set2_F2,N_Set2_F3,N_Set2_LB,N_Set2_LF,RdRp_B1c,RdRp_B2,RdRp_B3,RdRp_F1c,
RdRp_F2,RdRp_F3,RdRp_LB
```

The last file `alves21-genomes-ncbi-summary.txt` shows the summary of the analysis and coverages.

file of type summary.txt

```
primer set=alves21medrxiv, genome set=genomas-ncbi
Highest probe Tm E_Set1_LB Tm=70.569
Lowest probe Tm RdRp_F2 Tm=56.6261
Genomes tested=21665
ID Tm Coverage1(abs) Coverage2(abs) Coverage3(abs)
E_Set1_B1c 62.5157 99.1553196399723 (21482) 99.1599353796446 (21483) 99.1553196399723 (21482)
0,3,25,0,0,0,0,4,1,0,1,6,4,0,11,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0
E_Set1_B2 59.6919 98.5414262635587 (21349) 98.5414262635587 (21349) 98.5414262635587 (21349)
0,0,4,0,1,0,0,1,0,0,0,0,2,148,29,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0
E_Set1_B3 59.4842 99.4738056773598 (21551) 99.4738056773598 (21551) 99.4738056773598 (21551)
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,1,0,0,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0
```

The first line is the identification of primer and genome files, second and third lines are the highest and lowest primer with its name and T_m and the fourth is the number of genomes analysed. Each primer is listed with its name, melting temperature, the partially coverage `(delta Tm)`, the probe coverage `(delta probe)` and the strictly coverage `(delta match)`. In parentheses, the number of alignments for each coverage. The numbers list in the end of the lines is the number of alignments with mismatches in each position of the primer.

8 Files included in the script package

Here we list the files included in the `analyse_primers_lamp` package.

8.1 Documentation

The documentation file with the guidelines to carry out the AnalysePrimer workflow.

`analyseprimer-user-manual.pdf`

8.2 Bash and Perl scripts

`align_primer.sh` Running the alignments.

`calculate_primer_tm.pl` Calculating melting temperature of primers.

`calculate_alignments_tm.pl` Calculating melting temperature of all alignments.

`analyse_coverages.pl` Calculating coverages.

8.3 Examples of primer files

8.3.1 LAMP primer files

Examples of LAMP primer files located in `analyse_primers_lamp/lamp-primers-examples`.

`alekseenko21.fasta` primer file from Ref. [7]

`alves21.fasta` primer file from Ref. [8]

`daneshnia20.fasta` primer file from Ref. [9]

`diego21.fasta` primer file from Ref. [10]

`ganguli20.fasta` primer file from Ref. [11]

`garciavenzor21.fasta` primer file from Ref. [12]

`huang20.fasta` primer file from Ref. [13]

`jang21.fasta` primer file from Ref. [14]

`ji21.fasta` primer file from Ref. [15]

`lalli20.fasta` primer file from Ref. [16]

`lamb20.fasta` primer file from Ref. [17]

`lau20b.fasta` primer file from Ref. [18]

`manzano20.fasta` primer file from Ref. [19]

`mautner20.fasta` primer file from Ref. [20]

`mohon20.fasta` primer file from Ref. [21]

`park20.fasta` primer file from Ref. [22]

`reynes21.fasta` primer file from Ref. [23]

`tran20biorxiv.fasta` primer file from Ref. [24]

`wang20biorxiv.fasta` primer file from Ref. [25]

`yan20.fasta` primer file from Ref. [26]

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`zhang20b.fasta` primer file from Ref. [28]

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