IThOS

User Guide : version 2.2

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1. Introduction

IThOS is a software package dedicated to the design of primers. The input is a list of one or several DNA sequences (FASTA files) and the output is a list of primers fulfilling a list of criteria set by the user. Given a set of primers and a genome, IThOS can also determine putative hybridization sites.

IThOS works as a two-step procedure:

1. Primer design
2. Verification of hybridization sites

Each step relies on a specific program:

1 : `ithos_gen <genome> <param> <primers> [-c] [-s]`
2 : `ithos_chk <genome> <primers> <param> <pr_out> <pr_hyb> <pr_dic> <pr_pos>`

Both programs can be used separately and independently. A third program completes the package and enables the visualization of primer features:

- `ithos_viz <primers> <param>`

Parameters of the different programs are:

`<genome>` File containing one or several DNA sequences, FASTA format.

`<param>` File specifying criteria for primer selection.

`<primers>` Files containing a list of primers, FASTA format.
`<pr_in>`
`<pr_out>`
`<pr_hyb>`

`[-c]` Option enabling the search of primers on the complementary strand.

`[-s]` Option to get primers sorted by position on the genome

`<pr_dic>` Dictionary of primers

`<pr_pos>` Position of hybridizations

2. Generating primers: ithos_gen

Starting from one or several DNA sequences, ithos_gen generates primers fulfilling a list of criteria set by the user. ithos_gen considers all possible words having a size defined by the user. For each word, a suite of filters is applied. All words passing successfully through the filters are proposed as primer candidates. Each filter can be customized by the user to tune the primer selection according to the application. Six filters are implemented. They are described in the next paragraphs.

Filter 1: G+C %

For a primer of size T, filter 1 works as follows:

- Counts the number of G and C nucleotides (# GC)
- Calculates the percentage ➡ P = (# GC * 100) / T
- Discard the primer if: P < pcGCMin **or** P > pcGCMax

The default values are:

- pcGCMin = 40 [minimum percentage of GC]
- pcGCMax = 60 [maximum percentage of GC]

Filter 2: Tm (*Melting temperature*)

The nearest neighbor method is used to calculate the melting temperature of the primers (Santa Lucia et al., 1998). The method also takes into account the concentration of nucleotides (dnaConc) and the concentration of salt (saltConc).

This filter removes primers if:

- Tm < oligoTmMin
- Tm > oligoTmMax

The default values are:

- oligoTmMin = 57 °C [minimum Tm]
- oligoTmMax = 62 °C [maximum Tm]
- dnaConc = 500 nM [nucleotides concentration]
- saltConc = 50 nM [salt concentration]

Bibliography:

A Unified View of Polymer, Dumbbell, and Oligonucleotide DNA Nearest-Neighbor Thermodynamics, John SantaLucia, *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 95, No. 4 (Feb. 17, 1998), pp. 1460-1465

Filter 3: Number of repeats

This filter removes words with N consecutive identical nucleotides or dinucleotides (nbRepeat). For example, if nbRepeat = 4, the following primers will be removed:

1: GGGATGGACACGGA**TTTT**GGACCAGC
2: TTAGC**TATATATA**GGCAGGGATTAGG

The first primer presents a suit of 4 « T ». The second a suite of 4 « TA »

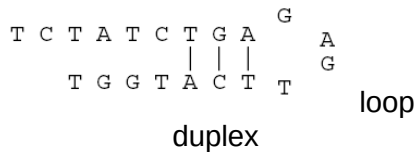
The default value is:

- nbRepeat = 5 [maximum number of repeats]

Filter 4: Hairpin

This filter removes words with hairpin loops that present the following features:

- hairpin duplex size ≥ maxHpDup
- hairpin loop size ≥ MaxHpLoop



The default values are:

- maxHpDup = 4 [maximum size of the hairpin duplex]
- maxHpLoop = 4 [maximum size of the hairpin loop]

Filter 5 : self-complementarity

This filter checks that primers must not auto hybridize during PCR. Primers that form a duplex with their complementary strand are removed. Criteria for selection are as follows:

- For the full length of the pirmer, authorized deltaG value must not exceed maxDeltaGAuto
- For the 3' end within a distance of sizeDeltaGAuto3p, authorized deltaG value must not exceed maxDeltaGAuto3p.
- The minimal size of a tested duplex is sizeDeltaGAuto

The default values are:

- maxDeltaGAuto = -10kcal/mol [max DeltaG for auto hybridization, full size]
- maxDeltaGAuto3p = -7kcal/mol [max DeltaG for auto hybridization, 3' extremity]
- sizeDeltaGAuto = 6 [min size for testing duplex]
- sizeDeltaGAuto3p = 8 [size of the 3' extremity]

Filtre 6 : thermodynamic stability at primer ends

This filter calculates deltaG on the 5' and 3' ends of the primers. The size of the 5' end to be considered is given by sizeExt5. The size of the 3' end to be considered is given by sizeExt3.

A primer is removed if:

- The value in 5' is above deltaG5
- The value in 3' is out of the interval [deltaG3Min,deltaG3Max]

The default values are:

- sizeExt5 = 5 [size of the 5' extremity to be considered]
- sizeExt3 = 5 [size of the 3' extremity to be considered]
- deltaG5 = - 4 kcal/mol [max deltaG value, 5' extremity]
- deltaG3Min = -6 kcal/mol [min deltaG value, 3' extremity]
- deltaG3Max = - 4 kcal/mol [max deltaG value, 3' extremity]

Software execution

The software is launched by the following command line:

```
ithos_gen <genome> <parameters> <primers> [-c] [-s]
```

input files

- `<genome>` is a text file (FASTA) that contains one or more DNA sequences.
- `<parameters>` is a text file that contains the parameters of the different filters

output files

- `<primers>` is a text file (FASTA format) that contains all the selected primers. Coordinates of the primers are given as comments between brackets ([ ]).

Option

- `[-c]` Primers are generated from the complementary strand only
  Without –c option, the leading strand is only considered

- `[-s]` Primers are sorted by position on the genome
  Without -s option, primers are alphabetically sorted

Note: the options can be set in any order

Example 1: search for primers on the leading strand

Suppose a FASTA file (ex1.fasta) with the 2 following DNA sequences

```
>sequence #1
CGATTAAAGATAGAAATACACGATGCGAGCAATCAAATTTCA
>sequence #2
GAAACAACAAAACCTTCTACTGAAACAACTGAGGATAAT
CATGTGCTTGGTAGAGAGCAATTCAATGCCC
```

Generating primers is run by the following command line:

```
ithos_gen ex1.fasta param.txt p1.fasta
```

With the default parameters, the file p1.fasta will contain:

```
>sequence #1   [17 41]
ACACGATGCGAGCAATCAAATTTCA
>sequence #1   [11 35]
AGAAATACACGATGCGAGCAATCAA
>sequence #2   [39 63]
CATGTGCTTGGTAGAGAGCAATTCA
>sequence #2   [38 62]
TCATGTGCTTGGTAGAGAGCAATTC
>sequence #2   [43 67]
TGCTTGGTAGAGAGCAATTCAATGC
```

The numbers inside the brackets indicate the coordinates – begin and end – of the primer in the sequence. Note that for each sequence, primers are alphabetically sorted. If the option –s is used, the primers will be sorted as follows:

```
>sequence #1   [11 35]
AGAAATACACGATGCGAGCAATCAA
>sequence #1   [17 41]
ACACGATGCGAGCAATCAAATTTCA
>sequence #2   [38 62]
TCATGTGCTTGGTAGAGAGCAATTC
>sequence #2   [39 63]
CATGTGCTTGGTAGAGAGCAATTCA
>sequence #2   [43 67]
TGCTTGGTAGAGAGCAATTCAATGC
```

Example 2: search for primers on the complementary strand (option –c)

The search for primers is done on the complementary strand of the genome sequence. The software generates exactly the same type of file. The only difference is that the primer coordinates are inverted. Thus, with the same input file (ex1.fasta), and running:

```
ithos_gen ex1.fasta param.txt ex1_p1cfasta -c
```

The file p1c.fasta will contain :

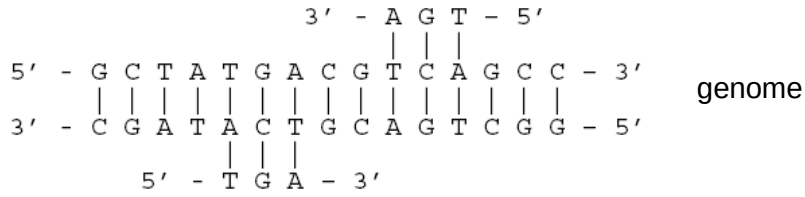
```
>sequence #1   [35 11]
TTGATTGCTCGCATCGTGTATTTCT
>sequence #2   [62 38]
GAATTGCTCTCTACCAAGCACATGA
>sequence #2   [67 43]
GCATTGAATTGCTCTCTACCAAGCA
>sequence #2   [68 44]
GGCATTGAATTGCTCTCTACCAAGC
>sequence #2   [69 45]
GGGCATTGAATTGCTCTCTACCAAG
>sequence #2   [63 39]
TGAATTGCTCTCTACCAAGCACATG
```

With the –s option, the file p1c.fasta will contain:

```
>sequence #1   [35 11]
TTGATTGCTCGCATCGTGTATTTCT
>sequence #2   [62 38]
GAATTGCTCTCTACCAAGCACATGA
>sequence #2   [63 39]
TGAATTGCTCTCTACCAAGCACATG
>sequence #2   [67 43]
GCATTGAATTGCTCTCTACCAAGCA
>sequence #2   [68 44]
GGCATTGAATTGCTCTCTACCAAGC
>sequence #2   [69 45]
GGGCATTGAATTGCTCTCTACCAAG
```

3. Checking the hybridization sites: ithos_chk

For each primer, the program checks that there are no secondary hybridization sites elsewhere on the genome. For example, the primer: 5′- T G A - 3′, requires that the following hybridization sites must be checked:



Detection of secondary hybridization sites are not based on the percentage of identity but on the thermodynamic stability of the duplex (cf. filter auto-complementarily).

A maximum deltaG is calculated on the whole primer length and a maximal deltaG in 3' is calculated on sizeDeltaGHybrid3 nucleotides. A deltaG value is calculated on consecutive matches, including putative mismatches (1 mismatch surrounded by 2 matchs).

A hybridization site is recognized if one of the two conditions is true:

- deltaG < maxDeltaGHybrid
- deltaG in 3' < maxDeltaGHybrid3

For example:

deltaG = max (G1,G2,G3) and deltaG in 3' = G3

The values for G1, G2 and G3 are the sum of the thermodynamic values between 2 consecutive nucleotide pairs.

The default values are:

- maxDeltaGHybrid = -16 kcal/mol
- maxDeltaGHybrid3 = - 9 kcal/mol
- sizeDeltaGHybrid3 = 8

Software execution

The software is launched by the following command line:

```
ithos_chk <genome> <primers> <param> <pr_out> <pr_hyb> <pr_dic> <pr_pos>
```

Input files

- <genome> is a text file (FASTA format) containing a genome sequence
- <primers> is a text file (FASTA format) containing a list of primers
- <param> is a text file containing the parameters for the different filters

Output file

- <pr_out> is a text file containing all the primers that have no hybridization sites
- <pr_hyb> is a text file containing all the primers that have at least one hybridization site. The program generates an additional file <pr_hyb.info> that indicates, for each primer of the file <pr_hyb> the positions of hybridization on the genome as well as the deltaG values.
- <pr_dic> is a text file containing the primer dictionary (alphabetically sorted)
- <pr_pos> is a text file containing all the sorted positions of the hybridization of the primer dictionary.

Example 3: Primer design on a genome and elimination of the primers that hybridize at other positions

If the following genome is considered and memorized in a file named ex2.fasta

```
>bank ex2
AAGATAGAAATACACGATGCGAGCAATCAAATTTCAGGTAGAAAGGATAGAACACGATCCGAGCAATCAGA
TTTCAGGTA
```

The design of primers is launched as follows:

```
ithos_gen ex2.fasta param.txt p2.fasta
```

The file p2.fasta will contain:

```
>bank ex2    [41 66]
AAAGGATAGAACACGATCCGAGCAAT
>bank ex2    [50 75]
AACACGATCCGAGCAATCAGATTTCA
>bank ex2    [42 67]
AAGGATAGAACACGATCCGAGCAATC
>bank ex2    [51 76]
ACACGATCCGAGCAATCAGATTTCAG
>bank ex2    [11 36]
ACACGATGCGAGCAATCAAATTTCAG
>bank ex2    [53 78]
ACGATCCGAGCAATCAGATTTCAGGT
>bank ex2    [13 38]
ACGATGCGAGCAATCAAATTTCAGGT
>bank ex2    [5 30]
AGAAATACACGATGCGAGCAATCAAA
```

```
>bank ex2    [48 73]
AGAACACGATCCGAGCAATCAGATTT
>bank ex2    [43 68]
AGGATAGAACACGATCCGAGCAATCA
>bank ex2    [46 71]
ATAGAACACGATCCGAGCAATCAGAT
>bank ex2    [52 77]
CACGATCCGAGCAATCAGATTTCAGG
>bank ex2    [12 37]
CACGATGCGAGCAATCAAATTTCAGG
>bank ex2    [49 74]
GAACACGATCCGAGCAATCAGATTTC
>bank ex2    [45 70]
GATAGAACACGATCCGAGCAATCAGA
>bank ex2    [44 69]
GGATAGAACACGATCCGAGCAATCAG
>bank ex2    [47 72]
TAGAACACGATCCGAGCAATCAGATT
```

Checking the hybridization sites is carried out by the command line:

```
ithos_chk ex2.fasta p2.fasta param.txt p2_ok p2_hyb p2_dic p2_pos
```

Four files are generated:

p2_ok

This file is empty since at least one hybridization site has been found.

p2_hyb

```
>bank ex2    [41 66]
AAAGGATAGAACACGATCCGAGCAAT
>bank ex2    [50 75]
AACACGATCCGAGCAATCAGATTTCA
>bank ex2    [42 67]
AAGGATAGAACACGATCCGAGCAATC
>bank ex2    [51 76]
ACACGATCCGAGCAATCAGATTTCAG
>bank ex2    [11 36]
ACACGATGCGAGCAATCAAATTTCAG
>bank ex2    [53 78]
ACGATCCGAGCAATCAGATTTCAGGT
>bank ex2    [13 38]
ACGATGCGAGCAATCAAATTTCAGGT
>bank ex2    [5 30]
AGAAATACACGATGCGAGCAATCAAA
>bank ex2    [48 73]
AGAACACGATCCGAGCAATCAGATTT
>bank ex2    [43 68]
AGGATAGAACACGATCCGAGCAATCA
>bank ex2    [46 71]
ATAGAACACGATCCGAGCAATCAGAT
>bank ex2    [52 77]
CACGATCCGAGCAATCAGATTTCAGG
>bank ex2    [12 37]
```

```
CACGATGCGAGCAATCAAATTTCAGG
>bank ex2    [49 74]
GAACACGATCCGAGCAATCAGATTTC
>bank ex2    [45 70]
GATAGAACACGATCCGAGCAATCAGA
>bank ex2    [44 69]
GGATAGAACACGATCCGAGCAATCAG
>bank ex2    [47 72]
TAGAACACGATCCGAGCAATCAGATT
```

p2_hyb.info

```
>bank ex2    [41 66]
AAAGGATAGAACACGATCCGAGCAAT
                                    seq      start      end dG max       dG 3'

5' AAAGGATAGAACACGATCCGAGCAAT 3' primer
   |||||||||||||||||||||||||
3' TTTCCTATCTTGTGCTAGGCTCGTTA 5' genome         41       66 -32394      -10934

5' AAAGGATAGAACACGATCCGAGCAAT 3' primer
   | |    |  ||||||| ||||||||
3' TCTATCTTTATGTGCTACGCTCGTTA 5' genome          1       26 -15934       -9094


>bank ex2    [50 75]
AACACGATCCGAGCAATCAGATTTCA
                                    seq      start      end dG max       dG 3'

5' AACACGATCCGAGCAATCAGATTTCA 3' primer
   |||||||||||||||||||||||||
3' TTGTGCTAGGCTCGTTAGTCTAAAGT 5' genome         50       75 -33074       -8614

5' AACACGATCCGAGCAATCAGATTTCA 3' primer
    ||||||| |||||||||| ||||||
3' ATGTGCTACGCTCGTTAGTTTAAAGT 5' genome         10       35 -23444       -4984


>bank ex2    [42 67]
AAGGATAGAACACGATCCGAGCAATC
                                    seq      start      end dG max       dG 3'

5' AAGGATAGAACACGATCCGAGCAATC 3' primer
   |||||||||||||||||||||||||
3' TTCCTATCTTGTGCTAGGCTCGTTAG 5' genome         42       67 -33114      -10734

5' AAGGATAGAACACGATCCGAGCAATC 3' primer
    |    |  ||||||| |||||||||
3' CTATCTTTATGTGCTACGCTCGTTAG 5' genome          2       27 -17574      -10734


>bank ex2    [51 76]
ACACGATCCGAGCAATCAGATTTCAG
                                    seq      start      end dG max       dG 3'

5' ACACGATCCGAGCAATCAGATTTCAG 3' primer
   |||||||||||||||||||||||||
3' TGTGCTAGGCTCGTTAGTCTAAAGTC 5' genome         51       76 -33554       -8564

5' ACACGATCCGAGCAATCAGATTTCAG 3' primer
   ||||||| |||||||||| |||||||
3' TGTGCTACGCTCGTTAGTTTAAAGTC 5' genome         11       36 -25344       -5984
```

```
>bank ex2    [11 36]
ACACGATGCGAGCAATCAAATTTCAG
                                    seq      start      end dG max       dG 3'

5' ACACGATGCGAGCAATCAAATTTCAG 3' primer
   ||||||| |||||||||| |||||||
3' TGTGCTAGGCTCGTTAGTCTAAAGTC 5' genome         51       76 -26294       -5984

5' ACACGATGCGAGCAATCAAATTTCAG 3' primer
   |||||||||||||||||||||||||
3' TGTGCTACGCTCGTTAGTTTAAAGTC 5' genome         11       36 -33924       -7984


>bank ex2    [53 78]
ACGATCCGAGCAATCAGATTTCAGGT
                                    seq      start      end dG max       dG 3'

5' ACGATCCGAGCAATCAGATTTCAGGT 3' primer
   |||||||||||||||||||||||||
3' TGCTAGGCTCGTTAGTCTAAAGTCCA 5' genome         53       78 -33654       -9074

5' ACGATCCGAGCAATCAGATTTCAGGT 3' primer
   ||||| |||||||||| |||||||||
3' TGCTACGCTCGTTAGTTTAAAGTCCA 5' genome         13       38 -25054       -9084


>bank ex2    [13 38]
ACGATGCGAGCAATCAAATTTCAGGT
                                    seq      start      end dG max       dG 3'

5' ACGATGCGAGCAATCAAATTTCAGGT 3' primer
   |||||||||||||||||||||||||
3' TGCTACGCTCGTTAGTTTAAAGTCCA 5' genome         13       38 -33634       -9084

5' ACGATGCGAGCAATCAAATTTCAGGT 3' primer
   ||||| |||||||||| |||||||||
3' TGCTAGGCTCGTTAGTCTAAAGTCCA 5' genome         53       78 -26394       -9074


>bank ex2    [5 30]
AGAAATACACGATGCGAGCAATCAAA
                                    seq      start      end dG max       dG 3'

5' AGAAATACACGATGCGAGCAATCAAA 3' primer
   |||||||||||||||||||||||||
3' TCTTTATGTGCTACGCTCGTTAGTTT 5' genome          5       30 -33224       -9564

5' AGAAATACACGATGCGAGCAATCAAA 3' primer
      |  ||||||| |||||||||| |
3' CTATCTTGTGCTAGGCTCGTTAGTCT 5' genome         45       70 -19454       -5354


>bank ex2    [48 73]
AGAACACGATCCGAGCAATCAGATTT
                                    seq      start      end dG max       dG 3'

5' AGAACACGATCCGAGCAATCAGATTT 3' primer
   |||||||||||||||||||||||||
3' TCTTGTGCTAGGCTCGTTAGTCTAAA 5' genome         48       73 -33204       -7864

5' AGAACACGATCCGAGCAATCAGATTT 3' primer
   |  ||||||| |||||||||| ||||
3' TTATGTGCTACGCTCGTTAGTTTAAA 5' genome          8       33 -20514       -4234
```

```
>bank ex2    [43 68]
AGGATAGAACACGATCCGAGCAATCA
                                    seq      start      end dG max       dG 3'

5' AGGATAGAACACGATCCGAGCAATCA 3' primer
   ||||||||||||||||||||||||||
3' TCCTATCTTGTGCTAGGCTCGTTAGT 5' genome         43       68 -33404       -9854

5' AGGATAGAACACGATCCGAGCAATCA 3' primer
   |     |  ||||||| ||||||||||
3' TATCTTTATGTGCTACGCTCGTTAGT 5' genome          3       28 -18964       -9954


>bank ex2    [46 71]
ATAGAACACGATCCGAGCAATCAGAT
                                    seq      start      end dG max       dG 3'

5' ATAGAACACGATCCGAGCAATCAGAT 3' primer
   |||||||||||||||||||||||||||
3' TATCTTGTGCTAGGCTCGTTAGTCTA 5' genome         46       71 -32294       -8424

5' ATAGAACACGATCCGAGCAATCAGAT 3' primer
     |  ||||||| |||||||||| ||
3' CTTTATGTGCTACGCTCGTTAGTTTA 5' genome          6       31 -18624       -4794


>bank ex2    [52 77]
CACGATCCGAGCAATCAGATTTCAGG
                                    seq      start      end dG max       dG 3'

5' CACGATCCGAGCAATCAGATTTCAGG 3' primer
   |||||||||||||||||||||||||||
3' GTGCTAGGCTCGTTAGTCTAAAGTCC 5' genome         52       77 -34974       -9634

5' CACGATCCGAGCAATCAGATTTCAGG 3' primer
   ||||||  |||||||||| ||||||||
3' GTGCTACGCTCGTTAGTTTAAAGTCC 5' genome         12       37 -26364       -8334


>bank ex2    [12 37]
CACGATGCGAGCAATCAAATTTCAGG
                                    seq      start      end dG max       dG 3'

5' CACGATGCGAGCAATCAAATTTCAGG 3' primer
   ||||||  |||||||||| ||||||||
3' GTGCTAGGCTCGTTAGTCTAAAGTCC 5' genome         52       77 -27714       -8334

5' CACGATGCGAGCAATCAAATTTCAGG 3' primer
   |||||||||||||||||||||||||||
3' GTGCTACGCTCGTTAGTTTAAAGTCC 5' genome         12       37 -34944       -9334


>bank ex2    [49 74]
GAACACGATCCGAGCAATCAGATTTC
                                    seq      start      end dG max       dG 3'

5' GAACACGATCCGAGCAATCAGATTTC 3' primer
   |||||||||||||||||||||||||
3' CTTGTGCTAGGCTCGTTAGTCTAAAG 5' genome         49       74 -33644       -8624

5' GAACACGATCCGAGCAATCAGATTTC 3' primer
     |||||||  |||||||||| |||||
3' TATGTGCTACGCTCGTTAGTTTAAAG 5' genome          9       34 -22154       -4994
```

```
>bank ex2    [45 70]
GATAGAACACGATCCGAGCAATCAGA
                                    seq      start      end dG max       dG 3'

5' GATAGAACACGATCCGAGCAATCAGA 3' primer
   ||||||||||||||||||||||||||
3' CTATCTTGTGCTAGGCTCGTTAGTCT 5' genome         45       70 -33574      -10144

5' GATAGAACACGATCCGAGCAATCAGA 3' primer
      |  |||||||  |||||||||| |
3' TCTTTATGTGCTACGCTCGTTAGTTT 5' genome          5       30 -18104       -6514


>bank ex2    [44 69]
GGATAGAACACGATCCGAGCAATCAG
                                    seq      start      end dG max       dG 3'

5' GGATAGAACACGATCCGAGCAATCAG 3' primer
   |||||||||||||||||||||||||
3' CCTATCTTGTGCTAGGCTCGTTAGTC 5' genome         44       69 -34214      -10164

5' GGATAGAACACGATCCGAGCAATCAG 3' primer
      |  |||||||  ||||||||||
3' ATCTTTATGTGCTACGCTCGTTAGTT 5' genome          4       29 -18674       -8364


>bank ex2    [47 72]
TAGAACACGATCCGAGCAATCAGATT
                                    seq      start      end dG max       dG 3'

5' TAGAACACGATCCGAGCAATCAGATT 3' primer
   |||||||||||||||||||||||||||
3' ATCTTGTGCTAGGCTCGTTAGTCTAA 5' genome         47       72 -33104       -7974

5' TAGAACACGATCCGAGCAATCAGATT 3' primer
    |  |||||||  |||||||||| |||
3' TTTATGTGCTACGCTCGTTAGTTTAA 5' genome          7       32 -19624       -4344
```

p2_dic

```
  0 AAAGGATAGAACACGATCCGAGCAAT
  1 AACACGATCCGAGCAATCAGATTTCA
  2 AAGGATAGAACACGATCCGAGCAATC
  3 ACACGATCCGAGCAATCAGATTTCAG
  4 ACACGATGCGAGCAATCAAATTTCAG
  5 ACGATCCGAGCAATCAGATTTCAGGT
  6 ACGATGCGAGCAATCAAATTTCAGGT
  7 AGAAATACACGATGCGAGCAATCAAA
  8 AGAACACGATCCGAGCAATCAGATTT
  9 AGGATAGAACACGATCCGAGCAATCA
 10 ATAGAACACGATCCGAGCAATCAGAT
 11 CACGATCCGAGCAATCAGATTTCAGG
 12 CACGATGCGAGCAATCAAATTTCAGG
 13 GAACACGATCCGAGCAATCAGATTTC
 14 GATAGAACACGATCCGAGCAATCAGA
 15 GGATAGAACACGATCCGAGCAATCAG
 16 TAGAACACGATCCGAGCAATCAGATT
```

p2_pos

```
1 0
2 2
3 9
4 15
5 14
5 7
6 10
7 16
8 8
9 13
10 1
11 3
11 4
12 11
12 12
13 5
13 6
41 0
42 2
43 9
44 15
45 14
45 7
46 10
47 16
48 8
49 13
50 1
51 3
51 4
52 11
52 12
53 5
53 6
```

4. Visualization of the primers features: ithos_viz

This utility displays the primer features. For each primer, it gives:

- the GC percent
- the melting temperature: Tm
- the maximal suite of identical nucleotides
- the size of the biggest stem-loop structure
- maximal deltaG for the complementary primer
- maximal deltaG in 3' for the complementary primer
- Stability in 5'
- Stability in 3'

Software Execution

The program is launched by the command line:

```
ithos_viz <primers> <parameters>
```

Results are displayed on monitor screen

Input files

- `<primers>` is a text file (FASTA format) that contains a primers list
- `<parameters>` is a text file that contains parameters of the different filters

Example 4: visualization of primer_ok file

The execution of the following command line:

```
ithos_viz p1.fasta param.txt
```

Displays on the screen:

```
sequence #1   [17 42]          %GC     Tm      #rep    Hpin    Cp_dG   Cp_dG3' Sta_5'  Sta_3'
ACACGATGCGAGCAATCAAATTTCA       40     58         3       3     1960      1960  -6500   -4750

>sequence #1   [11 36]          %GC     Tm      #rep    Hpin    Cp_dG   Cp_dG3' Sta_5'  Sta_3'
AGAAATACACGATGCGAGCAATCAAA      38     57         3       3     1960      1960  -4580   -4750

>sequence #2   [39 64]          %GC     Tm      #rep    Hpin    Cp_dG   Cp_dG3' Sta_5'  Sta_3'
CATGTGCTTGGTAGAGAGCAATTCAA      42     57         3       4      510      1960  -5220   -4750

>sequence #2   [38 63]          %GC     Tm      #rep    Hpin    Cp_dG   Cp_dG3' Sta_5'  Sta_3'
TCATGTGCTTGGTAGAGAGCAATTCA      42     58         3       4    -1820      1960  -5080   -4630

>sequence #2   [43 68]          %GC     Tm      #rep    Hpin    Cp_dG   Cp_dG3' Sta_5'  Sta_3'
TGCTTGGTAGAGAGCAATTCAATGCC      46     59         3       4     1960      1960  -5970   -6410
```

5. Parameters file

This is a text file that enables modifying the default parameters of the filters. The same file is read by the three programs.

Example

```
# IThOS PARAMETERS

# primers length

sizePrimer 25

# filter 1: GC percentage

pcGCMin 40
pcGCMax 60

# filter 2: tm

oligoTmMin 57
oligoTmMax 67
dnaConc 500
saltConc 50

# filter 3: hairpin

maxHpDup 4
maxHpLoop 4

# filter 4: repeat

nbRepeat 6

# filter 5: auto complementarity

maxDeltaGAuto -10000
maxDeltaGAuto3 -6000
sizeDeltaGAuto 8
sizeDeltaGAuto3 8

# filter 6: internal stability to 3' & 5' extremities

sizeExt5 5
sizeExt3 5
deltaG5 -4000
deltaG3min -6000
deltaG3max -3000

#site hybridation (only used by ithos_chk)

sizeDeltaGHybrid3 8
maxDeltaGHybrid3 -12000
maxDeltaGHybrid -18000
```