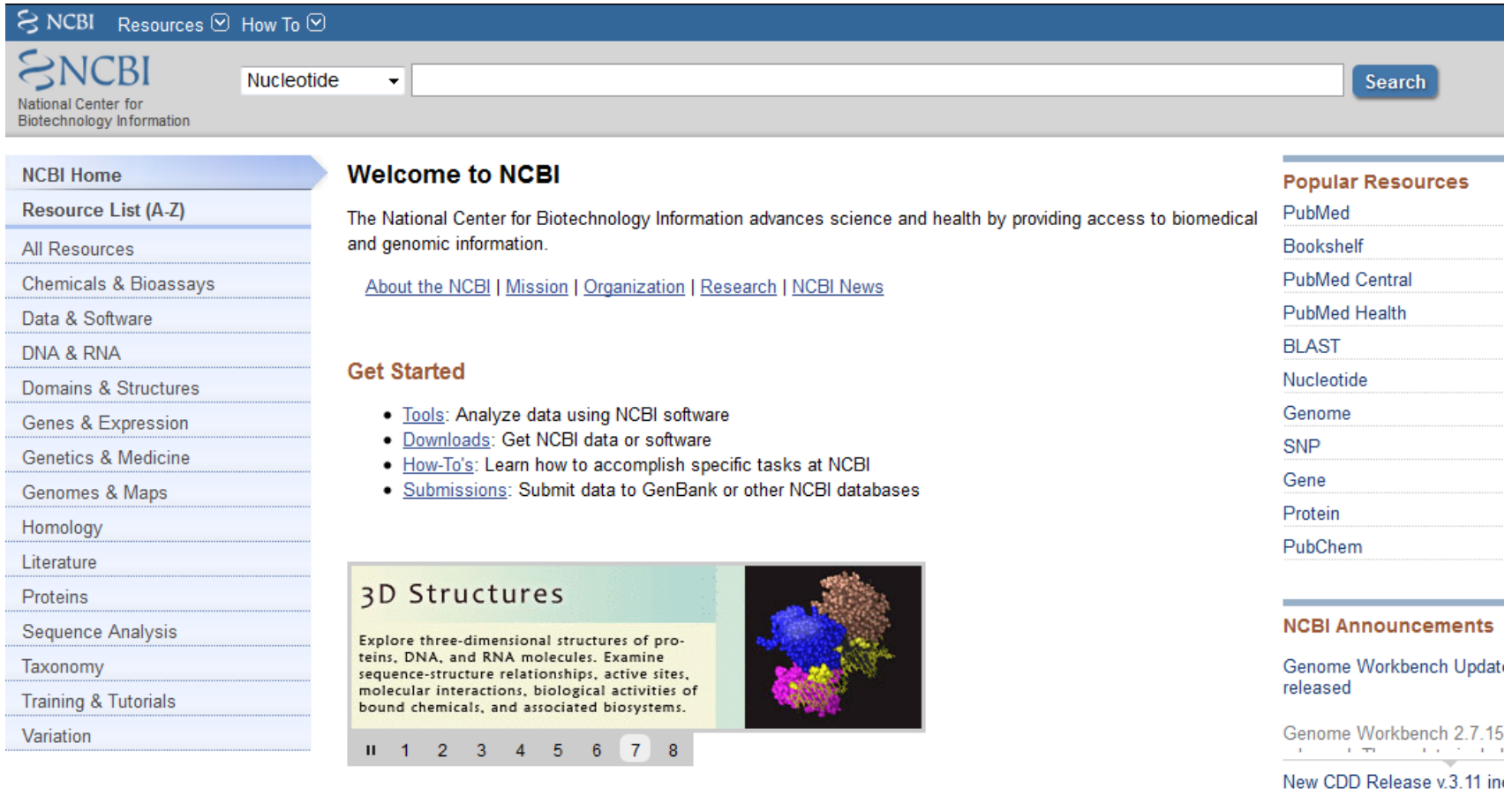
Choix d’amorces nucléotidiques par l’outil Bioinformatique

Le choix des amorces de PCR

The National Center for Biotechnology Information (NCBI)



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Nucleotide Human p53 Search

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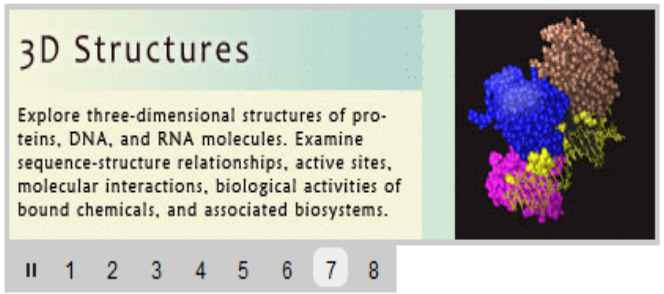

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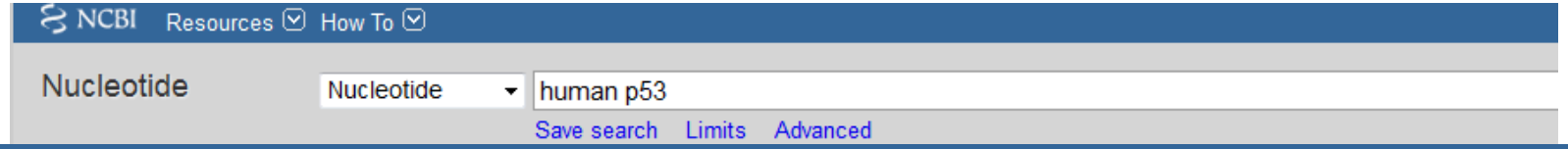
Genome Workbench Updat released

Genome Workbench 2.7.15

New CDD Release v.3.11 in



NCBI Resources How To

Nucleotide | Nucleotide | human p53 | Save search | Limits | Advanced

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Found 8593 nucleotide sequences. Nucleotide (8362) EST (196) GSS (35)

Results: 1 to 20 of 8362

<< First < Pre

1. **Homo sapiens** mRNA for **P53**, complete cds

2,451 bp linear mRNA

Accession: AB082923.1 GI: 23491728

GenBank FASTA Graphics Related Sequences

2. **Homo sapiens p53 (p53)** gene, exon 8 and partial cds

137 bp linear DNA

Accession: JF923573.1 GI: 349734071

GenBank FASTA Graphics Related Sequences

GenBank FASTA Graphics Related Sequences

6. **Homo sapiens p53 (p53)** gene, exon 4 and partial cds

279 bp linear DNA

Accession: JF923569.1 GI: 349734063

Homo sapiens mRNA for P53, complete cds

GenBank: AB082923.1

GenBank Graphics

>gi|23491728|dbj|AB082923.1| Homo sapiens mRNA for P53, complete cds

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ATTGAACAATGGTTCACTGAAGACCCAGGTCCAGATGAAGCTCCCAGAATGCCAGAGGCTGCTCCCCGCG
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TGTCCCTTCCCAGAAAACCTACCAGGGCAGCTACGGTTTCCGTCTGGGCTTCTTGCATTCTGGGACAGCC
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GCACAGAGGAAGAGAATCTCCGCAAGAAAGGGGAGCCTCACCACGAGCTGCCCCCAGGGAGCACTAAGCG
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TATATCCCATTTTTATATCGATCTCTTATTTTACAATAAAACTTTGCTGCCAAAAAAAAAAAAAAAAAAA
A

http://bioinfo.ut.ee/primer3/

Primer3web version 4.0.0 - Pick primers from a DNA sequence.

Select the Task for primer selection generic

Paste source sequence below (5'->3', string of ACGTNacgtn -- other letters treated as N -- numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector, .
Mispriming Library (repeat library) NONE

Pick left primer, or use left primer below	Pick hybridization probe (internal oligo), or use oligo below	Pick right primer, or use right primer below (5' to 3' on opposite strand)

Pick Primers | Download Settings | Reset Form

Sequence Id		A string to identify your output.
Targets		E.g. 50,2 requires primers to surround the 2 bases at positions 50 and 51. Or mark the source sequence with [and]: e.g. ...ATCT[CCC must flank the central CCCC.
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Primer3web version 4.0.0 - Pick primers from a DNA sequence.

Select the Task for primer selection generic

Paste source sequence below (5'->3', string of ACGTNacgtn -- other letters treated as N -- numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector,
Mispriming Library (repeat library) NONE

```
CGTGCTTTCCACGACGGTGACACGCTTCCCTGGATTGGCCAGACTGCCTTCCGGGTCACTGCCATGGAGG
AGCCGCAGTCAGATCCTAGCGTCGAGCCCCCTCTGAGTCAGGAAACATTTTCAGACCTATGGAAACTACT
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AAGTCTGTGACTTGCACGTACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAGACCTGCCCTG
```

Pick left primer, or use left primer below	Pick hybridization probe (internal oligo), or use oligo below	Pick right primer, or use right primer below (5' to 3' on opposite strand)

Pick Primers | Download Settings | Reset Form

Sequence Id		A string to identify your output.
Targets		E.g. 50,2 requires primers to surround the 2 bases at positions 50 and 51. Or mark the source sequence with [and]: e.g. ...ATCT[CCC must flank the central CCCC.
Overlap Junction List		E.g. 27 requires one primer to overlap the junction between positions 27 and 28. Or mark the source sequence with -: e.g. ...ATCTAC- must overlap the junction between the C and T.
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Included Region		E.g. 20,400: only pick primers in the 400 base region starting at position 20. Or use { and } in the source sequence to mark the beginning e.g. in ATC{TTC...TCT}AT the included region is TTC...TCT.

Primer3 Output

No mispriming library specified
Using 1-based sequence positions

OLIGO	start	len	tm	gc%	any th	3' th	hairpin	seq
LEFT PRIMER	542	20	59.02	50.00	6.70	0.00	0.00	TGGCCATCTACAAGCAGTCA
RIGHT PRIMER	753	20	59.02	55.00	0.00	0.00	0.00	GGTACAGTCAGAGCCAACCT

SEQUENCE SIZE: 2451
INCLUDED REGION SIZE: 2451

PRODUCT SIZE: 212,

```
   1 CGTGCTTTCCACGACGGTGACACGCTTCCCTGGATTGGCCAGACTGCCTTCCGGGTCACT

  61 GCCATGGAGGAGCCGCAGTCAGATCCTAGCGTCGAGCCCCCTCTGAGTCAGGAAACATTT

 121 TCAGACCTATGGAAACTACTTCCTGAAAACAACGTTCTGTCCCCCTTGCCGTCCCAAGCA

 181 ATGGATGATTTGATGCTGTCCCCGGACGATATTGAACAATGGTTCACTGAAGACCCAGGT

 241 CCAGATGAAGCTCCCAGAATGCCAGAGGCTGCTCCCCGCGTGGCCCCTGCACCAGCAGCT

 301 CCTACACCGGCGGCCCCTGCACCAGCCCCCTCCTGGCCCCTGTCATCTTCTGTCCCTTCC

 361 CAGAAAACCTACCAGGGCAGCTACGGTTTCCGTCTGGGCTTCTTGCATTCTGGGACAGCC

 421 AAGTCTGTGACTTGCACGTACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAG

 481 ACCTGCCCTGTGCAGCTGTGGGTTGATTCCACACCCCCGCCCGGCACCCGCGTCCGCGCC

 541 ATGGCCATCTACAAGCAGTCACAGCACATGACGGAGGTTGTGAGGCGCTGCCCCCACCAT
      >>>>>>>>>>>>>>>>>>>

 601 GAGCGCTGCTCAGATAGCGATGGTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAAGGA

 661 AATTTGCGTGTGGAGTATTTGGATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCC

 721 TATGAGCCGCCTGAGGTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAAC
                  <<<<<<<<<<<<<<<<<<<

 781 AGTTCCTGCATGGGCGGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGAC
```

Le Choix des amorces de QRTPCR

Le choix des amorces de PCR

Spécificité

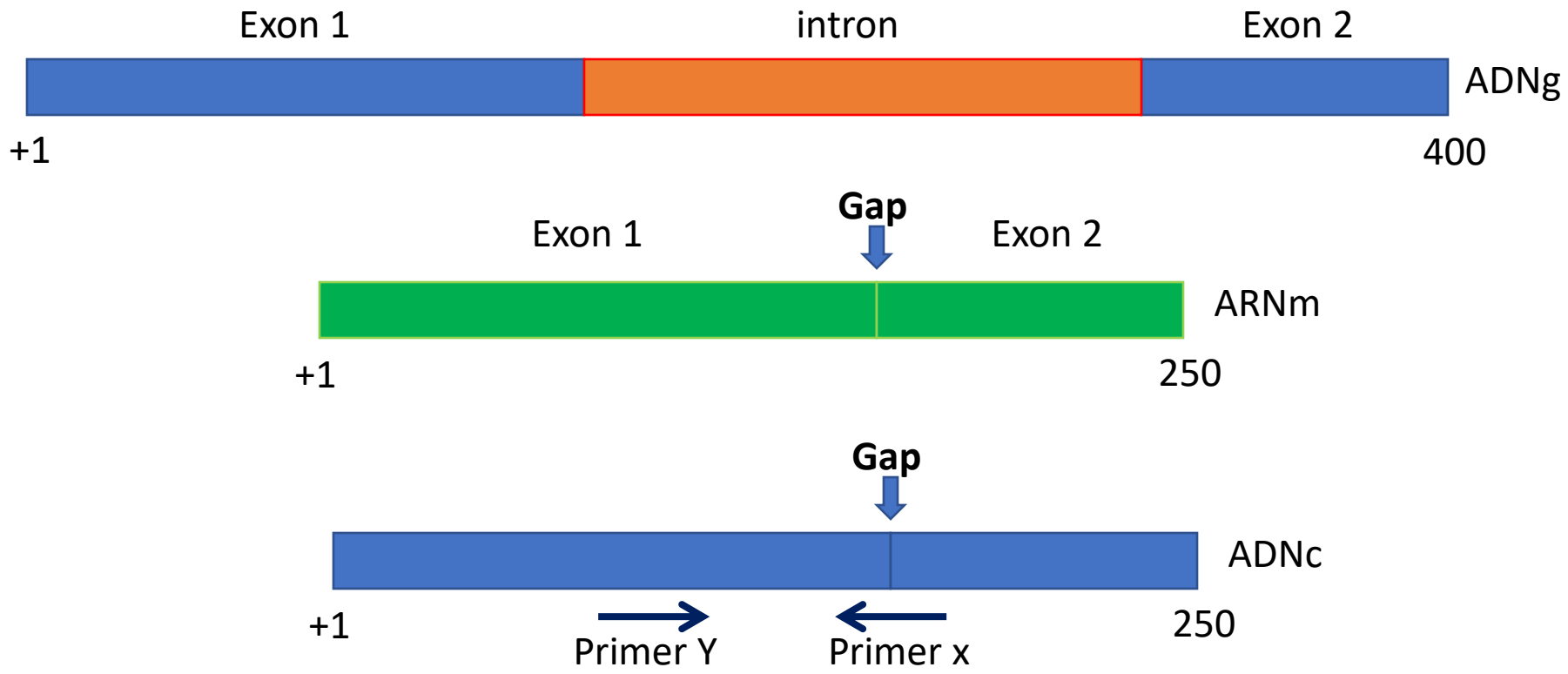
1. On ne veut amplifier que notre ADNc d'intérêt et pas un autre ADNc. Cet aspect est fondamental, en particulier si on s'intéresse à une famille multigénique ou à un transcrit alternatif précis
2. Même si notre échantillon d'ADNc est un peu contaminé par de l'ADN génomique, on aimerait que l'amplification de ce dernier ne soit pas possible
3. On ne veut pas que nos amorces puissent s'auto-amplifier en formant des dimères d'amorces

Outils

1. Connaissance de la séquence et des séquences qui présentent des homologies. Connaissance du ou des transcrits étudiés. GenBank, BLAST, Ensembl, PubMed, Multalin, Entrez…
2. Choix des primers sur deux exons différents séparés par un intron d'au moins 1-2 Kb. Ensembl, Entrez, BLAST

- **Comment choisir ses primers (amorces) pour éviter d'amplifier de l'ADN génomique (ADNg)?**

- **Comment choisir ses primers (amorces) pour éviter d'amplifier les introns si ils existent?**



Un des primer choisi doit chevaucher deux exons adjacents

Homo sapiens mRNA for P53, complete cds

GenBank: AB082923.1

GenBank Graphics

>gi|23491728|dbj|AB082923.1| Homo sapiens mRNA for P53, complete cds

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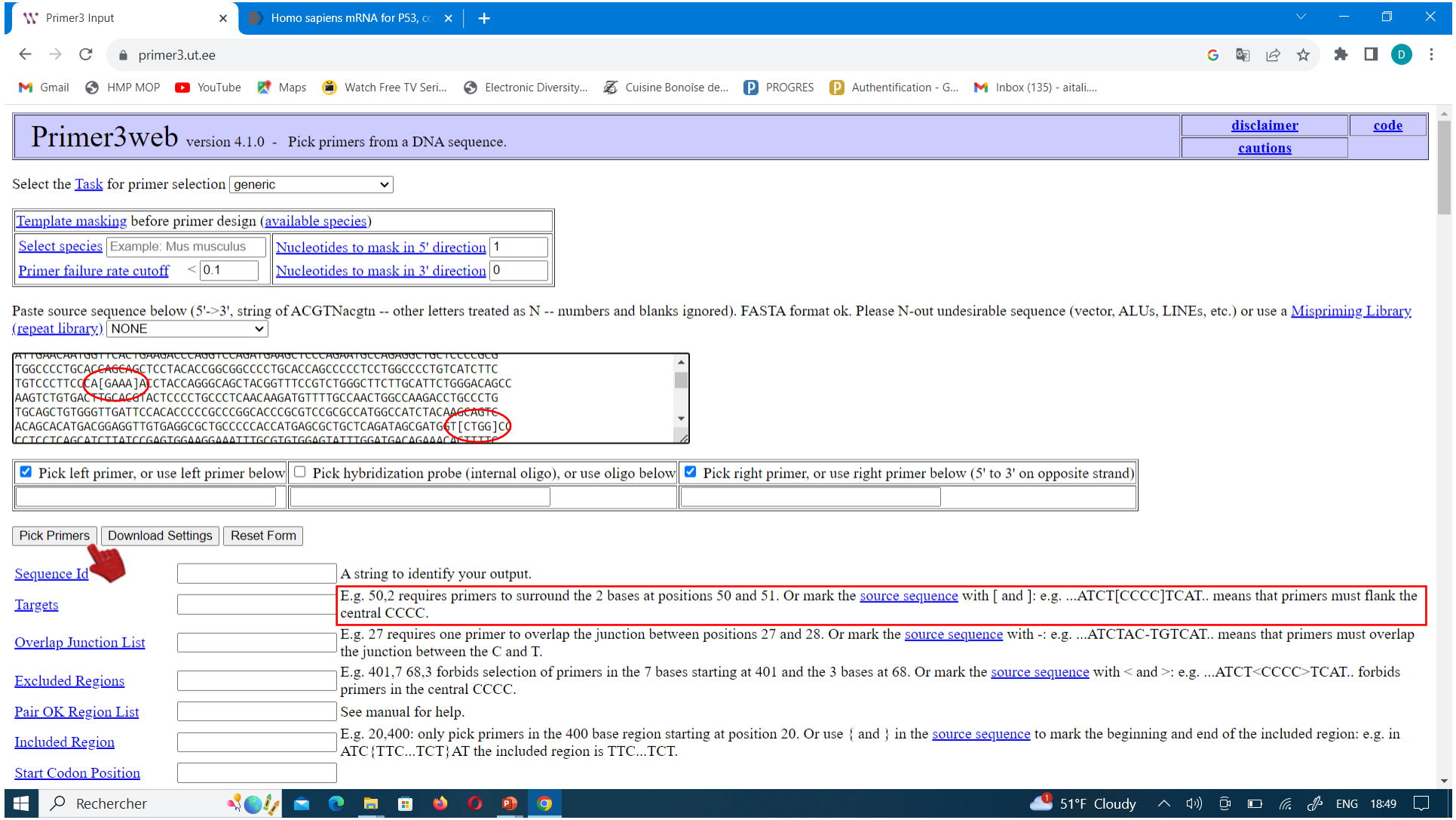
A

Délimiter la position des « gaps » en retrouvant (outil bioinformatique) la séquence des exons


Primer3 Input
Homo sapiens mRNA for P53, cc
primer3.ut.ee
Gmail
HMP MOP
YouTube
Maps
Watch Free TV Seri...
Electronic Diversity...
Cuisine Bonoise de...
PROGRES
Authentification - G...
Inbox (135) - aitali....
Primer3web version 4.1.0 - Pick primers from a DNA sequence.
disclaimer
code
cautions
Select the Task for primer selection generic
Template masking before primer design (available species)
Select species Example: Mus musculus
Primer failure rate cutoff < 0.1
Nucleotides to mask in 5' direction 1
Nucleotides to mask in 3' direction 0
Paste source sequence below (5'->3', string of ACGTNacgtn -- other letters treated as N -- numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector, ALUs, LINEs, etc.) or use a Mispriming Library (repeat library) NONE
TGGCCCCTGCACCAGCAGCTCCTACACCGGCGGCCCCTGCACCAGCCCCCTCCTGGCCCCTGTCATCTTC
TGTCCCTTCCCA[GAAA]ATCCTACCAGGGCAGCTACGGTTTCCGTCTGGGCTTCTTGCATTCTGGGACAGCC
AAGTCTGTGACTTGCACGTACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAGACCTGCCCTG
TGCAGCTGTGGGTTGATTCCACACCCCCGCCCGGCACCCGCGTCCGCGCCATGGCCATCTACAAGCAGTC
ACAGCACATGACGGAGGTTGTGAGGCGCTGCCCCCACCATGAGCGCTGCTCAGATAGCGATGGT[CTGG]CC
Pick left primer, or use left primer below
Pick hybridization probe (internal oligo), or use oligo below
Pick right primer, or use right primer below (5' to 3' on opposite strand)
Pick Primers
Download Settings
Reset Form
Sequence Id
A string to identify your output.
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Pair OK Region List
See manual for help.
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Start Codon Position
Rechercher
51°F Cloudy
ENG
18:49

Primer3web version 4.0.0 - Pick primers from a DNA sequence.

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```
CGTGCTTTCCACGACGGTGACACGCTTCCCTGGATTGGCCAGACTGCCTTCCGGGTCACTGCCATGGAGG
AGCCGCAGTCAGATCCTAGCGTCGAGCCCCCTCTGAGTCAGGAAACATTTTCAGACCTATGGAAACTACT
TCCTGAAAACAACGTTCTGTCCCCCTTGCCGTCCCAAGCAATGGATGATTTGATGCTGTCCCCGGACGAT
ATTGAACAATGGTTCACTGAAGACCCAGGTCCAGATGAAGCTCCCAGAATGCCAGAGGCTGCTCCCCGCG
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TGTCCCTTCCCAGAAAACCTACCAGGGCAGCTACGGTTTCCGTCTGGGCTTCTTGCATTCTGGGACAGCC
AAGTCTGTGACTTGCACGTACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAGACCTGCCCTG
```

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Pick Primers | Download Settings | Reset Form

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Primer3 Output

No mispriming library specified
Using 1-based sequence positions

OLIGO	start	len	tm	gc%	any th	3' th	hairpin	seq
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RIGHT PRIMER	753	20	59.02	55.00	0.00	0.00	0.00	GGTACAGTCAGAGCCAACCT

SEQUENCE SIZE: 2451
INCLUDED REGION SIZE: 2451

PRODUCT SIZE: 212,

```
   1 CGTGCTTTCCACGACGGTGACACGCTTCCCTGGATTGGCCAGACTGCCTTCCGGGTCACT

  61 GCCATGGAGGAGCCGCAGTCAGATCCTAGCGTCGAGCCCCCTCTGAGTCAGGAAACATTT

 121 TCAGACCTATGGAAACTACTTCCTGAAAACAACGTTCTGTCCCCCTTGCCGTCCCAAGCA

 181 ATGGATGATTTGATGCTGTCCCCGGACGATATTGAACAATGGTTCACTGAAGACCCAGGT

 241 CCAGATGAAGCTCCCAGAATGCCAGAGGCTGCTCCCCGCGTGGCCCCTGCACCAGCAGCT

 301 CCTACACCGGCGGCCCCTGCACCAGCCCCCTCCTGGCCCCTGTCATCTTCTGTCCCTTCC

 361 CAGAAAACCTACCAGGGCAGCTACGGTTTCCGTCTGGGCTTCTTGCATTCTGGGACAGCC

 421 AAGTCTGTGACTTGCACGTACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAG

 481 ACCTGCCCTGTGCAGCTGTGGGTTGATTCCACACCCCCGCCCGGCACCCGCGTCCGCGCC

 541 ATGGCCATCTACAAGCAGTCACAGCACATGACGGAGGTTGTGAGGCGCTGCCCCCACCAT
      >>>>>>>>>>>>>>>>>>>

 601 GAGCGCTGCTCAGATAGCGATGGTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAAGGA

 661 AATTTGCGTGTGGAGTATTTGGATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCC

 721 TATGAGCCGCCTGAGGTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAAC
                  <<<<<<<<<<<<<<<<<<<

 781 AGTTCCTGCATGGGCGGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGAC
```

Bad Primers:

- **Tm P1 et P2 = plusieurs dégrées de différences (Eviter les écarts de plus de 2 à 3°C)**

- **Eviter les primers avec région poly A/T ou région poly G/C**

Ex: AAAAAATTCGCATCCGAT GGAAAATTTACATCCGAT AAGGCGCGCGCATCCGAT

- **% G/C largement supérieur ou inferieur à 50**

- **Formation de « hairpin »**