REPUBLIQUE ALGERIENNE DEMOCRATIQUE ET POPULAIRE MINISTERE DE L'ENSEIGNEMENT SUPERIEUR ET DE LA RECHERCHE SCIENTIFIQUE UNIVERSITÉ ABOU BEKR BELKAID – TLEMCEN

FACULTÉ :SNV
Département de Biologie
Filière :Biologie moléculaire et cellulaire (M1)

Conception des Amorces et Réalisation d'un PCR in silico

Présenté Par : Dr SALMI Takwa

PCR(**p**olymerase **c**haine **r**éaction)

La réaction en chaîne par polymérase (PCR) est largement utilisée en biologie moléculaire,

Amplifier des fragments (morceaux) d'ADN pour pouvoir identifier des variations (mutations, SNP, polymorphismes)

La PCR comprend les trois étapes suivantes : dénaturation, hybridation, polymérisation

Toutes ces étapes sont sensibles à la température et le choix courant des températures est respectivement de 94 ° C, 60 ° C et 70 ° C.

PCR(**p**olymerase **c**haine **r**éaction)

Réaction biochimique de synthèse ADN réalisée *in vitro*

ce qui permet d'amplifier un fragment d'ADN à partir d'une matrice d'ADN qui contient ce fragment.

Etapes d’une PCR

Une réaction de PCR se déroule par répétitions successives des trois étapes suivantes :

1. dénaturation de la matrice d'ADN (94°C, 30 secondes)
2. hybridation de la matrice avec les amorces (entre 50 et 60)
3. polymérisation d'ADN à partir des amorces (72°C, entre 30 secondes et 5 minutes selon la longueur du fragment d'ADN qui doit être amplifiée).

Cet enchaînement d'étapes qui constitue un cycle d'amplification est reproduit successivement de 20 à 50 fois.

Outils d'une PCR

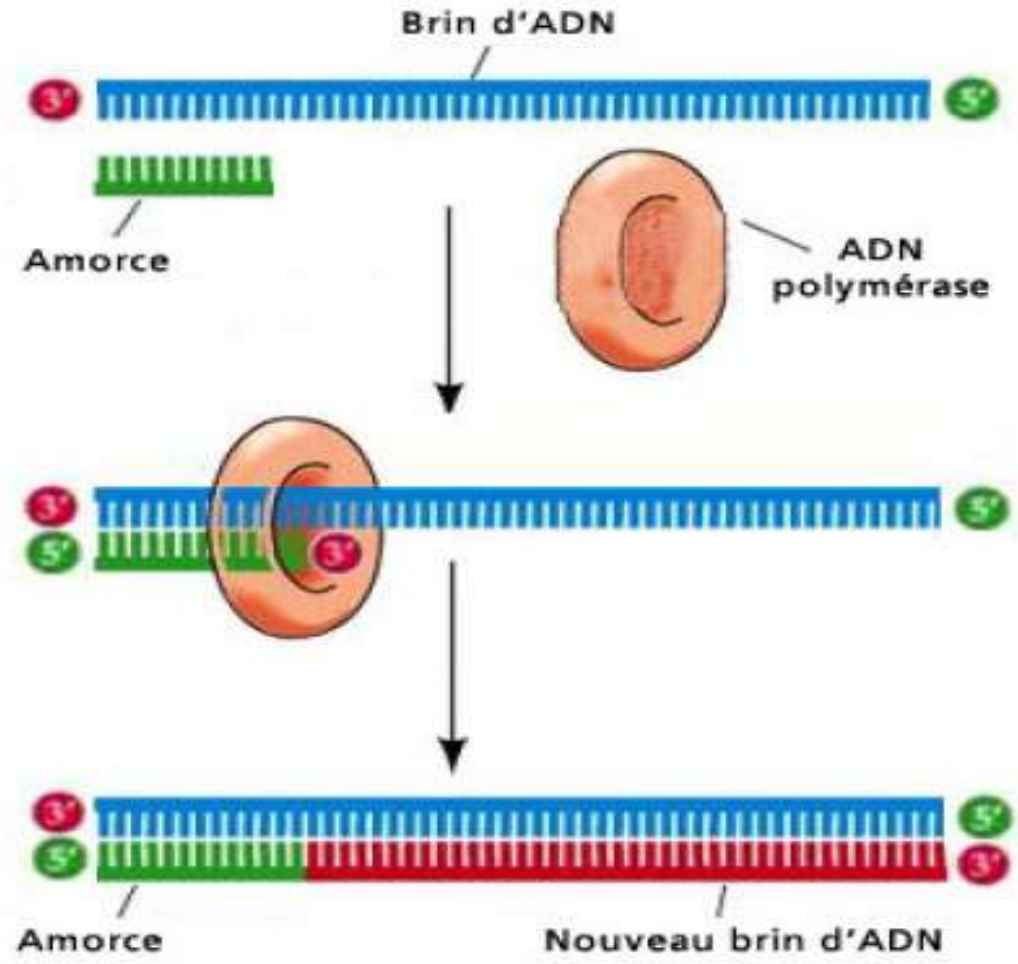
ADN

Enzyme « Taq polymerase »

Nucléotides

Amorces

Températures



Amorces

Des oligonucléotides → complémentaires → à la région matrice de l'ADN

Sont synthétisées chimiquement

Longueur entre 18-24 pb (si les amorces sont trop courtes, elles pourraient s'hybrider à des sites non cibles)

La composition en C/G doit être entre 40-60 %

La température de fusion (Tm) doit être entre 50-60°C (Si la température est trop élevée, aucune hybridation)

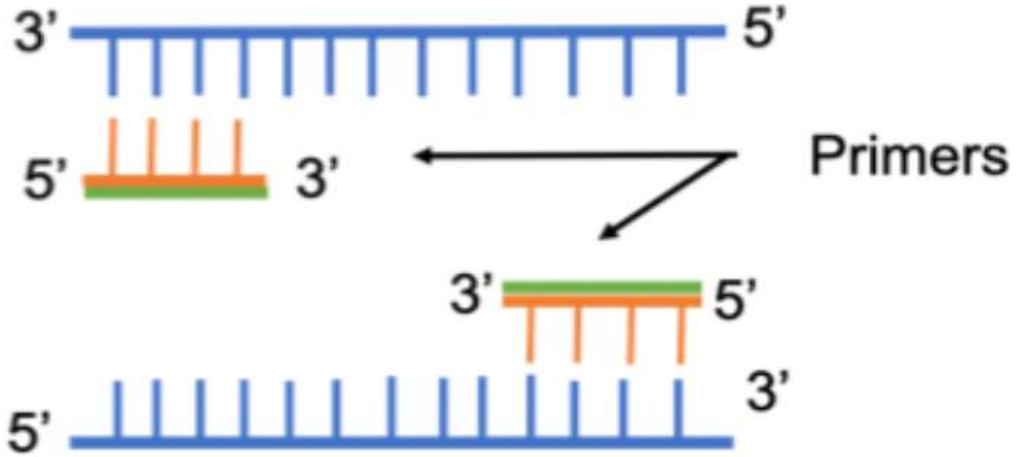
Les paires d'amorces ne doivent pas avoir de régions complémentaires

Amorces

sont la clé du succès ou de l’échec d’une expérience PCR

Si les amorces sont conçues correctement

l’expérience conduit à l’amplification d’un seul fragment d’ADN (correspondant à la région cible)



Amorces

Forwarde : 5’- 3’

AGAGTAGTCAGGAGGATT

Reverse : 5’ – 3’

TGCATGATGCAGTTATAATGC

2.5. Purification of CD4+CD25^{hi} Treg

CD4+CD25^{hi} Treg were purified from PBMC by magnetic bead cell sorting using the CD4+CD25^{hi} human regulatory T cell isolation kit (Miltenyi Biotech, Bergisch Gladbach, Germany). The purity of

allelic variant (C/T + T/T), together considered, were significantly more frequent in SSc patients than in healthy donors (Table 2). Accordingly, the frequency of the T variant allele was significantly higher in the studied SSc population than in control group (Table 3).

Table 1
Primers and temperatures used for PCR amplifications.

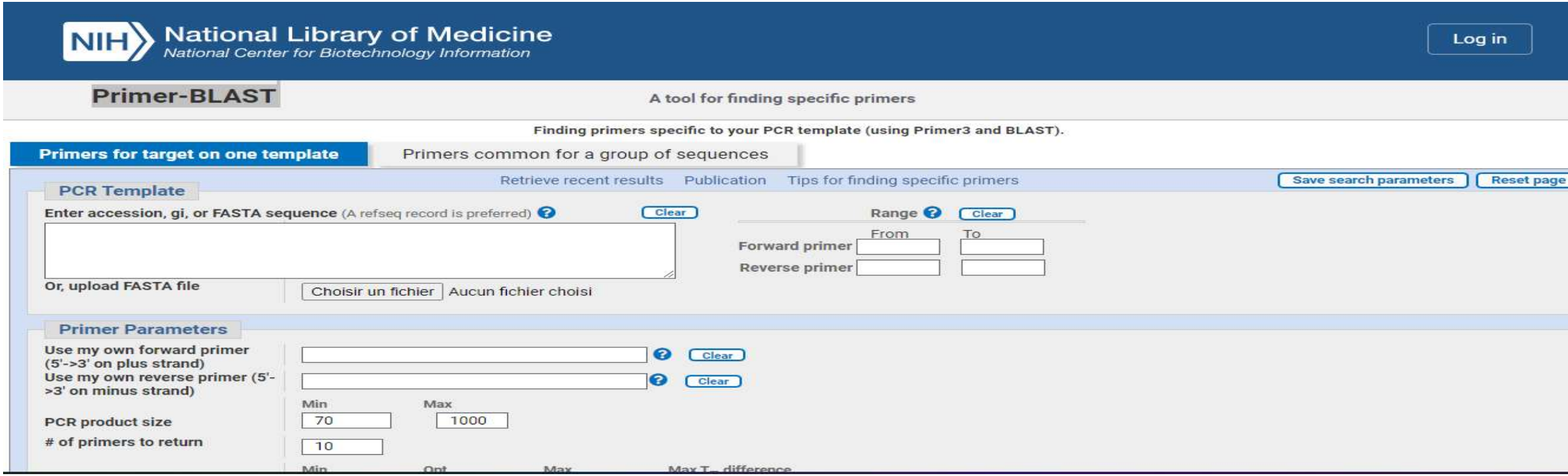
Exon	Intron	Forward primer 5′-3′	Reverse primer 5′-3′	Annealing temperature (°C)
1		CTCACTGCCCCTGTGATAAAC	AGTTAACTTGGCCAGGTAAGG	56
3		AGAGTAGTCAGGAGGATT	TGCATGATGCAGTTATAATGC	52
5	5–6	TGTAATGCCTACTGAAGAAAC	CTTAAATTATTTTTTGGCTGAATTCAA	54

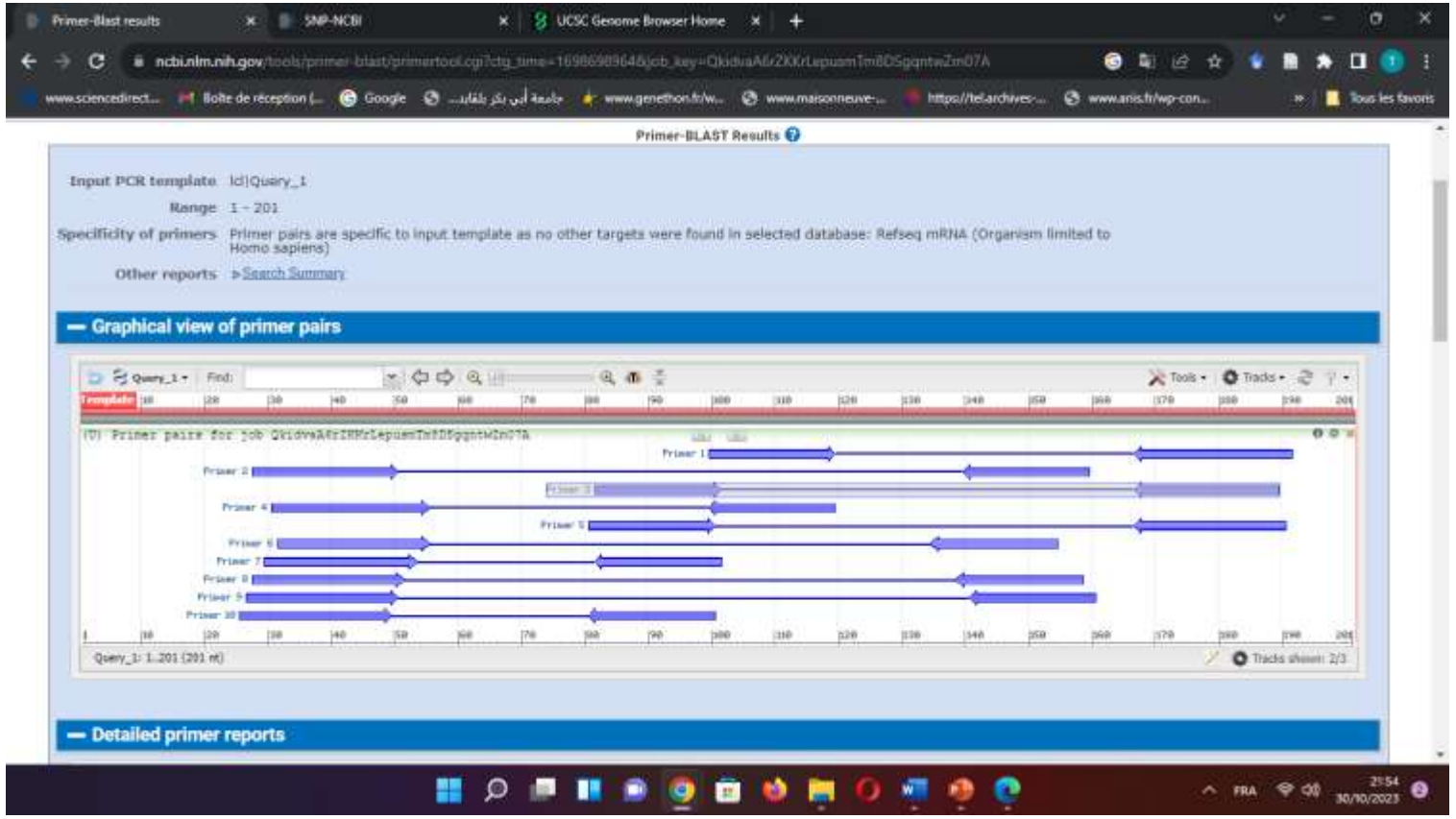
Table 2
Frequencies of genotypes carrying the rs7820268 SNP T allelic variant in SSc patients

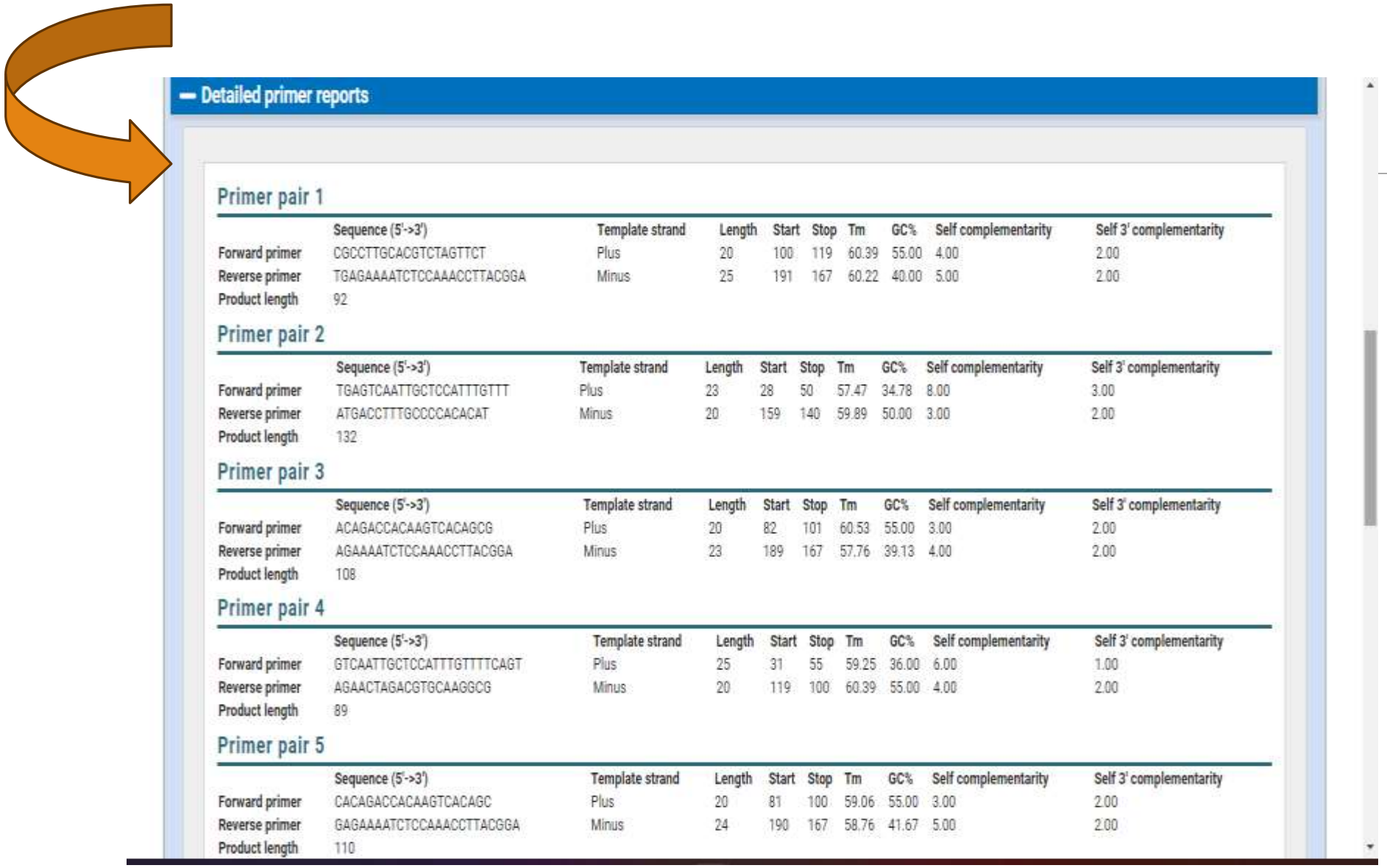
Table 4
CD8+ Treg suppression activity in SSc patients carrying or not the rs7820268 SNP T

Conception d'amorces à l'aide d'un logiciel

Primer-BLAST (NCBI) : https://www.ncbi.nlm.nih.gov/tools/primer-blast/




Primer-Blast results
SNP-NCBI
UCSC Genome Browser Home
Primer-BLAST Results
Input PCR template lcl|Query_1
Range 1 - 201
Specificity of primers Primer pairs are specific to input template as no other targets were found in selected database: Refseq mRNA (Organism limited to Homo sapiens)
Other reports Search Summary
Graphical view of primer pairs
Detailed primer reports



Detailed primer reports

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CGCCTTGCACGTCTAGTTCT	Plus	20	100	119	60.39	55.00	4.00	2.00
Reverse primer	TGAGAAAATCTCCAAACCTTACGGA	Minus	25	191	167	60.22	40.00	5.00	2.00
Product length	92								

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGAGTCAATTGCTCCATTTGTTT	Plus	23	28	50	57.47	34.78	8.00	3.00
Reverse primer	ATGACCTTTGCCCCACACAT	Minus	20	159	140	59.89	50.00	3.00	2.00
Product length	132								

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ACAGACCACAAGTCACAGCG	Plus	20	82	101	60.53	55.00	3.00	2.00
Reverse primer	AGAAAATCTCCAAACCTTACGGA	Minus	23	189	167	57.76	39.13	4.00	2.00
Product length	108								

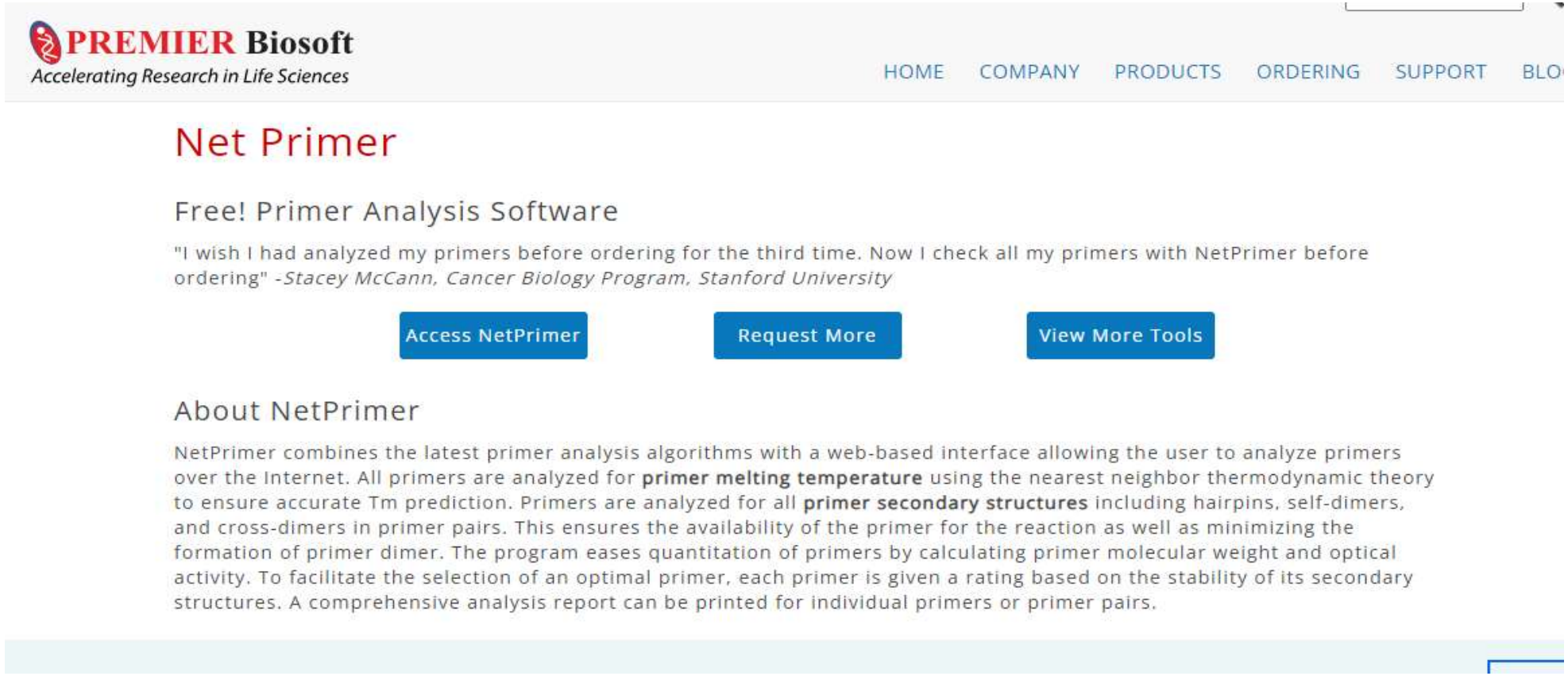
Primer pair 4

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GTCAATTGCTCCATTTGTTTTCAGT	Plus	25	31	55	59.25	36.00	6.00	1.00
Reverse primer	AGAACTAGACGTGCAAGGCG	Minus	20	119	100	60.39	55.00	4.00	2.00
Product length	89								

Primer pair 5

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CACAGACCACAAGTCACAGC	Plus	20	81	100	59.06	55.00	3.00	2.00
Reverse primer	GAGAAAATCTCCAAACCTTACGGA	Minus	24	190	167	58.76	41.67	5.00	2.00
Product length	110								

Net primer


PREMIER Biosoft
Accelerating Research in Life Sciences
HOME
COMPANY
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ORDERING
SUPPORT
Net Primer
Free! Primer Analysis Software
"I wish I had analyzed my primers before ordering for the third time. Now I check all my primers with NetPrimer before ordering" -Stacey McCann, Cancer Biology Program, Stanford University
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Request More
View More Tools
About NetPrimer
NetPrimer combines the latest primer analysis algorithms with a web-based interface allowing the user to analyze primers over the Internet. All primers are analyzed for primer melting temperature using the nearest neighbor thermodynamic theory to ensure accurate Tm prediction. Primers are analyzed for all primer secondary structures including hairpins, self-dimers, and cross-dimers in primer pairs. This ensures the availability of the primer for the reaction as well as minimizing the formation of primer dimer. The program eases quantitation of primers by calculating primer molecular weight and optical activity. To facilitate the selection of an optimal primer, each primer is given a rating based on the stability of its secondary structures. A comprehensive analysis report can be printed for individual primers or primer pairs.

Primer 3

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

☑ 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[CCCC]TCAT.. means that primers 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-TGTCAT.. means that primers must overlap the junction between the C and T.

Réalisation d’une PCR in silico

Réalisation d’une PCR in silico :

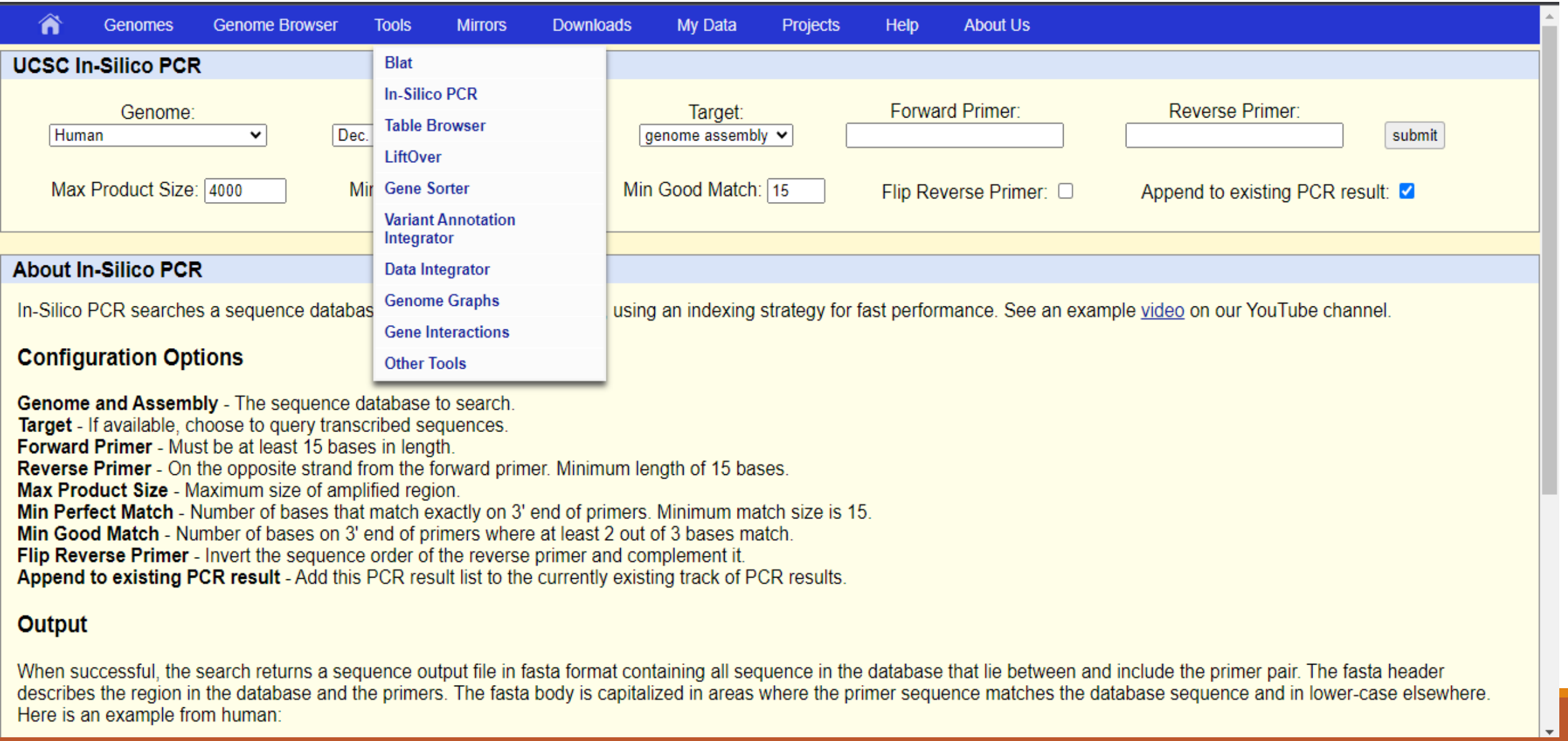
Pour vérifier si nous avons effectué une bonne conception des amorces :

on doit aller vers une PCR in silico élaborèe par l’université de California UCSC

- Cliquer sur l’option Tools
- Choisir l’option in silico PCR

Faire rentrer les amorces synthétisèes

Banque génomique élaborée par l'université de California


Genomes
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Projects
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Gene Sorter
Variant Annotation Integrator
Data Integrator
Genome Graphs
Gene Interactions
Other Tools
UCSC In-Silico PCR
Genome:
Human
Dec.
Target:
genome assembly
Forward Primer:
Reverse Primer:
submit
Max Product Size: 4000
Min Good Match: 15
Flip Reverse Primer:
Append to existing PCR result:
About In-Silico PCR
In-Silico PCR searches a sequence databas
using an indexing strategy for fast performance. See an example video on our YouTube channel.
Configuration Options
Genome and Assembly - The sequence database to search.
Target - If available, choose to query transcribed sequences.
Forward Primer - Must be at least 15 bases in length.
Reverse Primer - On the opposite strand from the forward primer. Minimum length of 15 bases.
Max Product Size - Maximum size of amplified region.
Min Perfect Match - Number of bases that match exactly on 3' end of primers. Minimum match size is 15.
Min Good Match - Number of bases on 3' end of primers where at least 2 out of 3 bases match.
Flip Reverse Primer - Invert the sequence order of the reverse primer and complement it.
Append to existing PCR result - Add this PCR result list to the currently existing track of PCR results.
Output
When successful, the search returns a sequence output file in fasta format containing all sequence in the database that lie between and include the primer pair. The fasta header describes the region in the database and the primers. The fasta body is capitalized in areas where the primer sequence matches the database sequence and in lower-case elsewhere. Here is an example from human:

Genomes | Genome Browser | Tools | Mirrors | Downloads | My Data | Projects | Help | About Us

UCSC In-Silico PCR

Genome: Human | Assembly: Dec. 2013 (GRCh38/hg38) | Target: genome assembly | Forward Primer: | Reverse Primer: | submit

Max Product Size: 4000 | Min Perfect Match: 15 | Min Good Match: 15 | Flip Reverse Primer: ☐ | Append to existing PCR result: ☑

About In-Silico PCR

In-Silico PCR searches a sequence database with a pair of PCR primers, using an indexing strategy for fast performance. See an example video on our YouTube channel.

Configuration Options

Genome and Assembly - The sequence database to search.
Target - If available, choose to query transcribed sequences.
Forward Primer - Must be at least 15 bases in length.
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Résultats d’une PCR in vitro

Génomes | Navigateur de génome | Outils | Miroirs | Téléchargements | Mes données | Projets | Aide | À propos de nous

PCR in silico UCSC

> chr8:39917600+39918311 712pb AGAGTAGTCAGGAGGATT TGCATGATGGCAGTTATAATGC
AGAGTAGTCAGGAGGATTttagtgaaacctttgaggtgaactttgaaaag
ctgcaaatggcagtgtttgaagagagaggaagaggtggaacacagagagg
gtcttcagccctgcgtgtgcagatgacaagacagaggctggtgtttccag
acaggtaagccatatgccagggcaacattgcacagaatggatgtgaaggc
aaggcatactatcagtgggaagccaaatctacaataactgctactactaa
ataaagatcttttttttttttcaaggaaaatctacctgatttttataatg
actggatgttcattgctaaacatctgcctgatctcatagagtctggccag
cttcgagaaagagttgagaaggtttgacatatgtattacatttgtcttct
tgtatagcttcttaacattgttaacttggttttgaagcataaaacattac
tgagattgatttgagtcaattgctccatttgttttcagttaaacatgctc
agcattgatcatctcacagaccacaagtcacagcgccttgcacgtctagt
tctgggatgcatcaccatggcatatgtgtggggcaaaggtcatggagatg
tccgtaaggtttggagattttctcagatttcttatgctatgtgacagatt
ttcatctaatttacatttaactttccaaaaattttctaaaaGCATTATAA
CTGCATCATGCA

Températures de fusion des apprêts

Avant : 45,1 C agagtagtcaggaggatt
Inverse : 57,3 C tgcatgatgcagttataatgc
Les calculs de température sont effectués en supposant une concentration de sel de 50 mM et une concentration d'oligo de recuit de 50 nM. Le code pour calculer la température de fusion provient de Primer3 , la formule de Rychlik W, Spencer WJ et Rhoads RE NAR 1990, qui peut être activée dans Primer3 avec PRIMER_TM_FORMULA=0.

Aide

Qu'est-ce que chr_alt et chr_fix ?
Réplication des résultats de PCR in-Silico sur une machine locale

Commande « Thermofisher »

Merci