PCR Primers Design

Mohammad-Sadegh Fallah, PhD Medical Molecular Genetics Kawsar Human Genetics Research Center

Testing Primer Specificity

- Most important factor affecting PCR: Choosing appropriate primers
- To amplify exactly the intended target and avoiding undesired amplification
- Primers should not have matches to other targets in certain orientations and within certain distances

Process of designing specific primers:

- Generating primers flanking the target region (manually or using software tools)
- Searching primers against an appropriate nucleotide sequence database
 - to examine the potential targets

- to examine many details between primers and targets, such as:
 - number and the positions of matched bases
 - primer orientations and distance between forward and reverse primers
- **Target specificity**: critical primer property

Primers dictate the successfulness of a PCR



- Mismatches towards the 3' end affect target amplification much more than mismatches towards the 5' end
- a two base mismatch at the 3' end generally prevents amplification
- A single base mismatch (even at the very 3' end), as well as a few mismatches in the middle or toward the 5' end, still allows amplification, though at a reduced efficiency for some cases

When is a "primer" a primer?



Primer BLAST

Basic Local Alignment Search Tools

- Searching primers against an appropriate nucleotide sequence database
 - potential targets
 - number and the positions of matched bases
 - primer orientations and distance between forward and reverse primers
- time-consuming and very difficult task for users, especially when the primers have a large number of hits
- Similarity Searching using BLAST program has been widely used for primer target detection
 - to compare a query sequence against a sequence database

Now: Primer Blast

- to design target-specific primers for PCR
- In-Silico PCR and Reverse ePCR vs Primer Blast:
 - Do not design primers
 - Not sensitive enough
 - to detect targets with a significant number of mismatches (potentially amplifiable)

Primer-BLAST

- incorporates BLAST with a global alignment algorithm
- a full primer-target alignment
- sensitive enough to detect targets that have a significant number of mismatches to primers
- design new target-specific primers in one step
- check the specificity of pre-existing primers
- very sensitive in detecting potential amplification targets
- capability to place primers based on exon/intron boundaries
- and excluding SNP sites in primers

http://www.ncbi.nlm.nih.gov/tools/primer-blast

highly sensitive default parameters to find seq potentially amplifiable in PCR

- Detect a target with up to 35% mismatches to the primer sequence
- default BLAST expect value cutoff is 30,000 for the primeronly case (3000 times higher than the standard BLAST program default)
- the higher the expect value cutoff, the more sensitive the search
- word size of seven (standard BLAST uses 11),

- 50,000 for the maximum number of database sequences (standard BLAST uses 250)
- 1 for match reward to mismatch penalty ratio (standard BLAST uses 1.5)

Check primers specificity

- Go to the <u>Primer BLAST</u> submission form.
- Enter one or both primer sequences in the Primer Parameters section of the form
 - If only one primer is available, a template sequence is also required
- In the Primer Pair Specificity Checking Parameters section, select the appropriate source Organism and the smallest Database that is likely to contain the target sequence.
 - For broadest coverage, choose the nr database and do not specify an organism.
- Click the "Get Primers" button to submit the search and retrieve template and specificity information.

PCR Template	Reset page Save search parameters Retrieve recent results	
Enter accession, gi, or FASTA	sequence (A refseq record is preferred) 😡 Clear Range	
	Form To Forward primer O	
Or, upload FASTA file	Browse	
Primer Parameters		
Use my own forward primer (5'>3' on plus strand) Use my own reverse primer (5'>3' on minus strand)	Image: Clear Image: Optimized constraints Image: Optimized constraints	
PCR product size		
# of primers to return	5	
	Min Opt Max Max T _m difference	
Primer melting temperatures (T _m)	57.0 60.0 63.0 3 9	
Exon/intron selection	A refseq mRNA sequence as PCR template input is required for options in the section 😡	
Exon junction span	No preference	
Exon junction match	Exon at 5' side Exon at 3' side	
International International	Minimai number of bases that must anneal to exons at the 5 or 3 side of the junction 😈	
Intron Inclusion	Primer pair must be separated by at least one intron on the corresponding genomic DNA	
ind on length range	1000 1000000 S	
		5
Primer Pair Specificity C	acking Parameters	(
Specificity check	Enable search for primer pairs specific to the intended PC ² template	
Database	Refseq mRNA	t
organism	Homo sapiens	_
	Add more organisms	5
Exclusion (opional)	🗆 Exclude predicted Refseq transcripts (accession with XM, XR prefix) 🗖 Exclude uncultured/environmental sample sequences 🥹	
Entrez query (optional)	9	
Primer specificity stringency	Primer must have at least 2 🗾 total mismatches to unintended targets, including	
	at least 2 💌 mismatches within the last 5 💌 bps at the 3' end. 🥹	
	Ignore targets that have 6 💌 or more mismatches to the primer. 🥹	C
Misprimed product size deviation	4000 💿	C
Splice variant handling	Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR template input) 😡	S
Get Primers	Show results in a new window Use new graphic view 🗕	t

Primer BLAST submission form.

Enter one or both primer sequences in the Primer Parameters section of the form

Primer Pair Specificity Checking Parameters section

Selecting the appropriate source Organism and the smallest Database that is likely to contain the target sequence.

Click the "Get Primers" button to submit the search and retrieve template and specificity information

Inter-BLAST A tool for finding specific primers Primer-BLAST: Finding primers specific to your PCR template (using Primer3 and BLAST). More Tips for finding specific primers PCR Template Resetbace Save search parameters Retrieve recent results Primer accession, gl, or FASTA sequence (A refseq record is preferred) (Primer Parameters) Range Or, upload FASTA file Choose File No file chosen Primer Parameters Use my own forward primer (5'-3' on plus strand) AGGTAGAAACGCCGGATCTCCTTACA (Primer Cear Min Max Min Opt Min Opt Min Opt Min Opt Min Opt	
Primer-BLAST: Finding primers specific to your PCR template (using Primer3 and BLAST). More Tips for finding specific primers PCR Template Enter accession, gi, or FASTA sequence (A refseq record is preferred) Cear Forward primer From To Forward primer From To Forward primer Cor, upload FASTA file Choose File No file chosen Primer Parameters Use my own forward primer (S'>3' on plus strand) Sequence AGGCTAACAGAAGGATTTGGTAGGTGTGCC Cear Min Max PCR product size for minus strand) Min Max Max Tm difference Min Opt Max Max Tm difference	ner-BLASI
Reset page Save search parameters Retrieve recent results PCR Template Reset page Save search parameters Retrieve recent results Enter accession, gi, or FASTA sequence (A refseq record is preferred) @ Clear Range Forward primer To @ Clear Pormer Parameters Reverse primer @ Clear Use my own forward primer (5'>3' on plus strand) AGGTAGAAACGCCGGATCTCCTTACA @ Clear Min Max Clear Min Opt Max Max Tm difference	 rimer-BLAST: Finding primers specific
PCR Template Interview Range Enter accession, gi, or FASTA sequence (A refseq record is preferred) (a) Clear Range Forward primer [] (a) Clear Forward primer [] (a) Clear Or, upload FASTA file Choose File No file chosen [] (a) Clear Primer Parameters [] (b) Clear Use my own forward primer (6'->3' on plus strand) [] (AGGTAGAAACGCCGGGATCTCCTTACA Use my own reverse primer (6'->3' on minus strand) [] (a) Clear PCR product size [] (a) (a) (a) # of primers to return [] (a) (b) (b) Min [] (b) (a) (b) (a) (b) (a) (b) Min [] (b) (a) (b) (a) (b) (a) (b) Min [] (b) (a) (b) (a) (b) (a) (b) (a) (b) Min [] (b) (a) (a) (b) (a) (a) (b) (a) (a) (b) (a) (a) (a) (b) (a) (a) (b) (a) (a) (a) (a) (a) (a) (a) (a) (a) (a	Reset of
Enter accession, gi, or FASTA sequence (A refseq record is preferred) Clear Range From To Forward primer From Primer Parameters Use my own forward primer (5'->3' on minus strand) PCR product size 70 1000 # of primers to return 5 Min Min Opt Max Max Tm difference	PCR Template
Or, upload FASTA file Choose File No file chosen Primer Parameters Use my own forward primer (5'~3' on plus strand) Use my own reverse primer (5'~3' on minus strand) PCR product size # of primers to return 5 Min Opt Max Max Max Min Opt Max Max Min Opt Max Max Max Max	Enter accession, gi, or FASTA sequen
Or, upload FASTA file Choose File No file chosen Primer Parameters Use my own forward primer (5'~3' on plus strand) Use my own reverse primer (5'~3' on minus strand) GAGCTAACAGAGGATCTCCTTACA Ø Clear (6'~3' on plus strand) Use my own reverse primer (5'~3' on plus strand) PCR product size 70 1000 # of primers to return 5 Min Opt Max Max Tm difference	
Or, upload FASTA file Choose File No file chosen Primer Parameters Use my own forward primer (5'->3' on plus strand) (5'->3' on minus strand) Min Min Min Min Min Min 0pt Max Max Tm difference	
Or, upload FASTA file Choose File No file chosen Primer Parameters AGGTAGAAACGCCGGATCTCCTTACA Clear Use my own forward primer (5'>3' on plus strand) AGGTAGAAACGCCGGATCTCCTTACA Clear Use my own reverse primer (5'>3' on minus strand) GAGCTAACAGAGGATTTGGTAGGTGTGC Clear Min Max Max PCR product size 70 1000 # of primers to return 5 Min Min Opt Max Min Opt Max Tm difference	
Primer Parameters Use my own forward primer (5'->3' on plus strand) Use my own reverse primer (5'->3' on minus strand) AGGTAGAAACGCCGGATCTCCTTACA GAGCTAACAGAGGATTTGGTAGGTGTGC Min Min Min Min Min Min Min Min Min Opt Max Max Tm difference	Or, upload FASTA file
Primer Parameters Use my own forward primer (5'->3' on plus strand) Use my own reverse primer (5'->3' on minus strand) AGGTAGAAACGCCGGATCTCCTTACA () Clear GAGCTAACAGAGGATTTGGTAGGTGTGC () Clear Min Max PCR product size 70 1000 # of primers to return 5 Min Opt Max Max Max Tm difference	
Use my own forward primer (5'>3' on plus strand) AGGTAGAAACGCCGGATCTCCTTACA Image: Clear Use my own reverse primer (5'>3' on minus strand) GAGCTAACAGAGGATTTGGTAGGTGTGC Clear Min Max PCR product size 70 1000 # of primers to return 5 Min Opt Max Max Max Tm difference	Primer Parameters
(5'>3' on plus strand) Controcercoconcreter race Clear Use my own reverse primer (5'>3' on minus strand) GAGCTAACAGAGGATTTGGTAGGTGTGC Clear Min Max PCR product size 70 1000 # of primers to return 5 Min Opt Max Min Opt Max	Jse my own forward primer
Use my own reverse primer (5'->3' on minus strand) GAGCTAACAGAGGATTTGGTAGGTGTGC Clear Min Max PCR product size 70 1000 # of primers to return 5 Min Opt Max Max Max	5'->3' on plus strand)
Min Max PCR product size 70 1000 # of primers to return 5 Min Opt Max Max Max Tm difference	5'->3' on minus strand)
PCR product size 70 1000 # of primers to return 5 Min Opt Max Max Tm difference	Min
# of primers to return 5 Min Opt Max Max Tm difference	PCR product size 70
Min Opt Max Max Tm difference	of primers to return 5
	Min
Primer melting temperatures 57.0 60.0 63.0 3	Primer melting temperatures 57.0

www.ncbi.nlm.nih.gov/tools/primer-blast/ C Exon/intron selection A refseq mRNA sequence as PCR template input is required for options in the section 📦 Exon junction span No preference ¥ 0 Exon junction match Exon at 5' side Exon at 3' side 7 4 Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction 👔 Intron inclusion Primer pair must be separated by at least one intron on the corresponding genomic DNA (a) Intron length range

	Note: Parameter values that differ from the default are highlighted in yellow
Primer Pair Specificity Ch	ecking Parameters
Specificity check	🗹 Enable search for primer pairs specific to the intended PCR template 🥹
Database	Genome (reference assembly from selected organisms) 💌 😡
Organism	Homo sapiens
	Enter an organism name, taxonomy id or select from the suggestion list as you type. 🔞
	Add more organisms
Exclusion (opional)	🗖 Exclude predicted Refseq transcripts (accession with XM, XR prefix) 🗖 Exclude uncultured/environmental sample sequences 🛞
Entrez query (optional)	
Primer specificity stringency	Primer must have at least 2 🚽 total mismatches to unintended targets, including
	at least 🛛 💌 mismatches within the last 5 💌 bps at the 3' end. 🕢
	Ignore targets that have 🛛 6 💌 or more mismatches to the primer. 🨡
Misprimed product size	4000
Splice variant handling	Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR template input) 🕑
Get Primers	🗹 Show results in a new window 🗹 Use new graphic view 📦

Advanced parameters

Note: Parameter values that differ from the default are highlighted in yellow

opyright | Disclaimer | Privacy | Accessibility | Contact | Send feedback on new interface

Min

1000

Max

1000000 😡

Checking the specificity of pre-existing primers

Primer pair 1

Se	equence (5'->3')	Length	Tm	GC%	Self complementari	ty Self 3' complementarity
Forward primer G	TAGGACTGCTCAGTTCAAACAT	23	52.81	43.48	6.00	2.00
Reverse primer A	CAGTTACTACACCCGTAAGGC	22	53.90	50.00	5.00	2.00
Products on target > <u>NM 001098494.1</u> product length Forward primer Template Reverse primer Template product length Forward primer Template	templates Homo sapiens zinc finger protein 4 = 444 1 GTAGGACTGCTCAGTTCAA 493 1 ACAGTTACTACACCCGTAA 936 = 780 1 GTAGGACTGCTCAGTTCAA 493	419 (ZNF4 ACAT 2 5 AGGC 22 91 ACAT 2 5	119), trar 3 15 5 3 15	ascript v	rariant 5, mRNA	 Default Perfect transcripting generate Some of 780 base
Reverse primer Template > <u>NM 001098491.1</u> product length Forward primer Template Reverse primer Template	1 ACAGTTACTACACCCCGTA 1272 CGGTT Homo sapiens zinc finger protein 4 = 444 1 GTAGGACTGCTCAGTTCAA 592	AGGC 2. 1. 419 (ZNF4 ACAT 2. 6 AGGC 2. 1.	2 251 19), trar 3 14 2 014	iscript v	variant 1, mRNA	 444 ba human access Howey
product length Forward primer Template Reverse primer Template	= 780 1 GTAGGACTGCTCAGTTCAA 592 1 ACAGTTACTACACCCGTA 1371CGGTT	ACAT 2. 6 AGGC 2: 1	3 14 2 350			the tary to prev non-sp
> <u>NM 198542.1</u> Hon product length Forward primer Template Reverse primer	ano sapiens zinc finger protein 773 = 444 1 GTAGGACTGCTCAGTTCAA 453A.GCA 1 ACAGTTACTACACCCGTAA	(ZNF773) ACAT 2 G. 4 AGGC 22	, mRNA 3 75			Users ca make ju experimentary

specificity parameters matches to the ZNF419 gene pt variant 5 and would e a 444 base amplicon.

ther potential amplicons:

- ase amplicon: in ZNF419 transcript
- ase amplicon: a different gene (i.e., n zinc finger protein 773, Genbank sion NM_198542.1)
- ver, there are up to 5 mismatches en at least one of the primers and gets, which is probably sufficient vent amplification interference or pecific amplification

an analyze this result and adgment based on their own experimental experiences.

Comparison to other primer design tools

- offers the ability to specify the **number of mismatches** that a specific primer pair must have to unintended targets
- custom 3' end region: number of mismatches must be present
- the specificity of a primer is typically judged by the number of mismatches it has to unintended targets
 - higher number of mismatches offer more specificity

- the locations of such mismatches (mismatches closer to 3' end offer more specificity)
- place primers on different exons (i.e., to span an intron) to avoid amplification of genomic DNA
- customization of the number of nucleotide matches on either side of an exon/exon junction
- Primer-BLAST presents detailed alignments between the primers and targets found

- high detection sensitivity
- capable of detecting potential amplification targets that have up to 5 mismatches to a primer
- by using highly sensitive BLAST parameters
- Primer-BLAST (with default parameters) will miss any targets that have 6 or fewer consecutive matches to a primer (since Primer-BLAST uses a word size of 7 by default).
 - For example, if a target has mismatches to a primer of 20 bases at positions 7 and 14 (assuming the 5' end is position one), the target will be missed by Primer-BLAST (with default parameters) even though it has only 2 mismatches.
 - Assuming a random distribution of mismatch locations, it is possible to calculate the number of possible arrangements of 18 matches and 2 mismatches.
 - There are 20*19 different ways to place the 2 mismatches among the 18 matches, but only 2 of these result in a word size shorter than 7, so the probability of missing a target with 2 mismatches to a primer of 20 bases is 2/(20*19) or about 0.5%.

← -	← → C 🗋 www.ncbi.nlm.nih.gov/tools/primer-blast/primertool.cgi?ctg_time=1361705656&job_key=JSID_01_88396_130.14.22.21_9003							€ ☆	-	
2	Primer-BLAST		Primer-Blast res	sults						
► NC	BI/ Primer-BLAST : results: Jok	id=JSID_01_88396	_130.14.22.21_9003 more							
	Input PCR template Specificity of primers Other reports	none Target templa Homo sapiens) ▶ <u>Search Sum</u> r	es were found in selected datab nary	oase: Genome	databa	se (ref	erence assembly only) for	selected species (Organism limited to		
	Detailed primer repor	<u>ts</u>								
	Primer pair 1									
		Sequence (5'->:	3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity		
	Forward primer	AGGTAGAAACG	CCGGATCTCCTTACA	26	65.17	50.00	7.00	1.00		
	Reverse primer	GAGCTAACAGA	GGATTTGGTAGGTGTGC	28	65.25	50.00	5.00	2.00		
	Products on targe	t templates								
	> <u>NT_030059.13</u> Horr	io sapiens chromos	ome 10 genomic contig, GRCh37	.p10 Primary A	ssembly	(
	product lengt	h = 241								
	Features asso <u>cytochrome</u>	ciated with <u>P450 2C9 p</u> r	this product: <u>ecursor</u>							
	Forward prime	r 1 47506464	AGGTAGAAACGCCGGATCTC	CTTACA 2	6 75064:	39				
	Reverse prime Template	r 1 47506224	GAGCTAACAGAGGATTTGGT	AGGTGTGC	28 4750	6251				

Primer pair 1							
	Sequence (5'->	3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AGGTAGAAACG	CCGGATCTCCTTACA	26	65.17	50.00	7.00	1.00
Reverse primer	GAGCTAACAGA	GGATTTGGTAGGTGTGC	28	65.25	50.00	5.00	2.00
Products on target	templates						
> <u>NT_030059.13</u> Homo	sapiens chromo:	some 10 genomic contig, GRCh37.	p10 Primary A	Assembl	У		
product length	= 241						
Features assoc cytochrome	iated with <u>P450 2C9 pr</u>	this product: <u>ecursor</u>					
Forward primer	imer 1 AGGTAGAAACGCCGGATCTCCTTACA 26						
Template	47506464GGGGC 47506439						
Reverse primer	1	GAGCTAACAGAGGATTTGGTA	AGGTGTGC	28			
Template	47506224			4750	6251		
product length	= 239						
Features assoc cytochrome	iated with <u>P450 2C19 p</u>	this product: recursor					
Forward primer	1	AGGTAGAAACGCCGGATCTCC	CTTACA 2	6			
Template	47339662	G	C 4	73396	37		
Reverse primer	1	GAGCTAACAGAGGATTTGGTA	AGGTGTGC	28			
Template	47339424			4733	9451		

rimer-BLAST) id=JSID_01_884	Primer-Blast n 05_130.14.22.21_9003 more	esults				
Input PCR template pecificity of primers Other reports Detailed primer repor	none Target temp Homo sapien ▶ <u>Search Su</u> ts	lates were found in selected dat. s) <u>mmary</u>	abase: Geno	me data	ıbase (r	eference assembly only) fo	r selected species (Organism limited
Primer pair 1					262214-10		
Forward astronom	Sequence (5'	' ->3')	Length	Tm CE 47	GC%	Self complementarity	Self 3' complementarity
Forward primer	GIGIGIGC	TOTTEGATEGATAAA	26 24	59.17 59.57	20.00	7.00	1.00
Broducts on targe	t tomplator						
NT 022778 16 Hon	n saniens chrom	osome 4 genomic contig. GPCb37	n10 Primary	Assemb	สง		
product lengt Features flan <u>64708 bp a</u> <u>849323 bp</u>	h = 733 king this p <u>t 5' side:</u> at 3' side:	product: trans-2,3-enoyl-CoA ro ephrin type-A recepto	eductase-	<u>-like</u> Form k) pred	cursor	
	r 1	CHCHCHCCCCHCHHHCAHCCA	1 000 24				
DAVARGA PRIMA	En (En)	GIGIGIGIGCCICITIGATGGA	1777 24 EEI	51152			
Template	5551175	, . A. A T A A		JTTC			
Reverse prime Template	5551175	A.ATAA		11102			

• NCBI/ Primer-BLAST : results: Job id=JSID_01_88578_130.14.18.128_9003 more...

put PCR template none cificity of primers Target templates were found in selected database: Genome database (reference assembly only) for selected species (Organism limited Homo sapiens)								
Other reports	▶ <u>Search Sum</u>	mary						
uled primer repor	<u>ts</u>							
Primer pair 1								
	Sequence (5'->	-3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	AGAAGGAGCA	TATAGTGGGCCTAGGT	26	64.38	50.00	6.00	4.00	
Reverse primer	GTGTGTGCCT	CTTTGATGGATAAA	24	59.54	41.67	4.00	4.00	
> <u>NT_030059.13</u> Hor product lengt:	o sapiens chromo: h = 60	some 10 genomic contig, GRCh	137.p10 Primary	/ Assem	bly			
Features flan <u>27127 bp a</u> <u>76 bp at 3</u>	king this pr <u>t 5' side: c</u> ' side: cyto	oduct: ytochrome P450 2C18 chrome P450 2C19 pre	<u>isoform 2</u> ecursor	prec	ursor	1		
Forward prime	r 1	AGAAGGAGCATATAGTGG	GCCTAGGT	26				
Template	47326791			47326	816			
Reverse prime	r 1	GIGIGIGCCTCTTTGATG	GATAAA 24					
Template	47326850		47	32682	7			

product length = 60 Features flanking this product: 85636 bp at 5' side: cytochrome P450 2C19 precursor 74 bp at 3' side: cytochrome P450 2C9 precursor Forward primer 1 AGAAGGAGCATATAGTGGGCCTAGGT 26 Template Reverse primer 1 GTGTGTGCCCCCCTTTGATGGATAAA 24 Template >NT 022778.16 Homo sapiens chromosome 4 genomic contig, GRCh37.p10 Primary Assembly product length = 733Features flanking this product: 64708 bp at 5' side: trans-2,3-enoyl-CoA reductase-like 849323 bp at 3' side: ephrin type-A receptor 5 isoform b precursor Reverse primer 1 GTGTGTGCCTCTTTGATGGATAAA 24 Template 5551175 ...A.A....T..A..A..... 5551152 Reverse primer 1 GTGTGTGCCTCTTTGATGGATAAA 24 Template 5550443 ...A.A.....T..A..A...... 5550466

-						-
$\boldsymbol{\nu}$	M 11	m	or.	na	112	
			U	pu		- -

	Sequence (5'->3	P)	Length	Tm	GC%	Self complementarity	Self 3' complementarity		
Forward primer	AGAAGGAGCAT	ATAGTGGGCCTAGGT	26	64.38	50.00	6.00	4.00		
Reverse primer	TATCTGTAGGA	GGATATTTCCAATCACTGGGA 29 61.32 37.93 6.00 5.00							
Products on target	templates								
> <u>NT_030059.13</u> Homo	sapiens chromos	ome 10 genomic contig, GRCh37.p10	Primary A	ssembly	/				
product length	= 271								
Features assoc: <u>cytochrome</u>	iated with [.] P450 2C19 p	this product: <u>recursor</u>							
Forward primer	lmer 1 AGAAGGAGCATATAGTGGGCCTAGGT 26								
Template	47326791		4′	73268	16				
Reverse primer	1	TATCTGTAGGATATTTCCAATCA	CTGGGA	29					
Template	47327061			473:	27033				
product length	= 269								
Features assoc: <u>cytochrome</u>	iated with <u>P450 2C9 pr</u>	this product: <u>ecursor</u>							
Forward primer	1	AGAAGGAGCATATAGTGGGCCTA	GGT 20	6					
Template	47502770	A	47	75027	95				
Reverse primer	1	TATCTGTAGGATATTTCCAATCA	CTGGGA	29					
Template	47503038			475	03010				

Primer pair 1

	Sequence (5'->3	')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TTTCAAATGGGA	VAAAGGGAGACCCTGG	27	65.12	48.15	6.00	3.00
Reverse primer	TTCTCATGAGCA	ATCTCTGGGGCTGTTTTCC	30	68.26	50.00	8.00	0.00
Products on target (emplates						
> <u>NT_030059.13</u> Homo	sapiens chromos	ome 10 genomic contig, GRCh37.p10 F	Primary As	sembly			
product length	= 329						
Features flank: 26264 bp at 670 bp at 3	ing this pro 5' side: cy ' side: cyt	oduct: ytochrome P450 2C18 isofo ochrome P450 2C19 precurs	rm 2 p: or	recur	<u>sor</u>		
Forward primer Template	1 47326256	TTTCAAATGGGAAAAGGGAGACCC	тдд 2' 4'	7 73262	30		
Reverse primer	1	TTCTCATGAGCATCTCTGGGGGCTG	TTTTCC	30			
Template	47325928	3 een een een een een eer		473	25957	7	
product length Features flank: <u>84809 bp at</u> <u>671 bp at 3</u>	= 290 ing this pro <u>5' side: cy</u> to 'side: cyto	oduct: ytochrome P450 2C19 precu ochrome P450 2C9 precurso	r <u>sor</u>				
Forward primer	1	TTTCAAATGGGAAAAGGGAGACCC	TGG 2	7			
Template	47502232		4'	75022	06		
Reverse primer	1	TTCTCATGAGCATCTCTGGGGGCTG	TTTTCC	30			
Template	47501943	T	c	475	01972	2	

Primer-BLAST

Primer-Blast results

NCBI/ Primer-BLAST : results: Job id=JSID_01_88610_130.14.18.128_9003 more...

I Please correct the following errors: Specified right primer exceeds built-in maximum of 36.

	Sequence (5'->3))	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TTTCAAATGGG	AAAAGGGAGACCCTGG	27	65.12	48.15	6.00	3.00
Reverse primer	TTCTCATGAGC/	ATCTCTGGG	20	56.70	50.00	8.00	4.00
Products on target t	emplates						
> <u>NT_030059.13</u> Homo	sapiens chromos	ome 10 genomic contig, GRCh37.p1	10 Primary	Assemb	ly		
product length Features flanki <u>26264 bp at</u> <u>670 bp at 3'</u>	= 329 .ng this pro <u>5' side: cy</u> side: cyto	oduct: <u>ytochrome P450 2C18 iso</u> ochrome P450 2C19 precu	form 2 ursor	preci	irsor		
Forward primer Template	1 47326256	TTTCAAATGGGAAAAGGGAGAC	CCTGG	27 47326	5230		
Reverse primer Template	1 47325928	TTCTCATGAGCATCTCTGGG	20 4732594	47			
product length Features flanki <u>84809 bp at</u> <u>671 bp at 3'</u>	= 290 .ng this pro <u>5' side: cy</u> side: cyto	oduct: /tochrome P450 2C19 pre ochrome P450 2C9 precur	<u>cursor</u> sor				
Forward primer Template	1 47502232	TTTCAAATGGGAAAAGGGAGAC	CCTGG	27 47502	206		
Reverse primer Template	1 47501943	TTCTCATGAGCATCTCTGGG	20 4750190	52			

```
product length = 295
Features flanking this product:
  20855 bp at 5' side: cytochrome P450 2C19 precursor
  64620 bp at 3' side: cytochrome P450 2C9 precursor
Forward primer 1
                       TTTCAAATGGGAAAAGGGAGACCCTGG 27
Template
              47437989 ..... 47438015
Reverse primer 1
                       TTCTCATGAGCATCTCTGGG 20
Template
              >NT_011512.11 Homo sapiens chromosome 21 genomic contig, GRCh37.p10 Primary Assembly
product length = 4870
Features flanking this product:
  195895 bp at 5' side: protein C-ets-2 isoform 1
  151939 bp at 3' side: proteasome assembly chaperone 1 isoform a
Reverse primer 1 TTCTCATGAGCATCTCTGGG 20
Template
              26057447 C.....G..T...G.... 26057428
Reverse primer 1 TTCTCATGAGCATCTCTGGG 20
              26052578 .G...C.A..... 26052597
Template
NT_008183.19 Homo sapiens chromosome 8 genomic contig, GRCh37.p10 Primary Assembly
product length = 1041
Features flanking this product:
```

17127 bp at 5' side: hydroxyacid-oxoacid transhydrogenase, mitochondrial 7130 bp at 3' side: uncharacterized protein C8orf46 >NT_011512.11 Homo sapiens chromosome 21 genomic contig, GRCh37.p10 Primary Assembly

```
product length = 4870
Features flanking this product:
  195895 bp at 5' side: protein C-ets-2 isoform 1
  151939 bp at 3' side: proteasome assembly chaperone 1 isoform a
Reverse primer 1
                         TTCTCATGAGCATCTCTGGG 20
                                              26057428
Template
               26057447 C....G..T...G...
Reverse primer 1
                       TTCTCATGAGCATCTCTGGG 20
Template
               26052578 .G...C.A..... 26052597
>NT_008183.19 Homo sapiens chromosome 8 genomic contig, GRCh37.p10 Primary Assembly
product length = 1041
Features flanking this product:
  17127 bp at 5' side: hydroxyacid-oxoacid transhydrogenase, mitochondrial
  7130 bp at 3' side: uncharacterized protein C8orf46
Reverse primer 1
                    TTCTCATGAGCATCTCTGGG
                                              20
Template
               19263154 GCAG...C.....
                                              19263135
Reverse primer 1
                         TTCTCATGAGCATCTCTGGG 20
```

19262114 AA.A....C...T 19262133

Template

					0_01_0	o, h_130.11.10.120_3003		
Primer-BLAST		Primer-Blast	results					
I/ Primer-BLAST : results:	Job id=JSID 01 907	47 130.14.18.128 9003 more						
Input PCR template	none							
Specificity of primers	l arget templ Homo sanien	lates were found in selected da s)	itabase: Genor	ne data	base (re	eterence assembly only) to	r selected species (Organism limited to	0
Other reports	▶ <u>Search Su</u>	<u>mmary</u>						
Detailed primer rep	orts							
Drimer pair	i.							
T Timer pui	Sequence (5'	->3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	AAATGAGGA	ACAATCAGGAGACGGCA	26	64.61	46.15	3.00	0.00	
Reverse primer	ATCTTGGGA	TGCTGAGTGCCTGGAGT	26	67.61	53.85	3.00	1.00	
Products on tai	get templates							
> <u>NT_004487.19</u> H	lomo sapiens chrom	osome 1 genomic contig, GRCh3	37.p10 Primary.	Assemb	ly			
product len	gth = 793							
PAST11FAC SC	sociated with	this product:						
filaggri	<u>n</u>							
Forward pri	n ner 1	AAATGAGGAACAATCAGGA	GACGGCA 2	6				
Forward pri Template	ner 1 3774288	AAATGAGGAACAATCAGGA	GACGGCA 2	6 77426	3			
Forward pri Template Reverse pri	ner 1 3774288 ner 1	AAATGAGGAACAATCAGGA	GACGGCA 2 3 CTGGAGT 2	6 77426	3			

```
product length = 4684
Features associated with this product:
  filaggrin
Forward primer 1 AAATGAGGAACAATCAGGAGACGGCA 26
Template 3774288
                                        3774263
                   Reverse primer 1 ATCTTGGGATGCTGAGTGCCTGGAGT 26
Template 3769605 C....C 3769630
product length = 1765
Features associated with this product:
  filaggrin
Forward primer 1 AAATGAGGAACAATCAGGAGACGGCA 26
      Template
                                        3774263
Reverse primer 1 ATCTTGGGATGCTGAGTGCCTGGAGT 26
Template 3772524 C....C....C.....C 3772549
product length = 3709
Features associated with this product:
  filaggrin
Forward primer 1
                  AAATGAGGAACAATCAGGAGACGGCA 26
Template 3774288
                                        3774263
                   Reverse primer 1 ATCTTGGGATGCTGAGTGCCTGGAGT
                                       26
```

3770580 C.....C.....C

3770605

Template

product length = 1765
Features associated with this product:
 <u>filaggrin</u>

Forward primer	1	AAATGAGGAACAATCAGGAGACGGCA	26
Template	3771369	T	3771344
Reverse primer	1	ATCTTGGGATGCTGAGTGCCTGGAGT	26
Template	3769605	CC	3769630

product length = 3709
Features associated with this product:
 <u>filaggrin</u>

Forward primer	1	AAATGAGGAACAATCAGGAGACGGCA	26
Template	3771369	$\ldots \ldots \mathbb{T}$	3771344
Reverse primer	1	ATCTTGGGATGCTGAGTGCCTGGAGT	26
Template	3767661	GC	3767686

```
product length = 790
Features associated with this product:
    <u>filaggrin</u>
```

Forward primer	1	AAATGAGGAACAATCAGGAGACGGCA	26
Template	3771369	T	3771344
Reverse primer	1	ATCTTGGGATGCTGAGTGCCTGGAGT	26
Template	3770580	CCC	3770605

```
product length = 4681
Features associated with this product:
  filaggrin
Forward primer 1 AAATGAGGAACAATCAGGAGACGGCA
                                         26
Template
                   3771369
                                         3771344
Reverse primer
                                         26
            1
                   ATCTTGGGATGCTGAGTGCCTGGAGT
Template
            3766689
                   G.A..... 3766714
product length = 2737
Features associated with this product:
  filaggrin
Forward primer 1
                   AAATGAGGAACAATCAGGAGACGGCA 26
            3771369
Template
                                         3771344
                   Reverse primer 1
                   ATCTTGGGATGCTGAGTGCCTGGAGT 26
Template
            3768633 C....C.T....C 3768658
product length = 790
Features associated with this product:
  filaggrin
Forward primer 1
                   AAATGAGGAACAATCAGGAGACGGCA 26
Template
            3767478
                   Τ....Τ
                                         3767453
Reverse primer 1
              ATCTTGGGATGCTGAGTGCCTGGAGT 26
Template
                                         3766714
            3766689
                   G.A...........
```

```
product length = 1762
Features associated with this product:
    <u>filaggrin</u>
```

Forward primer	1	AAATGAGGAACAATCAGGAGACGGCA	26
Template	3767478	ΤΤ	3767453
Reverse primer	1	ATCTTGGGATGCTGAGTGCCTGGAGT	26
Template	3765717	CCC	3765742

```
product length = 2734
Features associated with this product:
    <u>filaggrin</u>
```

Forward primer	1	AAATGAGGAACAATCAGGAGACGGCA	26
Template	3767478	$\mathtt{T},\ldots,\mathtt{T}$	3767453
Reverse primer	1	ATCTTGGGATGCTGAGTGCCTGGAGT	26
Template	3764745	CCC	3764770

```
product length = 3709
Features associated with this product:
    <u>filaggrin</u>
```

Forward primer	1	AAATGAGGAACAATCAGGAGACGGCA	26
Template	3773313	TT	3773288
Reverse primer	1	ATCTTGGGATGCTGAGTGCCTGGAGT	26
Template	3769605	CC	3769630

```
product length = 793
Features associated with this product:
    <u>filaggrin</u>
```

Forward primer	1	AAATGAGGAACAATCAGGAGACGGCA	26
Template	3770397	ΤΤ	3770372
Reverse primer	1	ATCTTGGGATGCTGAGTGCCTGGAGT	26
Template	3769605	CC	3769630

```
product length = 2737
Features associated with this product:
    <u>filaggrin</u>
```

Forward primer	1	AAATGAGGAACAATCAGGAGACGGCA	26
Template	3770397	$\mathtt{T}\ldots\ldots\mathtt{A}\ldots\ldots\mathtt{T}$	3770372
Reverse primer	1	ATCTTGGGATGCTGAGTGCCTGGAGT	26
Template	3767661	GC	3767686

```
product length = 790
Features associated with this product:
    <u>filaggrin</u>
```

Forward primer	1	AAATGAGGAACAATCAGGAGACGGCA	26
Template	3773313	TT	3773288
Reverse primer	1	ATCTTGGGATGCTGAGTGCCTGGAGT	26
Template	3772524	CCC	3772549

UCSC In Silico PCR

Testing Primer Specificity

In-Silico PCR:

UCSC In-Silico PC	CR			
Genome: Human	Assembly: Mar. 2006 💌	Forward Primer:	Reverse Primer:	submit
				19 - 19 - 19

Configuration Ontions

- Select genome
- Enter primers
- Minimum 15 bases
- Flip reverse primer?
- Submit

Genome and Assembly - The sequence database to search.
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.

(note: the tool does not handle ambiguous bases at this time—don't use Ns)

Home	Genomes	Blat	Tables	Gene Sorter	Session	FAQ	Help		
UCSC	In-Silico PO	CR							
Genome: Mouse			Assembly: Jul. 2007 Min	♥ Perfect Ma] tch: 18	Forward Primer: GCACCACCAaCTGCTT Min Go	Reverse Primer: GGATGCAGGGATGATG ood Match: 18	submit Flip Reverse Primer:	
A 1 4	L. Ciller D	CD							

About In-Silico PCR

In-Silico PCR searches a sequence database with a pair of PCR primers, using an indexing strategy for fast performance.

Configuration Options

Genome and Assembly - The sequence database to search.
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.

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:

>chr22:31000551+31001000 TAACAGATTGATGATGCATGAAATGGG CCCATGAGTGGCTCCTAAAGCAGCTGC

```
tatgacagctgaagttttccaggggctgatggtgagccagtgagggtaag
```

```
tacacagaacatcctagagaaaccctcattccttaaagattaaaaataaa
```



About In-Silico PCR

- In-Silico PCR searches a sequence database with a pair of PCR primers
- Configuration Options
 - Genome and Assembly: The sequence database to search
 - 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
 - **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.

- 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.
- The + between the coordinates in the fasta header indicates this is on the positive strand.

In-Silico PCR Results



- Genomic location shown, links to Genome Viewer
- Product size shown
- Your primers displayed, flipped if necessary
 - **Predicted genomic sequence shown**
- Primer molting temperatures provided

Copyright OpenHelix. No use or reproduction without express written consent

41

In Silico PCR Results



Primer Melting Temperatures

Forward: 66.7 C taacagattgatgatgatgatgatggg Reverse: 73.8 C cccatgagtggctcctaaagcagctgc The temperature calculations are done assuming 50 mM salt and 50 nM annealing oligo concentration. The code to calculate the melting temp comes from <u>Primer3</u>.

Melting temperature

		move moo o									
~	and the second second	more into o	2011 C. 101		- Antonio Statements	805000 (KR)	AND THE REAL PROPERTY.	ana an			
n	Genomes	Genome Browser	Tools	Mirrors	Downloads	My Data	About Us	Help			
UCSC Ir	JCSC In-Silico PCR										
No match	No matches to aggtagaaacgccggatctccttaca aggtagaaacgccggatctccttaca in Human Feb. 2009 (GRCh37/hg19)										
Primer Melting Temperatures											

Forward: 67.8 C aggtagaaacgccggatctccttaca Reverse: 67.8 C aggtagaaacgccggatctccttaca The temperature calculations are done assuming 50 mM salt and 50 nM annealing oligo concentration. The code to calculate the melting temp comes from <u>Primer3</u>.

UCSC In-Silico PCR

><u>chr10:96522327+96522386</u>60bp AGAAGGAGCATATAGTGGGCCTAGGT GTGTGTGCCTCTTTGATGGATAAA AGAAGGAGCATATAGTGGGCCTAGGTgattggccacTTTATCCATCAAAG AGGCACACAC

UCSC In-Silico PCR

>chr10:96522327+96522597 271bp AGAAGGAGCATATAGTGGGCCTAGGT TATCTGTAGGATATTTCCAATCACTGGGA AGAAGGAGCATATAGTGGGCCTAGGTgattggccactttatccatcaaag aggcacacacacttaattagcatggagtgttataaaaagcttggagtgca agctcacggttgtcttaacaagaggagaaggcttcaatggatccttttgt ggtccttgtgctctgtctctcatgtttgcttctcctttcaatctggagac agagctctgggagaggaaaactccctcctggccctactcctCCAGTG ATTGGAAATATCCTACAGATA

← ⇒ C	🗋 genome	e.ucsc.edu/cgi-bin/hgPc	cr?hgsid=32	7351193&org	=Human&db=hg	19℘_target=	=genome℘_f=	=TTTCAAATGGGAAAAGGGAGACCCTGG℘_r=TTCTCATGAGCATG
Â	Genomes	Genome Browser	Tools	Mirrors	Downloads	My Data	About Us	Нер
UCSC In	-Silico PC	R						
> <u>chr10:90</u> TTTCAAATO cacacago gcccatogi gacacaaata acaaaacaa ctgttcata GAAAACAGO	5521464-965 GGGAAAAGGAA Catagetgge Cggegeattat Lttgaaaaaaa Actteeaaaca Aaaacaggett CCCCAGAGATG	21792 329bp TTTCAA GACCCTGGgagaacagga agaactgggatttgagct ctcttacatcagagatgo aaatcgtttgctaaaact ttagttattctgaatata cacattaaatagaaccao CTCATGAGAA	AATGGGAAAA acacetgttg tgaggtette etttgagaac etttgttttag ataceacatt ettatttate	1GGGAGACCCT ygtgc :tgat :agaa gcaaa gcaaa .catc :taaG	GG TTCTCATGAGC	ATCTCTGGGGC	TGTTTTCC	

Primer Melting Temperatures

Forward: 71.8 C tttcaaatgggaaaagggagaccctgg

Reverse: 74.4 C ttctcatgagcatctctggggctgttttcc

The temperature calculations are done assuming 50 mM salt and 50 nM annealing oligo concentration. The code to calculate the melting temp comes from Primer3.

UCSC In-Silico PCR

No matches to agaaggaatatatagtgggcctaggt tatcaataggccatttccaatgactggg in Human Feb. 2009 (GRCh37/hg19)

Primer Melting Temperatures

Forward: 60.0 C agaaggaatatatagtgggcctaggt Reverse: 69.0 C tatcaataggccatttccaatgactggg The temperature calculations are done assuming 50 mM salt and 50 nM annealing oligo concentration. The code to calculate the melting temp comes from <u>Primer3</u>.

ñ	Genomes	Genome Browser	Tools	Mirrors	Downloads	My Data	About Us	Help				
UCSC I	JCSC In-Silico PCR											
> <u>chr10:</u> TTTCAAA cacacagu gcccatc ttagcaa acatta > <u>chr10:</u> TTTCAAA cacacagu gcccatcu gacacaaa acaaaacu ctgttca gaaaaca	CGGAAAAGGGAG CGGGAAAAGGGAG Ctcatagctggca gtggtgtattatc aataaaacaaact Ctctgttcataas Caggggaacagc OGS21464-9652 CGGAAAAGGAG Ctcatagctggca gtggcgcattatc atttgaaaaaaa acttccaaacat Caaaacaggcttc gccCCAGAGATGG	17768 290bp TTTCAA ACCCTGGgagaacagga ggaactgggatttgagct tccttacaccagagctgc tccaaacattagttatt acaggcttcacattaaa aCCAGAGATGCTCATGA 1792 329bp TTTCAA ACCTGGgagaacagga ggaactgggatttgagct tccttacatcagagatgc aatcgtttgctaaaact tagttattctgaatata acattaaatagaaccac TCATGAGAA	ATGGGAAAA cacctgttg gaggtcttc cttgagaac ctgaatata tagaacccc GAA ATGGGAAAA ATGGGAAAA gaggtcttc tttgagaac ttgttttag taccacatt ttatttatc	GGGIGICCCTC gtgc tgat aatt cacc ttat GGGIGICCCTC gtgc tgat agaa caaa catc taag	3G TTCTCATGAGC 3G TTCTCATGAGC	ATCTCTGG						

Primer Melting Temperatures

Forward: 71.8 C tttcaaatgggaaaagggagaccctgg

Reverse: 54.7 C ttctcatgagcatctctgg

The temperature calculations are done assuming 50 mM salt and 50 nM annealing oligo concentration. The code to calculate the melting temp comes from Primer3.

Â	Genomes	Genome Browser	Tools	Mirrors	Downloads	My Data	About Us	Неір	
		more more							

UCSC IN-SIICO PCR

>chr10:96697464-96697768 305bp TTTCAAATGGGAAAAGGGAGACCCTGG GAGAACTCTTATTTTTTCTCATGAGCATCTCTGG

Primer Melting Temperatures

Forward: 71.8 C tttcaaatgggaaaagggagaccctgg Reverse: 68.4 C gagaactcttatttttctcatgagcatctctgg The temperature calculations are done assuming 50 mM salt and 50 nM annealing oligo concentration. The code to calculate the melting temp comes from Primer3.

Sequence (5'>3')Length TmGC%Self complementaritySelf 3' complementarityForward primerTTTCAAATGGGAAAAGGGAGACCCTGG2765.1248.156.003.00Reverse primerGAGAACTCTTATTTTTTCTCATGAGCATCTCTGG3464.0938.248.002.00Products on target templates>NT_030059.13 Homo sapiens chromosome 10 genomic contig, GRCh37.p10 Primary Assemblyproduct length = 344Features flanking this product:26249 bp at 5' side: cytochrome P450 2C18 isoform 2 precursor670 bp at 3' side: cytochrome P450 2C19 precursorTTTCCAAATGGGGAAAAGGGGAGACCCTGG 27	Primer pair 1							
Forward primer TTTCAAATGGGAAAAGGGAGACCCTGG 27 65.12 48.15 6.00 3.00 Reverse primer GAGAACTCTTATTTTTTCTCATGAGCATCTCTGG 34 64.09 38.24 8.00 2.00 Products on target templates >NT_030059.13 Homo sapiens chromosome 10 genomic contig, GRCh37.p10 Primary Assembly 200 product length = 344 Features flanking this product: 26249 bp at 5' side: cytochrome P450 2C18 isoform 2 precursor 670 bp at 3' side: cytochrome P450 2C19 precursor		Sequence (5'->3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Reverse primer GAGAACTCTTATTTTTCTCATGAGCATCTCTGG 34 64.09 38.24 8.00 2.00 Products on target templates >NT_030059.13 Homo sapiens chromosome 10 genomic contig, GRCh37.p10 Primary Assembly	Forward primer	TTTCAAATGGGA	AAAGGGAGACCCTGG	27	65.12	48.15	6.00	3.00
Products on target templates >NT_030059.13 Homo sapiens chromosome 10 genomic contig, GRCh37.p10 Primary Assembly product length = 344 Features flanking this product: 26249 bp at 5' side: cytochrome P450 2C18 isoform 2 precursor 670 bp at 3' side: cytochrome P450 2C19 precursor Forward primer 1 TTTCAAATGGGAAAAGGGAGACCCTGG 27	Reverse primer	GAGAACTCTTAT	TTTTTCTCATGAGCATCTCTGG	34	64.09	38.24	8.00	2.00
>NT_030059.13 Homo sapiens chromosome 10 genomic contig, GRCh37.p10 Primary Assembly product length = 344 Features flanking this product: <u>26249 bp at 5' side: cytochrome P450 2C18 isoform 2 precursor</u> <u>670 bp at 3' side: cytochrome P450 2C19 precursor</u> Forward primer 1 TTTCAAATGGGAAAAGGGAGACCCTGG 27	Products on target	templates						
<pre>product length = 344 Features flanking this product: <u>26249 bp at 5' side: cytochrome P450 2C18 isoform 2 precursor 670 bp at 3' side: cytochrome P450 2C19 precursor Forward primer 1 TTTCAAATGGGAAAAGGGAGACCCTGG 27</u></pre>	> <u>NT_030059.13</u> Hom	o sapiens chromos	ome 10 genomic contig, GRCh37.p10 P	Primary Asser	nbly			
26249 bp at 5' side: cytochrome P450 2C18 isoform 2 precursor 670 bp at 3' side: cytochrome P450 2C19 precursor Forward primer 1 TTTCAAATGGGAAAAGGGAGACCCTGG 27	product length Features flank	n = 344	oduct:					
670 bp at 3' side: cytochrome P450 2C19 precursor	26249 bp at	5' side: c	ytochrome P450 2C18 isofo	rm 2 pre	curso	r		
Forward primer 1 TTTCAAATGGGAAAAGGGAGACCCTGG 27	<u>670 bp at 3</u>	3' side: cyt	ochrome P450 2C19 precurs	or				
	Forward primer	r 1	TTTCAAATGGGAAAAGGGAGACCC	TGG 27				
Template 47326256	Template	47326256		473	26230	l.		
Reverse primer 1 GAGAACTCTTATTTTTTCTCATGAGCATCTCTGG 34	Reverse primer	: 1	GAGAACTCTTATTTTTTCTCATGA	GCATCTCT	GG 3	4		
Template 47325913	Template	47325913		eneo eneo	4	7325	946	
reduct length = 200	nyaduat langth	- 205						
Features flanking this product:	Features flank	ing this pr	oduct:					
84794 bp at 5' side: cytochrome P450 2C19 precursor	<u>84794 bp at</u>	: 5 [°] side: c	ytochrome P450 2C19 precu	irsor				
671 bp at 3' side: cytochrome P450 2C9 precursor	<u>671 bp at 3</u>	<u> side: cyt</u>	<u>ochrome P450 2C9 precurso</u>	r				
Forward primer 1 TTTCAAATGGGAAAAGGGAGACCCTGG 27	Forward primer	: 1	TTTCAAATGGGAAAAGGGAGACCC	TGG 27				
Template 47502232 47502206	Template	47502232		475	02206	5		
Reverse primer 1 GAGAACTCTTATTTTTTCTCATGAGCATCTCTGG 34	Reverse primer	: 1	GAGAACTCTTATTTTTTCTCATGA	GCATCTCT	GG 3	4		
Template 47501928T.CC 47501961	Template	47501928	T.CC		4	7501	961	

	Sequence (5'->3')		Length	Tm	GC%	Self complementarity	Self 3' complementarity			
Forward primer	TTTCAAATGGGAA	27	65.12	48.15	6.00	3.00				
Reverse primer	GAGAACTCTTATT	TTTTCTCATGAGCATCTCTGGG	35	65.41	40.00	8.00	4.00			
Products on targe	t templates									
> <u>NT_030059.13</u> Horr	io sapiens chromos	ome 10 genomic contig, GRCh37.p10 Prima	iry Assem	bly						
product lengt Features flan <u>26249 bp a</u> <u>670 bp at</u>	h = 344 king this pro <u>t 5' side: cy</u> 3' side: cyto	oduct: ytochrome P450 2C18 isoform ochrome P450 2C19 precursor	2 prec	urso:	<u>r</u>					
Forward prime Template	rward primer 1 TTTCAAATGGGAAAAGGGAGACCCTGG 27 mplate 47326256									
Reverse prime Template	r 1 47325913	GAGAACTCTTATTTTTTTCTCATGAGCA	ТСТСТС	GG (35 47325	947				
product length = 305 Features flanking this product: <u>84794 bp at 5' side: cytochrome P450 2C19 precursor</u> <u>671 bp at 3' side: cytochrome P450 2C9 precursor</u>										
Forward prime Template	r 1 47502232	TTTCAAATGGGAAAAGGGAGACCCTGG	27 4750	2206						
Reverse prime Template	r 1 47501928	GAGAACTCTTATTTTTTTCTCATGAGCA	TCTCTG	GG (.T	35 47501	962				



Â My Data Genomes Genome Browser Tools Mirrors Downloads UCSC In-Silico PCR >chr1:152281938-152285646 3709bp AAATGAGGAACAATCAGGAGACGGCA ATCTTGGGATGCTGAGTGCCTGGAG AAATGAGGAACAATCAGGAGACGGCAccaggcactcagggtcacgtcatc atgaagetteeteteaggetgacagetetagacaeteacaggtgggecag ggacaatcatcggggcccaggacaagtaggaaccagggatccagtgttag ccaggacagtgacagtcagggacactcagaagactctgagaggtggtctg ggtctgcttccagaaaccatcatggatctgctcaggagcagtcaagagat ggctccagacaccccaggtcccatcacgaagacagagctggtcatgggca ctagtggacaggctgcgtcatcccatgaacaggcaagatcaagtgcagga gaaagacatggatcccgccaccagctccagtcagcagacagctccagaca ctcaggcactgggcacggacaagcttcatctgcagtcagagacagtggac accgagggtccagtggtagtcaggccactgacagtgagggacattcagaa gactcagacacacagtcagtgtcaggccatggacaggctggtcaccatca gcagagccaccaagagtccgcacgtgaccggtcaggggaaaggtctcgac

atagagccagtcatgggcactctgcagagagctccagacaatcaggcact cgtcatgcagagacttcctctggtggacaggctgcatcatcccaggaaca ggcaaggtcaagtccaggagaaagacatggatcccgccaccagcagtcag cagacagctccacagactcaggcactgggcgcagacaagattcatctgta gtcggagacagtggaaaccgagggtccagtggtagccaggccagtgacag cgagggacactcagaagagtcagacacagtcagtgtcagccacggac aggctgggccccatcagcagagccaccaagagtccacacgtggccagtca ggggaaaggtctggacgttcagggtctttcctctaccaggtggacactca tgaacagtctgagtccgccatggacgacagggcccagtgagaa gacaaagatcccgccacgagcaggacacgagacagCTCCAGGCACTCAGCg TCCCAAGAg

>chr1:152283882-152285646 1765bp AAATGAGGAACAATCAGGAGACGGCA ATCTTGGGATGCTGAGTGCCTGGAG

AAATGAGGAACAATCAGGAGACGGCAccaggcactcagggtcacgtcatc atgaagetteeteteaggetgacagetetagacaeteacaggtgggeeag ggacaatcatcggggcccaggacaagtaggaaccagggatccagtgttag ccaggacagtgacagtcagggacactcagaagactctgagaggtggtctg ggtctgcttccagaaaccatcatggatctgctcaggagcagtcaagagat ggetecagacaccccaggteccateacgaagacagagetggteatgggea ctagtggacaggctgcgtcatcccatgaacaggcaagatcaagtgcagga gaaagacatggatcccgccaccagctccagtcagcagacagctccagaca ctcaggcactgggcacggacaagcttcatctgcagtcagagacagtggac accgagggtccagtggtagtcaggccactgacagtgagggacattcagaa gactcagacacacagtcagtgtcaggccatggacaggctggtcaccatca gcagagccaccaagagtccgcacgtgaccggtcaggggaaaggtctcgac gttcagggtctttcctctaccaggtgagcactcataaacagtctgagtcc tcccatggatggacagggcccagcactggagtaagacaaggatcccacca tgagcaggcacgagacaactccaggcactcagcatcccaagatggtcagg acaccattcgtggacacccggggtcaagcagaagggaaggcaggggtcc caccacgagcaatcggtagataggtctggacactcagggtcccatcacag ccacaccacatcccagggaaggtctgatgcctcccgtgggcagtcaggat ccagaagtgcaagcagaacaacacgtaatgaggaacaatcaagagacggc tccaqqcactcaqqqtcacqtcaccatqaaqcttcctctcatqccqacat

>chr1:152284854-152285646 793bp AAATGAGGAACAATCAGGAGACGGCA ATCTTGGGATGCTGAGTGCCTGGAG AAATGAGGAACAATCAGGAGACGGCAccaggcactcagggtcacgtcatc atgaagetteeteteaggetgacagetetagacaeteacaggtgggeeag ggacaatcatcggggcccaggacaagtaggaaccagggatccagtgttag ccaggacagtgacagtcagggacactcagaagactctgagaggtggtctg ggtctgcttccagaaaccatcatggatctgctcaggagcagtcaagagat ggctccagacaccccaggtcccatcacgaagacagagctggtcatgggca ctctgcagacagetecagaaaateaggeaetegteacacacagaatteet ctagtggacaggctgcgtcatcccatgaacaggcaagatcaagtgcagga gaaagacatggatcccgccaccagctccagtcagcagacagctccagaca ctcaggcactgggcacggacaagcttcatctgcagtcagagacagtggac accgagggtccagtggtagtcaggccactgacagtgagggacattcagaa gactcagacacacagtcagtgtcaggccatggacaggctggtcaccatca gcagagccaccaagagtccgcacgtgaccggtcaggggaaaggtctcgac gttcagggtctttcctctaccaggtgagcactcataaacagtctgagtcc tcccatggatggacagggcccagcactggagtaagacaaggatcccacca tgagcaggcacgagacaaCTCCAGGCACTCAGCATCCCAAGAT

Primer Melting Temperatures

Forward: 69.7 C aaatgaggaacaatcaggagacggca

Reverse: 70.6 C atcttgggatgctgagtgcctggag

The temperature calculations are done assuming 50 mM salt and 50 nM annealing oligo concer <u>Primer3</u>.