

# Primers/Amorces

› Tumeur :

Gène	Séquence 5' - 3'	Référence GenBank <sup>®</sup>	Longueur de l'amplicon (pb)
<i>Glut1</i> ( <i>Slc2a1</i> )	Fwd : TGC CTT GGA TGT CCT ATC TG Rev : ACC AGG GCC TAC TTC AAA GA	NM_011400.3	71
<i>Hprt</i> ( <i>Hprt</i> )	Fwd : CAG GCC AGA CTT TGT TGG AT Rev : TTG CGC TCA TCT TAG GCT TT	NM_013556.2	147
<i>Ppia</i> ( <i>Ppia</i> )	Fwd : AGC ATA CAG GTC CTG GCA TC Rev : TTC ACC TTC CCA AAG ACC AC	NM_008907.1	127
<i>Tweak</i> ( <i>Tnfsf12</i> )	Fwd : GCT ACG ACC GCC AGA TTG GG Rev : GCC AGC ACA CCG TTC ACC AG	NM_011614.3	130
<i>Vegfa</i> ( <i>Vegfa</i> )	Fwd : CAC CCA CGA CAG AAG GAG AG Rev : TCT CAA TCG GAC GGC AGT AG	NM_001025250.3	87

Dr AMIROUCHE A.  
(2020/2021)

# Dessin Des Couples d'amorces Références- Primer

## Principe

Crée un couple d'amorces valide pour la quantification de l'expression d'un gène d'intérêt.

## Comment?

- 1- NCBI (National Center for Biotechnology Information) ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov))
  - Primer--BLAST ([www.ncbi.nlm.nih.gov/tools/primer---blast](http://www.ncbi.nlm.nih.gov/tools/primer---blast))
- 2- Integrated DNA Technologies® ([www.idtdna.com](http://www.idtdna.com))

## Protocole

› **Obtenir la sequence nucléotidique du transcript du gene (sur NCBI)**

:

1. Dans le menu déroulant, sélectionner «Gene». Dans la barre de recherche, taper le nom du gène d'intérêt, et cliquer sur «Search».
2. Sélectionner l'espèce désirée.
3. Cliquer sur «GenBank», puis sélectionner l'ARNm désiré dans «Features – mRNA».
4. Cliquer sur «FASTA», puis noter la sequence codante obtenue.

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30 Oct 2019

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- Structure

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GenBank release 234 is available

Gene

Un exemple d'un gène d'intérêt

Create RSS Save search Advanced

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See [KHSRP \(KSRP\) KH-type splicing regulatory protein](#) in the Gene database  
[ksrp](#) in [Homo sapiens](#) [Mus musculus](#) [Xenopus tropicalis](#) [All 5 Gene records](#)

Search results

Items: 1 to 20 of 60

See also 3 discontinued or replaced items.

Selectionner une espèce

<< First < Prev Page 1 of 3 Next > Last >>

Name/Gene ID	Description	Location	Aliases	MIM
<input type="checkbox"/> <a href="#">KHSRP</a> ID: 8570	KH-type splicing regulatory protein [ <i>Homo sapiens</i> (human)]	Chromosome 19, NC_000019.10 (6473102..6424811, complement)	FBP2, FUBP2, KSRP, p75	603445
<input type="checkbox"/> <a href="#">Khsrp</a> ID: 16549	KH-type splicing regulatory protein [ <i>Mus musculus</i> (house mouse)]	Chromosome 17, NC_000083.6 (57021049..57031507, complement)	6330409F21Rik, Fbp2, Fubp2, Ksrp	
<input type="checkbox"/> <a href="#">khsrp</a> ID: 100170586	KH-type splicing regulatory protein [ <i>Xenopus tropicalis</i> (tropical clawed frog)]	Chromosome 3, NC_030679.1 (130039451..130057575, complement)	VgRBP71, fbp2, fubp2, ksrp	
<input type="checkbox"/> <a href="#">khsrp.S</a> ID: 399189	KH-type splicing regulatory protein S homeolog [ <i>Xenopus laevis</i> (African clawed frog)]		XELAEV_18000829mg, VgRBP71, fbp2, fubp2, khsrp-b, ksrp	
<input type="checkbox"/> <a href="#">khsrp.L</a>	KH-type splicing	Chromosome 3L,	XELAEV_18019123mg,	

clear

Gene

Gene

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**KHSRP KH-type splicing regulatory protein [ *Homo sapiens* (human) ]**

Gene ID: 8570, updated on 3-Nov-2019

## Summary

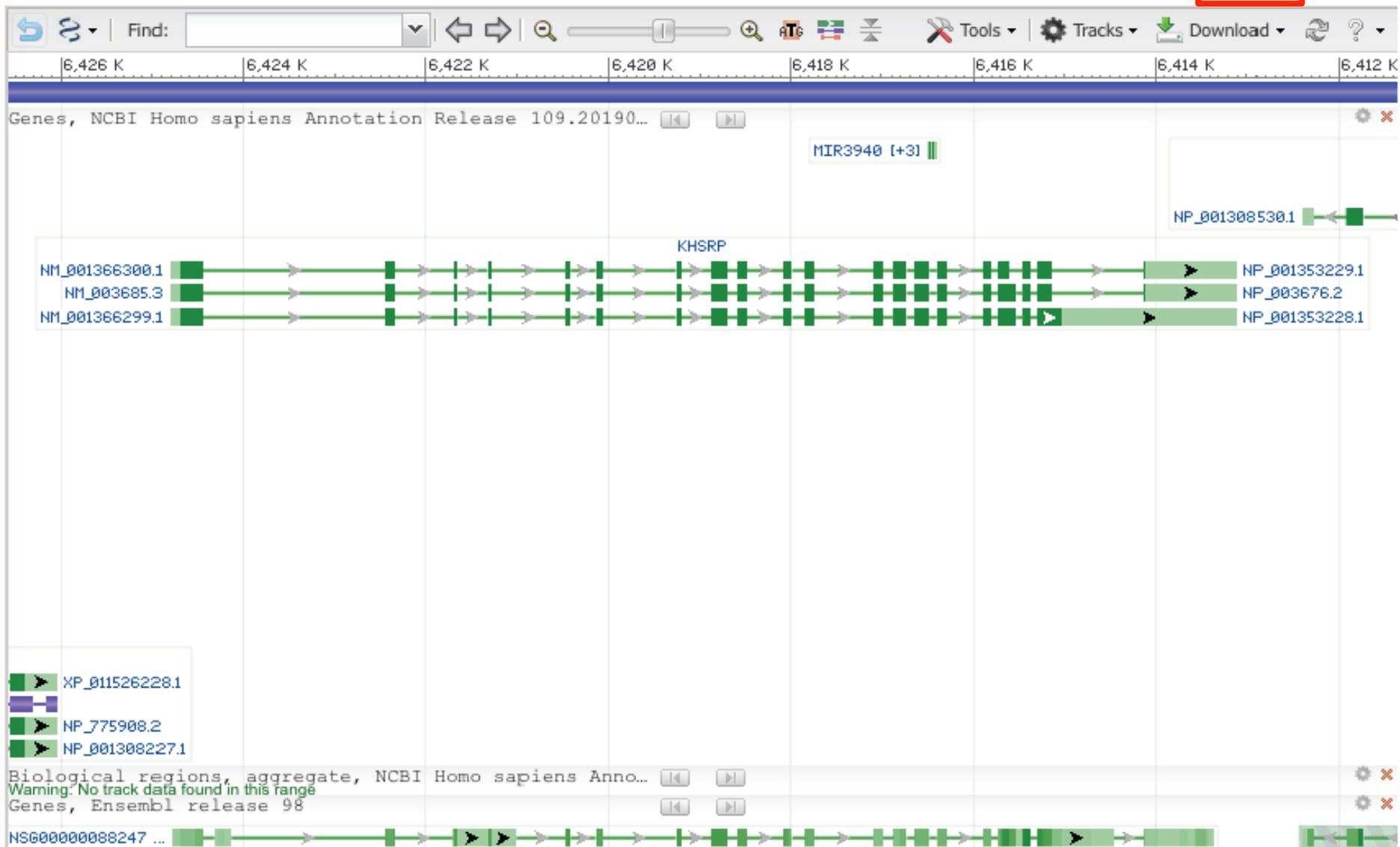


<b>Official Symbol</b>	KHSRP <small>provided by <a href="#">HGNC</a></small>
<b>Official Full Name</b>	KH-type splicing regulatory protein <small>provided by <a href="#">HGNC</a></small>
<b>Primary source</b>	<a href="#">HGNC:HGNC:6316</a>
<b>See related</b>	<a href="#">Ensembl:ENSG00000088247</a> <a href="#">MIM:603445</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	VALIDATED
<b>Organism</b>	<a href="#">Homo sapiens</a>
<b>Lineage</b>	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo
<b>Also known as</b>	p75; FBP2; KSRP; FUBP2
<b>Summary</b>	The KHSRP gene encodes a multifunctional RNA-binding protein implicated in a variety of cellular processes, including transcription, alternative pre-mRNA splicing, and mRNA localization (Min et al., 1997 [PubMed 9136930]; Gherzi et al., 2004 [PubMed 15175153]).[supplied by OMIM, Apr 2010]
<b>Expression</b>	Ubiquitous expression in testis (RPKM 37.2), endometrium (RPKM 25.1) and 25 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">mouse</a> <a href="#">all</a>

Go to [reference sequence details](#)

Genomic Sequence: NC\_000019.10 Chromosome 19 Reference GRCh38.p13 Primary Assembly

Go to nucleotide: [Graphics](#) **FASTA** [GenBank](#)



Nucleotide

Nucleotide

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GenBank

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## Homo sapiens chromosome 19, GRCh38.p13 Primary Assembly

NCBI Reference Sequence: NC\_000019.10

[FASTA](#) [Graphics](#)

LOCUS NC\_000019 11710 bp DNA linear CON 09-SEP-2019

DEFINITION Homo sapiens chromosome 19, GRCh38.p13 Primary Assembly.

ACCESSION [NC\\_000019](#) REGION: complement(6413102..6424811)

VERSION NC\_000019.10

DBLINK BioProject: [PRJNA168](#)Assembly: [GCF\\_000001405.39](#)

KEYWORDS RefSeq.

SOURCE Homo sapiens (human)

ORGANISM [Homo sapiens](#)Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;  
Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;  
Catarrhini; Hominidae; Homo.

REFERENCE 1 (bases 1 to 11710)

CONSRM International Human Genome Sequencing Consortium

TITLE Finishing the euchromatic sequence of the human genome

JOURNAL Nature 431 (7011), 931-945 (2004)

PUBMED [15496913](#)

REFERENCE 2 (bases 1 to 11710)

AUTHORS Grimwood,J., Gordon,L.A., Olsen,A., Terry,A., Schmutz,J.,  
Lamerdin,J., Hellsten,U., Goodstein,D., Couronne,O.,  
Tran-Gyamfi,M., Aerts,A., Altherr,M., Ashworth,L., Bajorek,E.,  
Black,S., Branscomb,E., Caenepeel,S., Carrano,A., Caoile,C.,  
Chan,Y.M., Christensen,M., Cleland,C.A., Copeland,A., Dalin,E.,  
Dehal,P., Denys,M., Detter,J.C., Escobar,J., Flowers,D.,  
Fotopoulos,D., Garcia,C., Georgescu,A.M., Glavina,T., Gomez,M.,  
Gonzales,E., Groza,M., Hammon,N., Hawkins,T., Haydu,L., Ho,I.,  
Huang,W., Israni,S., Jett,J., Kadner,K., Kimball,H., Kobayashi,A.,  
Larionov,V., Leem,S.H., Lopez,F., Lou,Y., Lowry,S., Malfatti,S.,

› **Dessiner les couples d'amorces sur Primer - Blast):**

1. Copier la sequence codante obtenue précédemment.
2. Dans la section «Primer Parameters», fixer les paramètres suivants: taille de l'amplicon, temperature d'hybridation de l'amorce ( $T_m$ ).
3. Dans la section «Exon/intron selection», fixer le parameter suivant: presence ou non d'un saut d'exon.
4. Cliquer sur «Get Primers» pour lancer la recherche.
5. Dans la section «Graphical view of primer pairs», verifier que l'amorce se trouve à cheval sur 2 exons.

Nucleotide

Nucleotide

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FASTA

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# Homo sapiens chromosome 19, GRCh38.p13 Primary Assembly

NCBI Reference Sequence: NC\_000019.10

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>NC\_000019.10:c6424811-6413102 Homo sapiens chromosome 19, GRCh38.p13 Primary Assembly

```

AGAGTGTCTCCGCGCCGTGTGGAGCGAGGCCTTGTTCGCCGCTTGAGCCGCCGCCGCCGCCGCCCTCC
TCAGCTTCAGCCTCCGCGCCAGGCCCGGCCCGCCGCGCCATGTCGGACTACAGCACGGGAGGACCCCG
CCCGGGCCCGCCCGCCCGCCCGGGCGGGGGGGGGGAGCCGGAGGCGCCGGGGAGGCCCTCCGCCGGCC
CGCCAGGCGCGGGGACCGGGCGGGCGGTCCCGCGCGCCCGCCCGGGCGGGGGTCCGCCGGGG
CCCCCTCTCAGCCACCCGGGAGGGCGCCCGGGAATCCGCAAGGACGCTTTCGCCGACGCCGTGCAGCGG
GCCGCCAGCTGAGGAGCGCGAGGGCTGAGGGCGCCCTCCGCCCGCCCGCCAGCCGGGGAGGGGGCG
GGGTCACGTGCGCGCGCGCGCAGAGGTCCCGAGGGGGCGGGGGCGTCCGGGGTACGTGGGCGG
TAGGCGGGGGCCTGGCCCTCCCGCTGCGCCCTCGAGTGGGATCCCAACGGGTGACCCCTCCAGGGAGTA
GGGGCGCACTGGGGTCAACAGGAGCGCGCCCTGGGTGTGCAGGCGCCCGGAGCTCGGAGACGCACATTT
TGGGACCCCCCTTAGCCCCCGCCCTTAGGACATGCCTGGAGGCAGGGTCCACCTTCCCGGGCGGAG
GGCCTTTACTCTCCCTCCATAACAGGCAAAAGGGCAGTGTTCGCCCAACCCAGTCCCATTTCTGTCC
CCTCTCTAGCAGAAAGCGACATTTGCGGGCTGGAGGACAGCTCCTTCTCTTCACTCGGAAACTGGAT
GGGAACTCTCTAGGGCTGAGCACTTGGCGAAATGGGAAAGACAGCATGGGACTGGCTCTTGGATGC
TTTGGTGGCTGAAAGCGTGGTCTGTCTCTTTGGGGAAAGAAGGGAGGGTCTCCCACCCCGTGTATTG
AGGCGTAATGACAAGGAAGTGTACATGCTGCTAGGACTGACCTTCCAGCTCCATTTGCAGCCTCCCCA
TGGCCTGGAGGGGTGGGGTCCGTAGAAATGGGAGGACTTTGCCCGCGACCGGCTCTGGGCTGTCCCAAT
GCCTCCGGGGCTAGAGACTGGCTCCTCCAAAGTGGGAGGTCCCGTTAAGTGAACGCTGTGTTCTGGCC
CTCTGTCTCTCTCTCACCCCTGGAGTTGGGTGGGTCTTAGCGTGCATTTGGTCTTATTGCCCATGGAAC
CGTCTGATTCTGGGACAGCTTGAAGGGAGGAAGCTCGTCTGTCTAGTTGGTGGGGACAGCCCCCTGTGG
GCATCCCATGAGTTTGGCTCTAGGGCAGCGTAGAAAAATGGGAGACTGGTGGAAAGGGAGATTCTTAACA
TGGTTTGGGTTTCTAAACCCAGACTCTGATCCTAAGTTTGGTGGGTTGAGGTTTCTTAAAGTTGCTCT
TCACGTGTCTGAGATGTAACCTTGTGCCATAGAGTCTTGGTTTTTGTGTTGTTGTTGTTTTTTGGAA
ACAGTCTCAATATCACCCAGGCGGGGTGCAGTGGCAGCATCTCAGCACACCACAGCCTCCACCTCTCCA
GTTGAAGTGTCTCTCTGCTCAGGCTCCGGAGTAGCTGGGATTATAAGTGTGCACCCACACAGCCAGCT
    
```

**Change region shown**

Whole sequence  
 Selected region

from:  to:

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## **6. Dans la section «Detailed primer reports», vérifier que:**

- Les amerses aient une taille d'environ 20 paires de bases (pb).
- La taille de l'amplicon soit comprise entre 70 et 120 (voire 150) pb.
- La température d'hybridation de l'amorce ( $T_m$ ) à la séquence codante soit le plus proche de 60°C (température optimale).
- La différence de  $T_m$  des amerses forward et reverse n'excède pas 4°C.
- L'amorce se termine par une base forte (G ou C) en 3'.
- Il n'y ait pas:
  - Plus de 2 bases fortes (G ou C) dans les 3 dernières bases en 3'.
  - Plus de 3 bases fortes (G ou C) dans les 6 dernières bases en 3'
  - Ou de G en 5'.
- Il y ait plus de C que de G dans l'amorce.
- Le pourcentage de GC soit inférieur à 60%.
- Le pourcentage de GC soit approximativement le même dans les amorces forward et reverse.

# Primer-BLAST

## A tool for finding specific primers

Finding primers specific to your PCR template (using Primer3 and BLAST).

### PCR Template

[Reset page](#)[Save search parameters](#)[Retrieve recent results](#)[Publication](#)[Tips for finding specific primers](#)

Enter accession, gi, or FASTA sequence (A refseq record is preferred)  [Clear](#)

NC\_000019.10

Range

Forward primer From  To   
Reverse primer   [Clear](#)

Or, upload FASTA file

aucun fichier sélé

### Primer Parameters

Use my own forward primer  
(5'→3' on plus strand)

[Clear](#)

Use my own reverse primer  
(5'→3' on minus strand)

[Clear](#)

PCR product size

Min  Max

# of primers to return

Primer melting temperatures  
(T<sub>m</sub>)

Min  Opt  Max  Max T<sub>m</sub> difference  

### Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section 

Exon junction span



Exon junction match

Exon at 5' side  Exon at 3' side

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 

# Amorces PCR

› Tumeur :

Gène	Séquence 5' - 3'	Référence GenBank <sup>®</sup>	Longueur de l'amplicon (pb)
<i>Glut1</i> ( <i>Slc2a1</i> )	Fwd : TGC CTT GGA TGT CCT ATC TG Rev : ACC AGG GCC TAC TTC AAA GA	NM_011400.3	71
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