TP GENOMA HUMANO

Medicina Molecular, Maestría en Biología Molecular Médica, 2011

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¿Qué es una base de datos?

- Una colección de **registros**, con información específica
- Cada registro tiene una **clave primaria.** Un identificador **único** que define al registro sin ambigüedad.

gi	Accession	version	date	Genbank Division	taxid	organims	Number of Chromosomes
6226959	NM_000014	3	06/01/2000	PRI	9606	homo sapiens	22 diploid + X+Y
6226762	NM_000014	2	10/12/1999	PRI	9606	homo sapiens	22 diploid + X+Y
4557224	NM_000014	1	02/04/1999	PRI	9606	homo sapiens	22 diploid + X+Y
41	X63129	1	06/06/1996	MAM	9913	bos taurus	29+X+Y

gi = Genbank Identifier: **Clave única : Clave primaria**

Cambia con cada actualización del registro correspondiente a la secuencia

Accession Number: **Clave secundaria**

Refiere al mismo locus y secuencia, a pesar de los cambios en la secuencia.
Se usa la misma en las diferentes bases de datos (Genbank, DDBJ, EMBL)

Accession + Version es equivalente al **gi** (representa un identificador único)

Ejemplo: AF405321.2 **Accession**: AF405321 **Version:** 2

Bases de datos

- **Primarias: datos derivados de un experimento o de conocimiento científico.**
  - **Genbank** (Repositorio de secuencias nucleotídicas)
  - **Protein DB, Swissprot**
  - **PDB**
  - **Pubmed** (literatura)
  - **Genome Mapping**
  - **Kegg** (Kyoto Encyclopedia of Genes and Genomes, base de datos de vías metabólicas)

- **Secundarias: información derivada de otras fuentes (primarias, entre otras).**
  - **Refseq (Colección curada de GenBank en NCBI)**
  - **Unigene (Clustering de ESTs en NCBI)**

Bases de datos : literatura

- **NCBI: Pubmed: toda la literatura biomédica.**
  - www.ncbi.nlm.nih.gov
  - Abstracts and links to publisher sites

- **Páginas web de los publishers**

Búsquedas simples

- El usuario tipea palabras libremente, sin restricciones
- Intentar “adivinar” la intención del usuario (sobre qué campo de la base de datos buscar)

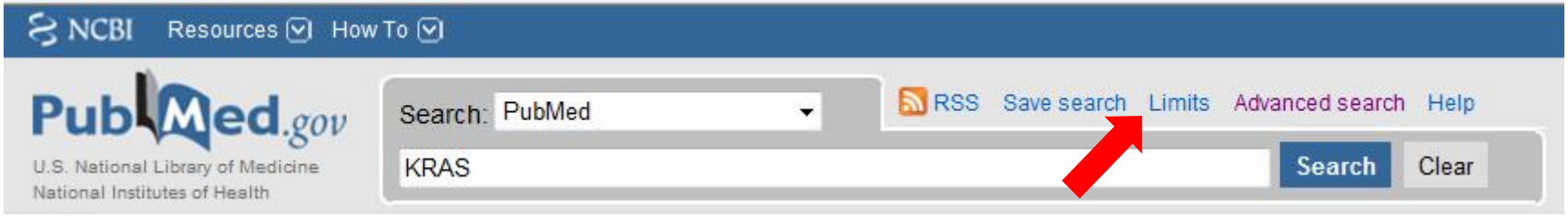
✓ Rápidas y fáciles de usar

❖ Pueden no “entender” lo que uno busca

Búsquedas avanzadas

- Hay que especificar sobre qué campos buscar:

  $\Rightarrow$ hay que conocer los campos
- Entrez: se especifican entre corchetes los tags predefinidos (hay que conocerlos)
  - `[organism],[publication type],[date]`
- Entrez provee además
  - **History:** una historia de las búsquedas que van realizando. En cualquier momento pueden combinar búsquedas o volver sobre alguna de ellas
  - **Preview/Index:** les permite probar una búsqueda (preview) y ver el número de registros que selecciona o ver los índices y el número de registros asociados a cada uno de ellos
  - **Details:** permite analizar la traducción que realizó Entrez de la búsqueda que realizamos (uso de sinónimos, límites, etc)
  - **Limits:** permite acotar los criterios de búsqueda a ciertos campos definidos



Limits

Dates

Published in the Last: Any date

Type of Article

- Clinical Trial
- Editorial
- Letter
- Meta-Analysis
- Practice Guideline

Languages

- English
- French
- German
- Italian
- Japanese

Species

- Humans
- Animals

Sex

- Male
- Female

Subsets

- AIDS
- Bioethics
- Cancer
- Complementary Medicine
- Core clinical journals

Ages

- All Infant: birth-23 months
- All Child: 0-18 years
- All Adult: 19+ years
- Newborn: birth-1 month
- Infant: 1-23 months

Text Options

- Links to full text
- Links to free full text
- Abstracts

Search Field Tags

Field: All Fields

- Editor
- Entrez Date
- Filter
- First Author
- Full Author Name
- Full Investigator Name
- Grant Number
- ISBN
- Investigator
- Issue
- Journal
- Language
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- Location ID
- MeSH Date
- MeSH Major Topic
- MeSH Subheading
- MeSH Terms
- Pagination
- Pharmacological Action
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- Publisher
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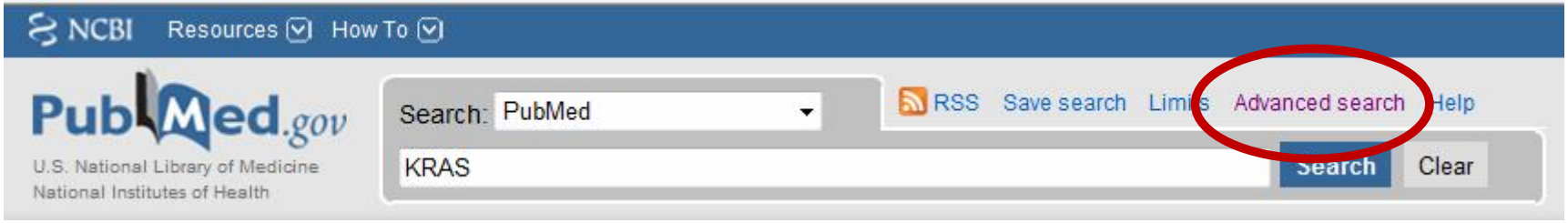
- Supplementary Concept
- Text Word
- Title
- **Title/Abstract**
- Transliterated Title
- Volume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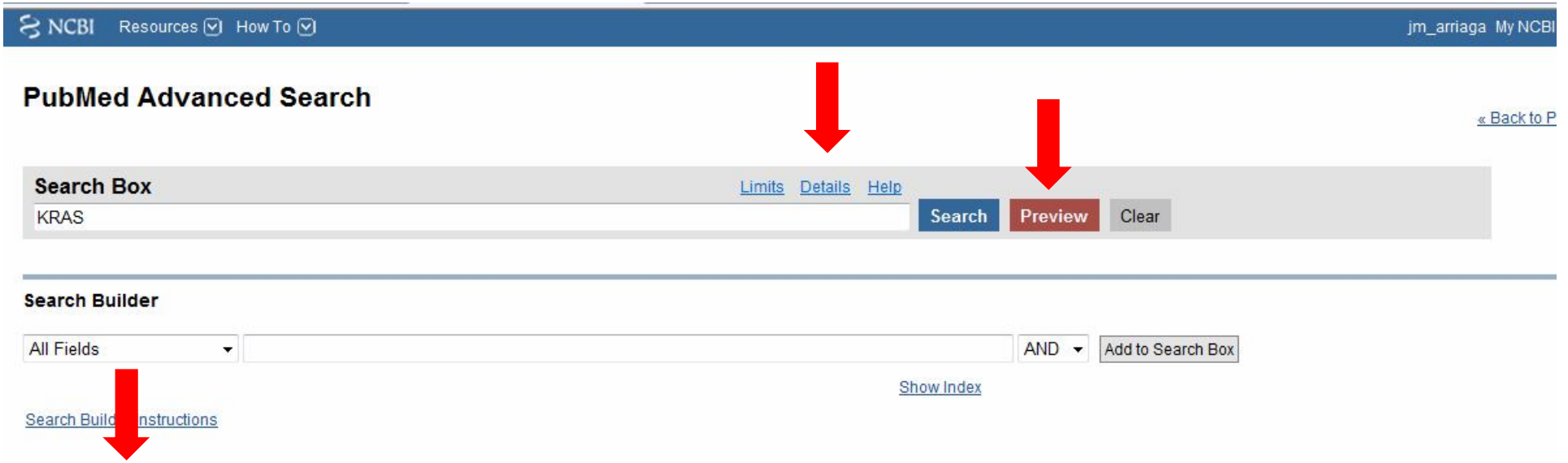
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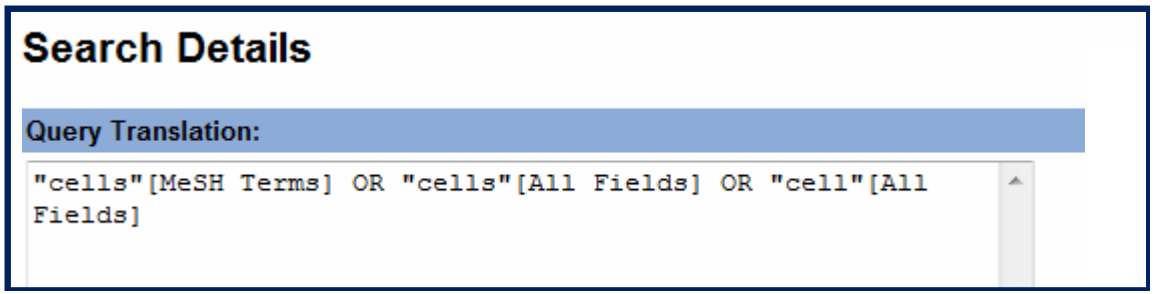
More Resources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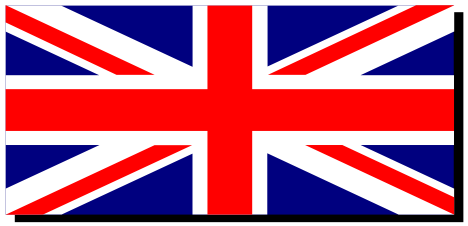
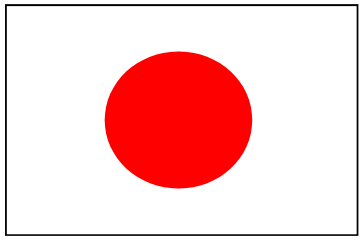
International Nucleotide Sequence
Database Collaboration (Base de datos primaria)

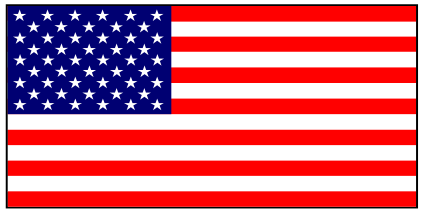




EMBL
Hinxton, UK
European Bioinformatics Institute

http://www.ebi.ac.uk

DDBJ
DNA Database of Japan
Mishima, Japan
National Institutes of Genetics
http://www.genome.ad.jp/



NCBI
Bethesdsa, MD
National Center for Biotechnology Information
National Institutes of Health

http://ncbi.nlm.nih.gov

GenBank

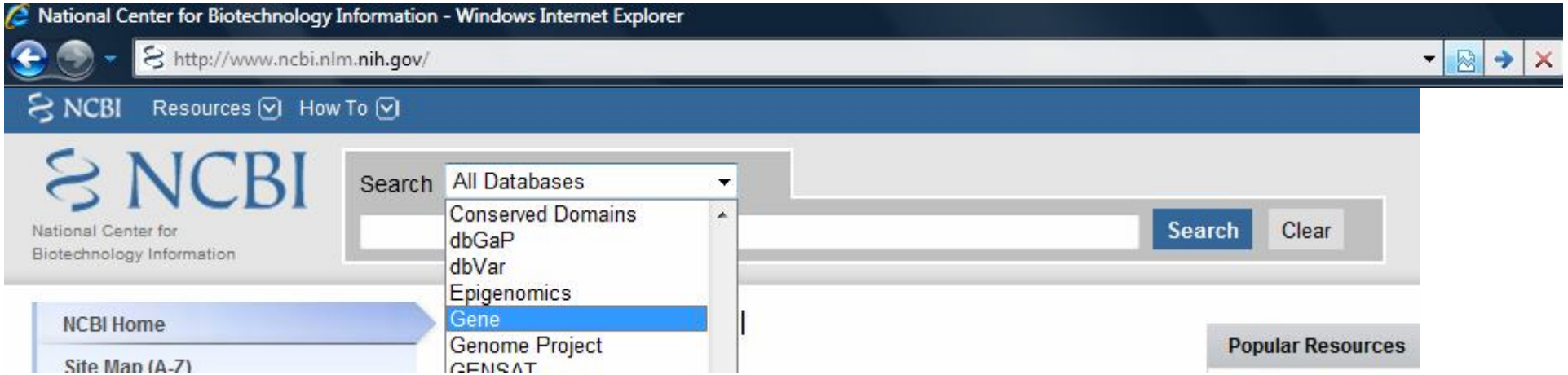
- **Es un banco: no se intenta unificar datos.**
- No se pueden modificar las secuencias sin el consentimiento del autor (submitter).
- Puede haber registros de diversas calidades de secuencia y diferentes fuentes: Redundante, posiblemente con errores
- Dificil de actualizar

RefSeq

- colección **curada** de registros de GenBank
- toma records de GenBank y los actualiza/corrije
- unifica para reducir redundancia
- Accession numbers del tipo XX_123456

A partir de una secuencia nucleotidica se puede:

- Secuencia DNA, mRNA, CDS (Secuencias codificantes)
- Traducir a proteinas
- Localización cromosómica y genes vecinos
- Sentido de transcripción
- UTRs
- Promotores
- SNPs
- Links a bases de datos de mutaciones (ej.: COSMIC)
- ESTs
- Diseño de Primers



KRAS

KRAS

3. **Official Symbol** KRAS and **Name:** v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog [*Homo sapiens*]

KRAS v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog [*Homo sapiens*]

Gene ID: 3845, updated on 10-Mar-2011

Summary

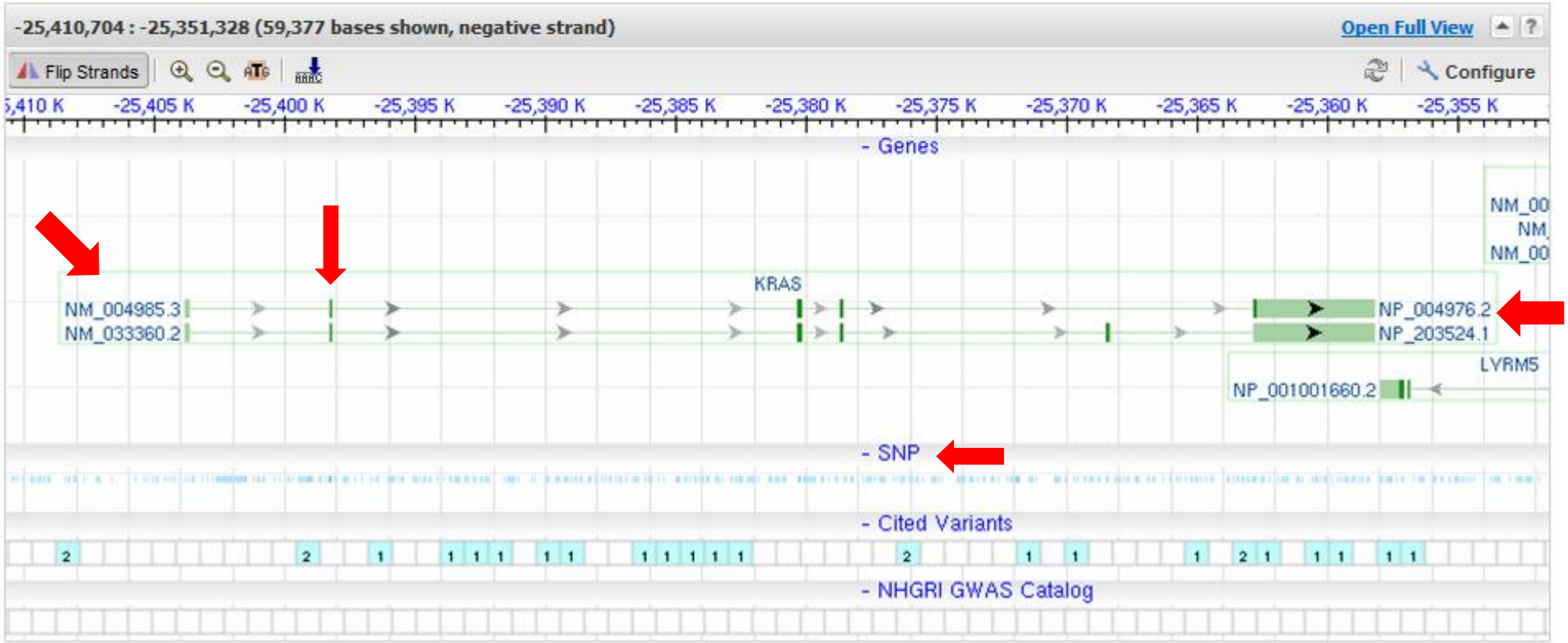
Official Symbol	KRAS provided by HGNC
Official Full Name	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog provided by HGNC
Primary source	HGNC:6407
See related	Ensembl:ENSG00000133703; HPRD:01817; MIM:190070
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Homo sapiens
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo
Also known as	NS; NS3; KRAS1; KRAS2; RASK2; KI-RAS; C-K-RAS; K-RAS2A; K-RAS2B; K-RAS4A; K-RAS4B; KRAS
Summary	This gene, a Kirsten ras oncogene homolog from the mammalian ras gene family, encodes a protein that is a member of the small GTPase superfamily. A single amino acid substitution is responsible for an activating mutation. The transforming protein that results is implicated in various malignancies, including lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas and colorectal carcinoma. Alternative splicing leads to variants encoding two isoforms that differ in the C-terminal region. [provided by RefSeq]

Genomic regions, transcripts, and products

Go to reference sequence details

Genomic Sequence NC_000012 chromosome

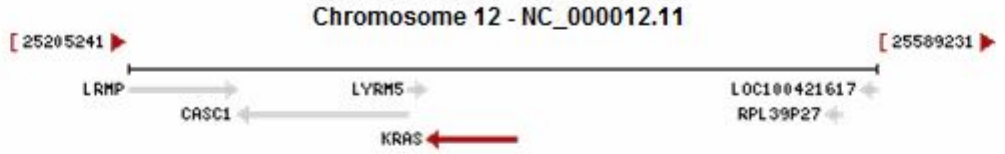
Go to nucleotide Graphics FASTA GenBank



Genomic context

chromosome: 12; **Location:** 12p12.1

See KRAS in MapViewer



Bibliography

Related articles in PubMed

1. Tubular adenomas with minor villous changes show molecular features characteristic of tubulovillous adenomas. Ishii T, *et al*. Am J Surg Pathol, 2011 Feb. PMID 21263241.
2. KRAS gene mutation in colorectal cancer is correlated with increased proliferation and spontaneous apoptosis. Liu X, *et al*. Am J Clin Pathol, 2011 Feb. PMID 21228365.
3. KRAS genotyping as biomarker in colorectal cancer: a comparison of three commercial kits on histologic material. Cavallini A, *et al*. Anticancer Res, 2010 Dec. PMID 21187522.
4. Prognostic and predictive implications of EGFR mutations, EGFR copy number and KRAS mutations in advanced stage lung adenocarcinoma. Bonanno L, *et al*. Anticancer Res, 2010 Dec. PMID 21187500.
5. Repression of the miR-143/145 cluster by oncogenic Ras initiates a tumor-promoting feed-forward pathway. Kent OA, *et al*. Genes Dev, 2010 Dec 15. PMID 21159816.

See all (688) citations in PubMed

GeneRIFs: Gene References Into Functions What's a GeneRIF?

1. Data show that KRAS mutation was detected in 201 tumours (39.4%).
2. 18/19 (94.7%) metastatic colorectal cancer smears were perfectly adequate for codon 12 and 13 KRAS mutational analysis by direct gene sequencing.
3. Multiple cellular proteins control the dynamics of membrane association and intercompartmental movement of K-ras to an important degree even under basal cellular conditions.
4. There was an incremental increase in KRAS mutation frequency with increasing villous compartment of colorectal neoplasms.
5. The KRAS mutation is a negative predictive factor for survival in patients rectal and colon cancer.
6. Compared with wild-type KRAS colorectal cancers, KRAS-mutated CRCs had a lower frequency of high microsatellite instabilities, a higher chance of having brisk mitosis and apoptosis, and a greater mean of mitotic figures and apoptotic cells

Interactions

Product	Interactant	Other Gene	Complex	Source	Pubs	Description
P01116	P10415	BCL2		HPRD	PubMed	
P01116	Calmodulin 1	CALM1		HPRD	PubMed	
P01116	P16452	EPB42		HPRD	PubMed	
P01116	P49354	FNTA		HPRD	PubMed	
P01116	P49356	FNTB		HPRD	PubMed	
P01116	P53609	PGGT1B		HPRD	PubMed	
P01116	P48736	PIK3CG		HPRD	PubMed	
P01116	P04049	RAF1		HPRD	PubMed	

Gene Ontology

Provided by GOA

Function	Evidence Code	Pubs
GDP binding	IEA	
GMP binding	IEA	
GTP binding	IEA	

Process	Evidence Code	Pubs
MAPKKK cascade	TAS	
Ras protein signal transduction	EXP	PubMed
activation of MAPKK activity	TAS	

Component	Evidence Code	Pubs
intracellular	IEA	
membrane raft	IEA	

NCBI Reference Sequences (RefSeq)

Genomic

1. **NG_007524.1 RefSeqGene**

Range	5001..50675
Download	GenBank, FASTA, Sequence Viewer (Graphics)

mRNA and Protein(s)

1. **NM_004985.3 › NP_004976.2 GTPase KRas isoform b precursor**

Description	Transcript Variant: This variant (b) is composed of five exons and lacks exon 4a which the longer transcript variant (a) includes. This predominant variant (b) has a cds that terminates in exon 4b and encodes isoform b.
Source sequence(s)	AC092794,AI539465,AK292510,BC010502,BC029545
Consensus CDS	CCDS8702.1
UniProtKB/Swiss-Prot	P01116
Related Ensembl	ENSP00000308495, ENST00000311936

Conserved Domains (1) summary

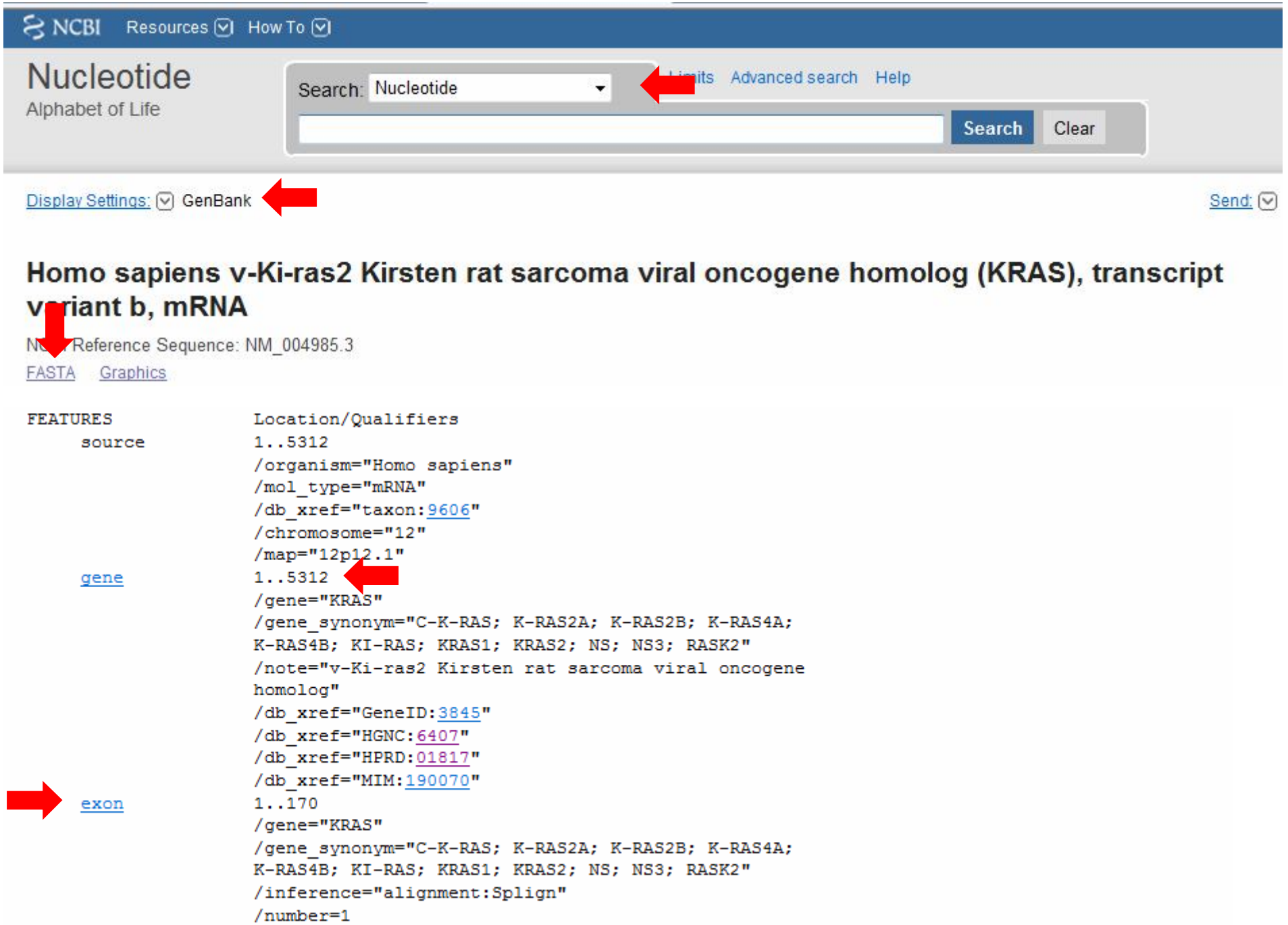
cd04138 Location:3 – 164 Blast Score: 833	H_N_K_Ras_like; H-Ras/N-Ras/K-Ras subfamily. H-Ras, N-Ras, and K-Ras4A/4B are the prototypical members of the Ras family. These isoforms generate distinct signal outputs despite interacting with a common set of activators and effectors, and are strongly associated...

2. **NM_033360.2 › NP_203524.1 GTPase KRas isoform a precursor**

Description	Transcript Variant: This variant (a) is composed of six exons, including exon 4a, which the shorter transcript variant (b) lacks. This rare variant (a) has a cds that terminates in exon 4a and encodes a unique C-terminus, compared to isoform a.
Source sequence(s)	AC092794,AI539465,AK292510,BC010502,BC029545
Consensus CDS	CCDS8703.1
UniProtKB/Swiss-Prot	P01116
Related Ensembl	ENSP00000256078, ENST00000256078

Conserved Domains (2) summary

COG1100 Location:1 – 185 Blast Score: 214	COG1100; GTPase SAR1 and related small G proteins [General function prediction only]
cd04138 Location:3 – 164 Blast Score: 829	H_N_K_Ras_like; H-Ras/N-Ras/K-Ras subfamily. H-Ras, N-Ras, and K-Ras4A/4B are the prototypical members of the Ras family. These isoforms generate distinct signal outputs despite interacting with a common set of activators and effectors, and are strongly associated...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Alphabet of Life
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Limits Advanced search Help
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FASTA

Homo sapiens v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), transcript variant b, mRNA

NCBI Reference Sequence: NM_004985.3

GenBank Graphics

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KRAS v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog [*Homo sapiens*]

Gene ID: 3845, updated on 10-Mar-2011

Summary

Official Symbol KRAS provided by HGNC
Official Full Name v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog provided by HGNC
Primary source HGNC:6407
See related Ensembl:ENSG00000133703; HPRD:01847; MIM:190070
Gene type protein coding

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KI-RAS; C-K-RAS; K-RAS2A; K-RAS2B; K-RAS4A; K-RAS4B; KRAS
homolog from the mammalian ras gene family, encodes a protein that is a member of the small GTPase
ubstitution is responsible for an activating mutation. The transforming protein that results is implicated in
ng adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas and colorectal carcinoma.
s encoding two isoforms that differ in the C-terminal region. [provided by RefSeq]

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SNP: GeneView
SNP: Genotype
SNP: VarView
Taxonomy
UniSTS
BioAssay
UniGene

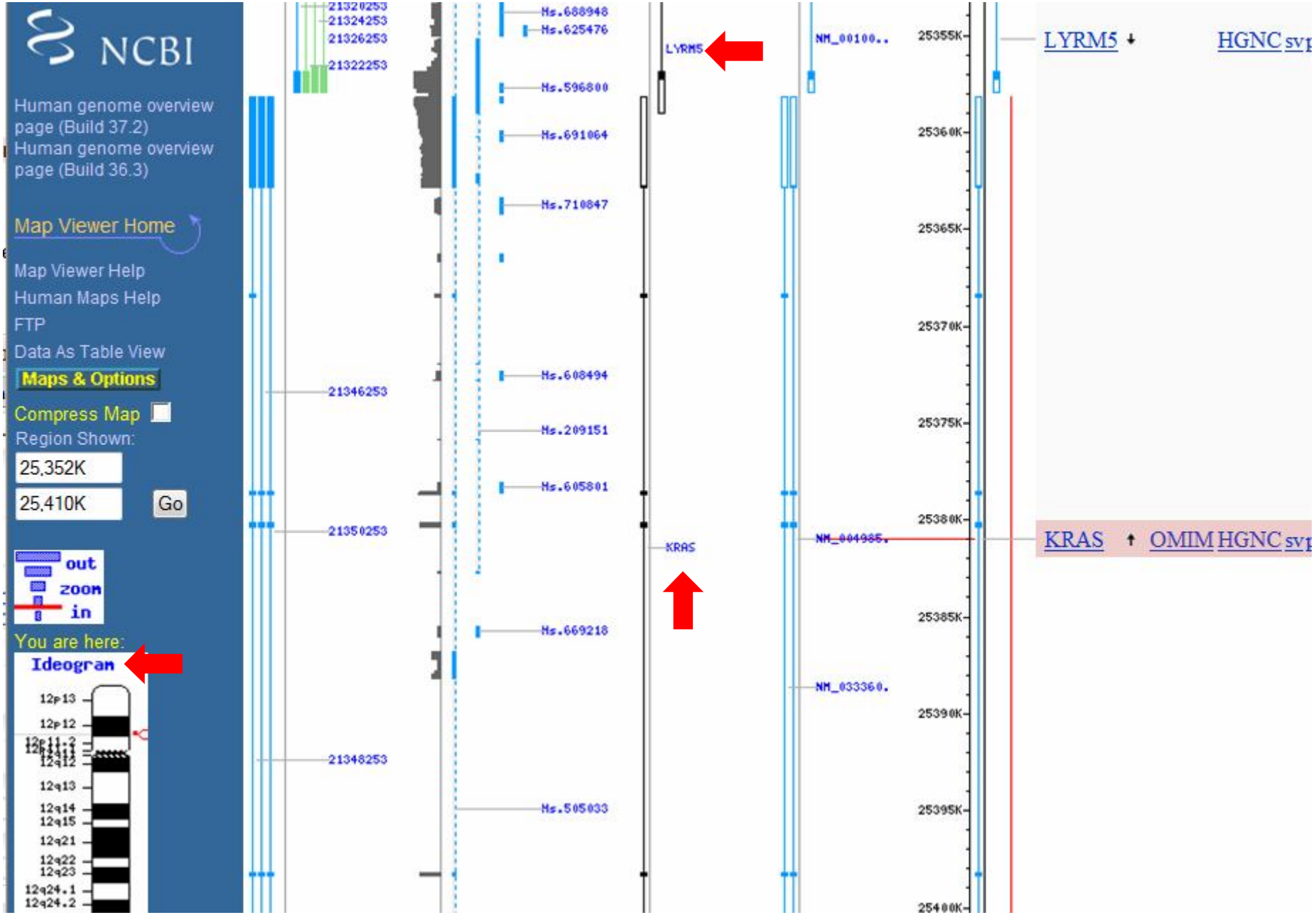
Al final de la página:

Additional Links

Additional Links

MIM 190070
Catalogue of Somatic Mutations in Cancer (COSMIC) KRAS
GeneTests for MIM: 190070
UCSC UCSC
UniGene Hs.505033

Map Viewer


NCBI
Human genome overview page (Build 37.2)
Human genome overview page (Build 36.3)
Map Viewer Home
Map Viewer Help
Human Maps Help
FTP
Data As Table View
Maps & Options
Compress Map
Region Shown:
25,352K
25,410K
Go
out
zoom
in
You are here:
Ideogram
12p13
12p12
12q12
12q13
12q14
12q15
12q21
12q22
12q23
12q24.1
12q24.2
21320253
21324253
21326253
21322253
21346253
21350253
21348253
Hs.688948
Hs.625476
Hs.596800
Hs.691064
Hs.710847
Hs.608494
Hs.209151
Hs.605801
Hs.669218
Hs.505033
LYRM5
KRAS
NM_00100..
NM_004985.
NM_033360.
25355K
25360K
25365K
25370K
25375K
25380K
25385K
25390K
25395K
25400K
LYRM5
HGNC
KRAS
OMIM
HGNC

DISEÑO DE PRIMERS PARA PCR

- CRITERIOS

1. **Especificidad**
2. **Longitud**: 18-24 nt
   a) Especificidad
   b) PCR: Tm y t annealing
3. **Temperatura melting** (Tm): 55-62°C
   a) Depende de longitud y secuencia (%GC)
   b) PCR: fundamental para elección T annealing (Tann < 5-8°C que Tm)
   c) ΔTm de los 2 primers no debe ser >2°C
   d) Fórmula de Wallace Tm=4(G+C) +2(A+T)

4. **% G/C**: 40-60%. (Tm)

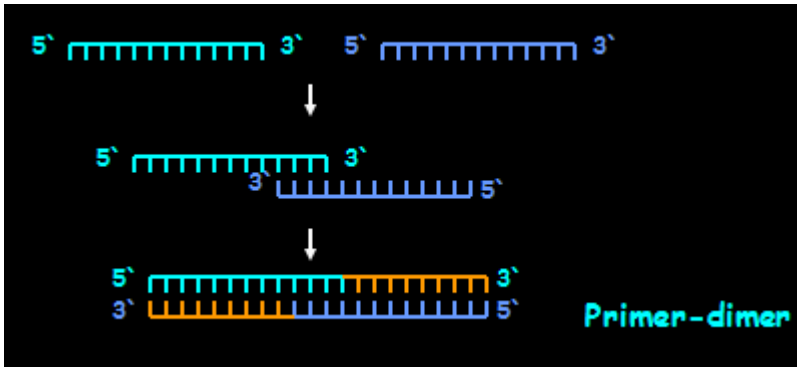
5. **Secuencias complementarias.** Evitar:

```
          5'  GGGAAA
                |||
3'  TATCTAGGACCTTA
```

   a) Homología INTRAprimer (hasta 3pb): hairpins

   b) Homología ENTRE primer, sobre todo en 3' (dímeros)

```
          5' GGGAAAATTCCAGGATCTAT 3'
               ||||   ||||
3' TATCTAGGACCTTAAAAGGG 5'
```



   c) Secuencias polinucleotídicas (>4)

6. **Terminal 3'**

   a. GC clamp: última base G o C y hasta 2 G/C en últimas 5pb

Consideraciones importantes

- Longitud fragmento de ADN a amplificar
- Tipo de templado adecuado: gDNA o cDNA. En este último caso, diseñar los primers para que el gDNA no amplifique o lo haga con otro tamaño más grande (primers en exones diferentes o en unión exón-exón)
- Diseño automático (softwares) o manual
- Objetivo final
- Corroborar especificidad primers: BLAST

http://biotools.umassmed.edu/bioapps/primer3_www.cgi

Primer3: WWW primer tool

pick primers from a DNA sequence

disclaimer | cautions | bugs? suggestions?

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 (vect etc.) or use a Mispriming Library (repeat library): NONE

PEGAR SECUENCIA AQUÍ (FASTA)

☑ 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'->3' on opposite strand).

Pick Primers | 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.. mea primers must flank the central CCCC.

Excluded Regions: E.g. 401,7 68,3 forbids selection of primers in the 7 bases starting at 401 and the 3 bases at 68. Or mark the source sequence with < and >: e.g. ...ATCT<CCCC>TCAT.. forbids primers in the central CCCC.

Product Size Min: 100 Opt: 200 Max: 1000

Number To Return: 5 Max 3' Stability: 9.0

Max Mispriming: 12.00 Pair Max Mispriming: 24.00

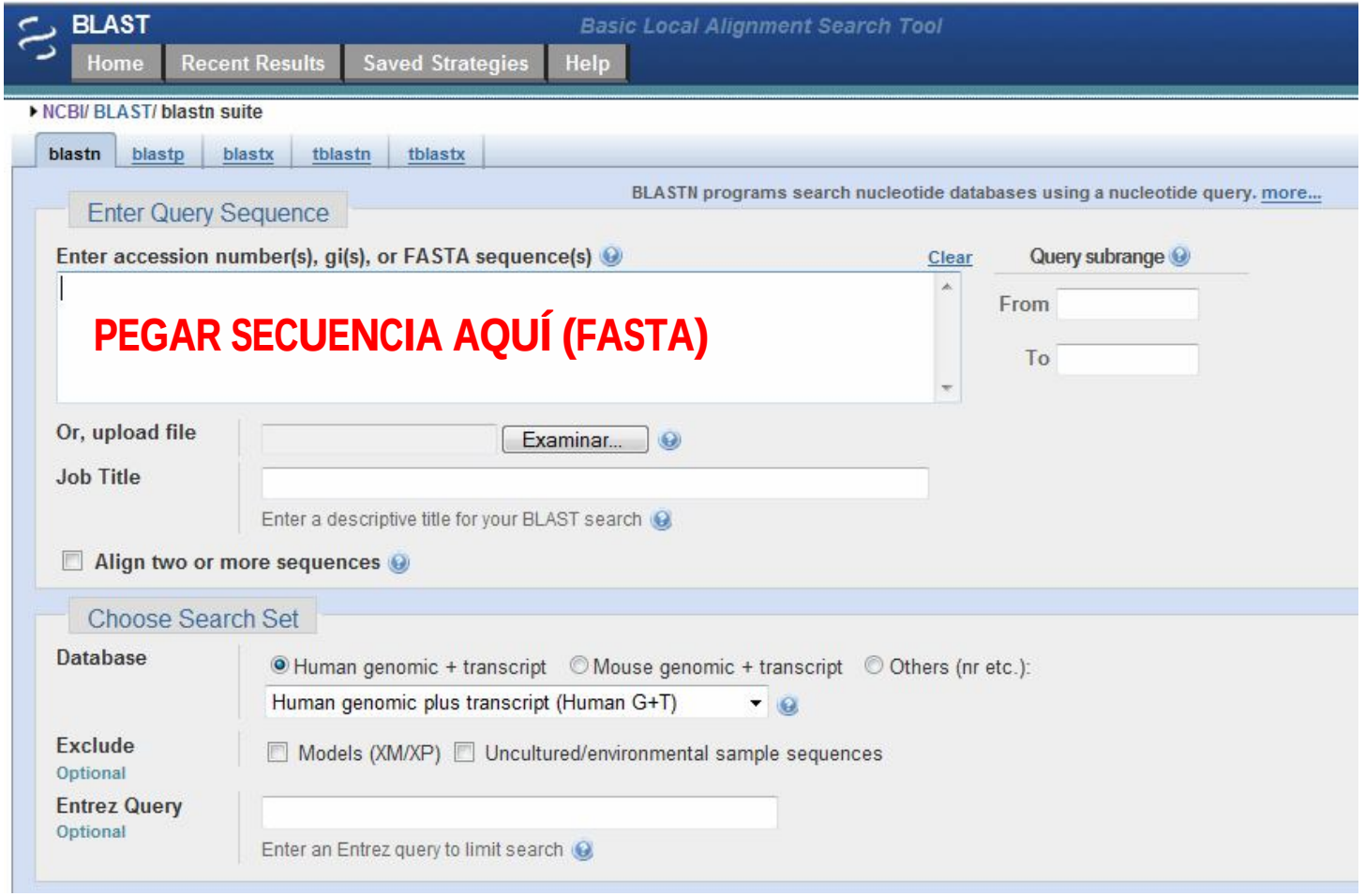
Pick Primers | Reset Form

General Primer Picking Conditions

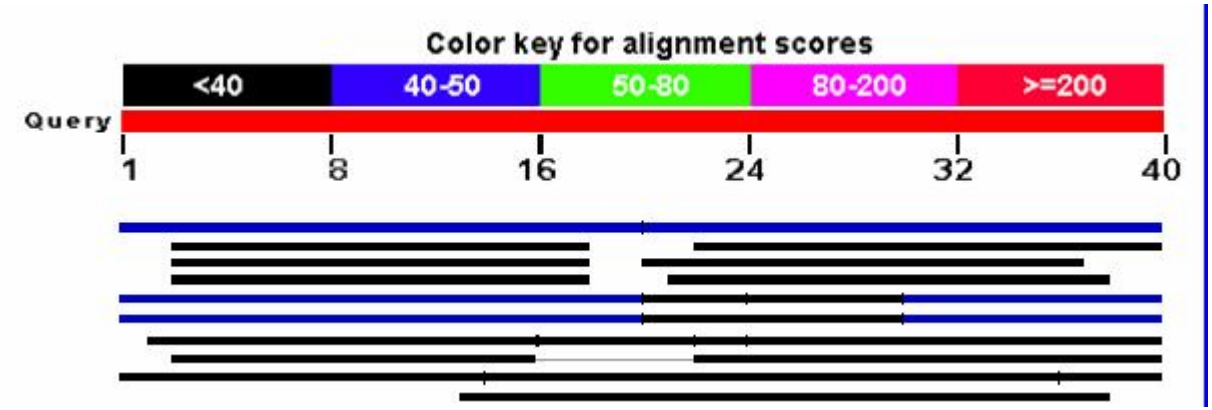
Primer Size	Min: 18	Opt: 20	Max: 27				
Primer Tm	Min: 57.0	Opt: 60.0	Max: 63.0	Max Tm Difference:	100.0		
Product Tm	Min:	Opt:	Max:				
Primer GC%	Min: 20.0	Opt:	Max: 80.0				
Max Self Complementarity:	8.00	Max 3' Self Complementarity:	3.00				
Max #N's:	0	Max Poly-X:	5				
Inside Target Penalty:		Outside Target Penalty:	0	Set Inside Target Penalty to allow primers inside a target.			
First Base Index:	1	CG Clamp:	0				
Salt Concentration:	50.0	Annealing Oligo Concentration:	50.0	(Not the concentration of oligos in the reaction mix but of those annealing to template.)			

☑ Liberal Base ☐ Show Debuging Info

Pick Primers | Reset Form


BLAST
Basic Local Alignment Search Tool
Home
Recent Results
Saved Strategies
Help
NCBI/ BLAST/ blastn suite
blastn
blastp
blastx
tblastn
tblastx
BLASTN programs search nucleotide databases using a nucleotide query. more...
Enter Query Sequence
Enter accession number(s), gi(s), or FASTA sequence(s)
Clear
Query subrange
From
To
PEGAR SECUENCIA AQUÍ (FASTA)
Or, upload file
Examinar...
Job Title
Enter a descriptive title for your BLAST search
Align two or more sequences
Choose Search Set
Database
Human genomic + transcript
Mouse genomic + transcript
Others (nr etc.):
Human genomic plus transcript (Human G+T)
Exclude
Optional
Models (XM/XP)
Uncultured/environmental sample sequences
Entrez Query
Optional
Enter an Entrez query to limit search

RESULTADO:


Color key for alignment scores
<40
40-50
50-80
80-200
>=200
Query
1
8
16
24
32
40

BLAST: algoritmos