Genetics Engineering (Zoo-455)

Lecture-2

Can we mix genes from one creature to another?

Mixing genes for medicine…

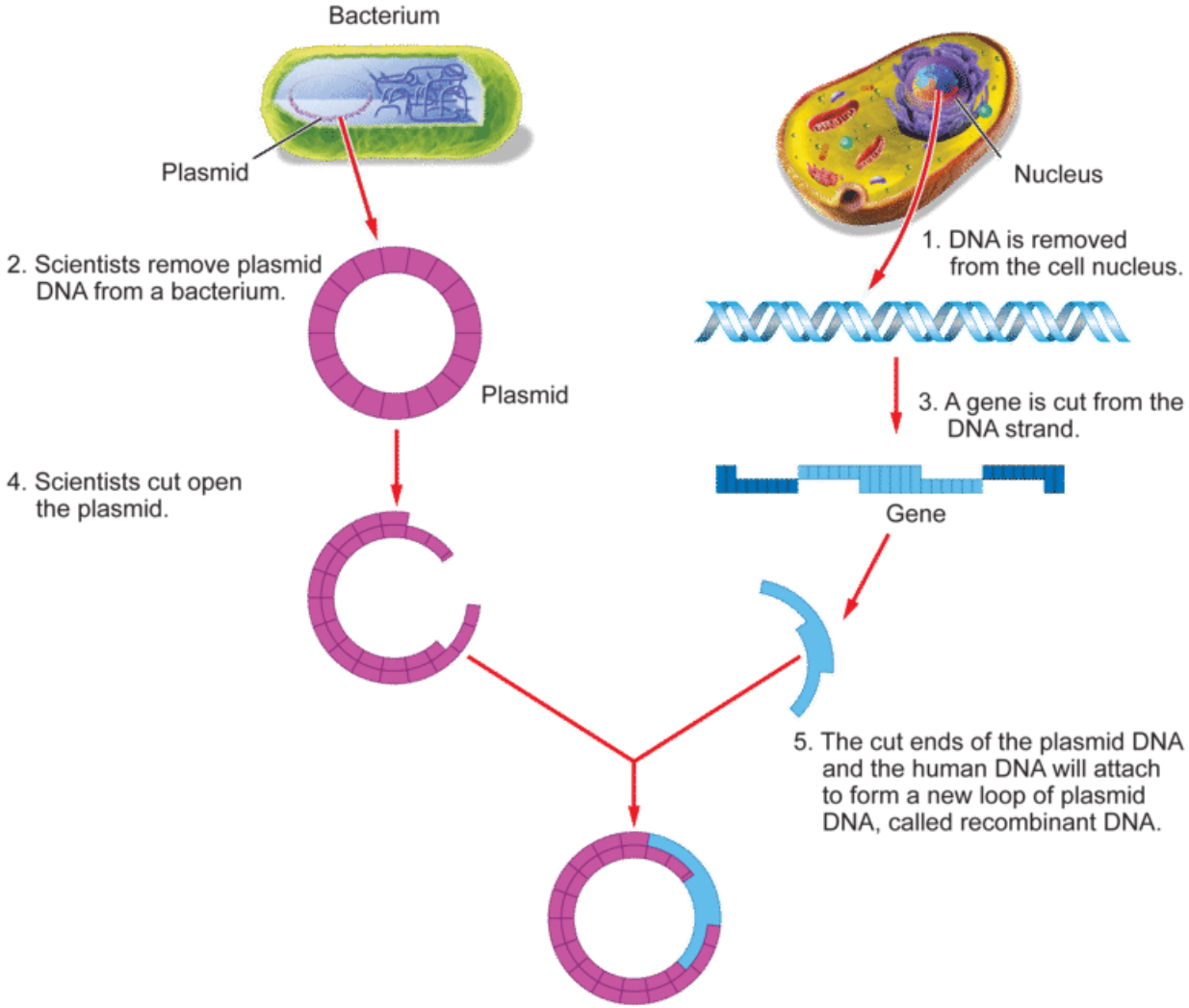
- Allowing organisms to produce new proteins

  bacteria producing human insulin

  bacteria producing human growth hormone

How do we do mix genes?

- Genetic engineering:
  1. Find the gene (NCBI database)
  2. Cut the DNA in both organisms
  3. Paste gene from one creature into other creature's DNA
  4. Insert new chromosome into organism
  5. Organism copies new gene as if it were its own
  6. Organism reads gene as if it were its own
  7. Organism produces NEW protein: Remember: we all use the same genetic code!



Cutting DNA:

- Enzymes that cut DNA
- <u>Restriction enzymes:</u>
  - Used by bacteria to cut up DNA of attacking viruses
  - EcoRI, HindIII, BamHI
  - Cut DNA at specific sites
  - Enzymes look for specific base sequences

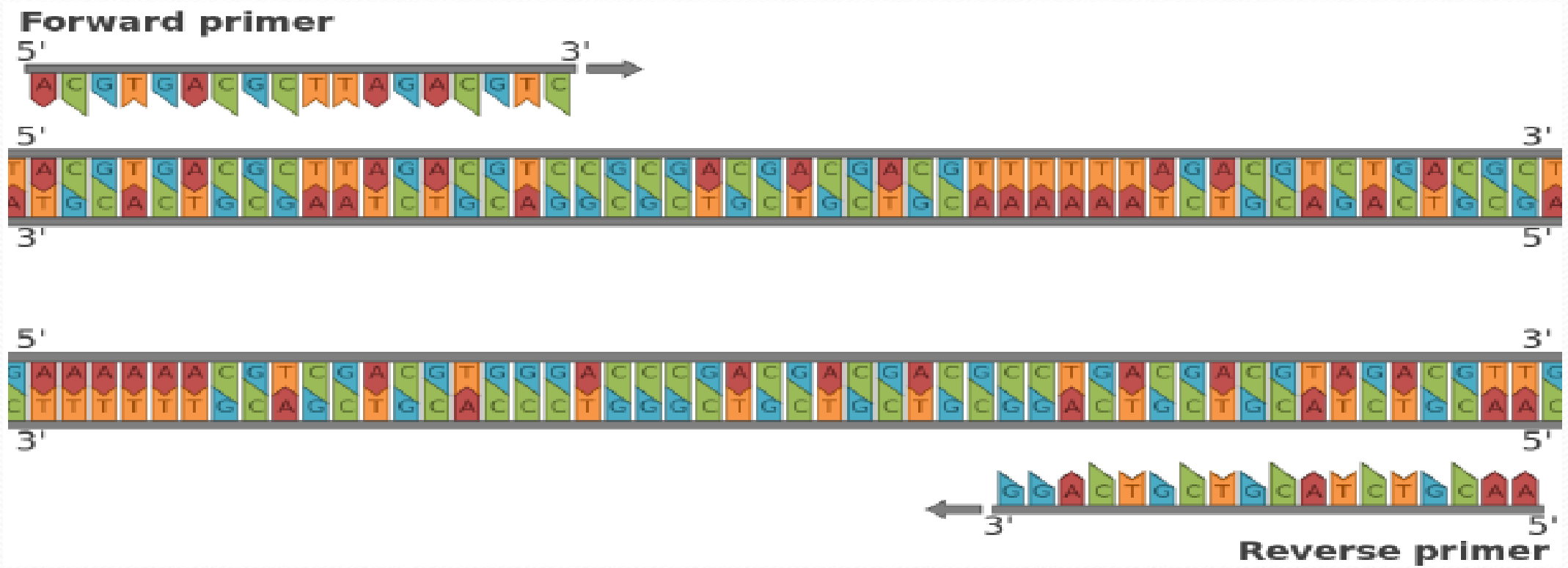
GTAACG▼AATTCACGCTT
CATTGCTTAA▲GTGCGAA

How do you cut the gene of interest by restriction enzyme?

- Primers design for cloning
- PCR Amplification using Phusion High Fidelity PCR Master Mix
- Loading of PCR product onto agarose gel electrophoresis
- DNA isolation from agarose gel
- Digestion of PCR inserts by restriction endonucleases

What is a Primer for cloning?

- It is a short oligonucleotide sequences, which complement to the target sequence and bind to the single-stranded DNA.
- It has a restriction enzyme site to the 5' end of the forward and reverse primers
- Taq DNA polymerases start replication at the 3’-end of the primer, and copies the opposite strand.


Forward primer
5'
3'
Reverse primer

Primer Design Guide for cloning:

- Design two primers: forward primer and reverse primer from 5' end to 3' end.
- The length of typical primers: from 18 to 24 nucleotides.
- GC content: design primers that contain between 40% and 60% GC.
- Melting Temperatures (Tm): the primers should be selected from 55°C to 60°C .
- Annealing Temperatures (Ta)= Tm - 5
- The two primers should be to have similar melting temperatures.

- The forward and reverse primers design in a 5'---3' orientation and contain a start codon (ATG) in the 5' primer and a stop codon (TTA) in the 3' primer.
- Use the following program to calculate the Tm for each primer http://biotools.nubic.northwestern.edu/OligoCalc.html
- Use the following program "Reverse Complement" to convert a DNA sequence into its reverse (https://www.bioinformatics.org/sms/rev_comp.html)

What is the tool that can predict restriction enzyme sites for the DNA cloning?

- Use the NEBcutter -- A restriction analysis tool (https://nc3.neb.com/NEBcutter/) to predict restriction enzyme sites for the DNA cloning.
- Enter a DNA sequence, or select from other options, to identify cut sites.
- Press submit.
- Choose a restriction enzyme that has a Zero cutter
- Add restriction enzyme sites to the 5' end of the forward and reverse primers before the CDS sequence.
- Add bases a couple of bases (GTG) to the 5' end of the primers to preserve restriction site during PCR.


Esc
F1
F2
F3
F4
F5
F6
F7
F8
F9
F10
F11
F12
Prt Sc
Sys Rq
Delete
Scr Lk
Insert
Num Lk
Pause
Break
Home
PgUp
PgDn
End
Caps
Lock
A
S
D
F
G
H
J
K
L
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E
R
T
Y
U
I
O
P
Z
X
C
V
B
N
M
Ctrl
Fn
Alt
Alt Gr
Ctrl

نشاط فردي: (ساعة)

Practice exercises on primer design for cloning

Question 1:

Design your cloning primers for the CDS of the human *ACTB* gene, after following the given criteria:

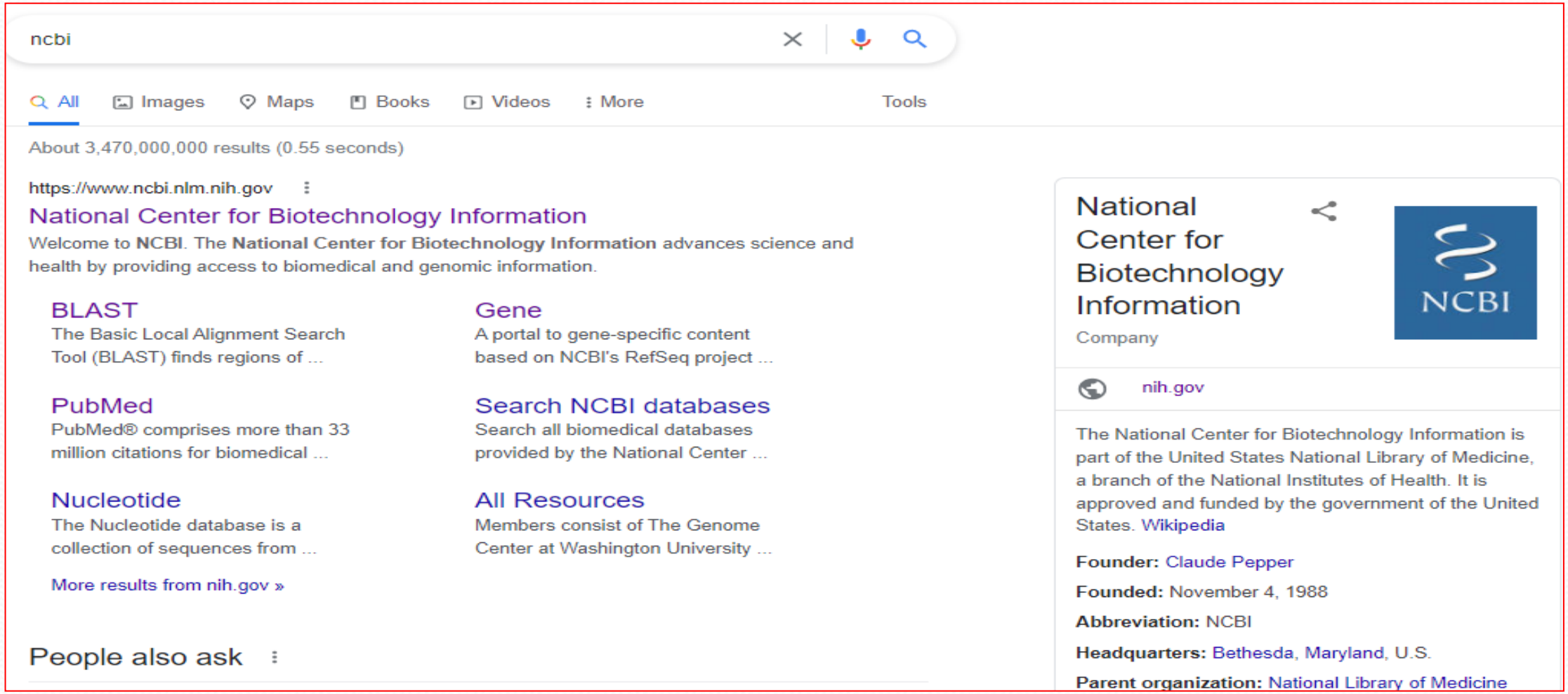
1) the length of each primer is between 18-22 bp
2) each primer must contain 50-60% GC content
3) the Tm of both primers is nearly identical
4) zero cutter of a restriction enzyme
5) add restriction enzyme sites to the 5' end of the forward and reverse primers
6) add bases a couple of bases (GTG) to the 5' end of the primers

Procedure of primer design for DNA cloning:

1) Switch the computer on and click on the internet explore icon.
2) Go to http://www.google.com, then type National Center for Biotechnology Information (NCBI).
3) On the NCBI page, click on Gene from the Popular Recourses menu.
4) Enter the gene name, which is looking for and type of human organism (*Homo sapiens)*. Then, click on search.
5) Click on the correct gene, then click on the CDS code, which is in front of consensus CDS.
6) Copy the CDS sequence of the gene and paste it on a new file of word document.

Nucleotide Sequence for CDS of ACTB gene (1128nt)

ATGGATGATGATATCGCCGCGCTCGTCGTCGACAACGGCTCCGGCATGTGCAAGGCCGGCTTC
GCGGGCGACGATGCCCCCCGGGCCGTCTTCCCCTCCATCGTGGGGCGCCCCAGGCACCAGGGC
GTGATGGTGGGCATGGGTCAGAAGGATTCCTATGTGGGCGACGAGGCCCAGAGCAAGAGAGGC
ATCCTCACCCTGAAGTACCCCATCGAGCACGGCATCGTCACCAACTGGGACGACATGGAGAAA
ATCTGGCACCACACCTTCTACAATGAGCTGCGTGTGGCTCCCGAGGAGCACCCCGTGCTGCTGA
CCGAGGCCCCCCTGAACCCCAAGGCCAACCGCGAGAAGATGACCCAGATCATGTTTGAGACCT
TCAACACCCCAGCCATGTACGTTGCTATCCAGGCTGTGCTATCCCTGTACGCCTCTGGCCGTACC
ACTGGCATCGTGATGGACTCCGGTGACGGGGTCACCCACACTGTGCCCATCTACGAGGGGTATG
CCCTCCCCCATGCCATCCTGCGTCTGGACCTGGCTGGCCGGGACCTGACTGACTACCTCATGAA
GATCCTCACCGAGCGCGGCTACAGCTTCACCACCACGGCCGAGCGGGAAATCGTGCGTGACATT
AAGGAGAAGCTGTGCTACGTCGCCCTGGACTTCGAGCAAGAGATGGCCACGGCTGCTTCCAGCT
CCTCCCTGGAGAAGAGCTACGAGCTGCCTGACGGCCAGGTCATCACCATTGGCAATGAGCGGTT
CCGCTGCCCTGAGGCACTCTTCCAGCCTTCCTTCCTGGGCATGGAGTCCTGTGGCATCCACGAAA
CTACCTTCAACTCCATCATGAAGTGTGACGTGGACATCCGCAAAGACCTGTACGCCAACACAGT
GCTGTCTGGCGGCACCACCATGTACCCTGGCATTGCCGACAGGATGCAGAAGGAGATCACTGCC
CTGGCACCCAGCACAATGAAGATCAAGATCATTGCTCCTCCTGAGCGCAAGTACTCCGTGTGGA
TCGGCGGCTCCATCCTGGCCTCGCTGTCCACCTTCCAGCAGATGTGGATCAGCAAGCAGGAGTA
TGACGAGTCCGGCCCCTCCATCGTCCACCGCAAATGCTTC**TAG**


ncbi
All
Images
Maps
Books
Videos
More
Tools
About 3,470,000,000 results (0.55 seconds)
https://www.ncbi.nlm.nih.gov
National Center for Biotechnology Information
Welcome to NCBI. The National Center for Biotechnology Information advances science and health by providing access to biomedical and genomic information.
BLAST
The Basic Local Alignment Search Tool (BLAST) finds regions of ...
Gene
A portal to gene-specific content based on NCBI's RefSeq project ...
PubMed
PubMed® comprises more than 33 million citations for biomedical ...
Search NCBI databases
Search all biomedical databases provided by the National Center ...
Nucleotide
The Nucleotide database is a collection of sequences from ...
All Resources
Members consist of The Genome Center at Washington University ...
More results from nih.gov »
People also ask
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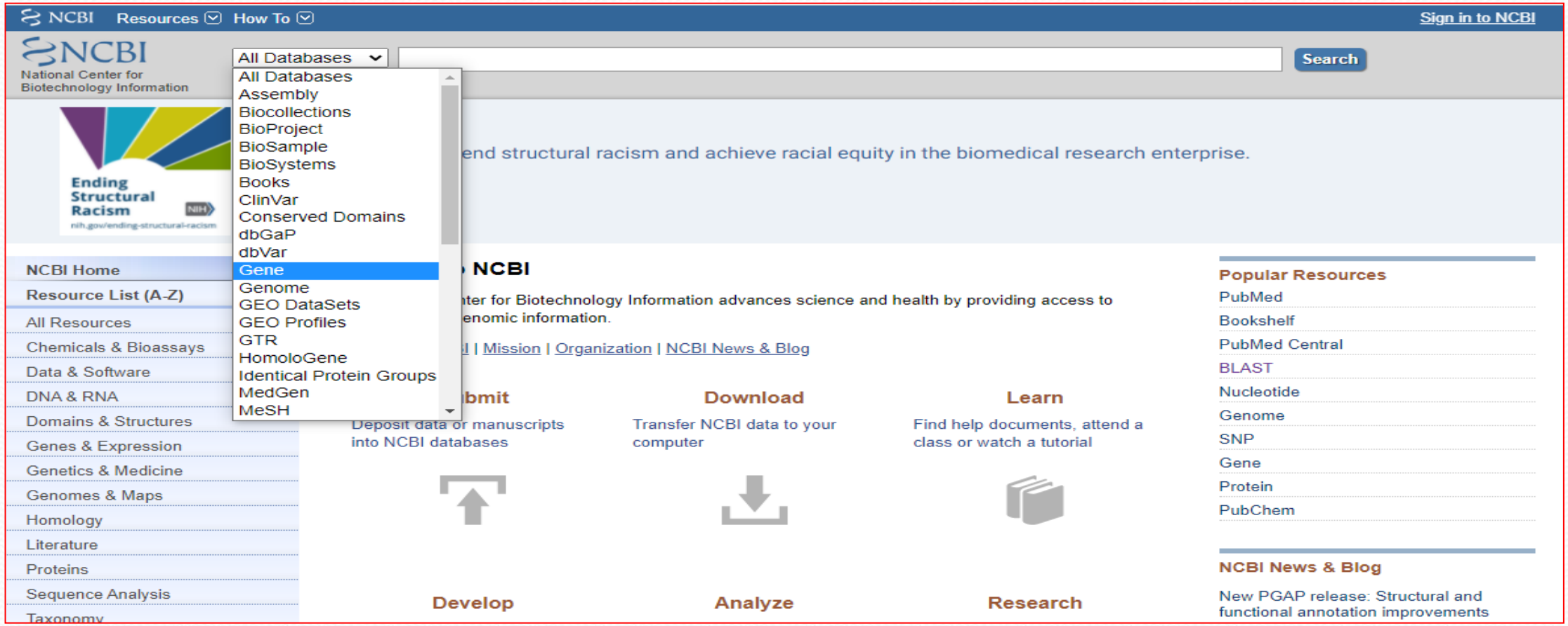
Company
NCBI
nih.gov
The National Center for Biotechnology Information is part of the United States National Library of Medicine, a branch of the National Institutes of Health. It is approved and funded by the government of the United States. Wikipedia
Founder: Claude Pepper
Founded: November 4, 1988
Abbreviation: NCBI
Headquarters: Bethesda, Maryland, U.S.
Parent organization: National Library of Medicine


NCBI
Resources
How To
Sign in to NCBI
NCBI
National Center for Biotechnology Information
All Databases
Search
All Databases
Assembly
Biocollections
BioProject
BioSample
BioSystems
Books
ClinVar
Conserved Domains
dbGaP
dbVar
Gene
Genome
GEO DataSets
GEO Profiles
GTR
HomoloGene
Identical Protein Groups
MedGen
MeSH
Ending Structural Racism
nih.gov/ending-structural-racism
end structural racism and achieve racial equity in the biomedical research enterprise.
NCBI Home
Resource List (A-Z)
All Resources
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Genomes & Maps
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Taxonomy
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ter for Biotechnology Information advances science and health by providing access to enomic information.
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bmit
Deposit data or manuscripts into NCBI databases
Develop
Download
Transfer NCBI data to your computer
Analyze
Learn
Find help documents, attend a class or watch a tutorial
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Genome
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Gene
Protein
PubChem
NCBI News & Blog
New PGAP release: Structural and functional annotation improvements

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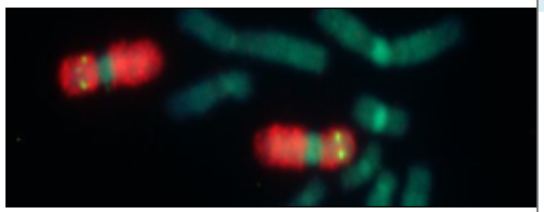
Gene

Gene ACTB

Search

Help

- Gallus gallus ACTB
- Homo sapiens ACTB
- ACTB orthologs
- Mus musculus Actb
- Gallus gallus ACTBL2
- Rattus norvegicus Actb
- Homo sapiens ACTBL2
- Danio rerio actb1
- Sus scrofa ACTB
- Bos taurus ACTB
- Drosophila melanogaster Actbeta
- Ovis aries ACTB
- ACTBL2 orthologs
- Oncorhynchus mykiss actb
- Cricetulus griseus Actb
- Danio rerio actb2
- actb1 orthologs
- Corvus cornix cornix ACTB
- Canis lupus familiaris ACTB
- Macaca mulatta ACTB



ature, Reference Sequences
nd locus-specific resources

Using Gene

Gene Quick Start

FAQ

Download/FTP

RefSeq Mailing List

Gene News

Factsheet

Representative queries

Gene

Gene | Search

Advanced | Help

Full Report | Send to: | Hide sidebar >>

ACTB actin beta [*Homo sapiens* (human)]

Download Datasets

Gene ID: 60, updated on 5-Dec-2021

Summary

Official Symbol	ACTB provided by HGNC
Official Full Name	actin beta provided by HGNC
Primary source	HGNC:HGNC:132
See related	Ensembl:ENSG00000075624 MIM:102630
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	BRWS1; PS1TP5BP1
Summary	This gene encodes one of six different actin proteins. Actins are highly conserved proteins that are involved in cell motility, structure, integrity, and intercellular signaling. The encoded protein is a major constituent of the contractile apparatus and one of the two nonmuscle cytoskeletal actins that are ubiquitously expressed. Mutations in this gene cause Baraitser-Winter syndrome 1, which is characterized by intellectual disability with a distinctive facial appearance in human patients. Numerous pseudogenes of this gene have been identified throughout the human genome. [provided by RefSeq, Aug 2017]
Expression	Ubiquitous expression in appendix (RPKM 2395.4), lymph node (RPKM 2072.0) and 24 other tissues See more
Orthologs	mouse all

Table of contents

Genomic

1. **NG_007992.1 RefSeqGene**

Range	5001..8454
Download	GenBank, FASTA, Sequence Viewer (Graphics), LRG_132

mRNA and Protein(s)

1. **NM_001101.5 → NP_001092.1 actin, cytoplasmic 1**

See identical proteins and their annotated locations for NP_001092.1

Status: REVIEWED

Source sequence(s)	AK130157, BC009636
Consensus CDS	CCDS5341.1
UniProtKB/Swiss-Prot	P60709
UniProtKB/TrEMBL	Q1KLZ0
Related	ENSP00000494750.1, ENST00000646664.1

Conserved Domains (1) summary

PTZ00281 Location:1 → 375	PTZ00281; actin; Provisional

CCDS Sequence Data

Blue highlighting indicates alternating exons.

Red highlighting indicates amino acids encoded across a splice junction.

Mouse over the nucleotide or protein sequence below and click on the highlighted codon or residue to select the pair.

Nucleotide Sequence (1128 nt):

ATGGATGATGATATCGCCGCGCTCGTCGTCGACAACGGCTCCGGCATGTGCAAGGCCGGCTTCGCGGGCG
ACGATGCCCCCCGGGCCGTCTTCCCCTCCATCGTGGGGCGCCCCAGGCACCAGGGCGTGATGGTGGGCAT
GGGTCAGAAGGATTCCTATGTGGGCGACGAGGCCCAGAGCAAGAGAGGCATCCTCACCCTGAAGTACCCC
ATCGAGCACGGCATCGTCACCAACTGGGACGACATGGAGAAAATCTGGCACCACACCTTCTACAATGAGC
TGCGTGTGGCTCCCGAGGAGCACCCCGTGCTGCTGACCGAGGCCCCCCTGAACCCCAAGGCCAACCGCGA
GAAGATGACCCAGATCATGTTTGAGACCTTCAACACCCCAGCCATGTACGTTGCTATCCAGGCTGTGCTA
TCCCTGTACGCCTCTGGCCGTACCACTGGCATCGTGATGGACTCCGGTGACGGGGTCACCCACACTGTGC
CCATCTACGAGGGGTATGCCCTCCCCCATGCCATCCTGCGTCTGGACCTGGCTGGCCGGGACCTGACTGA
CTACCTCATGAAGATCCTCACCGAGCGCGGCTACAGCTTCACCACCACGGCCGAGCGGGAAATCGTGCGT
GACATTAAGGAGAAGCTGTGCTACGTCGCCCTGGACTTCGAGCAAGAGATGGCCACGGCTGCTTCCAGCT
CCTCCCTGGAGAAGAGCTACGAGCTGCCTGACGGCCAGGTCATCACCATTGGCAATGAGCGGTTCCGCTG
CCCTGAGGCACTCTTCCAGCCTTCCTTCCTGGGCATGGAGTCCTGTGGCATCCACGAAACTACCTTCAAC
TCCATCATGAAGTGTGACGTGGACATCCGCAAAGACCTGTACGCCAACACAGTGCTGTCTGGCGGCACCA
CCATGTACCCTGGCATTGCCGACAGGATGCAGAAGGAGATCACTGCCCTGGCACCCAGCACAATGAAGAT
CAAGATCATTGCTCCTCCTGAGCGCAAGTACTCCGTGTGGATCGGCGGCTCCATCCTGGCCTCGCTGTCC
ACCTTCCAGCAGATGTGGATCAGCAAGCAGGAGTATGACGAGTCCGGCCCCTCCATCGTCCACCGCAAAT
GCTTCTAG

Translation (375 aa):

MDDDIAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEAQSKRGILTLKYP
IEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVL
SLYASGRTTGIVMDSGDGVTHTVPIYEGYALPHAILRLDLAGRDLTDYLMKILTERGYSFTTTAEREIVR
DIKEKLCYVALDFEQEMATAASSSSLEKSYELPDGQVITIGNERFRCPEALFQPSFLGMESCGIHETTFN
SIMKCDVDIRKDLYANTVLSGGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSILASLS
TFQQMWISKQEYDESGPSIVHRKCF

Oligo Calc: Oligonucleotide Properties Calculator

Enter Oligonucleotide Sequence Below
OD calculations are for single-stranded DNA or RNA

Nucleotide base codes

AGA AAA TCT GGC ACC ACA CC

Reverse Complement Strand(5' to 3') is:

GGT GTG GTG CCA GAT TTT CT

5' modification (if any) | 3' modification (if any) | Select molecule: ssDNA

50 nM Primer
50 mM Salt (Na^+)
1 Measured Absorbance at 260 nanometers

Calculate | Swap Strands | BLAST | mfold

Physical Constants

Length: 20 Molecular Weight: 6064 [4] GC content: 50 %

1 ml of a sol'n with an Absorbance of 1 at 260 nm
is 4.469 microMolar [5] and contains 27.1 micrograms.

Melting Temperature (T_M) Calculations

1	51.8	°C (Basic)
2	58.4	°C (Salt Adjusted)
3	51.27	°C (Nearest Neighbor)

Thermodynamic Constants Conditions: 1 M NaCl at 25°C at pH 7.

RlnK 33.404 cal/(°K*mol) | deltaH 164.6 Kcal/mol
deltaG 25.4 Kcal/mol | deltaS 432.8 cal/(°K*mol)

Deprecated Hairpin/self dimerization calculations

5 (Minimum base pairs required for single primer self-dimerization)
4 (Minimum base pairs required for a hairpin)

Check Self-Complementarity

Reverse Complement

Reverse Complement converts a DNA sequence into its reverse, complement, or reverse-complement counterpart. You may want to work with the reverse-complement of a sequence if it contains an ORF on the reverse strand.

Paste the raw or FASTA sequence into the text area below.

```
>Sample sequence
GGGGaaaaaaaatttatatat
```

SUBMIT CLEAR

- Convert the DNA sequence into its reverse-complement counterpart.

1

[home]

Reverse Complement

Reverse Complement converts a DNA sequence into its reverse, complement, or reverse-complement counterpart. You may want to work with the reverse-complement of a sequence if it contains an ORF on the reverse strand.

Paste the raw or FASTA sequence into the text area below.

```
AGGAAGGAAGGCTGGAAGAG
```

SUBMIT CLEAR

- Convert the DNA sequence into its reverse-complement counterpart.

2

[home]

The Sequence Manipulation Suite: Reverse Complement

Results for 20 residue sequence starting "AGGAAGGAAG".

```
CTCTTCCAGCCTTCCTTCCT
```

3

NEBcutter®

v3.0.17

WELCOME GUEST, SIGN IN OR SIGN UP | FEEDBACK | HELP

NEW ENGLAND BioLabs Inc. — be INSPIRED drive DISCOVERY stay GENUINE

en Recent Project

jects will be automatically
eted 7 day(s) after they were
t accessed.

amed

Disable cookies

Enter a DNA sequence, or select from other options, to identify cut sites. Once you submit a sequence, you may choose to customize your digest.

1. Input or choose sequence.

Text | File | GenBank | Plasmid Vector | Viral & Phage

Type or paste sequence

2. Set preferences.

Circular

Additional Preferences (enzymes, oligos, etc)

3. Name project (optional).

Enter project name

Submit

NEBcutter®

v3.0.17

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Create New Project

Unnamed

Download

- Graphical View
- Enzyme List
- Sequence
- ORF Summary
- Flanking Sites
- Custom Digest

Display

- [ ] Circular
- [x] Linear
- [ ] Alternative
- [x] 1 cutters
- [ ] 2 cutters
- [ ] 3 cutters

List 0 cutters

Cleavage

- Blunt-End Cut
- Cuts 1 strand
- 5′ Extension
- 3′ Extension

Supplier

- NEB
- Other supplier
- Not commercially available

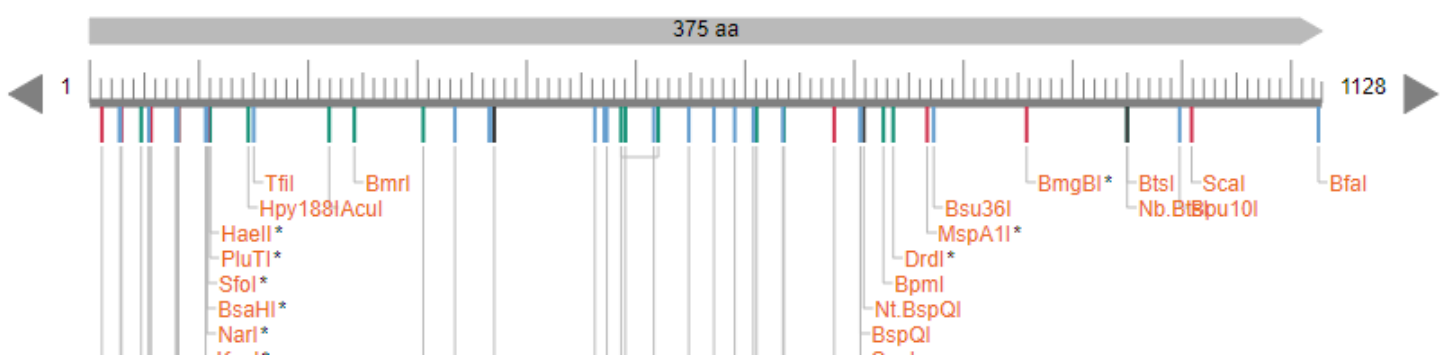
Site

- * Affected by CpG methylation
- # Afffected by other methylation
- () Ambiguous site

- [ ] Show flanks

Results For: Edit

- Enzymes:
  - Supplier: NEB
  - Type: Type I, II, III, Homing, Nicking
- ORFs:



Questions?