

Second Class

- To understand commonly used method to analyze DNA sequence on computer
- Sequence prediction of GFP-CaMKII fusion protein.
- No report. Send the sequence file and calculate molecular weight of the fusion protein by e-mail.

CaMKII: Ca²⁺/calmodulin dependent protein kinase

- Important enzyme for learning and memory
- Activated by Ca²⁺
- Change its localization by stimulation
- Four subtypes α , β , γ , and δ

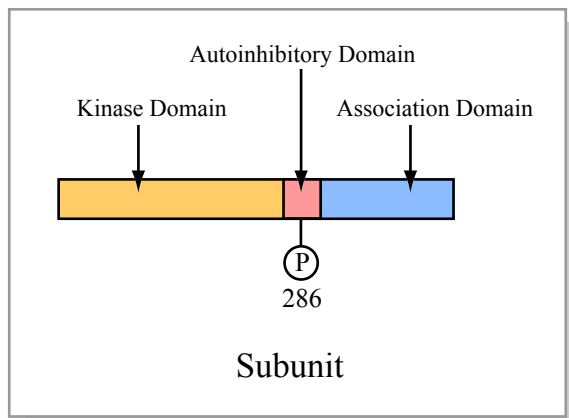


Figure by MIT OCW.

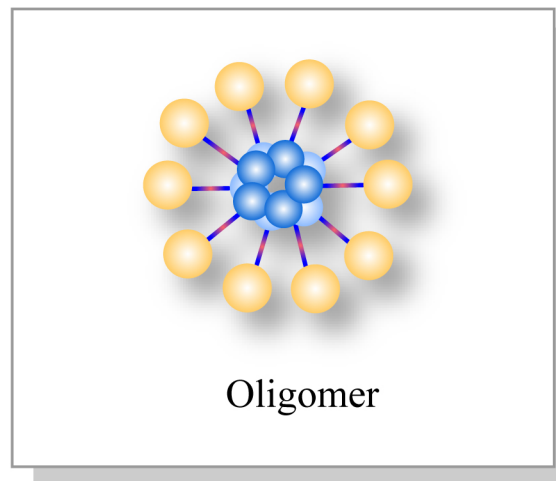


Figure by MIT OCW.

Image removed due to copyright restrictions.

GFP: Green Fluorescent Protein

Images removed due to copyright restrictions.
Jellyfish - source of GFP gene.
Neurons expressing GFP.

A neuron expressing fusion
protein of GFP and CaMKII

Neuronal activity dependent translocation of GFP-CaMKII

Movie by Bayer et al.

PCR (polymerase chain reaction)

CaMKII α cDNA



PCR

Amplify coding region

Add restriction enzyme site

XhoI

EcoRI

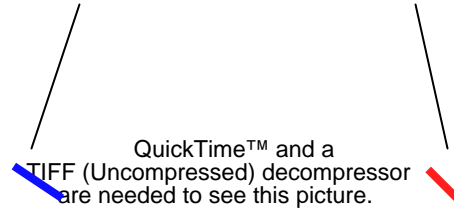


PCR

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Enzyme sites

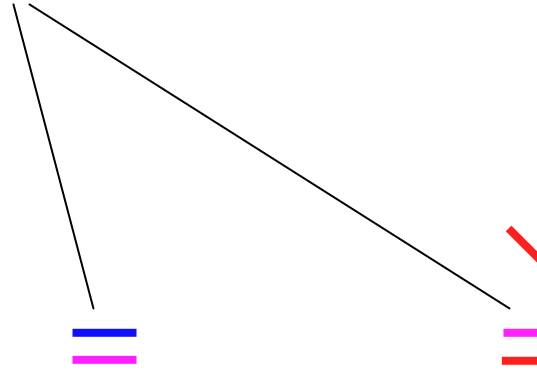
QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.



QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.



Enzyme sites are incorporated into the PCR product



QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.



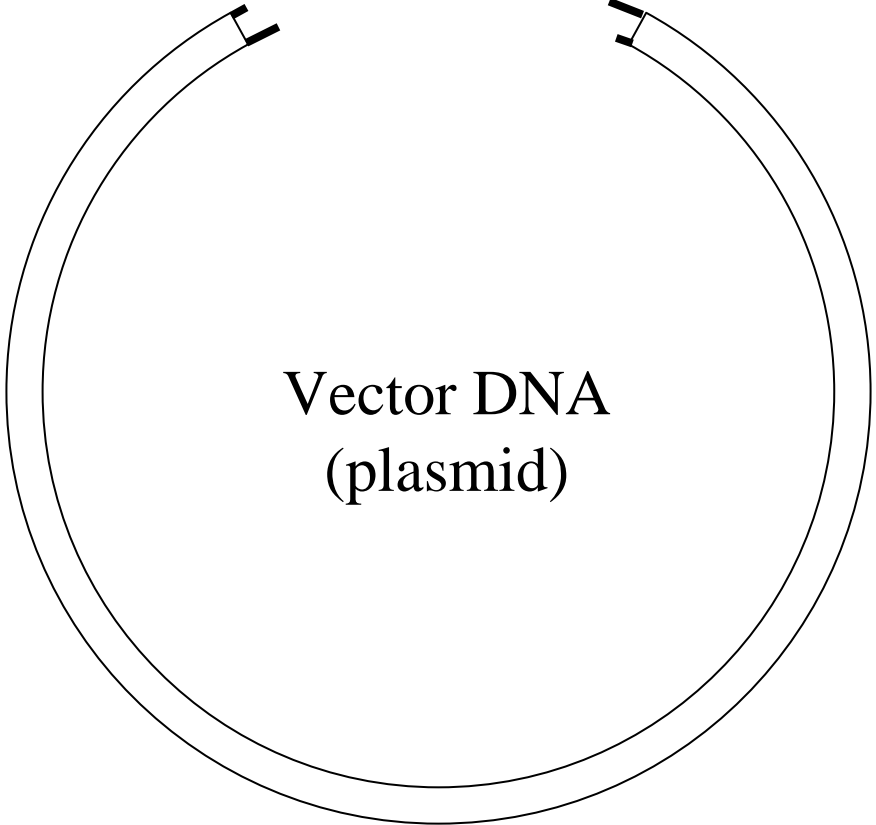


QuickTime™ and
TIFF (Uncompressed) decompressor
are needed to see this picture.

Enzyme digestion



ligation



QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

- A bit outdated, but enough for daily use
- Mac OS 9. Works on Classic mode on OS X
- No Intel Mac
- Vector NTI, DNASTar etc.

Download DNA sequence

- Goto <http://www.ncbi.nlm.nih.gov/>
- Choose "nucleotide" as database.
- Search for "Rat calcium/calmodulin-dependent protein kinase type II alpha-subunit mRNA"
- Find right record and click
- Copy nucleotide sequence from bottom of the record
- Open DNAsis
- File -> New Select DNA
- Paste DNA sequence
- Print-out the record for later reference

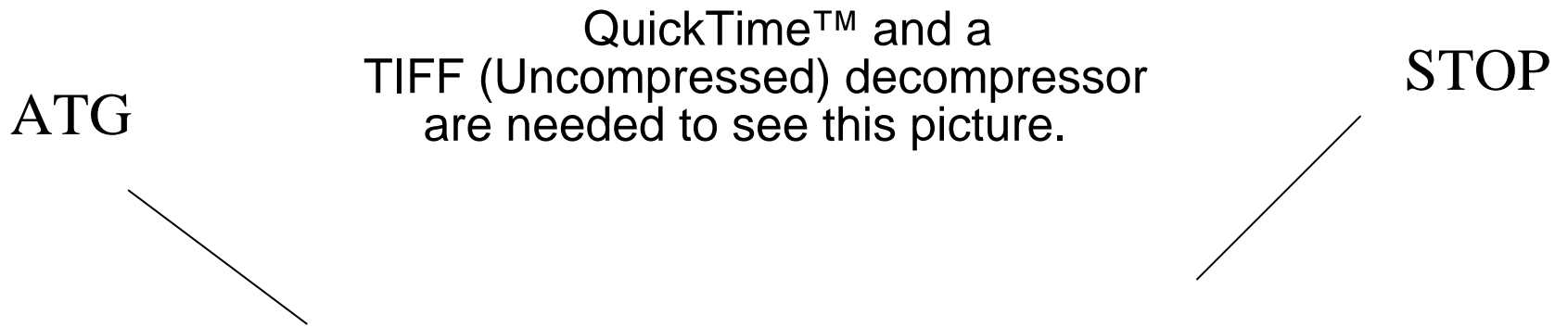
Image removed due to copyright restrictions.
Screenshot of DNAsis program.

Click "double
strand view"

Print-out sequence

Open-reading frame search

- How many reading frames are there?
- Function -> search -> open reading frame



- Click LUNGES 1 open-reading frame

Images removed due to copyright restrictions.
Screenshots of DNAsis program.

Select frame 1

The coding region and length of protein should match with record.

Primer Sequence

If you do not know
5' and 3' of DNA,
please talk to me.

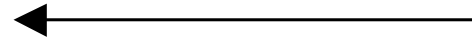
5' Primer GAT CCT CGA GCT ATG GCT ACC ATC ACC TGC ACC

CaMKII alpha

ATG GCT ACC ATC ACC TGC ACC
Met Ala Thr Ile Thr Cys Thr

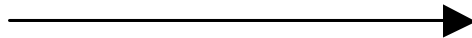
CaMKII alpha CTG CCC CAT TGA AGG ACC AGG CCA GGG
Leu Pro His ***

3' Primer GTA ACT TCC GTT GCC GGT CCC GTA CGCTT AAGC TAG



(usual writing, 5' -> 3')

GAT CGA ATT CGC ATG CCC TGG CCG TTG CCT TCA ATG



Expect the sequence of PCR product

- Search primer sequence: Sequence -> Find. Type in the 5' primer sequence. Do not include the restriction enzyme sequence.
- Do the same for the 3' sequence but *complementary strand*.
- Delete the sequence before and after the region amplified by PCR.
- Add primer sequence at both ends.

Note: what is the sequence you need to add 3'?

Restriction Enzyme Search

- Function -> Search -> Restriction Enzyme
- Select Enzyme, click "select"
- First click "none"
- Select EcoRI and XhoI, click OK
- Sequence type, select "linear"
- Click GO
- A table will open
- There must be two sites

Change view by
clicking icons.

Identify the same
place on the sequence
file (you can print out
if you want)

Image removed due to copyright restrictions.
Screenshot of DNAsis program.

Subcloning

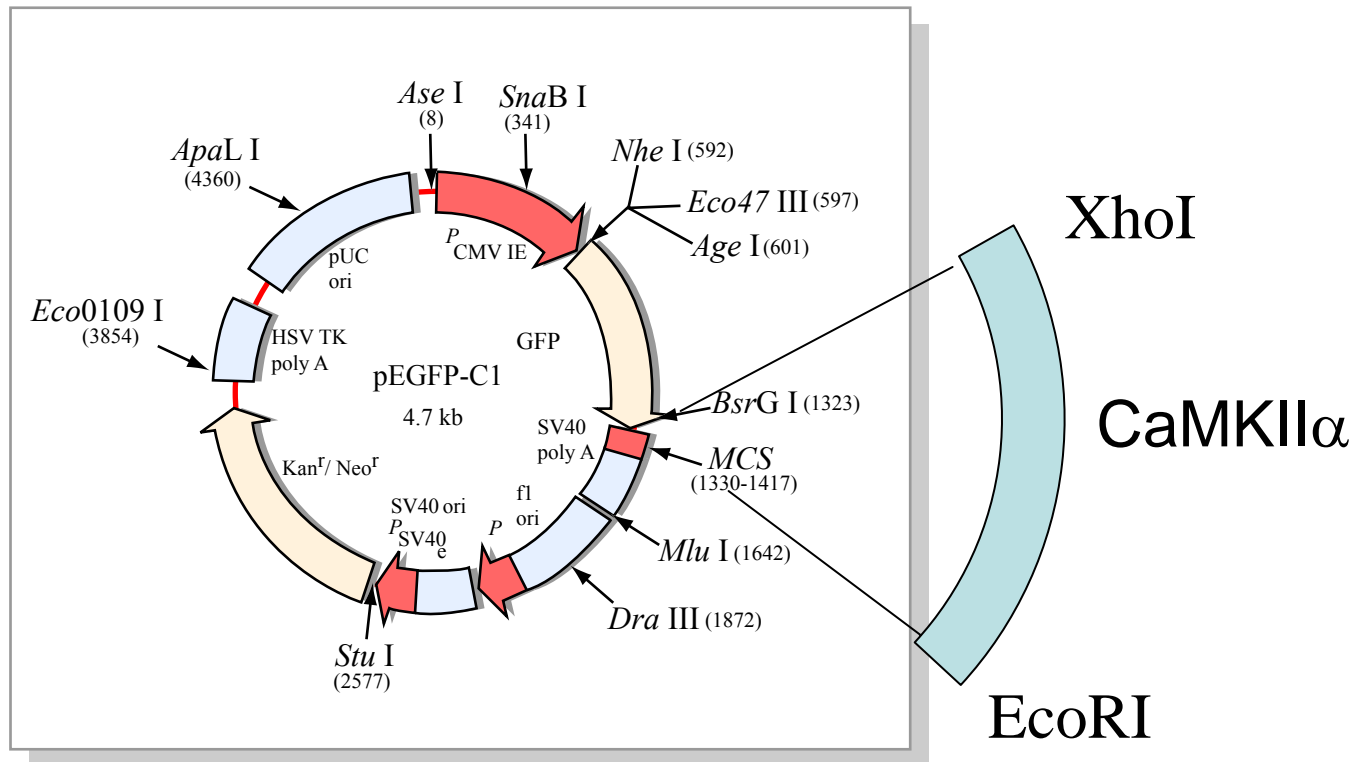


Figure by MIT OCW.

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

pEGFP-C1

- Download sequence from database.
- Search for EcoRI and XhoI sites.

Connect sequence of PCR product and pEGFP-C1

- Copy XhoI-EcoRI sequence of PCR product between XhoI-EcoRI of pEGFP-C1 by replacing the sequence in between.
- Make sure you leave only one XhoI and EcoRI site each.
- Save as a file
- Compare open reading frame of pEGFP-C1 and pEGFP-C1 with CaMKII. Open reading frame should be longer by the length of CaMKII.

Protein sequence analysis

- Amino acid content and molecular weight.
- Translate the sequence of fusion protein.
- Function->Content->Amino acid

Image removed due to copyright restrictions.

Screenshot of DNAsis program, "Amino Acid Content" window.

No report today

- Send sequence of
 - Expected PCR product
 - Expected ligation product between pEGFP-C1 and CaMKII
- Send molecular weight of the fusion protein

BLAST search

- Search identical or homologous sequence in database.
- Goto

<http://www.ncbi.nlm.nih.gov/>

- Click “Blast” (top of the page)

Image removed due to copyright restrictions.

Genome Blast

Image removed due to copyright restrictions.

Use CaMKII amino acid sequence as query.
Use TBLASTN program.

Protein Secondary Structure

- Use translated CaMKII sequence
- Function-> Prediction->Protein Secondary Structure

H: α -helix

S: β -sheet

T: β -turn

C: random coil

- Compare two methods

Hydrophobicity plot

- Hydrophobic amino acids: Leu, Ile, Val, Phe
- High in signal peptide and transmembrane domain
- Use translated CaMKII sequence
- Function -> Prediction -> Hydrophobicity
- Compare these two proteins
- If you want, check the identity of the protein by blast searching and think in relation to the protein of function of protein

Protein 3D structure

- Launch Cn3D
- File->GFP.cn3
- Click main screen and drag the structure to rotate
- Highlight amino acids 64-66 from sequence/alignment viewer
- Play around view and show/hide options