Second class: Sequence analysis and vector design

cDNA blasting

Open the ab1 file called fwd_CMVfwd.Seq.txt with the program editview
Open the sequence of nucleotide file
Copy the sequence (discarding the Ns)
Open NCBI BLAST website
Open the "nucleotide-nucleotide" link.
Paste you sequence in the window
Click "BLAST" button
In the next window, click the "format" button, and wait some 20-40 seconds
Study the sequences with the highest level of homology
(we will discuss the results of the search)

Sequence comparison

Open DNAsis
Start a new file (control N)
Select"DNA sequence"
Paste sequence of fwd_CMVfwd.Seq.txt, and save on the desktop
From the BLAST program, open the mouse homolog sequence and copy
Start a new file for this mouse homolog on DNASIS, and paste it there.
In DNASIS, go to "connect" in the menu bar
Select the desktop folder and click "go"
Go to "results" in the menu bar, and to "auto connection"
Choose parameters" 15 bp for minimal overlap and 50% for minimal matching"
Click select targets
Click on the mouse homolog and fwd_CMVfwd.Seq.txt
Click OK, click go
There should be a new sequence at the bottom of the window with the name
"fwd_CMVfwd.Seq.txt and mouse homolog"
Click on the new sequence, go to "result", and select "open editor"
Select "unmatching bases"
(we will discuss the results of the comparisons"

Genomic blasting

Google for "mouse genome resources"
Open "blast" and proceed as we did before with the blasting of cDNAS
Go to genome view
Open the chromosome that has the positive hit
(we will discuss the results of the genome search)

Identification of restriction sites

Open FUGW' with Strider program
Go to "enz' menu option, and check for absent sites, single sites, site usage and all sites
Check your notes from last week to figure out what kind of fragment you would expect using BamhI, BglII or both
(we will discuss results)

Identification of open reading frames

Open "partial CamkII"

Blast it to find the complete mRNA
Make a new file in strider and call it "complete mRNA"
Go to "AA" menu, go to "orf map"and try to find an open reading frame of approx 1200 bp.
One you figure out which frame is the right one, go to "AA", go to "translation 1 phase 3 letters to figure out the aminoacid content.

(we will discuss results)

Sequence identification
Open mistery sequence
Copy sequence (and exclude the final N's)
Blast this sequence in cDNA blast
Identify gene
Identify ORF
Identify restriction sites for digestions

Nucleotide cDNA search

Google NCBI pubmed
Select "nucleotide" option
Type name of gene identified in previous step
Find the mouse, human and rat cDNAs for this gene
Using strider, find the open reading frames
In DNASIS perform comparison of the nucleotide sequences
In DNAsis, convert to protein and perform comparison of aminoacids