Biol/Chem 475  Spring 2003
Analysis of DNA sequence data from DM cloning experiment

All students must analyze the following sequences:
Sequences #15 & 16  Carol nested PCR (#4):  S2-R1
(template: Derek’s L1.1-A3 PCR product -- gel purified ~ 190 kb band)

Sequence #44  Carol’s nested PCR (#5):  LN1-R1
(template: Derek’s L1.1-A3 PCR product -- gel purified ~ 190 kb band)

Sequence # 36  Derek’s nested PCR (#7):  LN1-R1
(template: Derek’s L1.1-A3 PCR product -- lots of bands of different sizes)

Student who don’t have their own sequence data to analyze should also
analyze the following data:
Sequence #  26 & 27  Carol’s nested PCR :  L1.1-R1
(template:CT’s L1.1-A3 PCR product product)

Suggestion for sequence analysis
1) Find sequence corresponding to PCR products by locating Eco RI restriction
sites (or other RE sites) that flank the insertion site
2) Locate primers to determine orientation in the vector
3) Align sequences to determine if they are the same PCR product – are #15 and
#16 the same sequence ?  Are #44 & #36 the same?
4) Is this a DM containing sequence?  If so which DM gene does it most closely
resemble?  (BLASTN and BLAST X) Record/copy info for TOP 3 matches
including E values.  To give you a point of reference in your interpretation
of the E values, be sure to run of BLASTX and BLASTN analysis of
NvDM1 and NvDM2.  [DM1 and DM2 sequences are available on web site].
5) If this clone has a DM domain, is this a repeat clone of Nv DM1 or DM2?  [For
a point of reference, align DM1 and DM2 with each other.]
6) If clone does not contain a DM domain, can you determine its identity by
finding a related sequence in one of the databases?  (BLASTN and BLAST X).
Record/copy info for TOP 3 matches including E values

Record results of your analysis of each clone and list your findings in a 1-2 pg
word document.  Don’t print out endless pages of BLAST analysis.