Here are the protein accession identification numbers for your MDH isozymes.

MDH Isozyme	Protein record	Important notes
Watermelon Glycoxysomal Vector: pQE60 (Qiagen)	P46488	The aa sequence shown in these records includes a 36 aa peptide that targets the protein to the glyoxysome. Our clone does not have this. Account for the change.
R153c E. Coli cytoplasmic	1IB6A gi:15988282	This is the A chain. Look at the links and you can find the whole protein. There is a PDB file for this enzyme. The clone we have is a R153S mutant.
Rat Liver Mitochondrial Vector: pRSET (Invitrogen)	P04636	There may not be a PDB for this file. The clone was cut into the vector using the BamHI site. This clone may not have a PDB file associated with it.
S. Cerevisiae Mitochondrial Vector: pET30 (Novagen)	NP_012838	The aa sequence shown here has 17 additional aas that are not included in our clone.

To get to the record, go to the NCBI web page and click on "structure" found in the bar at the top of the page. Then use the pull down menu to select "protein". Enter in your protein record. Be certain to look at the information there. You will find publications, related links (good source of material), BLink and Domains. These are the same links you used in the Cn3D tutorial.

Now what do you need to do?

1. Use the papers you have found on MDH structure and function to determine where the key amino acids are. Then look up what mutations we have. Don't forget if you want to compare wild-types you can. Hints: first think of the functions of the protein. What does it do what does it bind. Are the domains for these functions identified? If so which key amino acids have been found. Questions might include how can I increase or decrease the substrate specificity (can I make this bind other carboxylic acids?). Can I increase the thermal stability, and what does that mean. Might this enzyme bind NADPH in place of NADH? Is it converted to an LDH like enzyme. All of these are found if scientific papers.
2. Now you have to convert the amino acid from the protein you found in the literature to the amino acid in your clone. This can be done several ways. You can use a blast protein search of the sequence of the protein you found in the literature and align your clone with it after doing the search. You can also use VAST to do the alignment of the structure. You can use ExPASy. Alternatively, if you know where the amino acid is in both isoforms (from the paper you are referencing and the isozymes you wish to

mutate) you can determine where your mutation will need to be. For example, if there is a key amino acid three aa away from the catalytic His in your paper you wish to mutate in your protein, you can use the protein record to find the catalytic His and search from there. There are also ways to determine where the start site is from the NCBI info page. BIG POINT – the 107th amino acid of one clone may not be the same as the 107th amino acid on another, that is why you need to align the sequences. Remember to read the records and not just click away. You can miss a lot of good information that way.

3. Now that you have found the amino acids you wish to mutate and how you want to mutate them. You need to use the stratagene quickchange information to create that change. Congratulations, you are well on your way!

I have also included a couple of general links on MDH to help you along.

1. Chapman *et al.* (1999) Structural basis of substrate specificity in malate dehydrogenases: crystal structure of a ternary complex of porcine cytoplasmic malate dehydrogenase, alpha-ketomalonate and tetrahydoNAD. ***J Mol Biol* 285(2):703-12.**

2. Musrati *et al.* (1998) Malate dehydrogenase: distribution, function and properties. ***Gen Physiol Biophys* 17(3):193-210.**

3. Wilks *et al.* (1988) A specific, highly active malate dehydrogenase by redesign of a lactate dehydrogenase framework. ***Science* 242(4885):1541-4.**