A Project-Based Biotechnology Laboratory Course Using Isocitrate Dehydrogenase

Erin Mooney and A. Malcolm Campbell

Davidson College Biology Dept.
PO Box 1719 (US mail), 217 Concord Rd (shipping)
Davidson, NC 28036
email: macampbell@davidson.edu

***Abstract*:** We have utilized the yeast genome to let students discover how electronic databases are combined with laboratory procedures to clone a new gene. A semester-long laboratory course has been developed which models current research trends that utilize genomic data, and is similar to a rotation project for first year graduate students. Students are provided with amino acid sequences of the model enzyme isocitrate dehydrogenase (IDH) from species other than yeast and use the yeast database to electronically clone the yeast homolog. At the bench, they design PCR primers, amplify the gene from genomic DNA, clone the product, express yeast IDH in *Escherichia coli* (*E. coli*), and perform a western blot using an epitope tag. From this common beginning, students can perform a range of experiments such as running a native gel and staining for IDH activity, or performing a Southern blot on a range of species' DNA with the yeast *IDH* as a probe. This semester-long series of experiments provides students with a laboratory experience that has the feel of original research where results from one lab are needed for the next step in the process.

***Key Words*:** isocitrate dehydrogenase; IDH; unification of the laboratory curriculum; biotechnology laboratory; genome-based laboratory course, molecular biology

Introduction

The field of biotechnology is growing quickly; its tools are powerful and its impact is increasingly evident in news, medicine, and daily life. Biology teachers would like to share the process and the methods of biotech research with students, but the complicated procedures can make designing laboratory courses difficult. We have developed a molecular biology laboratory course for undergraduate students that can make it much easier to teach biotech methods and scientific thinking. This course utilizes current techniques as students progress through a semester-long cloning and expression project. The schedule requires eleven weekly three-hour sessions (Table I; Campbell, 1999), but provides many choices of preparation and analytical procedures. A range of optional activities may be added, depending on the resources, interests, and focus of the class. Students can research the project through genomic databases and articles before beginning the project and/or they can carry out several types of molecular analysis.

Development of this laboratory course is part of an ongoing effort to design a set of laboratory courses that focus on one enzyme, isocitrate dehydrogenase (IDH), (Campbell and Williamson, 1998). Cell, molecular, developmental, biochemistry and genetics laboratory courses can each analyze IDH using methods unique to that subdiscipline of biology. We hope that students will realize there are many ways to analyze a single gene product, and that it is important to retain knowledge from one laboratory experience to another. We selected IDH because it is well-characterized, ubiquitous, important to cellular function, and familiar to students who have completed an introductory biology course. It is an excellent protein to study in the laboratory because the assay for IDH activity is toxin-free, IDH is easy to purify, and has several testable variables. IDH catalyzes the oxidative decarboxylation of isocitrate to 2-oxoglutarate, providing both NADPH and 2-oxoglutarate for necessary lipid and amino acid synthesis activity. Yeast cells contain three genetically independent isoforms of IDH: IDP2, and two mitochondrial forms, NAD^+- and $NADP^+$ -dependent isocitrate dehydrogenases (IDH and IDP1, respectively); (Loftus et al., 1994). The particular isoform of IDH cloned in the project outlined below is yeast (*Saccharomyces cerevisiae*) cytosolic $NADP^+$ -dependent isocitrate dehydrogenase (IDP2).

This laboratory course allows students to be involved in all facets of the research process. The project's main purpose is to clone and express yeast IDP2 in *Escherichia coli* (*E. coli*). Unlike most eukaryotes, yeast genes do not contain introns, allowing genes to be cloned for expression directly from genomic DNA. Major steps in the process

include using polymerase chain reaction (PCR) (figure 1) to amplify the IDP2 from yeast genomic DNA, ligating it into a cloning vector, transferring the gene to an expression vector, then expressing and analyzing the recombinant protein. When the expression vector transcribes the IDP2 mRNA, a twelve codon chain is added to the 5' end, called a T7 tag. Translation of this mRNA produces an IDP2 fusion protein with a twelve amino acid T7 epitope-tag on the N-terminus for detection of the protein on an immunoblot.

Table I: *Schedule for Semester-long Laboratory to Clone and Express Yeast IDH.*

Week Number	Lab Description
1	Search Genbank, Determine Yeast IDH sequence
2	Perform PCR, Pour LB####
3	Clean PCR product, Ligate PC
4	Transorm E.Coli with Ligati#####
5	Miniprep Transformants, Digest and Electrop####
6	Purity PCR Fragment, Ligate into Express#####
7	Transform E. coli with Ligai
8	Miniprep Transformants, Digest and Electrop####
9	Transform Forward and Reverse Clones into Ex######
10	Express Recombinant IDH, Run SDS######
11	Detect IDH on
12-15	Additional weeks can be used to run native g##### assays with recombinant IDH

Materials and Methods

We will provide interested users with course specific recombinant plasmids (pCR2.1-IDP2 and pET5a-IDP2F and -IDP2R) as insurance. By providing pre-made constructs of recombinant vectors, we hope to create a safety net for teachers and students. Students' small, but critical, mistakes will not prevent the class from finishing the semester's "project," as there will always be a back-up construct with which to proceed. All materials which we do not provide can be purchased from major biotechnology or chemical companies.

Strains, Vectors and Reagents

The protease deficient yeast strain JYW2878 was obtained from Dr. John L. Woolford at Carnegie Mellon, though any wild-type strain will suffice. Reagents and protocols for polymerase chain reaction (PCR) and ligation of the pCR2.1 vector are components of the TA Cloning kit obtained from Invitrogen. Competent cloning strains of *E. coli*, Top10F' and JM109 were also from Invitrogen. The pET5 expression vector system, including BL21(DE3) expression cells, materials and protocols for transformation, expression and collection of protein were obtained from Novagen. All restriction enzymes and calf intestinal alkaline phosphatase were obtained from Promega. All other reagents are available from Sigma Chemical Company and/or Fisher Scientific unless otherwise noted.

Primer Design

The yeast cytosolic $NADP^+$ -dependent isocitrate dehydrogenase gene (IDP2) was previously isolated and cloned (Loftus et al., 1994). We used the gene sequence published at that time to create primers which would amplify the 1.2 kb IDP2 sequence using PCR. Primers were engineered to amplify the open reading frame of the gene, placing a BamHI site directly before the start codon (forward primer: 5' GCATGGATCCA TGACAAAGATTAAGGTAGC 3'), and a Hind III site directly after the stop codon (reverse primer: 5' TCCGAAGCTTTTACAATGCAGCTGCCTCGA 3'). In the future, we recommend that a BamHI site be used in both primers, as explained below.

Cloning into pCR2.1, the TA Cloning Vector

The only deviations from Invitrogen's PCR protocol which we found useful were the inclusion of 3% DMSO and the addition of Taq polymerase after the reaction mixture had been heated to 95°C for 4 minutes (hot start). Product from the primary PCR was used as template for a secondary PCR to further amplify the proportion of IDP2 in the product. This secondary PCR product was purified using a phenol/chloroform and ethanol extraction (Sambrook, et al., 1989). The PCR product was ligated directly into the pCR2.1 plasmid using the adenine overhangs added to the 3' ends of the PCR product by Taq polymerase. Top10F' *E. coli* competent cells were transformed with the pCR2.1-IDP2 ligation product, and plated on LB ampicillin (100 mg/ml) plates containing IPTG and X-gal (Sambrook, et al., 1989). Twelve white colonies were chosen for screening and grown overnight in 2 ml LB-ampicillin cultures. Plasmid DNA was isolated from these cultures using an alkaline lysis procedure (Sambrook, et al., 1989). The isolated plasmid DNA was analyzed by restriction digestion with BamHI and HindIII followed by agarose gel visualization to ascertain if the colony contained the 1.2 kb IDP2 gene sequence in the pCR2.1 vector. Colonies containing the recombinant plasmid were grown in 100 ml liquid cultures of LB-ampicillin overnight and the plasmid DNA was isolated using a Qiagen plasmid purification kit and stored at -20° C. Plasmid DNA concentration was determined using an OD_{260} measurement on a Perkin Elmer Lambda 3B UV/VIS spectrophotometer. During this phase, we discovered the Hind III restriction site was mutated and decided to use a BamHI site in the polylinker of pCR2.1 that was downstream of the IDP2 stop codon. This allowed us to clone IDP2 into the expression vector in two

orientations which provided us with a good negative control.

Cloning into pET5a, the expression vector

BamHI, separation from pCR2.1 by agarose gel electrophoresis and recovery by electroelution in a "V" apparatus (Medical Specialties, Baltimore). Briefly, the electroelution process uses current to draw the target DNA out of a small cube of agarose gel and into a vertical V-shaped well containing 7.5 M ammonium acetate. DNA is precipitated from the viscous ammonium acetate solution using glycogen in an ethanol precipitation procedure (Sambrook, et al., 1989). The pET5a vector was also digested with BamHI and treated with calf intestinal alkaline phosphatase (CIAP, Promega) to prevent self-ligation. The digested IDP2 fragment was ligated into the CIAP-treated pET5a plasmid and pET5a-IDP2 ligation product was used to transform JM109 *E. coli* competent cells by heat-shock. Transformed cells were plated on LB-ampicillin plates, and all colonies that appeared were screened, using an EcoR I digestion. Digestion with EcoRI was designed to reveal whether a colony contained a pET5a-IDP2 recombinant plasmid and in which orientation the IDP2 gene was ligated. The plasmid DNA of one "forward" clone and one "reverse" clone was harvested using a small scale alkaline lysis procedure (Sambrook, et al., 1989). This purified plasmid DNA was used directly to transform an expression strain of *E.coli*, BL21(DE3). These transformants were also plated on LB-ampicillin agar plates, and one colony from each transformation was screened to confirm the presence of the correct plasmid using an EcoR I digestion.

Expression and Isolation of IDP2 protein

The clone containing IDP2 in the reverse orientation was used as a negative control as it expresses a T7 virus epitope tag (Figure 5) but contains a stop codon 72 bases downstream, resulting in a 36 amino acid peptide. Three 2 ml liquid cultures of a forward clone and three 2 ml cultures of a reverse clone in exponential growth (OD_{595} = 0.5) were induced with 0.4mM IPTG to express the contents of the plasmid for one hour. Cells were centrifuged for 5 minutes in a microcentrifuge (13,000 rpm), and the medium of each was aspirated. Each pellet of cells was then resuspended in 0.2 ml cold 50 mM Tris HCl pH 8.0 and 2 mM EDTA. Lysozyme was added to a concentration of 100 mg/ml and Triton-X 100 to 0.1%. The cells were incubated in the lysing reagent for 15 minutes at 30° C. After a brief cooling period on ice, the lysates were treated to eliminate DNA contamination in pairs of forward and reverse products. DNase was added to 100 mg/ml to one set and incubated at 37° C for 45 minutes. A second set was treated with 10 mg/ml DNase and incubated at 37° C overnight (19 hours). A third set was mechanically sheared using a 27.5 gauge needle, through which the entire volume of lysate was drawn up into a 3 ml syringe and expelled 50 times. After the DNA degradation treatments, the lysate was precipitated by centrifugation for 15 minutes in a microcentrifuge (13,000 rpm). The protein pellets were resuspended in 10 µl distilled water and stored at 4° C for one week.

The pCR2.1-IDP2 DNA was prepared for ligation into the expression vector pET5a by digestion with

Protein Analysis by Immunoblot

Polyacrylamide gels were cast and run using Mini PROTEAN II equipment (BioRad). A 7.5% SDS-polyacrylamide resolving gel was cast under a 4% stacking gel (Deutscher, 1990). Proteins were prepared by addition of SDS sample buffer (0.06 M Tris HCl, pH6.8, 10% glycerol, 0.025% bromophenol blue, 1% SDS and 5% ß-mercaptoethanol). The samples and prestained low molecular weight markers were used (BioRad) were loaded onto the gel, run at 100V for approximately 20 minutes through the stacking gel, and at 200V for approximately 35 minutes through the resolving gel. The gel was equilibrated for 15 minutes in Western transfer buffer (25 mM Tris base, 1.9 M glycine, 70% water and 20% methanol). The transfer unit (BioRad) was assembled, run at 100V for 1 hour to transfer proteins onto a nitrocellulose membrane (Protran, Schleicher & Schuell), and stained with Ponceau S dye (0.1% Ponceau S and 1% acetic acid; Deutscher, 1990) to confirm the transfer was successful. The membrane was incubated in 10 ml Blotto (7% dry milk, 10 mM Tris base, pH 8.0, 1.5 M NaCl, 0.1% Tween 20 and 0.1% Antifoam A) for 20 minutes at room temperature with gentle agitation, incubated with the primary anti-T7 monoclonal mouse antibody (Novagen) for one hour at room temperature with gentle agitation, and rinsed three times for two minutes each in 1X Tris Saline (10 mM Tris base, pH 8.0 and 1.5 M NaCl). Ten milliliters of fresh Blotto containing 1:2500 dilution of a secondary antibody conjugated to horse radish peroxidase (a goat anti-mouse antibody; Kirkegaard and Perry Laboratories) was incubated with the membrane for an hour. The blot was washed three times for five minutes each in 1X Tris Saline, two milliliters of horse radish peroxidase substrate (True Blue; Kirkegaard and Perry Laboratories) were added to the drained blot to detect antibody binding and a Foto/Analyst Archiver (Fotodyne) was used to photograph the blot.

Activity Assays

Ten ml of BL21(DE3) cells (OD_{600} = 0.5) expressed the IDP2 for two hours, which was isolated using the protocol above and resuspended in a small volume of IDH assay buffer (0.2 M Tris, 1 mM $MgCl_2$, pH 8.0; Williamson *et al.*, 1980). Reactions were mixed in the 200 µl capacity microwells of a 96-well plate, and analyzed using a multi-well plate spectrophotometer (BioRad Model 3550-UV

Microplate Reader). Porcine heart $NADP^+$ -dependent isocitrate dehydrogenase (Sigma, product number I-2002) was used as a positive control. Pig IDH reaction solution consisted of 1:500 dilution of porcine heart IDH, 230 µM -isocitrate, 144 µM $NADP^+$ in assay buffer and experimental reaction solutions for IDP2 proteins (forward and reverse) contained the same reaction mixture. Negative controls lacked substrate to control for turbidity of enzyme extracts and spontaneous reduction of $NADP^+$ to NADPH. The OD_{340} of each reaction mixture was assessed every 30 seconds for a total of 5 minutes to measure the appearance of NADPH.

Results

Polymerase Chain Reaction

The first objective of the project was to amplify the IDP2 gene from yeast genomic DNA. The published IDP2 sequence (Loftus et al., 1994) was used to create primers. The primers were designed to amplify the 1.2 kb IDP2 gene sequence, adding a BamHI site upstream of the start codon, and a HindIII site downstream of the stop codon. Genomic DNA was purified for use as a template in PCR (Kaiser et al., 1994). After the PCR reaction was performed, the product was analyzed by agarose gel electrophoresis to confirm amplification of the expected 1.2 kb IDP2 gene (Figure 1). The gel revealed that the PCR amplified a band of the expected size, confirming that the reaction was successful.

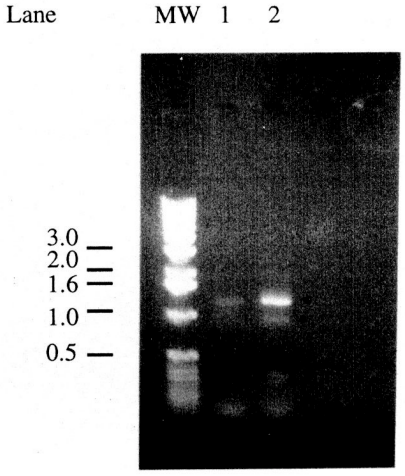


Figure 1. Comparison of primary and secondary PCR products. *Five microliters of "primary" PCR product from a reaction using yeast genomic DNA as template (lane 1) and a "secondary" PCR product which used 1 µl of a primary PCR product as template (lane 2). The 1.2 kb band corresponds to the amplified IDP2 gene, the light band at ~0.9 kb is a non-specific amplification product, and the diffuse bands below the 0.5 kb marker are most likely primer dimers. Markers have been labeled in kb.*

Analysis of pCR2.1-IDP2 clones

The second stage of the project was to clone the IDP2 PCR product directly into pCR2.1, the TA Cloning plasmid. The TA Cloning system takes advantage of the adenine overhangs added to the PCR product's 3' ends by Taq polymerase and pCR2.1 contains two single thymidine overhangs which allow direct ligation of PCR products. The pCR2.1 plasmid is capable of blue-white screening where colonies that contain plasmids lacking inserts appear blue, and those with inserts appear white. When TOP10F' cells were transformed with the pCR2.1-IDP2 ligation product, approximately 45% of colonies were white, suggesting clones with inserts. Twelve white colonies were chosen for restriction digestion analysis with BamHI and Hind III. Digestion products were visualized using gel electrophoresis (Figure 2). Of the twelve colonies, two appeared to contain inserts of 1.2 kb. Plasmid DNA from the colonies containing a 1.2 kb band was digested with BamHI, Hind III, and EcoR I to confirm the insert as IDP2, which contains an internal EcoRI site 359 bp downstream of the start codon. Gel electrophoresis revealed that in both colonies, the 1.2 kb band was digested into ~400 and ~800 bp bands, confirming the 1.2 kb band's identity as IDP2. A large scale preparation of pCR2.1-IDP2 plasmid DNA was isolated and digested with BamHI, and the gel purified for ligation into the pET5a expression vector. Digestion of pCR2.1-IDP2 with BamHI excises the entire IDP2 gene sequence in tandem with 36 bp of the pCR2.1 polylinker at its 3' end (see Materials and Methods). This relationship is maintained throughout cloning and expression in pET5a.

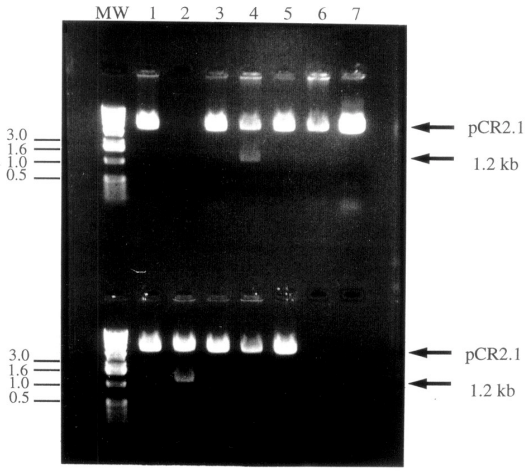


Figure 2. Digestion of colony plasmid DNA to identify cloned IDP2 PCR product. *Twelve white colonies were chosen for screening. The colonies' plasmid DNA was isolated and digested with BamHI and HindIII to release the 1.2 kb IDP2 PCR product. Most colonies contained pCR2.1 without the IDP2 insert (1a, 3a, 5a,6a, 7a, and 1b, 3b, 4b, 5b and 6b) or nothing (2a). Two clones appeared to contain the IDP2 insert (4a and 2b). Lanes 7b and 8b contained no sample. Molecular weight markers have been indicated in kb.*

Analysis of pET5a-IDP2 clones

In order to produce IDP2 protein for analysis, it was necessary to clone the IDP2 gene sequence in the expression vector pET5a. The BamHI digested and purified IDP2 sequence was ligated into BamHI-digested pET5a. Seven colonies appeared after JM109 cells were transformed with the pET5a-IDP2 ligation product. Because the IDP2 gene was ligated into pET5a using only a single restriction enzyme, two different ligation products could result (Figure 3). Some colonies could contain IDP2 ligated into pET5a in the forward orientation, for transcription of a sense RNA (pET5a-IDP2F). Others could contain IDP2 in the reverse orientation (pET5a-IDP2R) resulting in antisense RNA. The seven colonies which appeared were screened using an EcoR I digestion designed to determine if the colonies contained IDP2 and in what orientation (Figure 4). When digested with EcoR I, forward constructs released a ~950 bp band; reverse constructs released two bands of ~950 bp ~350 bp. One colony contained the reverse construct pET5a-IDP2R, while four colonies contained the forward construct, pET5a-IDP2F. Plasmid DNA was isolated from pET5a-IDP2F and pET5a-IDP2R for use in transforming BL21(DE3) expression cells.

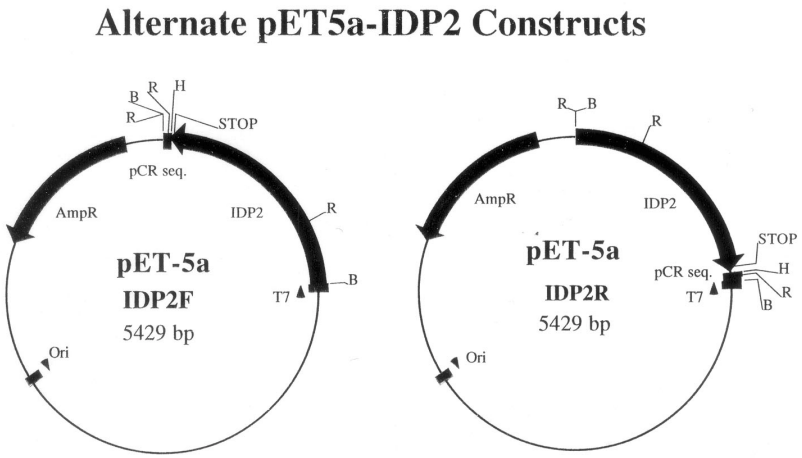


Figure 3. Representation of forward and reverse pET5a-IDP2 constructs. *T7 indicates the T7 tag region which initiates transcription of the T7 tag in tandem with the IDP2 coding region. pCR denotes sequence digested from pCR2.1 in tandem with the IDP2 gene. AmpR indicates the ampicillin resistance gene and Ori the origin of replication. R, B and H denote EcoR I, BamH I and Hind III restriction sites respectively. Notice the positions of stop codons and the result of an EcoRI digest on each construct.*

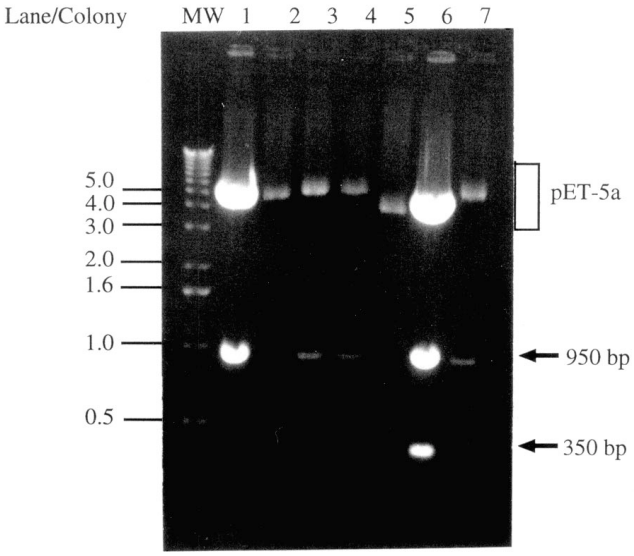


Figure 4. Digestion of pET5a plasmids to determine presence and orientation of IDP2. *All seven colonies which appeared after JM109 cells were transformed with the pET5a-IDP2 ligation product were screened using an EcoR I digestion. Forward clones (pET5a-IDP2F) digest to give a ~950 bp band (lanes 1,3,4 and 7), whereas reverse clones (pET5a-IDP2R) give a ~950 bp and a ~350 bp band (lane 6). The remaining two colonies did not contain an insert (lanes 2 and 5). Molecular weight markers are indicated in kb.*

Immunoblot Analysis

The T7 promoter region of pET5a initiates transcription upstream of the cloned insert. Translation of the resulting RNA produces a fusion protein with the 12 amino acid T7 epitope-tag on the N-terminus of the IDP2 protein. In pET5a-IDP2F, translation of the full length sense RNA transcript results in an IDP2 protein with the T7 tag on the N-terminus. Expression from pET5a-IDP2R results in a 36 amino-acid chain including the 12 amino acids of the T7 tag at the N-terminus and 24 amino acids encoded by the antisense transcript of the pCR2.1 polylinker and IDP2 (Figure 5). Induction of expression and purification of the resulting protein was performed in parallel with cells containing pET5a-IDP2F and cells containing pET5a-IDP2R. Protein from cells containing pET5a-IDP2F was used as a negative control in immunoblot analysis.

An immunoblot was performed to confirm that the protein expressed in pET5a-IDP2F was of the correct molecular weight (46,535 kDa), that no detectable protein was expressed in the pET5a-IDP2R clones, and to compare DNA removal methods (Figure 6). The blot provided evidence that the expressed protein is of the expected size (47,863 Da, Figure 7), no detectable protein is produced by the reverse construct, and that all three methods of DNA removal (short, long DNAse treatments, and mechanical shearing) are viable.

Activity Assay

Protein products from both forward and reverse constructs of pET5a-IDP2 were tested for $NADP^+$ -dependent isocitrate dehydrogenase activity. Through comparison to pig heart $NADP^+$ -dependent isocitrate dehydrogenase, the protein isolated from cells containing the forward T7-IDP2 construct was estimated to have 5.44×10^{-3} units of IDH activity /ml culture induced for 2 hours at $OD_{600} = 0.5$. Protein isolated from cells containing the reverse T7-IDP2 protein exhibited negligible activity, suggesting that the activity of protein of cells containing the forward construct is a result of the recombinant T7-IDP2 protein itself and not *E. coli* IDH (Figure 7).

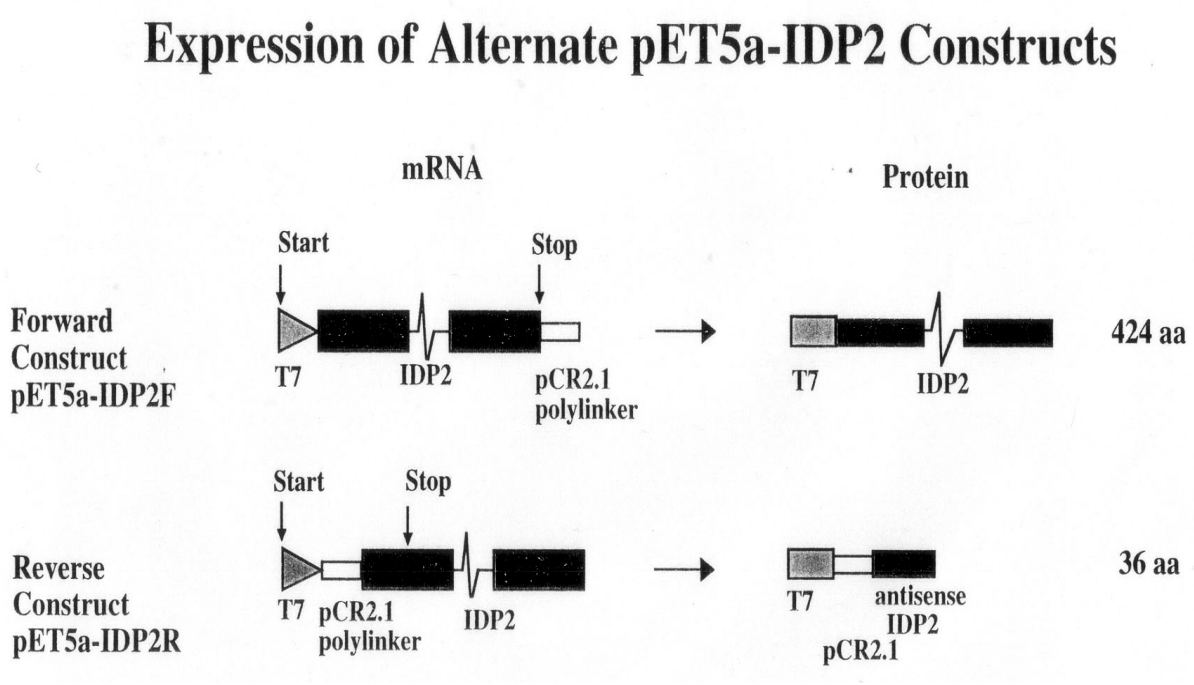


Figure 5. Expression of forward and reverse pET5a-IDP2 constructs. *Because a short sequence of polylinker was digested from pCR2.1 in tandem with the 3' end of the gene, the pCR2.1 fragment is transcribed in tandem with the IDP2 gene. In the forward construct (pET5a-IDP2F), a sense RNA is transcribed carrying the 12 codons of the T7 tag on the 5' end of the IDP2 transcript, and the pCR2.1 polylinker fragment on the 3' end. The pCR2.1 fragment is not translated, however, because IDP2 stop codon ends translation before reaching the pCR2.1 fragment. In the reverse construct (pET5a-IDP2R), the IDP2-pCR2.1 fragment was ligated into the pET5a plasmid in the opposite orientation. This ligation event placed the antisense DNA of pCR2.1 and IDP2 directly downstream of the T7 promoter and tag. The antisense RNA of pCR2.1 and IDP2 is translated into a very short amino acid chain (36 aa, approximately 4.0 kDa).*

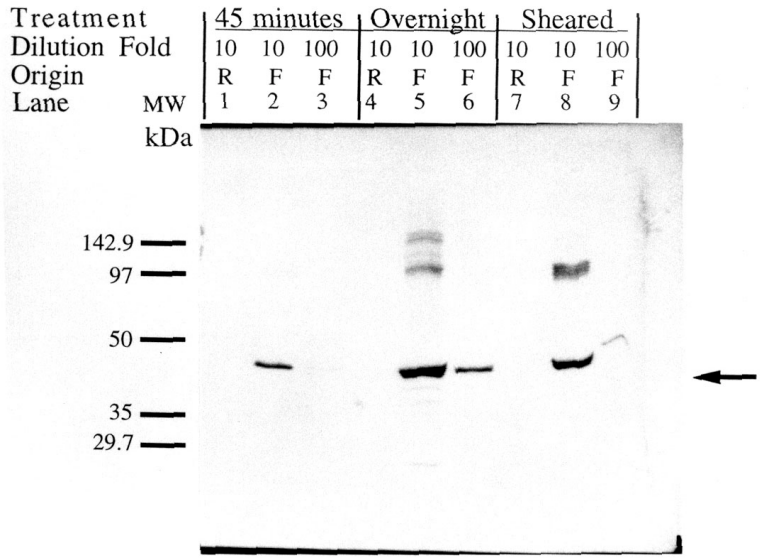


Figure 6. Verification of protein size and comparison of DNA degradation methods. *Expression was induced in both forward and reverse clones for one hour using 0.4 mM IPTG. Crude lysate protein was treated in three different ways to degrade contaminate DNA. Lanes 1-3 contain samples treated with 100 mg/ml DNAse for 45 minutes. Lanes 4-6 contain samples treated with 10 mg/ml DNAse for 19 hours. Lanes 7-9 contain samples whose DNA was degraded by means of mechanical shearing by 50 strokes of the entire lysate volume through a 27.5-gauge needle. The first lane in each set (1,4,7) contains a 1:10 dilution of the truncated antisense expression product (Reverse orientation). The second (2, 5, 8) and third lanes (3, 6, 9) in each set contain a 1:10 and 1:100 dilution, respectively, of the full-length sense expression product (Forward orientation). The IDP2 protein was calculated to be approximately 47 kDa, comparable to the expected size of 50 kDa.*

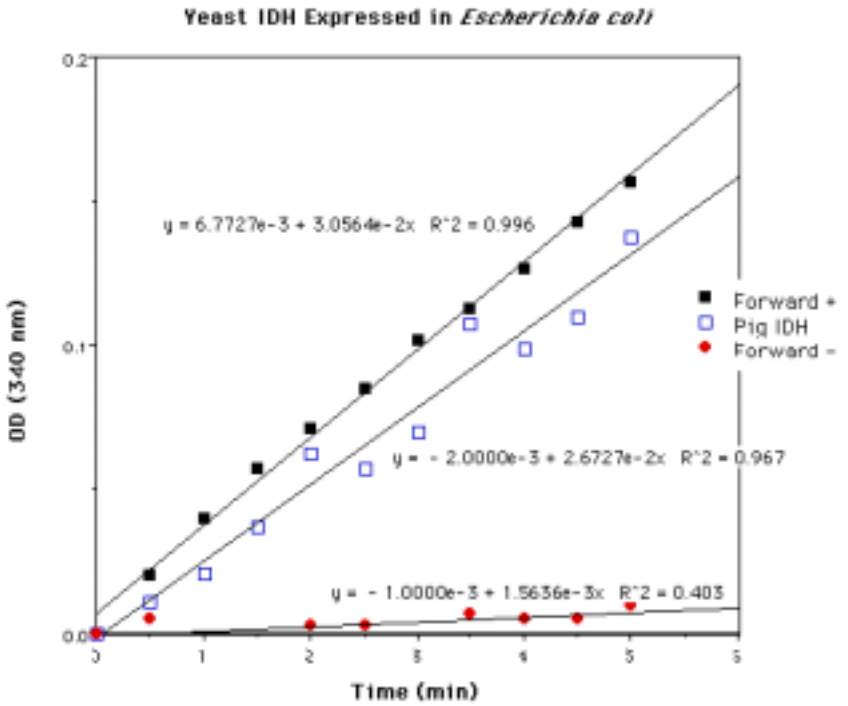


Figure 7: Determination of yeast IDH activity expressed in E.coli. *"Forward +" assay contained all reagents necessary for reaction; "Forward-" assay contained all reagents except isocitrate. Pig heart assay contained 0.0392 mg of IDH, constituting 0.01188 units of IDH activity. Comparison of the slopes of the pig IDH and "Forward +" assays allows estimation of 0.0136 activity units in the "Forward +" assay. The "Forward +" assay contained protein homogenate isolated from approximately 2.5 ml of E .coli cells induced with IPTG at* OD_{600} *= 0.5 and incubated for 2 hours.*

Discussion

This course integrates well with molecular biology and biotechnology classes, as different methods and objectives are addressed. Having an investigatory project associated with such courses effectively involves students in thinking about experimental design and what occurs at the molecular level as they work. Alternatively, the course could also be used independently to teach laboratory methods.

Valuable activities can be added during extra laboratory sessions, or when long incubation periods leave excess lab-time. Students could be involved in preliminary work, such as obtaining the IDP2 cDNA sequence from a yeast genome databank (Cherry et al., 1998), computer files containing the data from X-ray crystallography data (Campbell, 1997a) or NIH Genbank (Campbell, 1997b) and/or preparing the PCR primers and solutions. DNA and protein analysis could be introduced to students during the course, using software such as MacDNAsis (Hitachi Software) and/or CaseIt (BioQuest). MacDNAsis allows users to perform both DNA and peptide analysis, including searches for open reading frames or restriction sites, alignment of sequences, and prediction of peptides' primary and secondary characteristics. CaseIt provides a method to simulate and predict the results of DNA manipulation procedures such as PCR, restriction digestions and gel electrophoresis, and Southern Blots. After the yeast IDP2 has been cloned, students could analyze the recombinant IDP2 DNA, RNA or protein. Southern and Northern blots could be performed using nucleic acids isolated from a variety of tissues or species. For example, molecular biology students isolated genomic DNA from several species for use in a multi-species Southern blot (zooblot). This zooblot allowed students to compare interspecific differences between IDH genes and the importance of stringency conditions during probing (Campbell, 1998). Alternatively, Northern blots could be performed using RNA. The recombinant IDP2 gene could be used as a probe in such procedures.

Analysis of IDP2 activity is especially pertinent as the active site of IDP2 has not yet been localized. At least one study has shown the N-terminus of tobacco cytosolic $NADP^+$ -dependent isocitrate dehydrogenase is important to activity or adhesion to other subunits (Galvez, et al., 1995). Allowing students to view RasMol images of IDH molecules may aid students in making educated guesses about whether or not the T7-IDP2 recombinant protein is active. Activity can be determined using spectrophotometric activity assays or on a native polyacrylamide gel to determine the size of the functional holoenzyme (Davis, 1964). All of these final analyses allow students to perform investigatory labs where there is no "right" answer. In addition, faculty members can choose which procedures to perform. The recombinant yeast IDP2 protein could be used by students in a biochemistry course for purification and characterization. They could compare wild-type and recombinant enzymes. We believe this molecular laboratory module would work well in many different biology departments, and could be part of a departmental effort to unify the laboratory curriculum by adopting IDH as a model enzyme in a wide range of courses (Campbell and Williamson, 1998).

References

Campbell, A. M. (1997a). How to Find Your Favorite Protein (YFP) to be viewed by RasMol. Davidson College Biology Department. <http://www.bio.davidson.edu/Biology/Courses/Molbio/Rasearch.html> Accessed 1998 June 29.

Campbell, A. M. (1997b). How to search and retrieve from the NIH Genbank. Davidson College Biology Department. <http://www.bio.davidson.edu/Biology/Courses/Molbio/NIHsearch.htm> Accessed 1998 Mar 23.

Campbell, A.M. (1998). Here are some figures generated by Molecular Biology students (spring 1998). Davidson College Biology Department. <http://www.bio.davidson.edu/Biology/IDH/IDHmolecular.html> Accessed 1998 June 26.

Campbell, A.M. (1999). Laboratory Schedule for Molecular Biology. Davidson College Biology Department. <http://www.bio.davidson.edu/Biology/Courses/Molbio/Protocols/labsyllabus.html> Accessed 1999 August 31.

Campbell, A.M. and Williamson, J. H. (1998). IDH as a Model Enzyme for teaching biology. Davidson College Biology Department <http://www.bio.davidson.edu/Biology/IDH.html> Accessed 1998 May 4.

Cherry, J. M., Ball, C., Chervitz, S., Dwight, S., Harris, M., Hester, E., Juvik, G., Malekian, A., Roe, T., Weng, S., and Botstein, D. (1998). *Saccharomyces* Genome Database <http://genome-www.stanford.edu/Saccharomyces/> Accessed 1998 June 25.

Davis, B.J. (1964). Disc Electrophoresis II: Method and application to human serum proteins. Annals of the New York Academy of Science. 121:404-427.

Deutscher, Murray P. (Ed.) (1990). Guide to Protein Purification. Methods in Enzymology. Volume 182. San Diego: Academic Press Inc. 894 pages.

Galvez, S., Hodges, M., Bismuth, E., Samson, I., Teller, S., and Gadal, P. (1995). Purification and characterization of a fully active recombinant tobacco cytosolic $NADP^+$-dependent isocitrate dehydrogenase in *Escherichia coli*: Evidence for a role for the N-terminal region in enzyme activity. Archives of Biochemistry and Biophysics. 323(1): 164-168.

Kaiser, C., Michaelis, S., Mitchell, A. (1994). Methods in Yeast Genetics. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. 177 pages.

Loftus, T.M., Hall, L.V., Anderson, S.L., and McAlister-Henn, L. (1994). Isolation and characterization of the yeast gene encoding cytosolic $NADP^+$-specific isocitrate dehydrogenase. Biochemistry. 33: 9661-9667.

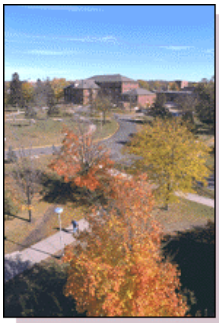
Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning. 2nd ed. USA: Cold Spring Harbor Laboratory Press. 1659 pages.

ACUBE 43rd Annual Meeting

University of Wisconsin -- River Falls
River Falls, Wisconsin

October 15-17, 1999

Integrating Process and Content: Flexibility for the Future



Call for Applications

John Carlock Award

This Award was established to encourage biologists in the early stages of their professional careers to become involved with and excited by the profession of biology teaching. To this end, the Award provides partial support for graduate students in the field of Biology to attend the Fall Meeting of ACUBE.

Guidelines:

The applicant must be actively pursuing graduate work in Biology. He/she must have the support of an active member of ACUBE. The Award will help defray the cost of attending the Fall meeting of ACUBE. The recipient of the Award will receive a certificate or plaque that will be presented at the annual banquet; and the Executive Secretary will provide the recipient with letters that might be useful in furthering her/his career in teaching.

Application:

Applications, in the form of a letter, can be submitted anytime during the year. The application letter should include a statement indicating how attendance at the ACUBE meeting will further her/his professional growth and be accompanied by a letter of recommendation from a member of ACUBE. Send application information to: Dr. William J. Brett, Department of Life Sciences, Indiana State University, Terre Haute, IN 47809; Voice -- (812)237-2392 FAX (812)237-4480; E-mail -- lsbrett@scifac.indstate.edu

If you wish to contribute to the **John Carlock Award** fund, please send check to: Dr. Marc Roy, Executive Secretary, ACUBE, Department of Biology, Beloit College, 700 College St. Beloit, WI 53511.