A Course-Based Undergraduate Research Experience (CURE) in an Introductory Molecular Biology Laboratory Class:

Investigating the Functional Defects of Human p53 Mutations in a Yeast Model System:

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The **Goal:**

An introductory biology class laboratory class that give students the opportunity to learn the methods and logic of molecular biology research by investigating an unknown question.

The **Students:**

- 250 students
- 60% sophomores (mostly biology majors)
- 40% juniors/seniors (mostly pre-med, various majors)
Characteristics of a research-based course that we have incorporated into Bio44X

**Longitudinal** research question with **unknown answer**

Student **choice** in designing experiments

High level of **collaboration** in lab pairs and between groups

Authentic **assessment**: poster presentation
The **hook:**

**p53**, a tumor suppressor that is mutated in ~50% of all human tumors
Tumor suppressors put the “brakes” on cell division

Proto-oncogene protein

Tumor Suppressor

Brake  Gas
p53 is a Tumor Suppressor

p53 mutation
(no brakes)
**p53 activates target gene transcription**

Cellular stress/DNA damage

- **Cell cycle arrest**
- **DNA repair**
- **Cell death**

Cellular stress leads to a rapid increase in **p53** levels

**p53** increases the expression of target genes whose products mediate cell cycle arrest, DNA repair and programmed cell death.
Course theme:

Use a *yeast* model to analyze a mutant allele of *p53*
identified in a tumor
and figure out if it works
(and, if not, why not)
Collaboration and Communication: “Mutant Group” Discussions

Lab groups – each pair of students working on a different mutant

Mutant groups – meet 3x, all students working on particular mutant
QUERY method

Question

Experiment

Results

Your interpretation
We use 2 different p53 reporter genes (
\textit{lacZ} and \textit{ADE2})
We use 3 different p53-REs to gauge how well p53-mut functions.

- Easiest to transactivate: Con
- Hardest to transactivate: Bax

Gene Reporter

Con Reporter Gene

p21 Reporter Gene

Bax Reporter Gene
9 p53 Reporter Yeast Strains

3 versions of p53:  
wt (pos control), mut & Δ (neg control)

3 p53-REs:  
Con > p21 > Bax
Does the p53 mutant have a transactivation defect?

Students perform **qualitative** assays using the yeast reporter strains. They score their spot: 3= like wt control (full transactivation), 1= like Δ control (no transactivation), 2=in-between (reduced transactivation)
How marked is the p53 mutant’s transactivation defect?

Students perform Quantitative p53-RE/lacZ reporter assays
Where is the **p53 mutation** located?

TAD = transcriptional activation domain

DBD = DNA binding domain

OD = oligomerization domain

Students use online computational tools to map their **mutation** within the p53 protein sequence
Where is the p53 mutation located?

Students use Jmol to highlight a mutated amino acid in p53’s DBD.
How well does our p53 mutant function as a transcriptional activator? Is the data reliable? What do the controls tell us? Why are replicates useful? Is there any previous research on our p53 mutant? If so, what does it tell us?

What is our best hypothesis so far?
Lab Discussion:

What could account for each p53 mutant’s transactivation defect?
3 possible **hypotheses** to explain mut#s transactivation defect(s)

1) p53-mut# protein is degraded more than p53-wt protein

2) p53-mut# protein doesn’t bind p53-RE DNA as well as p53-wt protein

3) p53-mut# protein doesn’t localize properly to the nucleus
3 Questions we will ask

Q1: Is the level of p53-mut# protein reduced?

Q2: Does p53-mut# protein show reduced binding to p53-RE DNA?

Q3: Does p53-mut# protein show reduced nuclear localization?
Q1: Is the level of p53 mutant protein reduced?

Students perform a western blot and quantify their results.
Mutant Group Discussion #2

Is the level of our p53 mutant protein reduced? If so, can it explain the p53 mutant’s transactivation defect?

What is our best hypothesis so far?

What conditions shall we test in the DNA-binding assay? Would it be better to test more conditions or have more replicates?
Q2: Does the mutant p53 display a DNA-binding defect?

Students perform an Epigentek DNA-binding assay.
Q2: Does the mutant p53 display a DNA-binding defect?

Students perform a DNA-binding assay and analyze their results.
Q3: Does p53-mut show reduced nuclear localization?

Students Transform Yeast with p53/GFP and verify transformants using PCR
Q3: Does p53-mut show reduced nuclear localization?

Students perform fluorescence microscopy on p53/GFP strains. Nuclei fluoresce red.
Mutant Group Discussion #3

Does our p53 mutant show reduced DNA binding? If so, can it explain the p53 mutant’s transactivation defect?

Does our p53 mutant show altered nuclear localization? If so, can it explain the p53 mutant’s transactivation defect?

Given all our data, what is our **best hypothesis** to explain our p53 mutant’s transactivation defect?
Assessment

Lecture

Prelab

Background, Protocols

Lab Day

Notebook

Postlab

Analysis, Interpretation

Final Poster

Class is graded P/NC to encourage a collaborative, congenial atmosphere. Quizzes included for accountability (>70% required to Pass)
Collaboration and communication: Final poster session
Are We Making a Difference?

Question: "please describe how your thinking like a scientist has changed."

“Scientists must use positive and negative controls (I used to think a positive control would be enough)"

“For data to be significant, we must do statistical tests and repeat our experiment many times “

“ I have learned how to frame questions better so that they are testable and produce a useful result”

“I now look at data with a really critical eye”

“At the beginning of the quarter I was simply following protocols, and now I feel myself actively engaged in understanding why each step is performed and how minor changes could affect outcomes of assays”

“This class has shown me how science is a collaborative effort, more so than I previously imagined”
Are We Making a Difference?

Students’ answers to the question: “What does it mean to think like a scientist?”

- Developing hypotheses
- Critical, logical thinking
- Using multiple approaches to answer a question
- QUERY/scientific method
- Learn from mistakes/failed experiments
- Need to repeat experiments
- Being skeptical of data
- Requires analyzing and interpreting data
- Involves collaboration

(* indicates significant change from pre to post survey results)
Recommendations for a Successful CURE

1) Central research question common to all students
2) Connection to a real world problem
3) Required introductory course
4) Variation in elements of the research project to create student ownership
5) Cross-sectional collaboration between students, sharing data to lead to more replicates of data
6) Community of collaborators rather than competitors
7) Instructors who think like scientists
8) Course assessments that are authentic to the research process
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