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Promoting Student Independence with Project Based Laboratories

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Plant Molecular Biology is an upper level elective course available to seniors in RIT's Life Science programs, students who will shortly be in graduate school or employment. Two key skills to future success are troubleshooting and time management key skills. This Project-Based Laboratory course seeks to enable increased independence and problem solving. Teams of 3-4 students are given an outline plan, a list of available reagents and web links to molecular biology protocols. The project involves PCR amplification, cloning and sequencing a gene from *Arabidopsis thaliana*. Teams produce a project plan and record their progress on the class wiki.

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Introduction

Hands-on, research based biology laboratories cultivate scientific thinking and allow students to participate in authentic activities of working scientists. (Vision and Change 2011) This project seeks to "throw away the cookbook" and to cultivate student responsibility, scientific thinking and time planning skills. Plant Molecular Biology is an upper level elective in RIT's Molecular Bioscience and Biotechnology program. Most students who take the course are seniors and will shortly be in graduate school or employment. This laboratory project encourages students to learn from their mistakes and to master some basic techniques used in molecular biology. Teams of 3-4 students researched, cloned and sequenced genes from *Arabidopsis thaliana*. Each team worked on a different gene. We used a wiki to record and showcase student laboratory work, and to encourage student collaboration.

When I first taught this course, I guided the students through cloning and sequencing genes from *Arabidopsis,* each week we did one step, and I did all the primer design, checked that everything worked, had back-up supplies of everything, and if things didn't work for some groups, I gave them the products to continue. Most of the time students got to the end of the 10 weeks with a sequence, but it was tremendously hard work for me, as the instructor!

I was fortunate to hear Carl Lundgren speak at RIT about "Throwing Away the Cookbook" for his Industrial Design labs. He sought to foster student confidence and independence. And so this experiment began. Could I "throw away the cookbook" for Plant Molecular Biology? Could the students devise their own cookbook? Would they learn from their mistakes?

Example Student Outline

Your task is to clone and sequence a gene from *Arabidopsis thaliana*. You will be provided with the following:

- An *Arabidopsis* plant,
- A gene number,
- Basic reagents
- Molecular Biology reagents,
- Protocols can be found on the manufacturer's websites, or from papers posted on the class website.
- Laboratory equipment.
- Access to online data bases such as
- TAIR, (The Arabidopsis Information Resource).
- NCBI (National Center for Biotechnology Information).
- Primer design tools (NCBI PrimerBlast).
- ApE (A Plasmid Editor- a free program for restriction digest analysis).
- CodonCode, (sequence analysis and contig assembly).
- Lecture sessions focused on background, on-line resources, bioinformatics, database handling.

Detailed student notes

Week 1

Your first task is to identify the gene you will be cloning from the gene number. Go to **http://www.arabidopsis.org**, and in the Search genes query box, type in the gene number. Find out about your gene. Which chromosome is it on? What is its function? What is the size of the genomic DNA? What is the intron-exon structure?

Your task for this quarter is to clone and sequence the gene from *Arabidopsis* genomic DNA. You will use PCR to amplify the gene of interest from genomic DNA, and use the plasmid cloning vector pNEB 193, a derivative of pUC19.

The first task is to make a restriction map of your gene, and using the restriction map of pNEB193 (available of the New England Biolabs website), devise a cloning strategy for your experiment. You need to identify two restriction endonucleases that have sites in the multiple cloning site of pNEB193, but do not have sites in the gene you are cloning. You can do this in ApE. Copy the genomic sequence of your gene and paste it into the ApE DNA page, and make a restriction site map.

Once you have identified restriction endonucleases to use for cloning, you can move on to designing the PCR primers to amplify your gene. One useful tool for primer design is the NCBI Primer Blast. In TAIR, find the GenBank accession number under "nucleotide sequence" on the Gene Model Data page for your gene. If you click this, you will be taken to NCBI. Click on Primer Blast and select the region of the gene you want to clone. You may have to adjust the parameters to find primers to amplify the whole gene. Aim for Tm of 55-60 oC.

Once you have some primers that will work, you need to add the restriction site sequences you identified earlier to the 5' ends of the primers. You can find restriction site information on manufacturer's websites- e.g. **http://NEB.com** (New England Biolabs) or **http://promega.com** (Promega). Now add 4 random bases to the 5' ends of your primer + restriction site sequences. This ensures that the restriction endonucleases will cleave the DNA correctly. Check your primer sequences and then submit them to your instructor for ordering.

Week 2

DNA extraction from *Arabidopsis* leaves and first attempt at PCR using student-designed primers. Direction for genomic DNA extraction are available on the class website. Solutions of 1M Tris HCl pH8.0, 0.5 M EDTA, 5M NaCl and 10% SDS are provided. Use these to make 100 ml Edward's buffer. (Edwards et al 1991).

Directions for PCR amplification are given in the Sigma-Aldrich protocol for JumpStart RedTaq. Use 50 ul reaction volumes. Don't forget to include a no-DNA control!

The class will design the PCR program based on the annealing temperatures for all of the primer sets being used.

Week 3

Electrophoresis of PCR products. Use 1 x TAE 0.8% agarose gels to visualize 10 ul of your PCR product. Include 1 ul of GelRed in your gel and check using the UV transilluminator. Record your results in your lab notebook, and save a copy of the gel image for your wiki entries.

If you were successful with you PCR amplification, proceed to cleaning up your PCR product and preparing the PCR product and pNEB193 vector for cloning.

If you were unsuccessful, discuss your results with your instructor or TA and repeat the PCR step.

Next steps

1. Clean up your PCR product and prepare both PCR product and plasmid vector for restriction digestion. Use the Cycle-pure kit for PCR product clean-up, and measure the amount of DNA on the Nanodrop spectrophotometer.
2. Prepare for ligation and transformation: prepare blue/white LB selection plates with antibiotics, calculate the amount of vector and insert DNA you will need for the ligation (the protocol is n the manufacturer's website) and think about which control reactions you need to set up.
3. DNA ligation and transformation of *E.coli* competent cells. Use the NEB5α cells from the -80°C freezer. Be careful to thaw them on ice and follow the directions exactly. Remember to check your plates after overnight incubation.
4. Subculture white colonies and grow up white, ampicillin-resistant colonies over night.
5. DNA isolation from white colonies. Use the EZNA plasmid kit for DNA isolation. Remember to measure the DNA yield on the Nanodrop spectrophotometer.
6. Design restriction digestions to check for presence of the inserted DNA.
7. Set up sequencing reactions using M13 primers.
8. Sequence analysis: Use CodonCode to clean up sequence, assemble contigs and align your sequences to the reference sequence from NCBI or TAIR.

Experimental Records: Wiki and Lab Notebooks

Wiki: One person from each group should update the wiki each week. Record your results (good and bad) and comment on what your group will do next. Be prepared to give an five-minute update during the pre-lab lecture session.

Lab Notebooks: Each group member should keep their own lab notebook, and record precisely what was done in the lab each week. You should write methods and record results during the lab. You should be prepared to show the instructor or teaching assistant your notebook at any time. If things go wrong, precise records made during the lab can help pin-point errors, and save you lots of time.

Notes for the Instructor

In traditional labs, we usually give detailed, step-by-step instructions, which allow the students to 'auto-pilot' through the lab exercise. Many don't really think about why they are performing a particular step. As detailed above, we often make the lab exercises "cookie-cutter" simplified, tested, and provide back-up materials. If the experiment doesn't work, well, it must be the instructor's fault, or the lab prep staff's fault. Students do not take responsibility for these experiments. It's easy for the students to "leave behind" a failed experiment if we constantly provide the materials for the next step, just so that the class stays on the same page. Often, re-examining failed experiments and identifying where they went wrong leads to greater learning.

The Learning Goals for this experiment were:

- Increase student responsibility.
- Promote troubleshooting skills.
- Durable laboratory records.
- Time management skills.
- Precision and accuracy.
- Planning skills

The project- based format of these labs allows each group of 2-4 students to work at their own pace over a 10 week quarter. They have to work hard at designing their own primers, and thinking the cloning experiment through before they even start DNA isolation. Some groups need more guidance than others. By week 3, when the first PCR products are visualized, there are some disappointed groups who need to re-examine their PCR parameters and some happy groups who are ready to move on to the next stage. All of the groups will at some point make mistakes. They misread the directions for restriction digests and incubate at the wrong temperature. They add the wrong antibiotic to their selection plates when performing *E.coli* transformation. They throw away their precious DNA during PCR product clean–up and have to start over. As beginning researchers, everyone makes mistakes, but we take responsibility and move on. I emphasize the importance of good laboratory notebooks and records, and frequently do spot checks. When called upon to assist a group who are having problems, my first question is always: "Show me exactly what you did- what did you record in your lab notebook?" If they can't tell me, then it's a wake-up call to improve their record keeping.

In addition to the lab notebooks, the students record their results (successes and failures) on our RIT PlantWiki. (Figs. 1-3). This allows the instructor and TAs to assess progress, address problems, and help with next steps. Each student is expected to contribute, and contributions can be monitored by the instructor and used for assessment. Students can see entries by previous classes, as well as entries by current groups. They can link to methods from papers, papers about their gene, class presentations, manufacturer protocols, and add images. I post comments weekly, and encourage students to make constructive comments about other group's wiki pages.

Lecture sessions explain the background behind each technique. On lab day, each group give a weekly progress report- what's happened and what are they doing in lab today. This allows the class to share common problems and devise strategies to solve them. Beyond week 3 of a 10-week quarter, groups are often at different stages in the procedure. Some groups needed much more help than others, and often lab started early as students arrived and prepared a gel, or prepared agar plates early in order to fit everything in. Office hours were often spent talking over problems, and occasionally an extra lab session was added instead of a lecture session.

Students became very confident with handling micropipettes, gel electrophoresis, PCR, restriction digestion and bacterial transformation. They usually get to perform each technique many times. Planning, team management and time management improved dramatically over the quarter. There were definitely high points and low points, but in the end most groups were successful in cloning and sequencing their gene, and feel proud.

Some Comments from Students:

Q: This year's lab section consisted of a self-paced project rather than weekly activities with detailed instructions. Which format do you prefer?
A: *I prefer detailed instructions when you first start out but a self paced project later in the quarter*

A: *I think I learned more applicable information in the self-paced project, because we had to deal with things not working rather than simply moving on to the next week's activities without learning how to actually perform the technique correctly.*

A: *The self-paced project was really useful because you couldn't go on 'autopilot 'with each week's protocol. You had to look ahead and read each protocol and remain organized.*

Q: Do you prefer writing up your results in a final report or posting them on the wiki?
A: *Posting on the wiki is a week-by-week task, not a burden at the end of the quarter.*

A: *Posting on the wiki is more interactive; you can look at other groups and learn from their lab work*.

Screen Shots of PlantWiki Entries

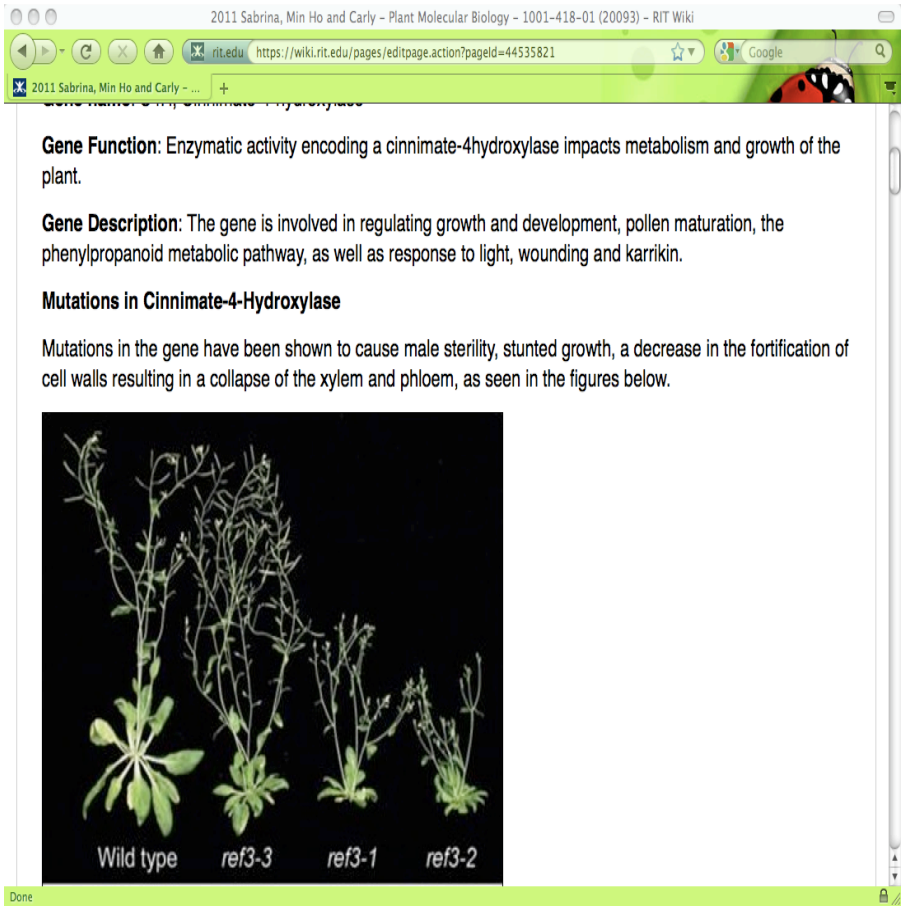

Figure 1. First page describing gene function and the effects of gene mutations.

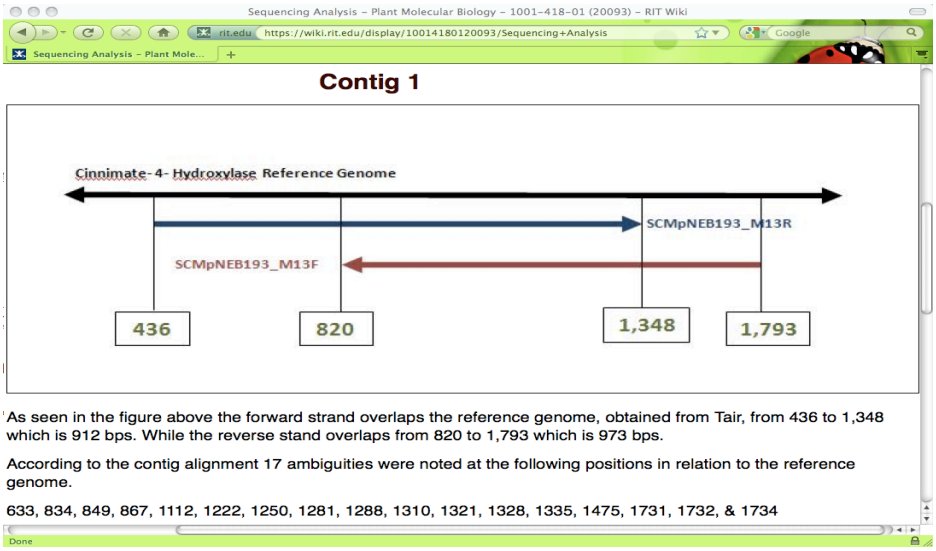

Figure 2. Final Contig assembly from CodonCode at the end of the project.

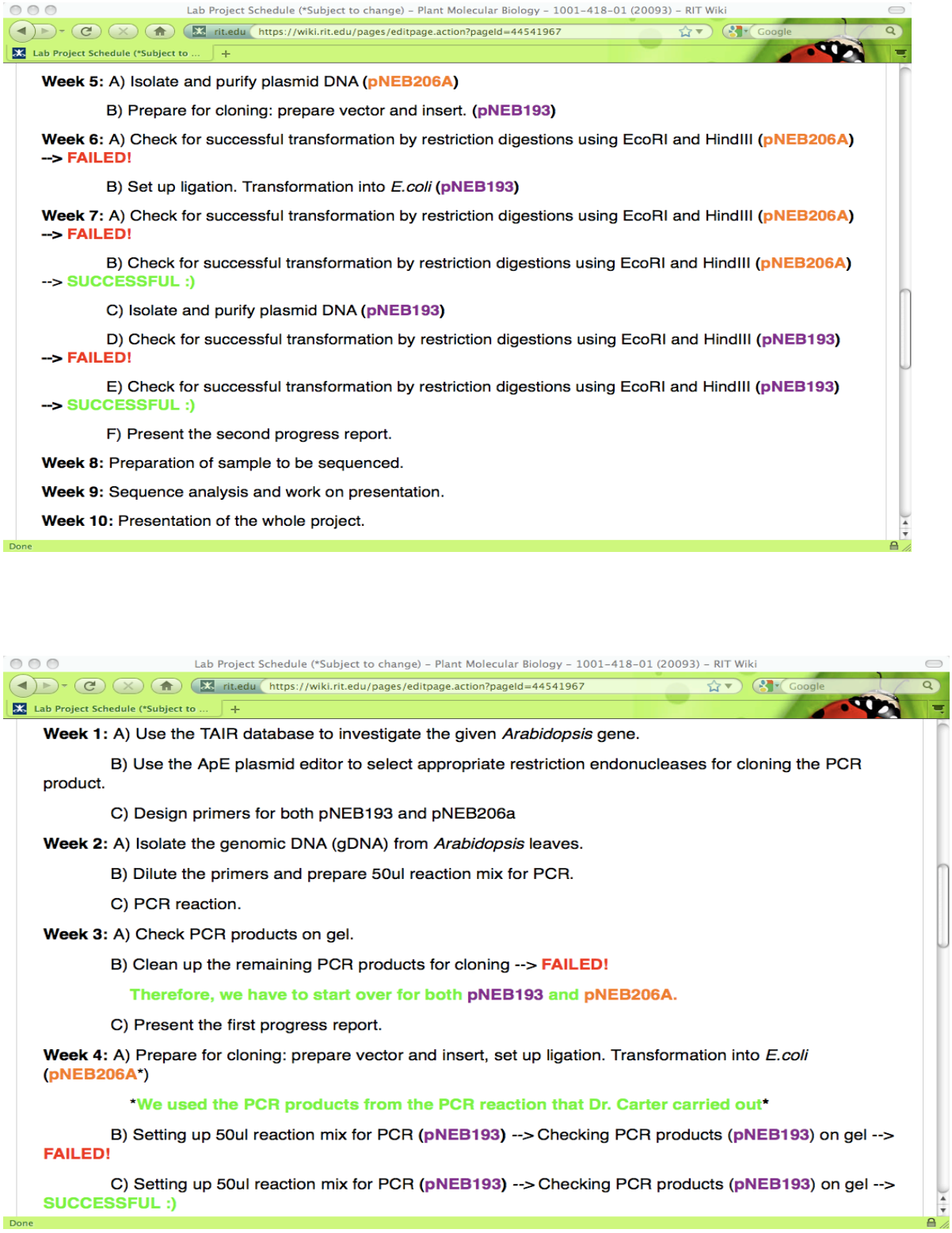

Figure 3. Excerpts from a student journal on the PlantWiki detailing successes and failures.

Assessment

Students were graded both individually and as teams. At the end of the quarter, each team prepared a PowerPoint presentation about their gene and their experiments. They researched and presented the function of their gene, and were challenged to find recent research about the gene function and interactions. In addition they presented the high points and low points of their progress and lessons learned. Both the instructor and their peers graded these presentations. Each student was expected to make at least two entries to the team wiki page, and to make at least two constructive comments on other teams' pages. The wiki structure allows the instructor to see the history of the student entries, and to summarize contributions from each team member. Teams were not graded on whether they successfully completed the task or not, rather they were assessed on the quality of their recordkeeping and the logical progression from one experiment to the next. Recording thoughts about why something went wrong ("I incubated the reaction at room temperature instead of than 37ºC") were encouraged.

Challenges

- Some teams needed to repeat work outside of designated laboratory period.
- Some groups needed extensive instruction in basic techniques early in the project.
- Frustration at early failures required instructor intervention to enhance morale and motivation.
- Project work does not allow for student absences!

In conclusion, students at RIT have embraced this project-based lab format, and have taken responsibility for their own successes and failures and worked on troubleshooting. Most teams enjoy posting results, both good and bad on the PlantWiki.

Resources

- *Arabidopsis* seed: Col 01 Lehle seeds (**http://arabidopsis.org**)
- PCR primers:**http://www.idtdna.com**
- ApE (A Plasmid Editor): free plasmid mapping software. Very useful for finding restriction sites, gel views of restriction digests, simple sequence alignments. **http://biologylabs.utah.edu/jorgensen/wayned/ape/**
- pNEB193: a pUC19 derivative – for traditional restriction enzyme/ ligase cloning. I have used other vectors (pCR2.1 TOPO, pNEB 206 (Uracil –based cloning), pJet) but the simple vectors still have a lot going for them in terms of learning about cloining methods. **http://www.neb.com/nebecomm/products/productn3051.asp**
- CodonCode: Easy-to-use sequence analysis software. Students can get a free one-month trial, and educational site licenses are available. In many cases the free trial is all you need. **http://www.codoncode.com/**
- JumpStart RedTaq- an almost student-proof product. Designed for high through-put PCR, this antibody linked taq polymerase can stand being left at room temperature whilst the last group gets their reactions set up, student's hot hands on the PCR tubes….Expensive, but worth it! **http://www.sigmaaldrich.com/catalog/product/sigma/p1107?lang=en&region=US**
- Molecular Biology kits: I have found that using commercial kits for PCR product clean-up and plasmid DNA preparation to be cost-effective and user-friendly. I use the inexpensive EZNA kits from Omega Biotek. **http://omegabiotek.com.**

Literature Cited

Edwards K, Johnstone C and Thompson C 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acid Research* 19(6) 1349.

Lundgren C 2010 **Scholarship, Teaching, Learning; Winning With All Three**: Create learning environments that facilitate learning AND student scholarship that informs and supports your scholarship. From project based learning through "skunkworks" labs, to undergraduates publishing their scholarship flowing from those learning experiences to collaborating with undergraduates on YOUR scholarship; some suggestions and observations from 30 years at RIT. Fall Faculty Learning Retreat, RIT .

Vision and Change in Undergraduate Biology Education- A Call to Action. **http://visionandchange.org/finalreport**

About the Author

Dr Dawn Carter teaches plant biology and freshman biology labs at RIT. Prior to teaching at RIT, she spent 15 years managing a plant tissue culture and biotechnology lab at Advanced Technologies (Cambridge) Ltd, a small plant biotechnology company in Cambridge, England.

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