Curriculum

The CURE for Cultivating Fastidious Microbes

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ABSTRACT

Course-based Undergraduate Research Experiences (CUREs) expand scientific educational benefits of research to large groups of students through a course setting. As part of an ongoing effort to integrate CUREs into freshman biology, we developed a microbiology CURE (mCURE) that uses a modified version of a dilution-to-extinction high throughput culturing protocol for isolating abundant yet fastidious aquatic bacterioplankton. Students learn to serially dilute and inoculate microbial cultures, perform DNA extractions and PCR, and identify microbial isolates via 16S rRNA gene sequences. The curriculum also includes exercises for learning to critically read and evaluate scientific literature, and emphasizes scientific communication through written and oral exercises that incorporate social media elements. In the first three semesters, the mCUREs achieved similar cultivability success as implementation of the protocol in a standard laboratory setting. The mCURE students have cultivated 43 unique bacterioplankton taxa, many of which occur as abundant taxa in the coastal environments from which the initial inoculum was sampled. Moreover, trends observed in the pre- and post-course survey data provide preliminary evidence that the mCURE format may improve a variety of scientific training objectives, based on the students' perceptions of the course. Our modular framework facilitates customization of the curriculum for use in multiple settings, and we provide classroom exercises, assignments, assessment tools, and examples of student output to assist with implementation.

INTRODUCTION

Undergraduate research experiences in STEM increase student retention in science majors; increase the proportion of students that go on to professional or graduate school; as well as improve critical thinking skills, data interpretation skills, content knowledge, and attitudes toward science (1-5). Typically, undergraduate research experiences are limited to relatively few students due to research lab size and funding, making these positions competitive, highly selective, and typically dominated by upperclassmen (4, 5). Course-based undergraduate research experiences (CUREs), in which students experience research as part of a course, can reach students early in their degree program and accommodate large numbers of students, thus increasing the diversity of students participating in research (4, 5). Despite these benefits, the time necessary to plan CURE projects and create assignments and rubrics can restrict their use (6). Fortunately, an increasing number of publications have shared CURE implementation strategies for a variety of settings (3, 7-9).

We recently outlined a flexible, modular CURE framework, including rubrics and course materials, that has facilitated conducting a variety of different research projects in the freshmen biology laboratory courses at Louisiana State University (LSU) (10). Using this framework, we have developed the microbiology CURE (mCURE) described herein that focuses on the cultivation of bacterioplankton from aquatic systems (**Figure 1**). Students execute a modified version of the protocol utilized by the Thrash Laboratory at LSU (11, 12) incorporating lessons on BSL2 safety, pipetting, serial dilution, sterile technique, DNA extraction, PCR, and basic sequence identification using BLAST. In addition, the mCURE emphasizes enhancing student skills in the areas of reading primary literature, communicating with a variety of audiences, and experimental design. The possibility of isolating new organisms provides a charismatic entry point into biological research and students experience a genuine excitement of discovery along with their laboratory and communication training.

Bacterioplankton occupy marine and freshwater environments at cell concentrations typically between 10^5-10^7 cells mL^{-1}. Within these mixed populations, taxa occupy a spectrum of frequencies, with some groups representing 10-20% of the total number of cells, and others occurring in much lower abundance. Regardless of their abundance, cultivation of many taxa from marine and fresh water has not been possible. Methods using traditional agar plating have only successfully cultivated 0.1-1% of the organisms present in a given sample (13), hampering our ability to understand the functions of a large majority of microorganisms. This Great Plate Count Anomaly (13) has led to the development of more sophisticated cultivation methods. One of these combined serial dilution of

samples with sterilized natural water as a medium (14). Many of the most abundant taxa in marine and freshwater systems have been successfully cultured using this approach. Examples included SAR11 *Alphaproteobacteria* (15-17), SUP05 *Gammaproteobacteria* (18), SAR116 *Alphaproteobacteria* (19, 20), and members of the so-called "Oligotrophic Marine *Gammaproteobacteria*" (21). Recently, a modification of these protocols demonstrated considerable success in cultivating many of the most abundant microorganisms in coastal systems using a set of artificial media (12). This advance was important because unlike sterilized natural water media, which requires access to and shipping of large volumes of water from environmental sources, artificial media can be made anywhere and easily modified (e.g., in salinity, carbon and nitrogen sources, etc.) to accommodate different environments. This advance also facilitated the adaptation of the protocol in teaching laboratories.

Learning time

We designed the mCURE for a semester timeline, with each single three-hour laboratory section meeting once a week for a minimum of 13 weeks. The project is divided into four major segments (color-coded in both **Figure 1** and **Table 1**). In weeks #2-4 (***orange***), students attempt to establish an initial culture of marine bacterioplankton using serial dilutions with the High-Throughput Culturing protocol (HTC; (11)). Transfer of the initial cultures to larger flasks for further growth occurs during weeks #5-6 (***green***). During weeks #7-9 (***blue***), students extract DNA from the successful cultures and amplify the 16S rRNA genes with PCR. The amplified products are then sequenced out of class for subsequent taxonomic identification of the microbe on week #10 (***yellow***). The remaining weeks (#11-13) are spent discussing poster construction and administering the final assessments for the semester. Note that the entire workflow does not require 13 weeks, but we have built in flexibility to allow for some elements of the protocol to be repeated in case of a failure in one or more of them (see Faculty Instructions below).

Intended audience and prerequisite student knowledge

This course is intended for college students at any level who have basic prerequisite training and proficiency in biosafety level 1 (BSL1) organisms and safety practices (22). No other prerequisites are required. Students receive training in many of the basic biology skills that they will utilize in other contexts, both at the bench and in communication of their results, and they receive training in biosafety level 2 (BSL2) protocols (see Safety Issues, below). We also provide suggestions for protocol modifications that could facilitate the use of this curriculum, albeit

with likely variations in the types of organisms cultivated, in settings for which the type of equipment we utilize is unavailable.

Learning objectives

By the end of the semester, students should be able to:

1. Describe the relationship between the research objectives, the high-throughput culturing approach, and the experimental design;
2. Find, read, and interpret relevant articles from the primary literature;
3. Work in groups to isolate, culture, and identify bacterioplankton from aquatic environments;
4. Communicate the methods, results and implications of their research to both scientific and non-scientific audiences using posters, writing assignments, and social media.

In addition to the learning objectives outlined above, the format of the mCURE sections incorporated aspects of three high-impact practices: Undergraduate Research, Collaborative Assignments, and Writing Intensive (23).

PROCEDURE

A summary of basic approach for the CURE is shown in **Figure 1**, along with a week-by-week breakdown of activities in **Table 1**. Table 1 also includes a summary of all class materials made available with this manuscript, as well as prep notes for faculty.

Materials

The required equipment and chemicals are the same as those previously published (11). Briefly, because most of the highly abundant aquatic microorganisms have oligotrophic lifestyles, occur in low cell densities (< 10^7 cells/mL), are very small (< 1μm), and will not grow on solid media, the cultivation approach makes use of liquid media and cell growth is measured using a benchtop flow cytometer (Millipore "Guava"). Alternative media, including detailed recipes and preparation instructions were previously published (12). To avoid trace-metal contamination, all reusable cultivation vessels should be made of polycarbonate plastic. Other major items include a thermocycler and PCR reagents, electrophoresis equipment and a gel viewing system (e.g., Bio-Rad Gel Doc), a DNA quantification

113 system (e.g. Qubit, ThermoFisher), DNA extraction kits (MoBio PowerWater), and pipettes/tips. The only
114 differences in the established protocol equipment (11) for the mCURE sections are the requirement for a biosafety
115 cabinet and disposable 2.1 mL 96-well plates (Thermo Nunc A/S) instead of those made from PTFE. For those
116 without access to some or most of this equipment, we provide alternatives in the Discussion that can improve
117 implementation flexibility.
118
119 ***Student instructions***
120
121 Segment 1 (*orange* in **Figure 1, Table 1**): In first two weeks of class, students are introduced to the overall mCURE
122 approach, coached on how to use pipets, and trained in BSL2 safety protocols. Each group of two students (a total of
123 12 per section) then dilutes and inoculates seven wells of a 96-well plate, containing low-nutrient medium, with the
124 diluted seawater (**Appendix 1**). An eighth well is inoculated with sterile media as a contamination control. Thus,
125 each section begins cultures in a 96-well plate, which is incubated at *in situ* temperature (depending on source water)
126 for 2-3 weeks, prior to being checked for growth using flow cytometry. During the incubation weeks, student
127 assignments focus on introducing effective reading of scientific literature, and experimental design and rationale.
128
129 Segment 2 (*green*): Each group selects 1-2 culture wells from those that were deemed positive via flow cytometry
130 (growth over 10^4 cells/mL) for transfers into larger volume growth flasks, and creates cryostocks for culture
131 preservation in 10% DMSO (**Appendix 2**). In our experience, most groups were able to perform at least one
132 transfer. The 2-3 groups (out of 12) that had no growth in any of their wells select an "extra" well from another
133 group (one that group isn't transferring). These flasks are then allowed to incubate for two weeks at the same
134 temperature as the initial cultivation plates. During the interim, students are introduced to scientific writing and give
135 "lightning talks" (see footnote #2 in **Table 1**).
136
137 Segment 3 (*blue*): Groups select at least one flask that shows growth and extracts DNA from the culture (**Appendix**
138 **3**). In the last three semesters of conducting this mCURE, the majority of groups observed growth in at least one
139 flask. The remaining 2-3 groups with no growth in any of their flasks use part of another group's culture for
140 extraction. Note this introduces redundancy in the final identification results. Over the next two weeks, they amplify

141 the 16S rRNA genes from their isolates using PCR and confirm the amplification product with gel electrophoresis.
142 Successful amplicons are then sequenced off campus at a sequencing facility (e.g., the RTSF Genomics Core at
143 Michigan State University or similar).
144
145 Segment 4 (*yellow*): Students learn to assemble forward and reverse (via the reverse complement) PCR
146 amplification sequence reads into a contig and identify their isolate sequences using NCBI BLASTN portal
147 (**Appendix 4**). Briefly, reads from both the forward and reverse primer, as well as the contig (if any), are searched
148 against the GenBank nt database with and without the exclusion of uncultured/environmental samples. The % ID,
149 Query coverage, E-value, and GenBank # for the top five BLAST hits (from all the runs) are recorded for all
150 isolates. Interpretation and contextualization of the results, including the similarity of isolates generated by the
151 students to those in the database, occurs via discussion with knowledgeable faculty/teaching assistants (TAs). These
152 results become part of the final data presented via posters.
153
154 ***Faculty instructions***
155
156 Segment 1 (*grey*, *orange* in **Figure 1, Table 1**): Prior to the beginning of the course, instructors must prepare the
157 following:
158 1) Collect seawater and measure the concentration of bacterioplankton using flow cytometry. The students use
159 this initial concentration to calculate the dilution factor required to inoculate ~1-5 cells per well. Collection
160 should occur as proximately to inoculation as possible to avoid microbial community change via bottle
161 effects.
162 2) Prepare the low-nutrient media (12) required to grow the microbes. This can be stored for a few days at 4˚C
163 if necessary. Keep wrapped in foil to reduce photodegradation of the vitamins. Aliquot ~1.7 mL of media
164 into each well of the 96-well plate just before class and allow time for equilibration to incubation
165 temperature.
166 3) Select ~12-15 scientific articles relevant to the project and create a reading guide for one of them for class
167 discussion (sample: **Appendix 5** for Connon & Giovannoni, 2002). The students may select one of the

168 remaining papers for their lightning talks (Table 1, weeks #4-5), and use any number of them as references
169 for their formal writing assignments.
170
171 Because of the considerably long incubation period (2-3 weeks) for the initial inoculations, we recommend that this
172 step involve at least one "holiday week" for the lab (see **Supplemental Table 1**). At the end of the incubation
173 period, instructors perform flow cytometry to check for growth in the 96-well plate and record the well numbers
174 positive for growth. As a reminder, since isolates will be unknown at this time, transfers from incubation plates to
175 counting plates (11) should be completed in a biosafety cabinet.
176
177 Segment 2 (*green*): Prior to the start of this segment, instructors must prepare more of the low-nutrient medium and
178 aliquot 50 mL into flasks, as well as prepare cryotubes with DMSO. Furthermore, students should have access to a
179 biosafety cabinet in which to handle all cultures. At the end of the 2-week incubation, instructors perform flow
180 cytometry again to check the flasks showing growth, and record cell concentrations for student use. For the scientific
181 writing discussion, we have made an activity available (**Appendix 6**) that familiarizes students with the content in
182 various sections of a paper (19).
183
184 Segment 3 (*blue*): We recommend that instructors aliquot the required amount of DNA extraction reagents (Power
185 Water DNA Isolation Kit; Mo Bio Laboratories) for each group of students to prevent cross-contamination. For
186 PCR, we propose that the instructor create a master mix with all PCR components (detailed in **Appendix 9**) as part
187 of a class demo, then have students add 1 µL of the isolated DNA to 49 µL master mix in a 50 µL PCR reaction. For
188 the gels, we suggest making an appropriate amount of agarose in a flask for each section, and allowing it to solidify
189 until class time. Then, prior to the start of class, the instructor can melt the agarose in the flask and have it ready for
190 students to pour their own gels.
191
192 Segment 4 (*yellow*): Before the BLAST lab, instructors need to have all successful 16S rRNA gene amplicons
193 sequenced, using both forward and reverse primers (we use 27F and 1492R, but this can be specified by the
194 instructor- see (24) for additional options), and the data available where the students can access it. Each sequencing
195 read needs to be labeled with the sample number and whether it is a "forward" or a "reverse" sequencing read. We

196 recommend the "BLAST behind the scenes" activity (**Appendix 7**) to introduce students to the concept of sequence
197 analysis.
198
199 Finally, instructors need to prepare for a departmental poster session at the end of the semester. Therefore,
200 the space for poster boards, display tables, and printing facilities need to be organized. However, as far as the graded
201 poster presentation is concerned, we recommend that the student groups present their posters electronically in class.
202 During this time, other students and the instructor can offer constructive criticism for the students to incorporate into
203 the final printed version of the poster.
204 Based on our experience performing this mCURE for the past three semesters, we anticipate at least 1-2
205 protocol failures per semester; hence, flexibility is built into the framework (**Table 1**, **Supplemental Table 1**).
206 Despite our anticipation of some failures and correcting these in subsequent semesters (e.g., students failing to
207 properly transfer and freeze their samples), each new semester has presented us with new and different failures (e.g.,
208 flow cytometer reagents on back-order, failed PCRs due to old reagents). Many non-experimental activities, such as
209 the lightning talks, can be easily performed during different time points in the course, amended to take less time, or
210 even completely eliminated. Similarly, other related activities may be added, such as peer review of initial formal
211 writing drafts and using social media for science outreach (*e.g.* we use the Twitter and Instagram hashtag
212 #LSUCURE for all CURE efforts in the Department of Biological Sciences at LSU; **Supplemental Table 1**). If
213 feasible, we recommend adding the following enhancements to further engage students in the course: (i) taking
214 students on a field trip, such as a one-day research cruise to collect water samples; (ii) demonstrating the use of
215 "behind the scenes" equipment, such as the flow cytometer, capillary sequencer, and/or modern microscopes used to
216 image bacteria.
217
218 ***Suggestions for determining student learning***
219 We used quizzes throughout the semester to gauge student learning, along with a set of written assignments (**Table**
220 **1**). While the quizzes and final exam were intended to test the students' knowledge about the course materials at
221 various levels of Bloom's taxonomy, the written and oral assignments were meant to hone their communication
222 skills. The students received instructor feedback for each informal and formal draft of their papers before submitting

223 the next one (**Appendices 8-11**). Finally, the course has a cumulative final exam (**Appendix 12**) and a poster
224 presentation by the student groups.
225 In addition to the course materials outlined above, we used pre/post surveys to assess self-evaluated student
226 research efficacy and perceptions of the course. The survey instrument consisted of 21 questions measuring student
227 attitudes toward science and biology labs (written by EWW and collaborators; 5 point Likert scale), twelve
228 questions from Lopatto's (25) instrument (5 point Likert scale), 27 questions from the Clinical Research Appraisal
229 Inventory (CRAI; 11 point Likert scale) (26), and demographic questions (**Appendix 13**). These surveys were
230 emailed to students in all research and traditional lab sections during the first week of the semester and again during
231 the last week of the semester. Students were allowed two weeks to complete the surveys; two reminder emails were
232 sent to students that had not yet completed the survey. Pre- and post-survey data were paired. We determined
233 significance in the pre/post semester differential (post response minus pre response) between research and traditional
234 students using the Mann-Whitney *U*-test implemented in R (27). Here we present a preliminary analysis of the data;
235 we will present a more thorough analysis of research and traditional lab student outcomes, perceptions, and self-
236 efficacy for all CUREs in our department in a future contribution.
237
238 ***Sample data***
239 Examples of formal writing assignment 1, final lab report, lightning talk, and student posters are included in
240 **Appendix 14** and are shared with permission from the students.
241
242 ***Safety issues***
243 Since the curriculum involves isolating unknown organisms, students must be proficient in BSL1 safety techniques
244 prior to taking the course. All elements of the course that involve handling live microorganisms should occur under
245 BSL2 safety protocols, as outlined by the JMBE Biosafety Guidelines for Handling Microorganisms in the Teaching
246 Laboratory (22). The specific elements of the curriculum that require BSL2 protocols are indicated in Table 1.
247 Additional safety measures must be taken for faculty during washing and preparation of medium mixture bottles and
248 growth flasks. See (11) for more details.
249
250 **DISCUSSION**

251

252 ***Field testing***

253 Thus far we have offered the mCURE during the fall 2015 and 2016 semesters in Biology 1207 (Honors: Biology
254 Laboratory for Science Majors) and spring 2016 in Biology 1208 (Biology Laboratory for Science Majors I). There
255 have been four sections per semester taught by two graduate TAs (two sections per TA), with up to 28 students per
256 section. Biology 1207 is only offered in the fall semester and consists of a total of four sections. Multiple (12-50)
257 sections of Biology 1208 are offered every semester, a few of which are typically offered as CUREs as outlined in
258 our previous publication (10); students do not know when they register for this course if their section will be in a
259 CURE or traditional format. We note that these previous sections of the mCURE were conducted with a BSL1
260 safety protocol. The current protocol occurring in this manuscript has been updated with BSL2 safety measures in
261 response to recommendations by ASM (22). We will offer the updated mCURE at LSU for the foreseeable future.

262

263 ***Experimental results***

264 Fall 2015, spring 2016, and fall 2016 average cultivability (14) was 9.9, 2.8, and 12%, respectively. These
265 cultivability numbers generally match the success rate of other HTC experiments (12), and demonstrate a significant
266 improvement over plate-based methods (13). The number of unique pure cultures that survived successive transfers
267 and were positively identified at the end of each course was 28 (fall 2015), 13 (spring 2016), and 23 (fall 2016). In
268 total, mCURE sections isolated 43 unique bacterioplankton during the first three semesters. Some courses isolated
269 taxa identified in a previous mCURE, so the overall total was smaller than the sum of the individual semesters.
270 Many of these have close relationships to organisms previously isolated using HTC techniques in the Thrash lab and
271 other labs, as indicated by taxonomic affiliations to strains with "LSUCC", "HTCC", "HIMB", or "IMCC" strain
272 numbers (**Table 3**). Importantly, many isolates represent abundant marine clades (12), and thus the results validate
273 the mCURE approach to produce valuable cultures with similar efficacy as HTC experiments conducted under more
274 typical laboratory settings.

275 Our curriculum development also provided anecdotal information that generated testable hypotheses about
276 cultivation conditions. During the first semester (fall 2015), the mCURE protocol utilized glass tubes instead of the
277 Thermo plates indicated above. This semester was also the only time we isolated numerous *Vibrio* spp., which are
278 rarely cultivated in HTC experiments with the same media and inoculated from the same source waters (12).

279 Although the lack of cultivation-independent data precludes us from knowing if the particular sample had a bloom
280 of *Vibrio* spp., thus increasing the probability of their isolation, the results of this experiment present the possibility
281 that glass preferentially selects for certain bacterioplankton. The finding corroborates our understanding of *Vibrio*
282 spp. as commonly having a surface-associated lifestyle (28). More importantly, the hypothesis that cultivation vessel
283 material selects for specific taxa can be tested experimentally in the mCURE setting, thus adding value to the course
284 design. During the fall 2016 semester, mCURE sections also experimented with using media of two different
285 salinities for the same inoculum. Sample site salinity was 17. Students inoculated cultures in our MHW2 and
286 MHW3 media with salinities of 23.2 and 11.6, and achieved 8.3 and 15.7% cultivability, respectively. Although
287 these results are inconclusive due to variability in student success with downstream protocol elements, future
288 experiments of this kind will provide additional data points and eventually strengthen the relationship between
289 media salinity, cultivability, and taxonomic identity of the isolates. Other examples of comparative cultivation
290 experiments easily facilitated with multiple concurrent mCURE sections include testing alternative carbon substrates
291 and alternative incubation temperatures while using the same inoculation source. Ideally, cultivation independent
292 data like 16S rRNA gene amplicons would also be collected to evaluate cultivation success (e.g., see (12)), but this
293 requires additional cost and analysis expertise.
294
295 ***Evidence of student learning***
296 Sample size and demographics of survey respondents who completed both pre- and post-surveys are outlined in
297 **Table 2**. We should note that these survey results reflect self-reported student perceptions of their confidence and
298 skills and do not necessarily reflect actual changes in student skills or competencies. Most research (mCURE)
299 students were freshmen with a few sophomores, whereas respondents in the traditional 1208 labs included students
300 at all undergraduate academic levels (**Table 2**). Despite these differences, mCURE students were demographically
301 similar to traditional lab students. In general, we found that students in both the traditional lab sections and research
302 lab sections had decreased confidence in their abilities at the end of the semester. However, we observed a non-
303 significant trend whereby research students lost confidence less and had less negative perceptions of the lab relative
304 to traditional sections (**Figure 2** and **Appendix 15**). These results are largely consistent with other similar studies
305 comparing freshmen level CUREs to traditional labs (3). Although all students in freshmen level biology labs at our
306 institution work in groups, we found that CURE students had significantly more positive (or at least less negative)

307 perceptions of collaboration and in their ability to work independently compared to traditional students (**Figure 2**).
308 In addition, research students increased confidence in their writing skills more than their traditional lab counterparts
309 (**Figure 2**).
310
311 ***Possible modifications***
312 We appreciate that many instructors may wish to implement the mCURE design but do not have access to some of
313 the more expensive equipment used in our protocol. Thus, we mention a few modifications to circumvent some of
314 these restrictions. Although a flow-cytometer works best for rapid counting of small bacterioplankton cells at typical
315 low growth densities, instructors can replace flow cytometry with direct microscopic counts, e.g., as in some of the
316 earlier iterations of the HTC protocol (19). For those without access to either a flow cytometer or a fluorescence
317 microscope, the protocol can still be completed using traditional agar-plate based methods. Our media can be
318 prepared with agar (11) or replaced with a classic marine medium like Difco 2216 (BD). Although solid media
319 generally select for different taxa than liquid media, for the purposes of a basic biology laboratory, this may not
320 matter. After streaking a seawater sample on plates, individual colonies can be picked, grown up in liquid culture to
321 increase cellular mass, or directly processed through DNA extraction. Colony PCR (29) may also be an attractive
322 alternative identification method, particularly because this also eliminates the time and cost associated with DNA
323 extraction. These last two steps may also help adapt the overall protocol for shorter time frames, e.g., academic
324 quarters instead of semesters. Please note that our protocol uses low-nutrient and low-carbon media that typically
325 selects for non-pathogenic, oligotrophic marine bacterioplankton. The use of rich media and plate-based methods
326 may increase the risk of cultivating pathogenic organisms. Finally, for those interested in freshwater environments,
327 the same protocol can be conducted with freshwater media, either artificial (30) or natural (31).
328
329 **ACKNOWLEDGMENTS**
330 We wish to thank Dr. Chris Gregg, Ann Dickey-Jolissaint, and Brooke Trabona for coordinating the labs and
331 prepping many of the lab materials behind the scenes. David Morris and Celeste Lanclos served as additional
332 graduate and undergraduate TAs, respectively. Andrew Flick and Dr. Paige Jarreau were instrumental in
333 incorporating social media assignments into the curriculum. The Socolofsky Microscopy Center provided images of
334 some of the microbes cultured by students and gave students tours of the facility. We thank the crew of the *R/V*

Acadiana and Murt Conover at the Louisiana Universities Marine Consortium for assisting with logistics and field training. We are grateful to Dean Cynthia Peterson for her support of CUREs in the Department of Biological Sciences. Funding for this project was awarded through the Student Excellence Fee from the College of Science.

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Table 1. The mCURE framework. Activities, associated assessments, faculty instructions, and the relevant supporting documents are detailed week-by-week. The various segments of the course are color coded (grey, orange, green, blue, and yellow), consistently with the flowchart in Figure 1.

Week	Topic	Quiz	Quiz Topic	In-class (Group) Activity	Assignments & Reminders	Instructor Prep Notes	Supporting Documents	Other Supporting Documents [a]
#1	Meet & greet; Basic intro to research question			Familiarize students with BSL-2 safety guidelines	Research paper (#1) reading assigned	Collect seawater & measure conc.; prep media and aliquot into 96-well plate	Course outline flowchart (Fig. 1)	
#2	Expt. design; pipetting & sterile technique; Scientific record-keeping			Research paper (#1) introduced; Dilute seawater & inoculate 96-well plates**		Incubate cultures under optimum conditions for 2-3 weeks.	Inoculation protocol; Reading guide	
#3	How to read scientific literature	1	Dilutions, pipetting & sterile technique	Decode & understand main points in a scientific paper; Other research papers introduced as part of in-class activity	HW based on research paper (#1) assigned[1]			How to Read a Scientific Paper
#4	Experimental design	2	Decoding scientific literature	Interactive discussion re: experimental design (compare & contrast methodology with paper #1)	HW based on research paper (#1) due; Student groups choose one from a pool of papers for the "lightning talks" [2]	Perform flow-cytometry to determine positive cultures[3]; prep media in flasks; prep cryostocks		
#5	Transfer & back-up; Discuss importance of back-ups; "Lightening talks" [2]	3	Expt. design	Transfer isolates to larger volume; prepare cryostocks**	Formal Writing #1 assigned; Informal Writing #1 assigned	Incubate flasks under optimum conditions for ~2 weeks	Formal Writing #1 guide & rubric; Informal Writing #1 guide & rubric	Writing Rubric
#6	Scientific Writing	4	Expt. design (contd.) – focus on transfers & back-ups	Order the sections of a scientific paper; Evaluate excerpts of scientific papers based on guide & rubric	Informal Writing #1 due	Perform flow-cytometry to determine positive cultures[3]; Prep aliquots of DNA extraction reagents	Order a scientific paper	Scientific Writing
#7	DNA extraction			Perform DNA extraction of isolates**	Feedback on Informal Writing #1 returned	Design & purchase 16S rDNA primers; aliquot PCR reagents		
#8	PCR; Primer choice & design	5	DNA extraction	Perform PCR of isolates	Formal Writing #1 due	Prep agarose gels		

#9	Gel electrophoresis & Sanger sequencing	6	PCR & primer design	Perform gel electrophoresis to confirm PCR products	Formal Writing #2 assigned; Informal Writing #2 assigned; Feedback on Formal Writing #1 returned	Perform Sanger Sequencing on positive samples	Formal Writing #2 guide & rubric; Informal Writing #2 guide & rubric	
#10	BLAST sequences & identify microbes	7	Electrophoresis & sequencing	Manually compare a set of sequences to identify the most closely related organisms; Identify the cultured microbes	Informal Writing #2 due; HW on poster critique assigned [4]		BLAST: How-to guide; BLAST behind the scenes	Poster Critique
#11	Elements of poster design; Poster development & critique	8	BLAST	Design rough drafts of posters; Peer poster critique session	HW on poster critique due; Feedback on Informal Writing #2 returned			Designing Scientific Posters
#12	Final Exam				Formal Writing #2 due			
#13	Poster Presentations; Peer Evaluation & Reflections							Poster Rubric; Peer Evaluation

[a] Available as Supplemental Materials from Bakshi, A., *et al*., *A Highly Scalable General Framework for Implementing Course-based Undergraduate Research Experiences (CUREs) in Freshman Biology Labs.* American Biology Teacher, 2016. **78**(6): p. 1-7.

[1] Basic questions to engage students in background information and the major take-home points from the research article

[2] Students give 5-minute presentations on a relevant research article of their choice from a pool of papers made available by the instructor (these papers are to be then used in the future as references in Formal Writings)

[3] Students are encouraged to make an appointment with the instructor to observe how the flow cytometer works.

[4] Evaluate publicly displayed posters within the department for clarity and style; designed to familiarize students with various poster designs

**BSL2 laboratory protocols required.

Abbreviations: HW – Homework; Expt. = Experimental; Prep = Prepare (for student use); Conc. = concentration

433 **Table 2.** Demographic data (percentages) for research (mCURE) lab and traditional lab survey respondents.

		Research (n=53)	Traditional (n=287)
Gender	Female	75.5	79.4
	Male	24.5	20.2
Class standing	Freshman	90.5	88.1
	Sophomore	9.5	6.6
	Junior	0	2.8
	Senior	0	1.4
	Other	0	0.7
Ethnicity	White	81.1	81.2
	Other	18.9	18.8

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436 **Table 3.** Bacteria cultured by mCURE students

Closest unique cultured relative	Major Taxonomic Group
Arthrobacter sp. 210_2	Actinomycetales; Actinobacteria
Marinobacterium sp. IMCC1424	Actinomycetales; Actinobacteria
Microbacterium esteraromaticum strain V45.13	Actinomycetales; Actinobacteria
Microbacterium sp. Ni17	Actinomycetales; Actinobacteria
Nocardioides exalbidus strain DS1-2B	Actinomycetales; Actinobacteria
Nocardioides hwasunensis strain XH199	Actinomycetales; Actinobacteria
Alteromonadales bacterium 3tb13	Alteromonadales; Gammaproteobacteria
Alteromonas macleodii	Alteromonadales; Gammaproteobacteria
Alteromonas tagae	Alteromonadales; Gammaproteobacteria
Marinomonas sp. SS8	Alteromonadales; Gammaproteobacteria
Porticoccus hydrocarbonoclasticus	Alteromonadales; Gammaproteobacteria
Pseudoalteromonas phenolica	Alteromonadales; Gammaproteobacteria
Pseudoalteromonas sp. A-3	Alteromonadales; Gammaproteobacteria
Shewanella sp. 49WBP	Alteromonadales; Gammaproteobacteria
Bacillus sp. L1(2012)	Bacillales; Firmicutes
Burkholderiales bacterium LSUCC0118	Burkholderiales; Betaproteobacteria
Limnobacter sp. MYOU6	Burkholderiales; Betaproteobacteria
Halieaceae bacterium LSUCC0247	Halieaceae; Gammaproteobacteria
Gamma proteobacterium SF293	OM182; Gammaproteobacteria
Gamma proteobacterium IMCC15037	OM252; Gammaproteobacteria
Gammaproteobacteria bacterium LSUCC0258	OM252; Gammaproteobacteria
Gammaproteobacteria bacterium LSUCC0272	OM252; Gammaproteobacteria

Marine gamma proteobacterium HTCC2080	OM60/NOR5; Gammaproteobacteria
Agrobacterium sp. TSH97	Rhizobiales; Alphaproteobacteria
Anderseniella baltica	Rhizobiales; Alphaproteobacteria
Anderseniella baltica strain BA141	Rhizobiales; Alphaproteobacteria
Rhizobium sp. MSSRF QS100	Rhizobiales; Alphaproteobacteria
Bacterium HIMB11	Rhodbacterales; Alphaproteobacteria
Rhodobacteraceae bacterium LSUCC0246	Rhodbacterales; Alphaproteobacteria
Rhodobacteraceae bacterium LSUCC0259	Rhodbacterales; Alphaproteobacteria
Roseobacter sp. strain WM2	Rhodbacterales; Alphaproteobacteria
Altererythrobacter ishigakiensi	Sphingomonadales; Alphaproteobacteria
Erythrobacteraceae bacterium LSUCC0210	Sphingomonadales; Alphaproteobacteria
Erythrobacteraceae bacterium LSUCC0236	Sphingomonadales; Alphaproteobacteria
Erythrobacteraceae bacterium LSUCC0240	Sphingomonadales; Alphaproteobacteria
Erythrobacteraceae bacterium LSUCC0267	Sphingomonadales; Alphaproteobacteria
Bacterium MH1	Vibrionales; Gammaproteobacteria
Vibrio chagasii	Vibrionales; Gammaproteobacteria
Vibrio pelagius	Vibrionales; Gammaproteobacteria
Vibrio proteolyticus	Vibrionales; Gammaproteobacteria
Vibrio sp. 0208F3	Vibrionales; Gammaproteobacteria
Vibrio sp. PaH3.31d	Vibrionales; Gammaproteobacteria
Vibrio sp. TP187	Vibrionales; Gammaproteobacteria

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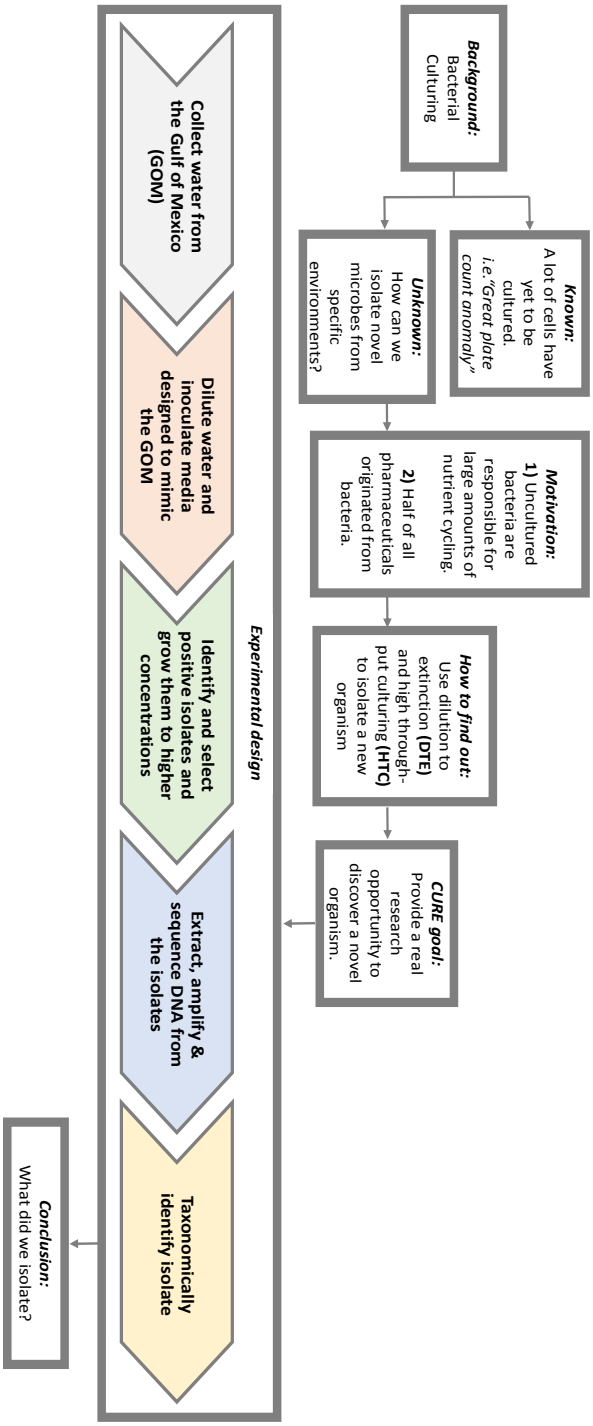
Background:
Bacterial Culturing
Known:
A lot of cells have yet to be cultured.
i.e. "Great plate count anomaly"
Unknown:
How can we isolate novel microbes from specific environments?
Motivation:
1) Uncultured bacteria are responsible for large amounts of nutrient cycling.
2) Half of all pharmaceuticals originated from bacteria.
How to find out:
Use dilution to extinction (DTE) and high through-put culturing (HTC) to isolate a new organism
CURE goal:
Provide a real research opportunity to discover a novel organism.
Experimental design
Collect water from the Gulf of Mexico (GOM)
Dilute water and inoculate media designed to mimic the GOM
Identify and select positive isolates and grow them to higher concentrations
Extract, amplify & sequence DNA from the isolates
Taxonomically identify isolate
Conclusion:
What did we isolate?

Figure 1. Flowchart of the mCURE background and experimental design. Using this flowchart, students are guided through the scientific process to gain an understanding of the relevance and importance of the project. Various segments of the course are color-coded (grey, orange, green, blue and yellow) corresponding to Table 1 where the week-by-week activities for each of these segments are described. This flowchart may be modified as needed to suit alternative projects using a similar protocol.

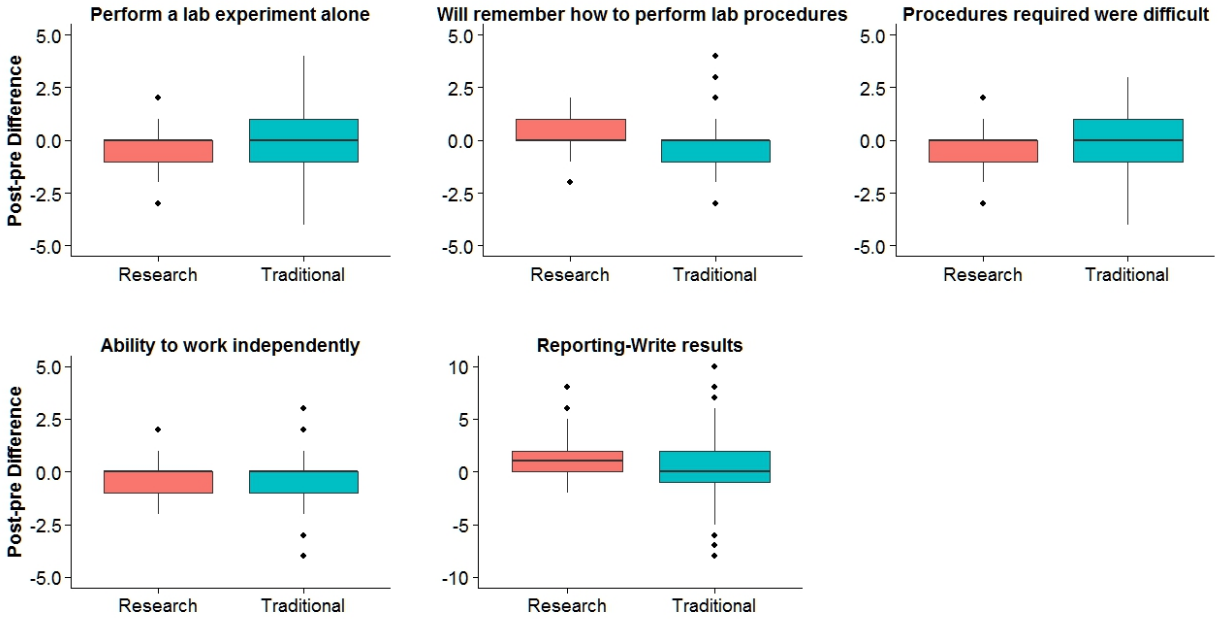

Figure 2. Survey results for questions with a significant difference (p-value ≤ 0.05) between research (mCURE) and traditional lab student pre/post perceptions. Note that the y-axis for "Reporting- Write results" is on a different scale than the other graphs.

Supplemental Materials

Supplemental Table 1. Example of a real implementation schedule of the idealized template in Table 1.

Appendix 1. Inoculation Protocol

Appendix 2. Transfer and Cryostock Protocol

Appendix 3. DNA Extraction Protocol

Appendix 4. BLAST How-to Guide

Appendix 5. Reading Guide

Appendix 6. Ordering a Scientific Paper

Appendix 7. BLAST Behind the Scenes

Appendix 8. Informal Writing 1

Appendix 9. Informal Writing 2

Appendix 10. Formal Writing 1

Appendix 11. Formal Writing 2

Appendix 12. Sample Final Exam

Appendix 13. Survey Instrument

Appendix 14. Example Student Assignments

Appendix 15. All Survey Instrument Data