Curriculum

The CURE for Cultivating Fastidious Microbes

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ABSTRACT

Course-based Undergraduate Research Experiences (CUREs) expand the scientific educational benefits of research to large groups of students in a course setting. As part of an ongoing effort to integrate CUREs into first-year biology labs, we developed a microbiology CURE (mCURE) that uses a modified dilution-to-extinction high throughput culturing protocol for isolating abundant yet fastidious aquatic bacterioplankton during one semester. Students learn common molecular biology techniques like nucleic acid extraction, PCR, and molecular characterization; read and evaluate scientific literature; and receive training in 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. 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.

30 **INTRODUCTION**

31 Undergraduate research experiences in STEM increase student retention in science majors;
32 increase the proportion of students that go on to professional or graduate school; as well as
33 improve critical thinking skills, data interpretation skills, content knowledge, and attitudes
34 toward science (1-5). Typical undergraduate research experiences are limited to relatively few
35 students due to research lab size and funding, making these positions competitive, highly
36 selective, and typically dominated by upper-level students (4, 5). Course-based undergraduate
37 research experiences (CUREs), in which students experience research as part of a course, can
38 reach students early in their degree program and accommodate large numbers of students, thus
39 increasing the diversity of students participating in research (4, 5). Despite these benefits, the
40 time necessary to plan CURE projects and create assignments and rubrics can restrict their use
41 (6). Fortunately, an increasing number of publications have shared CURE implementation
42 strategies for a variety of settings (3, 7-9). We recently outlined a flexible, modular CURE
43 framework, including rubrics and course materials, that has facilitated conducting a variety of
44 different research projects in first-year biology laboratory courses at Louisiana State University
45 (LSU) (10). Using this framework, we have developed the microbiology CURE (mCURE)
46 described herein that focuses on the cultivation of bacterioplankton from aquatic systems (**Fig.**
47 **1**).

48

49 Bacterioplankton occupy marine and freshwater environments at cell concentrations typically
50 between 10^5-10^7 cells mL^{-1}, however traditional agar plate methods usually only cultivate 0.1-1%
51 of the organisms present in a given sample (11), hampering our ability to understand the
52 functions of a large majority of microorganisms. An improved high-throughput cultivation

53 (HTC) method combines serial dilution of samples with sterilized natural water and/or artificial
54 seawater media (12-14). Many abundant taxa in aquatic systems have been successfully cultured
55 using this approach, for example SAR11 *Alphaproteobacteria* (15-18), SUP05
56 *Gammaproteobacteria* (19), SAR116 *Alphaproteobacteria* (12, 20), and members of the so-
57 called "Oligotrophic Marine *Gammaproteobacteria*" (21). Artificial media facilitates more
58 general application and modification (e.g., in salinity, carbon and nitrogen sources, etc.) to
59 accommodate different environments, as well as the adaptation of the protocol to teaching
60 laboratories. In the following mCURE, students execute a modified version of the HTC protocol
61 utilized by the Thrash Laboratory at LSU (14, 22). The possibility of isolating new organisms
62 provides a charismatic entrance into biological research where students experience a genuine
63 excitement of discovery combined with their laboratory and communication training.
64
65 ***Intended audience***
66 This course teaches basic laboratory skills and molecular biology methods, such as DNA
67 extraction and PCR, in the context of advanced microbial cultivation approaches and introduces
68 students to identification of microorganisms with molecular techniques. The curriculum also
69 includes exercises in reading and understanding primary literature and communicating science to
70 different audiences. The course is intended for undergraduates at the first- or second- year level
71 who are pursuing majors such as Biology and Microbiology.
72
73 ***Learning time***
74 We designed the mCURE for a semester timeline with a single three-hour laboratory section
75 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 HTC protocol (22). 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 cultures and amplify the 16S rRNA gene with PCR. Amplified products are then sequenced for subsequent taxonomic identification of the microbes in week #10 (***yellow***). The remaining weeks (#11-13) are spent discussing poster construction and administering the final assessments. Note that the entire workflow does not require 13 weeks, but we have built in flexibility to allow for repeating one or more elements in case of failure.

Prerequisite student knowledge

Students are required to have basic prerequisite training and proficiency in biosafety level 1 (BSL1) organisms and safety practices (23). No other prerequisites are required, however, high school biology and chemistry are recommended. Students receive training in many of the basic biology skills that they will utilize in other contexts and receive training in biosafety level 2 (BSL2) protocols (see Safety Issues, below).

Learning Outcomes

In addition to the learning objectives outlined below, the format of the mCURE sections incorporate aspects of three high-impact practices: Undergraduate Research, Collaborative Assignments, and Writing Intensive (24).

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

99 1. Properly handle and isolate microorganisms using serial dilutions with the HTC protocol;
100 2. Extract DNA and amplify 16S rRNA genes from pure cultures;
101 3. Use databases such as BLAST to identify unknown microorganisms;
102 4. Describe the relationship between the research objectives, the HTC approach, and the
103 experimental design;
104 5. Read and interpret relevant articles from the primary literature;
105 6. Communicate the methods, results, and implications of their research to both scientific
106 and non-scientific audiences.
107

108 **PROCEDURE**

109 A summary of the basic approach for the mCURE is shown in **Figure 1**, along with a week-by-
110 week breakdown of activities, materials, and prep notes in **Table 1**.
111

112 ***Materials***

113 The required equipment and chemicals have been previously published (22). Briefly, because
114 most highly abundant aquatic microorganisms have oligotrophic lifestyles, occur in low cell
115 densities (< 10^7 cells/mL), are very small (< 1µm), and will not grow on solid media, the
116 cultivation approach makes use of liquid media, and cell growth is measured using a benchtop
117 flow cytometer (e.g., the Millipore Guava easyCyte). The primary marine medium recipe,
118 MWH1, and our flow cytometer settings, are provided in **Appendices 1** and **2**, respectively.
119 Alternative media recipes and preparation instructions are available elsewhere (14, 18). To avoid
120 trace-metal contamination, all reusable cultivation vessels are made of polycarbonate plastic and
121 acid-washed in 10% HCl. Other major items include a thermocycler and PCR reagents,

122 electrophoresis equipment and a gel viewing system (e.g., Bio-Rad Gel Doc), a DNA
123 quantification system (e.g. Qubit, ThermoFisher), DNA extraction kits (MoBio PowerWater),
124 pipettes/tips, and incubators. The only differences in the established protocol equipment (22) for
125 the mCURE sections are the requirement for a biosafety cabinet and disposable 2.1 mL 96-well
126 plates (Thermo Nunc A/S). For those without access to some or most of this equipment, we
127 provide alternatives in the Discussion.
128
129 ***Student instructions***
130 Segment 1 (*orange* in **Figure 1, Table 1**): During the first two weeks of class, students are
131 introduced to the overall mCURE approach, pipetting, and trained in BSL2 safety protocols.
132 Each group of two students then dilutes their sample and inoculates seven wells of a 96-well
133 plate (**Appendix 3**) containing the medium. An eighth well is inoculated with sterile media as a
134 contamination control. Thus, a 24-student section initiates culturing in a 96-well plate. The plate
135 is incubated at *in situ* temperature (based on time/place of sampling) for 2-3 weeks and then
136 checked for growth using flow cytometry. During the incubation weeks, student assignments
137 focus on introducing effective reading of scientific literature and on the experimental design and
138 its rationale (**Table 1**).
139
140 Segment 2 (*green*): Each group selects 1-2 positive cultures (wells with > 10^4 cells/mL) for
141 transfer into larger volume growth flasks and creates cryostocks for culture preservation in 10%
142 DMSO (**Appendix 4**). In our experience, most groups usually have at least one positive well to
143 transfer. Those groups with no growth in any of their wells select an unused positive well from
144 another group. Inoculated flasks are incubated for two weeks at the same temperature as before.

145 During the interim, students are introduced to scientific writing and give "lightning talks" (**Table**
146 **1**).
147
148 Segment 3 (*blue*): Groups select at least one flask that shows growth and extract DNA
149 (**Appendix 5**). In the three mCURE semesters detailed here, the majority of groups in any given
150 section observed growth in at least one flask. Groups with no growth in any of their flasks use
151 part of another group's culture for extraction. Note that this introduces redundancy in the final
152 identification results. Over the next two weeks, students amplify the 16S rRNA genes from their
153 extracted DNA using PCR (**Appendix 6**) and confirm the amplification product with gel
154 electrophoresis. Successful amplicons are then sequenced (possibly off-campus, e.g., the RTSF
155 Genomics Core at Michigan State University).
156
157 Segment 4 (*yellow*): Students learn to assemble forward and reverse sequence reads into a contig
158 and identify their isolate using the NCBI BLASTN portal (**Appendix 7**). Briefly, reads from both
159 the forward and reverse primer, as well as the overlapping contig (if any), are searched against
160 the GenBank nt database with and without the exclusion of uncultured/environmental samples.
161 The % identity, Query coverage, E-value, and GenBank # for the top five BLAST hits are
162 recorded for all searches and isolates. Interpretation and contextualization of the results,
163 including the similarity of isolates generated by the students to those in the database, occurs via
164 discussion with knowledgeable faculty/teaching assistants. These results become part of their
165 final poster presentation.
166
167 ***Faculty instructions***

168 Segment 1 (*grey*, *orange* in **Figure 1, Table 1**): Prior to the beginning of the course, instructors
169 must prepare the following:
170 1) Collect seawater (≥ 1L) and measure the concentration of bacterioplankton using flow
171 cytometry (**Appendix 2**). The students use this initial concentration to calculate the
172 dilution factor required to inoculate ~1-5 cells per well. Collection should occur as
173 proximately to inoculation as possible to avoid microbial community change via bottle
174 effects.
175 2) Prepare the low-nutrient media (**Appendix 1**; ~200 mL per plate; 1 plate/12 groups).
176 Aliquot ~1.7 mL of media into each well of the 96-well plate just before class and allow
177 time for equilibration to incubation temperature.
178 3) Select ~12-15 scientific articles (examples in **Appendix 8**) relevant to the project and
179 create a reading guide for one of them for class discussion (sample: **Appendix 9** for
180 (12)). The students may select one of the remaining papers for their lightning talks
181 (**Table 1**, weeks #4-5), and use them as references for their formal writing assignments.
182
183 Because of the incubation period (2-3 weeks) for the initial inoculations, we recommend that
184 Segment 1 involve at least one "holiday week" (**Table S1**). At the end of the incubation period,
185 instructors count cells in the 96-well plate and record the well numbers positive for growth.
186 Since isolates will be unknown at this time, transfers from incubation plates to counting plates
187 (22) should be completed in a biosafety cabinet.
188
189 Segment 2 (*green*): Prior to the start of this segment, instructors must prepare more medium,
190 aliquot 50 mL into 125 mL flasks, and prepare cryotubes with DMSO. Prepare as many flasks

191 and cryotubes as the number of wells that show growth (with some extra on hand in case of
192 spillage). Students should have access to a biosafety cabinet in which to handle all cultures. At
193 the end of the 2-week incubation, instructors count flasks to determine growth and record cell
194 concentrations for student use. For the scientific writing discussion, we have made an activity
195 (**Appendix 10**) that familiarizes students with the content in various sections of a paper (12).
196
197 Segment 3 (*blue*): We recommend that instructors aliquot the required amount of DNA
198 extraction reagents (**Appendix 5**- Power Water DNA Isolation Kit; Mo Bio Laboratories) and
199 PCR reagents (**Appendix 6**- Taq, MgCl2, and buffer- ThermoFisher; 10mM AMRESCO dNTPs-
200 VWR Life Sciences; 27F/1492R primers) for each group to prevent cross-contamination. For gel
201 electrophoresis, gels are made with 1.5% agarose in DI MilliQ-filtered water. We suggest
202 making an appropriate amount of agarose in a flask for each section, and allowing it to solidify
203 until class time. Then, prior to the start of class, the instructor can melt the agarose in the flask
204 and have it ready for students to pour their own gels. We recommend gels contain enough wells
205 that each student has 1-2 wells to practice loading sample dye before loading their PCR product
206 into one of the remaining wells. Students combine 1 µL loading dye with 5 µL PCR products for
207 imaging. We typically employ a Lambda or 1 kb ladder. Gels are stained with SYBR green (1x)
208 and imaged using the Bio-Rad Gel Doc.
209
210 Segment 4 (*yellow*): Before the BLAST lab, instructors need to have all successful 16S rRNA
211 gene amplicons sequenced from a facility of their choice using both forward and reverse primers
212 (we use 27F and 1492R, but this can be specified by the instructor- see (25) for additional
213 options); the resulting sequences should be made available where the students can access them.

214 Label each sequence with the sample number and whether it is a "forward" or a "reverse" read.
215 We recommend the "BLAST behind the scenes" activity (**Appendix 11**) to introduce students to
216 the concept of sequence analysis. We have included the relevant lecture materials on molecular
217 characterization (**Appendix 12**) to aid the instructor. Briefly, we introduce PCR, the importance
218 of primers in PCR, describe the presence of conserved sequences flanking the hypervariable
219 regions within 16S rRNA genes, and how the primers must be designed to recognize the
220 conserved portion of the rRNA genes and amplify the hypervariable region they flank. We then
221 discuss how Sanger sequencing can be used to read the DNA code and compared to other
222 previously sequenced organisms using BLAST.
223
224 Finally, instructors need to prepare for a poster session at the end of the semester, including
225 organizing space for poster boards, display tables, and printing facilities. However, for grading
226 purposes, we recommend that the student groups present their posters electronically in class.
227 During this time, other students and the instructor can offer constructive criticism for the
228 students to incorporate into the final printed version of the poster.
229
230 Based on our experience implementing this mCURE for several semesters, we anticipate at least
231 1-2 protocol failures per semester; hence, flexibility is built into the framework (**Tables 1**, **S1**).
232 Despite our anticipation of some failures and correcting these in subsequent semesters (e.g.,
233 students failing to properly transfer and freeze their samples), each new semester has presented
234 us with new and different failures (e.g., flow cytometer reagents on back-order, failed PCRs due
235 to old reagents). Many non-experimental activities, such as the lightning talks, can be easily
236 inserted at different points in the course, amended to take less time, or even completely

237 eliminated. Similarly, other related activities may be added, such as peer review of initial formal
238 writing drafts and using social media for science outreach (*e.g.* we use the Twitter and Instagram
239 hashtag #LSUCURE for all CURE efforts in the Department of Biological Sciences at LSU;
240 **Table S1**). If feasible, we recommend adding the following enhancements to further engage
241 students in the course: (i) taking students on a field trip, such as a one-day research cruise to
242 collect water samples; (ii) demonstrating the use of "behind the scenes" equipment, such as the
243 flow cytometer, capillary sequencer, and/or modern microscopes used to image bacteria.
244
245 ***Suggestions for determining student learning***
246 The mCURE is an authentic research experience and therefore one important component is
247 communication of student findings for both scientific and non-scientific communities. Thus,
248 assessment of student learning is largely split between the students successfully completing the
249 protocols and the final poster presentation (**Table 2**). In order to complete the entire project,
250 students need to be able culture bacterioplankton with the HTC protocol, passage cultures to
251 larger volumes, extract DNA from these cultures, then successfully amplify and identify 16S
252 rRNA gene sequences. The final poster and presentation requires students to state the aims of the
253 project within the larger context of what is currently known about bacterioplankton in marine
254 environments, outline the basic methodologies used, clearly present their results, and discuss
255 these results in the context of their research question. Finally, the students suggest the next
256 logical question to explore. Each of the laboratory and communication elements has multiple
257 forms of evaluation (**Table 2** and **Appendices**).
258
259 ***Sample data***

260 Fall 2015, spring 2016, and fall 2016 average cultivability (13) was 9.9, 2.8, and 12%,
261 respectively. These cultivability numbers generally match the success rate of other HTC
262 experiments (14) and demonstrate a significant improvement over "traditional" methods (11).
263 The number of unique pure cultures that survived successive transfers and were positively
264 identified at the end of each course was 28 (fall 2015), 13 (spring 2016), and 23 (fall 2016). In
265 total, mCURE sections isolated 43 unique bacterioplankton during the first three semesters
266 reported herein. Some courses isolated taxa identified in a previous mCURE, so the overall total
267 was smaller than the sum of the individual semesters. Many of the isolates have close
268 relationships to organisms previously cultured using HTC in the Thrash lab and other labs, as
269 indicated by taxonomic affiliations to strains with "LSUCC", "HTCC", "HIMB", or "IMCC"
270 designations (**Table 3**). Importantly, many isolates represent abundant marine clades (14), thus
271 the results validate the mCURE approach to produce valuable cultures with similar efficacy as
272 HTC experiments conducted under more typical laboratory settings. Additional results are
273 provided in **Appendix 13**.
274
275 ***Safety issues***
276 Since the curriculum involves isolating unknown organisms, students must be proficient in BSL1
277 safety techniques prior to taking the course. All activities that involve handling live
278 microorganisms should occur under BSL2 safety protocols, as outlined by the JMBE Biosafety
279 Guidelines for Handling Microorganisms in the Teaching Laboratory (23). The specific activities
280 requiring BSL2 protocols are indicated in Table 1. Additional safety measures must be taken for
281 faculty during washing and preparation of medium mixture bottles and growth flasks. See (22)
282 for more details.

283

284 **DISCUSSION**

285

286 ***Field testing***

287 Here we report results from mCURE sections offered during the fall 2015 and 2016 semesters in
288 Biology 1207 (Honors: Biology Laboratory for Science Majors) and spring 2016 in Biology
289 1208 (Biology Laboratory for Science Majors I). There were four sections per semester taught by
290 two graduate teaching assistants (two sections each), with up to 28 students per section. Biology
291 1207 is only offered in the fall semester and consists of a total of four sections. Multiple (12-50)
292 sections of Biology 1208 are offered every semester, a few of which are typically offered as
293 CUREs as outlined in our previous publication (10); students do not know when they register for
294 this course if their section will be in a CURE or traditional format. We note that these previous
295 sections of the mCURE were conducted with a BSL1 safety protocol. The current protocol
296 offered in this manuscript has been updated with BSL2 safety measures in response to
297 recommendations by ASM (23). In each of these sections, some fraction of student groups (pairs)
298 were capable of successfully implementing the protocols from start to finish, while others had
299 failures that required they use cultures, DNA, or PCR products from other groups. In general, we
300 found that roughly a third of the groups could successfully complete the entire workflow
301 (however, failure at any given step did not preclude students from progressing to the next step,
302 albeit with successful cultures from a different group). This represents only one of the learning
303 outcomes. Other learning outcomes (**Table 2**) could be achieved regardless of students
304 experiencing failure at different stages (detailed below).

305

306 ***Evidence of student learning***

307 We provide evidence of student learning with example summative assessment of grade
308 distributions (**Fig. 2**), physical data (PCR products- **Fig. 3**), qualitative results of successfully
309 completed bacterioplankton isolation (**Table 3**), and examples of the range of student
310 communication outcomes (**Table 4**, **Appendix 22**).

311

312 **Figure 2** details the grade distributions across two sections from each semester during the 2015-
313 2016 school year, composed of students with differing levels of academic preparation. The fall
314 2015 sections consisted of Honors College students majoring in biology, many of whom were
315 already familiar with basic laboratory techniques. These students did not perform the original
316 dilution of the seawater before inoculation. This class generally performed well on quizzes,
317 which tested their proficiency in one or two of the major topics covered in the prior week of the
318 course. Nearly the entire class received a grade of either A or B on the cumulative final exam
319 (**Appendix 18**, **Fig. 2**). In spring 2016, we offered the mCURE in BIOL 1208R. Spring is the
320 "off" semester for this course such that students enrolled in it usually are not biology majors or
321 experienced some barrier to their enrollment or completion of the course in the preceding fall
322 semester. This semester we asked students to perform their own seawater dilution. Many
323 students found this difficult, as reflected in the Q1 and Q2 scores (**Fig. 2**). However, we note that
324 by the final exam most students were proficient in these calculations. At the end of the semester,
325 ~75% of the class received a passing grade (A-C) on the final exam, which is typical for the
326 traditional lab sections during the spring semester of this course.

327

328 In addition to demonstrating their knowledge on summative assessments, students became
329 proficient in laboratory techniques (Learning Outcomes 1-2) as evidenced by the vast majority of
330 student groups in both semesters who successfully extracted DNA from cultures and performed
331 PCR (e.g., **Fig. 3**). By the end of the semester, students were expected to understand and
332 interpret primary literature related to their research and describe their cultured microbe in the
333 final poster. Thus, the posters partially address Learning Outcomes 3-6, with other writing
334 assignments providing additional training (**Table 2**). **Table 4** provides excerpts from student
335 posters describing their isolated organism. The top performing students included detailed
336 description of scientific literature related to their organism and proposed future experiments to
337 expand our knowledge about their isolate. Their writing was concise while including all
338 important and relevant details and showed a thorough understanding of the experimental design.
339 We provide examples of formal writing assignment 2, lightning talks, and student posters in
340 **Appendix 22** (shared with permission from the students).
341
342 ***Possible modifications***
343 We appreciate that many instructors may wish to implement the mCURE design but may not
344 have access to some of the more expensive equipment used in our protocol. Here are a few
345 modifications to circumvent some of these restrictions. Instructors can replace flow cytometry
346 with direct microscopic counts, e.g., as in some of the earlier iterations of the HTC protocol (12).
347 For those without access to either a flow cytometer or a fluorescence microscope, the protocol
348 can still be completed using traditional agar-plate based methods. Our media can be prepared
349 with agar (22) or replaced with a classic marine medium like Difco 2216 (BD). Although solid
350 media generally select for different taxa than liquid media, for the purposes of a basic biology

351 laboratory, this may not matter. After streaking a seawater sample on plates, individual colonies
352 can be picked, grown up in liquid culture to increase cellular mass, or directly processed through
353 DNA extraction. Colony PCR (26) may also be an attractive alternative identification method,
354 particularly because this also eliminates the time and cost associated with DNA extraction. These
355 last two steps may also help adapt the overall protocol for shorter time frames, e.g., academic
356 quarters instead of semesters. Please note that our protocol uses low-nutrient and low-carbon
357 media that typically selects for non-pathogenic, oligotrophic marine bacterioplankton (14). The
358 use of rich media and plate-based methods may increase the risk of cultivating pathogenic
359 organisms. Finally, for those interested in freshwater environments, the same protocol can be
360 conducted with freshwater media, either artificial (18, 27) or natural (28).
361

362 **SUPPLEMENTAL MATERIALS**

363 **Table S1.** Example of a real implementation schedule of the idealized template in Table 1.
364 **Appendix 1.** MWH1 marine medium recipe
365 **Appendix 2.** Flow cytometry parameters
366 **Appendix 3.** Inoculation Protocol
367 **Appendix 4.** Transfer and Cryostock Protocol
368 **Appendix 5.** DNA Extraction Protocol
369 **Appendix 6.** PCR protocol
370 **Appendix 7.** BLAST How-to Guide
371 **Appendix 8.** Suggested literature
372 **Appendix 9.** Reading Guide
373 **Appendix 10.** Ordering a Scientific Paper
374 **Appendix 11.** BLAST Behind the Scenes
375 **Appendix 12.** Molecular biology lectures (as PowerPoint slides). If readers would like assistance
376 in developing other PowerPoints such as these, please contact the lead author.
377 **Appendix 13.** Supplemental results

Appendix 14. Informal Writing 1

Appendix 15. Informal Writing 2

Appendix 16. Formal Writing 1

Appendix 17. Formal Writing 2

Appendix 18. Sample Final Exam

Appendix 19. Lightning talk rubric

Appendix 20. Quizzes

Appendix 21. Poster rubric

Appendix 22. Example Student Assignments

Appendix 23. Example lightning talk and instructions (as PowerPoint slides).

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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; "Lightning 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 rRNA gene 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[4] 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 [5]		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] Students are required to find primary literature to include with this assignment

[5] 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

504 **Table 2. Determination of Student Learning**

Learning Outcome (artifact)	Assessment Method(s)*
1. Properly handle and isolate microorganisms using serial dilutions with the HTC protocol (isolated organisms).	Informal Writing 1 (Appendix 14), Formal Writing 1 (Appendix 16), successful completion of the protocols, results presented in the final poster (Appendix 21).
2. Extract DNA and amplify 16S rRNA genes from pure cultures (16S rRNA gene amplicons).	Informal Writing 2 (Appendix 15), Formal Writing 2 (Appendix 17), successful completion of the protocols, results presented in the final poster (Appendix 21).
3. Use databases such as BLAST to identify unknown microorganisms (taxonomic identity).	Formal Writing 2 (Appendix 17), successful completion of the protocols, results presented in the final poster (Appendix 21).
4. Describe the relationship between the research objectives, the HTC approach, and the experimental design.	Formal Writing 2 (Appendix 17), final poster (Appendix 21).
5. Read and interpret relevant articles from the primary literature.	Lighting Talks (Appendix 19), Formal Writing 2 (Appendices 17), final poster (Appendix 21).
6. Communicate the methods, results, and implications of their research to both scientific and non-scientific audiences (poster).	Lighting Talks (Appendix 19), final poster (Appendix 21).

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506 *Rubrics for both the writing assignments have been published previously (10).

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508 **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

511 **Table 4. Excerpts from students' posters describing the bacteria they cultured.** Students
512 were expected to identify and describe major points of interest regarding the bacteria they
513 cultured, supported by scientific literature references, relate that information back to the
514 experimental design, and identify a future direction for their work. Minor spelling and
515 grammatical errors have been fixed when reformatting the excerpts to fit the format of this table.

	Excerpts about the cultured organisms from students' posters
Excellent	*Pseudoalteromas phenolica* was originally found in 2003 by Alim Isnansetyo and Yuto Kamei in the waters near the islands of Japan. Species in the genus *Pseudoalteromas* are typically heterotrophic but [some may] be oligotrophic, which is what our experiment is designed to culture. . . . The most significant attribute of this organism, though, is that it produces anti-methicillin-resistant *Staphylococcus aureus* (MRSA) substances (Isnansetyo and Kamei 2003). . . . Because this species, *Pseudoalteromas phenolica*, produces anti-MRSA substances, more focus should be put on how effective these substances are against *Staphylococcus aureus*. Experiments should be done to see if this species can be grown easily in large quantities to be produce [the] antibiotic. *Several interesting attributes of the cultured bacteria and major points of significance are explained in detail with proper citations; future directions identified, and information related back to the experiment students conducted; demonstrates thorough understanding of experimental design.*
Good/ Acceptable	*Pseudoalteromonas phenolica*, found from B5-1, is significant because it can be used to treat MRSA, a bacterium that can cause skin infections, infected wounds and even pneumonia, that has resistance to many known antibiotics. It could possibly be used in a pharmaceutical product to treat illnesses caused by MRSA in the future. [In the future, we could] use the cryostocks to culture the organism . . . to confirm its identity . . . and attempt to find if our strain has anti-MRSA properties. *Organism's important attribute of scientific interest identified and its significance described but not cited; future directions identified, and information related back to the experiment students conducted; demonstrates thorough understanding of the experimental design.*
Needs Improvement	[*Pseudoalteromonas phenolica*] was first cultured in a lab near Tokyo, Japan in 2003. Strains are currently being researched for their antibiotic properties on anti-methicillin-resistant *Staphylococcus aureus*. 4 out of 11 groups at LSU cultured a *P. phenolica* showing that it is abundant in the Gulf of Mexico and readily grows through HTC. [Future directions include] identifying biological markers, studying its contributions to the ecosystem, and finding industrial, medical, and pharmaceutical applications. *Organism briefly described and important attributes mentioned without*

	expanding upon their significance or proper citations; future directions identified, but information not related back to the experiment conducted; demonstrates incorrect understanding of the experiment conducted (several students that semester characterized P. phenolica because not many cultures were initially successful, thus a few groups had to share the same initial broth cultures for the molecular analysis steps).

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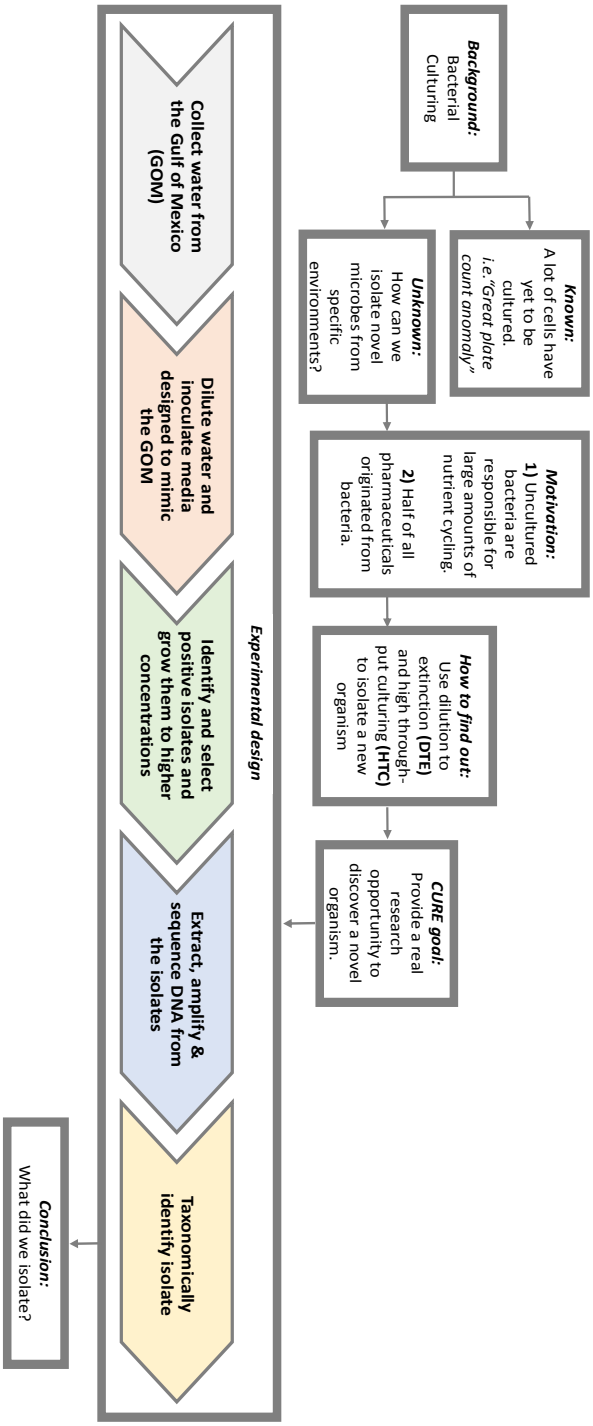

Figure 1. Flowchart of the mCURE background and experimental design. Using this flowchart, students are guided through the scientific process to gain an understanding of the relevance and importance of the project. Various segments of the course are color-coded (grey, orange, green, blue and yellow) 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.

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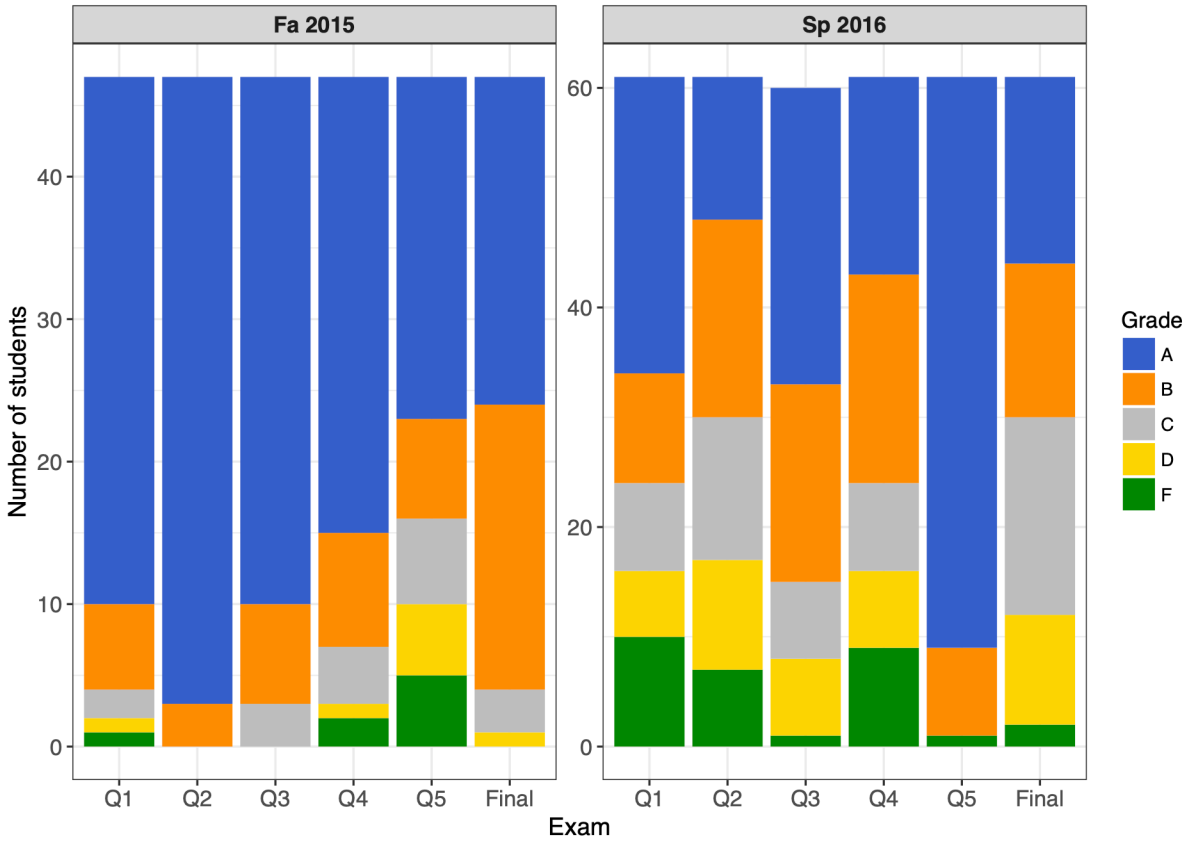

Figure 2. Grade distributions for two sections of mCURE students during each of two semesters in the 2015-2016 school year. Fall 2015 consisted of ~50 Honors College students majoring in biology. The topics for the five quizzes (Q1-Q5) were as follows: Q1 = Safety, Controls; Q2 = Experimental design, Scientific writing; Q3 = DNA extraction; Q4 = PCR; Q5 = Gel electrophoresis, Purpose of sequencing, Primer design. **Spring 2016** consisted of ~60 mostly non-biology major students. The topics for the five quizzes (Q1-Q5) were as follows: Q1 = Dilutions, Pipetting, Safety, Controls, Scientific writing; Q2 = Experimental design, Dilution, Pipetting, Controls; Q3 = DNA extraction; Q4 = PCR, Primer selection/design, Gel electrophoresis; Q5 = Purpose of sequencing, Sequence analysis. The grades for both semesters were assigned based on the following score criteria: A = 90-100%; B = 80-90%; C = 70-80%; D = 60-70%; F = <60%.

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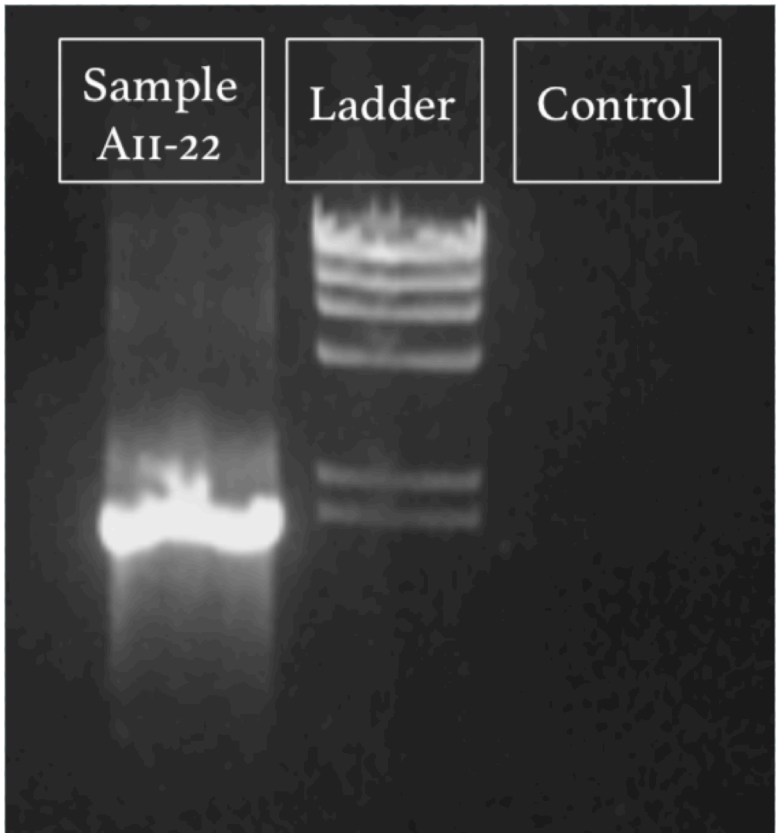

Figure 3. Example gel electrophoresis image of a successful 16S rRNA gene PCR amplification from fall 2015. Lanes labeled according to contents: "Sample A11-22" is the amplicon from isolate DNA (expected size 1466 bp); "Ladder" is Lambda HindIII digest ladder (NEB N3012S), with the lowest visible band at 2027 bp; "Control" is the negative control (water).