Supplemental Materials
for
Classroom Activities to Engage Students and Promote Critical Thinking about Genetic Regulation of Bacterial Quorum Sensing

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Appendix 1: Sample exam or quiz questions for instructors.

1. Define quorum sensing in one sentence.

2. List two examples of biological systems that incorporate bioluminescence.

3. Please match the following:

   ____ luciferase
   ____ regulator
   ____ autoinducer
   ____ luciferin

   a. A transcription factor, which first binds the autoinducer, and then to the _lux_ operon to initiate transcription.
   b. A small signal molecule that bacteria produce and exchange as a way of chemical communication.
   c. The complex that produces the aldehyde substrate, which is acted on to produce light.
   d. The enzyme that interacts with an aldehyde, FMH₂, and oxygen to produce light.

4. You are researching the _lux_ operon using genetic complementation. You have two strains of the bioluminescent bacteria _V. fischeri_. Strain 1 has a mutation in _luxAB_ that causes a defective luciferase enzyme. Strain 2 has a mutation in _luxR_ that causes a defective receptor protein. If you grow them on the same petri dish, what phenotype would you expect?

   a. Only Strain 2 will bioluminescence
   b. Only Strain 1 will bioluminescence
   c. Both strains will bioluminescence
   d. Neither strain will bioluminescence

5. You are researching the _lux_ operon using genetic complementation. You have two strains of the bioluminescent bacteria. Strain 1 has a loss-of-function mutation in _luxI_, and strain 2 is a wild type strain. If you grow them on the same petri dish, what phenotype would you expect?

   A. Only Strain 2 will bioluminescence
   B. Only Strain 1 will bioluminescence
   C. Both strains will bioluminescence
   D. Neither strain will bioluminescence

6. Which of the following would prevent bioluminescence from occurring?

   A. Overexpression of the _luxA_ and _luxB_ genes
   B. Cultivating bioluminescent bacteria under anaerobic conditions
   C. A point-mutation in the binding site of _luxR_ that prevents binding of AHLs
   D. Cultivating bioluminescent bacteria under aerobic conditions
   E. A and B
   F. B and C
   G. C and D

Answer Key: (1) density-dependent regulation of gene expression, answers may vary (2) angler fish, _Euprymna scolopes_ (bobtail squid), fireflies, answers may vary; (3) D, A, B, C; (4) D; (5) C; (6) F
Appendix 2: Genetic complementation worksheet.

The wild-type strain has a functional $\text{lux}$ operon and can produce bioluminescence. Strain A is lacking the $\text{luxR}$ gene. Strain B is lacking the $\text{luxI}$ gene. Strain C is lacking the $\text{luxR}$ gene, but has a promoter mutation that causes the $\text{lux}$ operon to be constitutively ON.

Problem 1. Draw what you predict would happen if we streaked the WT strain against Strain A. (Would we see luminescence? If so, where?)

Problem 2. Draw what you predict would happen if we streaked the WT strain against Strain B. (Would we see luminescence? If so, where?)

Problem 3. Draw what you predict would happen if we streaked the WT strain against Strain C. (Would we see luminescence? If so, where?)

Problem 4. Draw what you predict would happen if we streaked the Strain A against Strain B. (Would we see luminescence? If so, where?)
Answer Key: Genetic complementation worksheet

Problem 1: WT produces the autoinducer (AI), so its neighbors will detect it, and it can detect the AI produced by Strain A, so it will produce light. Strain A can’t detect any AI (not those from its neighbors or the WT) so it will not luminesce.

Problem 2: The WT produces AI, so its neighbors will detect it, and it can detect the AI produced by Strain A. Strain B can detect the AI produced by the WT, even though it cannot produce its own. Both will luminesce.

Problem 3: Strain C can’t detect AI due to the mutation in luxR. However, the Strain C lux operon is constitutively on, so it will still produce AI and transcribe the lux operon. WT can detect its own AI as well as the AI produced by C. Both will luminesce.

Problem 4: Strain B cannot produce AI, but it can detect and respond to the AI produced by strain A. Strain B will luminesce. Strain A can produce AI, but since it doesn’t have luxR, it cannot up-regulate the lux operon and thus cannot luminesce.
*Note: students may point out, correctly, that for some of the strains there may not be a uniform level of bioluminescence. For example, in problem #2, strain B may only bioluminesce where it’s nearest to strain A (basically, only where the AI from strain A has diffused into the strain B side of the plate).