Biotechnology

Dress Code		Attire appropriate to the occupational area
SLC Orientation		Event explained to the competitors and individual time cards handed out. Students will return to the event room at least 5 minutes before their allotted time. Students will have a secret scenario to solve when it is their turn to compete. Students should not share the secret scenario when they leave the event as it is an automatic disqualification.
Round # 1		Competitors must submit technical skill video to Montana HOSA by deadline. Additionally, competitors will take an online test during the testing window. Advisors will be informed of which competitors have moved on from Round 1 to qualify to participate in Round 2 at SLC.
Round # 2		Skill procedures from rubric guidelines will be performed by each competitor. Competitors may be asked to do one or multiple of the performance skills.
Scoring		Round 1 online testing scores will be combined with Round 2 skill procedures for a total score.

Official References

The below references are used in the development of the test questions and skill rating sheets. Bio-Rad has provided additional resources to support competitors in event preparation.

a. Brown, J. Kirk. *Biotechnology A Laboratory Skills Course*. Bio-Rad. Latest edition.
b. Starr and Taggart. *Biology: The Unity and Diversity of Life AP*. National Geographic Learning. Cengage. Latest edition.
c. Biotechnology Careers
d. Bio-Rad Resources

Round One Test

Test Instructions: The written test will consist of 50 multiple-choice items in a maximum of 60 minutes.

Written Test Plan

The test plan for Biotechnology is:

- Biotechnology industry practices and careers - 4%
- Biotechnology in health - 4%
- Governmental regulation of biotechnology - 4%
- Basic laboratory skills - 14%
  - PPE
  - Preparing solutions (calculations, use of balance and other equipment)
  - Pipetting
- Microbiology and cell culture - 12%
- DNA structure and analysis - 14%
- Bacterial transformation - 10%
- Polymerase chain reaction (PCR) - 14%

- Protein structure, function, and analysis - 14%
- Immunological applications - 10%

Basic handheld calculators (no graphing calculators) for addition, subtraction, division, multiplication, and square root calculations are allowed for the written test.

Sample Round One Test Questions

1. What type of bond connects nitrogenous base pairs and holds the two strands of a DNA molecule together? (Bio-Rad pg 114)
   A. **Hydrogen**
   B. Nitrogenous
   C. Oxygen
   D. Carbon

2. Which discipline of systems biology investigates the full complement of DNA in a cell? (Bio-Rad pg 5)
   A. Microbiomics
   B. Proteomics
   C. Genomics
   D. Metabolomics

3. What was the first bacterium used commercially to produce genetically engineered human insulin? (Starr pg 240)
   A. Saccharomyces
   B. E. coli
   C. Epstein-Barr
   D. Staphylococci

Round Two Skills

Round Two is the performance of a selected skill(s). The Round Two skills approved for this event are:

	Textbook (Bio-Rad)	**Time Allocated**	**Video Resource(s)**
Skill I: Using Micropipets, Transfer Pipets, and a Balance	pp. 50-53 (Part 3), 381, and 383 (use of balance)	15 min	Videos 1 and 2
Skill II: Restriction Digestion Reaction	p. 140 (Part 1)	15 min	none
Skill III: DNA Gel Electrophoresis	pp. 140-141 (Part 2) 391	20 min	Video
Skill IV: DNA Gel Interpretation	pp. 136-138, 142, 392	15 min	none
Skill V: Bradford Protein Quantitation Assay	pp. 254-255 (through step 10), 395	20 min	Video
Skill VI: Bacterial Transformation	pp. 167-171, 392	20 min	Video
Skill VII: Calculation of Transformation Efficiency	pp. 155-156, 393	10 min	none
Skill VIII: Qualitative ELISA	pp. 314-316, 400	20 min	Video

(FOR ALL SKILLS, ANY BODY FLUIDS WILL BE A SIMULATED PRODUCT)

The selected skill(s) will be presented to competitors as a written scenario at the beginning of the round. The scenario will be the same for each competitor. Where appropriate, scenarios will also provide protocols to those they could expect to see if working in an industry laboratory (these protocols will not be as detailed as those provided in the textbook; students are expected to know basic skills such as micropipet use, labeling of tubes, avoiding sample cross-contamination, lab hygiene, and basic

workflows). Some scenarios may involve the combination of multiple skill sheets, in which case some elements may not be scored due either to being duplicative or not appropriate within the scenario.
A specific Biotechnology sample scenario can be found HERE.

The scenario is a secret topic. Competitors MAY NOT discuss or reveal the secret topic until after the event has concluded or will face penalties per the GRRs.

Judges will provide information to competitors as directed by the rating sheets.-

Competitors must use all equipment correctly and safely. Judges will stop a student and not award points for a step if they see a competitor is about to cause a risk of harm or about to damage equipment or other supplies.

Selection of the correct micropipet for a specified volume is an essential skill. Though other micropipets exist (2, 10, or 100 µl, for example), the skills in this competitive event are to be performed using a 20, 20-200, or 100-1,000 µl micropipet (a p20, p200, or p1000).

The protocols for these skills follow protocols given in the Bio-Rad textbook, Biotechnology A Laboratory Skills Course (Brown JK). Note that, in skill VIII, the incubation times have been shortened to enable competitors to complete the skill and see color development within the allotted time.

Competitors must provide:

- Ruler (metric, w mm marks)
- Glasses, safety glasses, face shield or goggles
- Closed-toe shoes
- Disposable non-latex gloves
- Lab Coat (Optional)

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Though the 100-1,000 µl micropipet could be used for these steps, this skill specifies use of a 20-200 µl micropipet (p200).

Skill I: Using Micropipets, Transfer Pipets, and a Balance (Time: 15 minutes)	**Possible**	**Awarded**
1. Entered testing area wearing closed-toed shoes, then donned proper PPE: glasses/safety glasses/goggles, and gloves (lab coat is optional).	2 0	
2. Labeled 3 microcentrifuge tubes: for example, p200, p1000, and TP.	4 0	
3. Weighed each tube and recorded its mass on the paper provided. ***Judge verified competitor cleared the balance before weighing each tube.***	4 0	
Using the 20-200 µl micropipet[1]		
4. Pipetted 200 µl of the liquid provided to the tube labeled for p200 use. ***Judge verified competitor (i) selected 20-200 µl micropipet, (ii) set it to 200 µl, (iii) used an appropriate pipet tip, and (iv) transferred liquid without air bubbles or losing sample in the transfer process.***	4 0	
5. Repeated Step 4.	2 0	
6. Pipetted 100 µl of the liquid provided to the tube. Closed the tube tightly. ***Judge verified competitor (i) set the 20-200 µl micropipet to 100 µl, (ii) used a proper pipet tip, and (iii) transferred liquid without air bubbles or losing sample in the transfer process.***	4 0	
Using the 100-1,000 µl micropipet		
7. Pipetted 500 µl of the liquid provided to the tube labeled for p1000 use. Closed the tube tightly. ***Judge verified competitor (i) selected 100-1,000 µl micropipet, (ii) set it to 500 µl, (iii) used a proper pipet tip, and (iv) transferred liquid without air bubbles or losing sample in the transfer process.***	4 0	
Using the transfer pipet		
8. Transferred 500 µl of the liquid provided to the tube labeled for transfer pipet use. Closed the tube tightly. ***Judge verified competitor filled the transfer pipet to the appropriate line without air bubbles and transferred the entire volume to the tube.***	4 0	
Calculating mass of liquid		
9. Weighed all three microcentrifuge tubes and recorded mass of each on the paper provided. ***Judge verified competitor cleared the balance before weighing each tube.***	2 0	
10. Calculated the mass of the liquid in each tube (in g) and recorded it on the paper provided. ***Judge verified calculated mass and that the mass of liquid in each tube was ~0.50g (the mass of the liquid transferred using the transfer pipet may differ). Judge confirmed correct units (g) and number of significant figures were used (for example, 0.50 g).***	2 0	

[1]

Skill I: Using Micropipets, Transfer Pipets, and a Balance (cont.)	**Possible**	**Awarded**
Cleaning up		
11. Cleaned work area: a. Disposed of used pipet tips, microcentrifuge tubes, and transfer pipet in waste receptacle.	2 0	
b. Cleaned work area with surface disinfectant.	2 0	
c. Removed PPE.	2 0	
d. Washed hands or used alcohol-based hand-rub for hand hygiene. ***Judge verified steps 11 a-d were performed in the order written here.***	2 0	
TOTAL POINTS - SKILL I **70% Mastery for Skill I = 28.0**	**40**	

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Skill II: Set Up Restriction Digestion Reaction (Time: 15 minutes)	**Possible**	**Awarded**
1. Entered testing area wearing closed-toed shoes, then donned proper PPE: glasses/safety glasses/goggles, and gloves (lab coat is optional).	2 0	
2. Labeled 6 microcentrifuge tubes to match the labels on the DNA samples provided. ***Judge verified competitor labeled all 6 tubes correctly.***	2 0	
3. Pipetted 10 µl of each DNA sample into the corresponding labeled microcentrifuge tube. ***Judge verified competitor (i) used a 20 µl micropipet, (ii) set it to 10 µl, (iii) used an appropriate and clean pipet tip for the sample, and (iv) transferred liquid without air bubbles or losing sample in the transfer process.***	4 0	
4. Pipetted 10 µl of enzyme mix (ENZ) into each tube, then mixed by pipetting up and down 2-3 times. ***Judge verified competitor (i) used a 20 µl micropipet set to 10 µl, (ii) used a clean and appropriate pipet tip between each sample, and (iii) transferred liquid without air bubbles or losing sample in the transfer process.***	4 0	
5. Capped each tube tightly and mixed by flicking each tube with fingers.	2 0	
6. Pulse-spinned tubes in a microcentrifuge -or- tapped tubes on table to collect all liquid at the bottom of the tube. ***Judge verified competitor balanced the tubes in the microcentrifuge or tapped them on the table to collect liquid.***	4 0	
7. Verbalized one of the two options for incubation: (i) incubating reactions at room temperature overnight -or – (ii) incubating reactions at 37ºC for 45 min.	4 0	
Cleaning up		
8. Cleaned work area: a. Disposed of used pipet tips and microcentrifuge tubes containing the student-prepared reaction mixtures in waste receptacle.	2 0	
b. Cleaned work area with surface disinfectant.	2 0	
c. Removed PPE.	2 0	
d. Washed hands or used alcohol-based hand-rub for hand hygiene. ***Judge verified steps 8a-d were performed in the order written here.***	2 0	
TOTAL POINTS - SKILL II **70% Mastery for Skill II = 21.0**	**30**	

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**For all Judge verification steps, full points are only awarded if all components are accurate*

Skill III: DNA Gel Electrophoresis (Time: 20 minutes)	**Possible**	**Awarded**
1. Entered testing area wearing closed-toed shoes, then donned proper PPE: glasses/safety glasses/goggles, and gloves (lab coat is optional).	2 0	
Preparing samples		
2. Collected the liquid to the bottom of the 6 samples and DNA size standard by either (i) placing tubes into microcentrifuge or mini centrifuge and pulse-spinning for 5–10 seconds, or (ii) by tapping the tubes gently on the table. ***Judge verified competitor balanced the tubes in the microcentrifuge or tapped them on the table to collect liquid.***	4 0	
3. Pipetted 5 µl of sample loading buffer (SLB) into each tube. Pipetted up and down or flicking the tubes to mix. ***Judge verified competitor (i) used 20 µl micropipet w proper pipet tip, (ii) set micropipet to deliver 5 µl to all 7 tubes, (iii) used a fresh pipet tip for each sample, and (iv) transferred liquid without air bubbles or losing sample in the transfer process.***	4 0	
Loading the gel		
4. Placed the precast agarose gel into the electrophoresis chamber. ***Judge verified competitor placed* the wells of the agarose gel near the black (-) electrode (cathode).**	4 0	
5. Filled the electrophoresis chamber with 1x TAE buffer; added enough buffer to cover the gel and fill the wells.	2 0	
6. Loaded 10 µl standard into a well in the gel. ***Judge verified competitor (i) selected 20 µl micropipet and set it to deliver 10 µl, (ii) used a fresh pipet tip of the correct type, and (iii) loaded standard into the gel with no gel breakage or sample overflow into nearby wells.***	4 0	
7. Loaded 20 µl of each sample into separate wells of the gel. ***Judge verified competitor (i) selected 20 µl or 200 µl micropipet and set it to deliver 20 µl, (ii) used a fresh pipet tip of the correct type for each sample, and (iii) loaded samples into the gel with no gel breakage, piercing the bottom of wells, or sample overflow into nearby wells.***	4 0	
8. Recorded the order of sample loading on the sheet provided.	2 0	
9. Placed the lid on the electrophoresis chamber and connected the electrical leads to the power supply. ***Judge verified competitor connected red to red and black to black.***	1 0	
10. Turned on the power and ran the gel at 100 V. ***This step may be verbalized.***	2 0	
11. Verbalized "*run for 30 minutes*".	2 0	
12. Verbalized "*run has completed*", then turned off power and removed lid from chamber.	1 0	

Skill III: DNA Gel Electrophoresis (cont.)	Possible	Awarded
Cleaning up		
13. Cleaned work area: a. Disposed of used pipet tips, microcentrifuge tubes containing student-prepared electrophoresis samples in waste receptacle.	2 0	
b. Cleaned work area with surface disinfectant.	2 0	
c. Removed PPE.	2 0	
d. Washed hands or used alcohol-based hand-rub for hand hygiene. ***Judge verified steps 13a-d were performed in the order written here.***	2 0	
TOTAL POINTS - SKILL III **70% Mastery for Skill III = 28**	**40**	

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Skill IV: DNA Gel Interpretation (Time: 15 minutes)	Possible	Awarded
1. Using a ruler, measured the distance (in mm) that each of the DNA fragments or bands traveled from the well. Recorded results for each sample and standard in the table provided.	4 0	
2. Using the semilog graph paper provided, plotted the distance versus size for the bands in the standard.	6 0	
3. Drew a line of best fit through the points.	4 0	
4. Used the graph to estimate the fragment size for each band in the samples. Recorded estimates in the table provided.	6 0	
TOTAL POINTS - SKILL IV **70% Mastery for Skill IV = 14**	**20**	

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**For all Judge verification steps, full points are only awarded if all components are accurate*

Skill V: Bradford Protein Quantitation Assay (Time: 20 minutes)	**Possible**	**Awarded**
1. Entered testing area wearing closed-toed shoes, then donned proper PPE: glasses/safety glasses/goggles, and gloves (lab coat is optional).	2 0	
Preparing samples		
2. Labeled two empty microcentrifuge tubes for the sample dilutions (for example, **1/50 A** and **1/50 B**, or **A** and **B**)	1 0	
3. Pipetted 2 µl of sample A into the microcentrifuge tube labeled 1/50 A. ***Judge verified competitor (i) selected 20 µl micropipet, (ii) set micropipet to correct volume, (iii) used a clean pipet tip, (iv) used a proper pipet tip, and (v) transferred liquid without air bubbles or losing sample in the transfer process.***	2 0	
4. Pipetted 98 µl of 1x PBS into the microcentrifuge tube labeled 1/50 sample A. ***Judge verified competitor (i) selected (20-200 µl micropipet, (ii) set micropipet to correct volume, (iii) used a clean pipet tip, (iv) used a proper pipet tip, and (v) transferred liquid without air bubbles or losing sample in the transfer process.***	2 0	
5. Mixed well by pipetting, flicking, or vortexing.	2 0	
6. Pipetted 2 µl of sample B into the microcentrifuge tube labeled 1/50 B. ***Judge verified competitor (i) selected 20 µl micropipet, (ii) set micropipet to correct volume, (iii) used a clean pipet tip, (iv) used a proper pipet tip, and (v) transferred liquid without air bubbles or losing sample in the transfer process.***	2 0	
7. Pipetted 98 µl of 1x PBS into the microcentrifuge tube labeled 1/50 sample B. ***Judge verified competitor (i) selected 20-200 µl micropipet, (ii) set micropipet to correct volume, (iii) used a clean pipet tip, (iv) used a proper pipet tip, and (v) transferred liquid without air bubbles or losing sample in the transfer process.***	2 0	
8. Mixed well by pipetting, flicking, or vortexing.	2 0	
9. Labeled two cuvettes: Sample A and Sample B (or just A and B).	1 0	
10. Pipetted 20 µl of the 1/50 diluted samples into the corresponding cuvettes. ***Judge verified competitor (i) selected 20 µl micropipet, (ii) set micropipet to correct volume, (iii) used a clean pipet tip for each liquid, (iv) used a proper pipet tip, and (v) transferred liquid without air bubbles or losing sample in the transfer process.***	4 0	
Preparing standards		
11. Labeled eight cuvettes for the protein standards: Blank, 0.125, 0.250, 0.500, 0.750, 1.000, 1.500, 2.000	1 0	
12. Pipetted 20 µl of 1x PBS into the cuvette labeled Blank. ***Judge verified competitor (i) selected correct micropipet (20 µl), (ii) set micropipet to correct volume, (iii) used a clean pipet tip, (iv) used a proper pipet tip, and (v) transferred liquid without air bubbles or losing sample in the transfer process.***	4 0	

Skill V: Bradford Protein Quantitation Assay (cont.)	Possible	Awarded
13. Pipetted 20 µl of each protein standard into a corresponding cuvette. ***Judge verified competitor (i) selected correct micropipet (20 µl), (ii) set micropipet to correct volume, (iii) used a clean pipet tip for each standard, (iv) used a proper pipet tip, and (v) transferred liquid without air bubbles or losing sample in the transfer process.***	4 0	
Adding Bradford reagent		
14. Added 1 ml of the 1x Bradford reagent to all ten cuvettes. Mixed well by pipetting up and down. ***Judge verified competitor (i) used the correct micropipet (100-1,000 µl), (ii) set micropipet to correct volume, (iii) used a fresh pipet tip for each sample, (iv) used a proper pipet tip, (v) transferred liquid without air bubbles or losing sample in the transfer process, and (vi) pipetted up and down gently to mix (no liquid sucked into the barrel of the micropipet, for example).***	4 0	
15. Verbalized "*cuvettes incubate at room temperature for 5 minutes*".	1 0	
16. After visually comparing the cuvettes containing samples to the cuvettes containing the protein standard, verbalized an estimated protein concentration of the samples.	4 0	
Cleaning up		
17. Cleaned work area: a. Disposed of used pipet tips, microcentrifuge tubes containing the 1/50 dilutions, and cuvettes in waste receptacle.	2 0	
b. Cleaned work area with surface disinfectant.	2 0	
c. Removed PPE.	2 0	
d. Washed hands or used alcohol-based hand-rub for hand hygiene. ***Judge verified steps 17a-d were performed in the order written here.***	2 0	
TOTAL POINTS - SKILL V **70% Mastery for Skill V = 32.2**	**46**	

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**For all Judge verification steps, full points are only awarded if all components are accurate*

Skill VI: Bacterial Transformation (Time: 20 minutes)	**Possible**	**Awarded**
1. Entered testing area wearing closed-toed shoes, then donned proper PPE: glasses/safety glasses/goggles, and gloves (lab coat is optional).	2 0	
Preparing for heat shock		
2. Labeled one microcentrifuge tube +pGLO and another -pGLO.	1 0	
3. Pipetted 250 µl of transformation solution (0.05 M $CaCl_2$) into each tube, placed tubes on ice. ***Judge verified competitor (i) selected 100-1,000 µl micropipet, (ii) set it to deliver 250 µl, (iii) used a proper pipet tip, and (iv) transferred liquid without air bubbles or losing sample in the transfer process.***	4 0	
4. Used a sterile plastic inoculation loop to scrape 2–4 *E. coli* colonies from the surface of the starter plate.	2 0	
5. Transferred the loop into the +pGLO tube and swirled it in the transformation solution to disperse bacteria. Closed the tube and placed it back on ice.	2 0	
6. Disposed of loop into biohazard waste receptacle or onto a paper towel for disposal later.	2 0	
7. Used a second sterile plastic inoculation loop to scrape 2–4 *E. coli* colonies from the surface of the starter plate.	2 0	
8. Transferred the loop into the -pGLO tube and swirled it in the transformation solution to disperse bacteria.	2 0	
9. Disposed of loop into biohazard waste receptacle or onto a paper towel for disposal later.	2 0	
10. Pipetted 10 µl of pGLO plasmid into the +pGLO tube and mixed by pipetting gently up and down. ***Judge verified competitor (i) used 20 µl micropipet and set it to deliver 10 µl using a correct tip and (ii) did NOT add plasmid to the -pGLO tube.***	4 0	
11. Disposed of pipet tip into biohazard waste receptacle or onto a paper towel for disposal later.	2 0	
12. Placed both tubes back on ice, making sure the tubes were in full contact with the ice.	1 0	
13. Labeled agar plates with "+pGLO" or "-pGLO" as follows: LB/amp +pGLO LB/amp/ara +pGLO LB/amp -pGLO LB -pGLO ***Judge verified competitor labeled the bottoms of the plates and not the lids.***	4 0	
14. Verbalized "*10 min incubation on ice completed*".	2 0	

Skill VI: Bacterial Transformation (cont.)	Possible	Awarded
Performing heat shock		
15. Transferred the +pGLO and -pGLO tubes from the ice into a 42^0 C water bath for exactly 50 seconds making sure the tubes were in full contact with the water. Immediately placed tubes back on ice.	4 0	
16. Verbalized "*tubes remained on ice for 2 minutes*".	2 0	
17. Removed the tubes from ice and pipetted 250 µl of LB broth into each tube. ***Judge verified competitor (i) used 100-1,000 µl micropipet to deliver 250 µl and (ii) changed the pipet tip between each sample, (iii) used a proper pipet tip, and (iv) transferred liquid without air bubbles or losing sample in the transfer process.***	4 0	
18. Disposed of pipet tips into biohazard waste receptacle or onto a paper towel for disposal later.	2 0	
19. Verbalized "*samples incubated at room temperature for 10 min*".	1 0	
Plating the bacteria		
20. Mixed the tubes by inverting or flicking.	1 0	
21. Pipetted 100 µl of the transformation mixtures onto appropriately labeled agar plates (for example, a "+pGLO" mixture onto a "+pGLO plate", etc.). ***Judge verified competitor (i) used 20-200 µl micropipet to deliver 100 µl to each plate, (ii) applied correct sample to correct plate, (iii) changed the pipet tip between each sample, (iv) used a proper pipet tip, (v) transferred liquid without air bubbles or losing sample in the transfer process, and (vi) applied correct sample to correct plate.***	4 0	
22. Disposed of pipet tips into biohazard waste receptacle or onto a paper towel for disposal later.	2 0	
23. Used a sterile plastic inoculation loop to spread the bacteria over the entire surface of the plate in all directions. Disposed of loop into biohazard waste receptacle or onto a paper towel for disposal later.	4 0	
24. Repeated step 23 for each plate. ***Judge verified competitor used a new loop for each plate and the samples matched plates.***	10 0	
25. Stacked plates together with lids facing downward, agar side facing up. ***Judge verified competitor placed plates with agar side up.***	4 0	
26. Verbalized "*plates incubate at 37^0C for 16–24 hours*".	2 0	
Cleaning up		
27. Cleaned work area: a. Disposed of used pipet tips, the +pGLO and -pGLO tubes , and used loops into biohazard waste receptacle, cleaned area of any spilled liquid.	2 0	
b. Cleaned work area with surface disinfectant.	2 0	
c. Properly removed PPE.	2 0	
d. Washed hands or used alcohol-based hand-rub for hand hygiene. ***Judge verified steps 27a-d were performed in the order written here.***	2 0	
TOTAL POINTS - SKILL VI **70% Mastery for Skill VI = 56**	**80**	

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**For all Judge verification steps, full points are only awarded if all components are accurate*

Skill VII: Calculation of Transformation Efficiency (Time: 10 minutes)	**Possible**	**Awarded**
1. Entered testing area wearing closed-toed shoes, then donned proper PPE: glasses/safety glasses/goggles, and gloves (lab coat is optional).	2 0	
2. Counted the number of transformed colonies on the plate and recorded that number on the printed scenario or paper provided. If there are >~50 colonies, an estimation made by counting colonies in a quadrant on the plate is acceptable.	4 0	
3. Calculated how many micrograms of DNA were spread onto the plate.	4 0	
4. Expressed answer to #3 using correct units (µg).	2 0	
5. Calculated the transformation efficiency.	4 0	
6. Expressed answer to #5 using correct units (CFU/µg or colonies/µg).	2 0	
7. Removed PPE before leaving the area.	2 0	
TOTAL POINTS - SKILL VII **70% Mastery for Skill VII = 14**	**20**	

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**For all Judge verification steps, full points are only awarded if all components are accurate*

Skill VIII: Qualitative ELISA (Time: 20 minutes)	**Possible**	**Awarded**
1. Entered testing area wearing closed-toed shoes, then donned proper PPE: glasses/safety glasses/goggles, and gloves (lab coat is optional).	2 0	
2. Labeled a 12-well microplate strip: ● the first three wells with **+** for the positive controls ● the next three wells with a **–** for the negative control ● the next three wells with an **S** to indicate the sample.	2 0	
Antigen incubation		
3. Transferred 50 µl of purified antigen (AG) into each well. ***Judge verified competitor added AG to all 9 wells and (i) used a 20-200 µl micropipet, (ii) set it to 50 µl, (ii) used a proper pipet tip, and (iii) transferred liquid without air bubbles or losing sample in the transfer process.***	4 0	
4. Incubated the samples at room temperature for 2 min.	2 0	
5. Followed wash protocol: a. Tipped each microplate strip upside-down onto a short stack of paper towels and gently tapped strip a few times to drain the wells while making sure to avoid splashing sample back into wells.	2 0	
b. Discarded the wet paper towels.	2 0	
c. Used a transfer pipet (same transfer pipet can be used) to fill each well with wash buffer, taking care not to touch the well or spill the buffer into neighboring wells.	2 0	
6. Repeated step #5.	2 0	
Sample incubation (primary antibody)		
7. Transferred 50 µl of the positive control (+) into the three + wells. ***Judge verified competitor added + to only the first 3 wells labeled + and (i) used a 20-200 µl micropipet set to 50 µl, (ii) used a proper pipet tip, and (iii) transferred liquid without air bubbles or losing sample in the transfer process.***	4 0	
8. Transferred 50 µl of the negative control (-) into the three – wells. ***Judge verified competitor added - to only the 3 wells labeled - and (i) used a 20-200 µl micropipet set to 50 µl, (ii) used a proper pipet tip, and (iii) transferred liquid without air bubbles or losing sample in the transfer process.***	4 0	
9. Transferred 50 µl of the sample (S) into the corresponding three wells. ***Judge verified competitor added S to only the 3 wells labeled S and (i) used a 20-200 µl micropipet set to 50 µl, (ii) used a proper pipet tip, and (iii) transferred liquid without air bubbles or losing sample in the transfer process.***	4 0	
10. Incubated the samples at room temperature for 2 minutes.	1 0	
11. Performed wash protocol (step 5) two times.	2 0	

Skill VIII: Qualitative ELISA (cont.)	Possible	Awarded
Enzyme-linked antibody (secondary antibody) incubation		
12. Transferred 50 µl of enzyme-linked antibody (ELA) into each well. ***Judge verified competitor added ELA to all 9 wells and (i) used a 20-200 µl micropipet set to 50 µl, (ii) used a proper pipet tip, and (iii) transferred liquid without air bubbles or losing sample in the transfer process.***	4 0	
13. Incubated the samples at room temperature for 2 minutes.	1 0	
14. Performed wash protocol (step 5) three times.	2 0	
Substrate incubation and color development		
15. Transferred 50 µl of enzyme substrate (SUB) into each well. ***Judge verified competitor added SUB to all 9 wells and (i) used a 20-200 µl micropipet set to 50 µl, (ii) used a proper pipet tip, and (iii) transferred liquid without air bubbles or losing sample in the transfer process.***	4 0	
Cleaning up		
16. Cleaned work area: a. Disposed of used pipet tips, transfer pipets, and paper towels into waste receptacle, cleaned area of any spilled liquid, returned micropipette to rack (if available).	2 0	
b. Cleaned work area with surface disinfectant.	2 0	
c. Removed PPE.	2 0	
d. Washed hands or used alcohol-based hand-rub for hand hygiene. ***Judge verified steps 16a-d were performed in the order written here.***	2 0	
17. Observed and reported results. ***Judge verified (+) and S wells were blue, (-) was colorless; competitor confirmed sample was positive.***	4 0	
TOTAL POINTS – SKILL VI **70% Mastery for Skill VI = 39.2**	**56**	