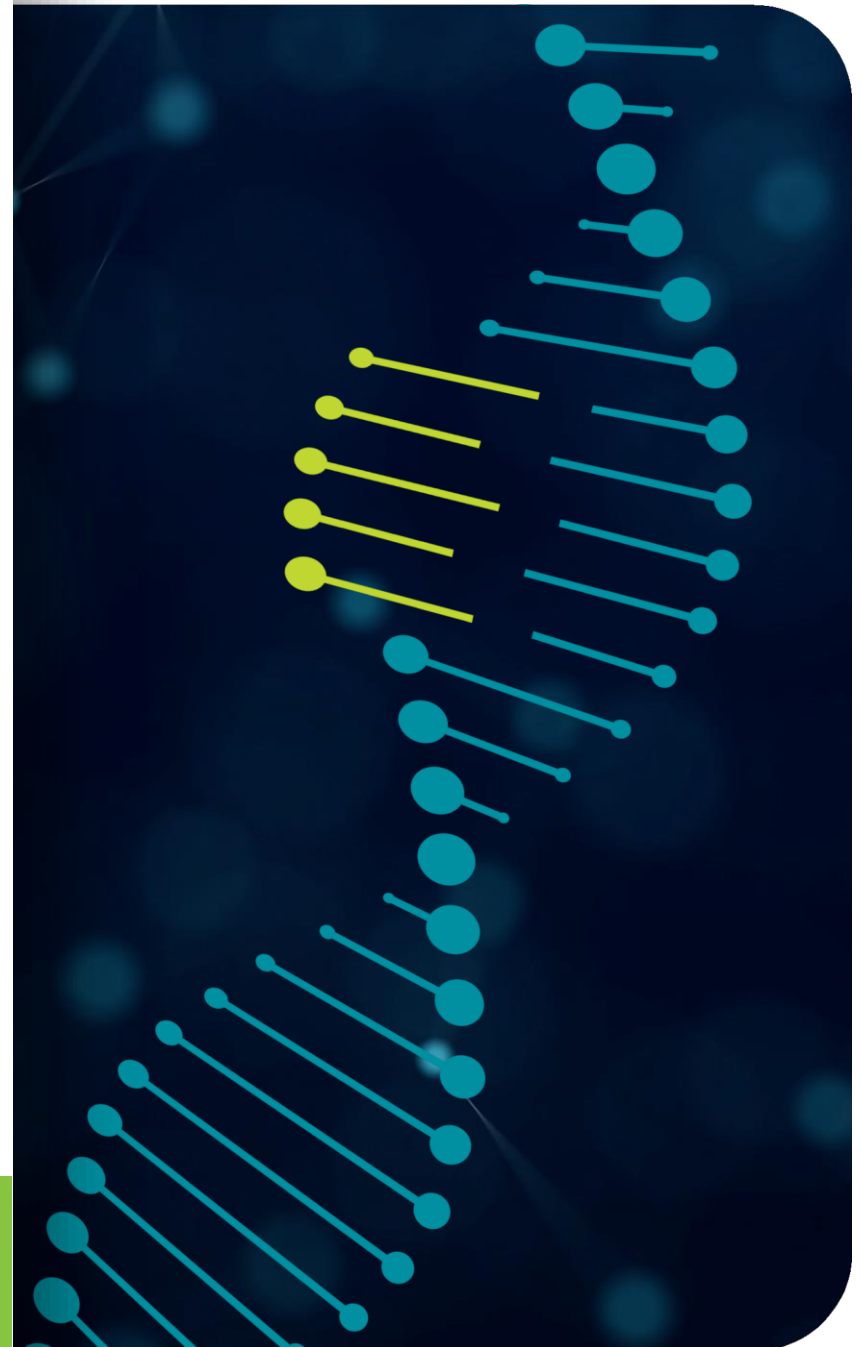




Building Confidence Using CRISPR with Biotechnical Lab Skills

Amanda Hewes
Education Program Manager

March 2025





Education Program Manager, Developer of CRISPR in a Box™

Amanda Hewes



University of Delaware

Bachelor's: Animal & Food Sciences

University of Delaware

Master's: Animal Sciences

- Studied: The effects of heat stress on the transcriptomics of day 28 spleens in Ross 708 and Illinois broilers.

Gene Editing Institute 2017 - Present

- Research Assistant
- Staff Scientist
- Education Program Manager



Education Program Coordinator, **Sarah LaTorre**



Delaware Technical Community College Associate's Biotechnology: 2018 - 2019

- Studied abroad to Belize focusing on biodiversity and conservation.

Wilmington University Bachelor's: Biology 2020-2024

- NASA Delaware Space Grant
- Delaware Environmental Institute
- Studied: genetic sequencing of corals to analyze stress genes, due to rising ocean temperatures and climate change

Gene Editing Institute: 2023 - Present

- Summer 2023 participant in Gene Editing 360™ Learning Lab
- Science Educator Intern: September 2023 – March 2024
- Education Coordinator: March 2024 – Current

Founded by
Molecular
Medicine & Gene
Editing Pioneer,
Eric Kmiec, Ph.D.

*ChristianaCare's Gene Editing Institute
seeks to **empower**, **inspire**, and
engage the next generation of
scientists committed to advancing
gene editing technology.*





Equity & Access

- **Enrich STEM education** at the high school and collegiate level by introducing a gene editing/CRISPR technology tool: CRISPR in a Box™.
- **Demystify gene editing** technology and application to innovations in healthcare.
- **Build trust** with communities historically excluded from cutting edge advances in biotechnology and healthcare.
- **Build talent** pipeline of future STEM scientists!





Building Confidence In Your Classroom

Why are you
interested in this
workshop?

Share with your partner!





CRISPR in a Box™

Teaches Trusted Techniques



Hands-on experiment



Live readout within non-infectious *E. coli* bacteria



Follows reaction from beginning to end



Lab safety skills

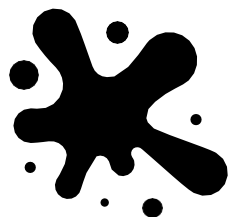


A chance to cut DNA with CRISPR/Cas technology





Lab Safety



Accidental Splash:

If solution gets into the eyes, **flush with water for 15 minutes.**



Skin Irritation:

Several components may cause skin irritation. In case of direct contact with the skin, **wash with abundant water for 10 minutes.**



Biohazard Bins:

Use biohazard waste bin for disposing anything with chemical residue.

Proper glove removal is important during this exercise



Our recommendations

Be Safe

- **Pay attention during demonstrations**
- Listening ears during discussions
- Ask questions

Be Ready

- **Follow the protocol**
- Clean up your workstation as you go

Be a Friend

- **Work together**
- Share materials & take turns



Micropipetting Practice





Micropipet breakdown

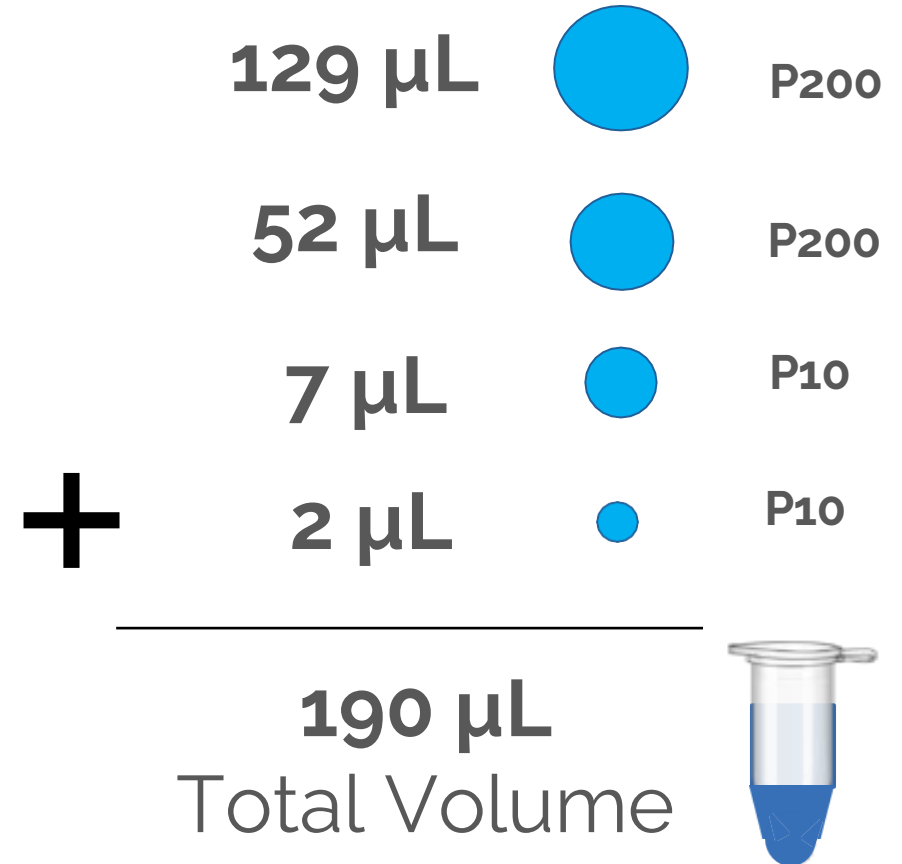


Each pipette has a volume range!



Activity #1: Instructions

1. Each participant must label a 1.5mL tube with their initials.
2. Pipet the largest volume, **129 μ L**, into your 1.5mL tube.
3. Pipet **52 μ L** into your 1.5mL tube.
4. Pipet **7 μ L** into your 1.5mL tube.
5. Pipet **2 μ L** into your 1.5mL tube.
6. Your final volume will be **190 μ L**.
7. **Finally:** Pipet **190 μ L of colored water** back into the large 5mL tube.





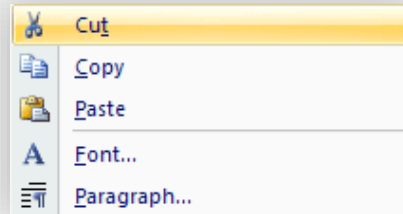
What is

Gene Editing?



Editing vs Gene Editing

Cutting



Deleting
Knock-out



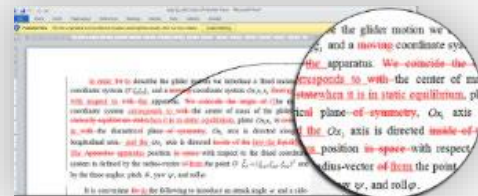
Pasting



Inserting
Transgenic



Changing



Replacing
Knock-in



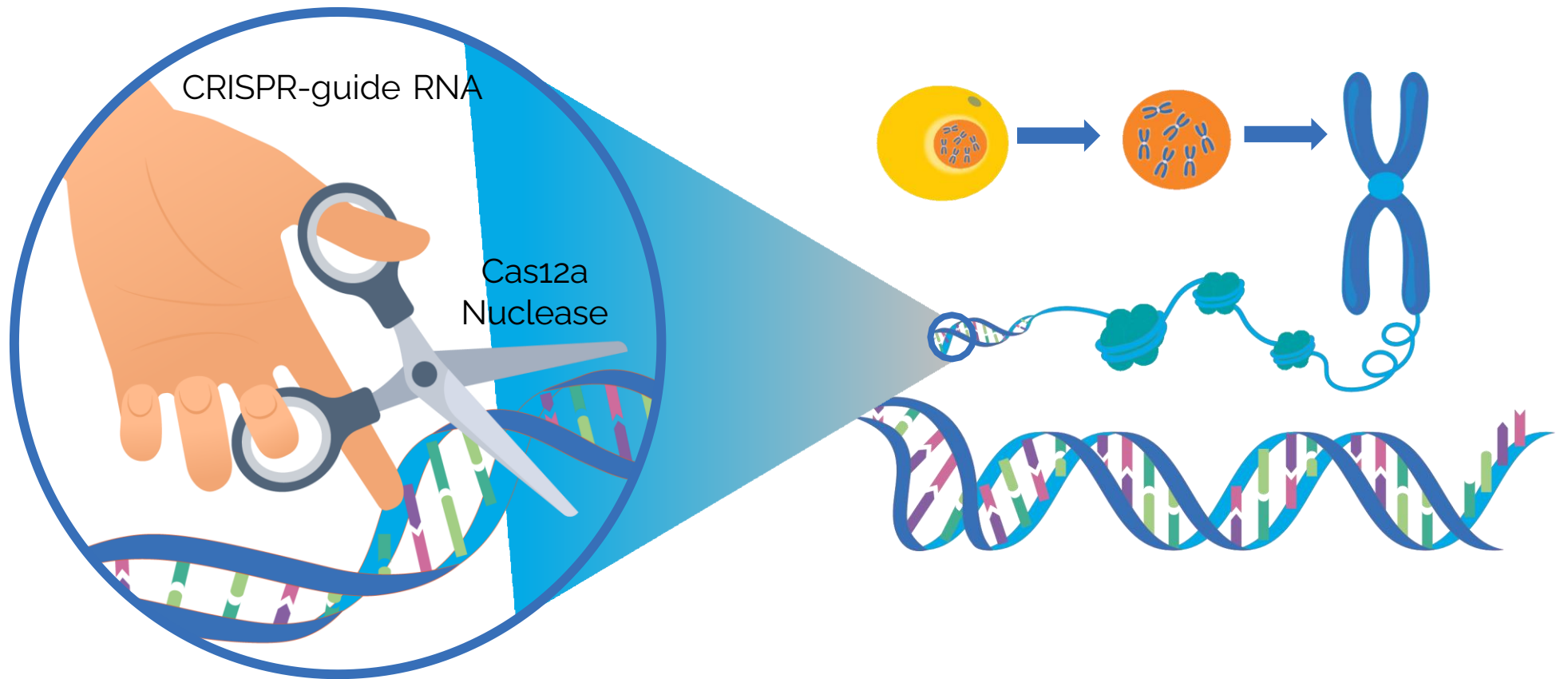


What is

CRISPR/Cas
technology?



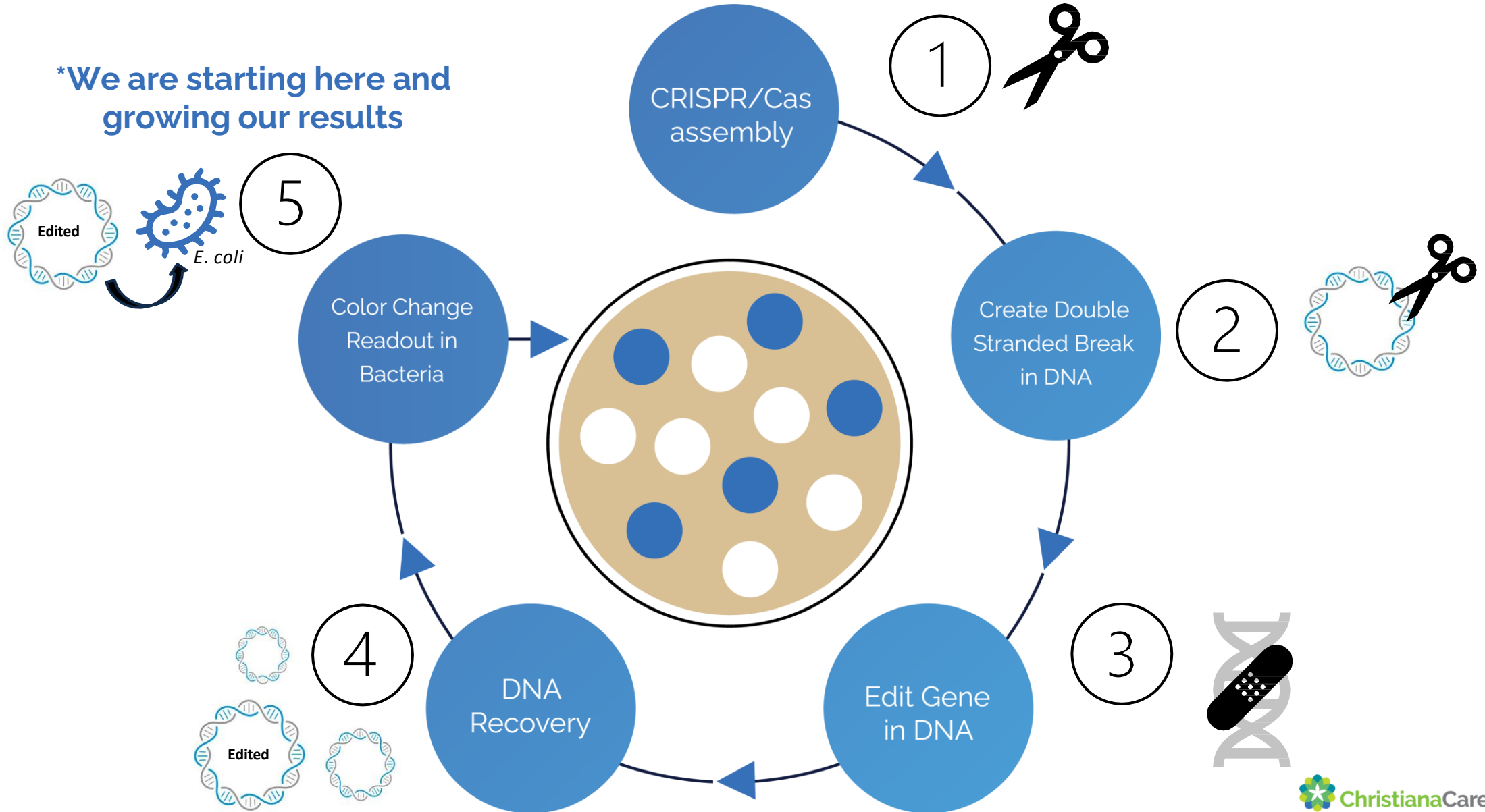
CRISPR/Cas12a is like a pair of **molecular scissors**





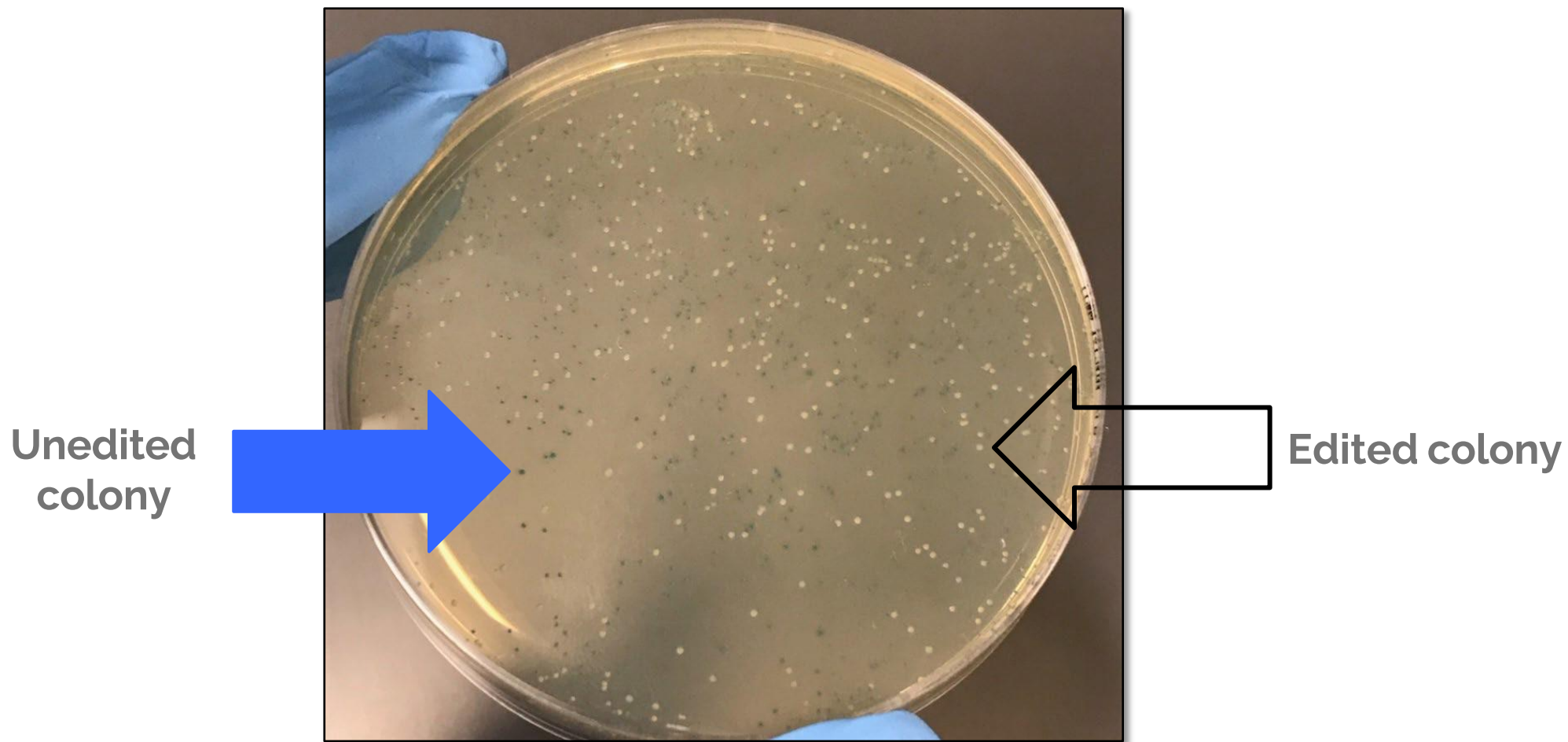
Today's experimental overview

*We are starting here and growing our results





Expected results

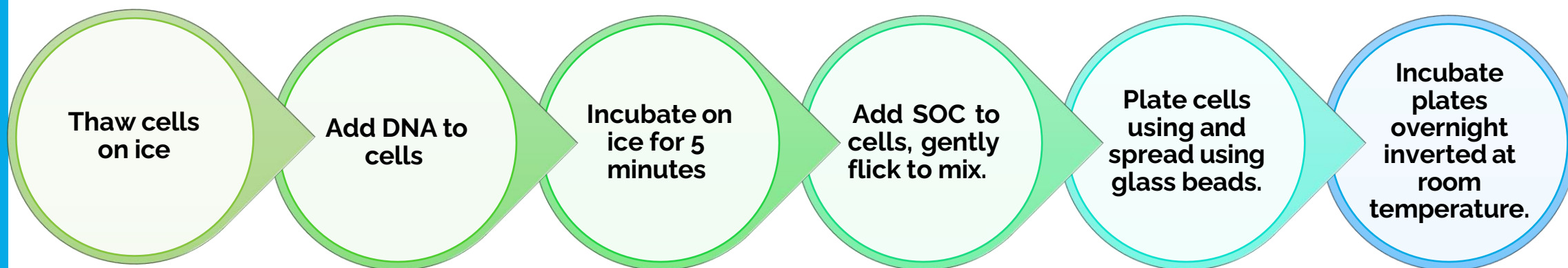


Unedited Colony = Blue
Edited Colony = White

*Note: The image above is a representation of the results from today's exercise. This transformation process is different in the CRISPR in a Box™ kit.



Activity #2: Bacterial Transformation



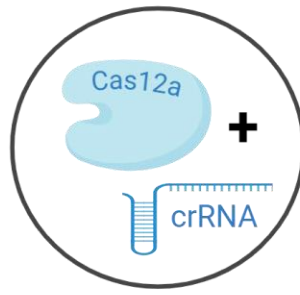
Results can be found at the Carolina Biological Booth #301 tomorrow.

*Note: Today's transformation has been modified to fit this workshop timeframe. CRISPR in a Box™ kit offers in-depth instructions on the phenotypic readout.



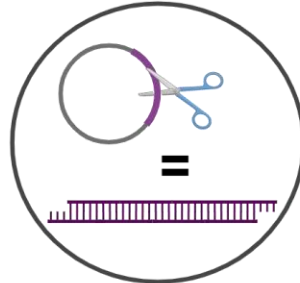
1

Form RNP by complexing Cas12a with crRNA



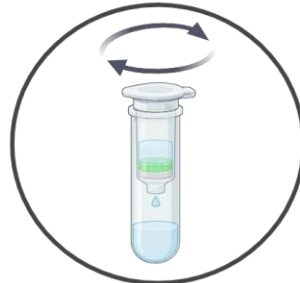
2

Cut plasmid DNA using the RNP



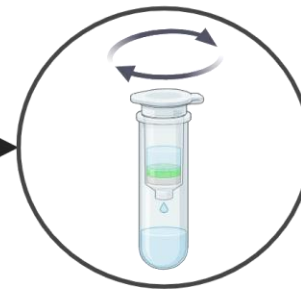
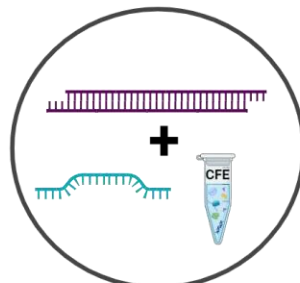
3

Clean DNA to obtain linearized DNA



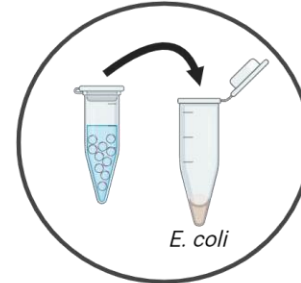
4

Rejoin plasmid with HDR oligo and human cell-free extract



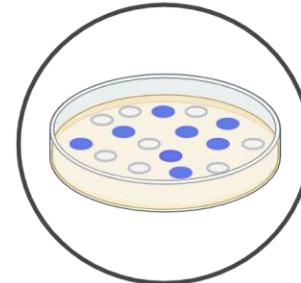
Clean DNA to obtain re-circularized DNA

5



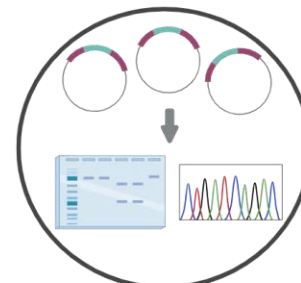
Transformed edited DNA into *E. coli*

6



Phenotypic Readout:
Visualize blue and white colonies

7



Genotypic Readout:
Restriction Digestion or Sequencing

8

We completed steps 6 and 7.

Education & Outreach

At the **heart** of Gene Editing 360™ is **CRISPR in a Box™**, a teaching toolkit providing students in high schools, community colleges and universities a unique, hands-on learning experience in CRISPR gene editing.



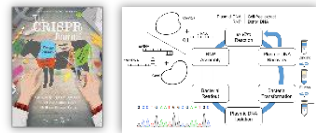
Gene Editing 360™

- CRISPR in a Box™ **bridges the gap** between classroom science experiments and cancer research at the institute.
- **We build confidence by connecting the hands-on biotechnical lab skills with CRISPR/gene editing**





History of CRISPR in a Box™



In vitro system and protocol

2018 **1**



CRISPR in a Box™ developed from existing system model

2019 **2**



Educational platform supports product & cause with DETV

2020 **3**



CIAB Complete Education Kit in action in classroom labs

2021 **4**



Learning Lab opens, students across the region attend CIAB workshops

2023 **5**



Local Impact with CRISPR in a Box™

CRISPR in a Box™ is a tried-and-true method of offering hands on scientific experiences for students local to the Greater Philadelphia region.

**Ask us about visiting Learning Lab at the
Q & A session!**

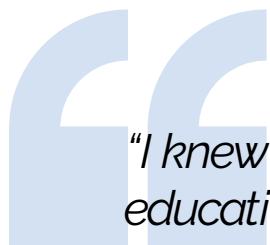
Program Reach

We've engaged over 1000 students this year, but where do they come from? From the boroughs of Philadelphia to the townships of Kent, we're proud to continue demystifying gene editing and building a pipeline of future scientists with fresh perspectives on the advancement of CRISPR.





Learning Lab is Making a Difference



"I knew that it would be educational, but I didn't anticipate how motivational it would be. My [students] came back believing in a future they didn't know they could dare to dream about."

– High School Teacher





ChristianaCare
Gene Editing Institute

Extending
the boundaries,
accelerating
the cure.

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the cure.

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Gene Editing Institute

Incorporating CRISPR into the Classroom



Chapter 1

A Complete Methodology for the Instruction of CRISPR-Based Gene Editing Using a Simplified Cell-Free Extract System with Genetic Readout in Bacteria

Kristen M. Pisarcik, Brett M. Sansbury, and Eric B. Kmiec

Table 2

Partial and complete semester curriculum for the in vitro gene editing method

Suggested short and long-term curriculum vitae	
Week	Laboratory activities
1	Aseptic technique; sterilization methods; medium preparation
2	Preparing an overnight culture; quadrant streaking; patch plate
3	Bacterial transformation; plating dilutions; discuss blue-white screening
4	Plasmid isolation
5	Restriction digestion, agarose gel electrophoresis
6	Polymerase chain reaction, gel electrophoresis
7	Designing CRISPR RNAs and homology directed repair oligonucleotide
8	In vitro reaction part I: Stop after first plasmid cleanup; gel electrophoresis of cleavage gel
9	In vitro reaction part II: Stop after second plasmid cleanup
10	In vitro reaction part III: <i>E. coli</i> transformation; medium preparation
11	In vitro reaction part IV: Plasmid isolation; restriction digestion; gel electrophoresis
12	In vitro reaction part V: Colony PCR; PCR purification; gel electrophoresis
13	In vitro reaction part VI: Prepare PCR for sequencing
14	Analyze sequencing results
15	Laboratory course wrap-up

Potential laboratory exercises and associated educational topics which can be covered utilizing this protocol. Instructors have the freedom to select topics that fit best within their established curriculum