Resource Pack for Running Educational Workshops

This pack is designed to contain all the core elements a team will need, should they desire to run outreach workshops, talks and practicals for middle and high school students. Here, we draw inspiration from sessions the reGELerate team delivered, to present five modules that can be mixed and matched to fit with the duration, venue and audience that your team wishes to present to.

Module	Activity	Requirements
1. Synthetic Biology Crash Course	An interactive talk where questions are posed to students throughout to assess their level of understanding and tailor the session as it is being delivered. Participation in iGEM at the high school, university and volunteer level can also be introduced.	10-20 minutes Any classroom/assembly hall
2. Design/Problem Solving Challenge	Students are split into teams of 4-5 and work together to build a genetic circuit from paper biobricks or biological parts to solve a real-world problem. A team representative then volunteers/is selected to pitch the solution.	30 minutes Any classroom Printouts required
3. Ethics Debate	Students are given statements on debated topics such as naturalness, gene drives, and alternative meats and must argue for or against it. If the classroom allows it, students are encouraged to stand on one side on the room depending on whether they agree or disagree to make the session more engaging.	10-15 minutes Any classroom/assembly hall
4. Introduction to Bacterial Transformation	Students work in pairs to perform a heat-shock transformation with appropriate controls that can be analysed the following day.	2-2.5 hours Biology/Chemistry/Physics lab Reagents required, from school or iGEM team
5. Introduction to DNA extraction	Students work in pairs to extract their own DNA from cheek cells and take it home if they wish to.	45 mins -1h Biology/chemistry lab Reagents required, from school or iGEM team

School outreach template

Provided below is a template used to reach out to high schools that received a successful response, and allowed us to deliver our talk. We encourage teams to consider through which type of science communication activity they wish to engage with students and adapt their emails accordingly.

I hope you are well.

My name is [full name], I am currently studying at [institution name]. I am part of this year's Imperial iGEM team, competing against 462 other teams across the globe in synthetic biology. iGEM is the world's largest synthetic biology competition. The goal of the competition is to develop scientifically backed synthetic biology solutions to real-world problems, such as infectious disease over the course of a year.

I am writing to you to inquire whether our team could visit your school and give a talk/workshop about iGEM and synthetic biology in the next two months. This could take the form of an assembly presentation or as part of a biology, chemistry or physics lesson. We would be very happy to adapt our outreach to your preference, towards areas such as sustainability or AI, which are tightly affiliated with iGEM.

Engaging with iGEM would give students insight into how biology, chemistry, engineering, AI and sustainability come together to solve real-world problems, while also exposing them to university-level research and STEM pathways. iGEM also runs a high-school competition, so students could potentially form their own team in future, as schools such as City of London School and Eton College already do.

Please let me know whether this initiative would be of interest, we are very open to having a short call to discuss this opportunity further. I have also attached a one-page summary of our project and pasted our project video link below:

https://linktoprojectsummaryandvideo

Choosing your Modules

After receiving interest from a school, consider the constraints that the teacher/organiser may encounter when trying to facilitate this collaboration. The following questions help to identify these:

1) How much time are we given for the session?
2) What type of room will we be in - a classroom, lecture hall, laboratory?
3) What year are the students in, and what topics have they subsequently covered?

Module Tip! The third question was a very important question to ask, as two of our sessions had been with Year 12 and 13 biologists, but one group had already been introduced to plasmid transformation whilst the other was still consolidating transcription and translation. Knowing this ahead of time allowed us to pitch each session at an adequate level to keep the students concentrated.

Modules can then be picked to keep these constraints in mind. The table below delineates the best modules per teaching level.

Module	**Activity**	**Watch out for:**
1. Synthetic Biology Crash Course	Any audience - the simplicity of the content should be adapted to the students' level	Can easily become a lecture, so make sure to build questions to ask the students into most slides.
2. Design/Problem Solving Challenge	Biobricks parts were chosen as a problem-solving workshop for Year 12 and 13 students as prior knowledge of transcription and translation was required. But these biological parts can be amended based on the student level, such as asking younger students to assign the bases of DNA and RNA correctly.	Requires an introductory presentation of its own explaining the real-world problem, its significance and how synthetic biology can be used to solve it. Also requires enough facilitators/teachers to move between teams and answer questions.
3. Ethics Debate	Recommended for Year 10 and upwards as discussion questions become a part of their school examinations at this stage. This activity is excellent for groups of students with different interests and backgrounds.	Requires a facilitator that can continue the discussion by questioning the arguments proposed by students, whilst keeping it balanced.
4. Introduction to Bacterial Transformation	Year 12 and 13 students, as will have some but limited	Prepare sufficient equipment for all teams so

	prior knowledge of bacterial biology.	that students are not waiting around and get distracted. Assign a facilitator/teacher per bench and ask students why each step is done before explaining.
5. Introduction to DNA extraction	Year 12 and 13	Obtain consent for cheek sampling. As stated above, prepare sufficient equipment for all teams so that students are not waiting around and get distracted. Assign a facilitator/teacher per bench and ask students why each step is done before explaining.

Planning Timeline

When	Task
4-8 weeks before	Contact the school and agree on a date, expected class size, age group and learning goals. Ensure that the teacher/organiser books a space to work in.
4 weeks before	If necessary, write and get a risk assessment/consent forms signed off according to the protocol designed. Ask for any medical assistance needed, such as allergies to reagents. Order consumables and kits needed.
2 weeks before	Practice a run of the session with the team, with handouts printed already.
1 week before	Confirm number of expected students. Ensure competent cells and all kit reagents/plates are ready from the team's side and the school's side.
After	Photograph and send the plate results to the team, collect feedback via QR code-scannable forms and write up for the Wiki. Improve the next session using the feedback.

Safety Tip! Transforming bacteria with a plasmid creates a genetically modified organism. In the UK, this is regulated under the Genetically Modified Organisms (Contained Use) Regulations 2014, and the rules may differ within other countries. Please ensure the following:

- Any practicals are run in high school or university labs covered by the institution's GMO risk assessment, with approval from a biological safety officer.
- A non-pathogenic laboratory strain of bacteria (likely *E. coli*) is used, such as the K-12 derivative, DH5-alpha, alongside a well-characterised plasmid
- Pipette tips and liquids should be disposed of into Virkon following use, in order to be disinfected.
- All tips, tubes and plates should then be disposed of in a bin that will be autoclaved. All individuals working in the lab must wash their hands before leaving the premises.
- Lab coats, gloves and eye protection should be provided to all participants.
- No food and drinks are allowed in the lab.
- Isopropanol and ethanol are flammable reagents. Therefore, no flames should be lit in the room.
- Ampicillin can cause allergic reactions, so allergies should be inquired for in advance. Any allergic students can observe rather than handle plates containing ampicillin.
- Teachers remain responsible for their students, so ensure that a teacher is present throughout and agrees ahead of time who will handle any behaviour issues and first aid.
- Obtain written consent from schools before photographing students, and do not photograph without it.
- Collecting a cheek cell sample is non-invasive, but some parents may still want to opt out - teams can offer a demonstrator-led video to watch ahead of time to decide whether they want their child to participate.

Module 1: Synthetic Biology Crash Course

This module should provide a 10-20 minute interactive talk that introduces synthetic biology as a field. The aim is for students to be able to explain what synthetic biology is, how engineers can design biological systems and what its applications in the real-world are. The clearest piece of feedback we received was that our first version of this talk was too passive - students informed us that they wanted to take part rather than just listen. We therefore recommend to build a question into most sections, such as asking them to name or predict a mechanism on the slide before it is revealed, or to explain why something works. This will make the session feel like more of an exchange, allowing students to integrate knowledge better. We also recommend using analogies to simplify and allow students to picture concepts more easily in relation to something they know (see table below). Finally, we recommend discussing numerous applications but also refer back to one specifically throughout the talk so students can see how the entire process of building a synthetic biology project leads to a specific product output. In this crash course, we recommend introducing the following topics:

- What is synthetic biology (the iGEM definition) and in simpler language?
- What is the central dogma of biology?
- What are chassis, and why is it important to consider different types?
- What is the DBTL cycle?
- What are biobricks, and why does standardisation matter?
- What are the diverse applications of synthetic biology?
- How is AI being integrated into synthetic biology?
- Why is it important to understand the public perception around synthetic biology?

Term	Plain language
Chassis	The vehicle that a design is inserted into; a cell chosen as a factory
Genetic circuit	A set of instructions wired together so that a cell produces an output once the right input conditions are met, eg: a light that only activates when someone walks into a room
Biobrick	A lego brick with standard studs so that parts from any team can click together
Promoter	The switch that decides whether a gene is read or not
DBTL cycle	Building a prototype, testing it, understanding why it failed and building a better one

At the end of the talk, because Year 12 and above are making subject and university decisions, lots of them might be interested in advice on how to get involved in the field. Teams can point out that:

- There is currently no UK undergraduate degree purely in synthetic biology, as the field is scattered across biochemistry, biology, biotechnology, bioengineering and microbiology degrees. Some of these degrees might offer modules dedicated to synthetic biology, whilst others will touch on the principles. So there are various routes into this field.
- The field is interdisciplinary: it also requires lawyers, policy experts, regulators, ethicists, designers and software engineers. This is useful information as students often assume that a lab career is the only option.

Module 2: Design/Problem Solving Challenge

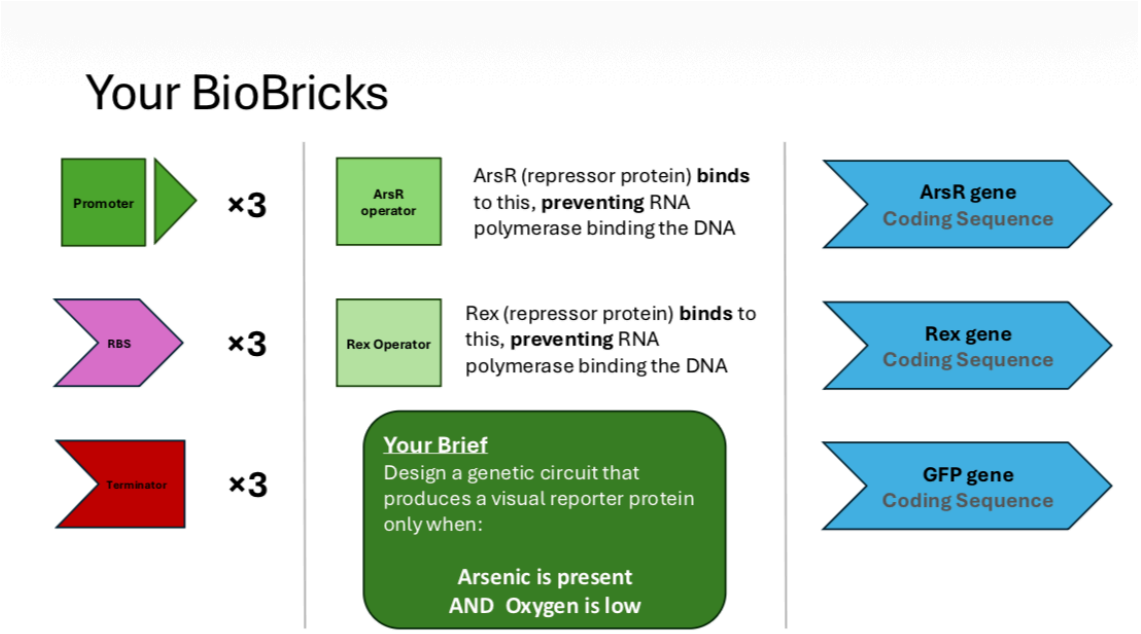
Teams of four or five can then be assigned a real-world problem explained by a facilitator, alongside a set of paper biobricks parts, and then given twenty minutes to design a genetic circuit that solves it. This is more appropriate for Year 12 and above, but the exercise can be simplified for years below. One representative from a team can then pitch the design to the room, where others indicate whether they agree or disagree with the proposed solution and may suggest their own. This will give students experience in firsthand designing a synthetic biology tool by working in a team and justifying it to facilitators walking by/to the entire class.

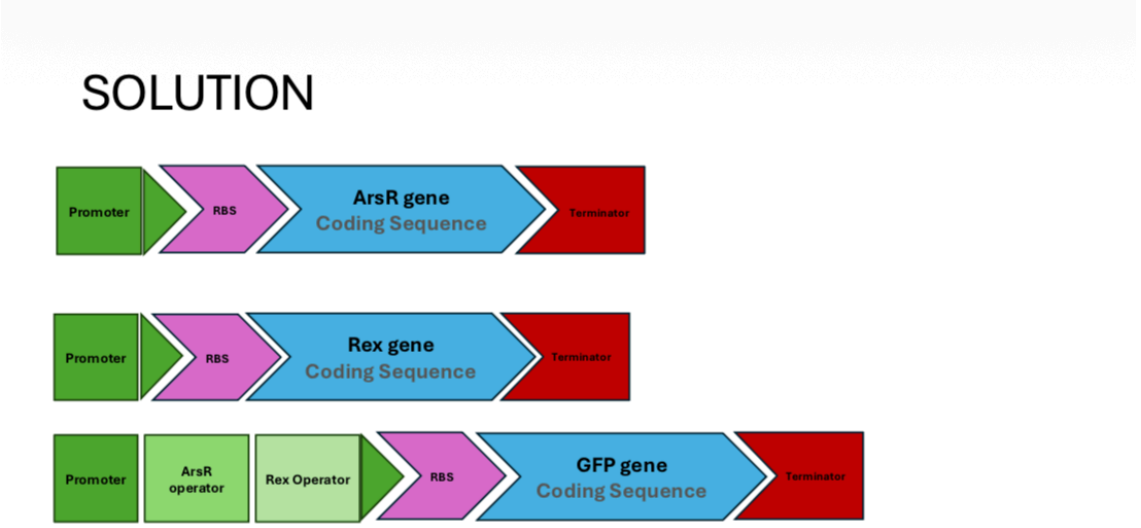
When designing your own brief to build a genetic circuit, the key features to include are 1) A 2-input logic requirement, 2) A problem that students care about that is contextualised, and 3) Room for several right answers, if possible, to encourage creativity. Before running the workshop, ensure that all facilitators can respond to common questions students may have and can give advice for common mistakes that might be made. We designed an example brief to teach students how to assemble a transcriptional unit on a plasmid backbone from a

promoter, ribosome binding site, coding sequence and terminator. We also introduced how operator and repressor sequences work, and how these elements can be combined to form a logic gate - a concept students might have already encountered in their physics classes. The brief is described below.

Groundwater arsenic contamination affects large populations across South and Southeast Asia. Arsenic is released from aquifer sediments under anoxic conditions (low oxygen content). Testing wells is slow and expensive, so creating a cheap biological sensor would be useful. Students can then be tasked with the following: **Design a genetic circuit that produces a visible reporter only when arsenic is present and oxygen is low. You have twenty minutes, and must be able to explain which part serves what function.** A list of printable parts is shown below.

Part	**Behaviour**
Constitutive promoter	Always switched on
ArsR coding sequence	Produces ArsR (repressor protein)
Rex coding sequence	Produces Rex (repressor protein)
ArsR operator	ArsR binds to this, preventing RNA polymerase from binding DNA
Rex operator	Rex binds to this, preventing RNA polymerase from binding DNA
Reporter coding sequence	Produces GFP protein
Ribosome binding site	Required before every coding sequence to regulate protein quantity produced
Terminator	Stops transcription at the end of each unit





Common mistakes made by students during this workshop were putting the ribosome binding site after coding sequences and trying to combine all biobricks into one single transcriptional unit, rather than three separate ones. Running this workshop as a discussion, rather than a marking exercise, whereby two different designs are selected and presented can be very valuable for the students to understand why their logic might have worked or not.

Module 3: Ethics Debate

This structured discussion is designed for students to develop their ability to articulate and defend a position on a contested application of biotechnology, in order to understand how nuanced engineering biology actually is in terms of ethics, social implications and legality.

We encourage students to respond to counter-arguments and adjust their stance should evidence that they weren't aware of change their mind. We recommend first reading a statement and asking students to move physically from one side of the room depending on whether they agree or disagree (or putting their hands up if they are in a classroom). This makes the split visible, and two or three reasons from each side can be asked for. The facilitator should rebuttal some of the statements, keeping the discussion calm and balanced and then ask if anyone has changed their perspective, inviting them to state why and move. Each statement can be closed by what current evidence, stakeholder and law settles the question. Examples of statements include:

- Something natural is safer than something made in a lab.
- Releasing engineered organisms into the wild is acceptable if it could eradicate malaria.
- Gene-editing for a disease is fine, but not for traits like height or intelligence.
- Any researcher that could be misused should not be published.
- Engineered crops should be judged by how they are made, not what they do.

This module is particularly important for students to learn how to spot a gap in current systems and analyse and synthesise evidence on the spot. Ingesting a new perspective and rebutting or building upon it is a core skill in all areas of life, not just the life sciences. By having students practice with a question they have likely never encountered before, this module introduces the field of synthetic biology but also what it means to think critically and make evidence-based decisions.

Module 4: Bacterial Transformation

In this practical, students will learn why and how bacteria take up foreign DNA in nature, then carry out a heat-shock transformation of competent *E. coli* with an ampicillin-resistance plasmid. They will use positive and negative controls to interpret the success of the transformation, predicting the outcome before observing the results the next day. After this workshop, students should also be able to describe the main elements of a plasmid vector (origin of replication, selectable marker, cloning site), explain how ampicillin kills bacteria and why a resistance gene would be needed.

Prior to the experiment, a slide deck can be delivered to introduce the following key concepts, once again using an interactive form where students are asked if they can first explain some of the terms:

- Why bacteria take up DNA, and what could go wrong
- Natural competence
- Artificial transformation
- Cloning and purification, with the example of human insulin
- Plasmid
- Ampicillin mode of action and the beta-lactamase gene

The protocol for the practical and troubleshooting approaches can be found at the end of this document. Students can be asked the following questions during the practical to keep them comprehending the practical and engaged at each stage:

Question	Answer
Why incubate on ice?	This prevents nucleases from degrading DNA, as enzymatic rates are slowed at cold temperatures Cold stiffens the membrane to set up a large temperature change during the heat-shock step.
What if the heat shock runs too long?	Cells might be damaged or killed as too many pores are created in the membrane, so fewer survive to form colonies and the transformation efficiency will drop. If the heat shock is too short, little plasmid DNA will be uptaken by the *E. coli* cell.
Why include recovery with LB?	After the creation of pores, cells will need to repair their membranes, and start expressing beta-lactamase protein from the plasmid before they encounter the antibiotic. They can only do this well if they are in recovery with LB, which is a nutrient-rich medium that promotes bacterial growth.
Why include AmpR in a construct?	It acts as a selectable marker: only cells that took up the plasmid survive on ampicillin, so this allows identification of the transformants. The antibiotic also keeps pressure on cells to keep the plasmid.
Why can recovery be shorter with ampicillin than other antibiotics?	Ampicillin only kills cells that are actively building a new cell wall, so it acts slowly on cells that have just been plated. Transformed cells have time to make beta-lactamase before they start dividing. Antibiotics such as kanamycin act faster and need a longer recovery step.

TIP! Ensure that students are briefed on the importance of labelling their tubes, as this is an easy way to mess up. Facilitators should be assigned to every desk to answer any questions and check that students are adding the correct volumes of reagents and using pipettes properly, as the volumes can be confusing when starting out. The day after the practical, students can analyse their plates to confirm whether their results matched their hypothesis and calculate transformation efficiency if colonies grew on the +DNA plate/ampicillin plate (colonies per ug of DNA).

Module 5: DNA Extraction

Students will collect cells from the inside of their cheeks, break them open, remove proteins and precipitate their own DNA, which will become visible as white strands. This practical is quick and allows students something to take home and might foster their fascination with

biology. After this practical, students are expected to be able to describe where DNA is found in a human cell, the purpose of each step in DNA extraction (lysis, protein digestion, salt and alcohol precipitation), explain how the properties of DNA (long, negatively charged, insoluble in alcohol) justify its extraction method and, finally, connect DNA extraction to real applications such as genetic testing, forensics and synthetic biology. An interesting hook to start off the practical could be to ask students to guess how long the DNA inside of one cell is. The 2 meters of DNA found per cell in the body means that unwinding all the DNA in our bodies and aligning it would allow us to reach the Sun and back many times!

The protocol for the practical and troubleshooting approaches can be found at the end of this document. The science behind each step, can be explained to students as such:

Step	**Reason**
Brushing	Cheek epithelial cells are constantly shed and replaced, so they can come off more easily. Increased cell count is proportional to increased DNA to extract.
Lysis buffer	Dissolves the lipid membranes protecting the cell and nucleus, which releases DNA into solution.
Protease at 50 degrees celsius	The protease digests proteins, include proteins wrapped around DNA called histones and nucleases that would degrade DNA. This temperature is close to the protease's optimal temperature, allowing DNA to be separated from histones and protected from degradation.
Salt	Positive sodium ions shield the negative phosphate backbone, so DNA strands can clump together instead of repelling each other.
Cold alcohol	DNA is insoluble in alcohol. When alcohol is combined with water, DNA comes out of the solution as white strands. Cold alcohol makes this precipitate form faster.
Gentle inversion	Long DNA strands tangle into a visible clump and shaking hard would shear them into pieces that would be too small for the naked eye to see.

Impact Evaluation

At the end of the sessions, students can be asked to rate themselves on a scale from 1 (strongly disagree) to 5 (strongly agree), in order to assess their understanding coming out of the workshop.

1. I can explain what DNA is and where it is found.

2. I can explain what genetic engineering or synthetic biology is.
3. I feel confident using laboratory equipment.
4. I would consider studying science after school.
5. I think synthetic biology can help to solve real-world problems.

They can then be asked about the most interesting thing they learned that day, and what unanswered questions they may still have to improve these sessions. This information can be gauged using a two minute online form linked to a QR code on the final slide, or on paper slips for those that might not have access to electronics. Overall, we recommend planning ahead and gauging the level of students ahead of the sessions and during them in order to deliver the most accessible synthetic biology material that can inspire the next generation of students to become engineering biologists. We hope this guide proves itself to be useful to your team!

Bacterial Transformation Workshop

The objective of this workshop is to familiarise students with common Molecular Biology techniques, crucial in synthetic biology workflows. The introductory talk session covers the basics of synthetic biology, such as the central dogma of molecular biology, as well as the purpose of transformation in synbio experiments. The practical session involves resuspending lyophilised (dry-frozen) empty plasmid vector DNA, preparing competent cells and transforming them via the heat shock method. The product of successful transformation would be scarce resistant bacterial cell colonies observed after an overnight incubation.

Materials (1 group)

- Resuspended DNA to be transformed (Provided by Imperial iGEM)
- Competent Cells (50µl per sample), 50 µL
- 2 x 1.5mL Microtubes
- LB Media (950µL per sample), 1900 µL
- Petri plates w/ LB agar and antibiotic (2 per sample): 2 x LB, 2 x LB-Amp

Equipment

- Floating Foam Tube Rack
- Ice & ice bucket
- Lab Timer
- Sharpie
- 42°C water bath
- 37°C incubator
- Sterile spreader or glass beads
- Pipettes and Tips (10µl, 20µl, 200µl recommended)
- Microcentrifuge (Provided by Imperial iGEM)

Method

1. Resuspend DNA in selected wells in the Distribution Kit with 10µl dH20. Pipet up and down several times, let sit for a few minutes. **(Done by demonstrators)**
2. Fill lab ice bucket with ice, and pre-chill 1.5ml tubes (one tube for each transformation, including your control) in a floating foam tube rack.
3. **Thaw competent cells on ice:** This may take 10-15min for a 260µl stock. Dispose of unused competent cells. Do not refreeze unused thawed cells, as it will drastically reduce transformation efficiency.
4. **Pipette 50µl of competent cells into two 1.5ml tubes.** Tubes should be labeled, pre-chilled, and in a floating tube rack for support. Keep all tubes on ice.
5. Label the tubes "+DNA" and "-DNA".
6. **Pipette 1µl of resuspended DNA into +DNA:** Pipette from well into appropriately labeled tube. Gently pipette up and down a few times. Keep all tubes on ice.
7. **Close 1.5ml tubes, incubate on ice for 30min:** Tubes may be gently agitated/flicked to mix solution, but return to ice immediately.
8. **Heat shock tubes at 42°C for 45 sec:** 1.5ml tubes should be in a floating foam tube rack. Place in water bath to ensure the bottoms of the tubes are submerged. Timing is critical.
9. **Incubate on ice for 5min:** Return transformation tubes to ice bucket.
10. **Pipette 950µl LB media to each transformation:** LB should be stored at 4°C, but can be warmed to room temperature before use. Check for contamination.
11. **Incubate at 37°C for 1 hour, using a water bath.** This step can be skipped or shortened, depending on timing.
12. **Pipette 100µL of +DNA onto an LB plate. Repeat for -DNA.** Spread with sterilized spreader or glass beads immediately.
13. **For -DNA and +DNA, spin down cells at 6800g for 3mins. This is done by a demonstrator. Discard 800µL of the supernatant, caring not to disturb the pellet. Resuspend the cells in the remaining 100µL, and pipette onto LB-Amp Petri plates** Spread with sterilized spreader or glass beads immediately. This increases the chance of getting colonies from lower concentration DNA samples.

Table 1. Plate map for the practical. Four plates are to be used, the participants are to fill this table during the recovery time (step 11)

Cheek Cell DNA Extraction: Laboratory Instructions

Capture Your Genetic Essence In a Bottle

Teacher's (Common) Station

Water bath at 50°C
Ice-cold bottle of 91% isopropanol or 95% ethanol on ice

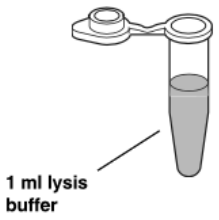
Students' Workstation (4 students per station)	Number required
Clear micro test tubes, each containing 1 ml lysis buffer	4
Blue micro test tube labeled "**prot**"	1
Pink micro test tube labeled "**salt**"	1
Clear, capless screw cap tubes	4
Assorted colored screw caps	4
Cytology brushes	8
5 ml round-bottom test tubes	4
Parafilm (small pre-cut pieces)	4
Disposable plastic transfer pipets	4
Foam micro test tube holder	1
Permanent marker	1
Disposable paper cup or beaker for waste collection	

Procedure for DNA Extraction and Precipitation

Steps 1 and 2: Collecting and breaking open cells

To get as many cheek cells as possible, you will use two brushes to collect the cells from your mouth. You will combine the cells you get from both brushes into one tube of detergent solution. Ample cell collection is critical for success. For best results, make sure you spend the recommended amount of time collecting and carefully transferring the cells.

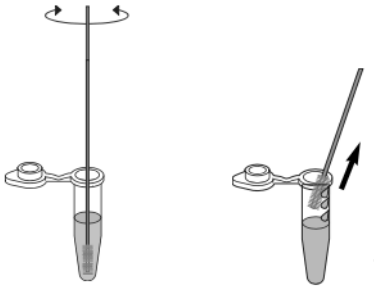
1. Obtain a clear micro test tube for yourself containing 1 ml of **lysis buffer**, and label it with your initials using a permanent marker.



2. Take the first brush and roll the bristles firmly along the inside of your right cheek and in the space between your cheek and gum for 1 minute. **For best results, make sure you spend the recommended amount of time collecting the cells**. Brush firmly, but don't hurt yourself.



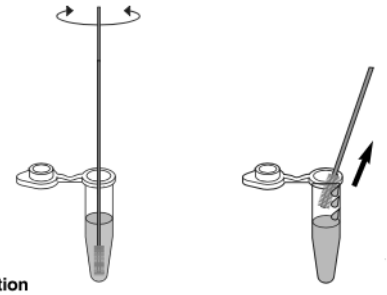
3. Place the brush with the cells into the tube containing lysis buffer. Swirl the brush around to release the cells from the brush into the buffer. Scrape the brush bristles across the top of the tube to transfer as much of the cells and liquid into the micro test tube as possible before disposing of your brush in the waste container.



4. Take a second, clean brush and collect cells from your left cheek, in between your cheek and gum, along the roof of your mouth, and under your tongue; again, try to collect as much cell material as possible.



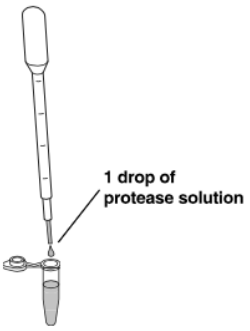
5. Place the brush with collected cells in the same tube as before, swirling the brush to release the cells and removing as much liquid as possible before disposing of the brush.



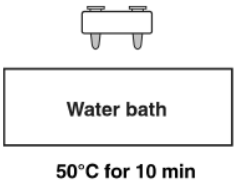
6. Cap the tube and gently invert it 5 times to mix.

Step 3: Removing proteins

1. Obtain the tube labeled "**prot**" and add 1 drop of protease solution (35 µl if you are using an adjustable micropipetor) to the micro tube containing your cell extract. Cap the cell extract tube and gently invert it 5 times to mix.

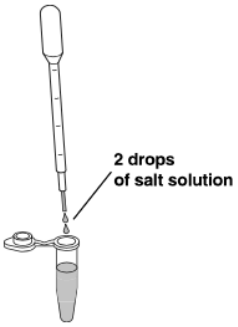


2. Place your cell extract tube in the foam micro test tube holder at your workstation and put the samples in a 50°C water bath (at the common workstation) for 10 minutes to allow the protease to work.

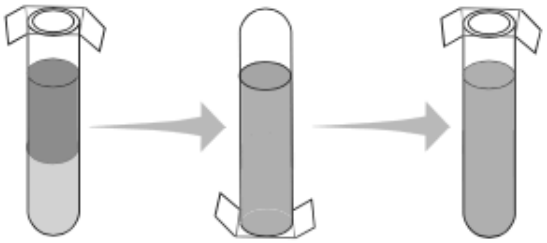


Steps 4 and 5: Making the DNA visible

1. Remove your micro test tube from the water bath and add 2 drops (70 µl if you are using an adjustable micropipetor) of "**salt**" solution. Cap the tube and gently invert it 5 times to mix.

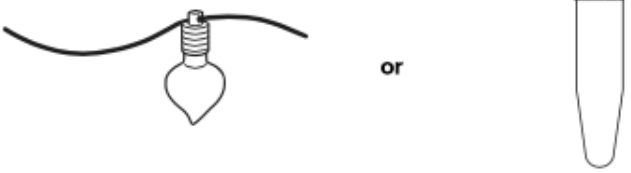


7. Place a piece of Parafilm over the top of the tube, put your thumb over it, and mix by inverting the tube 5 times. Look for any stringy, white or clear material. **This is your DNA!**



8. If you are going to make a DNA necklace, your teacher will provide you with a glass vial. With a plastic transfer pipet, carefully transfer the precipitated DNA along with approximately 750 µl to 1 ml of the alcohol solution into the vial. Then your teacher will show you how to seal the vial so you can complete the necklace.

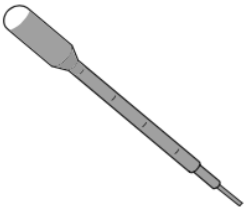
   If you are not going to make a DNA necklace, you can transfer and save your DNA in a screwcap tube. With a transfer pipet, gently withdraw your precipitated DNA along with about 500 µl of alcohol solution and transfer it into the screwcap tube. Tighten the cap and amaze your friends and family with your own DNA!



2. Label a 5 ml round-bottom test tube with your initials and transfer your cell extract into it.



3. (You may need to do this step at the common workstation. Consult your teacher for specific instructions.) Fill a transfer pipet with cold alcohol.



4. Tilt the round-bottom tube at a 45° angle and slowly add the alcohol, carefully letting it flow gently down the inside of the tube. You should be able to see two layers (upper and lower) forming. As you add the alcohol, pay close attention to the place where the alcohol and cell extract layers meet. Write down your observations.



5. Place your 5 ml tube upright either on the foam micro test tube holder or a test tube rack and **leave it undisturbed** at room temperature for 5 minutes.



6. After 5 minutes look again at the contents of your tube, especially in the area where the alcohol and cell extract layers meet. Do you see anything? Write down your observations. Compare your sample with those of your classmates.