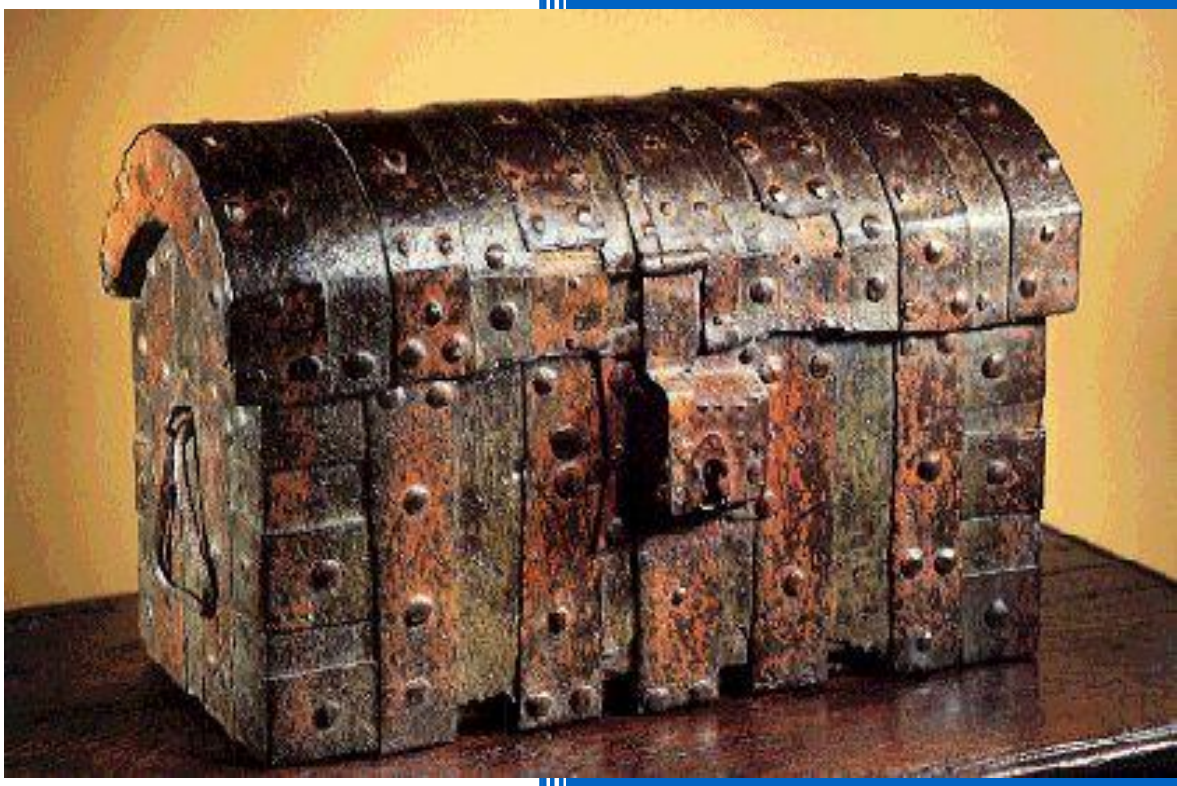
AMGEN® Biotech Experience

Scientific Discovery for the Classroom

United Kingdom

Puzzle rooms



AMGEN® Biotech Experience
Scientific Discovery for the Classroom
United Kingdom

Puzzle rooms

Metacognition Puzzle rooms

Metacognition is the ability of someone to consider the strategies that they use for learning. Those with good metacognitive abilities are more likely to do well in their studies, as they can identify a task, recognise strategies to complete it and adapt their approaches as required to complete the task. Since consideration of learning strategies and benefits is beneficial for learners, this short section explains how this 'Puzzle Room' activity can contribute to learning.

Cognitive science is research into how the mind processes thoughts and learning. Collaborations between cognitive scientists and education researchers has led to a better understanding of how students can remember things. It has been demonstrated that 'retrieval practice' helps to embed knowledge and skills in the long-term memory. Retrieval practice is the regular revisiting of key facts and ideas through practice, using a variety of quizzes and activities. The puzzle room has been designed to help with retrieval practice of the ideas, understanding and practical skills used through the ABE labs.

Additionally, leaving a gap between revisiting knowledge can help with memory. This is 'spaced learning' and is why you will probably use the puzzle room activity at a time distinct from doing the other ABE labs.

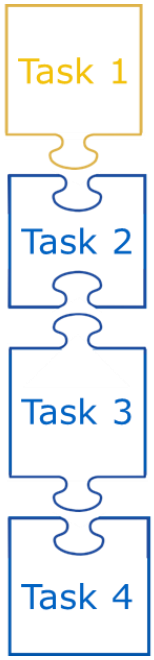
Background Puzzle rooms

Escape rooms originated in a virtual environment, with the first Escape Room video game being devised in 2004, before its transfer to a concrete Escape Room in 2007. Since then they have become extremely popular. In 2014 there were 22 companies running Escape Rooms in the US, in 2018 there were reportedly more than 2,000. Here, in the UK there are nearly 100 companies running Escape rooms. A BBC article summarised that, '*Games vary, not just in their themes, but also in their emphases.' 'It's clear from the way that the most popular sites have been selling out in advance for many months that there are hundreds of thousands of people who have enjoyed the genre over time in the UK*.' (https://www.bbc.co.uk/news/uk-england-35074346).

Within a school, the immersive experience, teamwork, focus, peer-learning and collaboration skills used in an Escape Room can be harnessed in a 'Puzzle Room' scenario. This allows development of a range of skills, whilst reviewing content and understanding in a fun way. In this particular puzzle room you will work in pairs, taking on the role of scientists working in a research lab. You will need to use your ingenuity, knowledge and practical skills to solve 4 puzzles, interpreting clues to rescue a poor defenceless creature and defeat a dastardly criminal mind.

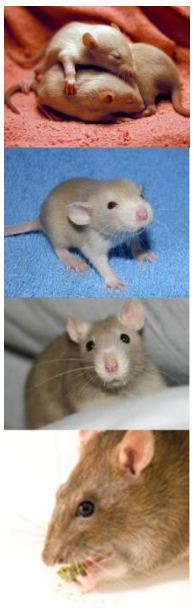
Task 1

Objective



Solve task 1 and use the answer to find the clue that will help you progress

Task 1 Cracking the code



As part of a team involved in ground-breaking research into genome mutation and **gene therapy*** you enter one morning to find the lab in disarray and Archie, one of the rats you have raised from a pup as one of a lively **mischief**** is missing.

Shocked, you look around and immediately notice a slip showing a series of 3-letter mRNA codons. Knowing that any thief audacious enough to get past your lab security and take one of your most valuable model organisms must be knowledgable in molecular biology you immediately deduce that this is a ransom note.

Aware that the 3-nucleotide mRNA codons each correspond to a single amino acid and that amino acids can be represented by a single letter code, you find your table that compares mRNA codons to amino acids produced. Using this you think that you should be able to decipher the ransom note.

* **Gene therapy** is introducing normal genes into cells in place of missing or defective ones in order to correct genetic disorders

** A **mischief** is a group of rats.

Task 1 Instructions

You need to use the information on mRNA codons, the amino acids that they encode during translation and their single letter code to decode the note. For example, to write 'cat' in mRNA codons you could have UGU (which is cysteine or C), GCG (which is alanine or A), then ACC (which is threonine or T).

Once you have deciphered the ransom note you need to use the correct location to find the clue to help you progress…

Task 1 Instructions (continued)

mRNA codon	Amino acid	Single letter code
AAU, AAC	Asparagine	N
AAA, AAG	Lysine	K
ACU, ACC, ACA, ACG	Threonine	T
AGU, AGC, UCU, UCC, UCA, UCG	Serine	S
AGA, AGG, CGU, CGC, CGA, CGG	Arginine	R
AUU, AUC, AUA	Isoleucine	I
AUG	Methionine	M
CAU, CAC	Histidine	H
CAA, CAG	Glutamine	Q
CCU, CCC, CCA, CCG	Proline	P
CUU, CUC, CUA, CUG	Leucine	L
GAU, GAC	Aspartic acid	D
GAA, GAG	Glutamate	E
GCU, GCC, GCA, GCG	Alanine	A
GGU, GGC, GGA, GGG	Glycine	G
GUU, GUC, GUA, GUG	Valine	V
UAU, UAC	Tyrosine	Y
UAA, UAG, UGA	STOP	
UGU, UGC	Cysteine	C
UGG	Tryptophan	W
UUU, UUC,	Phenylalanine	F
UUA, UUG	Leucine	L

A table to show mRNA codons, the amino acids that they translate to and the single letter code used to represent the amino acids.

Task 2

Objective

Solve task 2 and use the answer to reveal the first and second digits of the 6-number code

Task 2 How much is that?

Now you know is it a dastardly, twisted Biotech genius who has stolen Archie your ACHOO rat, you know that there is no option but to attempt the fiendish puzzles the rat-napper has set and work out the code for the padlock as quickly as you can to free Archie. Gathering your wits you prepare for the challenge to find an exact volume, with pen and paper at the ready to note down the first 2 digits of the code that unlocks the padlock.

Task 2 Instructions

To complete this task you will need to use your most accurate micropipetting skills.

Materials

EQUIPMENT	SUPPLIES
P-20 micropipette (2-20 µL) Disposable pipette tips Waste container 30 cm rulers Graph paper	Microfuge tube of food dye Piece of filter paper

Health and Safety

As good laboratory practice you should clear up any spills immediately and wash your hands well with soap after completing the practical work.

Methods

PART A: GATHERING DATA

1. Check that you have all of the materials.
2. Set the micropipette volume to 2.0 µl in the display window. Press a tip onto the micropipette and draw up the food dye, working at eye level.

Task 2 Instructions (continued)

⚠ Never set the P-20 micropipette lower than 2.0 µL or higher than 20.0 µL or you could damage the equipment.

⚠ When loading the micropipette, only press the plunger to the first stop or you will draw too much solution into the pipette tip.

3. Dispense the food dye onto the filter paper.

⚠ When dispensing liquid from the micropipette, press the plunger to the second stop in order to dispense all of the liquid.

4. Repeat steps 2 and 3 four further times, to dispense 5 µl, 10 µl, 15 µl and 20 µl volumes onto the same sheet of filter paper.
5. Once the food dye has dried sufficiently that it won't smudge, use a ruler to measure the height and width of the droplets. Record your results in a table like below.

Volume of droplet (µl)	Height (mm)	Width (mm)	Average diameter = (Height + width) ÷ 2
2			
5			
10			
15			
20			

PART B: PLOTTING A CALIBRATION CURVE

1. On graph paper, plot the volume of droplet (on the x axis) against the average droplet diameter (on the y axis).
2. Draw a line of best fit.

PART C: READING THE EXACT VOLUME

1. Calculate the average droplet diameter for the mystery droplet left by the rat-napper.

2. Use your calibration curve to determine the volume that corresponds to this average droplet diameter.

As you complete the task you discover what looks like it could be faint writing on the back of the droplet left by the rat-napper. Use the UV pen to reveal the message before proceeding.

Task 3

Objective

Solve task 3 and use the answer to reveal the third digit of the 6-number code

Task 3 How big is it?

Although you do, of course, have a full DNA profile for Archie with the size of 10 **VNTR*** sequences recorded, you don't know which one the rat-napper has chosen to amplify and purify. You realise you will need to determine the size of the VNTR by comparing the sample to a DNA ladder using gel electrophoresis. Missing his quivering whiskers and little sneezes, and galvanised by the photo of Archie as a pup with his mischief you immediately start preparing for gel electrophoresis

* **VNTR** stands for Variable Number Tandem Repeat: A location in the genome where a short nucleotide sequence is repeated. As the number of repeats varies between individuals, so does the length of the sequence of repeats. As the number of repeats at each site is so individual, VNTRs can be used to generate a DNA profile.

Task 3 Instructions

To complete this task you will need to use your ability to complete and interpret information from gel electrophoresis.

Materials	
EQUIPMENT	**REAGENTS**
P-20 micropipette (2-20 µL) Disposable pipette tips Waste container Microfuge tube rack Gel electrophoresis tank with pre-prepared 2% agarose gel Gel staining trays	Microfuge tubes containing: - 8 µL of Archie's VNTR - 4 µL loading dye - 10 µL of 100 bp DNA ladder 1 X sodium borate (SB) buffer Gel Red DNA stain

Task 3 Instructions (continued)

Health and Safety

In this laboratory you will be using biological materials, albeit in tiny amounts, and they should be used in a controlled way, using techniques that mirror the aseptic techniques used in microbiology. All of the materials that come into contact with the biological materials (pipette tips, microfuge tubes and agarose gels) will be autoclaved to denature the DNA before disposal.

The loading dye is low hazard, but can stain skin, so try to avoid contact with it as good practice would dictate. You should wear safety glasses and chemical-resistant gloves when using the sodium borate (SB) electrophoresis buffer, to avoid contact with the borate in the solution.

The electrophoresis chamber contains 1 X SB buffer at 130 volts. To prevent contact with the liquid during electrophoresis the tanks are designed so that the electric supply is cut off whenever the lid is removed. Additionally, all connections are plastic coated so that there is no possibility of touching a metal contact which is live at 130 volts.

GelGreen is used to stain the DNA. Whilst **not** mutagenic like some stains, this is a substance that can bind to DNA (of which you have lots), therefore you will need to wear chemical resistant gloves, a lab coat and eye protection if you are to complete this part of the procedure.

As good laboratory practice you should clear up any spills immediately and wash your hands well with soap after completing the practical work.

Methods

PART A: SAMPLE PREPARATION

1. Check that you have all of the materials.
2. Add 2.0 µl of loading dye to the 8.0 µl of Archie's VNTR left for you in the fridge by the rat-napper.

Task 3 Instructions (continued)

Methods

PART B: ELECTROPHORESIS

3. Make sure that the wells in your gel electrophoresis unit are located near to the negative (black) electrode.

If there are "dimples," add *very small* amounts of buffer to the electrophoresis box. While the gel needs to be completely under the buffer, you don't want too much buffer in the box, as this will allow the electrical current to run through the buffer and not the gel.

4. Using a fresh pipette for each sample, dispense 10.0 µl of 100 bp ladder into one lane of the agarose gel and 10.0 µl of Archie's VNTR with loading dye into the adjacent lane. As you are likely to be sharing this agarose gel with other pairs, do make a note of which lanes you have loaded your sample into.

Be careful not to place your pipette tip into the well or you might puncture the gel and lose your sample out the hole.

Only press the micropipette plunger to the first stop when dispensing samples to avoid getting air bubbles in the buffer. The sample will then sink into the well. When all the samples have been loaded, close the cover tightly over the electrophoresis box.

5. When all the samples have been loaded, close the cover tightly over the electrophoresis box. Lower the cover in a horizontal motion, so that the samples don't spill.

6. Connect the electrical leads to the power supply. Connect both leads to the same channel, with negative electrode to negative electrode (black to black) and positive electrode to positive electrode (red to red).

7. Turn on the power supply and set the voltage to 100 V.

8. After two or three minutes, check to see if the orange loading dye (Orange G) is moving toward the positive (red) electrode. If it's moving in the other direction—toward the negative (black) electrode—check the electrical leads to see whether they are plugged in to the power supply correctly.

Task 3 Instructions (continued)

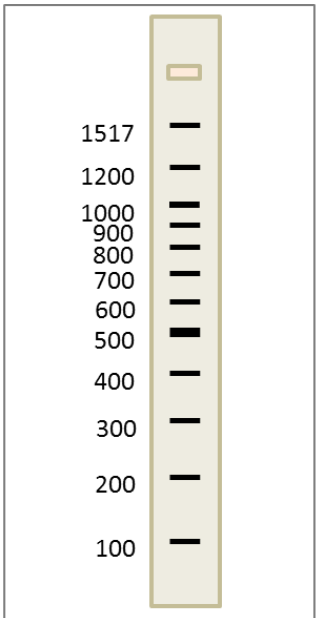
Methods

PART C: STAINING AND VISUALISATION

9. After 20 - 30 minutes electrophoresis, the gel needs to be stained to allow the DNA to be visualised. This will be done using GelRed. Whilst **not** mutagenic like some stains, this is a substance that can bind inside DNA (of which you have lots), therefore you will need to wear chemical resistant gloves, a lab coat and eye protection if you are to complete this part of the procedure.
10. Turn off the Powerpack. Carefully slide the gel from the electrophoresis gel tray into the plastic gel staining tray.
11. Cover the gel with GelRed 3 x solution and allow the gel to stain for 30 minutes.
12. Wearing chemical resistant gloves, carefully place the gel onto the glass plate of the UV transilluminator. You should be able to see the DNA stain fluorescing without using the hood and camera.
13. Use the hood and camera to take a photo of your gel for analysis.
14. Once you have obtained an image of the gel, place the gel in the box or autoclave bag on your teacher's desk for disposal.

PART D: WORKING OUT HOW BIG IT IS

15. Work out how big the VNTR from Archie is by comparison to the 100 bp DNA ladder, which contains fragments of known size.
16. The third digit of the code for the padlock is the number of 100 bp in the length of the VNTR. (The VNTR size divided by 100.)



A diagram of fragment sizes in the 100 bp DNA ladder

Task 4

Objective

Solve task 4 and use the answer to reveal the final 3 digits of the 6-number code

Task 4 BLAST, there's a change

Maverick with his aviator shades in Top Gun, Iron Man with his Von Zipper sunglasses. Possibly they were just deflecting the Sun. Or maybe, along with 1 in 4 of the population Tom Cruise and Robert Downey Jr are Sun sneezers who carry the ACHOO SNP.

To help sun-sneezing superheroes throughout the universe you must recover Archie the ACHOO rat, who is part of a personalised medicine trial testing a cure.

You try to remember as much as you can about bioinformatics: how the **N**ational **C**entre for **B**iotechnology **I**nformation (NCBI) runs a database of biological information; how, like iTunes you can use search functions to find information and link to other, related items of interest; how there is a **B**asic **L**ocal **A**lignment **S**earch **T**ool (BLAST), that can compare sequences to find mutations… Recalling what you know about bioinformatics you prepare for the final task.

Task 4 Instructions

You will be performing a Nucleotide BLAST (matching up a reference 'normal' DNA sequence to the DNA sequence from Archie). This should allow you to find any differences between the DNA and to locate the ACHOO mutation. Hopefully this contains the ACHOO SNP to find the position of the mutation).

Task 4 Instructions (continued)

1. Make sure that you can open the DNA sequence file, containing DNA sequence from a reference sample and from Archie.
2. Go to the NCBI BLAST website
3. http://blast.ncbi.nlm.nih.gov/Blast.cgi
4. Select '**nucleotide blast**', since we will be comparing a DNA sequence (sequence of nucleotides) to a DNA sequence (sequence of nucleotides).
5. From the nucleotide blast page, click the box to choose the option to '**Align two or more sequences**'.
6. A second text box will appear.
7. Copy the reference sequence for ACHOO from the file, including the ">" symbol and the name, and paste it into the top text box. This is the box underneath where it says '**Enter Query Sequence**'.
8. Copy the DNA sequence from Archie from the file, and paste it into the bottom text box, again including the ">" symbol and the name. This is the box underneath where it says '**Enter Subject Sequence**'.
9. Click "BLAST."
10. When your search is complete, you will see a window with the BLAST results. As you scroll down it shows a graphic summary, dot matrix view, descriptions and alignments of the two DNA sequences you entered above.
11. To make it easy to find any differences between the 2 sequences, click the '**Formatting Options**' link located near the top of the page.
12. Find the **Alignment View** and use the drop-down menu to choose '**Query-anchored with dots for identities**'. (This refers to the reference sequence as the query and will then display the reference sequence at the top with Archie's sequence aligned below. Dots are used to show nucleotides that are identical and **letters are used to show nucleotides that differ**.
13. Click the '**Reformat**' button.
14. Scroll down the page to the '**Alignments**' section, to see if there are positions where Arche's sequence differs from the reference sequence, by looking for a place where there is a letter instead of a dot, showing that there's been a change in the nucleotide at that position. Note: the numbers at the ends of the lines refer to the position of the nucleotide.

Task 4 Instructions (continued)

Analysis

Are there any differences between the DNA sequences?

At what position on chromosome 2 does the single nucleotide polymorphism occur?

Armed with the last 3 digits of the single nucleotide polymorphism position (the location of the ACHOO mutation on chromosome 2) you now have all 6 digits required to open the box and complete the puzzle room.

AMGEN® Biotech Experience
Scientific Discovery for the Classroom
United Kingdom

Reflection

Metacognition Puzzle rooms

To gain greatest benefit from the Puzzle Room activity you need to reflect upon the learning experience. Employing metacognition (considering the strategies that you have used for learning) will let you recognise the skills and strategies that you have developed and which are applicable to other parts of your studies.

What social skills did I use whilst completing the Puzzle Room activity?	
How could these skills be of benefit in other parts of my studies?	
What scientific skills did I use whilst completing the Puzzle Room activity?	
What scientific skills would I benefit from practising?	
What knowledge did I use whilst completing the Puzzle Room activity?	
What knowledge would I benefit from revising again?	
What were the benefits of completing the Puzzle Room activity?	