הפקולטה לחינוך
Faculty of Education
אוניברסיטת בר-אילן
Bar-Ilan University

הפקולטה לחינוך
Faculty of Education

משרד החינוך, המזכירות הפדגוגית
הפיקוח על הוראת הביולוגיה
המרכז לפיתוח ותמיכה במעבדות הביולוגיה
בבתי הספר

A genetically engineered bacterium as a biosensor for presence of pollutants and changes in temperatures

Use of engineered bacteria to identify substances in the environment that cause damage to DNA and activate DNA repair mechanisms.

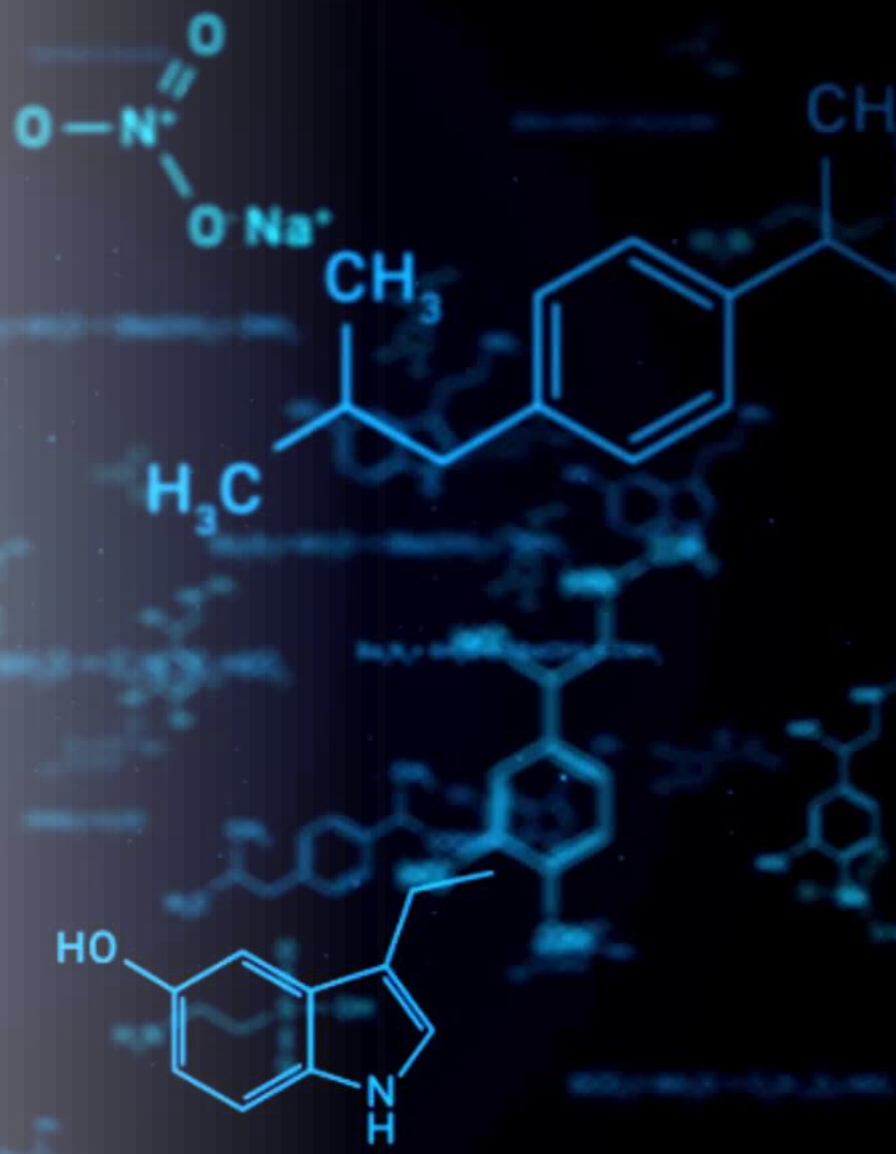
The bacteria were engineered with a plasmid containing control areas: a reporter gene, a selector gene ~~and a~~ that are activated when DNA damage occurs.

The selector gene - confers resistance to the antibiotic ampicillin and the reporter gene allows us to quantify the expression level of the promoter which corresponds to the level of damage caused to the DNA.

In this activity we will test the effect of increasing concentration of ethanol on the level of expression of these genes.

It is possible to test the effect of other conditions or substances on the expression of the reported gene:

exposure to high temperature,
exposure to salt, pollutants, toxins...



Suitable for teaching subjects such as:	Enzyme activity.
	Antibiotic resistance
	from DNA to protein.
	Genetic engineering and gene expression.
Prior knowledge required:	Control of gene expression in prokaryotes
	lactose operon
	Genetic engineering and transformation in bacteria.

Laboratory skills:

- Using a micropipette

- Preparation of different concentrations of Ethanol

- Using color scale editing features / applications

- Seeding and growing of bacteria in liquid medium and on solid medium.

- **Recommended schedule:**

- The duration of the laboratory is about an hour and a half.

In addition:

- It is possible to add tests of additional influencing factors.

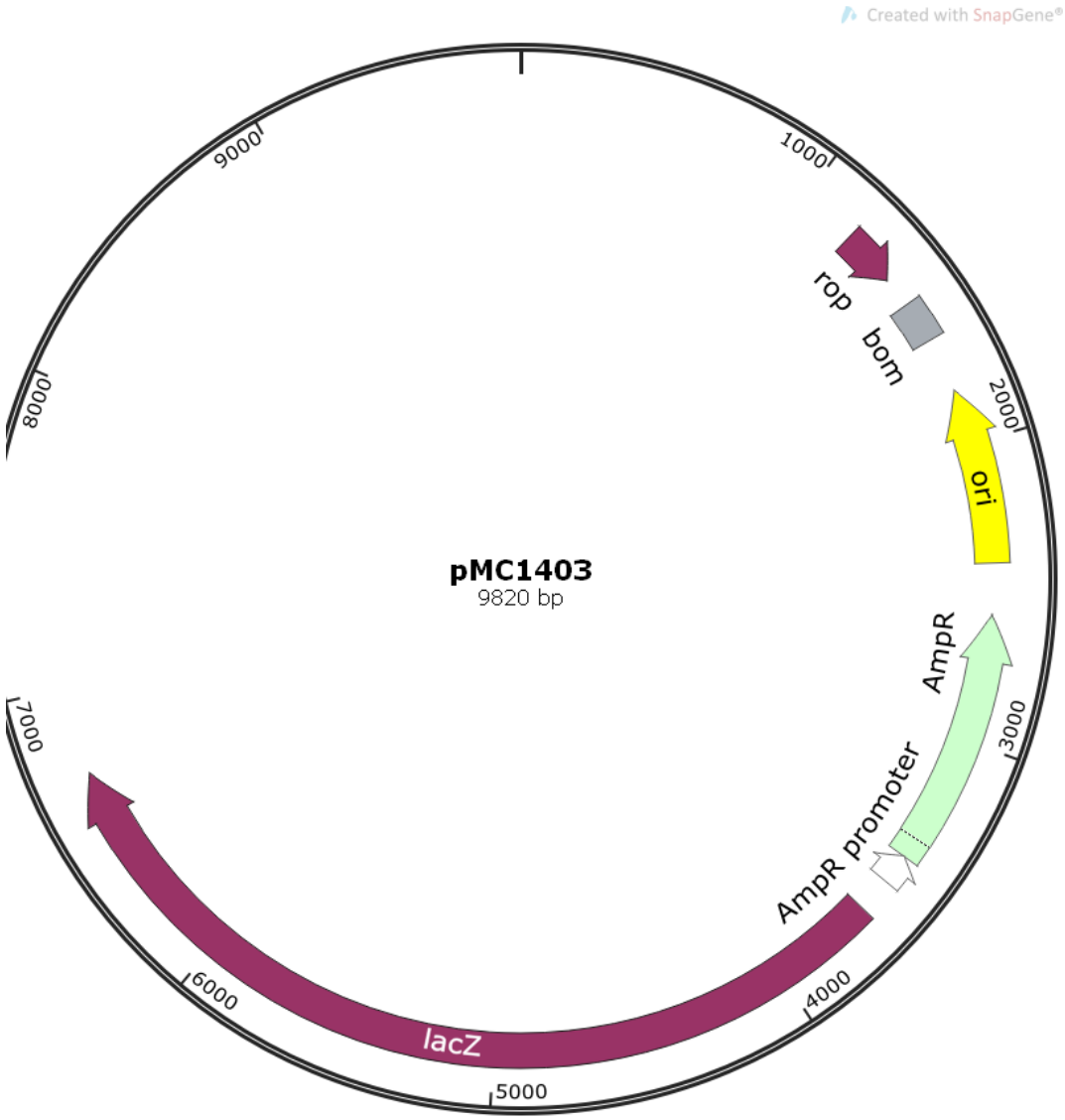
- It is possible to engineer (transform) bacteria to learn about the transformation prosses.

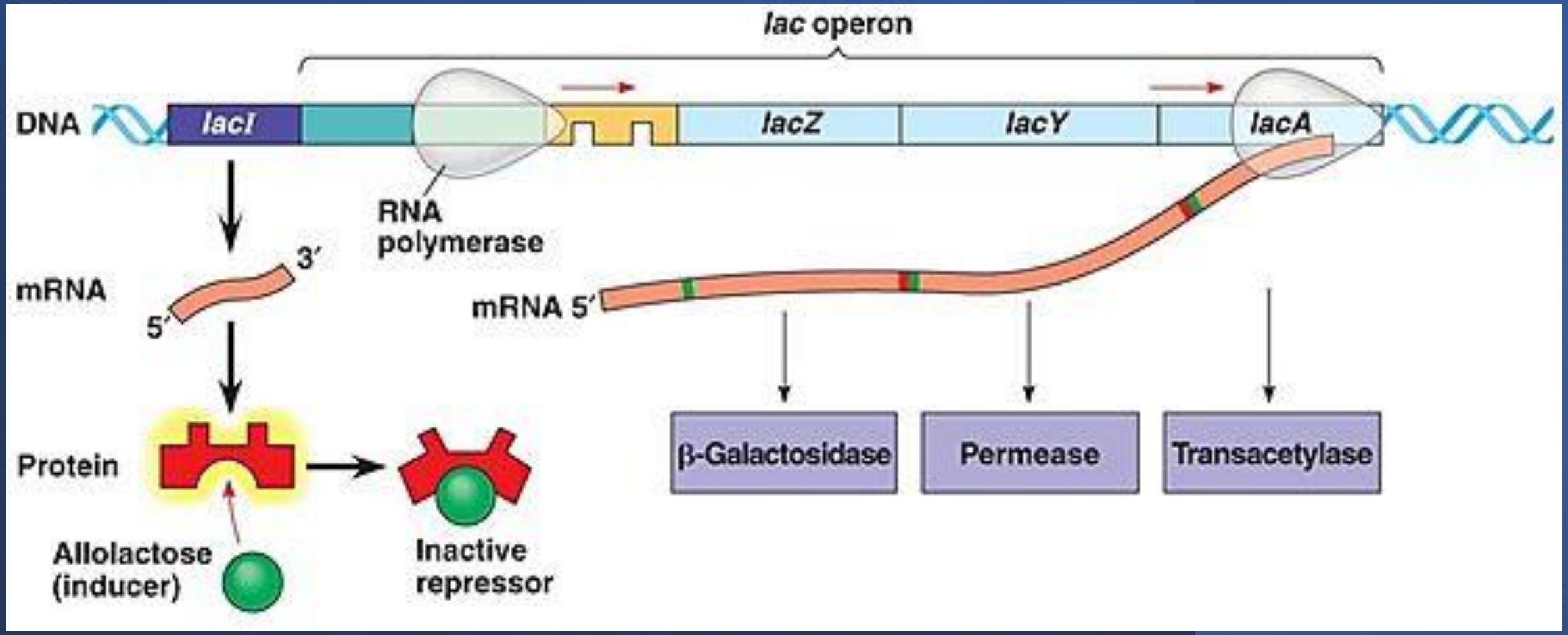
Type of bacteria: E.coli
The plasmid: pMC1403 contains:

Promoter for genes that allow DNA repair. Activated when DNA damage occurs. stress-responsive promoter sequence.

The lacZ gene - part of the lactose operon. Codes for an enzyme β-galactosidase, cleaves lactose into monosaccharides. Possible substrates are: lactose, x-gal.

The X-GAL artificial substrate: the degradation of X-gal by the enzyme creates a blue colored product. The intensity of the blue color indicates the level of the β-galactosidase enzyme and its rate of action.


Created with SnapGene®
pMC1403
9820 bp
1000
2000
3000
4000
5000
6000
7000
8000
9000
rop
bom
ori
AmpR
AmpR promoter
lacZ


lac operon
DNA
lacI
lacZ
lacY
lacA
RNA
polymerase
mRNA
3′
5′
mRNA 5′
Protein
β-Galactosidase
Permease
Transacetylase
Allolactose
(inducer)
Inactive
repressor

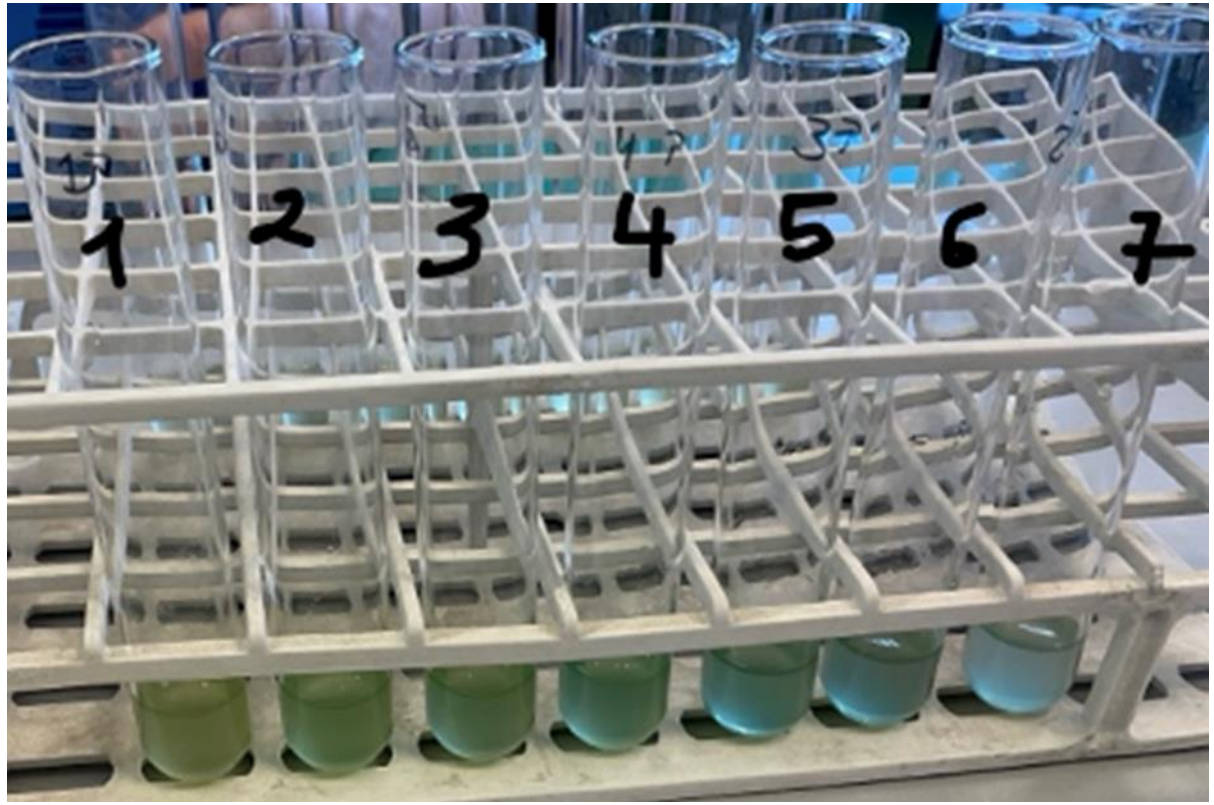
Preparing the experiment :

Preparation the bacteria:

- Design a plasmid
- Insert the plasmid to an E. coli
- Grow the bacteria with antibiotics

- Preparation of different concentrations of ethanol with LB
- Adding bacteria
- Adding X gal
- Waiting 30 minutes
- Color observation

Table 1. Preparing the experiment				
Tube number	Volume of bacteria (µL)	Volume of ethanol 99% (µL)	Volume of pure LB (µL)	Final concentration of ethanol (%)
1	1000	0	1000	
2	1000	100	900	
3	1000	200	800	
4	1000	400	600	
5	1000	600	400	
6	1000	800	200	
7	1000	1000	0	



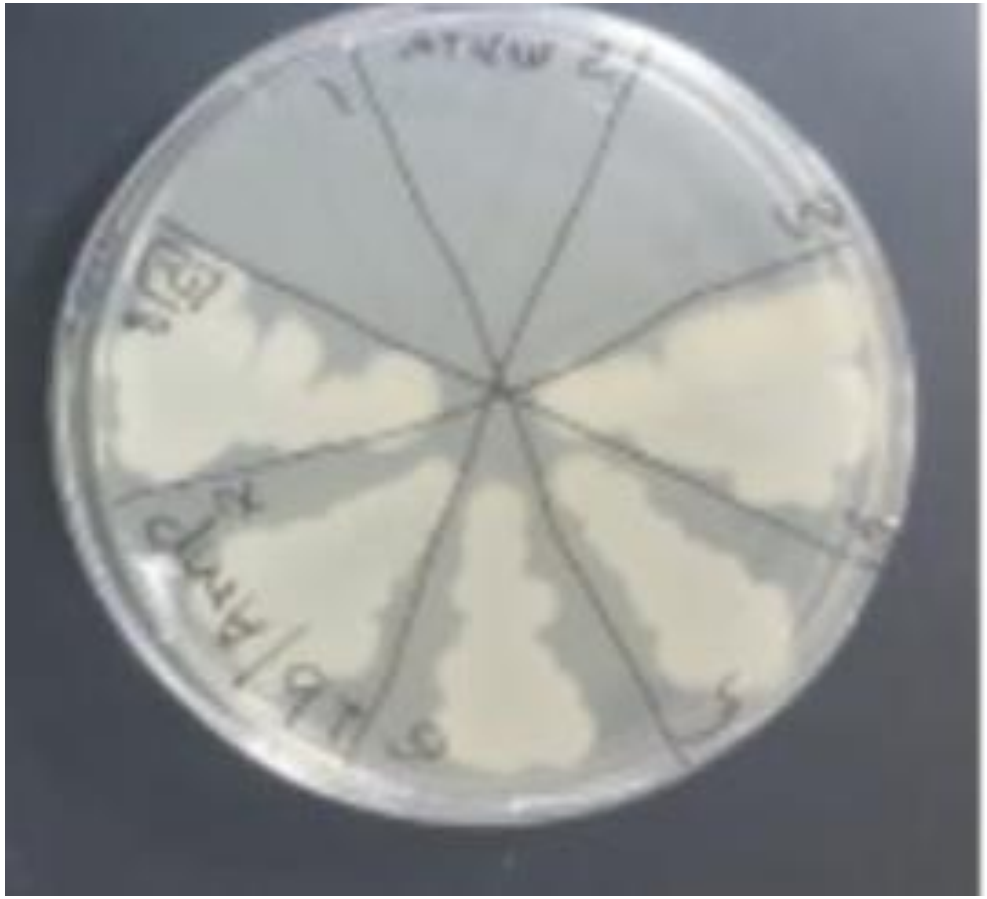
The measurement method:

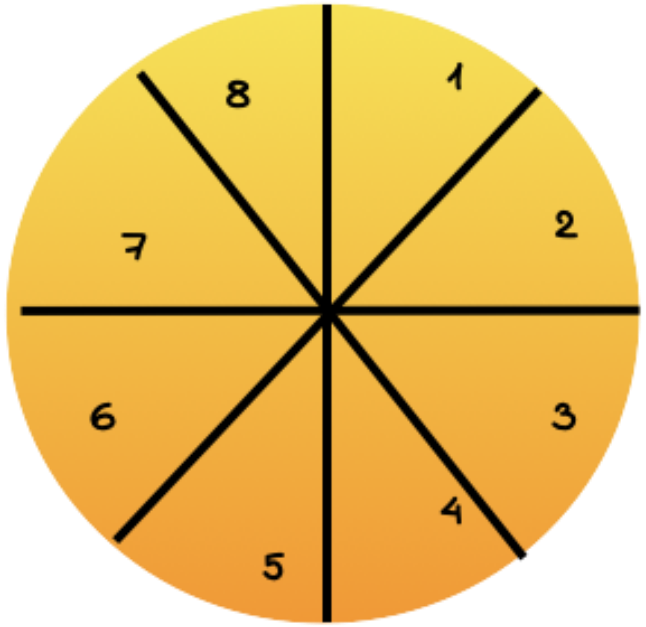
Using a color scale:

Using certain applications, the color obtained in each treatment is sampled (from a photograph) and compared to a color scale. With the RGB method, the resulting color can be quantified into quantitative values. or can be compared to a reference color.

Ethanol concentration

Activation of the selective resistance gene through exposure to varying concentrations of ethanol





- Discussion of results, research skills:

  - An explanation of the results of each of the treatments.
  - Importance of the control test tubes.
  - Constant factors kept in the experiment and their importance in the experimental setup.
  - Are the results quantitative or qualitative?

Other influencing factors that activate DNA repair mechanisms:

- Ethanol
- High temperature
- stress conditions (salt, oxigen, radiation)
- toxins and pollutants

Applications in synthetic biology and citizen science:

Building a system that regularly samples water sewage, and polluting factories and checks for pollutant and toxins.