Introduction: from maintaining our body metabolism to keeping

Literature, Play

Introduction: Genetic Engineering in one of the most evolved concept in science today that has open many doors to new scientific discoveries.

It has enabled mankind to modify an organism's DNA to change any aspect of an organism for a particular purpose. By conducting bioluminescent experiment an organism can be given access to a new DNA on a plasmid through the process of DNA transformation. A plasmid is a small circular piece of DNA that is not associated with the chromosomes of host cell. The experiment requires 2 days with 10 hours of work. The experiment can be easily completed over the weekend if bacterial cultures are prepared on a Friday night.

What is Bioluminescence? Proteins are the most important component for the survival of bacteria and all living organisms. These are the tiny nano-machines that control everything in our body; from maintaining our body metabolism to keeping our heart beating. Cells use DNA code to make protein in our bodies whereas; bacterial bioluminescence is created by bacterial luciferase which is a protein that causes chemical reaction with a Flavin MonoNucleotide (FMN) molecule. Light is created when the chemical reaction takes places.

Even if your experiment is not successful, you will get bioluminescence as we have included a strain of E. coli on the plate that has pJE202 plasmid DNA. Content for Experiment 1 – LB Amp Agar 40g/L Concentration (15mL tube to mix with 150mL water) 1 – LB Agar 40g/L Concentration (15mL tube to mix with 150mL water) 8 – Tubes LB Amp Broth 23 g/L Concentration (15mL tube to mix with 200mL water) 1 – 250 mLglass bottle for pouring

plates (fill with 150mL water)5 – 1mLSyringes (100uL = 1/10 mL)1 – 5blunt end tips14 -Petri Plates1 –Micro-centrifuge tube rack 5 – Inoculation Loops / Plate spreader / Pairs of Nitrile Gloves in plastic bag 25~ -micro-centrifuge tubes 6 – 1. 5mL micro-centrifuge tubes containing LB 50mL centrifuge tube for measuring liquid volume 1 – 1. 5 mL w/ bacterial transformation buffer 25mM CaCl2, 10% PEG 3550, 5% DMSO Perishable content · E. coli BL21/DH10B/DH5? strain(non-pathogenic) · pJE202 plasmid Ampicillin Resistant enough for 5 reactions· Bacterial Strain transformed with pJE202 plasmidPreparation Time· 1 hour to make plates (you will need more time if it's your first time making plates)· 1 minute to streak out bacteria on an LB Agar plate· 12-18 hours for bacteria to grow (easiest ways is to leave it overnight) Day of Experiment· Approximately 5 minutes in mixing together sample plasmids and transformation mix· Refrigeration time of 30 minutes (do not Freeze)· Heat shock of 30 seconds in warm water with temperature of (42ºC/108ºF)· Addition of LB media in cell solution, which will take one hour· Incubation period of 1 hour, for 3 hours if leaving it in room temperature· 10 minute or plate preparation of bacterial solution+ 10 minutes to let it dry· 24 hours for incubation of plates at 37ºC (99ºF) for 12-16 hours or room temperature for 16-24 hoursPlatesMaking plates take approximately 1 hour. Agar plates are an important part of this experiment; they provide a solid media nutrient source for bacteria to grow.

Usually LB media is used which contains a carbon source, a nitrogen source and salt. Most of the bacteria like salt. Plates making process 1. Take

Agar media and put it in a 250mL glass bottle. LB agar is used to grlw bacteria, yeast, and other micro-organism. You can easily but it from Amazon online store. https://www.

amazon.
com/Nutrient-Lysogeny-Preparation-Plates-Project/dp/B075GZTZF3/ref=
sr_1_4? s= industrial&ie= UTF8&qid= 1513404312&sr= 1-4&keywords=
Agar+media#feature-bullets-btf 2. Add 15 mL of water to the glass bottle. Measure the exact amount of water with the help of conical tube that is used for measuring liquids. https://www.

amazon.
com/EarthOx-Sterile-Centrifuge-Tubes-12000xg/dp/B0141KBAXK/ref=
sr_1_7? s= industrial&ie= UTF8&qid= 1513404450&sr= 1-7&keywords=
conical+tube 3. Heat the bottle for 30 seconds in a microwave. Be very careful while doing it and make sure you don’t over boil it. Also, don’t close the lid tightly, just place it on top of the glass bottle and give it a slight turn. 4.

When the liquid turns yellow and somewhat see-through it means it’s done. It will take 2-3 minutes approximately. Take the bottle out of the oven and leave it for at least 30 minutes to cool down. Give it a swirl after every few minutes so that it doesn’t solidify. 5. Pour the LB agar in plates while it is still warm. Remove the lid of the plates one by one and pour the LB Agar. Cover the plates after pouring the mixture in them.

Make sure to only pour enough LB Agar to cover the bottom of the plates only. Get easy to handle petri plates that are also breakage proof. https://www. amazon. com/SEOH-Petri-Dish-Sterile-Vented/dp/B0015T0LZO/ref= sr_1_3? s= industrial&ie= UTF8&qid= 1513403720&sr= 1-3&keywords= Petri+Plates

6. Leave the plates for at least 1 hour to cool down. If you want you can put the plates in refrigerator but don't freeze. Plates should be solid at the time of use.

If possible, leave the plates out for couple of hours preferably overnight to get rid of condensation. After that, store it upside down in your fridge (at 4 ºC) so that any condensation doesn't drip in plates either. It is highly important to take care of condensation because it can permit rogue bacteria and yeast to grow. If you store these plates in fridge they can last for up to a month. Competent Bacterial Cells for Transformation Competent bacteria is that bacteria or yeast cell that allows foreign DNA to permeate. Cell walls normally do not allow anything foreign from entering the cell but by creating a chemical reaction we can change that.

The bioluminescent plasmids will work once the bacteria cells we created enter the cell. This is called the transformation process. Through this process we trick the bacteria by putting all the synthetic material in to a DNA and make the bacteria think that this DNA is it's on. Once the bacteria think that it is their own DNA, they start making luciferase protein and perform the chemical reaction releasing a photon and creating light. Components of

bacterial transformation mix: Now make the bacterial transformation mixture by adding:• 10% Polyethylene Glycol (PEG) 3350https://www. amazon.

com/Basic-Care-ClearLax-Polyethylene-Laxative/dp/B074F2X43S/ref= sr_1_cc_4_a_it? s= aps= UTF8= 1513405001= 1-4-catcorr= Polyethylene+Glycol+3350 • 25mM Calcium Chloride (CaCl2)https://www. amazon.

com/Calcium-Chloride-Midwest-Winemaking-Supplies/dp/B015X6LGJY/ref= sr_1_5? s= industrial= UTF8= 1513404759= 1-5= Calcium+Chloride · 5% Dimethyl Sulfoxide (DMSO)https://www. amazon. com/Liquid-DMSO-99-995-Dimethyl-Sulfoxide/dp/B01MFDSL6J/ref= sr_1_4? s= industrial= UTF8= 1513404810= 1-4= Dimethyl+Sulfoxide PEG 3350 plays an important role in the transformation process. Both DNA and cell walls are negatively charged hence, they reject each other. PEG 3350 functions to shield DNA charge and make it easier to infiltrate the cell wall. It also helps transport DNA into the cell and makes cell membrane more absorbent.

Just like PEG 3350, CaCl2 also neutralize the negative charge of DNA and makes it easier to permeate the cell. DMSO also serves the function of making the cell wall more prone to infiltration. Also, sometimes when DNA folds in to complex structure which is difficult to pass, DMSO helps to break such complex structures. 1. Use an inoculation loop to scrape bacteria from a bacteria plate and streak it on to a new plate. Now take out a tube of dried E. coli BL21 and add water to the top. Shake the tube until the content is well dissolved.

2. Next, put 100 uL of bacteria solution on to a new LB plate with the help of your pipette. Use your inoculation tube with a fresh tip to gently spread the bacteria.

Make sure to put the plates in warm and consistent temperature and not in area of high temperature variation. It is also advisable to use fresh bacteria for transformation as it will greatly increase the success of your experiment.

3. Take out a new centrifuge tube and pipette 100 uL of transformation mix.

https://www. amazon. com/Transfer-Pipette-Graduated-Karter-Scientific/dp/B005Z4QVZ4/ref= sr_1_6? s= industrial= UTF8= 1513405367= 1-6= pipette. 4. After that, use an inoculation loop to scrape some bacteria off of the fresh plate that you made and mix it with the transformation mix. Make sure to scrape the bacteria until it is completely fills the hole of the loop before adding it in the transformation mixture.

Mix until all the clumps have disappeared and it turns smooth. This will require gentle pipetting for few minutes in up and down motion. Avoid creating any bubbles; make sure that transformation mixture is very cloudy.

If not, add more bacteria in to it until it turns translucent and you cannot see through the liquid anymore. Make as many tubes, as many experiments you want to perform and store them at 4ºC (39ºF) in the fridge. https://www.

amazon. com/SEOH-INOCULATING-LOOP-3wire-handle/dp/B0018RRNBS/ref= sr_1_4? s= industrial= UTF8= 1513403901= 1-4=-+inoculating+loops 5. Now take out 10 uL of pJE202 plasmid from DNA tube while using pipette and add it to your competent cell mixture. 6. Leave this tube in the fridge for 30 minutes. 7. After that, incubate the tube in warm water of 42ºC (108ºF) temperature for 30 seconds. 8.

Take 1. 5 mL of room temperature water and pour it in one of the LB media micro-centrifuge and then shake it well to dissolve the LB. you can also fill the tube till the top if you don’t want to do the measuring. 9. Now add 500 uL of LB media in the competent mixture.

Use your pipette to do that. 10. Incubate this tube for 1 hours at 37C (99F) or for 2 hours if you plan to put it at room temperature. This is an important step for bacteria to recover and replicate the DNA and perform the process. 11.

Take out the LB Amp plate from the fridge and put it at room temperature. 12. Add 200 uL of LB Media in to competent cell mixture in the LB Amp plate with the help of a pipette. 13.

Just like before, flip the plate upside down to avoid condensation from forming on your bacteria. 14. Incubate the plate for 16-24 hours at below 30ºC (80ºF) or for 24-48 hours at room temperature. 15. If little white dots start appearing on your plate, it means that your genetic engineering experiment is a success. If not, you can give it another try.

If your experiment is a success take your plate in a dark room and you might be able to see small glowing dots on your plate. The glowing whitish or yellowish dots are actually the bacterial colonies that were successfully edited by you and have survived and replicated settlements. You can also scrape these colonies to new plate or suspending broth. In a contamination free environment one should be able to re-plate these colonies once a month while kept in fridge to maintain a healthy and functioning glowing eco-system. Just make sure to not let the temperature rise above 80F (30C). The best way to keep your bacteria safe from any temperature fluctuation is to store it in Styrofoam container that is easily available in the markets and online. Protocols for Liquid Culture · Put the content of 1 LB Amp media tube to a glass bottle and then add 200 mL of clean water in it.

· Slightly skate the bottle. Once all the powder is dissolved. Scrape small amount of bacteria off of the plate with the help of an inoculation loop and mix it in the bottle. Don’t seal tight the bottle to let the oxygen pass.

If you don’t see any bacteria growth after half a day, add some more bacteria in it. The intensity of the glow is dependent upon the amount of bacteria you add. · Take extra care to avoid contamination because contamination means no glow at all in this experiment.· Once the bacteria starts glowing, keep it in a dark place. This way, its glow will last for longer period of time. Note: Never share the bottle or tubes while incubating · Protocols for Plates· Transfer bacteria to a fresh LB Amp media plate with the help of an inoculation loop.

· Keep the plate for 4-12 hours at below 30C (80F) temperature or until growth is visible. · Take the plate in a dark room to see the glowing bacteria.· Keep the bacteria in a dark and cold place once it starts glowing.· You can also store the plate for few weeks in your fridge to increase its life. · When you want to make it glow, take it out of the fridge and warm it up a little bit.

Re-streak to a new plate to renew the glow.