Table of Contents - Brian Wiley

Tuesday, August 21, 2018 8:08 AM

8/20/2018 - Lab 1: Use of Micropipettes

Table of Contents

Tuesday, August 21, 2018 8:05 AM

Objective:

Practice using micropipettes to add and remove fluids within a 10% level of accuracy.

Today's Topics:

- Micropipettes
- Balance
- Centrifuge

Protocol:

Part 1: Pipettes with dye

Materials:

- Microcentrifuge tubes containing 1500 µl dye. (3 tubes per student)

Procedure:

1. Obtain solutions from instructor.
2. Obtain a box of yellow and blue micropipette tips.
3. Using the p20, transfer the following volumes Onto a paraffin paper (wax paper): 1 µl, 3 µl, 10 µl.
4. Once the fluid has all been transferred, adjust the p20 to 14 µl and remove all the fluid from the microcentrifuge tube.
5. Observe your accuracy. Check if there is air in the bottom of tip or fluid remaining in the microcentrifuge tube.
6. 3 times with the p20 using numbers of your own.
7. Repeat the entire protocol with the p200 and p1000 micropipettes.
    a. For the p200 use the volumes 30 µl, 80 µl, and remove 110 µl.
    b. Also perform with p200 using the volumes 30 µl, 80 µl, 150 µl, 200 µl and remove 460 µl using the p1000.
    c. For the p1000 use the volumes 100 µl, 300 µl, 800 µl and remove 1200 µl.
    d. Also perform with p1000 using the volumes 100 µl, 400 µl, 900 µl, and remove 1400 µl.

Part 2: Pipettes with water

Materials:

- Microcentrifuge tubes containing DI water
- Micropipettes, P1000, P200
- Pipet tips for P1000, P200
- Balance with +/- 0.001g precision
- Piece of wax paper

Procedure:

1. Turn on weighing balance.
2. Cut a piece of wax paper and place onto balance. Tear (0/T) the balance.
3. Using P1000, set the pipet to 450uL.
4. Transfer 450uL of water and pipet onto wax paper. Record the weight.
5. Wipe off the water Or tear the balance.
6. Repeat steps 3-4 Two additional times (a total of three measurements)
7. Generate a table and record all data

Calculate % error using the following equation:

$$\%\ error = \frac{mass\ measured\ - mass\ expected}{mass\ expected} \times 100$$

The expected mass of 450uL of water is calculated using the density of water of 1g/mL

Same protocol is repeated using P200. To pipet 450uL using p200, set the pipet to 150uL and do repeat pipetting three times (3x150uL = 450uL)

Results:

Part 1: Pipettes with dye

ID	Pipette Size	Volume Added	Volume Added	Volume Added	Volume Added	Volume Removed	Observations
1-p#	P20	1 µl	3 µl	10 µl	---	14 µl	The first effort did not put in 14 µl so there was air. The second effort was perfect.
2-p#	P200	30 µl	80 µl	---	---	110 µl	Missed on the first effort but perfect on the second effort. Notice that 110 µl almost filled the smaller tip used for the P20 experiment just prior.
3-p#	P200	30 µl	80 µl	150 µl	200 µl	460 µl (Use p1000)	First effort there was still a lot dye left in microcentrifuge. The second effort again was perfect.
4-p#	P1000	100 µl	300 µl	800 µl	---	1200 µl (Used p1000)	Used two withdraws of 600 µl to remove the 1200 µl but was never able to successfully remove all the liquid. The error rate for 500 µl using P100 is ±4% or 20 µl so for 600 µl twice at 4 % would give an absolute error of 48 µl. I would like to assume the error rate would be positive or negative on both withdraws since the setting remains the same so the setting could have had a negative error.
5-p#	P1000				---		Missed this one. Will on complete on Wednesday

Part 1: Pipettes with dye

ID Try	Pipette Size	Volume Removed	Mass	Error Rate	Observation
1	**P1000**	**450** µl	**.449** g	**-.222%**	
2	**P1000**	**450** µl	**.455** g	**1.111%**	
3	**P1000**	**450** µl	**.458** g	**1.777%**	
4	**P200**	**3×150** µl	**.450** g	**0%**	
5	**P200**	**3×150** µl	**.450** g	**0%**	
6	**P200**	**3×150** µl	**.452** g	**.444%**	**P200 pipettes were more accurate**

Conclusion:

With respect to part one in the lab, the measurements with the smaller micropipettes were more successful and being spot on however if you averaged all the fails the relative error does seem to converge to being the same for all sizes as the error charts state for relative error.

With respect to part two, the relative error is around 4% for P1000 micropipettes according to Artel for 500 µl, so it would be just 4% for 450 µl. For P200 the error rate is between 2% and 4% for 150 µl so the results align with the data.

Sources:

[1] Artel. Lab Report 5: Setting Tolerances for Pipettes in the Laboratory. 2018. https://www.artel-usa.com/resource-library/setting-tolerances-for-pipettes-in-the-laboratory/

8/22/2018 - Lab 2: Preparation of Reagents and Bacterial Media

Table of Contents

Saturday, August 25, 2018 5:03 PM

Objective:

Prepare reagents for making chemical competent bacteria and also prepare bacteria media. Autoclave all to purify reagents and bacteria media.

Today's Topics:

- Reagents
- Molarity and Molecular Weight
- Scale
- Bacteria Media
- Magnetic Stirrer
- Autoclave

Protocol:

Part 1: Create Reagents

Materials:

- Calcium Chloride Dihydrate $CaCl_2(H_20)_2$
- Magnesium Chloride Hexahydrate $MgCl_2(H_20)_6$
- 100 mL Graduated Cylinder
- 3 - 150 mL Square Reagent Bottles
- 15 mL Glycerol
- Scale
- Wax Paper
- Stretch Tape
- Aluminum Foil
- Autoclave Tray

Procedure Calcium Chloride Reagent:

1. Obtain reagents from your instructor.
2. Create 100mL of 100mM solution Calcium Chloride:
    a. Weigh 1.47 grams of Calcium Chloride Dihydrate on scale
    b. Add Calcium Chloride Dihydrate to 100 mL water in 100mL graduated cylinder
    c. Use stretch tape on top of graduated cylinder to turn graduated cylinder upside down to mix solution in water
    d. Remove stretch tape and pour solution into 500 mL square reagent bottle
    e. Place cap on bottle lightly with aluminum foil to allow autoclave
3. Place bottle in autoclave tray

Procedure Calcium Chloride Solution with Glycerol:

1. See procedure above but instead of 100 mL water in 2b., add 15 mL glycerol and 85 mL water.

Procedure Magnesium Chloride Reagent:

1. See procedure above but instead of 1.47 grams of Calcium Chloride Dihydrate in 2a., weigh 2.03 grams of Magnesium Hexahydrate.

Part 2: Create Luria Broth

Materials:

- Luria Powder
- 250 mL Beaker
- 100 mL Graduated Cylinder
- Scale
- Wax Paper
- 500 mL Erlenmeyer flask
- Magnet
- Magnetic Stirrer
- Aluminum Foil
- Autoclave Tray

Procedure:

1. Create 2.5% (w/v) solution of Luria Broth (LB):
    a. Measure 2.5 grams LB and add to 250 mL beaker
    b. Add magnet to solution and cover with aluminum foil
    c. Place beaker on BioRad magnetic stirrer and turn up to 750 rpm
    d. Move rpm up and down until Luria powder is completely dissolved in solution
    e. Transfer LB to 500 mL Erlenmeyer flask and cover with foil
2. Place bottle in autoclave tray.

Part 3: Autoclave

Materials:

- Reagents and LB from Part 1 & 2
- Autoclave Tray
- Water
- Autoclave

Procedure:

1. Add water to base of autoclave tray which holds reagents and LB so that temperature will boil water and not lose any solution.
2. Set autoclave to liquid setting at 121°C at 15 psi for 30 minutes.
3. Insert autoclave tray into autoclave and press Start.
4. Return in 30 minutes for retrieval and storage.

Results:

None

Conclusion:

None

8/27/2018 - Lab 3: Steak for Isolation

Table of Contents

Saturday, August 25, 2018 5:03 PM

Objective:

Prepare media for isolation of bacteria colonies using streak for isolation technique. Using aseptic technique with goal to avoid contamination in sample and to grow bacteria colonies that can be isolated within media.

Today's Topics:

- Streak for Isolation
- Aseptic Technique
- Media
- Bacteria Culture / Pure Culture
- Cell Colonies
- Genus and Species

Protocol:

Part 1: Transfer Bacteria to Liquid and Solid Media

Materials:

Stock Cultures:

- Slant culture E. Coli
- Broth culture Stapylococcus Carnosus
- Mixed broth culture E. Coli and Stapylococcus Carnosus
- Culture Micrococcus Luteus in broth? **(Need type of media here)**
- Culture Micrococcus Roseus in broth? **(Need type of media here)**

Media:

- 1 Sterile trypticase soy agar slant (TSA)
- 4 Sterile trypticase soy agar plates (TSA)

Equipment:

- Inoculation Loop
- Test tube rack
- Bacticinerator
- Permanent marker
- Labeling tape

Procedure:

Aseptic transfer E. coli from slant media to agar plate:

1. Label agar plate with name, class, date and culture E. coli
2. Sterilize inoculation loop by inserting in bacticinerator until loop is red hot and remove immediately.
3. Let inoculation loop cool for 5-10 seconds by holding still.
4. Remove cap from slant culture of E. coli, holding cap, slant, and inoculation loop in hands.
5. Revolve top of slant tube around just outside of bacticinerator to sterilize.
6. Touch tip of inoculation loop onto culture growth of E. coli in slant. Only obtain tiny amount of culture.
7. Revolve top of slant around bacticinerator and replace cap.
8. Remove lid from top of TSA plate and hover over top of TSA media.
9. Lightly drag tip across center of first section back and forth making sure not to puncture media.
10. Replace lid back on top of TSA plate and rotate plate 45 to 90 degrees.
11. Sterilize inoculation loop by inserting in bacticinerator until loop is red hot and remove immediately.
12. Let inoculation loop cool for 5-10 seconds by holding still.
13. Remove lid from top of TSA plate and hover over top of TSA media.
14. Lightly drag tip of inoculation loop through first section across to second section of TSA plate and drag back and forth lightly in second section. Do not cross back into first section while dragging across second section.
15. Replace lid back on top of TSA plate and rotate plate 45 to 90 degrees.
16. Sterilize inoculation loop by inserting in bacticinerator until loop is red hot and remove immediately.
17. Let inoculation loop cool for 5-10 seconds by holding still.
18. Remove lid from top of TSA plate and hover over top of TSA media.
19. Lightly drag tip of inoculation loop through second section across to third section of TSA plate and drag back and forth lightly in third section. Do not cross back into first section or second section while dragging across third section.

Aseptic transfer Stapylococcus Carnosus broth culture to agar plate:

1. Repeat steps above except where in step 6 touching tip of inoculation loop onto culture growth of E. coli in slant, dip tip into Stapylococcus Carnosus broth and remove. Light film should be visible in loop.

Aseptic transfer mix Stapylococcus Carnosus and E. coli broth culture to agar plate:

1. Repeat steps above except where in step 6 touching tip of inoculation loop onto culture growth of E. coli in slant, dip tip into mix Stapylococcus Carnosus and E. coli broth and remove. Light film should be visible in loop.

Aseptic transfer Micrococcus Roseus broth culture to agar plate:

1. Repeat steps above except where in step 6 touching tip of inoculation loop onto culture growth of E. coli in slant, dip tip into Micrococcus Roseus broth and remove. Light film should be visible in loop.

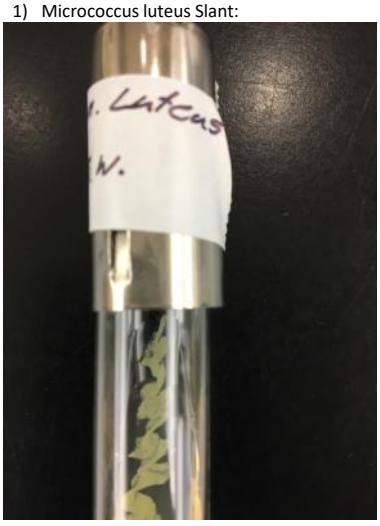
Aseptic transfer Micrococcus Luteus broth culture to agar slant:

1. Repeat steps 1-14 above except where in step 6 touching tip of inoculation loop onto culture growth of E. coli in slant, dip tip into Micrococcus Roseus broth and remove. Light film should be visible in loop. Instead of where in step 14 dragging tip across agar plate, lightly drag tip back and forth from bottom of slant to top of slant.

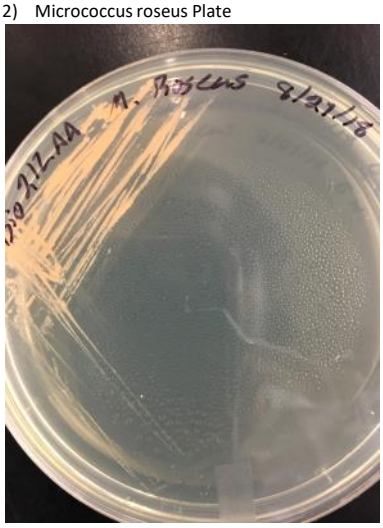
Results:

Images:

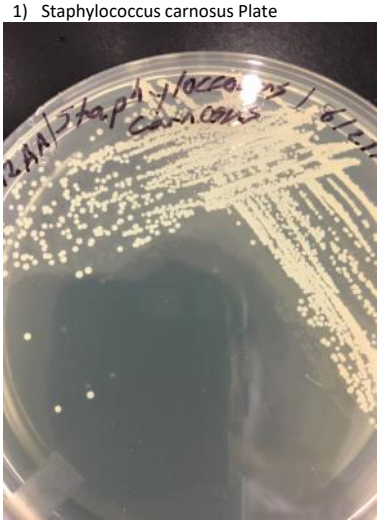
1) Micrococcus luteus Slant:



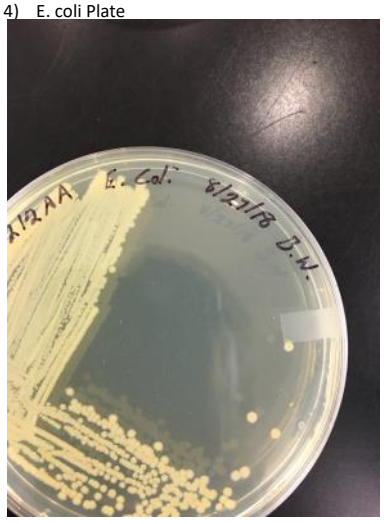
2) Micrococcus roseus Plate



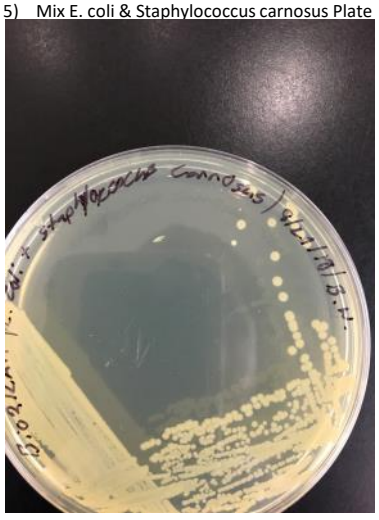
1) Staphylococcus carnosus Plate



4) E. coli Plate



5) Mix E. coli & Staphylococcus carnosus Plate



Conclusion:

I was able to successfully isolate colonies in the streak for isolation of individually E. coli and Staphylococcus carnosus on the agar plates as well as isolate the mixture of the two on a third plate and be able to distinguish individual colonies by the streak on the third section. I was unsuccessful in isolating individual colonies on the agar plate streaking for Micrococcus roseus and on the agar slant for Micrococcus luteus or the colonies are just too small to see. I was however more successful when isolating the micro species on the plate over the slant because of sectional streaking.

8/29/2018 - Lab 4: Culturing and Counting Bacteria and Creating LB Agar Plates

Table of Contents

Saturday, August 25, 2018 5:03 PM

Objective:

Culture bacteria growth and measure cell count with OD600 spectrophotometer.
Spread for isolation bacteria on TSA plates.
Pour Miller LB agar plates as media to culture bacteria.

Today's Topics:

- Bacteria reproduction
- Cell culture
- Escherichia coli
- Making Miller LB agar plates
- Spectrophotometer
- Spreading for isolation
- Bacteria growth curve
- Counting Bacteria

Protocol:

Part 1: Growing bacteria, diluting culture solution, and counting cells.

Materials:

- Liquid broth E. coli culture
- 100 mL Miller Luria broth
- Micropipettes
- Test tube rack
- Microcentrifuge tubes
- Microcentrifuge tube holder
- 11 Trypticase soy algar (TSA) plates
- Bio Rad spectrophotometer
- 5 Spectrophotometer cuvettes
- 5 mM diameter glass spreader
- 95% Ethanol

Procedure at 0 hour:

1. Obtain 100 mL LB from instructor and place in test tube rack.
2. Obtain pre-warmed 100 mL LB made from Lab 2 one week prior in Erlenmeyer glass.
3. Using P1000 pipette add 1mL of E. coli culture to pre-warmed 100mL LB. Swirl solution around to mix culture in broth.
4. Using P1000 micropipette remove 500 µL from new 100mL LB mixed with E. coli and add to cuvette.
5. Add 500 µL of pure LB to the cuvette to form a 1:2 ratio.
6. Using blank cuvette with 100% LB and 0% E. coli culture, place cuvette in cuvette chamber of Bio Rad spectrophotometer and blank out OD600 spectrophotometer. Select OD600 and press "Read Blank." Confirm 0 optical density on screen.
7. Remove blank cuvette from cuvette chamber and replace with 1:2 ratio E. coli culture with LB. Press read sample and obtain optical density reading from screen. Multiply read by 2 to account for 1:2 ratio and record number in chart (see Results).
8. Remove cuvette from cuvette chamber and place 100mL LB mixed with E. coli Erlenmeyer glass in shaker at 37°C for 30 minutes at 200 rpm to grow culture.
9. Using P200 micropipette remove 100 µL of 1:2 ratio E. coli culture with LB from cuvette and add to microcentrifuge tube and with P1000 micropipette mix with 900 µL pure LB to form 1:20 ratio.
10. Again dilute by another 1/10th in another microcentrifuge tube to form 1:200 ratio.
11. Label TSA plate with name, class, date, dilution factor, and culture E. coli.
12. Using P200 micropipette transfer 100 µL of 1:200 diluted E. coli culture to middle of TSA plate.
13. Place spreader in 95% ethanol to sterilize. Remove and add flame to sterilize and remove ethanol.
14. Using spreader, apply lightly to spreader out 100 µL of 1:200 diluted E. coli culture around TSA plate in while rotating plate in circular motion.
15. Place top on TSA plate, turn over, and place aside for storage at 37°C to grow culture.
16. Flame spreader to sterilize before placing back in ethanol.

Procedure at 30 minutes:

1. Remove 100mL LB mixed with E. coli Erlenmeyer glass from shaker.
2. Repeat steps 4 through 16 above except after step 10 repeat step 10 again to create a 1:2000 ratio E. coli culture with LB.

3. Repeat steps 11 to 16 for 1:2000 ratio.

Procedure at 60 minutes:
1. Repeat steps for 30 minutes.

Procedure at 90 minutes:
1. Remove 100mL LB mixed with E. coli Erlenmeyer glass from shaker.
2. Repeat steps 4 through 16 above except where in steps 5 & 6 creating a 1:2 ratio, create a 1:4 ratio with 250 µL LB mixed with E. coli and 750 µL pure LB and after step 10 repeat step 10 two more times to create a 1:4000 ratio E. coli culture with LB and 1:40000 ratio.
3. Repeat steps 11 to 16 for 1:4000 ratio and 1:40000 ratio.

Procedure at 120 minutes:
1. Repeat steps for 90 minutes except repeat step 10 three more times to create a 1:40000 ratio and 1:400000.
2. Repeat steps 11 to 16 for 1:40000 ratio and 1:400000 ratio.

Part 2: Create Miller LB Agar for media plates.

Materials:

- Miller LB Agar made up of:
    - 15 g/L Agar
    - 10 g/L Tryptone
    - 10 g/L NaCl
    - 5 g/L Yeast Extract
- 500 mL Erlenmeyer Flask
- Large scale tray
- Scale
- Lab Spatula
- Deionized water
- Magnet
- Magnetic Stirrer
- Aluminum Foil
- Autoclave Tray
- Autoclave
- Clean uncracked Petri dishes

Procedure:
1. Obtain LB Miller agar from instructor.
2. Add scale tray to scale and tare.
3. Measure 10 g total of LB agar for 250 mL solution.
4. Add 50 mL deionized water to Erlenmeyer flask and add 10 g of LB agar to Erlenmeyer flask.
5. Continue to add deionized water to Erlenmeyer flask until total volume is 250 mL.
6. Insert magnet into Erlenmeyer flask and place flask on magnetic stirrer. Increase rpm to 750 until LB agar is completely dissolved.
7. Remove magnet from Erlenmeyer flask and place aluminum foil on top.
8. Place Erlenmeyer flask in autoclave clay to be placed in autoclave.
9. Add water to base of autoclave tray so that temperature will boil water and not lose any solution.
10. Set autoclave to liquid setting at 121°C at 15 psi for 30 minutes.
11. Insert autoclave tray into autoclave and press Start.
12. Return in 30 minutes for retrieval.
13. Remove clean uncracked petri dishes in sets of three.
14. Add LB agar one dish at a time starting from the bottom. Remove lid and cover while adding LB agar to spread over entire bottoms of dish.
15. Repeat for rest of petri dishes and allow agar to cool and form solid.
16. Place agar plates back into bag and store.

Part 3: Counting Cell Colonies

Materials:

- Plate cultures from Part 1
- Permanent market
- Clicker counter

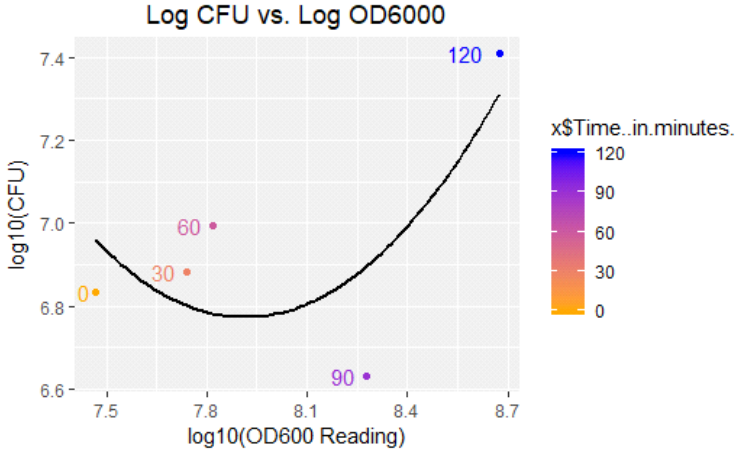
Procedure:

1. Retrieve all plates from storage in Part 1.
2. For one plate in each time period count the colonies that have formed. If there is a very large amount divide plate into sections with a ruler and thin permanent marker, count one section and multiply by number of sections.
3. Count cell colonies, from bottom/agar side of plate, using a marker to dot each colony that was counted and a clicker counter.
4. Record the total number of colonies for the CFU calculation.
5. Calculate the CFU for each time period and graph it vs. the actual on the OD600 count.

Results:

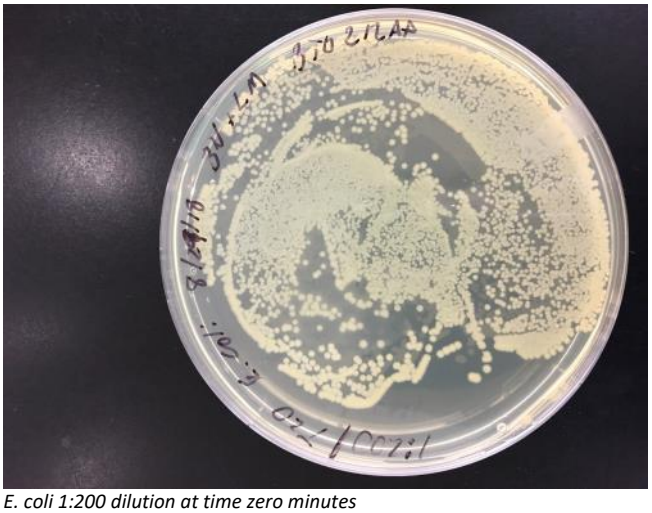
Growing bacteria, diluting culture solution, and counting cells.

Time (min)	Read (including 2x/4x)	OD600 Count	CFU Dilution Added to Agar Plates	CFU = $\frac{Colonies}{DF*Vol\ in\ mL}$
0	.058	2.92×10^7	1/200	6.80×10^6
30	.092	5.58×10^7	1/200 & 1/2000	7.64×10^6
60	.262	1.31×10^8	1/200 & 1/2000	9.84×10^6
90	.376	1.89×10^8	1/4000 & 1/40000	4.28×10^6
120	.948	4.74×10^8	1/40000 & 1/400000	2.56×10^7



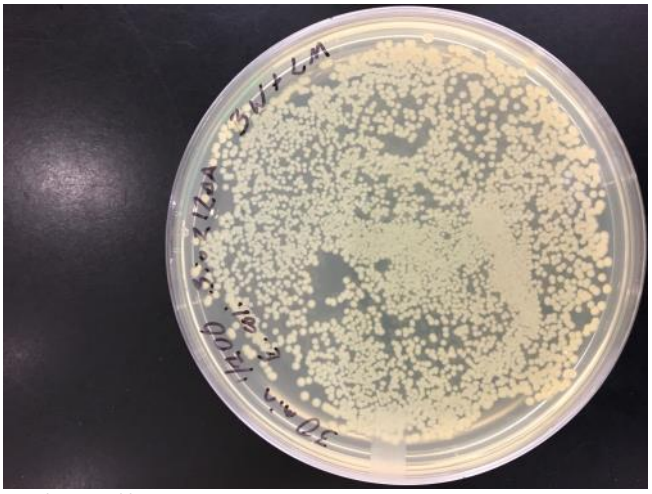
Part 3 Plates:

Time 0:

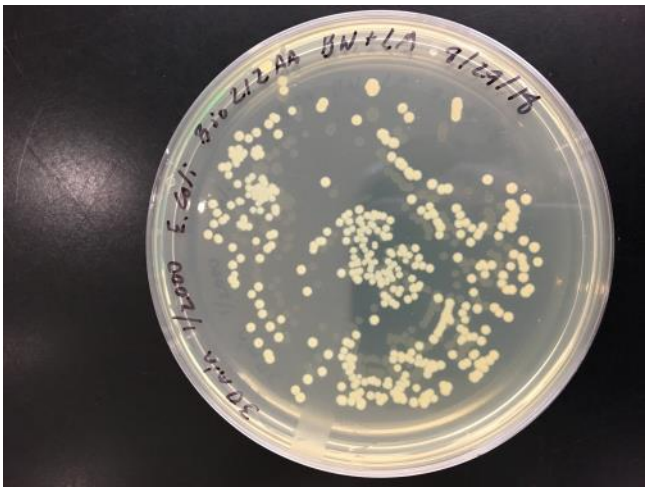


E. coli 1:200 dilution at time zero minutes

Time 30 minutes:

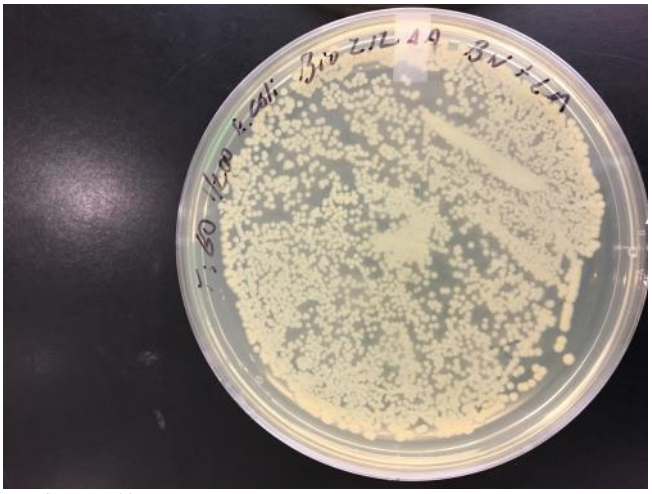


E. coli 1:200 dilution at time 30 minutes

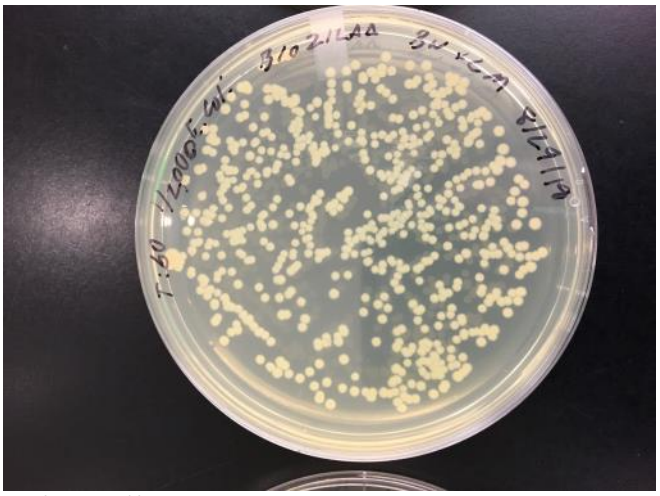


E. coli 1:2000 dilution at time 30 minutes

Time 60 minutes:

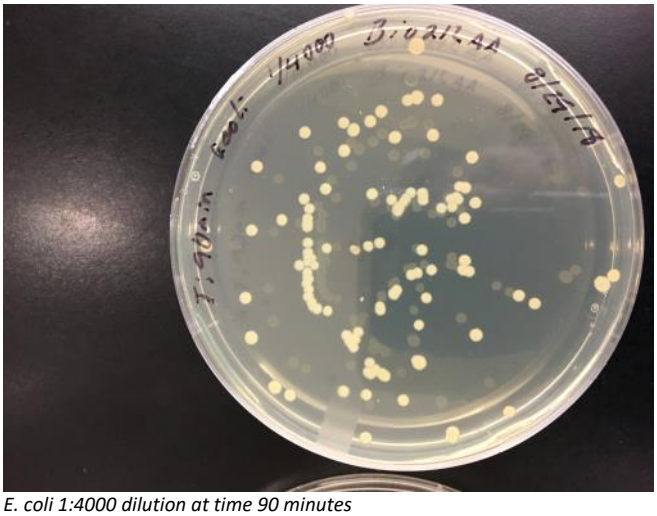


E. coli 1:200 dilution at time 60 minutes

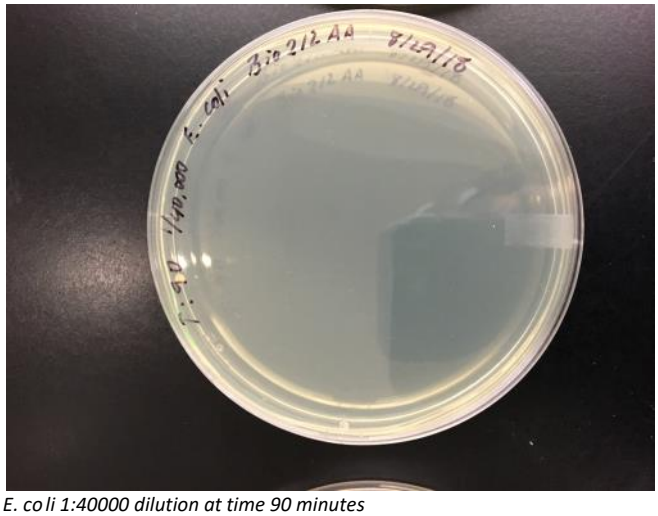


E. coli 1:2000 dilution at time 60 minutes

Time 90 minutes:

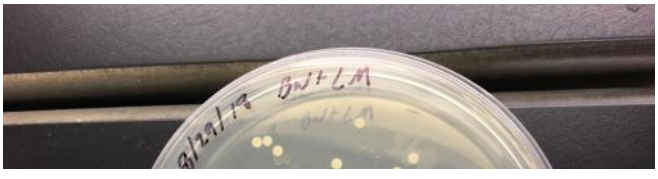


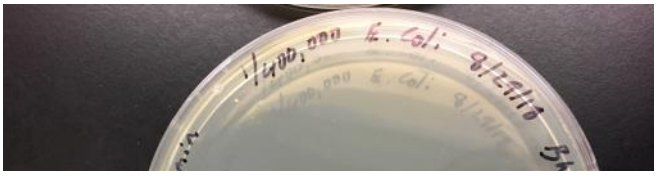
E. coli 1:4000 dilution at time 90 minutes

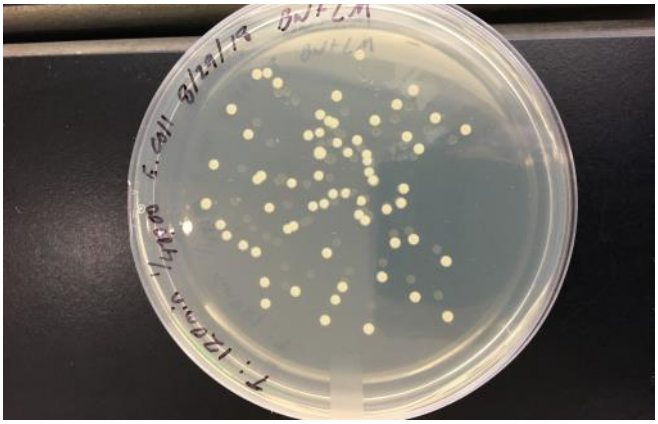


E. coli 1:40000 dilution at time 90 minutes

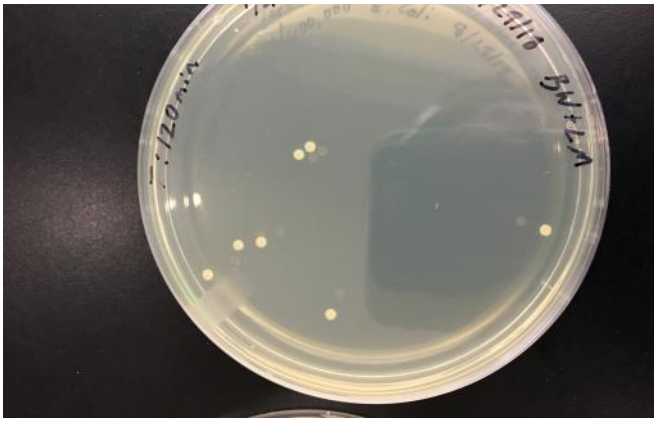
Time 120 minutes:







E. coli 1:40000 dilution at time 120 minutes



E. coli 1:400000 dilution at time 120minutes

Conclusion:

Results were off by a factor of 10 when comparing CFU to OD600 reading. Reason for results may be not mixing the solution again before spreading as well as flaming the spreader too long.

9/5/2018 - Lab 5: Making Cells Competent

Table of Contents

Saturday, August 25, 2018 5:03 PM

Objective:

Growing competent cells to store at -80 C to later use to insert antibiotic resistant DNA vector for transformation.

Today's Topics:

- Growing Competent Cells
- Using Reagents in Making Competent Cells
- Storing Competent Cells
- E. coli strain DH5α

Protocol:

Part 1: Growing Competent Cells
Batch 1 - 9/5/2018

Materials:

- E. coli in solution (DH5α)
- Pre-made 100mL LB broth in Erlenmeyer Flask
- Shaker
- Separate LB broth in Testube
- Microcentrifuge Tubes
- OD600 Cuvettes
- BioRad Spectrophotometer
- Micropipettes
- 20 mL Magnesium Chloride
- 20 mL Calcium Chloride
- 1 mL Calcium Chloride with Glycerol
- 2 – 50 mL Centrifuge Tubes
- Ice Bucket
- BioRad Centrifuge
- 4 – 1.5 mL Cryogenic vials

Procedure:

1. Obtain E. coli (DH5α) from your instructor
2. Using a P1000 micropipette transfer 1 mL of E. coli to 100 mL LB broth in Erlenmeyer flask and swirl flask.
3. Take OD600 reading at time zero.
4. Shake for 30 minutes and remove.
5. Mix 500 mL pure broth and 500mL from Erlenmeyer flask with E. coli in cuvette.
6. Take OD600 reading.
7. Repeat steps 4-6 until OD600 reading reaches .4
8. Once OD600 reading is .4, transfer half (50 mL) to one 50 mL centrifuge tube, and the other half to another 50 mL centrifuge tube.
9. Incubate on ice for 20 minutes.
10. After 20 minutes spin tubes in centrifuge at 1600 RCF for 7 minutes at 4° C.
11. Remove centrifuge tubes from centrifuge and pour out liquid in waste, leaving the bacteria at the bottom of the tube.
12. Add 1 mL $MgCl_2$ first to each tube to mix bacteria with P1000 micropipette and then add remaining 9 mL of $MgCl_2$ to each tube.
13. Centrifuge tubes at 1100 RCF for 6 minutes at 4° C.
14. Repeat step 11-12, except in 12 where adding 1 mL of $MgCl_2$ followed by 9 mL, add $CaCl_2$.
15. Combine both centrifuge tubes together using large pipette and discard empty tube.
16. Incubate on ice for 20 minutes.
17. After 20 minutes spin tubes in centrifuge at 1100 RCF for 6 minutes at 4° C.
18. Repeat steps 11-12, except in 12 where adding 1 mL of $MgCl_2$ followed by 9 mL, add only 1 mL of $CaCl_2$ with glycerol.
19. Aliquot total solution to 4 cryogenic vials, with about 250 µl each and store vials at -80° C.

Batch 2 - 9/10/2018
See materials and methods in protocol above.

Batch 3 - 9/12/2018
See materials and methods in protocol above.

Results:

Batch 1

Time (minutes)	OD600 Count
0	.058
30	.094
60	.212
90	.522

Batch 2

Time (minutes)	OD600 Count
0	.054
20	.08
40	.126
60	.274
80	.398

Batch 3

Time (minutes)	OD600 Count
0	.028
30	.052
60	.194
90	.290
110	.516

Conclusion:

None

9/10/2018 - Lab 6: Transformation

Table of Contents

Saturday, August 25, 2018 5:03 PM

Objective:

Insert DNA gene of interest from various plasmids into E. coli strain DH5α to transform E. coli.

Today's Topics:

- Plasmids and vectors
- Enzymes
- DNA Replication and Origin of Replication
- Transformation
- Antibiotics
- E. coli strain DH5α

Protocol:

Part 1: Transformation

Transformation 1 - 9/10/2018

Materials:

- E. coli stored at -80° C (DH5α)
- Ice
- 3 - 1.5 mL Microcentrifuge tubes
- Temperature controlled water-bath
- Pre-warmed LB at 37°C
- IPTG
- X-gal
- Shaker
- 40 µL antibiotic 1
- 120 µL antibiotic 2
- 3 Agar plates
- Spreader
- Ethyl alcohol
- Flame lighter

Procedure:

1. Obtain batch 1 competent E. coli (DH5α) from -80° C freezer.
2. Thaw competent cells on ice for 5 minutes or until thawed.
3. Transfer 80 µL of E. coli or an even amount to 3 microcentrifuge tubes. Keep tubes on ice.
4. Immediately transfer 1-2 µL of plasmid pUC19 (100 pg mass, 1 ng mass, 10 ng mass) to each microcentrifuge containing E. coli.
5. Incubate on ice for 30 minutes. Flicker tubes intermittently.
6. After 30 minutes of incubation, transfer tubes to prewarmed bath for heat shock at 42° C for 60 seconds.
7. Place back on ice for 2 minutes.
8. Transfer 920 µL of LB to each tube and place tubes in shaker.
9. Run shaker for 30 minutes at 200 RPM at 37°C.
10. While cells are being agitated prepare agar plates with antibiotics. Add 40 µL X-gal
11. and spread across place with spreader. Then add 120 µL IPTG and allow to dry for 30 minutes.
12. Transfer 100 µL from each tube containing E. coli and DNA plasmid to a separately labeled agar plate and use spreader to spread across the plate.
13. Allow to dry before placing in incubator.
14. Incubate plates upside down for 16-24 hours at 37°C.
15. After 24 hours remove plates and count colonies formed. Record total number and compute transformation efficiency for each plate.

Transformation 2 - 9/12/2018

See materials and methods in protocol above. Instead of using batch 1 use batch 2 and instead of using plasmid pUC19 use plasmid pMIG.mk13. Do not add IPTG or Xgal to agar plate.

Transformation 3 - 9/17/2018

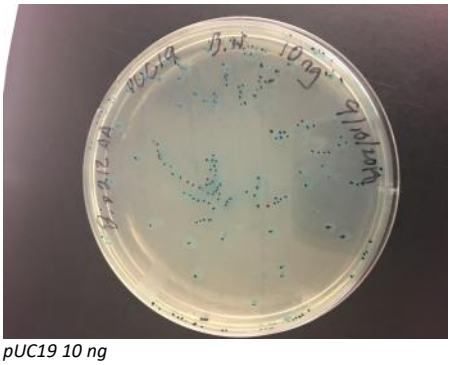
See materials and methods in protocol above. Instead of using batch 1 use batch 3 and instead of using plasmid pUC19 use plasmid pCIP. Do not add IPTG or Xgal to agar plate.

Results:

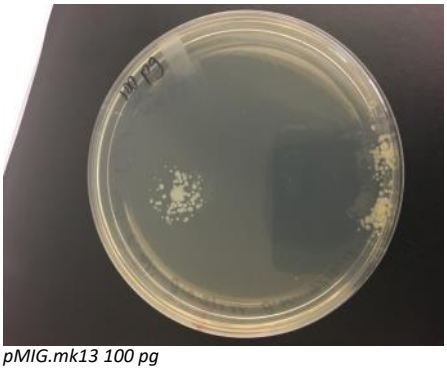
Transformation Efficiency = $\frac{Colonies}{DNA\ in\ \mu g * Vol\ in\ mL}$

Transformation	Competent Cells	Batch #, Date	Transformation Plasmid, Date	Amount of DNA in µg	Transformation Efficiency
#1	E. coli DH5α	1; 9/5/2018	pUC19; 9/10/2018	1.0×10^{-4}	0
#1	E. coli DH5α	1; 9/5/2018	pUC19; 9/10/2018	1.0×10^{-3}	9.0×10^{4}
#1	E. coli DH5α	1; 9/5/2018	pUC19; 9/10/2018	1.0×10^{-2}	1.92×10^{5}
#2	E. coli DH5α	2; 9/10/2018	pMIG.mk13; 9/12/2018	1.0×10^{-4}	3×10^{5}
#2	E. coli DH5α	2; 9/10/2018	pMIG.mk13; 9/12/2018	1.0×10^{-3}	8.6×10^{5}
#2	E. coli DH5α	2; 9/10/2018	pMIG.mk13; 9/12/2018	1.0×10^{-2}	2.184×10^{6}
#3	E. coli DH5α	1; 9/12/2018	pCIP; 9/17/2018	1.0×10^{-4}	1.0×10^{5}
#3	E. coli DH5α	1; 9/12/2018	pCIP; 9/17/2018	1.0×10^{-3}	9.0×10^{4}
#3	E. coli DH5α	1; 9/12/2018	pCIP; 9/17/2018	1.0×10^{-2}	2.15×10^{5}

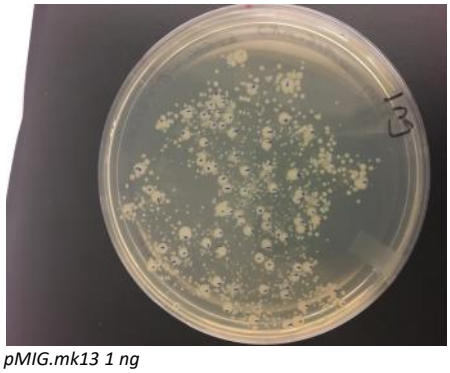
Photos of Transformation Plates:



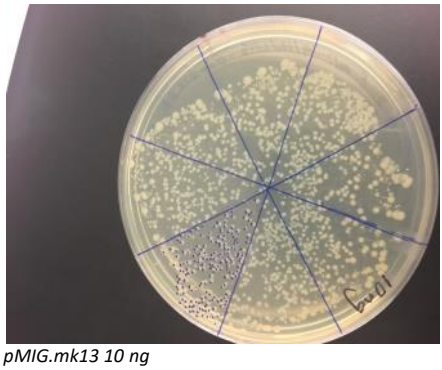
pUC19 10 ng



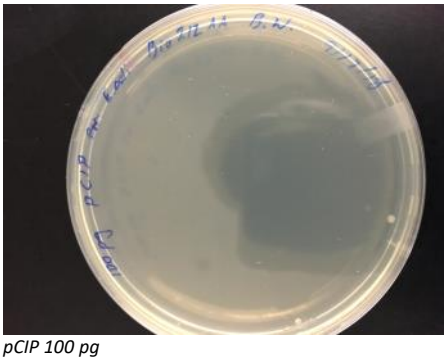
pMIG.mk13 100 pg



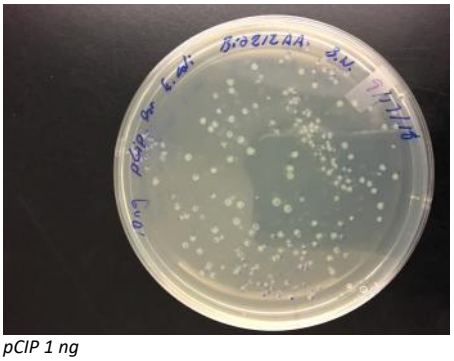
pMIG.mk13 1 ng



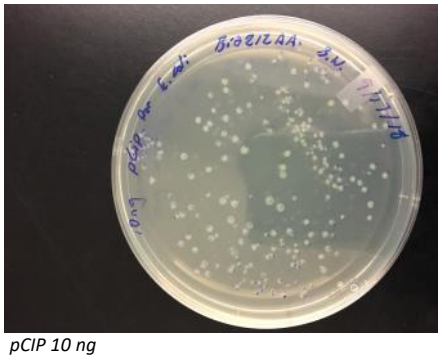
pMIG.mk13 10 ng



pCIP 100 pg



pCIP 1 ng



pCIP 10 ng

Conclusion:

None

9/19/2018 - Lab 7: Mini Prep Plasmid DNA

Table of Contents

Saturday, August 25, 2018 5:03 PM

Objective:

Isolate plasmid DNA from transformation of E. coli DH5α.

Today's Topics:

- Transformation
- E. coli strain DH5α
- Plasmids pUC19, pMIG.mk3, pCIP
- Recombinant DNA
- Nano Drop
- Alkaline Lysis Miniprep
- Miniprep Kit
- DNA isolation

Protocol:

Part 1: Mini Prep of Plasmid DNA w/o Kit

Materials:

- 4 mL E. coli (DH5α) transformed with pUC19 plasmid in LB
- 4 mL E. coli (DH5α) transformed with pMIG.mk3 plasmid in LB
- 4 mL E. coli (DH5α) transformed with pCIP plasmid in LB
- Ice
- 3 - 2 mL Microcentrifuge tubes
- 3 - 1.5 mL Microcentrifuge tubes
- Fisher Scientific Micro centrifuge
- 200 µl GTE
- 400 µl NaOH/SDS
- 300 µl Potassium Acetate
- 95% Ethanol
- 70% Ethanol
- DEPC H_20
- Ice bucket

Procedure:

1. Obtain prepped sample of E. coli transformed with pUC19 plasmid in LB.
2. Add 2 mL of sample to 2 mL microcentrifuge tube and centrifuge at 9000 xg for 1-2 minutes.
3. Decant supernatant.
4. Repeat step 2 and decant supernatant.
5. Add 200 µL of GTE and resuspend cells in GTE.
6. Incubate at room temperature for 5 minutes.
7. Add 400 µL NaOH/SDS, cap tubes and invert 4-6 times.
8. Place tube on ice for 5 minutes.
9. Add 300 µL, cap tubes and invert 4-6 times.
10. Centrifuge at 15000 xg for 3 minutes.
11. Transfer 750 µL of supernatant to 1.5 mL tube.
12. Add .8 mL 95% ethanol to tube and incubate at room temperature for 2 minutes.
13. Centrifuge at 15000 xg for 1 minute.
14. Pour out 95% ethanol.
15. Add 1mL of 70% ethanol and centrifuge again at 15000 xg for 1 minute.
16. Decant 70% ethanol and let pellet air dry for 30 minutes.
17. Add 30-50 µL of DEPC water and set it ice.
18. Using the nano drop, place 1 µL on the nano drop and measure and record concentration, 260/280 ratio, and 260/230 ratio.
19. Repeat steps 1-18 for each E. coli transformed with pMIG.mk13 plasmid and pCIP plasmid.

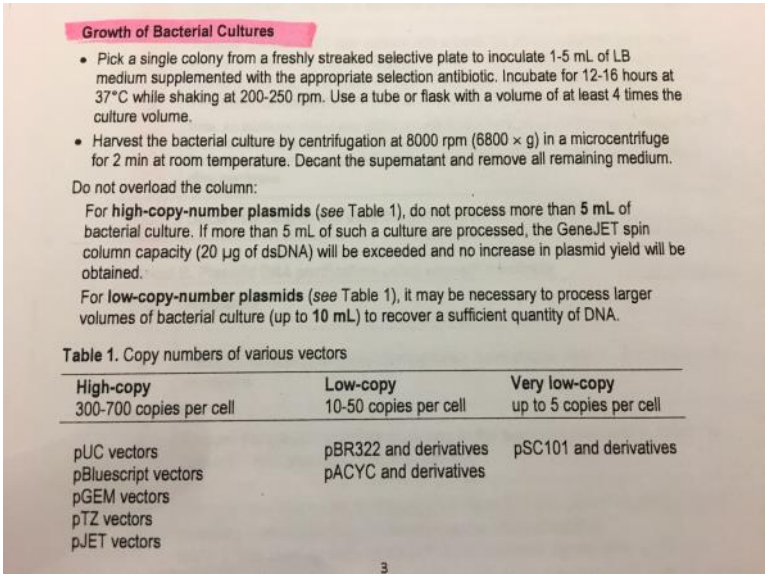
Part 2: Mini Prep of Plasmid DNA with Kit

Materials:

- See above for same materials
- Mini Prep Kit

Procedure:

1. See images below for procedure. Repeat procedure twice for each E. coli transformed with pUC19 plasmid, pMIG.mk13 plasmid, and pCIP plasmid.
2. Record same nano drop results as with procedure above for alkaline lysis miniprep.



Growth of Bacterial Cultures

- Pick a single colony from a freshly streaked selective plate to inoculate 1-5 mL of LB medium supplemented with the appropriate selection antibiotic. Incubate for 12-16 hours at 37°C while shaking at 200-250 rpm. Use a tube or flask with a volume of at least 4 times the culture volume.
- Harvest the bacterial culture by centrifugation at 8000 rpm (6800 × g) in a microcentrifuge for 2 min at room temperature. Decant the supernatant and remove all remaining medium.

Do not overload the column:

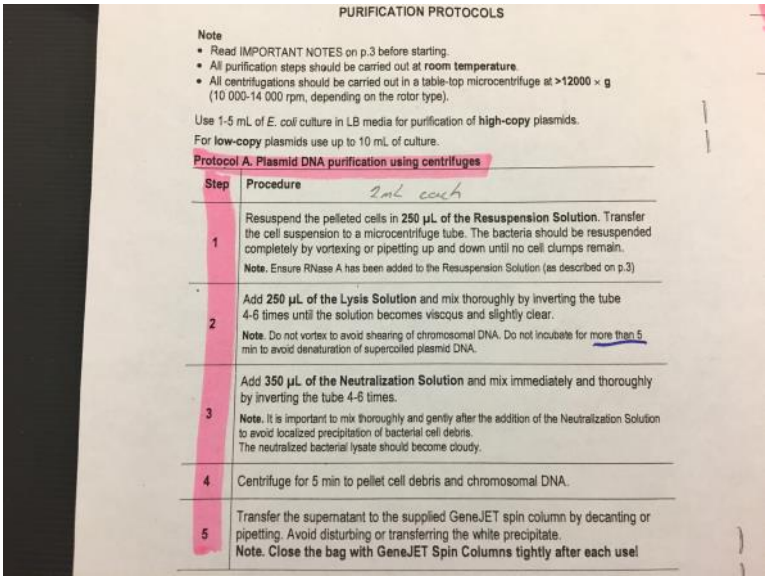
For **high-copy-number plasmids** (*see* Table 1), do not process more than **5 mL** of bacterial culture. If more than 5 mL of such a culture are processed, the GeneJET spin column capacity (20 µg of dsDNA) will be exceeded and no increase in plasmid yield will be obtained.

For **low-copy-number plasmids** (*see* Table 1), it may be necessary to process larger volumes of bacterial culture (up to **10 mL**) to recover a sufficient quantity of DNA.

Table 1. Copy numbers of various vectors

High-copy 300-700 copies per cell	Low-copy 10-50 copies per cell	Very low-copy up to 5 copies per cell
pUC vectors pBluescript vectors pGEM vectors pTZ vectors pJET vectors	pBR322 and derivatives pACYC and derivatives	pSC101 and derivatives

3



PURIFICATION PROTOCOLS

Note

- Read IMPORTANT NOTES on p.3 before starting.
- All purification steps should be carried out at **room temperature**.
- All centrifugations should be carried out in a table-top microcentrifuge at **>12000 × g** (10 000-14 000 rpm, depending on the rotor type).

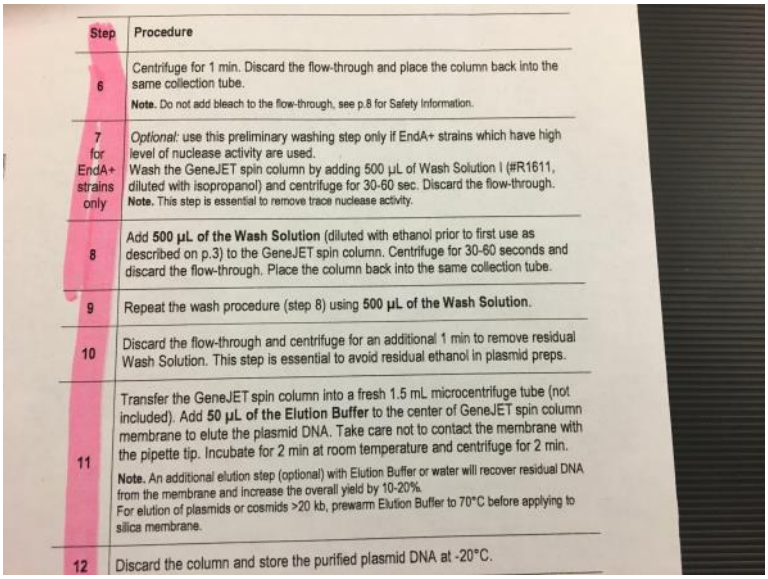
Use 1-5 mL of *E. coli* culture in LB media for purification of **high-copy** plasmids.

For **low-copy** plasmids use up to 10 mL of culture.

Protocol A. Plasmid DNA purification using centrifuges

2mL each

Step	Procedure
1	Resuspend the pelleted cells in **250 µL of the Resuspension Solution**. Transfer the cell suspension to a microcentrifuge tube. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain. **Note.** Ensure RNase A has been added to the Resuspension Solution (as described on p.3)
2	Add **250 µL of the Lysis Solution** and mix thoroughly by inverting the tube 4-6 times until the solution becomes viscous and slightly clear. **Note.** Do not vortex to avoid shearing of chromosomal DNA. Do not incubate for more than 5 min to avoid denaturation of supercoiled plasmid DNA.
3	Add **350 µL of the Neutralization Solution** and mix immediately and thoroughly by inverting the tube 4-6 times. **Note.** It is important to mix thoroughly and gently after the addition of the Neutralization Solution to avoid localized precipitation of bacterial cell debris. The neutralized bacterial lysate should become cloudy.
4	Centrifuge for 5 min to pellet cell debris and chromosomal DNA.
5	Transfer the supernatant to the supplied GeneJET spin column by decanting or pipetting. Avoid disturbing or transferring the white precipitate. **Note. Close the bag with GeneJET Spin Columns tightly after each use!**



Step	Procedure
6	Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube. **Note.** Do not add bleach to the flow-through, see p.8 for Safety Information.
7 for EndA+ strains only	*Optional:* use this preliminary washing step only if EndA+ strains which have high level of nuclease activity are used. Wash the GeneJET spin column by adding 500 µL of Wash Solution I (#R1611, diluted with isopropanol) and centrifuge for 30-60 sec. Discard the flow-through. **Note.** This step is essential to remove trace nuclease activity.
8	Add **500 µL of the Wash Solution** (diluted with ethanol prior to first use as described on p.3) to the GeneJET spin column. Centrifuge for 30-60 seconds and discard the flow-through. Place the column back into the same collection tube.
9	Repeat the wash procedure (step 8) using **500 µL of the Wash Solution**.
10	Discard the flow-through and centrifuge for an additional 1 min to remove residual Wash Solution. This step is essential to avoid residual ethanol in plasmid preps.
11	Transfer the GeneJET spin column into a fresh 1.5 mL microcentrifuge tube (not included). Add **50 µL of the Elution Buffer** to the center of GeneJET spin column membrane to elute the plasmid DNA. Take care not to contact the membrane with the pipette tip. Incubate for 2 min at room temperature and centrifuge for 2 min. **Note.** An additional elution step (optional) with Elution Buffer or water will recover residual DNA from the membrane and increase the overall yield by 10-20%. For elution of plasmids or cosmids >20 kb, prewarm Elution Buffer to 70°C before applying to silica membrane.
12	Discard the column and store the purified plasmid DNA at -20°C.

Results:

Transformation	Transformation Plasmid, Date	Miniprep Method	Concentration	260/280 Ratio DNA/RNA	260/230 Ratio Phenol, Salt
#1	pUC19; 9/10/2018	Alkaline Lysis	437.4 ng/µl	1.72	2.43
#1	pUC19; 9/10/2018	Miniprep Kit #1	474.4 ng/µl	1.90	2.07
#1	pUC19; 9/10/2018	Miniprep Kit #2	478.5 ng/µl	1.92	2.20
#2	pMIG.mk13; 9/12/2018	Alkaline Lysis	311.7 ng/µl	1.69	2.49
#2	pMIG.mk13; 9/12/2018	Miniprep Kit #1	499.5 ng/µl	1.87	1.85
#2	pMIG.mk13; 9/12/2018	Miniprep Kit #2	493.7 ng/µl	1.91	2.14
#3	pCIP; 9/17/2018	Alkaline Lysis	40.9 ng/µl	1.69	2.06
#3	pCIP; 9/17/2018	Miniprep Kit #1	380.2 ng/µl	1.94	2.11
#3	pCIP; 9/17/2018	Miniprep Kit #2	231.9 ng/µl	1.97	2.13

Conclusion:

None

Table of Contents

9/26/2018 - Lab 8: Gel electrophoresis

Saturday, August 25, 2018 5:03 PM

Objective:

Perform gel electrophoresis on pure DNA isolation for E. coli transformed with 3 different plasmids.

Today's Topics:

- DNA isolation
- Gel electrophoresis

Protocol:

Materials:

- 9 total samples of isolated DNA for E.coli transformed with pUC19, pMIG.mk13, and pCIP stored in -20°C
- Mini electrophoresis system
- 15-tooth comb
- .28 grams of agarose
- 40 mL SB buffer
- 200 mL Erlenmeyer Flask
- Microwave
- 4 µL SYBR Safe staining dye
- DEPC H_20
- Chemidoc instrument (Biorad)
- Gloves or rubber holder
- Ice bucket
- Pipettes and tips
- 1.5 mL centrifuge tubes
- Centrifuge
- 5X loading dye

Procedure:

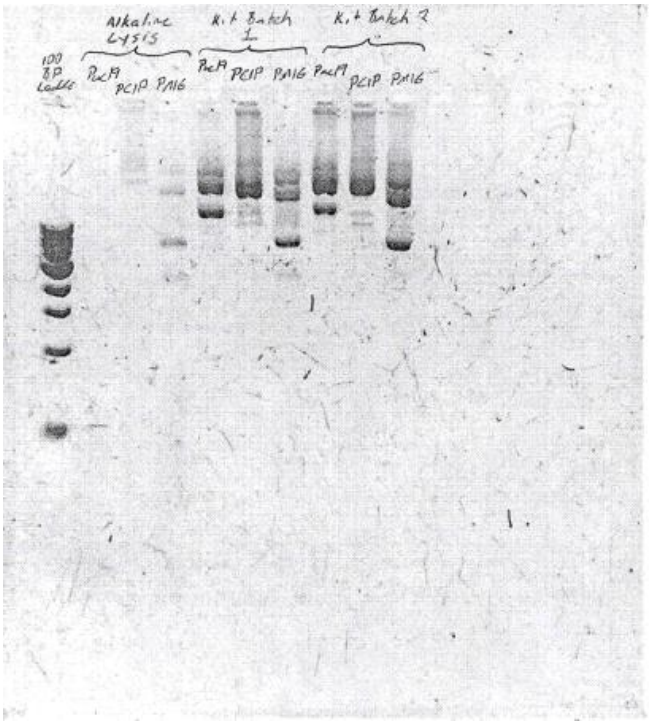
1. To make .7% agarose gel, add .28 g of agarose to 40 mL 1X SB buffer in 200 mL Erlenmeyer flask and swirl.
2. Microwave for 1 minute, swirl and check for boiling. Repeat for 30 more seconds if needed.
3. Remove Erlenmeyer flask from microwave with glove or rubber holder.
4. Swirl flask and confirm solution is clear.
5. After cooling add 4 µL of SYBR staining dye.
6. Setup mini electrophoresis system with dams and pour gel solution into cassette.
7. Add the 15-tooth comb to the system.
8. Allow to gel for 20 minutes.
9. Prepare samples to add to gel (see Table 2):
    a. Each sample volume will be 10 µL comprising of pDNA, 2 µL 5 X loading buffer, and DEPC water.
    b. Determine amount of pDNA needed for 250 ng.
    c. Label each 1.5 mL centrifuge tube.
10. Prepare a 10th tube with 1 Kb DNA Ladder.
11. After samples are prepared, centrifuge for 10 seconds.
12. Remove comb from gels, and place gel so that so that wells face negative electro node.
13. Add 1X SB buffer to systems to cover the gel at least 1mm above gel.
14. Load each well with corresponding samples.
15. Place top of mini electrophoresis system and connect nodes to power supply.
16. Run your gel at 100V for 30-40 minutes or until bromophenol blue lane reaches end of gel.
17. Visualize gel on Chemidoc instrument and print image of gel.

Table 2: List of Samples for Gel Layout

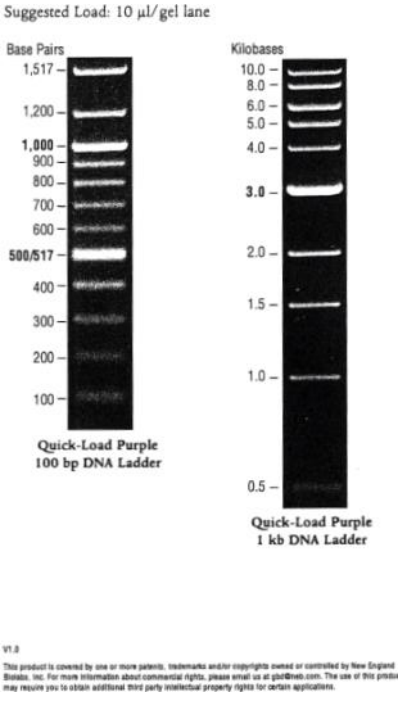
Gel Lane	Sample #	Volume	Amount of	Amount of DEPC	Concentration	Total Amount	Alkaline	Batch

#		pDNA Used (µL)	5X Loading Buffer (µL)	H_2O (µL)	Ng/µL	of DNA µg	Lysis or Kit	
1	100 bp DNA Ladder	None	None	None	None	None	None	
2	pUC19	.572	2	7.4	437.4	250	AL	
3	pCIP	6.11	2	1.9	40.9	250	AL	
4	pMIG.mk13	.804	2	7.2	311.7	250	AL	
5	pUC19	.527	2	7.5	474.4	250	Kit	1
6	pCIP	.658	2	7.3	380.2	250	Kit	1
7	pMIG.mk13	.501	2	7.5	499.5	250	Kit	1
8	pUC19	.522	2	7.5	478.5	250	Kit	2
9	pCIP	1.078	2	6.9	231.9	250	Kit	2
10	pMIG.mk13	.506	2	7.5	493.7	250	Kit	2

Results:



Chemidoc output of .7% agarose gel



Conclusion:

The concentration of DNA from the mini prep done by Alkaline lysis was not as pure at the samples done with the mini prep kits as seen in lanes 2-4. The corresponding lanes with respect to each plasmid DNA look similar across both kit batches.

10/1/2018 - Lab 9: Restriction Digest using EcoR1

Table of Contents

Saturday, August 25, 2018 5:03 PM

Objective:

Compare DNA cut with single EcorRI restriction enzyme of two plasmids with that of uncut plasmids.

Today's Topics:

- DNA isolation
- Gel electrophoresis
- Restriction Enzymes
- Restriction Enzyme Buffers
- Circular DNA
- Linearized DNA

Protocol:

Materials:

- 1.5 mL Microcentrifuge tubes
- pUC19 and pCIP plasmids stored in -20°C.
- Ice buckets
- Mini electrophoresis system
- 8-tooth comb
- .28 grams of agarose
- 40 mL SB buffer
- 200 mL Erlenmeyer Flask
- Microwave
- 4 µL SYBR Safe staining dye
- DEPC H_20
- Chemidoc instrument (Biorad)
- Gloves or rubber holder
- 4 µL of 20,000 U/mL EcorR1
- 20 µL of 10x enzyme buffer
- Pipettes and tips
- Centrifuge
- 5X loading dye

Prepare Samples see (Table 2):

1. Setup reaction volume of 25 mL for uncut DNA:
    a. Add in water, 0.5 µg DNA, and 5 µL of 5X loading dye in accordance with concentration of DNA.
    b. Keep samples on ice
2. Setup reaction volume of 60 mL for cut DNA:
    a. Add in water first in accordance with DNA concentration.
    b. Add in 5 µL of 10X buffer for EcoR1.
    c. Add in volume of DNA in accordance with concentration to contain 1 µg of DNA.
    d. Add 1 µL of EcoR1 enzymes to each sample.
3. Once cut samples are prepared, quick spin tubes in centrifuge to pellet down.
4. Incubate cut samples in warm water bath at 37° C for 1 hour.
5. Began preparation of agarose gel for Electrophoresis (see below).
6. After one hour of incubation for cut samples, add 10 µL of 5X loading dye to samples and quick spin in centrifuge.
7. Load 100 bp ladder, 25 µL from each uncut sample, and 30 µL for each cut sample into each lane of gel in accordance to procedure below for agarose gel.

Procedure Agarose Gel:

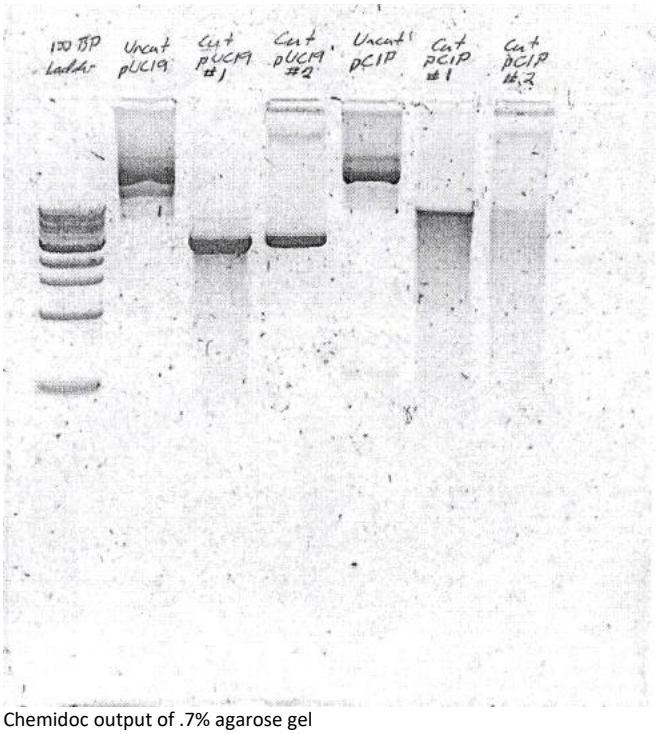
1. See procedure from Lab 8: Gel Electrophoresis for setting up, loading and running .7% agarose gel.

Table 2: List of Samples for Gel Layout

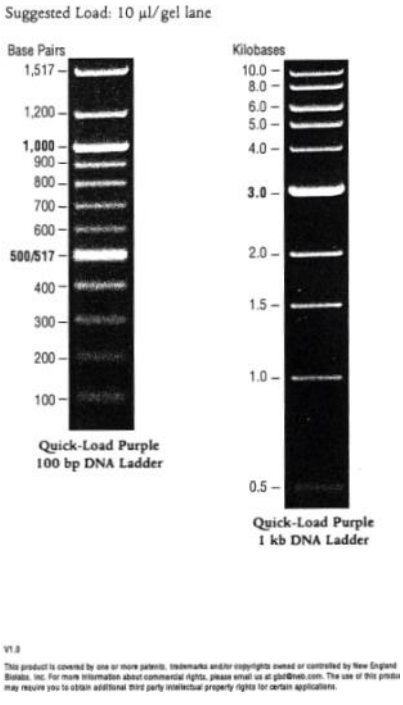
Gel Lane	Sample #	Volume	Amount of	Amount of	Amount of 5X	Enzyme (10-20	Concentration	Total Amount

#		pDNA Used (µL)	10X Enzyme Buffer (µL)	DEPC H_2O (µL)	Loading Buffer (µL)	Units/Reaction)	Ng/µL	of DNA µg
1	100 bp DNA Ladder	None	None	None	None	None	None	None
2	Uncut pUC19	1.1	None	18.9	5	None	474.4	500 Ng
3	Cut pUC19 Batch #1	2.1	5	41.9	10	1	474.4	1 µg
4	Cut pUC19 Batch #2	2.1	5	41.9	10	1	478.5	1 µg
5	Uncut pCIP	1.3	None	18.7	5	None	380.2	500 Ng
6	Cut pCIP Batch #1	2.6	5	41.4	10	1	380.2	1 µg
7	Cut pCIP Batch #2	4.3	5	39.7	10	1	231.9	1 µg

Results:



Chemidoc output of .7% agarose gel



Conclusion:

In analyzing the output from the electrophoresis we can see that the circular DNA of the uncut samples is larger and moves slower than the cut linear DNA. EcoR1 only has one cut site in both sequence so both cut and uncut DNA is same size but the cut DNA is linear instead of circular.

Table of Contents

10/8/2018 - Lab 10: Restriction Digest of λ DNA using EcoRI and HindIII

Saturday, August 25, 2018 5:03 PM

Objective:

Compare length of DNA fragments using combination of different restriction enzymes in Lambda DNA.

Today's Topics:

- DNA isolation
- Gel electrophoresis
- Restriction Enzymes: EcoR1 and HindIII
- Restriction Enzyme Buffers
- Linearized DNA
- Lambda DNA
- Bacteriophage

Protocol:

Materials:

- 1.5 mL Microcentrifuge tubes
- Lambda DNA plasmid stored in -20°C.
- Ice buckets
- Mini electrophoresis system
- 8-tooth comb
- .28 grams of agarose
- 40 mL SB buffer
- 200 mL Erlenmeyer Flask
- Microwave
- 4 µL SYBR Safe staining dye
- DEPC H_20
- Chemidoc instrument (Biorad)
- Gloves or rubber holder
- 2 µL of 20,000 U/mL EcorR1 restriction enzyme
- 2 µL of 20,000 U/mL HindIII restriction enzyme
- 10X NeBuffer EcoR1
- 10X Cutsmart Buffer
- Pipettes and tips
- Centrifuge
- 5X loading dye

Prepare Samples see (Table 2):

1. Setup reaction volume of 31 mL for uncut DNA:
    a. Add in water, .5 µg DNA, (I also added in 2.5 µL which probably should not have done but won't affect results) and 6 µL of 5X loading dye in accordance with concentration of DNA.
    b. Keep sample on ice
2. Setup reaction volume of 25 mL for cut DNA:
    a. Add in water first in accordance with DNA concentration and volume of enzymes being used.
    b. Add in 2.5 µL of 10X NEbuffer for EcoR1 to 2nd sample, and add 2.5 µL of 10X Cutsmart buffer for HindIII to 3rd sample as well as to 4th sample for EcoR1 with HindIII.
    c. Add 2 µL of λDNA in accordance with concentration to contain 1 µg of DNA.
    d. Add 1 µL of EcoR1 enzyme to sample 2, 1µL of HindIII enzyme to sample 3, and 1 µL of each EcorR1 and HindIII to sample 4.
3. Once cut samples are prepared, quick spin tubes in centrifuge to pellet down.
4. Incubate cut samples in warm water bath at 37° C for 1 hour.
5. Began preparation of agarose gel for Electrophoresis (see below).
6. After one hour of incubation for cut samples, add 6 µL of 5X loading dye to samples and quick spin in centrifuge.
7. Load 31 µL from each cut and uncut sample into each lane of gel in accordance to procedure below for agarose gel.
8. Analyze results to expected cut site results from NEBcutter 2.0 tool (see Table 3).

Procedure Agarose Gel:

1. See procedure from Lab 8: Gel Electrophoresis for setting up, loading and running .7% agarose gel.

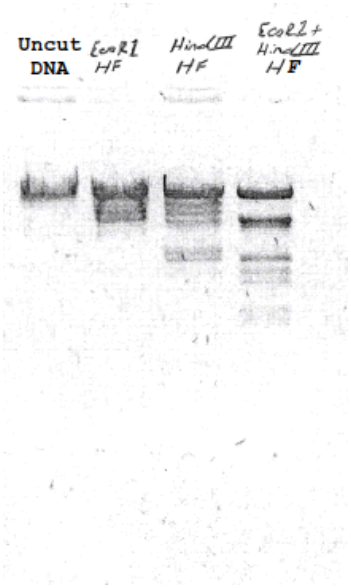
Table 2: List of Samples for Gel Layout

Gel Lane #	Sample #	λDNA Used (µg)	Volume λDNA From Stock (µL)	10X Enzyme Buffer (µL)	Enzyme (10-20 Units/Reaction) (µL)	Enzyme Buffer Used	Amount of 5X Loading Buffer (µL)	Amount of DEPC H_2O (µL)
1	λDNA Uncut	0.5	1	2.5	0	NeBuffer EcoR1	6	21.5
2	λDNA + EcoR1	1.0	2	2.5	1 of EcoR1	NeBuffer EcoR1	6	19.5
3	λDNA + HindIII	1.0	2	2.5	1 of HindIII	Cutsmart Buffer	6	19.5
4	λDNA + EcoR1 + HindIII	1.0	2	2.5	2 (1 of EcoR1 & 1 of HindIII)	Cutsmart Buffer	6	18.5

Table 3: Expected Length/Size (bp) of Fragments for λDNA

EcorR1	HindIII	EcoR1 + HindIII
21226	23130	21226
7421	9416	**5148**
5804	6682	**4973**
5643	4361	4268
4878	**2322**	3530
3530	**2027**	**2027**
	564	**1904**
		1709
		1375
		947
		831
		564

Results:



Chemidoc output of .7% agarose gel

Conclusion:

For the sample cut with just EcoR1 there should have seen a band on the top and then seen 4 more bands because bands 3 and 4 would overlap. I only see 3 bands below the top band maybe because the 5th band of 4878 is close to bands 3 and 4. For the sample cut with HindIII there are 7 bands total but 6 should be seen since the 5th and 6th band are pretty close. I only see 5 bands and I do not see the smallest band of 564 bp. For the sample cut with both there are 12 bands total but I am presuming we will only see 8 due to close overlaps of band lengths. I can make out all 8 if I count the second band as two since there seems to be a faint band just below the second band. After band 4 I see 4 more faint bands.

10/10/2018 - Lab 11: Generate Restriction Maps for Plasmid DNA

Table of Contents

Saturday, August 25, 2018 5:03 PM

Objective:

Generate restriction maps that are unique fingerprints for certain plasmids. These can be used to identify unknown samples when using the same restriction enzymes used in the mapping.

Today's Topics:

- DNA isolation
- Gel electrophoresis
- Restriction Enzymes
- Restriction Enzyme Buffers
- Circular DNA
- Linearized DNA
- Restriction Mapping

Materials:

- 1.5 mL Microcentrifuge tubes
- pUC19, pCIP, and pMIG.mk3 plasmids stored in -20°C.
- Ice buckets
- Mini electrophoresis system
- 8-tooth comb
- .28 grams of agarose
- 40 mL SB buffer
- 200 mL Erlenmeyer Flask
- Microwave
- 4 µL SYBR Safe staining dye
- DEPC H_20
- Chemidoc instrument (Biorad)
- Gloves or rubber holder
- 3 µL of 20,000 U/mL HindIII restriction enzyme
- 10X NeBuffer EcoR1
- 10X Cutsmart Buffer
- Pipettes and tips
- Centrifuge
- 5X loading dye

Prepare Samples see (Table 2):

1. Setup reaction volume of 25 mL for cut DNA:
    a. Add in water first in accordance with DNA concentration and volume of enzymes being used.
    b. Add in 2.5 µL of 10X Cutsmart buffer for HindIII to all 3 DNA samples.
    c. Add volume of DNA in accordance with concentration to contain 1 µg of DNA.
    d. Add 1 µL of HindIII enzyme to all 3 DNA samples.
2. Once cut samples are prepared, quick spin tubes in centrifuge to pellet down.
3. Incubate cut samples in warm water bath at 37° C for 1 hour.
4. Began preparation of agarose gel for Electrophoresis (see below).
5. After one hour of incubation for cut samples, add 6 µL of 5X loading dye to samples and quick spin in centrifuge.
6. Load 31 µL from each cut sample into each lane of gel in accordance to procedure below for agarose gel.
7. Analyze results with respect to expected cut site results from NEBcutter 2.0 tool (see Table 3).

Procedure Agarose Gel:

1. See procedure from Lab 8: Gel Electrophoresis for setting up, loading and running .7% agarose gel.

Table 2: List of Samples for Gel Layout

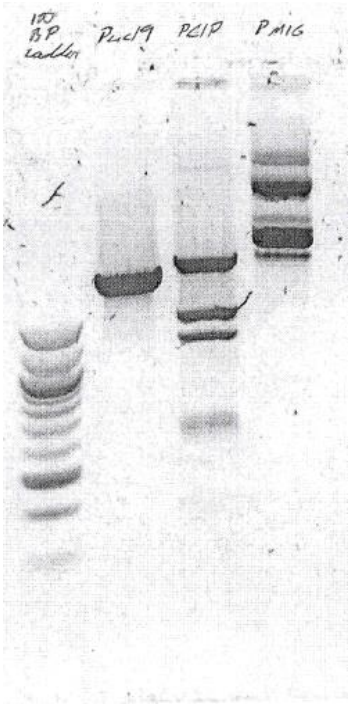
Gel Lane #	Sample #	DNA Used (µg)	Volume DNA From Stock (µL)	DNA Concentration Ng/µL	10X Enzyme Buffer (µL)	Enzyme (10-20 Units/Reaction) (µL)	Enzyme Buffer Used	Amount of 5X Loading Buffer (µL)	Amount of DEPC H_2O (µL)
1	100 bp DNA Ladder	None	None	None	None	None	None	None	None

2	pUC19	1.0	2.1	474.4	2.5	1 of HindIII-HF	Cutsmart Buffer	6	19.4
3	pCIP	1.0	2.6	380.2	2.5	1 of HindIII-HF	Cutsmart Buffer	6	18.9
4	pMIG.mk3	1.0	2.0	499.5	2.5	1 of HindIII-HF	Cutsmart Buffer	6	19.5

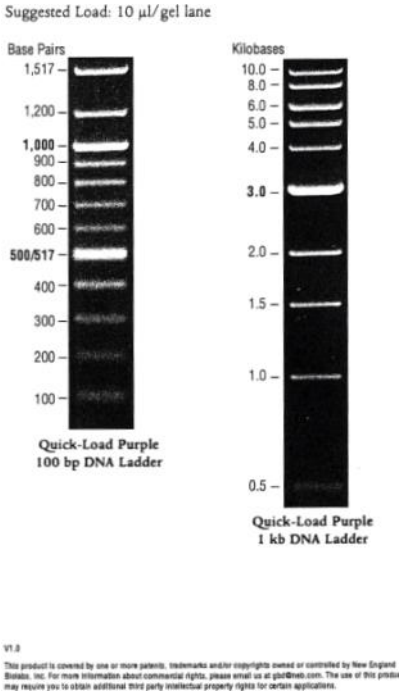
Table 3: Expected Length/Size (bp) of Fragments for DNA
* Using only HindIII Enzyme

pUC19	pCIP	pMIG.mk3
2635	3424	5967
	1436	1096
	1157	
	583	
	533	
	348	

Results:



Chemidoc output of .7% agarose gel



Conclusion:

The bands in the gel matched the mapping for pUC19 with only one band at 2635 bp and for pCIP which had a band above pUC19 at around 3424 bp and then two more bands at around 1100 and 1400. The last 3 bands for pCIP were faint and close together. pMIG.mk3 sample should have only had two bands, one which we see as the first band of around 5967 bp but it looks like there are bands below that one and above the 3400 bp band of pCIP so this indicates there are some bands between 3400 and 5900 for pMIG.mk3 which is not the case of the mapping from the NEB tool.

10/22/2018 - Lab 12: Testing Food for GMO

Table of Contents

Saturday, August 25, 2018 5:03 PM

Objective:

Extract DNA from foods in which it is unknown if they are genetically modified organisms (GMO). Identify through PCR by adding Plant and GMO primers to the foods. Run DNA in gel to confirm if there are genetically modified DNA bands.

Today's Topics:

- PCR
- Genetically Modified Organisms (GMO)
- Plant primers
- GMO primers
- Instagene
- DNA replication

Protocol:

Materials:

- Microcentrifuge tubes
- 8 0.5 mL PCR tubes
- 2 grams Non-GMO food
- 2 grams Soy protein isolate
- 2 grams Papaya
- 40 µL GMO+ food
- Ice buckets
- Mini electrophoresis system
- 15-tooth comb
- .28 grams of agarose
- 40 mL SB buffer
- 200 mL Erlenmeyer Flask
- Microwave
- 4 µL SYBR Safe staining dye
- DEPC H_20
- Chemidoc instrument (Biorad)
- Gloves or rubber holder
- Pipettes and tips
- Centrifuge
- 80 µL Plant Master Mix
- 80 µL GMO Master Mix
- 80 µL Orange G loading dye

Procedure Day 1:

1. Label 3 screw caps "Non-GMO", "Soy", and "Papaya".
2. Starting with Non-GMO first, weigh 2 grams and place in mortar.
3. Add 10 mL of water to the mortar.
4. Grind with pestle until a slurry is created.
5. Pipette sample into capped tube and place on ice.
6. Repeat steps 2-5 for both Soy and Papaya.
7. For each sample pipette 50 µL slurry into tube containing 500 µL of Instagene.
8. Cap tubes and place in 95° C water bath for 5 minutes.
9. After 5 minutes centrifuge at max speed for 5 minutes.
10. Label 8 PCR tubes in accordance with rows 1-8 in Table 2 below.
11. In PCR tubes 1, 3, 5, and 7 pipette 20 µL of Plant Master Mix (green) and in PCR tubes 2, 4, 6, and 8 pipette 20 µL of GMO Master Mix (red).
12. Place all tubes in a capless microtube adapter and place the adapter deepened into the ice.
13. Using a fresh tip each tim,e pipette 20 µL from the top of each of samples into the first 6 PCR tubes, twice for each sample, making sure to not pipette from pellet matrix at bottom of centrifuge tube. Pipette up and down to mix sample.
14. In PCR tubes 7 and 8 pipette in 20 µL from GMO+ DNA. Pipette up and down to mix sample.

15. Place PCR tubes in thermal cycle for 4 hours to replicate DNA.

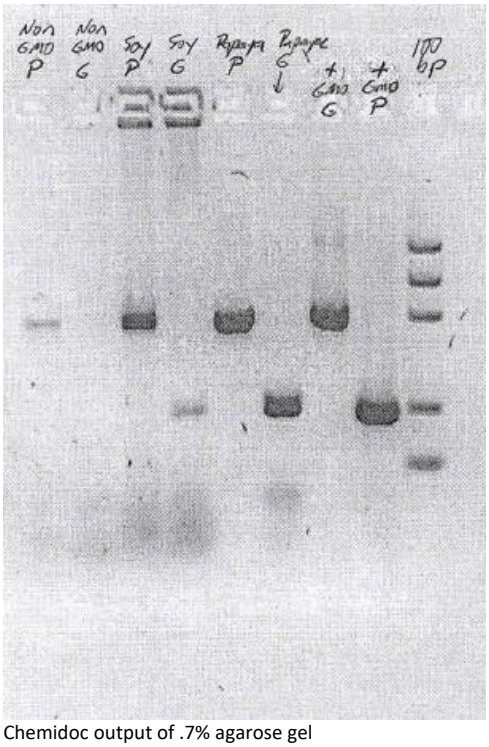
Procedure Day 2:

1. Set up gel electrophoresis system. See procedure from Lab 8: Gel Electrophoresis for setting up, loading and running agarose gel. Instead of using .7 % gel in SB buffer make 3% agarose gel in 1X TAE buffer. Dilute 50X TAE with 10 mL of 50 TAE with 490 mL of water. Weight out 1.2 g of agarose from 40 mL of 1X TAE to make 3% gel.
2. Obtain PCR tubes from thermal cycler.
3. Pulse spin tubes in centrifuge for 3-6 seconds.
4. Using a fresh tip each time, add 10 µL Orange G loading dye to each of the 8 tubes and pipette up and down.
5. Load each gel lane in accordance to order in Table 2.
6. Instead of running gel for 40 minutes run for only 20 minutes at 100 V.
7. Analyze results under Chemidoc instrument.

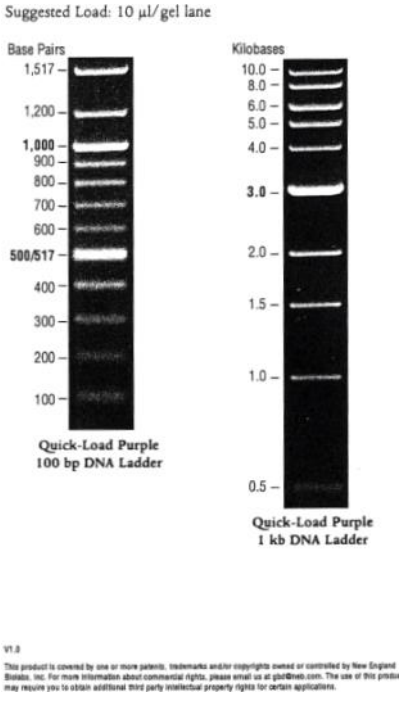
Table 2: List of Samples for Gel Layout

Gel Lane #0	Sample #	Volume DNA Used (µL)	Master Mix Used	Amount of Master Mix (µL)	Amount of Orange G loading dye (µL)
1	Non-GMO PMM	20	Plant	20	10
2	Non-GMO GMM	20	GMO	20	10
3	Soy PMM	20	Plant	20	10
4	Soy GMM	20	GMO	20	10
5	Papaya PMM	20	Plant	20	10
6	Papaya GMM	20	GMO	20	10
7	Papaya PMM	20	Plant	20	10
8	Non-GMO GMM	20	GMO	20	10
9	100 bp DNA Ladder	10	None	None	None

Results:



Chemidoc output of .7% agarose gel



Conclusion:

Both the Soy protein isolate and Papaya had 200 bp fragments at the same spot which the GMO+ 200 bp fragment was so both foods are GMO.

10/29/2018 - Lab 13: Preparation of Reagents and Transformation for pcDNA.EGFP

Table of Contents

Saturday, August 25, 2018 5:03 PM

Objective:

Prepare Luria Broth from growing cultures.
Prepare LB Agar plates for growing colonies.
Prepare DNA transformation of E. coli with pcDNA.EGFP

Today's Topics:

- Plasmid DNA
- Gene of Interest
- Insert Vectors
- Gene Cloning

Protocol:

Materials:

- See Lab 6: Transformation for materials for transformation
- 230 mL Luria Broth
- 250 mL LB Agar
- 5 mL transfer pipettes
- Culture tubes
- Autoclave
- Shaker
- Erlenmeyer Flasks
- 10 cm petri plates
- Ampillicin (100 mg/ml) stock concentration

Procedure pcDNA.EGFP transformation **(we may have done this on the 24th)** :

1. See procedure from Lab 6: Transformation but instead of using pUC19 (100 pg mass, 1 ng mass, 10 ng mass) use only 1 ng of pcDNA.EGFP.
2. Do not add IPTG or Xgal to agar plate.

Procedure preparation of reagents:

1. Preparation of LB media
    a. Prepare 230 mL Luria Broth in Erlenmeyer flask
    b. Transfer 5 mL to 25 culture tubes
    c. Add remaining LB to Erlenmeyer flask
    d. Place cap on top and autoclave culture tubes and rest of LB

2. Preparation Miller LB-Agar Plates:
    a. Prepare 250 mL Miller LB-Agar in Erlenmeyer flask
    b. Autoclave using liquid cycle
    c. Add to 50 °C water bath to allow cooling to 50 °C
    d. Add 25 mg (250 µL of stock) ampicillin to achieve 100 µg/mL concentration
    e. Pour around 20 mL of LB-Agar plus ampicillin into 10-cm petri dishes and allow cooling before storing at 4 °C
3. Preparation plasmid starter cultures: **(We only did this for pcDNA.EGFP)**
    a. Obtain tubes of 5 mL LB broth
    b. Add 5 µL of ampicillin to each tube to achieve 100 µg/mL concentration
    c. Pick plates with transformed bacteria and drop into LB and ampicillin
    d. Place in shaker for 12-16 hours

Results:

I must not have taken a picture of the pcDNA.EGFP transformation because I don't have the plates.

Conclusion:

None

10/31/2018 - Lab 14: Midiprep Plasmids and Vectors for Cloning Project

Table of Contents

Saturday, August 25, 2018 5:03 PM

Objective:

Make high concentration of plasmid DNAs and plasmids DNAs for vector insert isolation.

Today's Topics:

- Plasmid DNA
- Gene of Interest
- Insert Vectors
- Gene Cloning

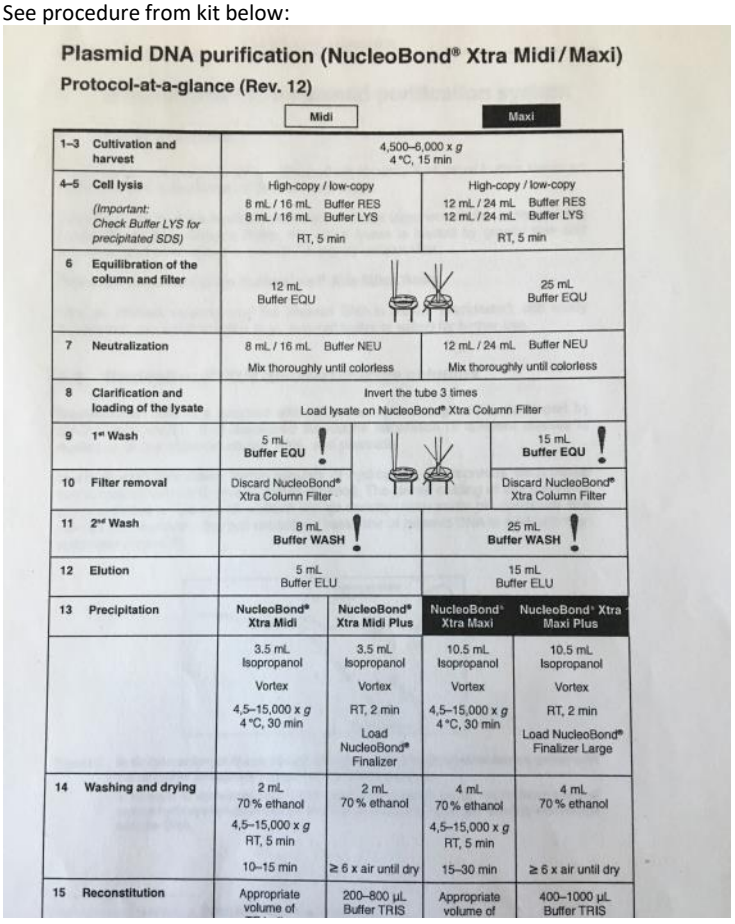
Protocol:

Materials:

- NucleoBond Xtra Midi Plasmid DNA Purification Kit
- Preparation of starter cultures which have been transformed with pUC19, pCIP, pMIG.mk3, and pcDNA.EGFP
- DEPC Water
- Nano drop
- 1.5 mL microcentrifuge tubes
- Pipettes and tips
- Centrifuge
- Ice bucket

Procedure:

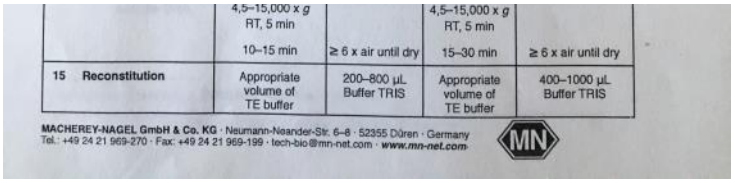
1. See procedure from kit below:



Plasmid DNA purification (NucleoBond® Xtra Midi / Maxi)

Protocol-at-a-glance (Rev. 12)

		Midi		Maxi	
1–3	Cultivation and harvest	4,500–6,000 x *g* 4 °C, 15 min			
4–5	Cell lysis *(Important: Check Buffer LYS for precipitated SDS)*	High-copy / low-copy 8 mL / 16 mL Buffer RES 8 mL / 16 mL Buffer LYS RT, 5 min		High-copy / low-copy 12 mL / 24 mL Buffer RES 12 mL / 24 mL Buffer LYS RT, 5 min	
6	Equilibration of the column and filter	12 mL Buffer EQU		25 mL Buffer EQU	
7	Neutralization	8 mL / 16 mL Buffer NEU Mix thoroughly until colorless		12 mL / 24 mL Buffer NEU Mix thoroughly until colorless	
8	Clarification and loading of the lysate	Invert the tube 3 times Load lysate on NucleoBond® Xtra Column Filter			
9	1st Wash	5 mL **Buffer EQU** !		15 mL **Buffer EQU** !	
10	Filter removal	Discard NucleoBond® Xtra Column Filter		Discard NucleoBond® Xtra Column Filter	
11	2nd Wash	8 mL **Buffer WASH** !		25 mL **Buffer WASH** !	
12	Elution	5 mL Buffer ELU		15 mL Buffer ELU	
13	Precipitation	NucleoBond® Xtra Midi	NucleoBond® Xtra Midi Plus	NucleoBond® Xtra Maxi	NucleoBond® Xtra Maxi Plus
		3.5 mL Isopropanol Vortex 4,5–15,000 x *g* 4 °C, 30 min	3.5 mL Isopropanol Vortex RT, 2 min Load NucleoBond® Finalizer	10.5 mL Isopropanol Vortex 4,5–15,000 x *g* 4 °C, 30 min	10.5 mL Isopropanol Vortex RT, 2 min Load NucleoBond® Finalizer Large
14	Washing and drying	2 mL 70 % ethanol 4,5–15,000 x *g* RT, 5 min 10–15 min	2 mL 70 % ethanol ≥ 6 x air until dry	4 mL 70 % ethanol 4,5–15,000 x *g* RT, 5 min 15–30 min	4 mL 70 % ethanol ≥ 6 x air until dry
15	Reconstitution	Appropriate volume of	200–800 µL Buffer TRIS	Appropriate volume of	400–1000 µL Buffer TRIS



		4,5–15,000 x *g* RT, 5 min 10–15 min	≥ 6 x air until dry	4,5–15,000 x *g* RT, 5 min 15–30 min	≥ 6 x air until dry
15	**Reconstitution**	Appropriate volume of TE buffer	200–800 µL Buffer TRIS	Appropriate volume of TE buffer	400–1000 µL Buffer TRIS

MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6–8 · 52355 Düren · Germany
Tel.: +49 24 21 969-270 · Fax: +49 24 21 969-199 · tech-bio@mn-net.com · www.mn-net.com

2. Use nano drop to measure DNA concentration and DNA/protein ratio
3. Store plasmids in -20 °C

Results:

Table 1: Nano drop measurements:

Plasmid DNA	Concentration ng/µL	A 260/280 Ratio
pcDNA.EGFP	4071	1.79
pCIP	185.5	1.91
pMIG.mk3	3366.7	1.84
pUC19	1686.7	1.92

Conclusion:

When we re-ran the Nano drop again a few weeks later all the concentrations dropped.

11/5/2018 - Lab 15: PCR Amplification of GOI from plasmid DNAs and Restriction Digest of Plasmid Vector DNA

Table of Contents

Saturday, August 25, 2018 5:03 PM

Objective:

Using Polymerase Chain Reaction (PCR) and forward and reverse primers to amplify DNA in order to restriction digest DNA to isolate Gene of Interest (GOI). Digest Plasmid vector with same restriction enzymes as

Today's Topics:

- Plasmid DNA, Plasmid Vectors
- Gene of Interest
- Insert Vectors
- Gene Cloning
- Polymerase Chain Reaction
- DNA Primers
- Restriction Digest
- DNA Amplification

Protocol:

Materials PCR:

- PCR Thermal Cycler
- .2 mL PCR tubes and PCR tube rack
- 1.5 mL microcentrifuge tubes
- Ice Bucket
- Micropipettes and **filtered** tips
- Template DNA - pcDNA.EGFP and pMIG.mk3
- Forward and Reverse Primers for each Template DNA (10 mM stock)
- dNTPs (10 mM stock)
- Taq DNA Polymerase (5000 U/mL stock)
- Taq polymerase buffer (10X stock)
- PCR grade water

Materials for Plasmid Vector Digest:

- See Lab 9: Restriction Digest
- Plasmids pCIP and pUC19
- Restriction Digest Enzmyes
    - EcoR1-HF (20,000 U/mL)
    - BamH1-HF (20,000 U/mL)

Procedure PCR:

1. Mix and quick spin reagents in Master Mix Table 1 below except for Taq polymerase
2. Create master mix for 8 samples for temperature gradient according to Master Mix Table 1 for pcDNA.EGFP
3. Transfer 50 µL of Master Mix to 8 PCR tubes
4. Transfer tubes to PCR thermal cycler
5. Run thermal cycler according to program below:
    a. 95 °C > 2 min
    b. 95 °C > 30 sec
    c. 65 to 55 °C gradient > 30 sec
        i. 65 °C
        ii. 64.2 °C
        iii. 63 °C
        iv. 61.1 °C
        v. 58.7 °C
        vi. 56.9 °C
        vii. 55.7 °C
        viii. 55 °C
    d. 68 °C > 2 min
    e. Repeat b through d 34 times
    f. 68 °C > 5 min
    g. 4 °C > Hold

Master Mix Table 1:

**Table is for 8 samples for each pcDNA.EGFP and pMIG.mk3

	50 µL Reaction (µL)	Master mix 8.5X (µL)
Template DNA (10 ng/µL)	1	8.5
Forward Primer	2	17
Reverse Primer	2	17
dNTPs	1	8.5
10x Taq Buffer	5	42.5
H_2O	38.6	328.1
Taq Enzyme	0.4	3.4

Procedure for Restriction Digest:

1. See Lab 9: Restriction Digest except use Table 3: reaction setup below instead of Table 2 from Lab 9

Table 3: Reaction Setup (See Lab 14: Midiprep for Concentration)

	pUC19 Tube (units µL)	pCIP Tube (units µL)
Plasmid DNA (3 µg)	1.1	16.2
10X Cutsmart Buffer	5	5
DI Water	41.9	26.8
EcoR1-HF (20,000 U/mL)	1	1
BamH1-HF (20,000 U/mL)	1	1

Results:

None - Need to perform restriction digest in next lab of PCR results

Conclusion:

None

Table of Contents

11/7/2018 - Lab 16: Electrophoresis of EGFP PCR Reactions, Purification of Cut Plasmid Vectors, Phosphatase Treat Cut Plasmid Vectors

Saturday, August 25, 2018 5:03 PM

Objective:

Confirm Polymerase Chain Reaction (PCR) worked for EGFP gene by running PCR reactions on gel. Purify cut Plasmid vectors using DNA purification kit with silica column. Treat

Today's Topics:

- Plasmid DNA
- Gene of Interest
- Insert Vectors
- Polymerase Chain Reaction
- DNA Primers
- Restriction Digest
- DNA Amplification
- DNA Purification

Protocol:

Materials:

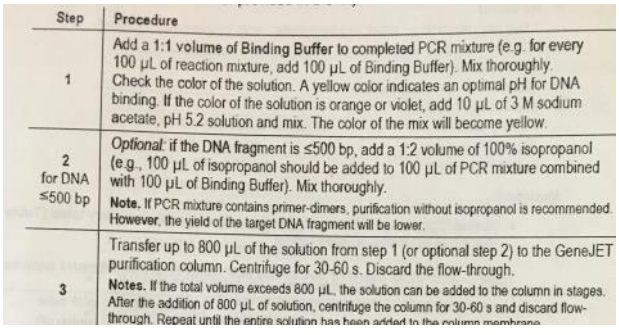
- Mini electrophoresis system
- 15-tooth comb
- .28 grams of agarose
- 40 mL SB buffer
- 200 mL Erlenmeyer Flask
- Microwave
- 4 µL SYBR Safe staining dye
- DEPC H_20
- Chemidoc instrument (Biorad)
- Gloves or rubber holder
- Wax Paper
- Ice Bucket
- Loading Dye
- 1KB Ladder
- Silica Column Purification Kit
- 1.5 mL Microcentrifuge tubes
- Calf Intestinal Phosphatase enzyme (10,000 U/mL)
- 10X Cutsmart buffer

Procedure Confirm PCR on Gel for EGFP Insert:

1. See process from Gel Electrophoresis lab
2. Load gel with all 8 columns from EGFP PCR reactions
    a. Apply 5 µL of each column to wax paper
    b. Add 1 µL loading dye to each drop
    c. Load 6 µL in 8 different lanes
    d. Load 1KB ladder
3. Run Gel electrophoresis and interpret results
4. Store PCR reactions at -20 °C

Procedure for Purification of Plasmid Vector and Phosphatase Treatment:

1. See procedure below except in step 6 elute with 43 µL DEPC water



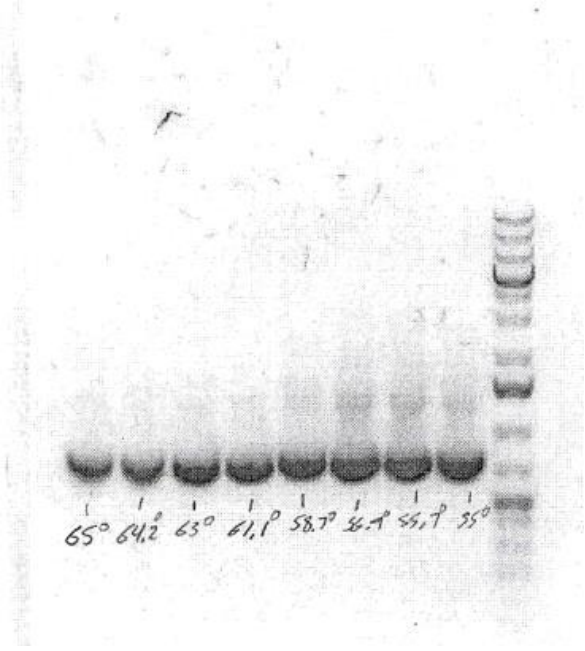
Step	Procedure
1	Add a 1:1 volume of Binding Buffer to completed PCR mixture (e.g. for every 100 µL of reaction mixture, add 100 µL of Binding Buffer). Mix thoroughly. Check the color of the solution. A yellow color indicates an optimal pH for DNA binding. If the color of the solution is orange or violet, add 10 µL of 3 M sodium acetate, pH 5.2 solution and mix. The color of the mix will become yellow.
2 for DNA ≤500 bp	*Optional:* if the DNA fragment is ≤500 bp, add a 1:2 volume of 100% isopropanol (e.g., 100 µL of isopropanol should be added to 100 µL of PCR mixture combined with 100 µL of Binding Buffer). Mix thoroughly. **Note.** If PCR mixture contains primer-dimers, purification without isopropanol is recommended. However, the yield of the target DNA fragment will be lower.
3	Transfer up to 800 µL of the solution from step 1 (or optional step 2) to the GeneJET purification column. Centrifuge for 30-60 s. Discard the flow-through. **Notes.** If the total volume exceeds 800 µL, the solution can be added to the column in stages. After the addition of 800 µL of solution, centrifuge the column for 30-60 s and discard flow-through. Repeat until the entire solution has been added to the column membrane.

3	Transfer up to 800 µL of the solution from step 1 (or optional step 2) to the GeneJET purification column. Centrifuge for 30-60 s. Discard the flow-through. **Notes.** If the total volume exceeds 800 µL, the solution can be added to the column in stages. After the addition of 800 µL of solution, centrifuge the column for 30-60 s and discard flow-through. Repeat until the entire solution has been added to the column membrane. **Close the bag with GeneJET Purification Columns tightly after each use!**
4	Add **700 µL** of **Wash Buffer** (diluted with the ethanol as described on p. 3) to the GeneJET purification column. Centrifuge for 30-60 s. Discard the flow-through and place the purification column back into the collection tube.
5	Centrifuge the empty GeneJET purification column for an additional 1 min to completely remove any residual wash buffer. **Note.** This step is essential as the presence of residual ethanol in the DNA sample may inhibit subsequent reactions.
6	Transfer the GeneJET purification column to a clean 1.5 mL microcentrifuge tube (not included). Add **50 µL** of **Elution Buffer** to the center of the GeneJET purification column membrane and centrifuge for 1 min. **Note** • For low DNA amounts the elution volumes can be reduced to increase DNA concentration. An elution volume between 20-50 µL does not significantly reduce the DNA yield. However, elution volumes less than 10 µL are not recommended. • If DNA fragment is >10 kb, prewarm Elution Buffer to 65 °C before applying to column. • If the elution volume is 10 µL and DNA amount is ≥5 µg, incubate column for 1 min at room temperature before centrifugation.
7	Discard the GeneJET purification column and store the purified DNA at -20 °C.

2. Add 5 µL 10X Cutsmart buffer and 2 µL Calf Intestinal Phosphatase
3. Mix and quick spin
4. Incubate in 37 °C Water bath for 1 hour
5. Repeat purification steps above and elute with 50 µL water
6. Store at -20 °C

Results:

PCR worked for EGFP gene.



Conclusion:

None

11/14/2018 - Lab 17: Purify EGFP PCR Reactions and Perform Restriction Digest, Run on Gel pMIG.mK3 PCR Reactions, Setup Ligation Reactions and Perform Ligation

Table of Contents

Saturday, August 25, 2018 5:03 PM

Objective:

Digest vector insert to prepare for insertion into Plasmid vector. Setup ligation reactions using DNA ligase to perform ligation of digested GOI into the recipient plasmid vector.

Today's Topics:

- Plasmid DNA
- Gene of Interest
- Insert Vectors
- Polymerase Chain Reaction
- DNA Primers
- Restriction Digest
- DNA Amplification
- DNA Purification
- Ligation

Protocol:

Materials:

- Mini electrophoresis system
- 15-tooth comb
- .28 grams of agarose
- 40 mL SB buffer
- 200 mL Erlenmeyer Flask
- Microwave
- 4 µL SYBR Safe staining dye
- DEPC H_20
- Chemidoc instrument (Biorad)
- Gloves or rubber holder
- Wax Paper
- Ice Bucket
- Loading Dye
- 1KB Ladder
- Silica Column Purification Kit
- 1.5 mL Microcentrifuge tubes
- Restriction Digest Enzmyes
    - EcoR1-HF (20,000 U/mL)
    - BamH1-HF (20,000 U/mL)
- 10X Cutsmart buffer
- T4 DNA Ligase (400,000 U/mL, NEB)
- 10X ligation buffer

Procedure for Purification of EGFP Insert:

1. See Purification procedure in Lab 16: Purification except in instead of adding Calf Intestinal Phosphatase enzyme adding 1 µL of each EcoR1-HF and BamH1-HF

Procedure for Restriction Digest of EGFP Insert:

1. See Restriction digest procedure from Lab 9: Restriction Digest
2. Repeat Purification above and store on ice for ligation preparation

Procedure Confirm PCR on Gel:

1. See process from Procedure Confirm PCR on Gel for EGFP Insert on Lab 16 except run for mk3 PCR reactions

Procedure Setup and Perform Ligation

1. Before setting up ligation reaction first run both Vector and Insert on Nano drop to determine concentration
2. Run both Vector and Insert on agarose gel to verify DNA is present **(I don't have these gel results so not sure if we did this)**
3. Setup DNA ligation reactions according to Table 4 using formula below:

$$Insert = Mass\ Plasmid * \frac{Length\ Insert}{Length\ Plasmid} * Mole\ Ratio$$

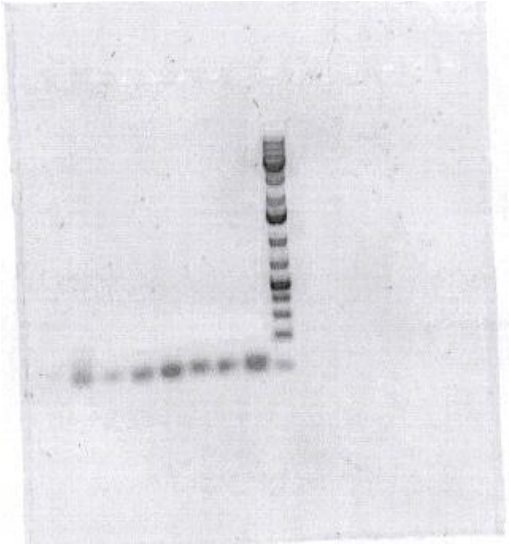
4. Label 3 microcentrifuge tubs according to reaction setup in Table 4
5. After adding reagent quick spin
6. Incubate at room temperature for 30 minutes
7. Heat inactivate T4 ligase by placing in 65 °C bath for 10 minutes
8. Store at -20 °C

Table 4: DNA Ligation Setup EGFP and pUC19

Tubes	Vector Only	Vector : Insert 1 : 3 (µL)	Vector : Insert 1 : 7 (µL)
pUC19 Vector (50 ng)	1.82	1.82	1.82
Insert EGFP	0	0.38	0.89
Ligase 10x Buffer	2	2	2
H_2O	15.18	14.8	14.29
DNA Ligase	1	1	1

Results:

PCR did not work for mk3 gene on 11/14. See last page for Summary of mk3 PCR.



Conclusion:

None

Table of Contents

11/19/2018 - Lab 18: Transformation of E. coli using Ligation Reactions and Run PCR for mk3 using DMSO

Saturday, August 25, 2018 5:03 PM

Objective:

Transform E. coli bacteria with ligation reactions of Plasmid insert and vector. Plate transformed bacteria and grow colonies so that we can screen for colonies that have taken new plasmid with GOI inserted.

Today's Topics:

- Plasmid DNA
- Gene of Interest
- Insert Vectors
- Polymerase Chain Reaction
- DNA Primers
- Restriction Digest
- Gene Cloning
- Ligation
- Transformation and Blue/White Screening
- DMSO for high G-C NTP content

Protocol:

Materials:

- See materials from Lab 6: Transformation
- Ligation reactions from Lab 17: Ligation Reactions
- See materials from Lab 15: PCR mk3
- DMSO

Procedure Transformation Using Ligation Reactions:

1. See procedure from Lab 6: Transformation except instead of using pUC19 using ligation reactions with insert of EGFP.

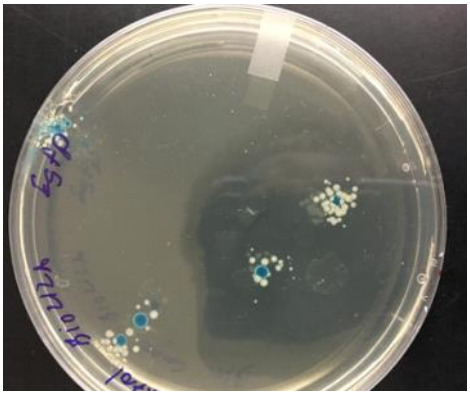
Procedure PCR of mk3:

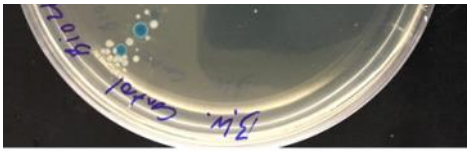
1. See procedure for setting up and running PCR reactions for GOI in Lab 15: PCR mk3 except instead of using 38.6 µL of H_2O used 37.6 µL and 1 µL DMSO for final DMSO concentration of 2%

Results:

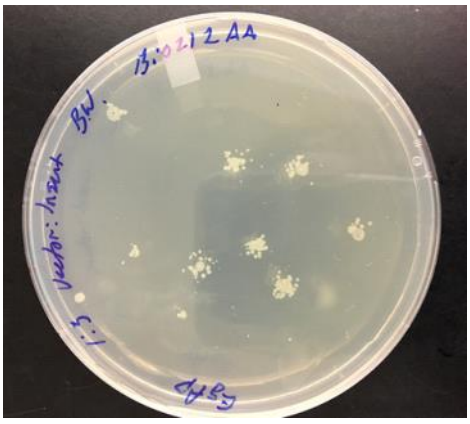
Transformation using EGFP in pUC19

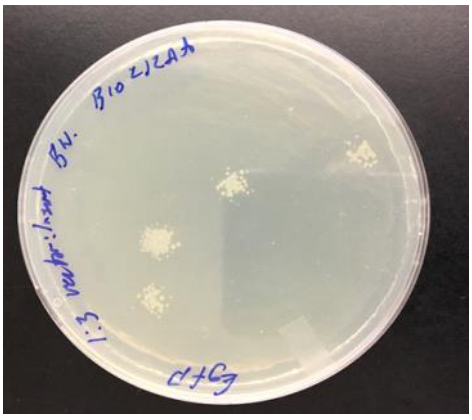
Control No Insert



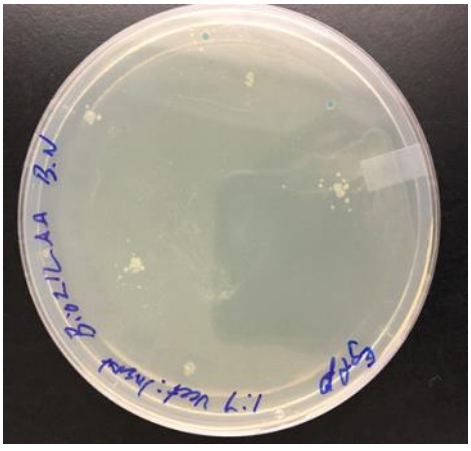


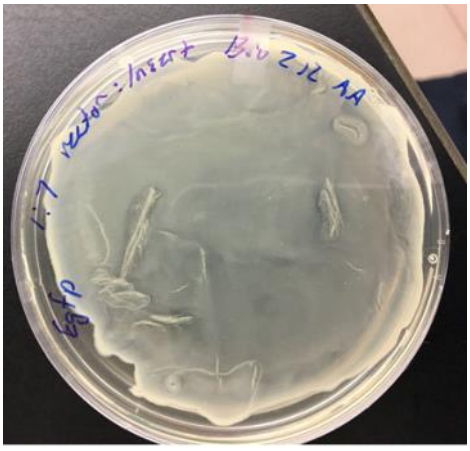
1 : 3 Insert to Vector Ratio



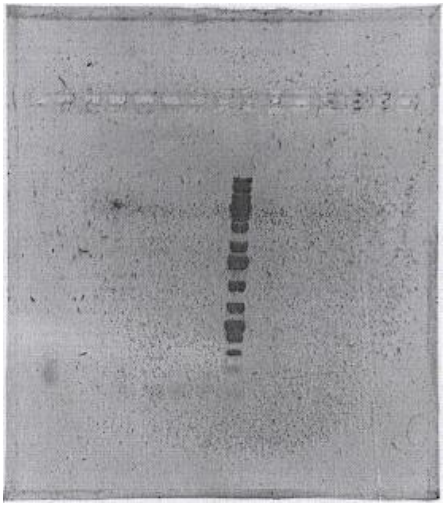


1 : 7 Insert to Vector Ratio





PCR mk3 with 2% DMSO



Conclusion:

White colonies grew on all plates and blue colonies grew on the control. The white colonies as expected on the plate with insert because of the interruption inside the lac-Z operon. There must have been a contamination issue on last plate for 1:7 ratio.

PCR failed again for mk3. We eventually tried again with more DMSO concentration.

Table of Contents

11/21/2018 - Lab 19: Perform Miniprep of Picked Colonies and Perform Restriction Digest

Saturday, August 25, 2018 5:03 PM

Objective:

White colonies were picked from the plate in Lab 19 which we assumed to have taken up the pUC19 Vector with EGFP insert. Cultures were grown and Miniprep followed by restriction digest and gel electrophoresis to determine the length of the GOI against the control with no GOI insert.

Today's Topics:

- Plasmid DNA
- Gene of Interest
- Insert Vectors
- Polymerase Chain Reaction
- DNA Primers
- Restriction Digest
- Gene Cloning
- Miniprep

Protocol:

Materials:

- See materials from Lab 7: Miniprep with kit

Procedure Grow Culture from Transformation of Ligation Reactions

1. Using 4 colonies from each 1:3 Vector to Insert and 1:7 Vector to Insert ratio pick colonies and grow in LB at 37 °C for 12-16 hours

Procedure Miniprep with Kit:

1. See procedure from Lab 7: Miniprep with kit

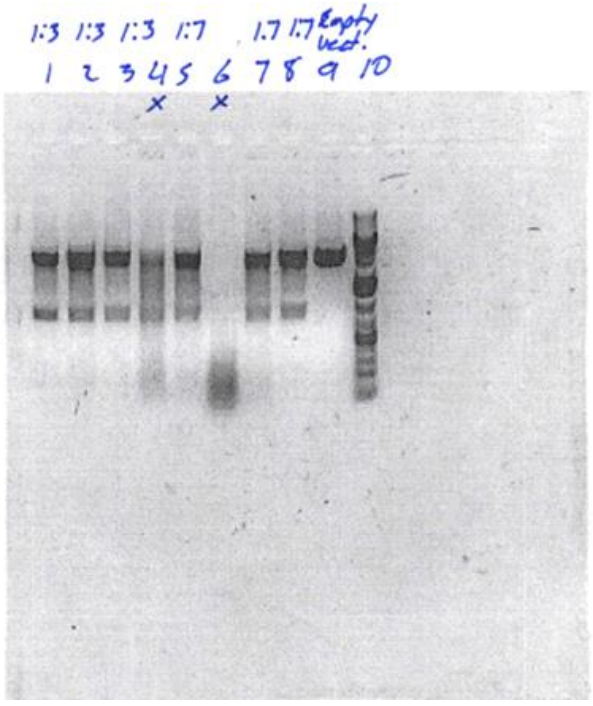
Procedure Restriction Digest of Isolated DNA of Transformed Bacteria:

1. See procedure for Lab 9: Restriction Digest except use EcoR1-HF (20,000 U/mL) and BamH1-HF (20,000 U/mL) in accordance with Table 5 below:

Table 5: Setup Restriction Digest Reactions for Transformed Bacteria with GOI

Sample		ug/uL	uL	enzyme	buffer	water
1:3 #1	1	0.547	1.8	2	2.5	18.7
1:3 #2	2	0.481	2.1	2	2.5	18.4
1:3 #3	3	0.43	2.3	2	2.5	18.2
1:3 #4	4	0.448	2.2	2	2.5	18.3
1:7 #1	5	0.421	2.4	2	2.5	18.1
1:7 #2	6	0.35	2.9	2	2.5	17.6
1:7 #3	7	0.475	2.1	2	2.5	18.4
1:7 #4	8	0.454	2.2	2	2.5	18.3

Results:



Conclusion:

After running restriction digest again the control with empty vector in Lane 9 it shows a band of around 700 bp in lanes 1, 2, 3, 5, 7, and 8 where this confirms the pUC19 vector took up the EGFP insert.

11/26/2018 to 12/03/2017 - Trouble Shoot PCR for mk3 and redo EGFP cloning into pCIP Vector

Table of Contents

Saturday, August 25, 2018 5:03 PM

Objective:

Trouble shoot PCR with mk3 by adding more DMSO and also changing temperature gradient. Performing entire gene cloning process from Lab 15 to Lab 19 with EGFP into pCIP instead of pUC19.

Today's Topics:

- Plasmid DNA
- Gene of Interest
- Insert Vectors
- Polymerase Chain Reaction
- DNA Primers
- Restriction Digest
- Gene Cloning
- Miniprep

Protocol:

Materials:

- See Lab 15 through 19

Procedure cloning GOI

1. See Lab 15 through 19 except instead of using pUC19 as vector use pCIP to insert EGFP
2. During transformation do not spread IPTG and X-gal when plating transformed bacteria

Procedure PCR mk3 11/26:

1. See procedure from Lab 18: PCR of mk3 with DMSO except instead of 2% DMSO concentration use 3% and 5%

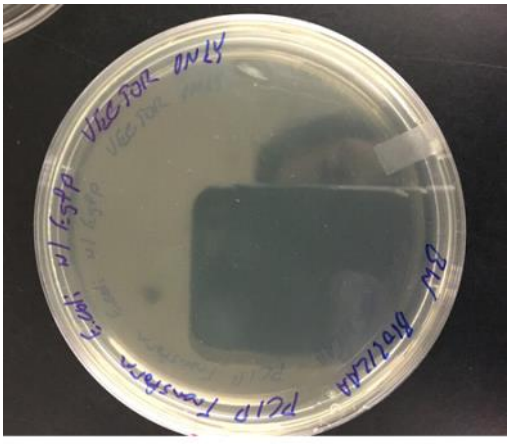
Procedure PCR mk3 12/3:

1. See procedure from Lab 18: PCR of mk3 with DMSO except change temperature gradient in °C (60, 58.9, 57, 54.1, 50.5, 47.9, 46, 45) as well as use new template of pMIG.mk3

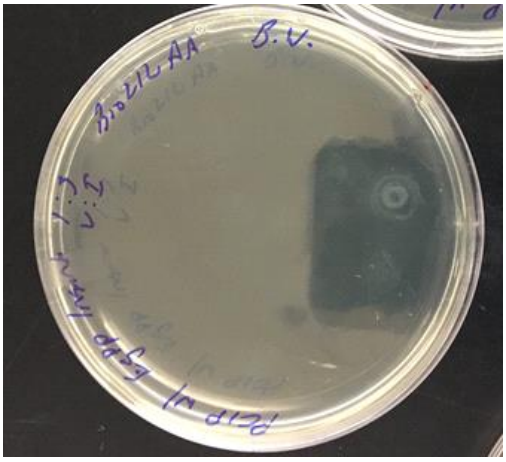
Results:

Transformation using EGFP in pCIP

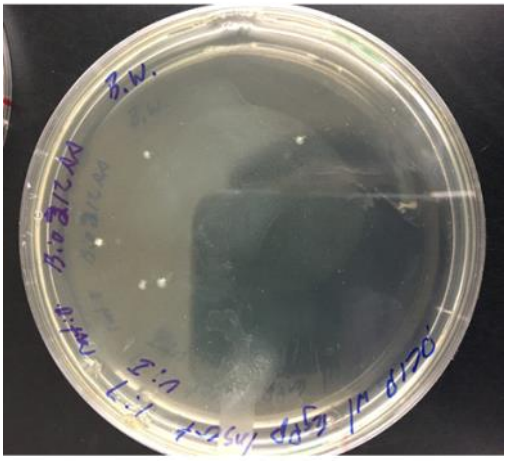
Control No Insert



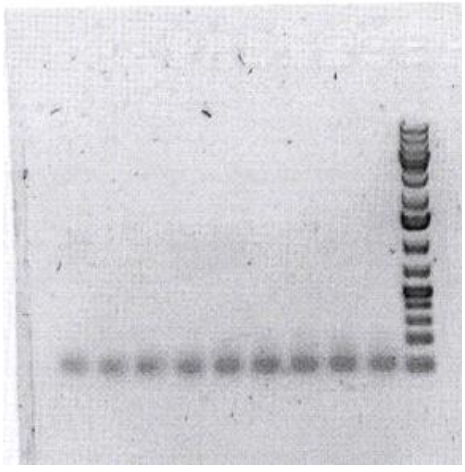
1 : 3 Insert to Vector Ratio



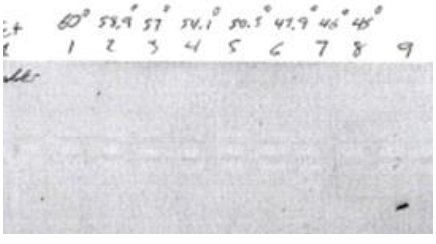
1 : 7 Insert to Vector Ratio

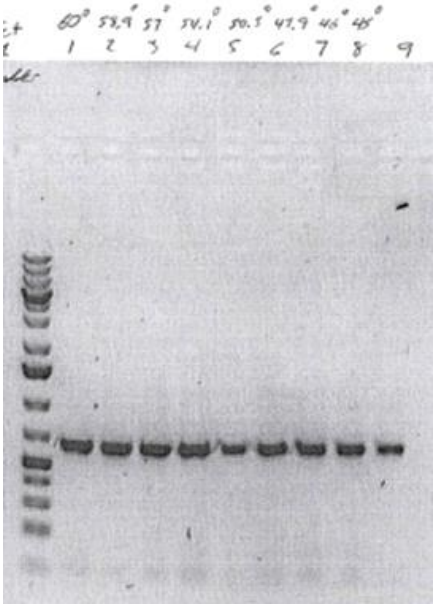


PCR mk3 with 3% and 5% DMSO 11/26



PCR mk3 with new temperature gradient 12/3





Conclusion:

Only one plate with 1:7 Vector to Insert grew colonies. After picking colonies and growing in culture we had issues since no bacteria grew. This could have been due to picking some agar with the colonies which interrupter cell growth.

PCR for mk3 worked with the new temperature gradient which was the issue.