

Transformation & Plasmid Mini-Prep

Rapid Colony Transformation (Part 1)

Supplies

2 -1.5ml microfuge tubes
P200 + tips
P20 + tips
A sterile disposable pipets
CaCl₂ solution
pAMP plasmids
Ice in a styrofoam cup
Plate of *E. coli* MM294

Procedure

1. **LABEL EMPTY TUBES:** Label one microfuge tube +pAMP and the other tube -pAMP. Plasmid DNA will be added to the +pAMP tube and not to the -pAMP tube. Label both tubes with the date, your name, etc.
2. **ADD CaCl₂:** Use a micropipettor (P200) to add 200ul of cold CaCl₂ solution to each tube and place the tubes on ice. This will make the cells “competent”.
3. **PUT *E. Coli* cells INTO THE TUBES:** Use an inoculating loop to transfer several large (3mm+) colonies of *E. coli* MM294 to each tube. Use a sterile pipet to re-suspend the cells (pipet up and down gently a few times). Make sure all the cells fall into the tube. Place tubes back on ice.
4. **ADD pAMP PLASMIDS to the “+” tube:** Keeping the tubes on ice, use a P20 to add 10ul of pAMP plasmid solution to the tube labeled +pAMP. Tap tube gently with your finger to mix- do not create bubbles or mix vigorously. Immediately return to ice.
5. **KEEP THEM ON ICE:** Incubate both tubes on ice for 15 minutes. During this time, obtain and label 2 LB and 2 LB-amp plates along with other supplies listed on next page.
6. **HEAT SHOCK THE CELLS:** Carry the ice bucket with your tubes to the counter near the 42degC water bath. Remove tubes from ice and immediately immerse them in hot water for exactly 90 seconds.
7. **PUT THEM BACK IN ICE:** Return tubes to ice for at least one minute. Then place them at room temp for 5 minutes prior to plating them.

Rapid Colony Transformation (Part 2)

Supplies

2 LB plates
2 LB/amp plates
Small tube of LB broth
Hockey stick
P200 + tips

Procedure

1. Obtain and label the plates as follows:
 - LB/amp with +pAMP (this is the experimental plate)
 - LB/amp with -pAMP (this is the negative control)
 - LB with +pAMP (this is a positive control)
 - LB- with pAMP (this is a positive control)
2. Use a P200 to add 200ul of LB broth to each tube. Tap tubes gently to mix.
3. Use a P200 to inoculate the plates. Inoculate as follows:

Agar Used	+pAMP	-pAMP
LB/amp	100ul	100ul
LB	100ul	100ul

4. Use a hockey stick to spread the cells evenly over the surface of the agar. Let plates sit for a few minutes before taping together, inverting and placing in the incubator at 37degC.
5. Incubate until the next lab period.

Day 2: Analysis & Culture

1. Obtain your plates from the refrigerator. Observe and record the number of colonies on each plate. If too dense record as “lawn” or as “TMTc”. Where the results as expected? For each plate explain why it did or did not grow (from the point of view of the cell).

Supplies:

2 tubes LB/amp broth

1. Label the tubes pAMP+ .
2. Aseptically transfer a large colony from your LB/amp+pAMP plate to each LB/amp tube. This tube will contain cells that have been transformed and contain the plasmid.
3. Incubate at 37degC for 24-48hrs.
4. Place the plates into the refrigerator.

Day 3: Plasmid Mini-Prep

1. Get your tubes from the incubator or refrigerator and label them #1 and #2. Place both tubes into the centrifuge (exactly opposite each other) with the hinges facing outwards. Spin at maximum rpm for 2 minutes to pellet the cells.
2. VERY CAREFULLY pour off supernatants (broth) into a coffee can without disturbing cell pellet. Invert tubes onto paper towel and tap gently to drain. You do not have to get all the broth out of the tube.
3. Use a P200 to add 150ul GTE to **tube #1 only**. Pipet in and out several times to make sure the cells are re-suspended.
4. Transfer all the material from tube #1 into tube #2. Pipet in and out several times to make sure the cells are re-suspended. Discard the tube labeled #1.
5. Use a P1000 to add 300ul lysis buffer (SDS/NaOH) to the tube. Close the lid and invert rapidly 5 times to mix. This will break the cells open.
6. Immediately place tubes on ice for 5 minutes.
7. Use a P1000 to add ice-cold KOAc to the tube. Close the lid and invert gently and rapidly 5 times to mix. A white precipitate will form. This is the cellular debris and chromosomal DNA.
8. Place the tube on ice for 5 minutes.
9. Spin the tube in the centrifuge (hinges out) at maximum rpm for 5 minutes to pellet the cell debris.
10. Obtain and label an empty microfuge tube "pAMP".
11. Carefully, making sure not to disturb the pellet, use a p200 (**not a P1000**) to remove the supernatant from the tube #2. Use three aliquots of 200ul each to transfer 600ul of the supernatant into the tube labeled "pAMP". Discard the old tube with the pellet remaining in it.
12. Make you will have a centrifuge available before completing this step. Use a P1000 to add 600ul of isopropanol to the tube. Close the lid and invert several times to mix. Incubate at room temperature for exactly 2 minutes.
13. Spin in centrifuge at maximum rpm for 5 minutes to pellet the nucleic acids, then carefully, pour off the supernatant. Do not disturb the pellet. Invert tube over a paper towel to drain.

14. Use a P1000 to add 300ul ice-cold ethanol to the tube and invert the tube gently a couple times. This is a cleaning step. Do not re-suspend pellet.

FREEZE TUBES UNTIL NEXT LAB

Day 4: Restriction Digest

1. Obtain your tube from the freezer. Centrifuge at maximum for 3 minutes.
2. Carefully, pour off supernatant. Gently invert tube onto a paper towel to dry completely.
3. Centrifuge tube again for ~30seconds and then use a P20 set on 5ul to carefully remove any pooled ethanol. Let air dry 10 minutes.
4. Before continuing, label this tube #1 on the side and the lid.
5. Add 16ul TE (tris-EDTA) buffer and re-suspend pellet by vortexing. Flick tube to get all material to the bottom.
6. Add 2.6ul reaction buffer to the tube and then add 4ul *EcoRI* to the tube. Pipet up and down gently to mix. Close tube and flick solution to bottom of tube.
7. Obtain a second microfuge tube (label it #2 on the side and the lid) and aliquot 11.3ul from tube #1 into it.
8. Add 2ul TE to tube #1.
9. Add 2ul *HindIII* to tube #2.
10. Incubate tubes at 37degC for 60 minutes.
11. While tubes are incubating, we will prepare the gels (see below).
12. Refrigerate until the next lab.

Gel preparation:

Supplies:

- Gel tray
- Agarose (1% in TBE buffer) - molten in a 65C water bath.
- Flask or beaker

Procedure:

1. Raise the gates on the gel tray and gently tighten the screws at both ends.
2. Measure 30ml agarose in a beaker and pour it into the tray.
3. Immediately place the 12-tooth comb at one end.
4. When the gel is firm, place your gel into the storage container with TBE buffer.

Day 5: Electrophoresis

1. Obtain your tubes from last lab. Spin them in the centrifuge for a few seconds to get all the liquid to the bottom.
2. Add 2ul of loading dye to each tube. Pipet in and out to mix.

3. Get your agarose gel from the storage container. Loosen (do not remove!) the screws. Lower the gates and gently tighten the screws. Place it into the electrophoresis chamber so that the comb is at the negative (black) end.
4. Use a beaker to measure and add 300ml 1X TBE buffer to the chamber.
5. Remove the plastic comb, dry it and return it to storage.
6. Assign wells to each table and draw a diagram to show where your samples are.
7. Load 10ul of each sample into the wells using a P20.
8. When all samples are loaded, close lid carefully and plug in leads.
9. Turn on power supply and slowly adjust it to 130V. Check the milliamps (it should be about 50mA). Record the time.
10. Turn off power supply when the loading dye gets just past the middle of the gel.
11. Remove gel from chamber and place into a large weigh boat.
12. Stain gel in Ethidium bromide for 10 minutes.
13. Observe and photograph or diagram results.