Supplemental Material

CBE—Life Sciences Education

Wu *et al.*

Supplementary data one: Teacher-designed experimental protocol for P-Two

LSM 1102 P-two: Plasmid extraction and Transformation

I - Extraction of plasmid DNA from *E. coli*

Introduction

The High-Speed Plasmid Mini Kit (Geneaid Biotech Ltd) is designed for rapid isolation of plasmid or cosmid DNA from 1-4 ml bacterial cultures. Modified alkaline Lysis method (1) and RNase treatment are used for obtaining clear cell lysate with minimal genomic DNA and RNA contaminants. In the presence of a chaotropic salt, the plasmid DNA in the lysate binds to the glass fiber matrix in the spin column (2). The contaminants are washed off with an ethanol contained Wash Buffer and the purified plasmid DNA is eluted by a low salt Elution Buffer or water. The procedure does not require DNA phenol extraction or alcohol precipitation. Typical yields are 20-30 μg for high-copy number plasmid or 3-10 μg for low-copy number plasmid. The entire procedure can be completed within 30 minutes. The purified plasmid DNA is ready to use for restriction enzyme digestion, ligation, PCR, and sequencing reactions.

Materials

1. *E. coli* (pUC18) culture
2. Geneaid Spin Column with glass fiber matrix
3. Microcentrifuge tubes
4. Buffer PD1(50 mM Tris-HCl pH 8.0; 10 mM EDTA; 10 μg/ml RnaseA)
5. Buffer PD2 (200 mM NaOH; 1% SDS (w/v))
6. Buffer PD3 (guanidinium hydrochloride and acetic acid)
7. W1Buffer (guanidine hydrochloride and isopropanol)
8. Wash Buffer (70% ethanol)
9. Elution Buffer (10 mM Tris-HCl, pH8.5 at 25°C)

Procedure

Do the following before you start to extract plasmid DNA

- Add provided RNase A to PD1 Buffer and store at 4°C, if precipitates have formed in PD2 Buffer, warm the buffer in a 37°C water bath to dissolve.
- Add absolute ethanol to Wash Buffer prior to initial use (see the bottle label for volume).

1. Harvesting *E. coli* cells.

- Transfer 1.5 ml of bacterial culture to a 1.5 ml microcentrifuge tube. Spin for 1 minute in a microcentrifuge and discard the supernatant. If more than 1.5 ml of bacterial culture is used, repeat the Harvesting Step.

2. Re-suspension
   - Add 200 µl of PD1 Buffer (RNase A added) to the tube and resuspend the cell pellet by vortex or pipetting.
3. Lysis
   - Add 200 µl of PD2 Buffer and mix gently by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA.
   - Let stand at room temperature for 2 minutes or until the lysate is homologous.
4. Neutralization
   - Add 300 µl of PD3 Buffer and mix immediately by inverting the tube 10 times. Do not vortex.
   - Microcentrifuge for 3 minutes.
5. DNA Binding
   - Place a PD Column in a 2 ml Collection Tube.
   - Add the supernatant from Step 4 to the PD Column and microcentrifuge for 30 seconds.
   - Discard the flow-through and place the PD Column back in the 2 ml Collection Tube.
6. Wash
   - Add 400 µl of W1 Buffer into the center of the PD Column.
   - Microcentrifuge for 30 seconds.
   - Discard the flow-through and place the PD Column back in the 2 ml Collection Tube.
   - Add 600 µl of Wash Buffer (ethanol added) into the center of the PD Column.
   - Microcentrifuge for 30 seconds.
   - Discard the flow through and place the PD Column back in the 2 ml Collection Tube.
   - Microcentrifuge again for 3 minutes to dry the column matrix.
7. DNA Elution
   - Transfer the dried PD Column to a new 1.5 ml microcentrifuge tube.
   - Add 50 µl of Elution Buffer or water into the center of the column matrix.
   - Let stand for 2 minutes or until the Elution Buffer or water is absorbed by the matrix.
   - Microcentrifuge for 2 minutes to elute the DNA.

References

(1) Birnboim, H. C. and Doly, J. (1979) Nucleic Acids Res. 7, 1513.
(2) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Additional Information about the High-Speed Plasmid Mini Kit

- Buffer PD1 (Tris-buffer, EDTA and RNase)
  Tris-buffer: to maintain pH
  EDTA: chelating agent, to chelate divalent metal ions which are co-factor for nucleases, so indirectly inactivates nucleases to protect plasmid DNA
  RNase: to remove RNA
- Buffer PD2 (NaOH and SDS)
  NaOH: to provide alkaline condition. Strong alkaline could denature proteins. More importantly, at pH 12-12.5, most chromosomal DNAs are denatured.
  SDS: to interact with membrane lipids and break down plasma membrane.
- Buffer PD3 (Guanidine hydrochloride and acetic acid)
  Guanidine hydrochloride: strong denaturant of proteins
  Acetic acid: to rapidly bring down the pH. As a result the denatured chromosomal DNA would attempt to renature into dsDNA. However, because these molecules are large and the pH change is rapid, the chromosomal DNA would not renature properly, ended up as a tangled mass and precipitated out. The small plasmid molecules will renature correctly and remain in supernatant, thus are separated from the chromosomal DNA
- The spin column used contains a silica membrane that binds nucleic acid. Both the plasmid and the bacterial chromosomal DNA are nucleic acids. How to eliminate the bacterial chromosomal DNA from plasmid prep?

  A major reason is that chromosomal DNA has been precipitated and spun down with cell membrane debris in earlier step (after addition of buffer PD3), so only plasmid DNA is left to bind onto the silica membrane.

- Why is it necessary to wash the silica membrane with two different buffers before the elution step?
  W1 Buffer (Contains guanidine hydrochloride, isopropanol)
  Guanidine hydrochloride-strong denaturant of protein: to remove trace nucleases (if any)
  Isopropanol: DNA is not able to dissolve in isopropanol, using isopropanol as solvent prevented DNA loss during the washing step
  Wash Buffer (70% ethanol)
  75% ethanol: to remove salt efficiently ➔ after removal of salt, plasmid not so tightly bound to silica membrane ➔ could be easily eluted out
- Prior to the elution step, the plasmid DNA molecules are bound to the silica membrane. However, during the elution step the DNA molecules dissolved in water and are eluted down. What cause the difference?
  Salt concentrations. At a high salt concentration, DNA is bound to the silica membrane. After the removal of salt by wash buffer, DNA could be eluted in Tris-EDTA buffer or water.

Effective Learning (1)

Group/Bench No._____; Matriculation No._____

Answer the following questions with True/False

Plasmid DNA is one type of genetic material which can pass to the next generation during *E. coli* replication.	
All plasmid DNAs carry ampicillin resistant gene.	
Bacterial chromosomal DNA attaches to cell membrane, so it can be co-precipitated with insoluble complexes during plasmid purification.	
DNA size is the only factor which affects its migration rate during agarose gel electrophoresis.	
One *E.coli* cell contains one copy of plasmid and one copy of chromosomal DNA.	
Extensive and complete lysis of bacteria increases the yield of plasmid.	
The DNAs are negatively charged but proteins are positively charged.	
DNA loading buffer allows the DNA be negatively charged so it can move toward anode during electrophoresis.	
1% agarose gel is prepared by melting 1 gram agarose into 100 ml distilled water.	
The higher of OD260/280, the purer of isolated plasmid DNA.	
Q1. What is the main function of buffer PD3?	
Q2. What impressed you most in this practical?	
Q3. What's your comment to this practical or your suggestions to TA/instructor?	

Your Question (s)

II - Transformation

Materials

1. 1 ml of competent *E. coli* cells in 100mM CaCl2
2. Plasmid DNA (pUC18) _prepared in practical 2
3. Double distilled (dd) sterile water
4. LB broth
5. LB agar plates (one plate for each pair of students)
6. LB + ampicillin agar plates (5 plates for each pair of students)

Procedure

1. Measure plasmid concentration using nanodrop. Calculate plasmid amount in your sample (and you can decide how much you want to use in your "mutated" protocol).
2. Set up 3 microfuge tubes with 300 µl competent cells in each tube.
3. Add 5 µl and 15 µl plasmid DNA to the first two tubes, and 10 µl dd water to the third (negative control).
4. Leave **on ice** for 30 min.
5. **Heat shock at 42°C for 90 seconds**.
6. Add 1 ml LB broth and incubate at 37°C for 20 min. Invert the tube at 5 min intervals to mix.
7. Centrifuge at 12,000 rpm for 2 min. Discard all supernatant.
8. Re-suspend in 300 µl LB broth. Make a 10^{-1} dilution of only the **transformed cells**.
9. Spread 100 µl of the neat and 10^{-1} suspensions of transformed cells on LB + ampicillin agar plates (a total of 4 plates).
10. Spread 100 µl neat of negative control (using water instead of plasmid DNA) to each of LB and LB+ampicillin plates (a total of 2 plates)
11. Incubate overnight at 37°C.

III - Transformation (continued)

Examine the agar plates. Note the relative number of transformants on the different plates and any other results you think you should.

IV-Complete survey forms

Effective Learning (2)

Group/Bench No._____; Matriculation No._____

Answer the following questions with <u>T</u>rue/<u>F</u>alse

Bacteria in different phases of growth are equally competent for taking foreign DNA.	
Calcium ions improve the transformation efficiency by neutralizing the negative charges on DNA molecules and phospholipids.	
The ampicillin-resistance gene codes for the enzyme β-lactamase that hydrolyzes ampicillin. The enzyme is secreted into the cell's environment, so that the antibiotics are broken down before they even enter the cell.	
Transferred gene must integrate into the bacterial chromosome DNA to be transcribed and then translated.	
After the heat shock step, transformed intact plasmid DNA molecules can replicate in bacterial cells.	
The number of cells transformed per 1 microgram of DNA is called the transformation efficiency.	
Linear plasmid DNA gives higher transformation efficiencies than supercoiled plasmid DNA during heat-shock transformation.	
Human/animal cells can also be transformed with a foreign DNA.	
If Mammalian cells take up the plasmid pUC18, the cells can also produce β-lactamase to hydrolyze ampicillin.	
Transferring a gene into a bacterial cell will surely change its phenotype, because bacteria are haploids.	
Q1. How are bacterial cells containing plasmid DNA selected?	
Q2. What impressed you most in this practical?	
Q3. What's your comment to this practical or your suggestions to TA/instructor?	
Your Question (s)	

Supplementary data two: a list of proposed mutations and hypotheses by students

Proposed mutations	Detail of mutations	Hypothesis	Ideas/mechanisms tested	Brief Comments of TAs
Cell lysis to release plasmid DNA				
Insertion	Addition of Potassium Acetate into lysis buffer PD2	Potassium Acetate enhances the precipitation of SDS, thus improve the purity of plasmid DNA.	Investigate the salt effects on plasmid DNA purification and bacterial transformation	How to prove SDS is reduced? What data going to be collected?
	Add one step (vortex) to extensively break up cells	Extensive lysis of cells will not affect the yield of plasmid but may increase the contamination from genomic DNA	Plasmid DNA would not be effectively transformed into competent cells due to contamination of chromosomal DNA	Good idea to test, do you collect direct data of more genomic DNA released?
Deletion	Omit the 1% SDS solution from lysis buffer PD2 containing NaOH and SDS	Removal of SDS will result in incomplete cell lysis, less plasmid DNA will be obtained	Examine the role of SDS in cell lysis and plasmid yield	Good to test, is the difference significant?
	Omit suspension buffer PD1 containing Tris, EDTA and RNase.	Directly proceeding to cell lysis may produce similar amount of plasmid DNA compared to standard protocol.	I doubt about the importance of buffer PD1. Enzymes may not function in the lysis condition, thus EDTA to inhibit DNase and RNase to remove RNAs are not necessary and efficient.	Very interesting, cannot wait to see your result
	Skip lysis of cells using PD2	No lysis of *E. coli* cells, no or less plasmid released	Are the cell's boundary structures so durable and stable?	Do you still perform the neutralization step?
Substitution	Replace the use of PD2 with heating at 95°C for 5 min	The concentration of chromosomal DNA in extracted Plasmid DNA will be higher because heating helps to release all DNA.	Compare the effects from chemical and physical lysis on plasmid DNA purity.	Do you consider the effects of PD2 on the following steps?
	Replace the 10mM EDTA in the buffer PD1 with H_2O	Without EDTA, nucleases would cleave and fragment the plasmid DNA.	Examine the role of EDTA, does it really function to protect DNA?	Believe it or not, let's see the proof.
	Replace Tris buffer with distilled water	If RNase A is unable to function in water, we expect to have more RNA contamination.	"test of essentiality" of buffer for RNase function	Data supporting? RNase may not loss its activity in distilled water
	Decrease the concentration of NaOH in PD2 from 200 mM to 8 mM	Lower concentration of NaOH may not denature chromosomal DNA and result its contamination and thus affect the efficiency of transformation	Determine whether or not the presence of chromosomal DNA in the purified plasmid DNA will affect the efficiency of transformation	Great idea to test! It is unclear so far.
	Use five-fold diluted PD2 cell lysis buffer	Using diluted PD2 increases the contamination of chromosomal DNA and decrease the transformation efficiency.	Chromosomal DNA is not denatured sufficiently and would not be entirely precipitated in the subsequent steps	Do you measure the pH of diluted PD2? Why do you think you?
	Elongate the lysis duration to 5 min instead of 2 min in the	Proper lysis time ensures the yield of plasmid while minimizing its exposure to	Investigating effects of time duration of lysis on plasmid yield and quality	Intensive lysis may cause more contamination from

	standard protocol	denaturing conditions.		genomic DNA
	Elongate the lysis duration to 10 min instead of 2 min in the standard protocol	Elongated lysis will cause chromosomal DNA contamination , but it may not affect transformation of plasmid DNA	Chromosomal DNA is much larger and has different property from plasmid DNA. It may not compete with plasmid.	Controversial to many others' opinions, great to test! You may be Right.
	Vortexing instead of inverting the tube gently during lysis.	Vortexing leads to the extraction of impure plasmid DNA with genomic DNA contaminants.	Strong vortex releases genomic DNA. Contaminated plasmid may impair the efficiency of transformation.	Many others also test this idea, let's see whether your data are consistent.
	Replace room temperature water with heated water to 60 to 70°C to elute plasmid DNA	Heated water gives better elution of plasmid DNA as the higher temperature would denature the remaining debris or cellular contaminates	Test whether the elution buffer at 60 to 70°C brings out a higher yield and purer plasmid DNA	Hypothesis is good, but explanation does not make much sense
Reverse	RNase added after cell lysis	If RNase cannot diffuse through the plasma membrane, then RNase would not be able to denature the RNA.	The addition of RNase before the lysis of bacteria cells is less effective than after the lysis of the cell.	Can you read more about RNase?
Others	Increasing the amount of bacteria for plasmid extraction	More bacteria used, more plasmid DNA obtained.	The capacity of the column for each binding is limited, but it can be repeatedly used for several cycles of DNA binding and elution.	You may get more plasmid DNA, but what is it for? Or are you trying to recycle the column?
Plasmid DNA extraction				
Deletion	Remove neutralization (Step 4) from the standard protocol	Without neutralization, single-stranded DNA with multiple molecular masses will be produced.	Test whether the strong alkaline in lysis buffer PD2 will denature and fragment DNA.	How are you going to examine the denaturation and fragment of DNA?
	Omission of adding PD3 neutralization buffer	Omission of adding PD3 buffer will not cause the rapid pH change. Both genomic DNA and plasmid DNA may re-nature.	Test whether both genomic and plasmid DNA can re-nature during washing step	How are you going to examine the renaturation?
	Removing the washing step with a buffer (W1) containing guanidine hydrochloride and isopropanol	The removal of W1 wash step will not affect the yield of DNA plasmid but the plasmid DNA sample may not be pure.	To confirm the idea that the washing is to remove protein residues.	Washing not only function to remove proteins, but also facilitate DNA binding to the column,
	Removing the washing step with the buffer (W1)	Without washing, a low yield and impurity of plasmid extracted	Test the mechanism that plasmid molecules will be tightly held to the membrane as the salts are still present	How do you set the control and collect data to test your hypothesis?
	Removing the washing step with the buffer (W1)	If skip the wash step, a larger mass of 'DNA protein complex' may be obtained	RNase and other proteins are not denatured and remain their association with DNA.	How does your data directly correlate to your hypothesis?
	Omit washing step with 70% ethanol	Without washing with 70% ethanol, most of the plasmid DNA will not be eluted, causing a lower yield of plasmid DNA	Test the working principle that guanidine hydrochloride salt remains in the mixture without washing. The external environment remains hydrophobic and plasmid DNA remains attached to the	Wash Buffer is essential in improving the yield and purity of plasmid DNA.

			PD column. Hence	
Substitution	Replace acetic acid with water	If acetic acid is not added to bring the pH down rapidly, both chromosomal and plasmid DNA remain denatured.	Test whether the strong alkaline will cause denature and precipitation of DNA	How can your data tell?
	Replace guanidine hydrochloride with water	pH plays an important role in the denaturation of proteins, replacing Guanidine Hydrochloride with water will not affect plasmid extraction	Demonstrate that addition of guanidine hydrochloride is not essential for plasmid extraction	Misunderstanding the kit working principles, hypothesis makes no sense.
	Increase concentration of NaOH and SDS in buffer PD2 while reduce use of W1buffer	A higher pH increase the solubility of DNA, thus increase the yield of plasmid DNA	Study the effect of change in pH on DNA re-naturation	More than one variables involved,
	Replace the isopropanol in the W1 buffer with distilled water	Without isopropanol, DNA will be lost during wash	Test the DNA binding affinity to the column	Not scientifically sound
	Dilute the 70% ethanol to 10% ethanol	A lower concentration of ethanol used may result in a lower yield of DNA.	Study effects of concentration of ethanol on the yield of DNA	Not scientifically sound
	Increased the ethanol concentration to 95%	A higher concentration of ethanol in washing step decreases DNA purity while increase plasmid DNA yield	Less DNA is lost during washing process, and less salts are washed away	Does it cause a significant difference?
	Cool wash buffer (70% ethanol) to -20°C	Washing DNA with Wash Buffer cooled to -20°C increases eluted DNA yield.	Decreasing the temperature would cause an increase in DNA precipitation as it prevents denaturation of plasmid DNA.	Rationale not correct, and most likely, no much difference
Plasmid DNA elution				
Insertion	Incubate the DNA-column for 30 min on ice before centrifuge	Prolonged incubation on ice may prevent denaturation and increase DNA binding to the column, thus an increase of extracted plasmid DNA is expected	Optimize the DNA binding conditions and prevent denaturation	Good try, curious to see its effects
Substitution	Increase the volume of elution buffer from 50 µl to 150 µl	An increase in the buffer volume would increase the yield of plasmids extracted	To increase the total yield of plasmids extracted from *E. coli* cells	It decreases the concentration too.
Competency of *E. coli* cells				
Deletion	Remove the 30min pre-cooling step	Eliminating of ice cooling results in the decreased efficiency of transformation	It reduces cells' competency towards the uptake of plasmids	Rationale needs to be corrected. Read more.
	No cooling of the competent cells before the heat shock treatment	Without pre-cooling of competent cells, transformation efficiency will drop.	Lack of the icing treatment would in fact cause a decrease in fluidity from cell surface to extra-cellular medium.	It sounds interesting, but how can you collect data to examine it?
Substitution	Replace the competent cell buffer with modified glucose concentration	Glucose helps the survival of cells and increases the transformation efficiency.	Add glucose to the buffer to maintain osmotic pressures to prevent lysis of cells.	Great to test, hope you collect the data of osmosis effects on cell's survival?

	Change competent cell buffer pH to a slightly acidic condition	Extremely acidic conditions are unfavorable to competent cells but a slightly acidic medium may enhance the uptake of DNA.	How changes in pH will affect the vitality and competency of cells.	What bases your hypothesis? Does the collect data justify your hypothesis?
Insertion	Add 75mM $CaCl_2$ and 20% glycerol into the buffer of competent cells	The transformation efficiency increases as the concentration of $CaCl_2$ increases	To re-test the effects of $CaCl_2$ on transformation efficiency	So why do you need glycerol?
	add 2µl 0.1M $CaCl_2$ to each of the 3 test tubes before leaving on ice for 30min.	Increase the competence of cells by introducing Ca^{2+} molecules at the incubating stage	Investigate the effect of calcium ions on *E. coli* bacterial transformation	Well referenced hypothesis, hope you get anticipated results
	Addition of ice cold 5% v/v DMSO into competent cell buffer	Incubation of competent cells with DMSO in an ice bath will increase the transformation efficiency	Test DMSO to increase its transformation efficiency	Positive, no or negative effects, eager to see your results
Transformation by heat shock				
Insertion	Additional step of putting the bacterial cells back immediately into the ice after heat shock	Cooling will freeze the plasma membrane and protect the cells from the damage caused by heat shock. This allows more successful cases of transformed cells.	Cooling protects the cells from the damage caused by heat shock	Well studied factor on transformation,
Deletion	Remove the heat-shock steps	There are none or less colonies growing on the LB ampicillin plate due to the absence of the heat shock step.	Examine whether the heat-shock is critical for the efficiency of transformation	Do you see any transformed cells? If yes, how do you explain?
Substitution	Prolonged heat-shock from 90s to 120s	Increase the duration of heat shock from 90 seconds to 120 seconds will decrease the transformation efficiency	Prolonged thermal agitation causing the cell to fall apart and die	Do you set up a fair control?
	Shorten the duration from 90s to 45s	A decrease in the duration of heat shock will lead to an increase in the transformation efficiency	Longer time at high temperature will lead to exit of DNA from the cells	Optimize the time duration in a case-by-case situation
	Heat shock at 30, 35, 42, 45, 50 and 60°C for 30s and 90s respectively.	Uptake of plasmid DNA is affected by temperature and time duration of heating. Different time and temperature lead to different transformation efficiency.	Optimize heat treatment for the best transformation efficiency.	Low temperature at 30 and 35°C may not produce shock
	Substitute circular plasmid with linearized plasmid	Linear DNA gives lower transformation efficiency as compared to circular plasmid DNA.	Which conformation of plasmid gives higher transformation efficiency by heat shock method	There are controversial hypothesis and also controversial results, most likely depending on the concentration used for transformation.

** The content highlighted in Red is the example used to elaborate marking rubrics*

Supplementary data three: student evaluation on teaching assistants

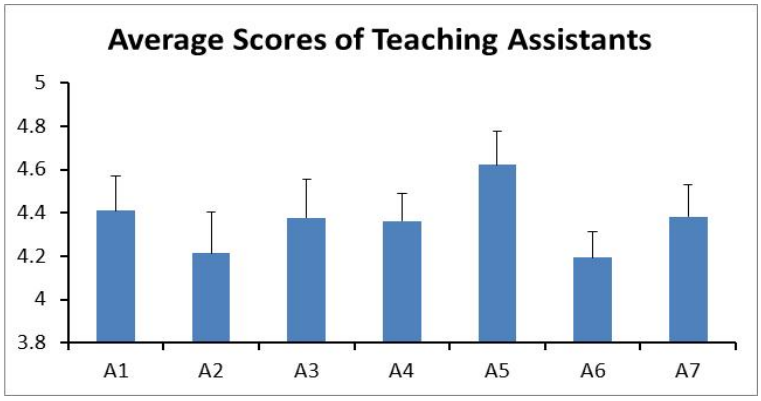
Group No:_____ Bench No :_____ Teaching Assistant: _____

The major objective of this survey is to aid in improving teaching effectiveness. Your responses provide valuable feedback to instructors, administrators and other students. The results will be used for pedagogical research and reform of our lab education. Please indicate the extent to which you agree with the following statements.

Please score (with a ✓) your Teaching Assistant (TA) for the following questions according to this scale:

(1) Strongly Disagree (2) Disagree (3) Neutral (4) Agree **(5) Strongly Agree**

Form Three	1 2 3 4 5
A1: TA is well prepared and knowledgeable (competence in knowledge).	☐☐☐☐☐
A2: TA explains the concepts clearly (articulating and presenting).	☐☐☐☐☐
A3: TA demonstrates sufficient skills/techniques (skill).	☐☐☐☐☐
A4: TA ensured we learnt and performed practical techniques correctly.	☐☐☐☐☐
A5: TA is friendly/approachable for consultation (willingness and attitude).	☐☐☐☐☐
A6: TA encourages active learning and application.	☐☐☐☐☐
A7: Overall, teaching of TA is effective.	☐☐☐☐☐



Supplementary data three: The average score of students' evaluation on teaching assistant's performance over three semesters (AY1011 Semester I, AY1112 Semester I and Semester II).