Bring a Molecular Cell Biology Laboratory into the Classroom of HKUST

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Outline

- Objectives
- Teaching materials generated in this project
- Methods of applying these materials into teaching
- Demonstrations
- Evaluation Results

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Rational and Objectives

- A very effective way to teach biological courses is to combine the classroom teaching with laboratory practice.

- In this project, we propose to record detailed experimental process of DNA cloning on videos.

- These videos will allow students to visualize how experiments are conducted in the laboratory, thus increase their understandings and leaning efficiency on the fundamental concepts of Molecular and Cell Biology.

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Introduction on DNA Cloning

- DNA cloning is a commonly used technique in both Molecular Biology and Biotechnology.

- It can be used to make many identical copies of a DNA molecule or separate a particular gene from the genome of a cell.

- DNA cloning includes basically five steps as shown below.

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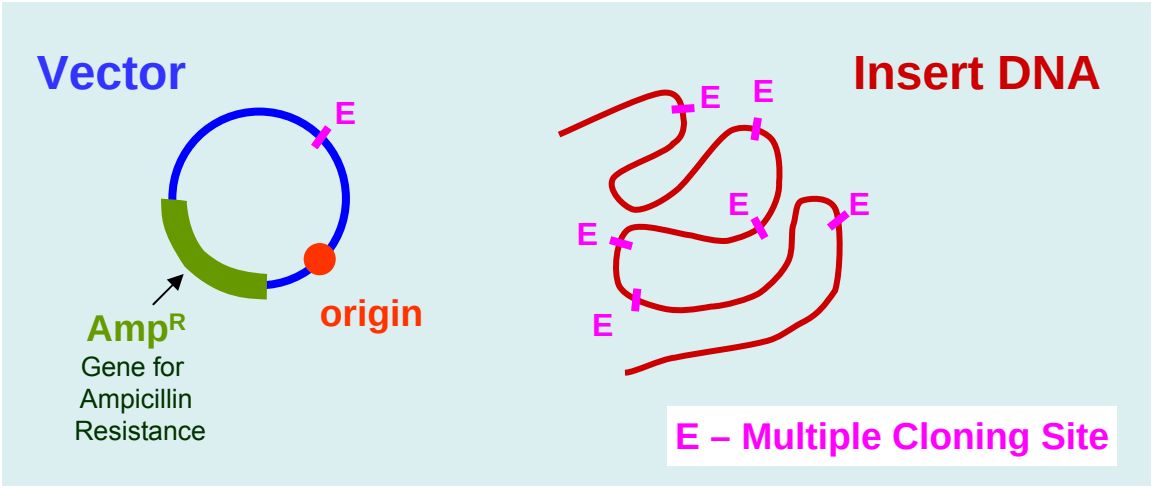
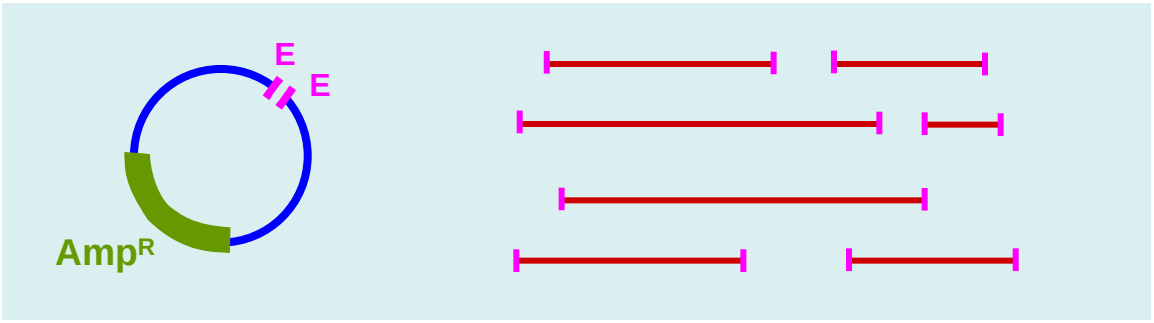
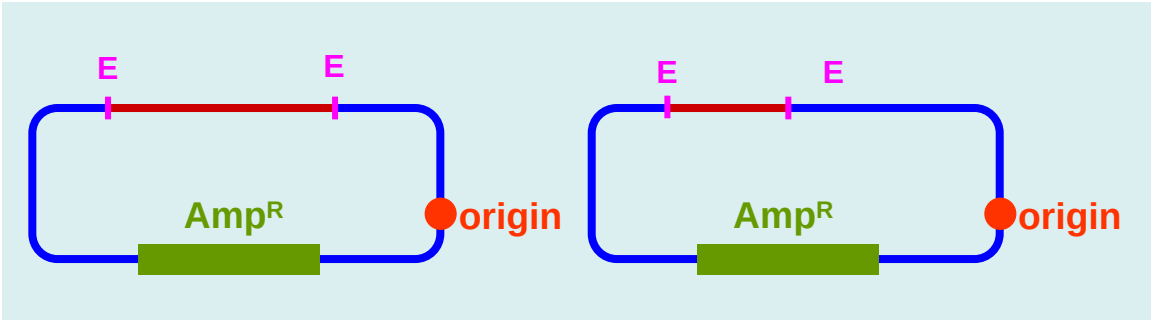
Cloning DNA using a plasmid vector







1) Digest both the vector and the insert DNA with restriction endonuclease, *Eco* RI.
2) Mix the vector DNA and the insert DNA and join them together with a DNA ligase. This results in the formation of novel combinations of DNA molecules – recombinant DNA.
3) Transfer these new plasmids into bacteria, usually *E. coli*. This process is know as transformation.

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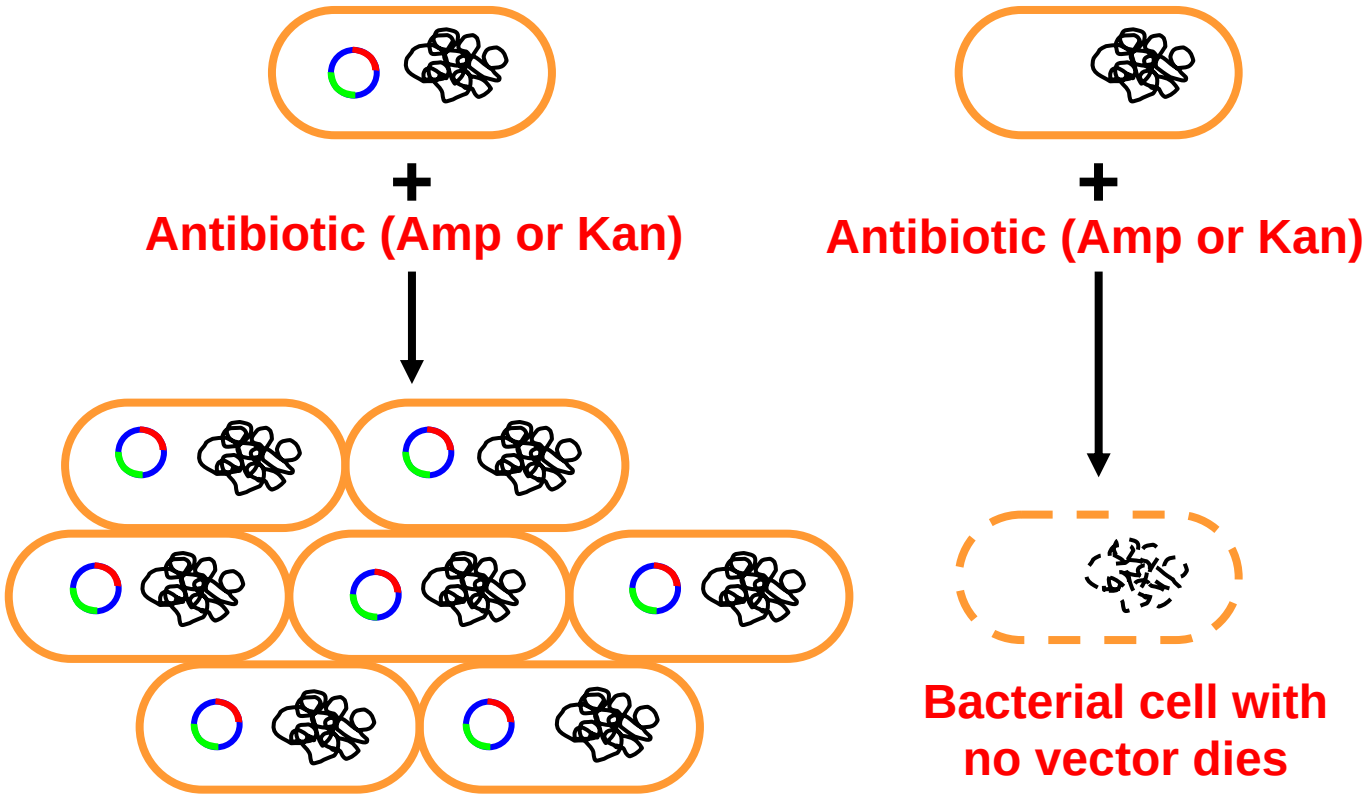
4) Select bacteria that contain plasmids by growing the bacteria in the presence of an antibiotic



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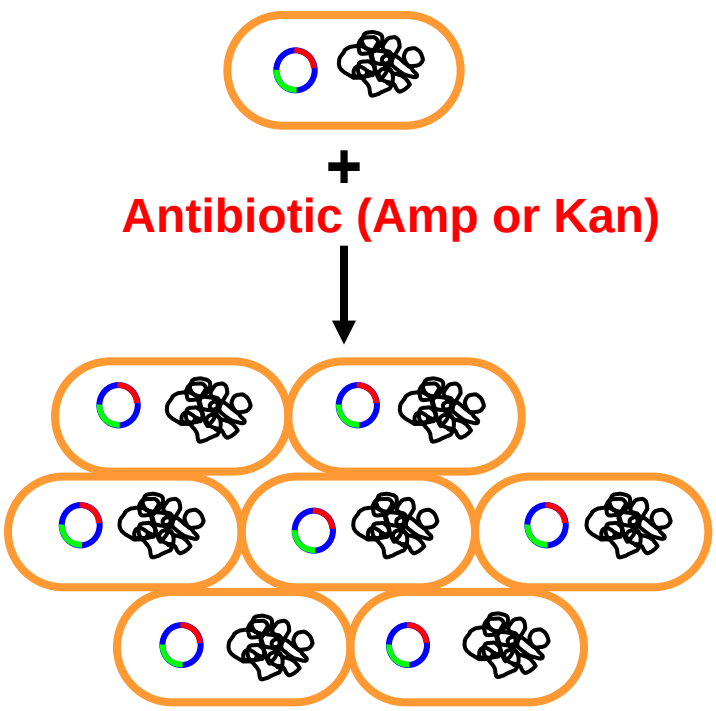
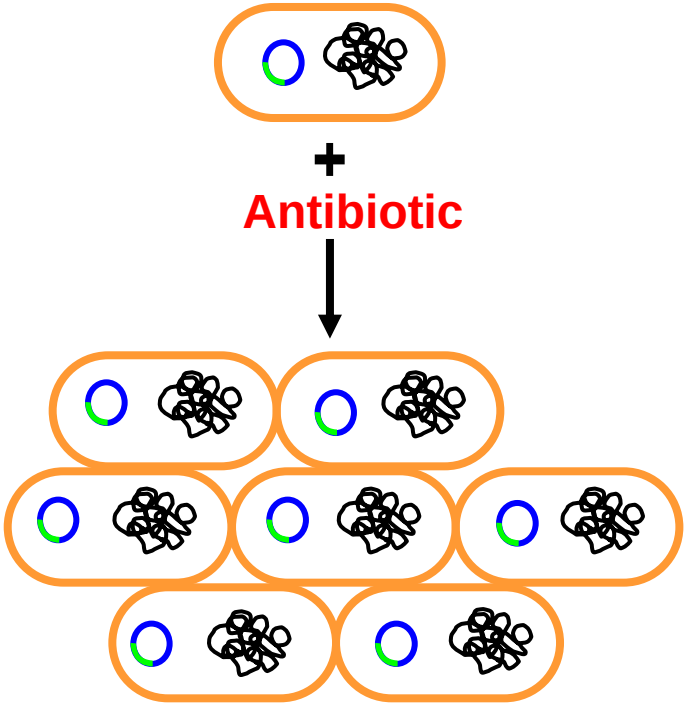
5) Analysis recombinant clones by restriction analysis and DNA sequencing



Bacteria with recombinant vectors can proliferate



Bacteria with empty vectors can also proliferate

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Produced teaching materials

- Course materials for three lectures at 3 hours each
- Thirteen videos demonstrating essential techniques of DNA cloning
- Numerous drawing and animations
- One Lab manual covering both theory and experimental procedures for DNA cloning

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BIEN501: Molecular Biology for Bioengineering

Lecture 9: DNA Cloning-Part I

Lecture contents	Videos
• Introduction	
• Basic features of plasmid	
• Basic components of a cloning vector	
• Aseptic techniques	1. Autoclave 2. Filtration 3. Biosafety cabinet
• Bacterial culture	4. Liquid medium 5. Agar plate 6. Streaking a plate 7. Bacterial inoculation

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BIEN501: Molecular Biology for Bioengineering

Lecture 10: DNA Cloning-Part II

Lecture contents	Videos
• DNA purification	8. Isolating plasmid DNA using a miniprep DNA kit
• Determining DNA concentration	9. Measuring DNA concentration using a spectrophotometer
• Restriction digestion	10. Digest plasmid DNA using restriction enzymes
• Separation of DNA	11. Separating DNA fragments by agarose gel electrophoresis

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BIEN501: Molecular Biology for Bioengineering

Lecture 11: DNA Cloning-Part III

Lecture contents	Videos
• Ligation	
• Transformation-introducing plasmid DNA into bacteria by physical and chemical methods	12. Electroporation 13. Calcium chloride/ heat shock
• Antibiotic selection	
• Detailed procedures of DNA cloning and important factors for determining a cloning strategy	

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Example of integrating the course material of plasmid DNA Isolation with animation & video

- Plasmid DNA can be isolated from bacterial cells using **alkaline/SDS** based mini-plasmid purification method.

- The method of isolating plasmid DNA using **alkaline lysis with SDS** was first reported by Birnboim HC and Doly J. in 1979 *(Nucleic Acids Res. 1979 Nov 24;7(6):1513-23).* "A rapid alkaline extraction procedure for screening recombinant plasmid DNA".

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Lecture 6. Isolation of plasmid DNA

- This method has been used for almost 30 years to isolate plasmid DNA from *E. coli.*
- It is very simple and permits the analysis of 100 or more clones per day by gel electrophoresis.
- The plasmid DNA is pure enough to be digestible by restriction enzymes.

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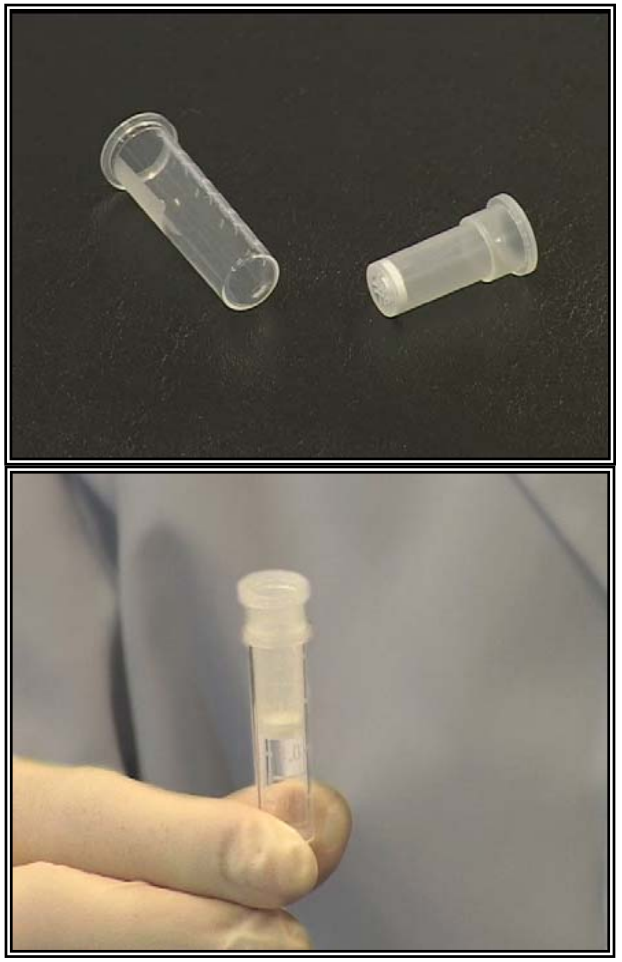
Lecture 6. Isolation of plasmid DNA

- About 10-15 years ago, this method was modified so that very pure plasmid DNA can be purified using a column specially packed with glass fiber.

- The plasmid DNA purified through those columns can be used directly for PCR, cloning, sequencing, *in vitro* transcription, synthesize of labelled hybridization probes, microinjection, electroporation and transfection, etc.



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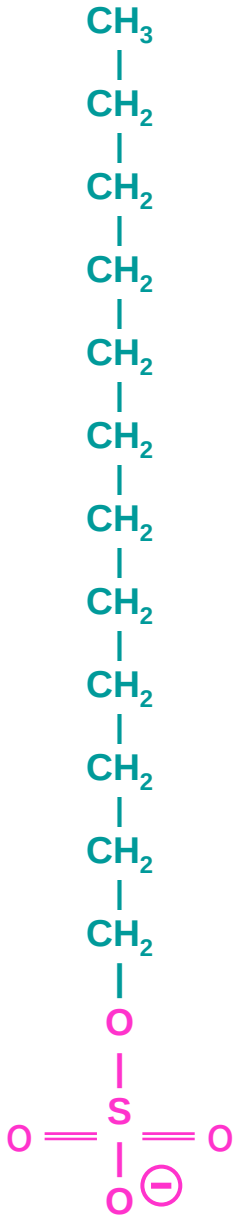

Sodium dodecyl sulfate (SDS)

Please go to the next slide to replay the animation.

- SDS is a strong ionic detergent with a **negative charge.**
- At high pH, SDS can open the bacterial cell wall, denature chromosomal DNA and proteins, and release plasmid DNA into the supernatant.

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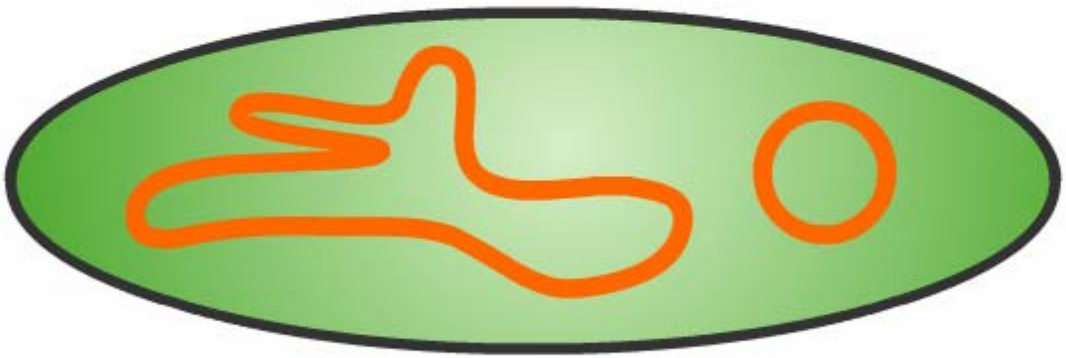

Click on animation to start playing

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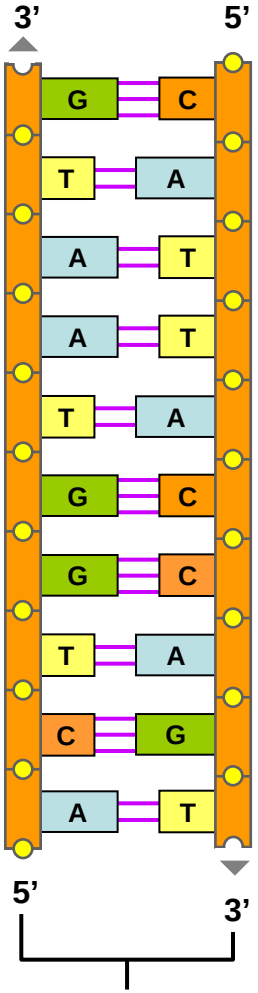
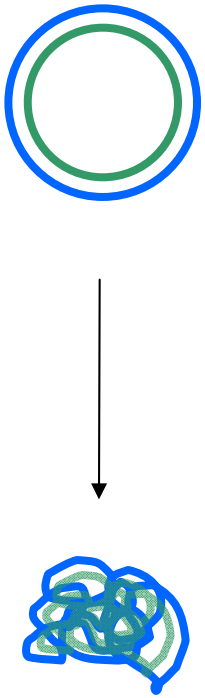
- SDS/alkaline solution can denature chromosomal DNA by disrupting the hydrogen bonds between the two complementary strands of nucleotides.

- However, this treatment cannot destroy the covalently closed circular plasmid DNA because they are topologically intertwined.



Hydrogen-bonded base pairs

Circular dsDNA



dsDNA in supercircular form

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Lecture 6. Isolation of plasmid DNA

- During SDS/alkaline lysis, bacterial proteins, broken cell walls, and denatured chromosomal DNA form large complexes that are coated with SDS. These complexes are efficiently precipitated from solution during neutralisation process when sodium ions are replaced by potassium ions.

- Once the pH returns to neutral, the two strands of plasmid DNA anneal again, native plasmid DNA can be purified by applying the supernatant onto glass fiber located inside of the purification column.

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Plasmid DNA isolation by Miniprep DNA kit

1) Obtain two 3 ml of overnight bacterial cultures.
2) Centrifuge for 10 min at 4,000 rpm.
3) Discard the supernatant and dry the pellet by gently tapping on a piece of paper towel.
4) Resuspend pellet completely in 250 µl of Cell Resuspension Solution by pipetting up and down.
5) Add 250 µl of Cell Lysis Solution, mix by inversion.
6) Add 10 µl of RNase Solution and mix by inverting the tube 4 times.
7) Incubate the tube for 5 min at room temperature. Do NOT EXCEED 5 min. Longer incubation time may result in contamination from bacterial genomic DNA.

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Plasmid DNA isolation by Miniprep DNA kit (2)

8) Add 350 µl of Neutralization Solution and mix by inverting the tube 4 times. DO NOT VORTEX!
9) Leave the tube at room temperature for 5 min.
10) Centrifuge the bacterial lysate at 14,000 rpm in a microcentrifuge for 8 min at room temperature to precipitate the pellet.
11) Insert a Wizard spin column into a 2 ml collection tube.
12) Transfer clear bacterial lysate into Wizard spin column (Avoid disturbing or transferring any of the white precipitate with the supernatant)

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Plasmid DNA isolation by Miniprep DNA kit (3)

13) Stand for 1 min, centrifuge at maximum speed in a microcentrifuge for 1 min at room temperature.
14) Remove the spin column from the tube and discard the flow through from the collection tube. Reinsert the spin column into the collection tube.
15) Add 750 µl of Column Wash Solution to the spin column, stand the column for 2-5 min and centrifuge for 1 min.
16) Discard the flow through and repeat the wash procedure using 250 µl of Column Wash Solution.
17) Centrifuge for 2 min to completely remove the Column Wash Solution.

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Plasmid DNA isolation by Miniprep DNA kit (4)

18) Transfer the spin column to a clean 1.5 ml microcentrifuge tube. This time, the supernatant is collected.
19) Add 50 µl of Nuclease free autoclaved ddH_2O to the spin column and let stand for 2 min.
20) Centrifuge at 14,000 g for 1 min at room temperature.
21) Collect the plasmid DNA eluted from the column.

Video #8: Isolation of plasmid DNA

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Evaluation results from 23 students

	Questions		Frequency	Percent
1	Have you had any laboratory experience in molecular cloning?	Yes	10	43.5
		No	13	56.5
2	Do you think the videos of the following topics help enhance your understanding?			
	2a. Aseptic techniques	Yes	23	100.0
	2b. Bacterial culture	Yes	23	100.0
	2c. Isolation and quantification of DNA	Yes	23	100.0
	2d. Restriction analysis	Yes	22	95.7
		No	1	4.3_{23}

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Comments from the students

- Greatly help the student understand lab practical. Very good
- All in all, it is really useful, must need to keep them.
- It is a very good tool to help us learn, and it's quite good
- They really helped me a lot because of my lacking of molecular biology background
- The video shows techniques needed in Biology labs in a detailed & clear way
- The videos give clear explanation and instruction of experimental skills, which text and pictures could not accomplish
- Very good and detailed, instructor, Dr. Luo is very nice
- Very helpful

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