HiPer® Plasmid Curing Teaching Kit

Product Code: HTBM037

Number of experiments that can be performed: 10

Duration of Experiment: 6 days

Day 1- Revival of Host
Day 2- Inoculation of culture
Day 3- Treatment with curing agent
Day 5- Dilution of culture and plating
Day 6- Observation and interpretation

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store *E. coli* Host and ampicillin at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

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Aim:

To learn the process of curing (elimination) of plasmid from host.

Introduction:

Plasmid is an extra-chromosomal DNA molecule different from the chromosomal DNA which is capable of independent replication. The term "Plasmid" was first introduced by the American molecular biologist Joshua Lederberg in 1952. Plasmid size varies from 1 to over 1000 kilo base pairs (kbps). Plasmids are mostly circular and double-stranded and are found in a wide variety of bacterial species.

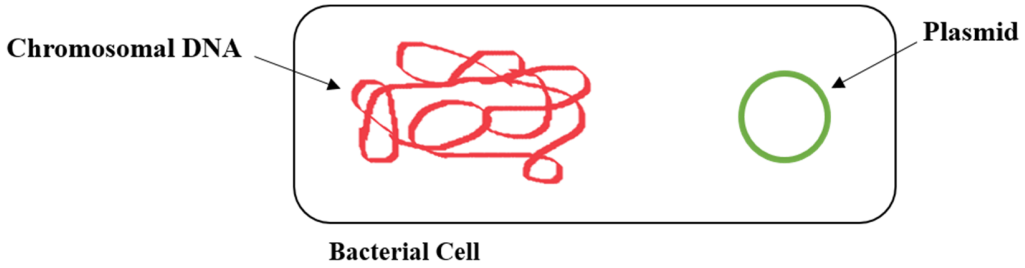


Fig1: A bacterium showing that the plasmids are not part of the chromosomal DNA

Bacterial plasmids are known to harbor genes for resistances to antibiotics and have a major impact on metabolic functions. Curing of plasmids from bacterial strains is a way to eliminate the bacterial plasmid from the host cell. There are several methods of plasmid curing (elimination) in *E.coli* involving use of chemical agents such as ethidium bromide (EB), acridine orange (AO), and sodium dodecyl sulphate (SDS), and physical agent. Ethidium bromide is an intercalating agent which resembles a DNA base pair. Due to its unique structure, ethidium bromide can easily intercalate into DNA strand. The mechanism of plasmid curing starts from the inhibition of plasmid replication resulted from a single nick, outside of the replication origin of the superhelical structure. The process leads to further relaxation of plasmid DNA, an increase in melting point and circular dichroism. The intercalating agents would then break the superhelical form of plasmid DNA subsequently forming an open circular or linear form. Thus, inhibiting its replication.

Principle:

Ethidium bromide have been successfully used in curing bacterial plasmids. The mode of action of intercalating agents are through preferential inhibition of plasmid replication. Curing agents at a concentration ranging from 0.1 to 0.5mg/ml is added to the culture broth. The concentration depends on the organism and curing agent used. The cultures are then incubated for 48 hours at 37°C under constant agitation. Post treatment the culture is plated on both LB and LB-ampicillin plates, further incubated for 24 hours. The cured cells will grow only in the absence of ampicillin, as the antibiotic resistance is lost due to elimination of plasmid.

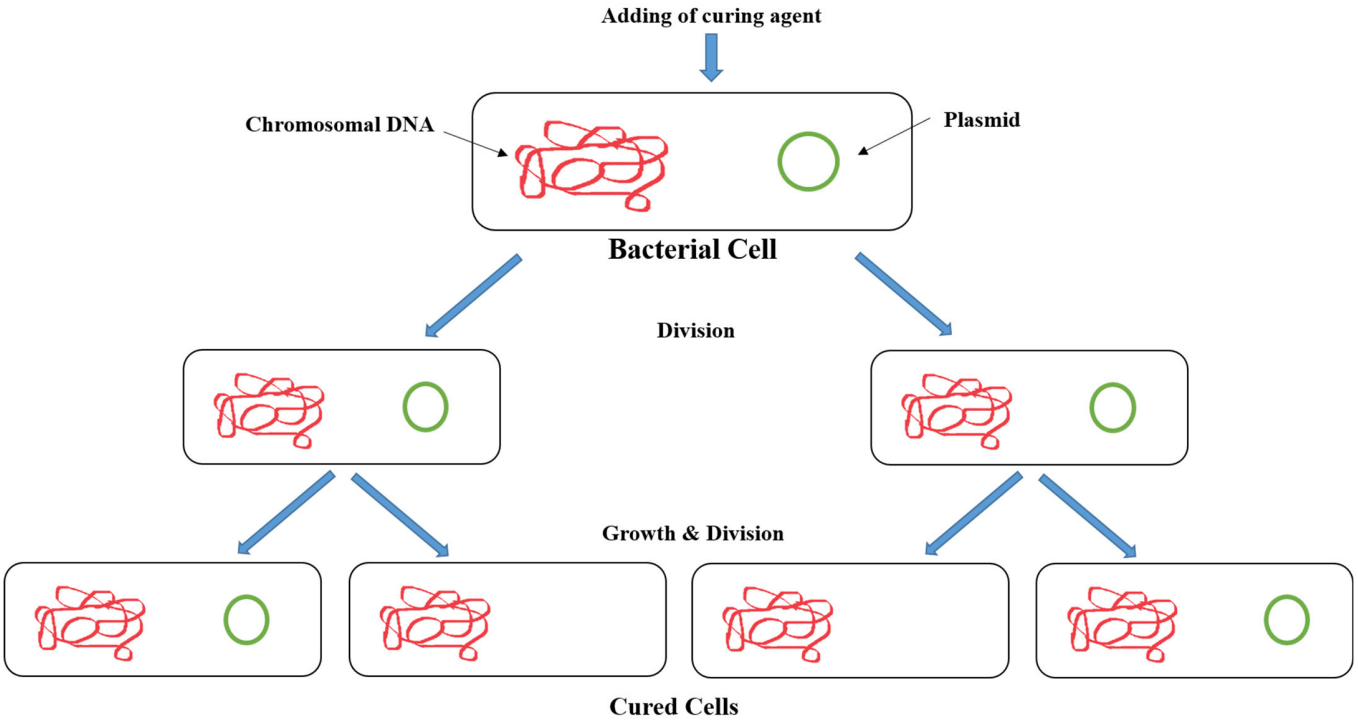


Fig2: Process of plasmid curing in bacterial cell

Kit Contents:

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials provided	Quantity	Storage
			10 expts	
1	MB104	Ampicillin	0.25 g	2-8°C
2	TKC434	*E. coli* Host	1 No.	2-8°C
3	M1245	Luria Bertani (LB) Broth	15 g	R T
4	M1151	Luria Bertani Agar	325 g	R T
5	MB074	Ethidium bromide solution (10 mg/ml)	1.25 ml	R T
6	MB023	Sodium chloride	2 gm	R T
7	PW1152	Screw cap tubes with 'O' Ring (2.2 ml)	180 Nos	R T

Materials Required But Not Provided:

Glasswares: Conical flask, Measuring cylinder, Beaker
Other requirements: Micropipettes, Tips, 37°C Incubator, 37°C Shaker, Centrifuge, Sterile loops and spreader, Sterile Petri plates.

Storage:

HiPer® Plasmid Curing Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. On receipt, store *E.coli* Host and ampicillin at 2-8° C. All other kit contents can

be stored at room temperature (15-25°C).

Important Instructions:

1. Read the entire procedure carefully before starting the experiment. The entire procedure should be carried out under sterile conditions.
2. Be very careful while handling with Ethidium bromide. Wear gloves while handling ethidium bromide solution and discard the gloves after use.
3. Sterilize the 2.2 ml screw cap tubes by autoclaving before use.
4. **Preparation of 0.85% saline (Sterile) (100 ml):** Dissolve 0.85 gm of Sodium chloride in 100 ml distilled water. Sterilize by autoclaving. Store at 2-8°C.
5. **Preparation of LB (Luria Bertani) broth (50 ml):** Dissolve 1.25 g of LB media in 50 ml distilled water. Sterilize by autoclaving.
6. **Preparation of LB (Luria Bertani) agar plates (400 ml):** Dissolve 16 g of LB agar in 400 ml of sterile distilled water. Sterilize by autoclaving and pour on sterile petri plates.
7. **Preparation of Ampicillin:** Dissolve 250mg of ampicillin powder in 5 ml of sterile double distilled water to give a concentration of 50 mg/ml. Store in aliquots at -20 C.
   NOTE: Avoid repetitive freeze thawing of ampicillin solution once prepared.
8. **Preparation of LB (Luria Bertani) Agar plates containing Ampicillin (400 ml):** Dissolve 16 g of LB agar media in 400 ml of distilled water. Sterilize by autoclaving and allow the media to cool down to 40-45°C. Add 400 µl of ampicillin to it, mix well and pour on sterile petri plates.

Procedure:

Read important instructions before starting the experiment.

Day 1: Revival of Host

1. Open the vial containing culture and resuspend the cells with 0.25 ml of LB broth.
2. Pick up a loopful of culture and streak onto LB agar plate with ampicillin.
3. Incubate overnight at 37°C.

Day 2: Inoculation of culture

1. Pick up a single colony from LB agar plate and inoculate in 10 ml of LB broth containing 10 µl of ampicillin.
2. Incubate at 37°C shaker at 250 rpm overnight.

Day 3: Treatment with curing agent

A. Cell suspension:

1. Measure the O.D of overnight grown culture at 600 nm. Concentration of culture is equal to O.D X 10^6 cells/ml.
   Example: If O.D of culture is 3.0, then the concentration of culture is 3 X 10^6 cells/ml.

2. Dilute the culture to the cell count of 10^3 cells/ ml using fresh LB broth.
3. Use this cell suspension for treatment.

B. Treatment with Ethidium bromide:
(Final concentration of ethidium bromide for the experiment is 300 µg/ ml)

1. Take 3 tubes and label them as negative control (NC), positive control (PC) and Test (300µg/ml).
2. Add the following components as per table:

Tube	Cell suspension (ml)	Ethidium bromide solution (10 mg/ml) (µl)	Ampicillin (50 mg/ml) (µl)
Negative control	2	-	-
Positive control	2	60	2
Test	2	60	-

3. Incubate at 37°C shaker at 150 rpm for 48 hours.

Day 5: Dilution of culture and plating

1. Take 5 sterile 2.2 ml tubes and label them as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} for each reaction tube.
2. After incubation, take out the negative control (NC) labelled tube, mix properly using pipette and perform 10 fold serial dilution upto 10^{-5} as follows:

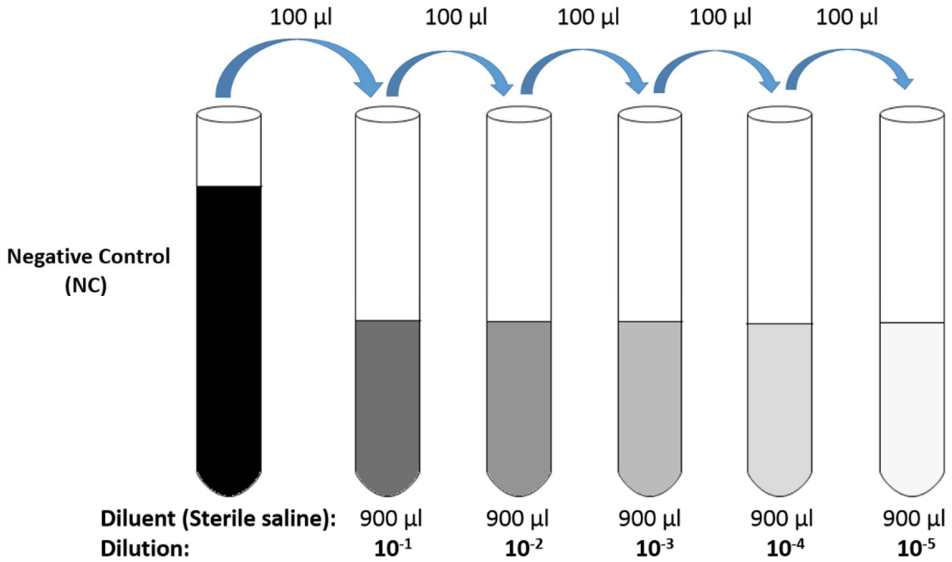


Fig 1: Serial dilution for spread plate method

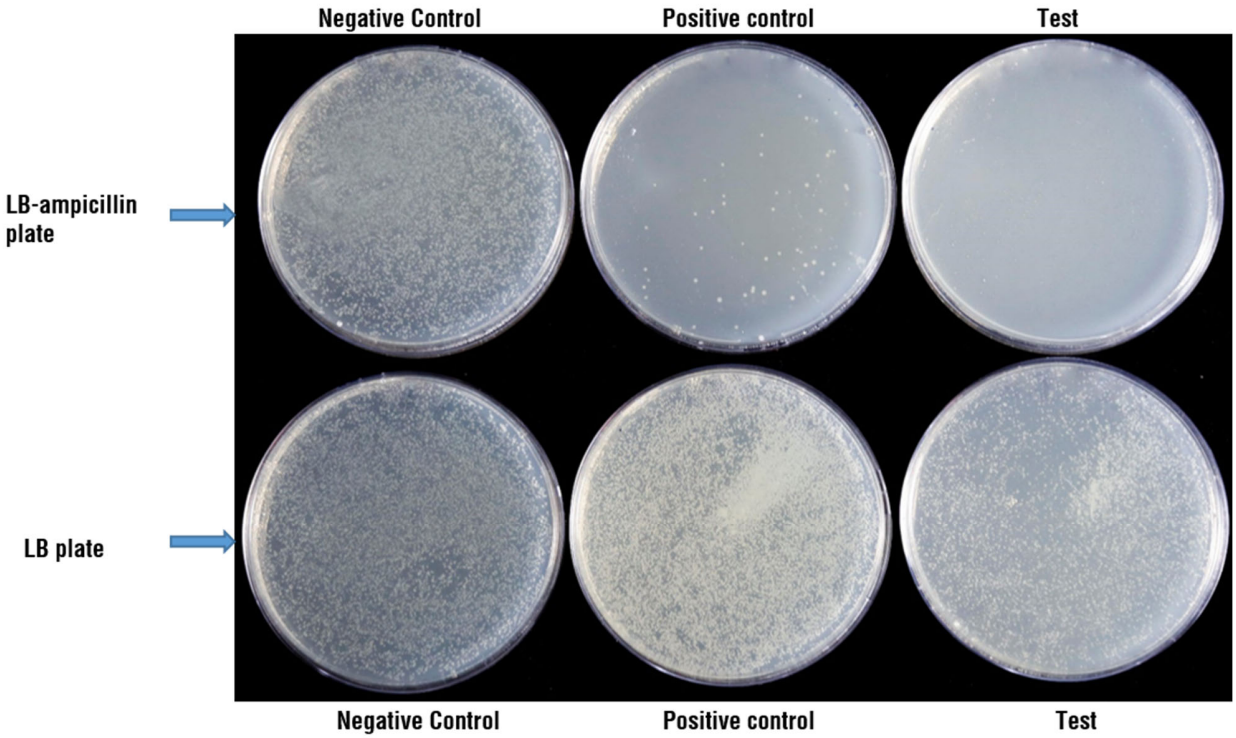
3. Spread plate 0.1 ml of each dilution of each concentration on LB plate and LB-ampicillin plates.
4. Incubate at 37°C for 24 hours.
5. Repeat the steps 1-4 for Positive Control (PC) and Test.

Day 6: Observation and Results

1. After incubation, observe for bacterial growth on LB plates and no growth on LB-ampicillin plates for test.
2. For negative control, observe for bacterial growth on both LB plates and LB-ampicillin plates.
3. For positive control, observe for bacterial growth on LB plates and no or few colonies on LB-ampicillin plates.

	LB plate	LB plate with ampicillin
Negative control	+	+
Positive control	+	Few or no colonies
Test	+	-

Note: Growth: (+), No growth: (-)



Interpretation:

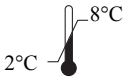
When the host cell is cured using Ethidium bromide solution, the antibiotic resistance marker is lost as plasmid is eliminated. Therefore, cured cells will not grow in presence of ampicillin. Ethidium bromide is an intercalating agent and it prevents plasmid DNA replication by specifically binding to it. As a result, plasmid DNA is not transferred to consecutive generation which leads to loss of plasmid from host cells.

Troubleshooting Guide:

Sr.No.	Problem	Possible Cause	Solution
1	Observed bacterial growth on test plate containing ampicillin	Agitation condition not maintained properly.	Agitation condition should be maintained at 150 rpm. Increase in agitation condition may lead to growth of plasmid containing cells.
		Ampicillin is inactivated	Add ampicillin to the media after it cools down to 40-45°C
		Ineffective curing of plasmid	Insufficient or excess cell concentration used for treatment with ethidium bromide
2	Contamination observed on plates	Sterility not maintained during the experiment	Make sure that the entire procedure is performed aseptically
		Plates may contain excess moisture	Make sure the plates are dry before use
3	Isolated colonies not observed	Plating is not performed properly	Plating should be performed uniformly
	No growth observed for any of the dilution	Serial dilution of culture was not carried out properly	Properly mix the culture with pipetting after treatment with ethidium bromide. Carry out serial dilution with proper mixing for each dilution

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com



Storage temperature

Do not use if package is damaged

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