

Biotechnology and genetic analysis practical assessment

[Technology](#)



**ASSIGN
BUSTER**

Answers: 2. The following table refers to experiments investigating the plasmid content of bacteria recovered on MLSA medium from River Taff sediment at two sites. One set of results is from sediment at the source of the river, the other is class results obtained from river sediment beside UWIC in Cardiff. Explain the results.

(100 words).

SITE

% BACTERIA CARRYING PLASMIDS

% PLASMIDS LARGER THAN 35Kb

SOURCE

9%

5%

UWIC

76%

73%

Answer:

The plasmid composition of two bacteria samples were found to be significantly different and this observation is mainly due to the composition and water quality of two different sites. At the origin of the river, water quality must be quite good without much polluted water contamination and hence the observed bacterial community doesn't require diverse metabolic capability to detoxify pollutants. As most of these kinds of catabolic genes found on plasmids observed in the bacterial population obtained from the origin of the river have very few plasmids. While in the case of the second sample which was obtained from the nearby site of UWIC having a large number of plasmid-bearing bacteria. This is

mainly attributed to accumulation of pollutant along the path of river and hence bacterial population need to evolved catabolic capability to survive and hence more plasmid bearing bacterial population were found in second sample. Similarly most of the plasmid was found to be in size range more than 35 KB clearly indicates most of them are Conjugative plasmids as this group of plasmid has more number of genes compared to non-conjugative plasmid to carryout conjugation process and hence larger the size.

(Source: NEIL F. BURTON, MARTIN J. DAY, AND ALAN T. BULL, Distribution of Bacterial Plasmids in Clean and Polluted Sites in a South Wales River APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1982, p. 1026-1029)

4. Explain exactly what is happening in steps 2, 3, 4 and 5 in the " Wizard" preparation (protocol 3), and how this begins the separation of plasmid DNA from chromosomal DNA.

Answer:

Step 2: Pellet suspended in to re-suspension solution

Re-suspension solution constitutes of glucose, EDTA and Tris each have its own role. Glucose provides osmotic stress and EDTA as chelating agents which binds to heavy metals and helps in disintegration of cell wall. Tris act as buffering agent and maintains pH of over all reactions to avoid any pH dependent side reaction. In this stage cells become highly fragile and some are break open.

Step 3: Lysis solution

This solution is mixture of SDS and NaOH. Here NaOH provides alkaline condition which helps in cell lyses and denaturation of DNA while SDS dissolves cell wall constituents and induces extensive cell lyses. It also helps

in proteins denaturation and precipitation. In this stage most of cell constituents get denatured including genomic DNA, But as plasmid is in its CCC (covalently closed circular) forms will not denatured completely and most of them remains in its native configuration.

Step 4: Neutralisation Solution

Here potassium acetate and acetic acid act as neutralizing agent to bring back the pH to normal. Similarly it induces the renaturation of DNA. Because of larger size most of the Genomic DNA remains denatured and mingled with proteins remains with cell debris while plasmid being smaller molecule except out to supernatant .

Step 5: centrifugation at high speed;

During this stage all cell debris along with genomic DNA settled at the bottom of tube and being smaller in size plasmid remains in supernatant. Which subsequently purified and used for transformation.

5. Attach the original photo of your restriction digest, with an explanation of what is shown in each lane. (Always number the lanes in a gel photo or diagram.) If your own experiment did not provide a complete set of results you should still attach a copy of your photo and try to explain the appearance of each lane, but use the one attached below to construct the curve etc. (If using the photo below you should note that it was run under different electrophoresis conditions to those we used in the prac and the relative positions of supercoiled and linear plasmid may be different.)

Answer:

Lane 1: This lane represents the digested product of lambda genomic DNA by enzyme pst1. There are 28 restriction site for pst1 on lambda genomic

DNA and hence in properly made gel one can get 29 bands. The size of fragments ranges between 11501bp to 247bp.

Lane 2:

Here the lambda genomic DNA was digested with enzyme HindIII which leads to generation of 8 fragments ranging between 23130 bp to 213bp

Lane 3:

This lane represents undigested lambda genomic DNA and hence we can see bright slightly migrated DNA band.

Lane 4:

Uncut native plasmid pGEM3Z was shown having molecular weight of around 2750 bp. Similarly we observe three bands represents three different form of plasmid configuration (i. e. CCC)

Lane 5

Here the band represents linearised pGEM3Z and hence we can see only single band representing linear form of pGEM3Z. e travel by

Molecular weight determination of DNA fragments:

Plot a graph size v/s RF value(distance travelled by DNA fragment from origin). Here Lane 2 (lambda DNA digested with HindIII) taken as standard molecular weight of each fragment was plotted against the RF value. Once standard curve will be made size of unknown fragment can be calculated.

Table 1:

Molecular weight of DNA fragment generated by HindIII digetion

Distance migrated (mm)

23, 130

9420

6560

4360

2320

2030

560

Example:

7. Protocol 6. Transformation.

The photos of plates below are from a similar experiment. The plate to the right is from tube 2, step 7. Plate to the left is from tube 3, step 7. Explain precisely the mechanisms that have led to the difference in appearance of the two plates. (Use diagrams to assist). When you recorded the appearance of your plates there were a lot of satellite colonies; can you offer an explanation.

Answer:

Here we have two different observations 1) colonies from tube 2 grown as blue coloured colonies 2) while from tube 3 there is a mixture of blue and white. This can be explained as follows. In the case of tube 2 there is only the vector pGEM3Z used for transformation. The plasmid pGEM3Z has the lacZ gene as a marker which codes for an enzyme called beta-galactosidase. After transformation, cells were plated on LA supplemented with X-gal and IPTG. Now in the presence of IPTG, expression of lacZ is induced and leads to the synthesis of beta-galactosidase which subsequently acts on X-gal and cleaves it to a chromogenic end product that gives rise to a blue colour.

While in case of tube 3 there was plasmid vector along with insert gene (ligation mixture) and plated on similar plate after transformation. As vector pGEM3Z having MCS (multiple cloning sites) within the lacZ gene any insertion or recombination leads to inactivation of lacZ (insertional inactivation). Inactive lacZ will not code for functional beta glycosidase and hence colonies having insertion give rise to white colors. In another scenario where cut plasmid re-ligated without any insertion during ligation process preserves functional lacZ and hence post transformation give rise to blue colonies.

Micro colonies appeared near to white colonies is mainly due to diffusion of beta lactamase which cleaved ampicillin and reduced its concentration but not at extent where bacterium will grow like other bacterium having plasmid coding for beta- lactamase.

8. How would you prove that the white colonies on the plate from tube 3 contained recombinant plasmids

Answer;

There are many ways by which one can prove recombinant plasmid some of them are as follows:

1) As the vector pGEM3Z have lac Z as marker and MCS (multiple cloning site) located within this gene any recombination or insertion makes lac Z inactive (insertional inactivation). So white colonies on X-gal, IPTG plates serves as primary indication of recombinant plasmid. But any cells which transformed by unligated cells gives white colonies and hence needs to be confirm.

2) The second and confirmatory test is isolating the plasmid from white

colonies and run on to the agarose gel electrophoresis. Increase in Size equivalent to inserted gene clearly indicates recombinant plasmid.

3) Similarly one can confirm it by PCR based method where gene of interest will be amplified by primers deigned against flanking sequence available on plasmid. Any amplification indicates recombination or insertion.