Identifying the mystery plasmids based upon their characteristics

ASSIGN BUSTER

Identification of Mystery Plasmids Abstract Plasmids serve as important tools in genetics and biotechnology labs, where they are commonly used make many copies of a particular gene. The aim of this experiment was to identify three mystery plasmids based upon their characteristics; such as size, antibiotic resistance, lacZ profile and conjugative properties. The results obtained showed that plasmid number2 was the pDSK519 plasmid and its size was 26229.

58 Bp. Plasmid pDSK519 also was found to be resistant to kanamycin only and it possessed the LacZ gene. DSK519 had the oriT gene but it did not have the tra gene. Plasmid number 22 was found to be pHSG298 as it had a size of 9767. 08 Bp and it was found to be resistant to kanamycin only.

It also possessed the LacZ gene and conjugation results showed that it did not possess the oriT gene the tra gene. Plasmid number 12 was found to be pRP1:: Tn501 as it was resistance to all three antibiotics. It was also found out that this plasmid did not possess the LacZ gene as evidenced by the white colonies. pRP1:: Tn501 had oriT gene as well as the tra gene as shown in the conjugation results. The use of plasmids in very beneficial towards humans and these types of experiments contribute greatly towards this.
Introduction A plasmid is an extra-chromosomal DNA molecule separate from the chromosomal DNA which is capable of replicating independently of the chromosomal DNA (Chandrasegaran, 2008).

In many cases, it is circular and double-stranded. Plasmids usually occur naturally in bacteria, but are sometimes found in eukaryotic organisms. Plasmid size varies from 1 to over 200 kilo base pairs (Russell and Sambrook,

2001). Plasmids can be considered to be independent life-forms that tend to form symbiotic relationships with their hosts, as they endow their hosts with useful packages of DNA to assist mutual survival in times of severe stress. For example, plasmids can convey antibiotic resistance to host bacteria, who may then survive along with their life-saving guests who are carried along into future host generations.

Moreover, plasmids can be considered to be mobile genetic elements that can move between genomes since they are often associated with conjugation, a mechanism of horizontal gene transfer (Barkay and Smets, 2005). Conjugation, or mating, is a process of genetic transfer that requires cell-to-cell contact (Barkay and Smets, 2005). The genetic instructions for conjugation are encoded on a double-stranded, circular piece of DNA. The circular DNA exists in the bacterial cell entirely separate from the much larger bacterial chromosome. This is known as the conjugative plasmid or the fertility factor (Snyder et al. , 1997).

Cells that possess it are donor or male cells, and those that lack a conjugative plasmid are recipient or female cells (Snyder et al. , 1997). There are multiple genes involved in the process of conjugation. Some of the genes code for a surface structure found on donor cells, the sex pilus (Snyder et al. , 1997). This is a threadlike tube made of protein.

The sex pilus recognizes a specific attachment site on a recipient cell. When the donor cell comes near a recipient, the sex pilus attaches to the specific site and begins to retract, pulling the two cells together (Snyder et al. , 1997). As the two cells draw close, their connection stabilizes and their outer

membranes fuse together to allow the transfer of DNA from one cell to the other (Snyder et al. , 1997).

Only one of the two strands of DNA making up the plasmid passes through the fused membranes into the recipient cell. Thus, DNA synthesis must occur in both donor and recipient to replace the missing strand in each (Snyder et al. , 1997). The genes encoding the enzymes responsible for this part of the conjugative process are also found on the plasmid. Once passage and synthesis are successfully completed, both donor and recipient cells contain a whole double-stranded, circular, conjugative plasmid (Snyder et al.

, 1997). Therefore, there are now two donor cells when before there was only one. This process is so efficient that it can quickly change an entire population to donor cells (Snyder et al. , 1997).

Cconsequently, this gives researchers the ability to introduce genes into a given organism by using bacteria to amplify the hybrid genes that are created in vitro. This tiny but mighty plasmid molecule is the basis of recombinant DNA technology. There are some minimum requirements for plasmids that are useful for recombination techniques. These encompass a plasmid to have an origin of replication (ORI), that is, they must be able to replicate themselves or they are of no practical use as vectors (Fukuhara et al.

, 2007). Secondly, they must have a marker in order to select for cells that have certain genes and, thirdly, there should be restriction enzyme sites in non-essential regions (Fukuhara et al, 2007). In addition to these necessary requirements, there are some factors that make plasmids either more useful

or easier to work with. These include the plasmid being small as it will easier to isolate, handle, and transform (Yau et al.

, 2008). Likewise, multiple restriction enzyme sites give researchers greater flexibility in cloning and, finally, multiple origins of replication allow two genes to be inserted into the same plasmid (Yau et al. 2008). Plasmid pDSK519 was made by modifying plasmid pDSK509 and incorporating a LacZ gene into the plasmid (Keen et.

al. , 1988). This gave the plasmid several additional cloning sites, improved their antibiotic resistance and facilitated sub cloning and mobilization into various gram negative bacteria (keen et. al.

, 1988). The size of the plasmid was found to be 8. 1 kbp (Keen et. al. , 1988). The plasmid contained kanamycin resistant genes in the KmR vector site.

As the plasmid contained a LacZ gene, it was helpful in identifying the gene in this experiment as a blue colour appears when x-gal is broken down by ? galactosidase, which is a product of the LacZ gene. Figure 1: Genetic maps of plasmids pHSG298 and pDSK519. oriR is the origin of replication and oriT is the origin of transfer. Plasmid pHSG298 is a high copy number vector and is of 2. 68 kbp in size (Takeshita et al.

, 1987). It is a ? -complementation plasmid vector which contains a kanamycin-resistance gene (KmR) and the plasmid also has a pUC18 replicon. When developed all the cloning sites except for the HindIII and

SmaI sites in the KmR vectors were unique to each individual (Takeshita et. al. , 1987).

Plasmid pRPl:: Tn5Ol was developed by the insertion of the mercury resistance transposon Tn501 into broad-host-range plasmid RPl (Pemberton and Brown, 1981). This greatly enhanced the ability of this plasmid to promote chromosome transfer in the photosynthetic bacterium Rhodopseudomonas sphaeroides (Pemberton and Brown, 1981). Plasmid pRPl:: Tn5Ol promoted polarized transfer of chromosomes from one or perhaps two origins on the chromosome, giving rise to two linkage groups. The plasmid contained an ampicillin resistance gene, a kanamycin resistant gene and a tetracycline resistant gene (Pemberton and Brown, 1981).

Figure 2: The genetic map of plasmid pRPl:: Tn5Ol. DNA was extraction was used to collect DNA for subsequent molecular analysis. There are three basic steps in a DNA extraction procedure; firstly, the cells are broken open to expose the DNA within, by grinding or sonicating the sample; secondly, the lipid membrane lipids are removed by adding a detergent; thirdly, the DNA is precipitated with an alcohol, usually ethanol or isopropanol, to aggregate all the DNA together, which gives a pellet upon centrifugation (Wehausen et. al.

2004). Agarose gel electrophoresis is a method used in biochemistry and molecular biology to separate DNA, or RNA molecules by size. This is achieved by moving negatively charged nucleic acid molecules through an agarose matrix with an electric field (electrophoresis). Shorter molecules move faster and migrate farther than longer ones (Russell and Sambrook,

2001). Antibiotic resistance is the ability of a microorganism to withstand the effects of antibiotics.

It is a specific type of drug resistance (Cirz et. al. , 2005). As each of the plasmids contained different antibiotic resistant genes, they were tested on three types of antibiotics, and if showed that they were resistant to the antibiotic they grew, and if they were sensitive they did not grow.

The presence of the LacZ gene was also tested by growing each of the plasmids in X-gal plates. The presence of LacZ gene could be identified by the blue color of the colonies present as x-gal is broken down by ? -galactoxidase, which is the product of the LacZ gene. Alkaline lysis is the method of choice for isolating circular plasmid DNA, or even RNA, from bacterial cells. It is probably one of the most generally useful techniques as is a fast, reliable and relatively clean way to obtain DNA from cells.

If necessary, DNA from an alkaline lysis prep can be further purified. Alkaline lysis depends on a unique property of plasmid DNA. It is able to rapidly anneal following denaturation. This is what allows the plasmid DNA to be separated from the bacterial chromosome (Ciccolini, 2000). Conjugation techniques were used to identify the mystery plasmids as they contained had different genetic maps. A helper plasmid, pRK600, was also added to the plasmids to give one of them the tra gene to be able to conjugate.

As the genetic maps of the plasmids were known, the results obtained were compared to these and the identities of the mystery plasmids could be known. For conjugation to occur, the presence of the tra gene as well as the oriR gene was necessary. The overall aim of the experiment was to

determine the identities of the mystery plasmids based upon their characteristics; such as size, antibiotic resistance, lacZ profile and conjugative properties. Three hypotheses were drawn up. The first one was that plasmid pDSK519 would travel the least furthest in agarose gel electrophoresis as it was the biggest, that it would not undergo conjugation without the helper plasmid, that it would be resistant to kanamycin, and that it would show blue colonies in the x-gal plates.

The second, hypothesis was that plasmid pHSG298 would travel the second furthest in the agarose gel electrophoresis as it was the second largest plasmid, that it would not undergo conjugation with or without the helper plasmid, that it would be resistant to kanamycin and that it would show blue colonies in the x-gal plates. The third hypothesis was that plasmid pRP1:: Tn501 would travel the furthest in the agarose gel electrophoresis as it was the smallest plasmid, that it would undergo conjugation with or without the helper plasmid, that it would show resistance towards all three types of antibiotics used and that it would show white colonies in the x-gal plates. Method As per pages 40 to 52 of the practical manual. Biol 215 Introductory Genetics Manual (2008). Pages 40-52. Results Agarose Gel Electrophoresis Agarose gel electrophoresis was run on the mystery plasmids together with the standard markers.

Results obtained were then compared to identify the sizes of the mystery plasmids. Figure 3: Agarose gel electrophoresis of purified mystery plasmid DNA. Lane 11 contained plasmid number 2: pDSK519. Lane 12 contained plasmid number 22: pHSG298. Lane 13 contained plasmid Number 12: pRP1:: Tn501. Molecular weight markers (in Bp) are indicated on the left.

It was found out that plasmid number 2 had a size of 26229. 58 Bp, plasmid number 12 had a size of 9767. 08 Bp and plasmid number 22 had a size of 1176. 04 BP.

Figure 4: Sizes of the plasmid as found by comparing the distances moved by the plasmids through the gel against the distance moved y the standard markers through the gel. The sizes of the markers together with their sizes were plotted and this standard curve was used to find the sizes of the plasmids. Plasmid Plasmid NumberDistance Moved (cm) Size (Bp) pDSK519 2 1. 7 26229.

58 pHSG298 22 2. 4 9767. 08 pRP1:: Tn501 12 3. 9 1176. 04 Antibiotic Resistance The mystery plasmids were checked for resistance or sensitivity to kanamycin, ampicillin and tetracycline.

If the colonies were blue, this indicated that the plasmids had the LacZ gene. Figure 5: Table showing the characteristics of the 3 mystery plasmids along with the results obtained for the resistance or sensitivity towards the three types of antibiotics: kanamycin, ampicillin and tetracycline. oriR refers to the origin of replication; oriT refers to the origin of transfer; tra refers to the presence of conjugal transfer genes; and antibiotic resistance corresponds to the resistance of the plasmids to the kanamycin, ampicillin and tetracycline antibiotics. Plasmid Number oriR oriT tra Antibiotic Resistance LacZ Size (Bp) 2 yes yes noyes only to Kanamycin es – Blue colonies 26229. 58 22 yes no noyes only to kanamycin yes – Blue colonies 9767. 08 12 yes yes yesyes to all antibioticsno- white colonies 1176.

04 Conjugation The results indicated that plasmid 2 had no conjugation without the helper plasmid, pRK600, however, with the helper plasmid, plasmid 2 underwent conjugation. Plasmid 12 showed results of conjugation both with the helper plasmid and without it. Plasmid 22 had no conjugation results for the two variations in the methods. Figure 6: Conjugation results for the three mystery plasmids. Two methods were carried out to get the conjugation results.

The first method was done without the helper plasmid, pRK600, and the second method entailed the use of the helper plasmid. The conjugation frequency was calculated in each case. TMTC means too many to count. Plasmid NumberNumber of transconjugants per mL without helperNumber of recipients per mL without helper Conjugation frequency without helper plasmidNumber of transconjugants per mL with helper plasmidNumber of recipient cells per mL with helper plasmidConjugation Frequency with helper plasmid 2 01.

x10501. 65×1037. 0x1040. 024 12 5. 0 x1072. 0x1072.

54. 0x1074. 0 x1071 22 0TMTC001. 0x1040 Discussion: The different characteristics of plasmids make them very useful in molecular science. The three mystery plasmids were identified by comparing the results obtained with their genetic maps, which were previously known.

Plasmid number 2 moved the least furthest in the agarose gel electrophoresis. This indicated that this plasmid was the largest amongst the three studied. Moreover, plasmid number 2 was only resistant to the kanamycin antibiotic. This meant that the plasmid contained the KmR gene.

Plasmid number 2 also appeared as blue colonies in the x-gal plates, thus, it was stated that it contained the LazZ gene.

Furthermore, plasmid number 2 did not undergo conjugation without the helper plasmid, however, it did conjugate with the helper plasmid. This led to the assumption that plasmid number 2 had the oriT gene but not the tra gene. All these observations were put together to conclude that plasmid number 2 was the pDSK519 plasmid. Plasmid number 22 moved the second furthest in the agarose gel electrophoresis.

This indicated that this plasmid was the second largest amongst the three studied. Moreover, plasmid number 22 was only resistant to the kanamycin antibiotic. This meant that the plasmid contained the KmR gene. Plasmid number 22 also appeared as blue colonies in the x-gal plates, thus, it was stated that it contained the LazZ gene.

Furthermore, plasmid number 22 did not undergo conjugation with or without the helper plasmid, indicating, that it had no oriT gene or tra gene. All these observations were put together to conclude that plasmid number 22 was the pHSG298 plasmid. Plasmid number 12 moved the furthest in the agarose gel electrophoresis. This indicated that this plasmid was the smallest amongst the three studied. Moreover, plasmid number 12 was resistant to all three antibiotics, signifying that the plasmid contained the KmR , ApR and TcR genes. Plasmid number 12 also appeared as white colonies in the x-gal plates, thus, it was stated that it lacked the LazZ gene.

Furthermore, plasmid number 12 underwent conjugation with and without the helper plasmid, indicating, that it had the oriT gene as well as the tra

gene. All these observations were put together to conclude that plasmid number 12 was the pRP1:: Tn501 plasmid. The experiment was successful as the mystery plasmids were identified properly. Possible reasons for failure of the experiment include chromosomal contamination which would have given unreliable results. The deoxyribonucleic acid extraction procedure should be done with great care as chromosomal contamination could occur. The extracted DNA’s should be stored properly to ensure that they do not degrade as the experiment is long and would take a few days to complete.

Using new pipettes and tubes, as well as sterilizing the equipment properly would ensure in more reliable results. The results obtained for plasmid pDSK519 conveyed with those results obtained by Keen et al. (1988). The researchers found that the plasmid was resistant to the kanamycin antibiotic as it did not grow in the agar plates containing kanamycin. They also found that the plasmid had the LacZ gene present because blue colonies were observed after the plasmid was incubated with bacteria in x-gal plates (Keen et.

al. , 1988). All of these observations were also made in this experiment. For pHSG298 plasmid, the results were compared to those obtained by Takeshita et al. (1987).

The researchers found that they had developed ? -complementation plasmid vectors that contained the kanamycin (KmR) resistant gene and a polylinker cloning site within the region of the LacZ gene. They also identified that plasmid pHSG298 contained the polylinker, pUC18, in the LacZ gene as blue colonies were observed when the plasmid was incubated with bacteria in x-

gal plates (Takeshita et. al. , 1987). All of these observations related to the observations obtained in this experiment.

The results obtained for plasmid pRP1:: Tn501 were compared to the results obtained by Pemberton and Brown (1981). These two researchers found that the plasmid pRP1:: Tn501 contained ApR, TcR and KmR genes as colonies of bacteria containing the plasmid were observed growing in plates containing the three types of antibiotics. They also found that the plasmid contained the two necessary genes required for conjugation to take place; these were the oriT and tra genes (Pemberton and Brown, 1981). These results agreed with the results obtained in the experiment. This type of genetic engineering experiment is important so that it is possible to identify the different properties a plasmid possess, thus, enabling humans to use them in situations where they would provide scientific assistance. We can use bacteria as little factories to make more DNA, as they replicate, or to make protein, by transforming them with genes for proteins we want to make.

For example, say you know someone who is diabetic. They have to take the protein insulin to be healthy. We can put the insulin gene into a plasmid and then insert that plasmid into bacteria. These bacteria will make insulin for diabetics to use. Before genetic engineering was invented, we used to have to kill pigs to get their insulin.

Now we can use bacteria to make human insulin instead of using pig insulin, especially important if someone is allergic to pigs, and we don’t have to kill any animals to do it. In conclusion, these types of experiments help to identify those plasmids which are capable of working in the required

environments, thus, enabling scientists to incorporate sought after genes into bacteria for production. Questions). The procedure starts with the growth of the bacterial cell culture harboring the plasmid.

When sufficient growth has been achieved, the cells are then pelleted by centrifugation to remove them from the growth medium. The supernatant is discarded as pieces of cell wall are released from the bacteria and are floating around in the supernatant. These cell wall pieces can inhibit enzyme action on the final DNA, so it is important to get rid of all of the supernatant. The cells are then lysed with sodium hydroxide (NaOH) and sodium dodecyl sulfate (SDS). The cells are then mixed by gentle inverting and incubating on ice for five minutes (but no longer, or the DNA will be irreversibly denatured).

Three things happen during this stage: firstly, SDS pops holes in the cell membranes; secondly, NaOH loosens the cell walls and releases the plasmid DNA and sheared cellular DNA; and thirdly, NaOH denatures the DNA. Cellular DNA becomes linearized and the strands are separated. Plasmid DNA is circular and remains topologically constrained. The plasmids are then renatured and the supernatant discarded. The addition of sodium acetate (NaAc) does three things: firstly, circular DNA is allowed to renature and sheared cellular DNA remains denatured as single stranded DNA (ssDNA); secondly, the ssDNA is precipitated, since large ssDNA molecules are insoluble in high salt; and finally, adding sodium acetate to the SDS forms KDS, which is insoluble.

This allows for the easy removal of the SDS from the plasmid DNA. The cell is then centrifuged to remove centrifuge to remove cell debris, KDS and

cellular ssDNA. The plasmid DNA is in the supernatant, while all of the wastes are in the pellet. The plasmid DNA is precipitated by alcohol precipitation (ethanol) and a salt (sodium acetate) and spun. DNA is negatively charged, so adding a salt masks the charges and allows DNA to precipitate.

This will place your DNA in the pellet. The pellet was air-dried to remove the ethanol. The pellet was then resuspended in buffer (dH2O) and to cleave any remaining RNA. The DNA was back in the solution. 2) The addition of the helper plasmid, pRK600, to plasmid pDSK519 causes the plasmid to undergo conjugation because the helper plasmid incorporates a tra gene into the pDSK519 plasmid.

As both the oriT and tra genes were present, pDSK519 underwent conjugation. Moreover, the addition of pRK600 to pHSG298 does not affect its ability to conjugate because it does not possess the oriT gene. Even though, the helper plasmid gave the tra gene to pHSG298, it could not conjugate as it did not possess the necessary oriT gene. Furthermore, the addition of pRK600 did not have any effect on the conjugation properties of pRP1:: Tn501 as the plasmid already possessed the oriT gene as well as the tra gene necessary to undergo onjugation.

3) The medical significance of plasmids that are capable of degrading antibiotics is to measure the antibiotic resistance of bacteria and to come up with new vaccines to viruses in order to combat them. The medical significance of plasmids degrading lactose is that lactose-fermenting salmonellae may arise and plasmids are used to carry genes that enhance

the host organism to degrade the lactose and pass the harmful substances in their feces. Additionally, the medical significance of plasmids that degrade sucrose is glucan, which is synthesized from the glucose moiety of sucrose, plays a prominent role in the cariogenicity of the dental pathogen Streptococcus mutans. In order to develop genes which combat this, plasmids are used. Appendix References Barkay, T. and Smets, B.

F. (2005). Horizontal gene flow in microbial communities. ASM News 71: 412-419.

Ciccolini, L. A. S. , Ayazi Shamlou, P. , Titchener-Hooker, N. J.

, Ward, J. M. and Dunnil, P. (2000).

Time Course of SDS-alkaline lysis of recombinant bacterial cells for plasmid release. Biotechnology and Bioengineering 6: 768-770. Cirz, R. T. , Chin, J. K.

, Andes, D. R. , de Crecy-Lagard, V. , Craig, W.

A. and Romesberg, F. E (2005). Inhibition of mutation and combating the evolution of antibiotic resistance.

PLoS Biol. 3 (6): 176. Fukuhara, Y. , Naoi, T. , Ogawa, Y. , Nishikawa, M.

and Takakura, Y. (2007). Plasmid DNA uptake and subsequent cellular activation characteristics in human monocyte-derived cells in primary culture. Journal of Pharmaceutical Sciences 96: 1576-1584.

Holmes, R. K. and Jobling, M. G.

(1996). Genetics: Conjugation. in: Baron's Medical Microbiology. Univ of Texas Medical Branch Kandavelou, K. and Chandrasegaran, S.

(2008). Plasmids for Gene Therapy. Plasmids: Current Research and Future Trends. Caister Academic Press.

Watson, J and Crick, F. (1953). Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. Nature 171 (4356): 737–8. Russell, D.

W. and Sambrook, J. (2001). Molecular cloning: a laboratory manual. Cold Spring Harbor, N.

Y: Cold Spring Harbor Laboratory. Keen, T. N. Tamaki, S. , Kobayashi, D.

and Trollinger, D. (1988). Improved broad-host- range plasmids for DNA cloning in gram-negative bacteria. Gene 70: 191-197.

Pemberton, J. M. and Bowen, St. G. A. R.

(1981). High-Frequency Chromosome Transfer in Rhodopseudomonas sphaeroides Promoted by Broad-Host- Range Plasmid RP1 Carrying Mercury Transposon Tn501. Journal of Bacteriology 147: 110-117. Snyder, L. and Champness, W. (1997).

Molecular Genetics of Bacteria. Washington, DC: ASM Press. Takeshita, S. , Sato, M.

, Toba, M. , Masahashi, W. and Hashimoto-Gotoh, T. (1987). High copy-number and low-copy-number plasmid vectors for lacZ alpha-complementation and chloramphenicol- or kanamycin-resistance selection.

Gene 61: 63-74. Wehausen, J. D. , Ramey, R. R. and Epps, C.

W. (2004). Experiments in DNA Extraction and PCR Amplification from Bighorn Sheep Feces: the Importance of DNA Extraction Method. Journal of Heredity 95(6): 503-509. Yau, Y.

S. , Keshavarz-Moore, E. and Ward, J. (2008). Host strain influences on supercoiled plasmid DNA production in Escherichia coli: Implications for efficient design of large-scale processes. Biotechnology and Bioengineering 101: 529-544.