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Why the cloning was done:

Streptococcus pneumonia is a gram-positive bacteria that can cause pneumonia and ear infections among other pathologies. However, because it is very hard to trace the bacteria through the infection process, scientists have not been able to identify the exact mechanism through which these bacteria produce their pathogenic effects.

In order to better study the actions of *Streptococcus pneumonia*, researchers engineered a plasmid that expresses green fluorescent protein in the presence of maltose. Previously, plasmids had been developed (pLS1GFP and pLS1RGFP) that contained *gfp*, but it was difficult to regulate the expression of *gfp*. These previously developed plasmids contained both malM and malR gene sequences. The protein created by MalR suppresses the function of MalM, which also effects the transcription of *gfp*. One of the goals in the construction of this new plasmid was to create plasmid that did not contain the malR gene. In this new plasmid, researchers hoped to develop a product where the expression of *gfp* could be easily regulated in the presence of maltose.

It also was important to the researchers that the plasmid be moblilizable because they wanted to be able to follow the action of this bacteria after it was conjugated. The fact that researchers were able to create a cloning process that allowed them to create a mobilized plasmid containing the gene for gfp was very significant; before the publication of this paper, this type of cloning and transformation process had only been available when applied to gram-negative bacteria.

How the cloning was done:

pMV158 is the natural streptococcal plasmid. pLS1GFP is a plasmid that was engineered using pMV158 and contains the gene for gfp. In pLS1GFP, the P_M promoter regulates *gfp* expression. In this cloning procedure, researchers used pLS1PMGFP, which is a derivative of the pLS1GFP. They cut this plasmid with *AvaI* and *SalI*. *AvaI* and *SalI* were used because the erythromycin resistance gene has an *AvaI* restriction site on one end and a *SalI* restriction site on the other end. Researchers wanted to cut this DNA sequence out of the plasmid because they wanted to replace the gene for erythromycin resistance with the *mobM* gene. After cutting the plasmid with *AvaI* and before cutting it with *SalI*, *PolIK* was used to create sticky ends that would match up with the sticky ends on either side of the *mobM* gene.

Researchers used polymerase chain reaction to make copies of the pMV158-*mobM* gene. This gene fragment was then inserted into the pCR2.1-TOPO plasmid. This step was important because the *mobM* gene needed to be flanked with the necessary restriction sites if it was going to be inserted into the new plasmid. On one end of the *mobM* gene there was an *XhoI* restriction site, and on the other end there was a *SmaI* restriction site. These restriction enzymes were used to cut the plasmid on either end of the *mobM* gene. After cutting with these two restriction enzymes, researchers were left with the *mobM* DNA sequence and the promoter for this gene. It was important to insert the mob gene into the plasmid, because only plasmids containing the mob family of genes have the ability to be mobilized.

In the resulting plasmid, the erythromycin resistance gene was exchanged for the *mobM* gene. Because the plasmid contained *mobM*, it was able to be mobilized. The plasmid also contained *gfp* under the regulation of the P_M promoter.

Overall Summary of Findings

To test the success of the construction of the new plasmid (pMV158gfp), researchers had to make sure that the plasmid both glowed and was mobilizable. pMV158 does not have a mob gene and is therefore not mobilizable. However, pMV158 can be mobilized using an auxiliary plasmid. To check if the plasmid was mobilizable, they compared the transfer of pMV158gfp to the transfer of pMV158 with an auxiliary plasmid. The researchers observed the transfer between S. *pneumoniae lactis* and E. *faecalis.* pAMB1 was used as the auxiliary plasmid to move pMV158. The rate of conjugal transfer for pMV158gfp was very similar to the rate of conjugal transfer for pMV158 with pAMB1. This result indicates that the *mobM* gene was successfully incorporated into the new plasmid.

Researchers also had to make sure that the new plasmid contained the gene for *gfp* and that this gene could be easily regulated. When researchers treated bacteria with pMV158GFP, florescence was observed. This showed that the gene for *gfp* was successfully incorporated into the plasmid. When pneumonoccal cells were grown in a medium with maltose, researchers observed that the transconjugates glowed. This showed that even when the plasmid was mobilized, it still had the ability to glow when it was grown with maltose.

The researchers were able to achieve their two main goals in the construction of the new plasmid: to make a plasmid that was not only mobilizable, but also capable of expressing *gfp* through a simple regulation process.

Research has shown that this plasmid can be transferred into many different types of bacteria. Because of this, the new plasmid will allow scientists to study a variety of different bacteria and their processes. For example, they could better study the process of infection of pneumococcal cells, or study lactic acid bacteria in dairy products.

Work Cited:

Nieto, Concepción and Manuel Espinosa, Construction of the mobilizable plasmid pMV158GFP, a derivative of pMV158 that carries the gene encoding the green fluorescent protein, Plasmid, Volume 49, Issue 3, May 2003, Pages 281-285