PCR-Targeting of a Plasmid for Heterologous Expression of Thalassospiramides

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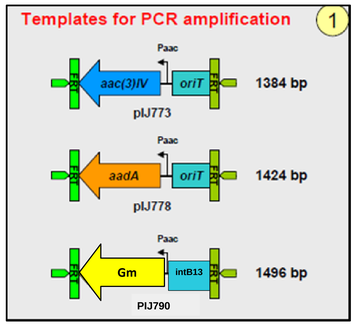
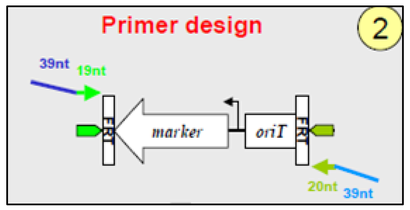
Abstract

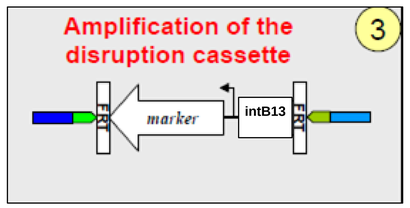
Sequences of DNA are responsible for the biosynthesis of small molecules. The heterologous expression of biosynthetic gene clusters are synthesized by a living cell to create thalasosspiramides[1]. With the isolated plasmid, the introduction of the integrase intB13 allows the plasmid to reintroduce itself into the genome with specified borders through PCR-targeting. The introductions occur within different strains of *E.coli* JM109, NEB10β, and BW25113 cells. The amplification of the intB13 gene was successful in all strains, and colonies grew. However, when screened, the intB13 PCR product did not integrate where expected. Ultimately, the integration of the enhanced plasmid could lead to the heterologous expression of the biosynthetic gene clusters present in the plasmid. In turn, the organism could become a thalassospiramide producer, and those thalassospiramides can be used for medicinal and therapeutic purposes.

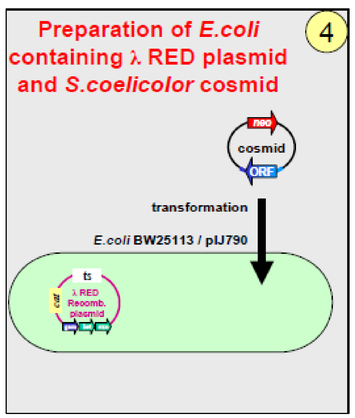
Methods

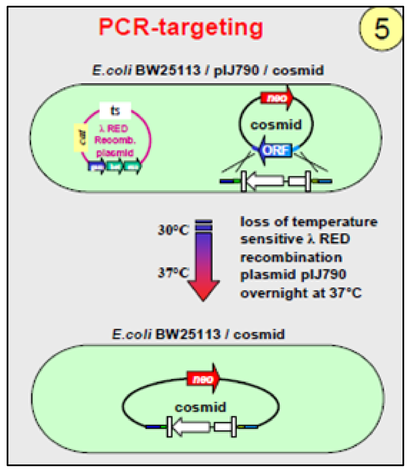




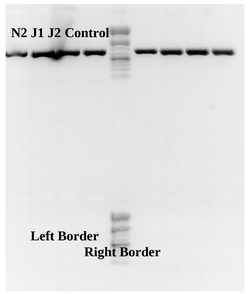
Gene disruption by PCR-Targeting[2]

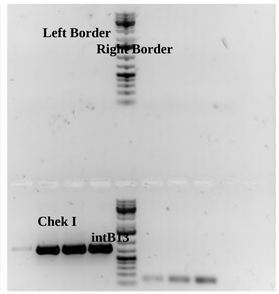






Results





To confirm that the plasmid was successfully transformed into the genome, primers are virtually designed and sent in for sequencing. They are then tested on the DNA and run on a gel to determine if the PCR product is present. The two primer sequences above represent the borders of where the plasmid is expected to have integrated. However, as seen in the next image, there was no PCR product present within those borders.

Primer Sequences

intB13-pBTttc_3607-F
CGTTAATCGAATGTATATCTATTTAATCTGCTTTTCT
TGTTGCTGGGTGTGGGTCTATGG

intB13-pBTttc_3607-R
TCCGAGCTGGCGTAACAGATGAGGGCAAGCGGATG
GCTGAATGCGCTCACGCAACTGGTC

intB13-pBTttc_LB_753-F
GCAGCGCATCGCCTTCTATC

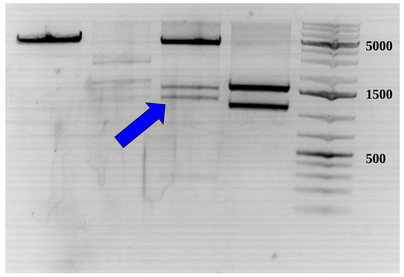
intB13-pBTttc_LB_753-R
AAGCCGCCTCACCCGTATTC

intB13-pBTttc_RB_664-F
CCCGCATGGATTTGACTTGG

intB13-pBTttc_RB_664-R
CGTTGGAATGCGGAGTAGAG

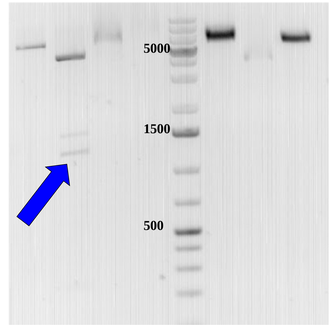
When the primers were run through a PCR, the bp readings did not show any indication that the plasmid had integrated within the borders. Due to this new and unsuspected information, another PCR was performed, but this time with Chek I primers: these primers indicate whether or not the plasmid integrated. The Chek I primers came back positive, with bp readings above 500. In addition, intB13 was run to tell if it was present within the genome.

BW25113/JM109/PKD20 organisms



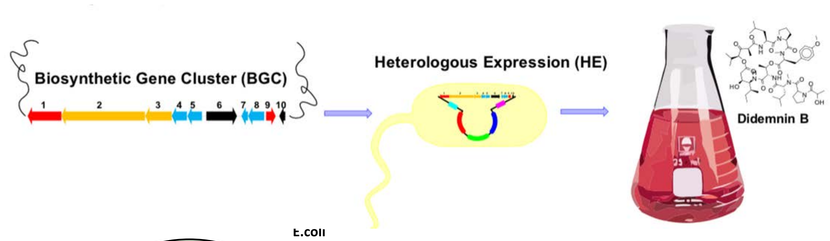
Within the PIJ790/PKD20 strain, there appeared to be an extra band of DNA that was not supposed to be there. Although none of the bp readings of the gel were completely accurate, the extra band was out of ordinary. There was a possibility that the plasmid was contaminated by foreign DNA.

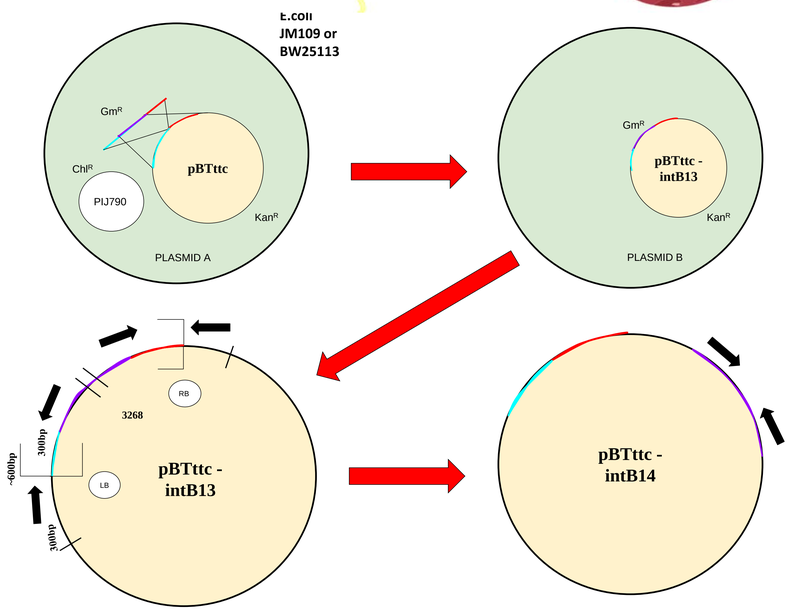
PKD20 digested with BAM1, ECOR1, NCO1, and PST1 enzymes



In order to figure out why there was an extra band present within the PIJ790/PKD20 strain, PKD20 was run through another PCR: this time, however, with four different enzymes. Enzymes cut the DNA differently, and after the gel was run once again, the extra band was still present within the ECOR1. This rules out the possibility that there was a contamination of foreign DNA.

Heterologous Expression of Natural Products





Discussion and Conclusions

The PCR-targeting to the intB13 integrase gene into the pBTttc plasmid was successful. This was confirmed through PCR analysis. However. it appears that the integrase cassette inserted at an unknown place because the plasmid could not be amplified with the primers for the borders on the plasmid backbone and with the second primer in the integration cassette. This unexpected results lead to a hypothesis that the integrase *was* transferred into the plasmid but in a different location. Currently, we are trying to determine where the integrase was inserted in the plasmid and we are attempting to conjugate the plasmid into the host organism, *Pseudomonas putida.* Ultimately, our goal is to be able to genetically modify this Pseudomonas strain to produce the thalassospiramides in order to better study these biomedically important molecules and possibly make new anti-cancer drugs.

References

1. Ross AC, *et al.* (2013) Biosynthetic multitasking facilitates thalassospiramide structural diversity in marine bacteria. *J Am Chem Soc* 135(3):1155-1162.

2. Gust B, Kieser T, & Chater K (2002) PCR targeting system in Streptomyces coelicolor A3 (2). *John Innes Centre* 3(2):1-39.

Acknowledgments

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