5-2022

RNA-Protein Interactions Influencing Ribosomal Subunit Assembly

Caroline Curll
cecurll@gmail.com

CAROLINE CURLL

(Cell & Molecular Biology: Biochemistry)
RNA-Protein Interactions Influencing Ribosomal Subunit Assembly

Sponsor: Steven Gregory (Cell & Molecular Biology)

The ribosome is an essential component of the cellular gene expression machinery, and its assembly from some 50 proteins and large RNA molecules remains only poorly understood. This project entailed reviewing past research investigating ribosomal subunit assembly and the nature of RNA-RNA and RNA-protein interactions involved in this process. After researching the scientific literature on the topic of ribosome assembly, I used the software program PyMOL to view crystal structures of the ribosome and identify higher-order interactions that might be important for ribosome assembly. Specifically, after consulting my sponsor, I chose to look at interactions between ribosomal protein uS15 and 16S ribosomal RNA (rRNA). The experimental aspect of my project entailed creating a gene knockout mutant of the model bacterium *Thermus thermophilus*. This was done in two phases. First, the region of DNA flanking the *rpsO* gene (encoding ribosomal protein uS15) was made using PCR. Gibson Assembly was then used to rapidly insert these DNA fragments into the *E.coli* cloning vector pUC18. This included a drug-resistance marker replacing the uS15 coding sequence. From there, this plasmid with the knockout of the *rpsO* gene encoding region of DNA was used to transform *E.coli* where it was allowed to grow on agar plates. The knockout plasmid was then sequenced to confirm the correct structure of the plasmid. Next, plasmid was used to transform *Thermus Thermophilus*, and genomic DNA was sequenced to confirm the successful deletion of *rpsO*. The knockout mutant was characterized for growth and ribosome assembly.