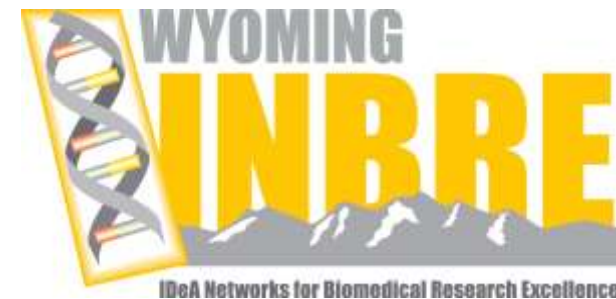
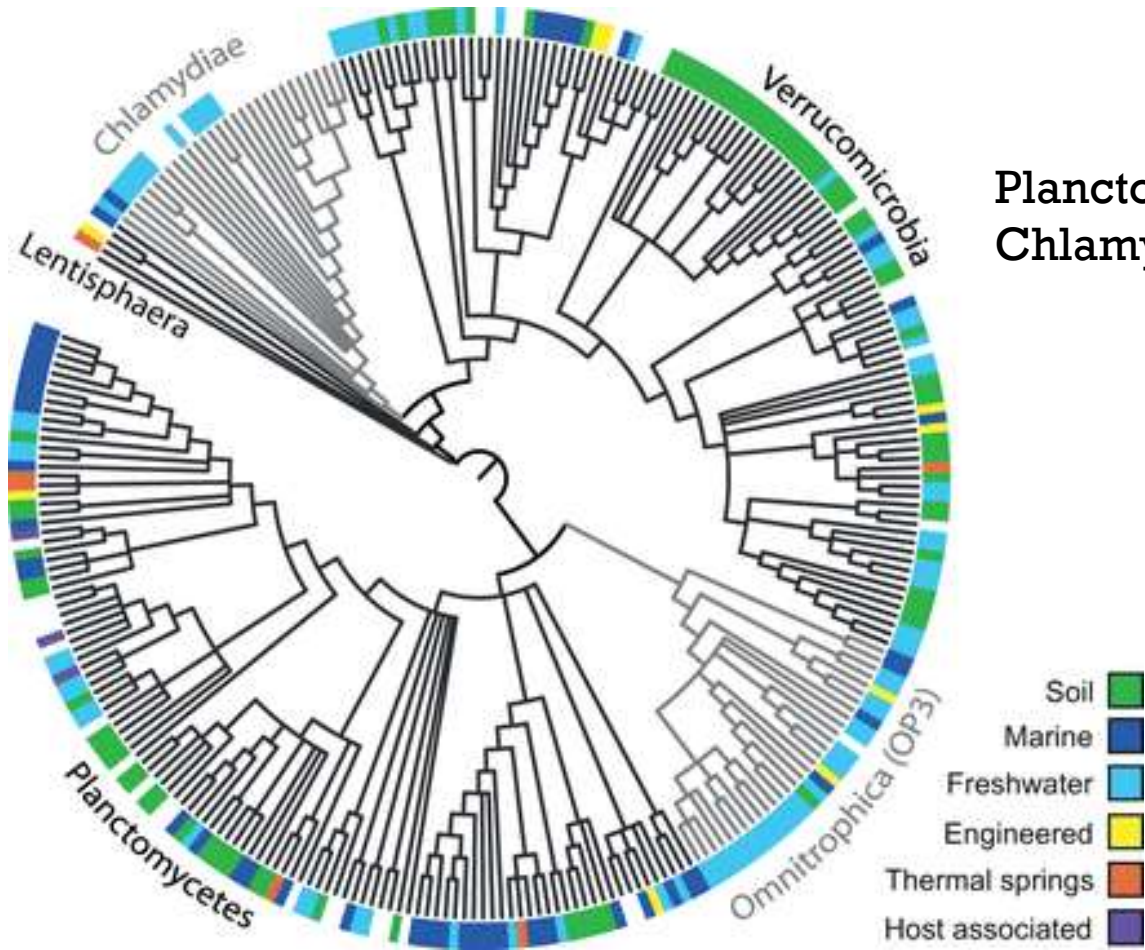


Exploring Sterol Function in Gemmata Obscuriglobus

By: Emily Armitage



+ PVC Superphylum



Planctomycetes, Verrucomicrobia,
Chlamydiae superphylum

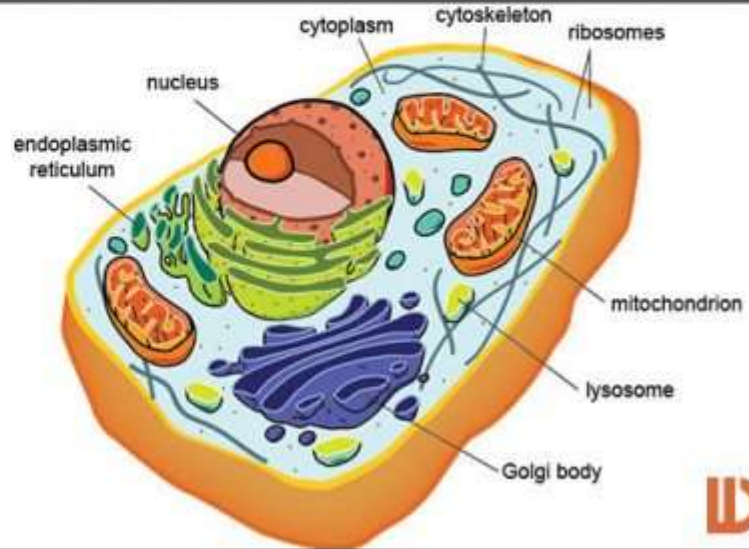
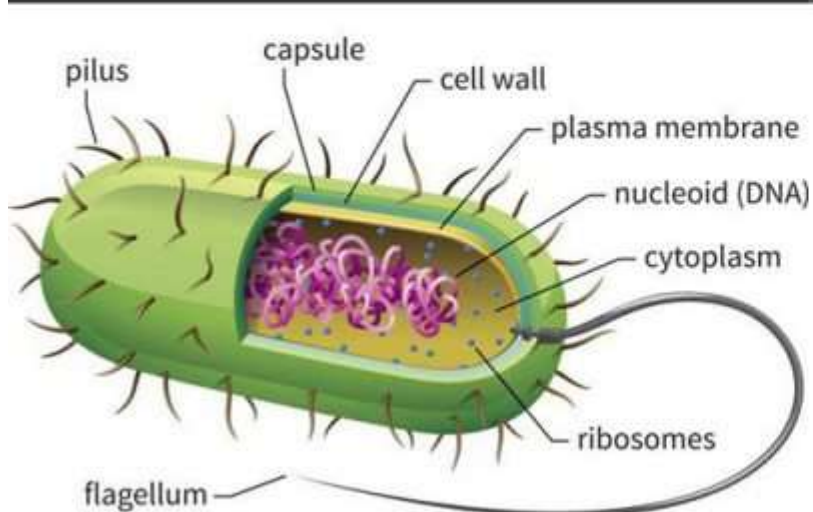
+ Background



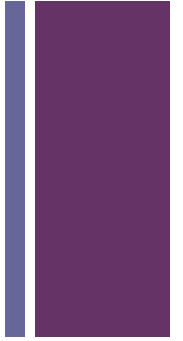
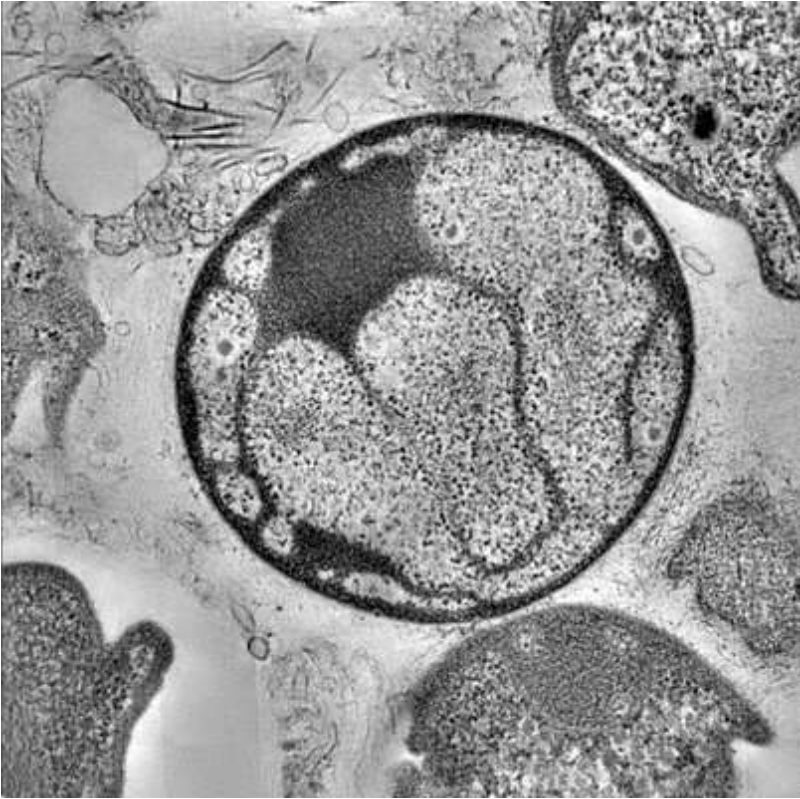
PROKARYOTIC CELL

VS

EUKARYOTIC CELL



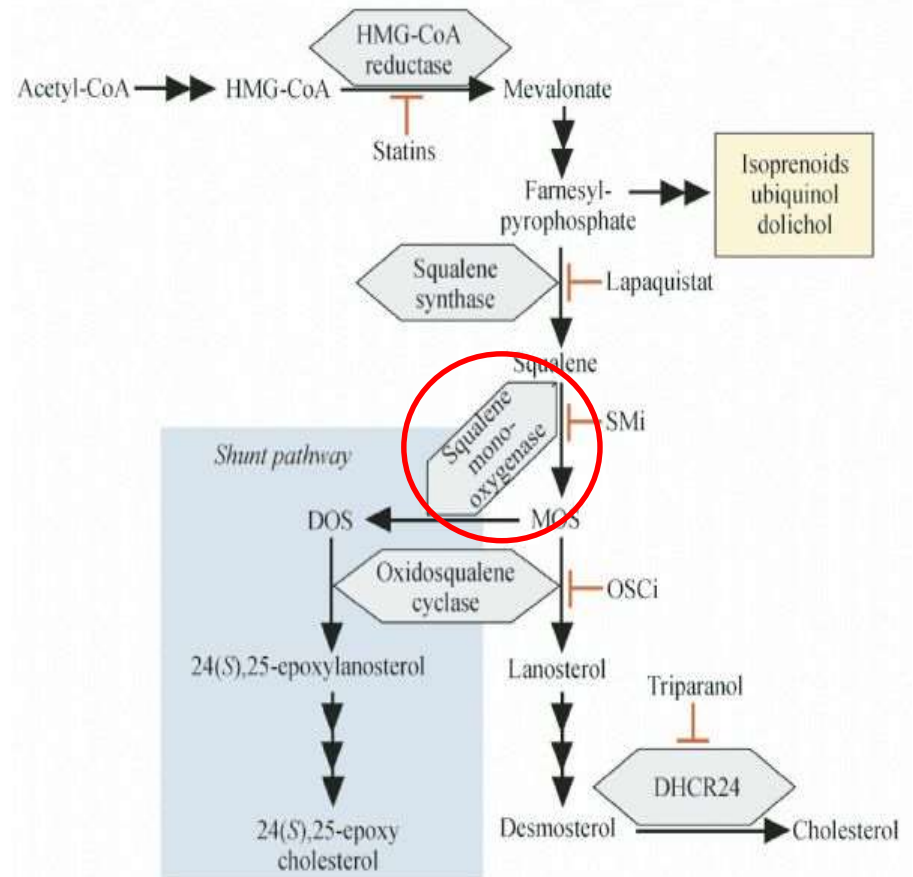
+ *Gemmata obscuriglobus*



+ Question

- Sterol depletion by terbinafine causes severe cellular replication defects, suggesting sterols are necessary for survival, the first instance in a bacterium.
- What is the function of sterols in *G. obscuriglobus*?

Sterol Synthesis:





+ Methods



- We are conducting **RNA sequencing** on treated vs. untreated cells to determine the global effect of sterol depletion on gene expression.
- Conduct **quantitative real-time polymerase chain reactions** on the best hits to verify the RNA sequencing results
- Utilize **Triparental mating** to further elucidate function of the confirmed RNA sequencing best hits in response to sterol depletion

+ RNA purification

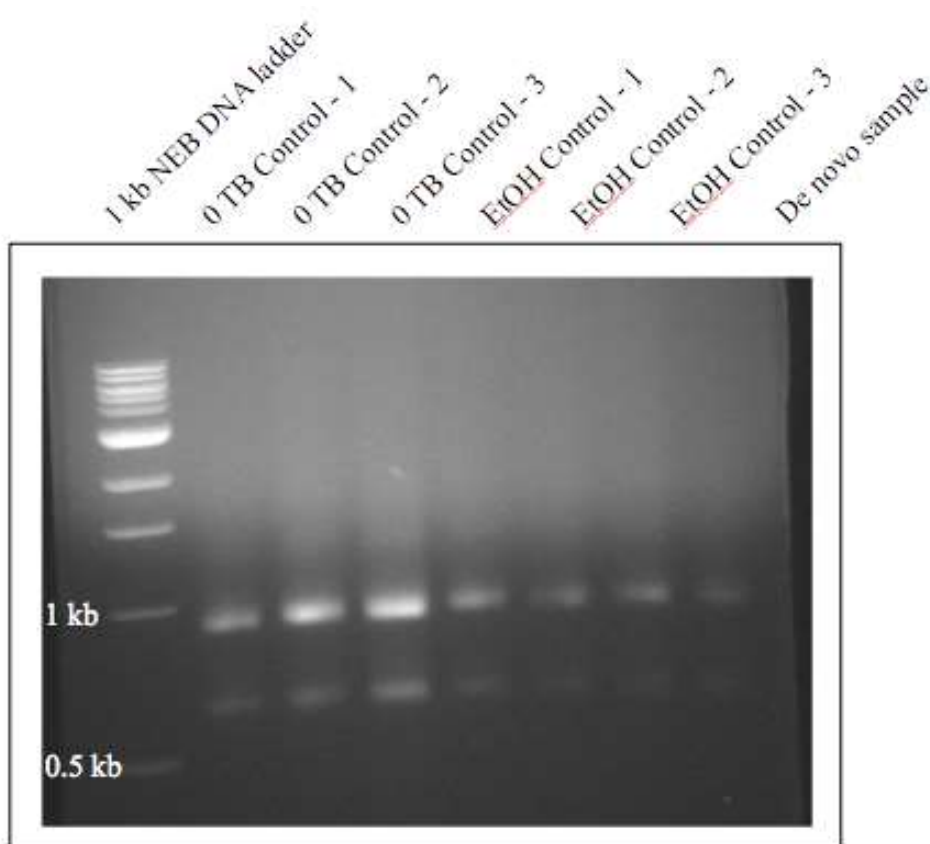
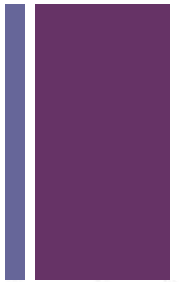


Figure 3: Non-denaturing gel electrophoresis to determine RNA presence and quality. Samples include the untreated control, the ethanol vehicle control, and the sample representing all possible transcriptomes for *de novo* transcriptome synthesis. The upper band represents the 23S rRNA and the lower band represents the 16S rRNA.

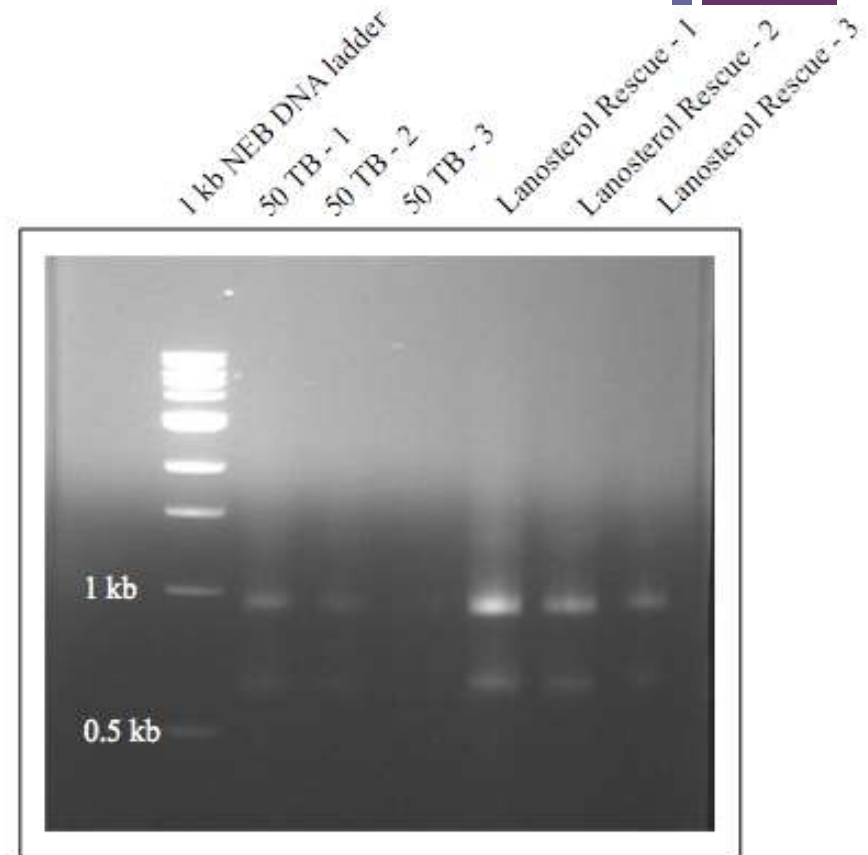
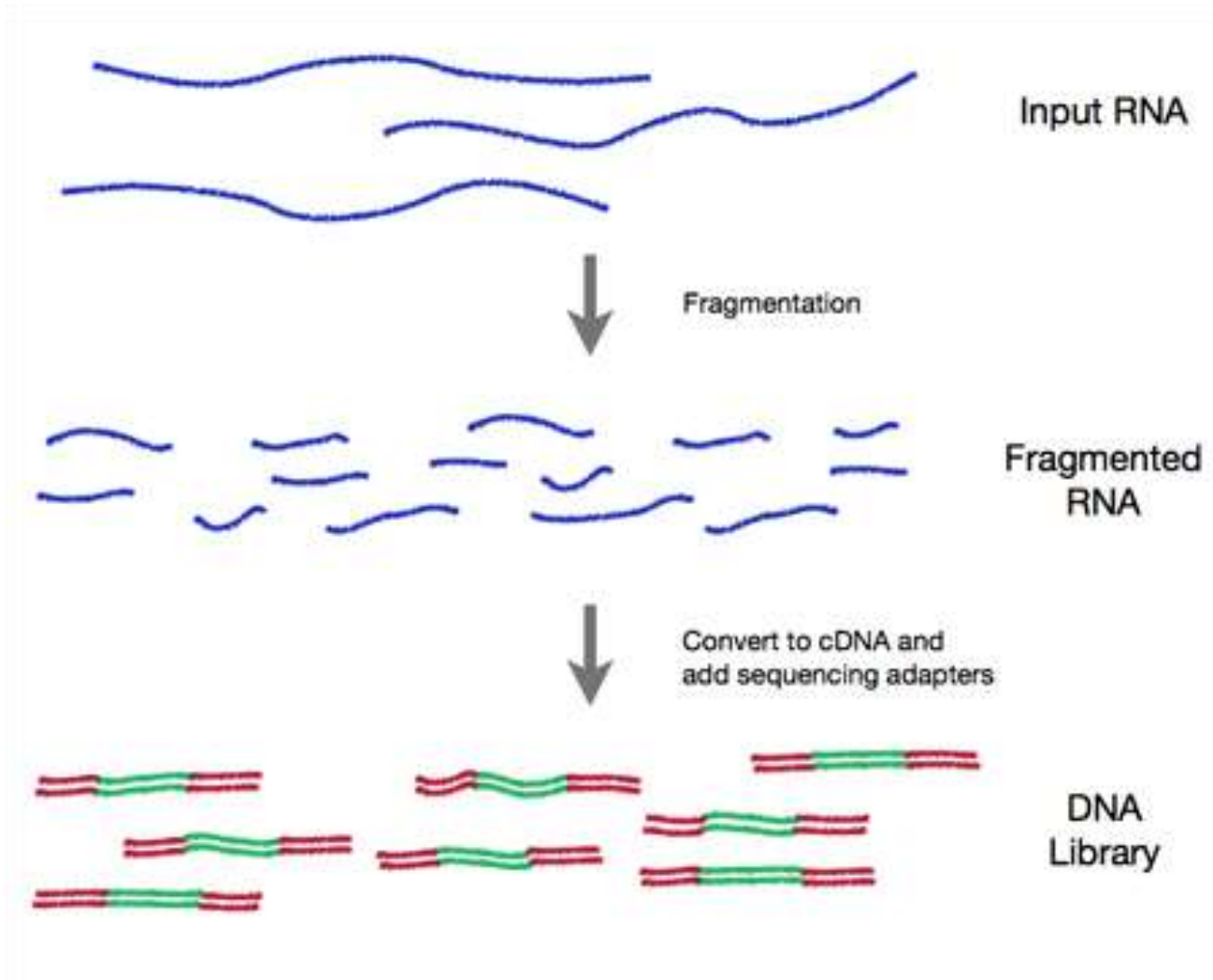


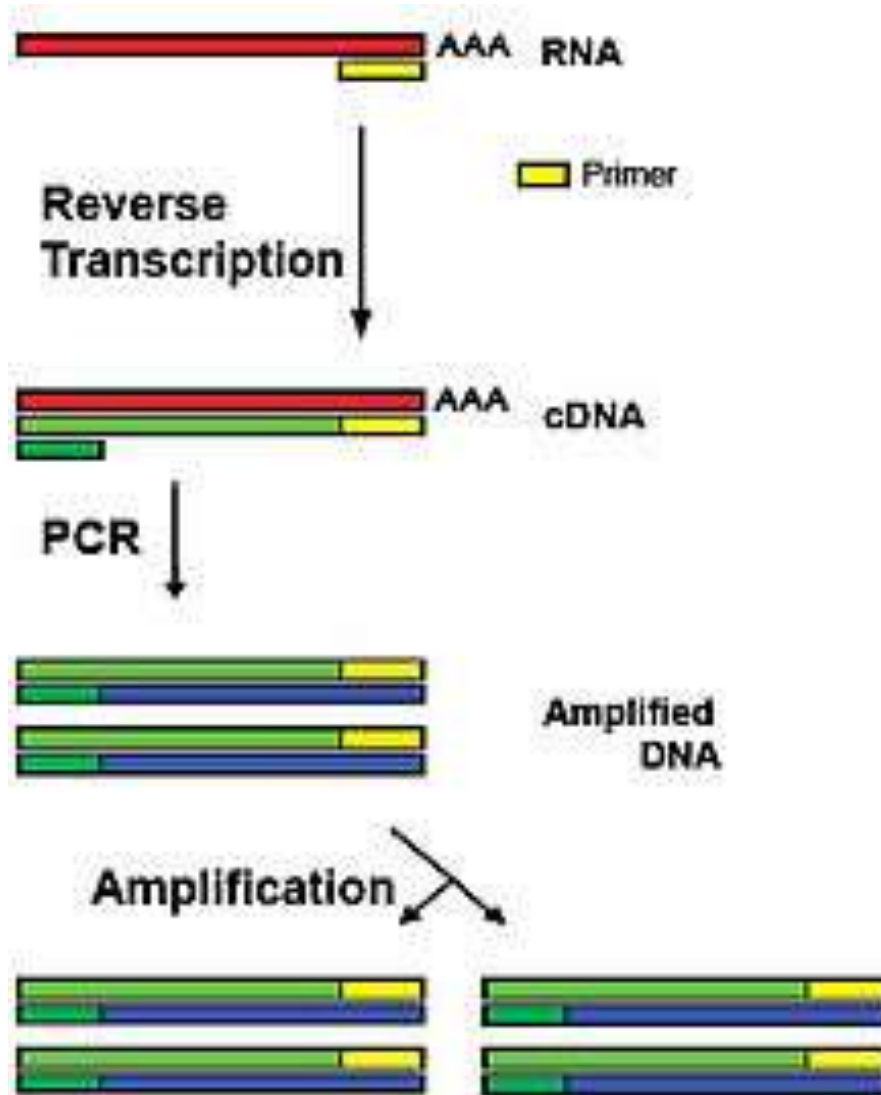
Figure 4: Non-denaturing gel electrophoresis to determine RNA presence and quality. Samples include the terbinafine treated sample (50 $\mu\text{g}/\text{mL}$) and the lanosterol rescue samples (50 $\mu\text{g}/\text{mL}$ terbinafine and 1 $\mu\text{g}/\text{mL}$ lanosterol). The upper band represents the 23S rRNA and the lower band represents the 16S rRNA.



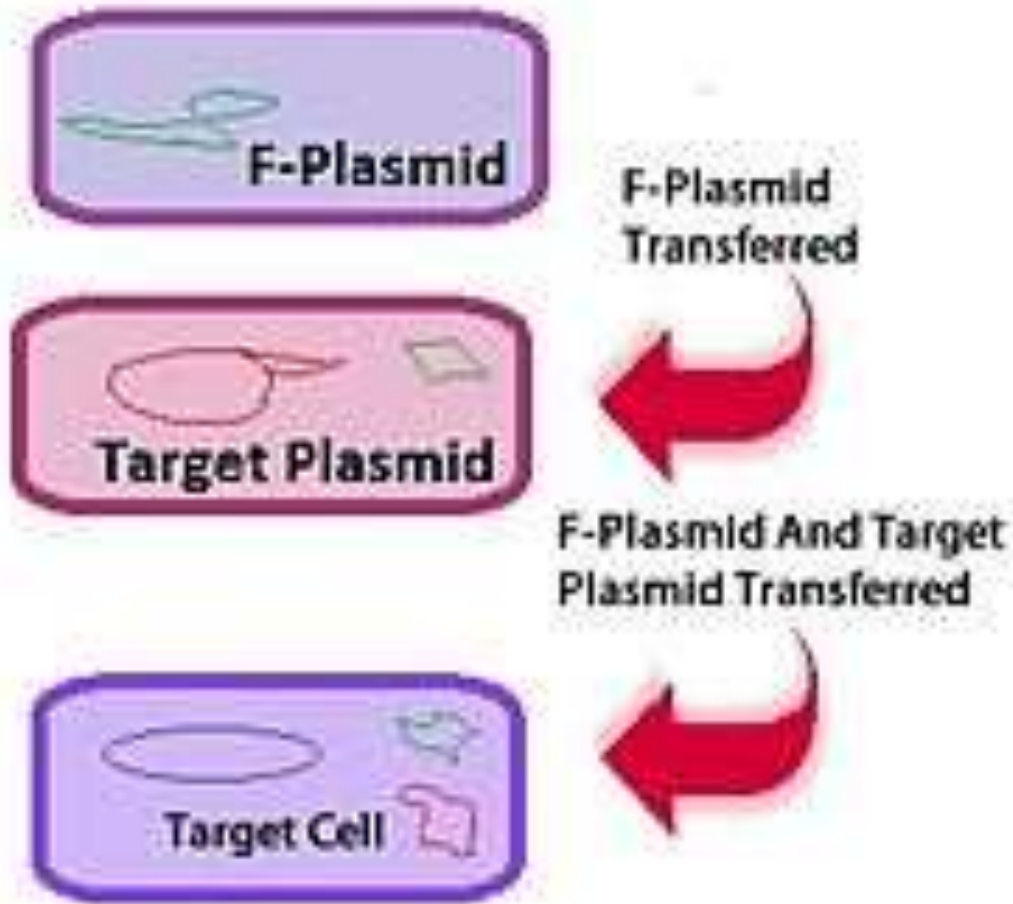
+ RNA Sequencing



+ Verify hits with qRT-PCR



+ Triparental Mating





Possible Results



- A possible gene from the RNA-seq analysis: gene encoding squalene-hopene cyclase
- It is a necessary gene in the production of bacterial hopanoids, and *G. obscuriglobus* seems to have the capacity to synthesize both sterols and hopanoids.
- The protein cyclase could be attempting to mitigate the effect of sterol depletion. Other lipid-modifying enzymes that may be attempting to mitigate the membrane effects of sterol depletion could come from the analysis as well.



Works Cited



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