

Transfection of a Marine Alga: Examining Lipid-DNA Interactions

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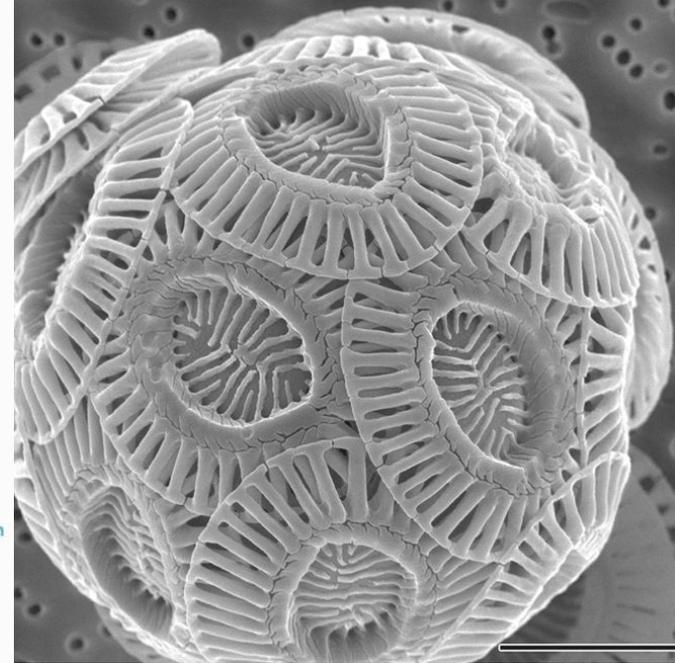
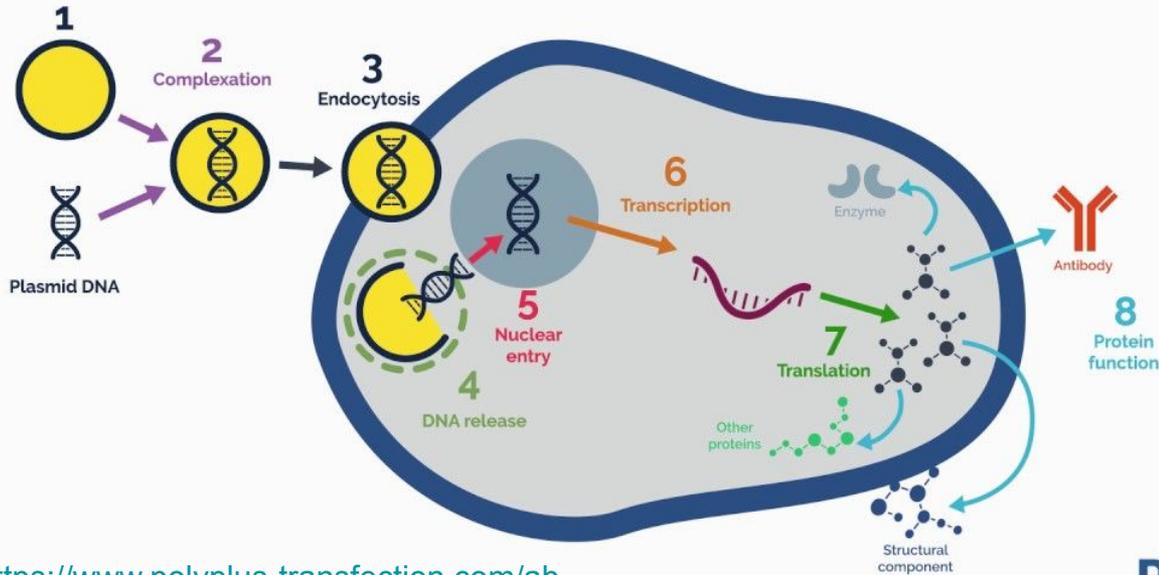
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The Overall Goal: Transfection of *Emiliana Huxleyi*

Chemical transfection process

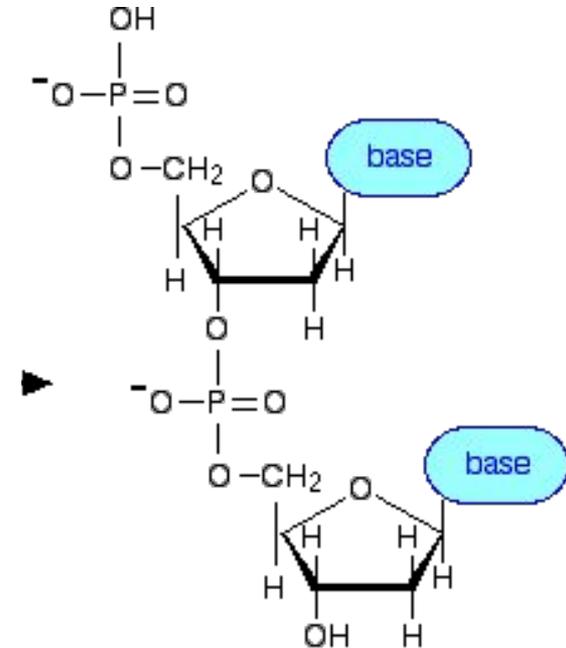
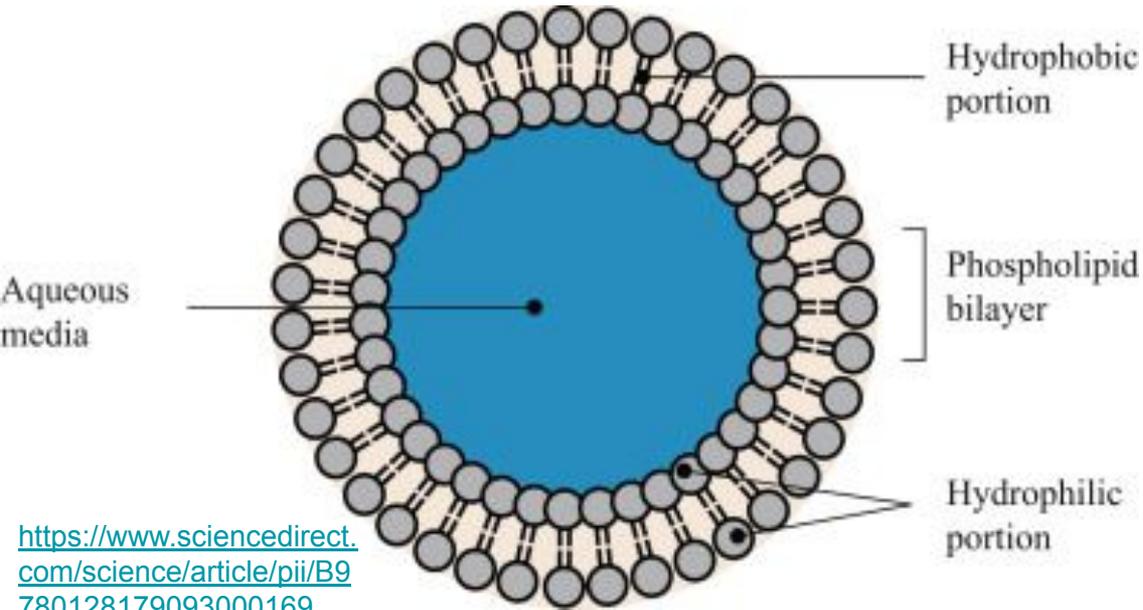


<https://www.polyplus-transfection.com/about-us/what-is-transfection/>

Polyplus
transfection⁺

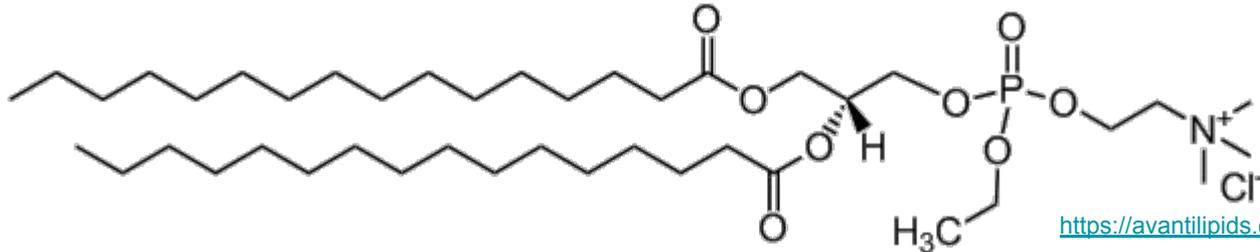
https://en.wikipedia.org/wiki/Emiliana_huxleyi

Liposome and DNA Interactions



<https://www.sciencedirect.com/science/article/pii/B9780128179093000169>

18:0 3-Ethyl Phosphocholine (EPC)

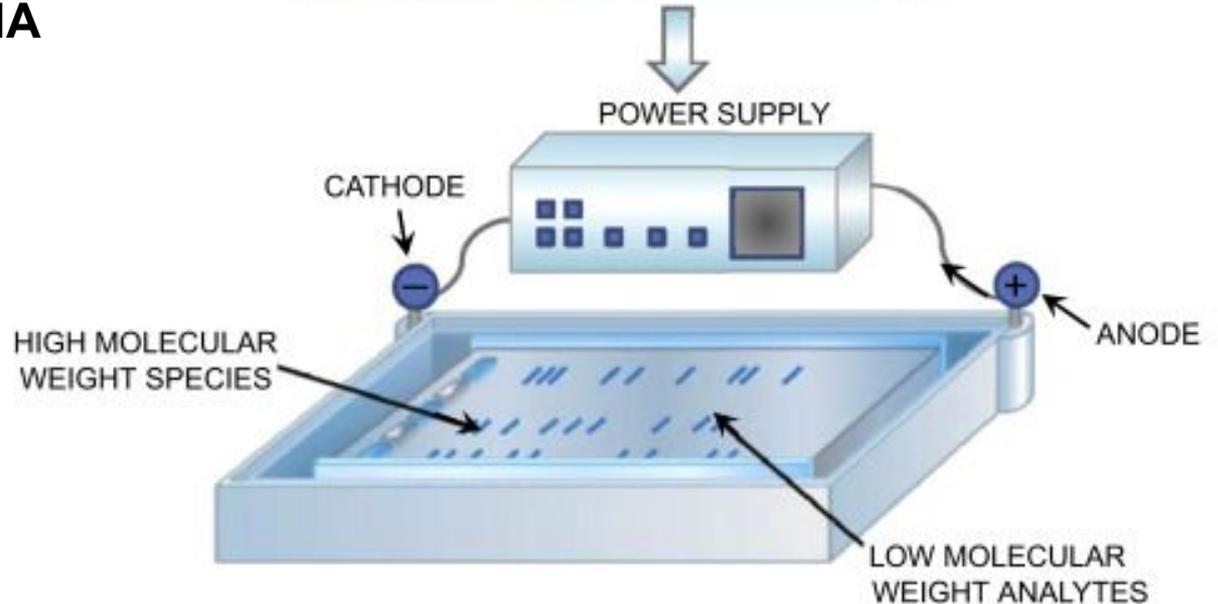
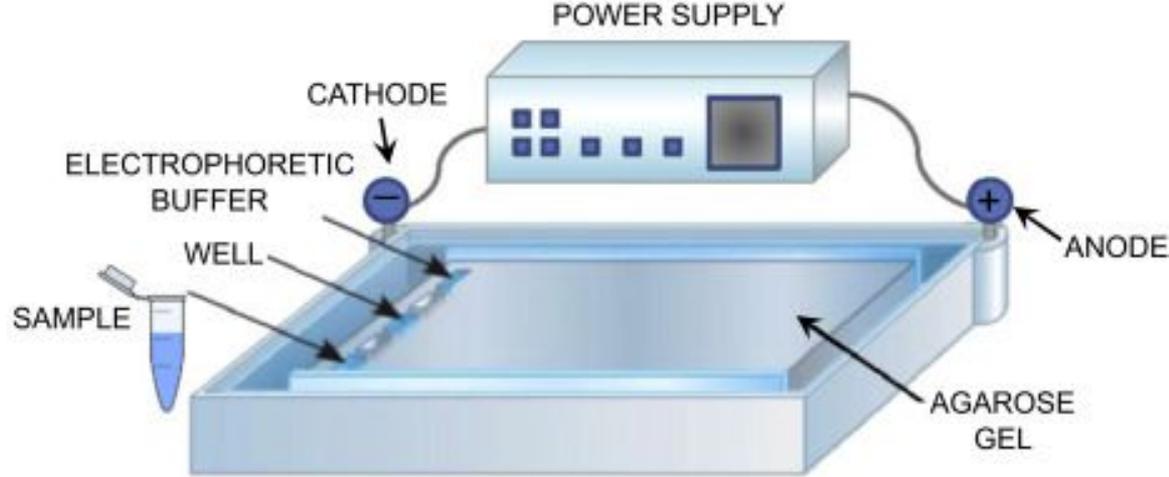


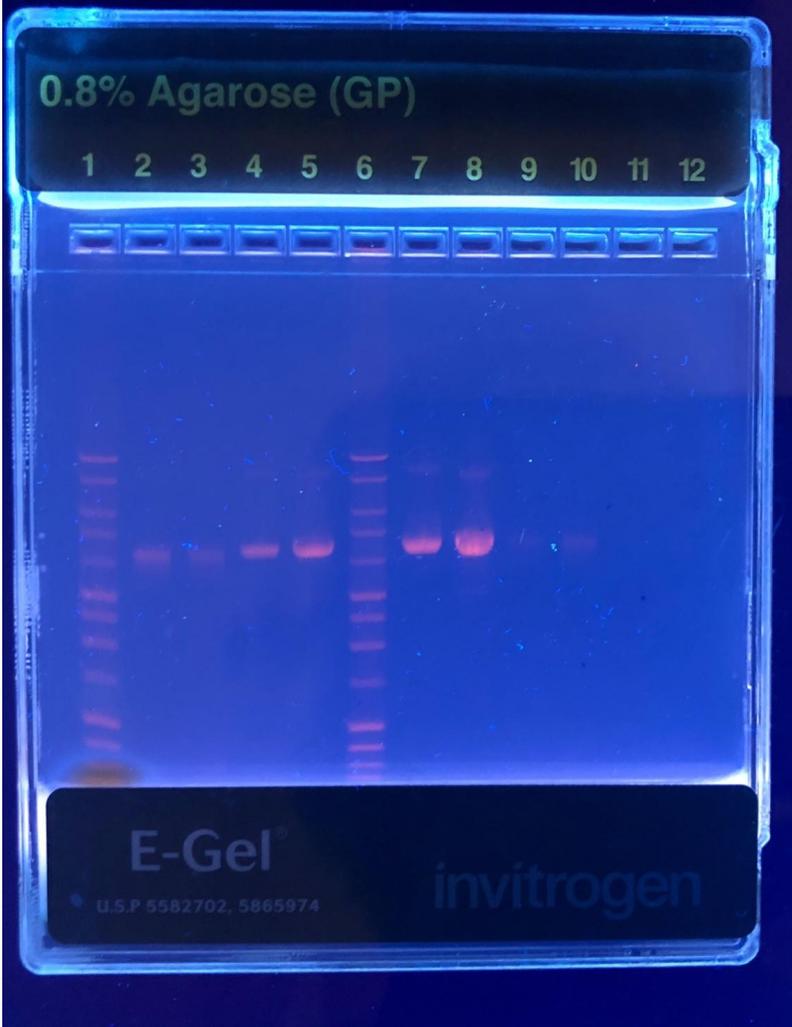
<https://www.chemguide.co.uk/organicprops/aminoacids/dna1.html>

<https://avantilipids.com/product/890704>

Experimental Approach: Examining Lipid and pDNA Interactions with Gel Electrophoresis

Main Question: Is there tight complexing between pDNA and Liposomes?





Determining the Optimal DNA Mass for Gel Electrophoresis (HH1-210325A)

Lane 1 +6: MW Ladder

Lane 2: 4 μ L of 26 ng/ μ L pDNA

Lane 3: 3 of 33 ng/ μ L pDNA

Lane 4: 1 μ L of 400 ng/ μ L pDNA after 4x Dilution

Lane 5: 1 μ L of 500 ng/pDNA after 5x fold dilution

Lane 8+9: 200 ng pDNA

Lane 10: 33 ng pDNA

Lane 11: 66 ng pDNA

Lane 12: TE Buffer (negative control)

} 100 ng pDNA total in each of these lanes

0.8% Agarose (GP)

1 2 3 4 5 6 7 8 9 10 11 12

Testing Varying Ratios of Liposome to pDNA (HH1-210401)

Lane 1: Loading Dye

Lane 2: MW Ladder

Lane 3: 100 ng pDNA (Positive Control)

Lane 4: Liposomes (Negative Control)

Lane 5+6: 1:1 Cationic Lipid:pDNA Charge Ratio

Lane 7: 2:1 Cationic Lipid:pDNA Charge Ratio

Lane 8: 3:1 Cationic Lipid:pDNA Charge Ratio

Lane 9: 4:1 Cationic Lipid:pDNA Charge Ratio

Lane 10+11: 5:1 Cationic Lipid:pDNA Charge Ratio

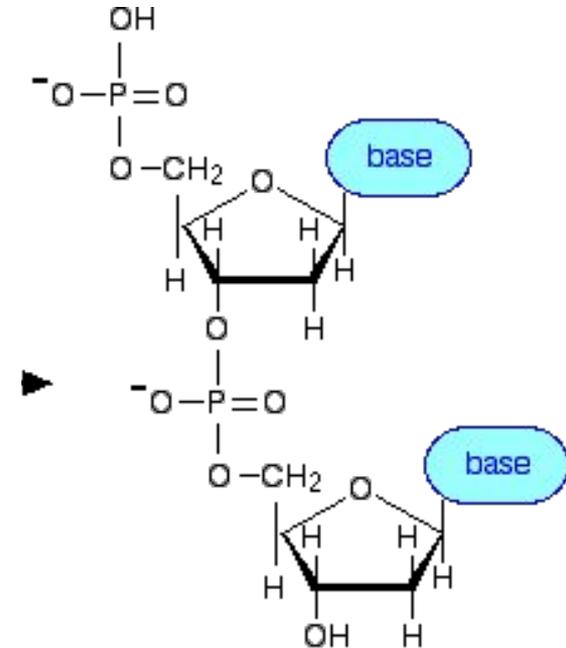
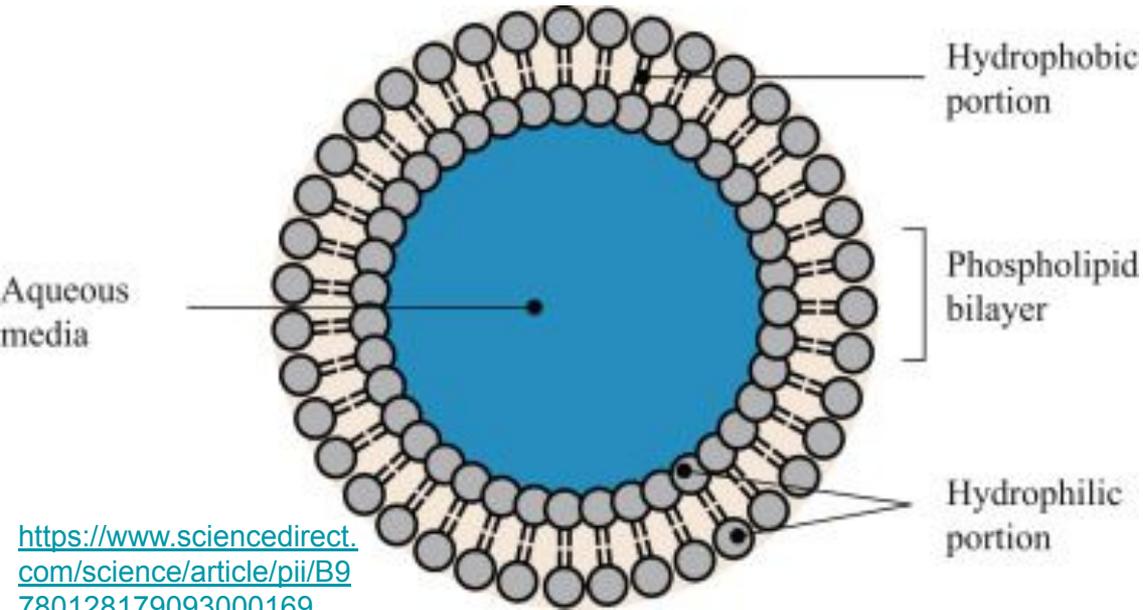
Lane 12: Negative Control

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U.S.P. 5582702, 5865974

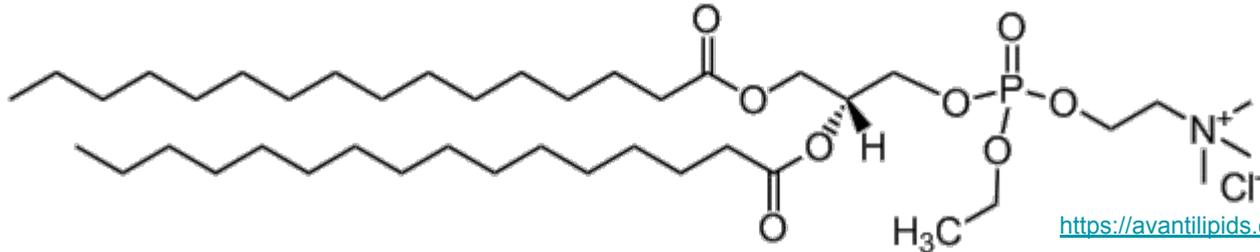
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Liposome and DNA Interactions



<https://www.sciencedirect.com/science/article/pii/B9780128179093000169>

18:0 3-Ethyl Phosphocholine (EPC)



<https://avantilipids.com/product/890704>

<https://www.chemguide.co.uk/organicprops/aminoacids/dna1.html>

0.8% Agarose (GP)

1 2 3 4 5 6 7 8 9 10 11 12

Testing Lower Charge Ratios of Liposome:pDNA Samples (HH1-210405)

Lane 2: 0.5:1 Cationic Lipid:pDNA Charge Ratio

Lane 3: 0.75:1 Cationic Lipid:pDNA Charge Ratio

Lane 4: 1:1 Cationic Lipid:pDNA Charge Ratio

Lane 5: 1.25:1 Cationic Lipid:pDNA Charge Ratio

Lane 6: 1.5:1 Cationic Lipid:pDNA Charge Ratio

Lane 7: 2.0:1 Cationic Lipid:pDNA Charge Ratio

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Figure 7- Examining Fluorescent Lipids (0.2% Oregon Green) (HH1-210415)

Tested Fluorescent Lipids and 250 ng pDNA per lane

Lane 1: MW Ladder

Lane 2+8: 0.5:1 Cationic Lipid: pDNA Charge Ratio

Lane 3+9: 0.75:1 Cationic Lipid: pDNA Charge Ratio

Lane 4+10: 1:1 Cationic Lipid: pDNA Charge Ratio

Lane 5+11: 1.5:1 Cationic Lipid: pDNA Charge Ratio

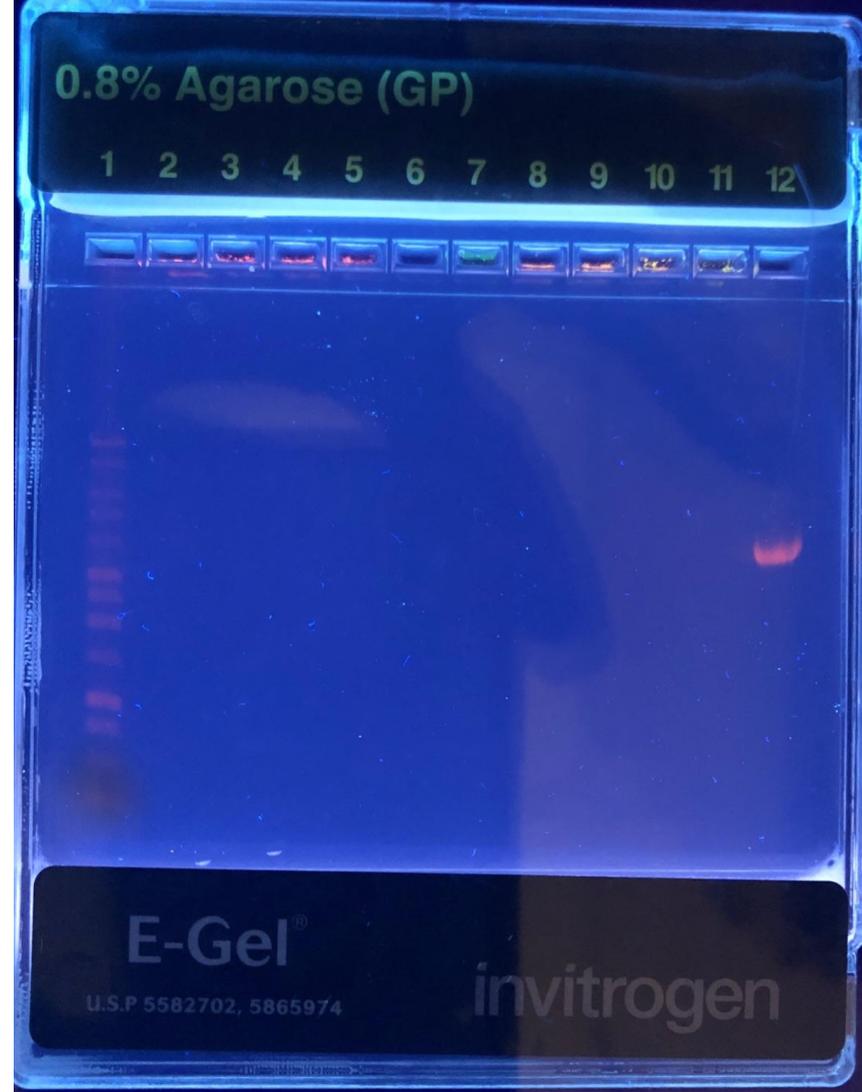
Lane 6: Liposome (Negative Control)

Lane 7: Fluorescent Liposome (Negative Control)

Lane 12: 250 ng pDNA (Positive Control)

Interesting Findings

- No Changes with Fluorescent Lipids
- Fluorescence can be seen in some of the wells
- Presence of Bands right outside of the wells



Summary of Findings

- Ethidium Bromide in our gels is sensitive to 100 ng pDNA for optimal results (tight, visible bands)
- Tight liposome and pDNA binding was seen for a broad range of ratios, ranging from 0.25:1 cationic lipid:pDNA to 5:1 cationic lipid:pDNA ratios
- Fluorescent liposomes did not differ significantly in binding pDNA as compared to non-fluorescent liposomes.

Interesting Questions

- What is the size of the liposome-pDNA complexes?
- How much DNA is present in the visible bands?
- Is Ethidium Bromide a sensitive enough gel to detect lower levels of pDNA migration?



Potential Next Steps

- Cast our own gels with lower agarose concentration and analyze by Flow Cytometry
- Excise bands and dissolve in solution. Remove liposomes with detergent. Densitometry.
- Cast our own gels and utilize other DNA dyes such as GelRed

Acknowledgements

- The Hirsh Research Group: Fizzah Ehsan and Bianca Asaro (Liposome Preparation), Shreya (Plasmid DNA Preparation), Chris Wu (Gel Electrophoresis)
- TCNJ Department of Chemistry Staff: Pam Schmierer and Marc Brescia

Questions?