Can Liposomes be Used to Transfect Tetrahymena Thermophila for CRISPR/Cas9 Studies?

Heather G. Kuruvilla
Cedarville University, heatherkuruvilla@cedarville.edu

Arianna Bland
Cedarville University, abland@cedarville.edu

Miki Fath
Cedarville University, mikifath@cedarville.edu

Caleb Faul
Cedarville University, cfaul@cedarville.edu

Abigail Hall
Cedarville University, abigailhall@cedarville.edu

See next page for additional authors

Presenters

Heather G. Kuruvilla, Arianna Bland, Miki Fath, Caleb Faul, Abigail Hall, Samuel Johnson, Sarah Kinder, Katarina Mills, Abigail Misselbeck, Chukwuemelie Ojukwu, Nathan Spottswood, Emily Wachter, Ethan Wang, and Samantha Wolff

Progress toward a "peaceful" protocol for transfection of *Tetrahymena thermophila* with plasmid DNA

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Abstract

CRISPR-Cas9 editing is a powerful tool for making genome changes in organisms. The free-living ciliated protozoan, *Tetrahymena thermophila*, has been used for decades as a model system for eukaryotic genetic and epigenetic phenomena. Because of this, there is a great deal of interest in adapting the CRISPR/Cas9 system to *Tetrahymena*. Plasmids containing the gene for Cas9 nuclease are currently available for transfection into *Tetrahymena*. However, the transfection protocols which are currently used in this organism, electroporation or biolistic transformation using a gene gun, are both expensive and inefficient. We are attempting to use liposomes in order to transfect *Tetrahymena* with a gene for the Cas9 nuclease. Our hope is to develop a protocol that would allow for a more efficient, less expensive transfection platform, facilitating further development of CRISPR-Cas9 editing in this system.

Conclusions

- *Tetrahymena* exposed to plasmid in the presence of $CaCl_2$ do not take up and express the plasmid: no Cas9 expression was seen under these conditions.
- Neither $CaCl_2$-containing buffers nor liposomes are toxic to *Tetrahymena*.
- **Liposomes appear to allow transfection of plasmid DNA into *Tetrahymena*** (Figure 4).
- **Transfection and total DNA extraction needs to be repeated alongside a control containing plasmid DNA and *Tetrahymena* in the absence of liposomes** in order to **confirm that DNA has entered the cells** and is not merely associating with the membrane**.**

Acknowledgements

- We would like to thank Dr. Douglas Chalker and the *Tetrahymena* Stock Center for providing information about the pC9T plasmid and designing guide RNAs in this organism.
- We would like to thank Zymo Research for providing samples of competent cells and FuGENE, Polyplus, LifeSct, EZ Biosystems, and ThermoFisher Scientific for providing the samples of liposomes that we used in our transfection experiments.
- We would like to thank Dr. Tracy Collins for PCR reagents and Dr. Kaleb Pauley for helping us program the PCR instrument.

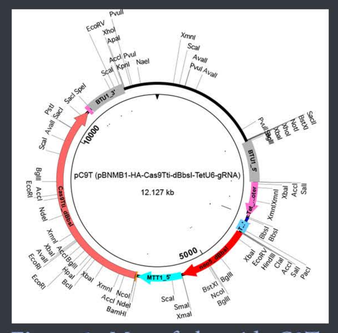


Figure 1. Map of plasmid pC9T

Figure 2. *E. coli* transformed with pC9T plasmid

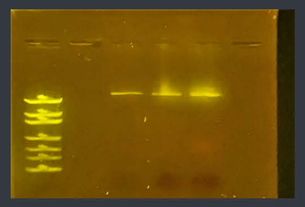


Figure 3. Purified plasmid from transformed *E. coli.*

Purchased plasmid pC9T from *Tetrahymena* Stock Center containing genes for Cas9, ampicillin resistance (for selection in *E. coli*) and paromomycin resistance (for selection in *Tetrahymena*)

Transformed *E. coli* with the pC9T plasmid and grew transformants in liquid culture to amplify DNA

Purified DNA from transformed bacteria and attempted to transfect *Tetrahymena* with it using either endocytosis (Table 1) in buffer, or liposomes (Table 2)

Table 1. Unlike prokaryotes, *Tetrahymena thermophila* do not take up DNA when exposed to plasmid in the presence of $CaCl_2$

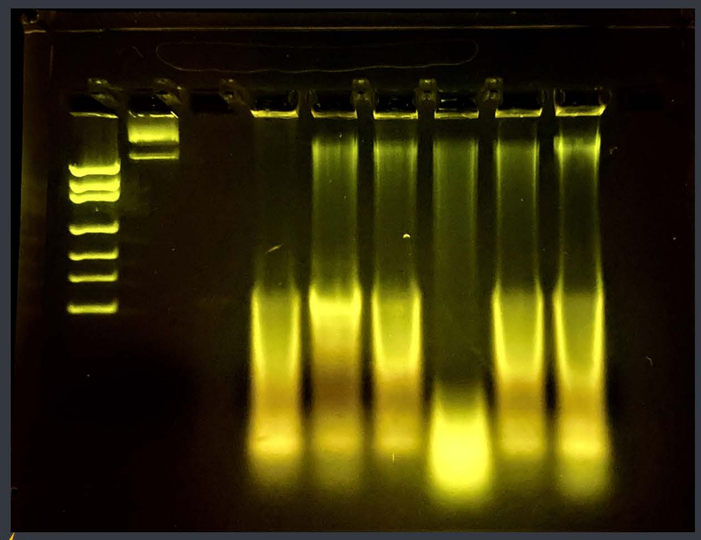
Buffer	Viability after 24 hours in buffer + DNA	Western Blot Results
Behavioral Buffer (contains 1 mM $CaCl_2$, pH 7.0)	Approximately 100%	Negative for Cas9 expression
BIORAD™ Transformation buffer (contains 50 mM $CaCl_2$, pH 6.1)	Approximately 100%	Negative for Cas9 expression
Yeast Transformation Buffer from The ODIN (contains surfactant)	No survivors	N/A

- **Viability (Table 1 and Table 2) was assayed by observing cell motility under a compound microscope**
- **Presence of plasmid DNA in *Tetrahymena* (Table 2) was suggested by gel electrophoresis (Figure 4)**

Table 2. *Tetrahymena thermophila* appear to take up plasmid DNA when transfected in the presence of liposomes. Paromomycin resistance was used to select for transfectants. A DNA band the size of the pC9T plasmid was seen in whole cell extract taken from transfected *Tetrahymena* (Figure 4).

Liposome	Viability after 24 hours in Liposomes alone	Paromomycin Selection 1 mg/ml	Paromomycin Selection 0.1 mg/ml	Plasmid DNA seen in DNA extract from *Tetrahymena*
Penefect™	Approximately 100%	No survivors after 24 hours	Cells are alive after 24 hours	Plasmid band seen
jetOPTIMUS™	Approximately 100%	No survivors after 24 hours	Cells are alive after 24 hours	Faint plasmid band seen
Fugene4K™	Approximately 100%	No survivors after 24 hours	Cells are alive after 24 hours	Faint plasmid band seen
Lipofectamine 3000™	Approximately 100%	No survivors after 24 hours	Cells are alive after 24 hours	Faint plasmid band seen
Avalanche Everyday™	Approximately 100%	No survivors after 24 hours	Cells are alive after 24 hours	Plasmid band seen

Figure 4. Total DNA extracted from transfected *Tetrahymena thermophila*. Lane 1: DNA Ladder, Lane 2: pC9T plasmid, Lane 3: empty, Lane 4: Negative control, Lane 5: Avalanche Everyday™, Lane 6: JetOPTIMUS™, Lane 7: FuGene4K™, Lane 8: Lipofectamine 3000™, Lane 9: Penefect™. All of the liposomes appeared to be somewhat successful in getting DNA into *Tetrahymena.*



MOLECULAR BIOLOGY
CEDARVILLE UNIVERSITY

Contact: heatherkuruvilla@cedarville.edu