Development of GFP-Tagged Akata EBV Cell Line via BACmid System

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Background

Epstein-Barr Virus (EBV) is a human herpesvirus that has infected >90% of adults globally.

EBV is causatively associated with a variety of different conditions, including Burkitt's Lymphoma (BL), specific types of gastric cancer, and many nasopharyngeal cancers.

Within the body, EBV exists in two main phases: lytic and latent.

- Lytic phase: active replication characterized by production of infective viral particles (epithelial and B cells)
- Latent phase: viral DNA existing in episome within cell nucleus characterized by four distinct patterns of protein production (B cells)

The Akata strain of EBV is associated with a tumor cell line established from an individual with BL.

When inserted in plasmids alongside viral genomes, the gene encoding green fluorescent protein (GFP) serves as an effective way to track viral infections on a cell-by-cell basis, as gene expression can be observed via fluorescent microscopy.

Objective

This project aims to establish a stable, immortalized B cell line infected with GFP-tagged Akata EBV.

Project Schematic

Replication of plasmid in *E. coli*

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Collection of plasmid in solution

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Transfection of HEK-293 cells

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Induction of lytic phase

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Collection of viral supernatant

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Infection of primary B cells

Methodology

Miniprep. Minipreparations of plasmid DNA were used to verify the presence of the plasmid within the DNA collected from cultured *E. coli*. Protocol was adapted from Zhang of Texas A&M University. Plasmids cultured in *E. coli* were gifted by Lin Lab of Tulane University School of Medicine.

QIAGEN Maxiprep. Maxipreparations of plasmid DNA were used to collect large and extremely pure quantities of plasmid. QIAGEN Large Construct kit was used for maxipreps, and the protocol was provided by the manufacturer.

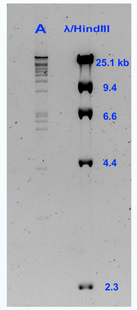
Transfection. jetPRIME transfection reagent was used to infect human epithelial cells with the collected plasmid. Protocol was provided by the manufacturer.

Induction. 12-O-tetradecanoylphorbol-13-acetate (TPA) and butyric acid induction was used to induce lytic replication within the virally-infected epithelial cells.

Results

Miniprep

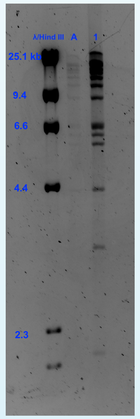
After running an EcoRI digest and performing gel electrophoresis for 12 h at 40V in a 0.75% agarose gel, the collected plasmid exhibited the exact 13-band pattern expected based upon the genomic composition of the plasmid. Spectrophotometry confirmed high concentration and purity of DNA.



	Sample 1	Sample 2
260	2.145	2.114
280	1.175	1.138
260/280	1.825	1.857
ng/μL	2145.4	2114.1

Maxiprep

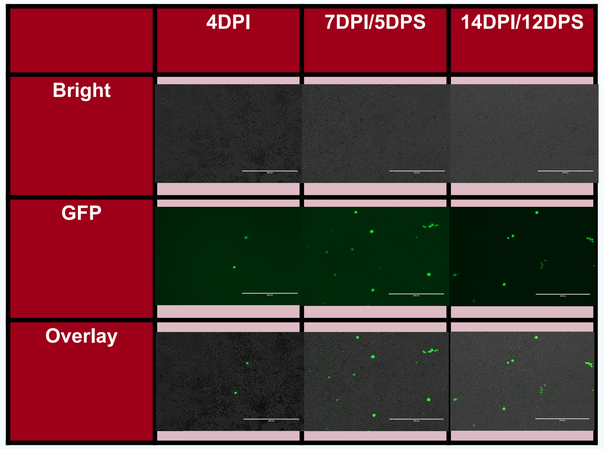
The *E. coli* cultures used for the maxipreps originated from the same colonies as those verified to be properly infected through the miniprep process. As expected, the maxipreps yielded high quantities of plasmid, the purity of which was once again measured via spectrophotometry.



	SPL1	SPL2	
1	0.041	0.038	260
	0.022	0.019	280
	1.859	2.005	260/280
	40.982	38.347	ng/μL
	SPL1	SPL2	
A	0.076	0.084	260
	0.039	0.043	280
	1.951	1.936	260/280
	75.981	83.81	ng/μL

Transfection

After transfection, HEK 293 cells were cultured in DMEM for several days before selection with G418 to eliminate non-transfected cells. As the non-transfected cells were killed and the transfected cells were allowed time to multiply, the GFP-tagged cell density increased.



Conclusion & Future Steps

Collection of the viral supernatant, verification of collected virus, and infection of primary B cells are yet to be performed.

Once this cell line is fully established, Akata EBV infections can be tracked on a cell-by-cell basis, which will allow further research to be conducted on the dynamics of Akata infection and replication.

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