Results and Discussion

Double Digestion:

L S

Nanoluc vector

L = ladder
S = sample

Our Nanoluc vector was isolated at 9,500 kb. We proceeded to use this section for DNA isolation and ligation.

Transformation and Ligation:

The plate with the ligated plasmid had significantly more colonies than the control plate due to antibiotic selection. This indicates that the ligation was successful.

Transduction and Infection:

The IFNβ-Nluc plasmid was transduced into the CHME cells, and we infected the CHME cells with a virus containing icRNA which causes the secretion of IFNβ-Nluc. This secretion allows us to measure IFN-β response through luciferase expression.

Methods

Plasmid Creation and Cloning

- Isolate the promoter from the IFIT2-Nluc plasmid using an Afe1 + BamH1 double digestion
- Use ligation to join the Nanoluc vector and the IFN-β promoter
- Use bacterial transformation to create more copies of the plasmid
- Isolate the plasmid using miniprep

Transfection + Transduction

- Transfect plasmid into HEK 293T cells to create the lentivector
- Transduce the lentivector into CHMEs
- Select for successfully transduced cells using antibiotic resistance

Because the sensitivity of the CHMEs with IFNβ-Nluc is lower than other CHME cell lines, additional studies need to be conducted to determine the most effective luciferase cell line.

References


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