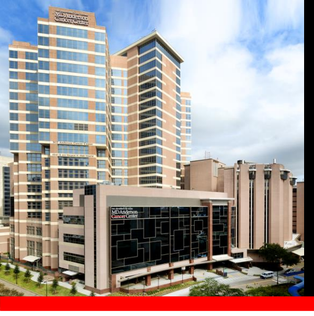

Generating Artificial Antigen Presenting Cells for Adoptive T-Cell Therapy

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Making Cancer History®

Introduction:

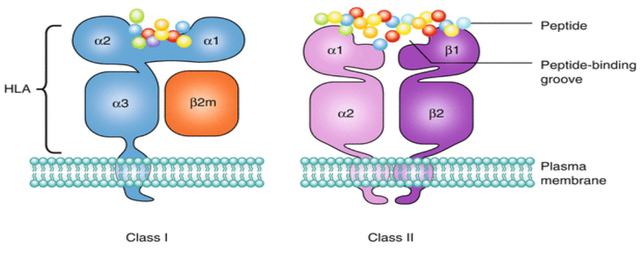
The transformation of cancers are commonly as a result of a series of hereditary and somatic DNA mutations that change healthy cells into cancerous ones.

Therapeutic cancer vaccines have potential to activate and strengthen the patient's immune system against cancer cells. For instance, the vaccination of patients with tumors can induce strong antigen-specific anti-tumor T cell responses that lead to cancer clearance.

Recent development in next generation sequencing of the human genome project paved the pathway to identify and alter genes/ mutated genes. Using bioinformatic analysis now it is possible to predict mutated peptides that can activate the immune system.

The Major Histocompatibility Complex (MHC) proteins, or the Human Leukocyte Antigens (HLA) system in humans, plays an important role in presenting these mutated peptides to the immune system.

Figure 1- Depicting HLA class I and class II with peptide binding groove (attachment that binds to T-cells and activates immune response)



Objective:

To achieve this aim, we propose to develop a cell-based tool for validating HLA binding partner of the predicted neoantigens.

We develop artificial antigen-presenting cells (APCs) by expressing HLA subtypes into HLA negative cell line K562. This will provide an opportunity to study:

(i) Peptide and HLA specific interaction and
(ii) Specific T-cell interaction with cancer peptide

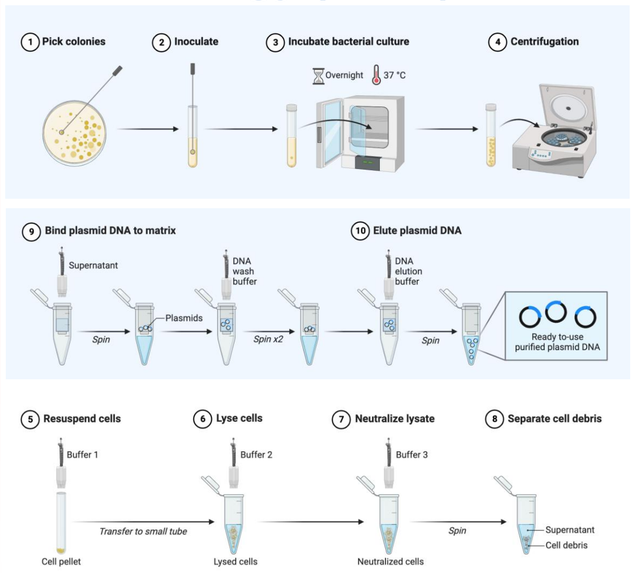
Methodology:

Bacterial Transformation:

Bacteria is mixed with plasmid DNA and briefly exposed to an elevated temperature; allowing plasmid DNA to enter the bacteria and be amplified.

- First, the bacteria is incubated with DNA on ice for 5–30 minutes in an Eppendorf tube.
- After the incubation period heat shock is performed at 37–42° C for 25–45 seconds as appropriate for e. Coli.
- Heat-shocked cells are then returned to ice for ≥2 minutes before culturing overnight prior to the Maxiprep kit.

Methodology (cont.):



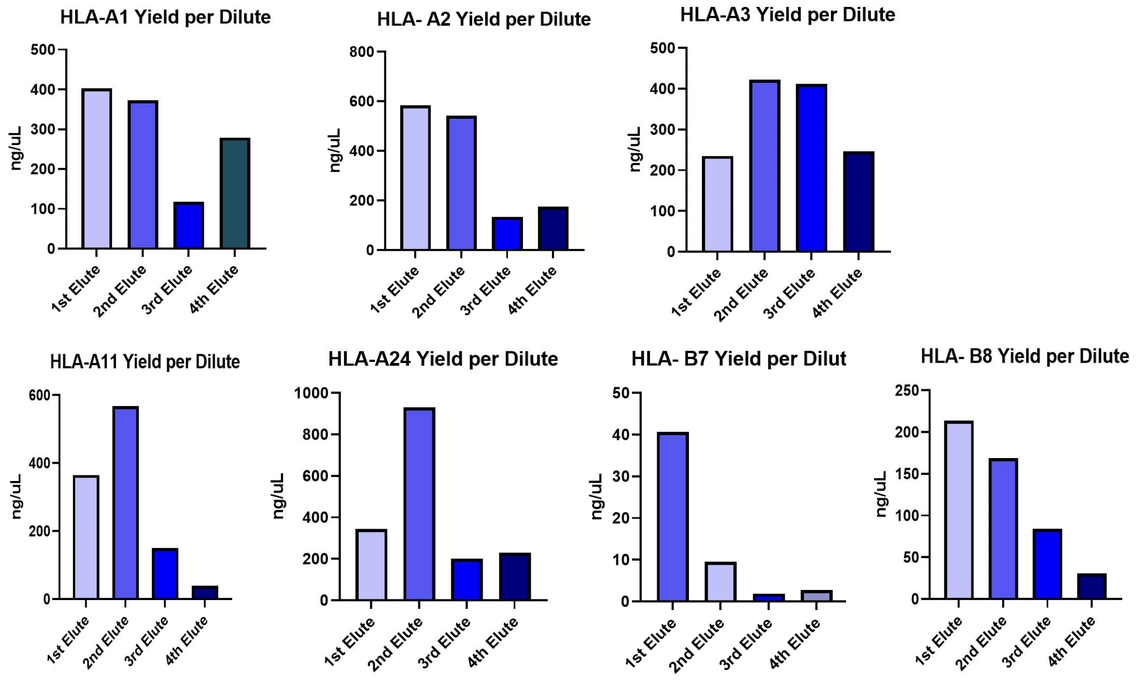
HiSpeed Maxiprep:

High Speed Plasmid Maxiprep Kit provides a simple and fast silica membrane-based method to isolate high quality plasmid DNA from large recombinant E. coli culture.

- The ultrafast purification protocol, based on the remarkable selectivity of patented QIAGEN resin, allows the isolation of ultrapure, supercoiled plasmid DNA with high yields.
- Maxi prep plasmid purification protocols are based on a modified alkaline lysis procedure.
- Followed by binding of plasmid DNA to QIAGEN resin under appropriate low-salt and pH conditions.
- Plasmid DNA is eluted using TE buffer and then concentrated and desalted by isopropanol precipitation.
- HiSpeed Tip is packed with QIAGEN resin HS and is designed to operate by gravity flow.
- DNA binding, washing, and elution steps proceed markedly, and the increased capacity enables higher yields from high copy plasmids.

Results:

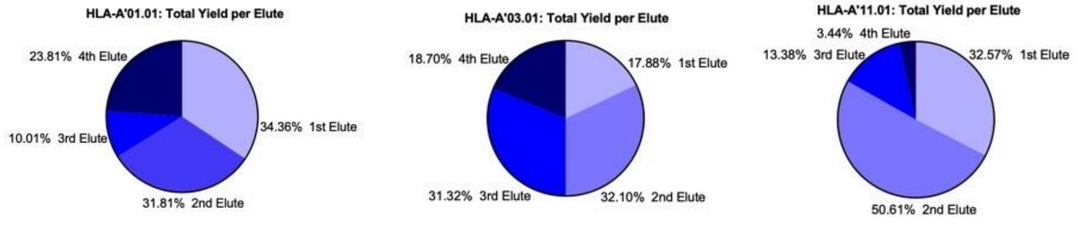
Figures below depict HLA- A1/2/3/11/24 and HLA- B7/8 yield per elute using TE buffer.



HLA Yield Per Dilute:

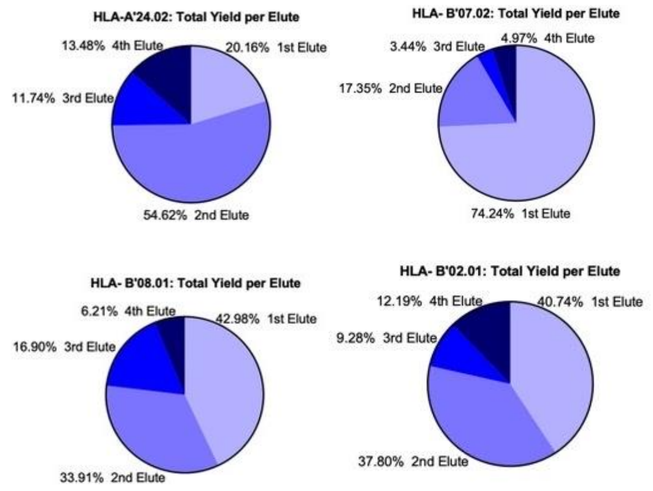
Figures HLA-A A1/2/3/11/24 and HLA- B7/8 show the concentration of purified plasmid DNA after four elutes using 500ul – 1,000ul of TE buffer. Figure HLA- A1 used 1,000ul of TE buffer, in contrast to the rest of the HLAs in which the recommended 500ul of TE buffer was used to elute the HLAs.

Figures below depict the total percentage yield per elute per HLA.



Results (cont.):

Figures below depict the total percentage yield per elute per HLA.



HLA Percentage Yield Per elute:

Figures HLA-A A1/2/3/11/24 and HLA- B7/8 show the percentage per elute for each HLA using TE buffer.

Discussion:

- We amplified 7 HLAs using bacterial transformation.
- We then isolated them and purified them using the Plasmid MaxiPrep kit.
- From the results obtained from using 500ul of the elution buffer, there were higher DNA concentrations (conc. 567-931 ul), which led to a more purified DNA concentration (average 280/260: 1.857), after using the recommended volume of the elution buffer, which signifies high purity in the samples obtained.
- However, using more than 1,000ul of the elution buffer did not stepwise increase the purity of the DNA products with significantly compromised the total yield.

Conclusions

We were able to isolate high quality plasmid DNA. These plasmid DNA will be used to express HLAs on the K562 cells to generate artificial antigen presenting cells.

References

1) Gfeller, D., & Bassani-Sternberg, M. (1AD, January 1). *Predicting antigen presentation-what could we learn from a million peptides?* Frontiers. Retrieved August 1, 2022.

2) Po*Plasmid isolation*. MyBioSource Learning Center. (2017, August 30). Retrieved August 1, 2022.