Background
Respiratory syncytial virus (RSV) is a common infection that affects most infants by the age of two, as well as a large percentage of the elderly population. For infants under one year, RSV is the most common cause of bronchiolitis and pneumonia, and each year RSV leads to about 58,000 hospitalizations and 2.1 million outpatient visits for children under five in the United States alone. On a global scale, 6.7 percent of deaths are due to complications from RSV, which is the highest fatality rate among all pathogens except malaria. Additionally, about 177,000 adults over 65 are hospitalized and 14,000 within this age group die annually in the U.S. due to the virus. Currently, the majority of prevention lies in the hands of the caretakers: wash hands frequently and before touching babies, avoid contact with babies if sick or have babies wear masks, keep young children away from babies, and avoid crowds during outbreaks. A monoclonal antibody has been approved by the Food and Drug Administration, and has shown reduced hospitalization rates of 45 to 55 percent. However, this method requires monthly injection during RSV season, requiring about five doses. In order to improve the lives of so many, this project aims to develop a vaccine for RSV by focusing on the fusion (F) glycoprotein in the trimer form. This protein is necessary for the virus to infect, as it assists the virus in fusing to the plasma membrane of the target cell, and therefore assists in the insertion of viral RNA into target cells and in spreading the virus to other cells.

Methods
1. Manufacture recombinant F in the trimer form
   - Clone F – grow up bacteria from colonies
   - Miniprep – isolate the plasmids (F and baculovirus shuttle vector)
   - Cut – cut plasmids with restriction enzymes
   - Zap – break down bacteria cell membrane to integrate gene
   - Gel electrophoresis – separate DNA fragments by size
   - Purification – extract desired DNA from gel
   - Plate – grow colonies with F insert
   - PCR – confirm F gene insertion into vector

2. Express high quantities of F
   - Clone F – grow up bacteria from colonies
   - Midiprep – isolate the plasmids
   - Transfections – expose F to various cell lines
   - Baby hamster kidney (BHK) and insect cell lines
   - SDS-PAGE – separate protein fragments by size
   - Western Blot – transfer gel to membrane to identify proteins present
   - Histidine purification by Ni-resin for recombinant F protein

3. Test immunogenicity of F in mice with adjuvants
   - Vaccinations – inject F (2x 2-5x2 apart) and adjuvants (daily)
   - Bleed mice – take blood samples from mice two weeks post-vaccination
   - ELISA – quantify antibodies against F from blood samples

Results
- Figure 1: Crystal structure of the F protein, monomer form. Segment A, acting as a membrane anchor, was removed to make F soluble, and a trimerization domain was added in its place. Segment B binds to the cellular receptor and should be targeted by the immune system.
- Figure 2: Proteins separated by SDS-PAGE. Reference bands on the left display the location of specified molecular weights. F is expressed, as it is slightly less than 69kDa. Lane 1 was the ladder, Lane 2 was a negative, Lane 3 was cell extract after Ni-resin, and Lane 4 was from after resin release.
- Figure 3: DNA gel electrophoresis performed on the F gene, the pOET vector, and F inserted into the vector. The two bands in the pOET + F show the F gene was present in the baculovirus shuttle vector. The shuttle vector was then used in insect cell (S21) transfections to make recombinant virus. The virus was amplified in more insect cells and titered for infection.
- Figure 4: Antibody titers present in mice following injection. Adjuvants metformin (Met) and methyl pyruvate (Methyl) act on the mitochondria, and were administered with F monomer. F alone (Sham) was also tested as a control for typical F vaccines. The end titer represents total viral-specific antibodies; neutralization titer represents antibodies functional against RSV entry.

Conclusions
Many studies have concentrated on F protein vaccine but have failed in clinical trials
- We hypothesized that an F protein that "looks" more native to the viral structure would be more efficacious but would likely not protect fully
- We tested whether we could enhance and broaden the immune response to F monomer protein through use of mitochondrial adjuvants
- We sought to make a recombinant F protein that folds into the native trimeric form

Future Directions
- Further testing on mice using additional mitochondrial adjuvants will be done to determine an optimal choice
- Perform confirmation studies using native protein gels and chromatography to confirm our recombinant protein is trimeric
- Vaccination with trimeric F will be substituted for monomeric F to determine whether we can get enhanced neutralizing antibodies
- Challenge studies are planned to determine whether mice are protected from the virus and whether any protection correlates with neutralizing antibody titers

References