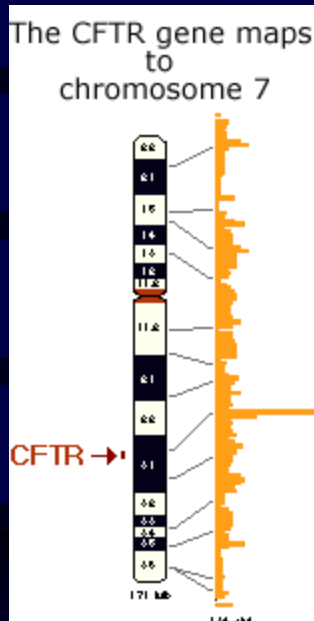


# CFTR Genotyping Cutting Lab



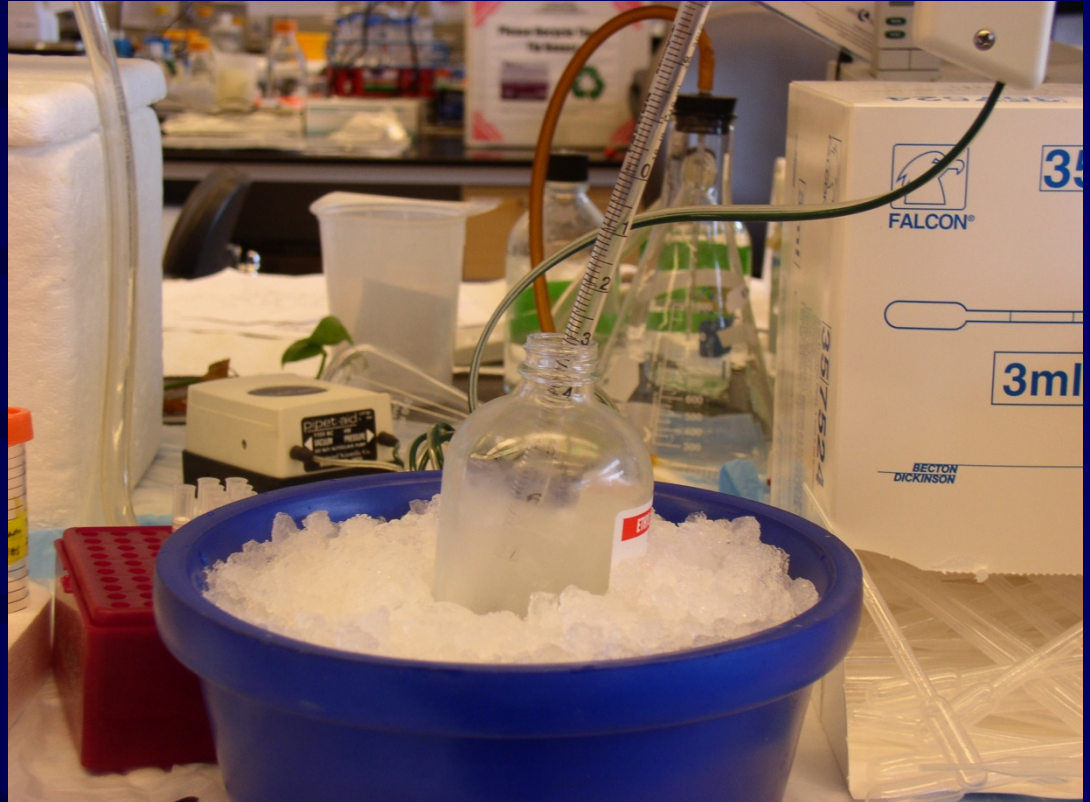
## CFTR Genotyping Cutting Lab

When we receive blood from a study participant, we refrigerate it for 2-3 weeks which enables better DNA extraction from the cells.



# CFTR Genotyping Cutting Lab

Preparing Buffers and  
Solvents to add to  
blood cells.



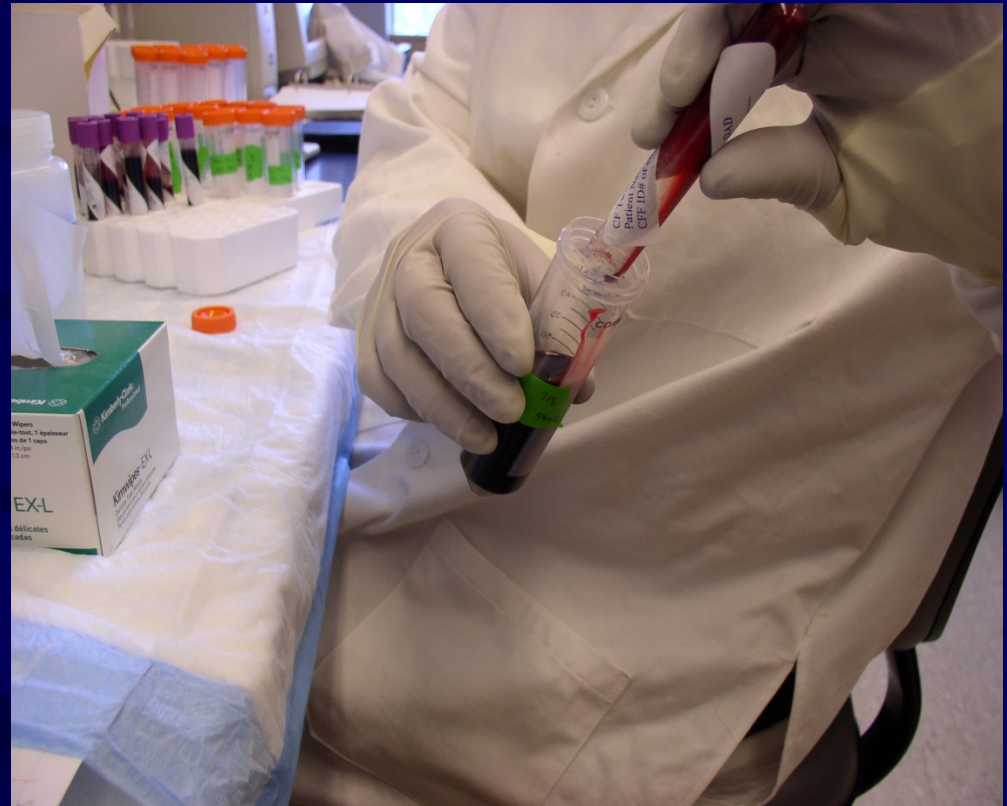
# CFTR Genotyping Cutting Lab

After the completion of several steps in the DNA extraction process, a “pellet” of blood cells is collected in the a Falcon tube for additional processing.



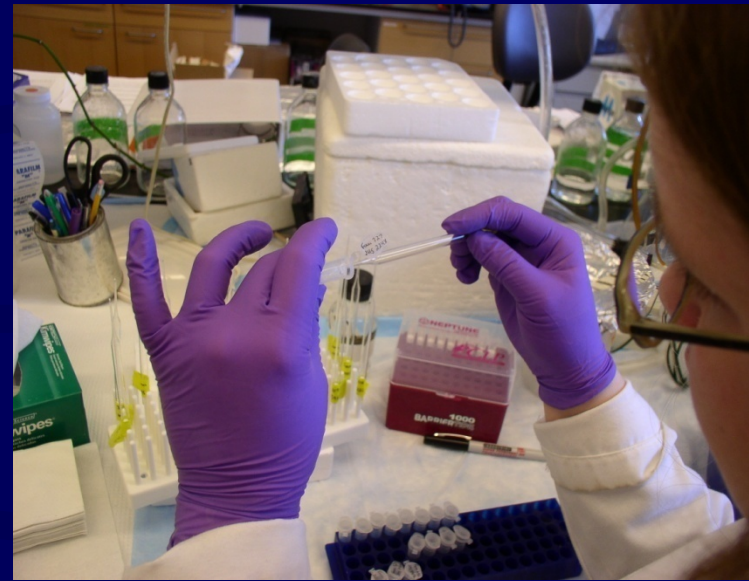
# CFTR Genotyping Cutting Lab

The blood sample is mixed with special buffer, and the blood cells “burst”, releasing the DNA inside the cell.



## CFTR Genotyping Cutting Lab

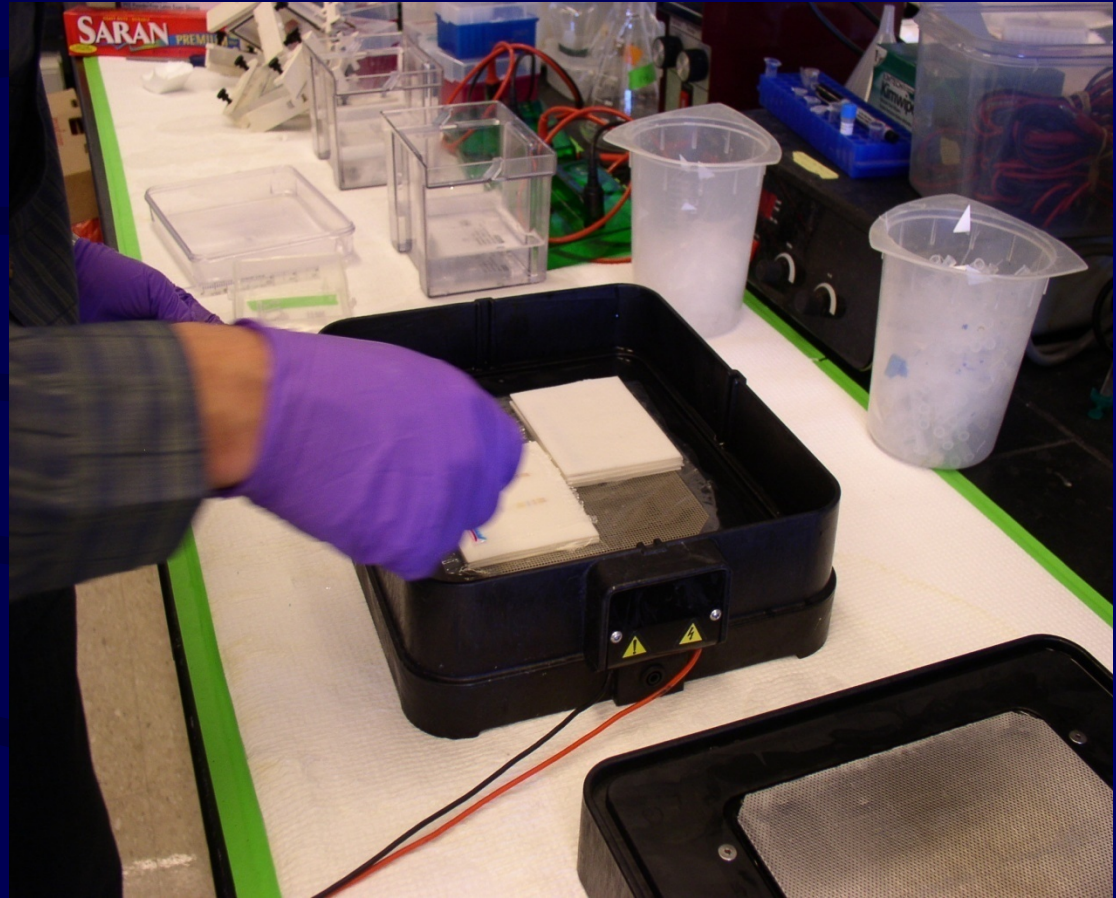
Finally, after several more steps, we can see the DNA suspended in solution. We then collect the DNA onto glass pipettes, and ready it for its final preparation.



The white material on the end of this pipette is dried DNA, which is then placed in a final solution for lab experiments.

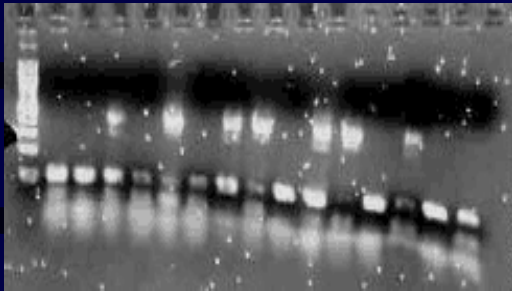
## CFTR Genotyping Cutting Lab

To begin testing for CFTR mutations, we first do a Polymerase Chain Reaction (PCR) using the DNA from the participant's blood sample.



[http://en.wikipedia.org/wiki/Polymerase\\_chain\\_reaction.](http://en.wikipedia.org/wiki/Polymerase_chain_reaction)

## CFTR Genotyping Cutting Lab

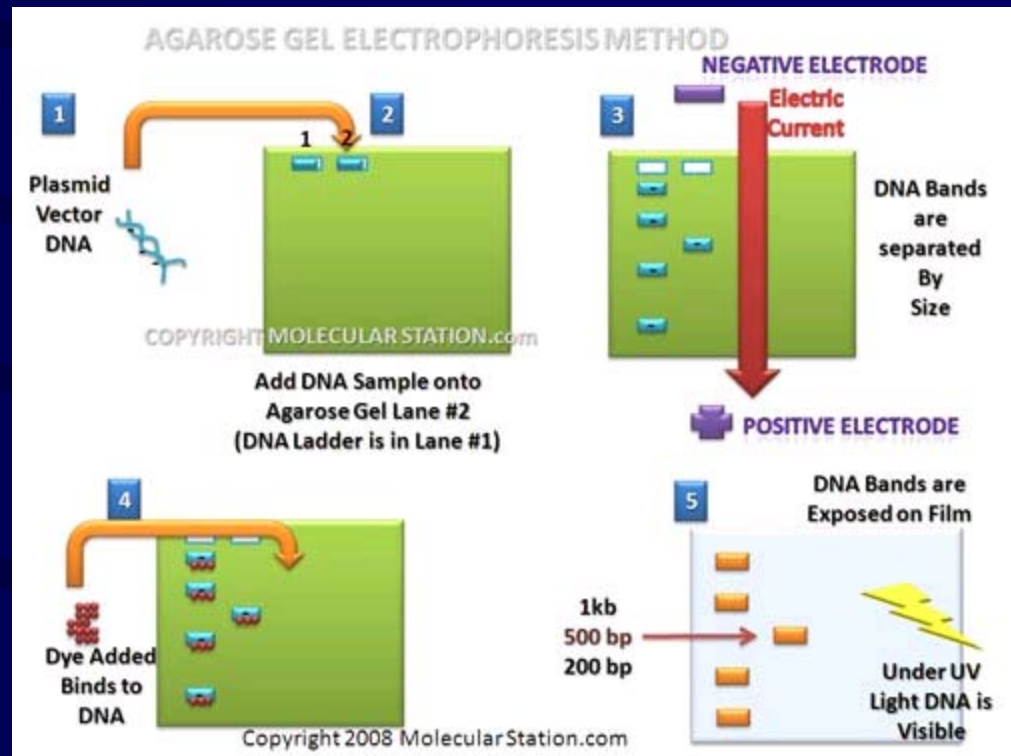


- We use Gel Electrophoresis to confirm that there is a PCR product
- There are specific reagents in the PCR for the 31 most common CFTR mutations (see table below) that were designed to look for the “sites” in the CFTR gene where the mutations occur and amplify these bits of DNA.



# CFTR Genotyping Cutting Lab

The gel contains a chemical that clings to the DNA strands. When we look at the gel under UV light, the DNA luminesces. The electric current that passes through the DNA during electrophoresis separates the amplified pieces of DNA by size (small moves faster and farther).



# CFTR Genotyping Cutting Lab

The DNA is treated with a chemical that causes the strands to unwind. This DNA is washed over a strip. The strip has been treated with an array of probes that are specific to the normal and mutated DNA at each of the 31 most common potential mutation sites in CFTR.



# CFTR Genotyping Cutting Lab

There are 2 probes for each possible mutation: one for normal DNA and one for the mutant. If the bit of DNA is present, it “hybridizes” to its probe and “sticks” to the strip. After processing the strip with chemicals, a blue color complex is formed at each place on the strip where DNA has hybridized. These blue lines are read by aligning the strip with a guide that maps the location of each probe on the strip.

