National Science Foundation Plant Genome

Cereal Plant Transformation Workshop

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Microprojectile Bombardment

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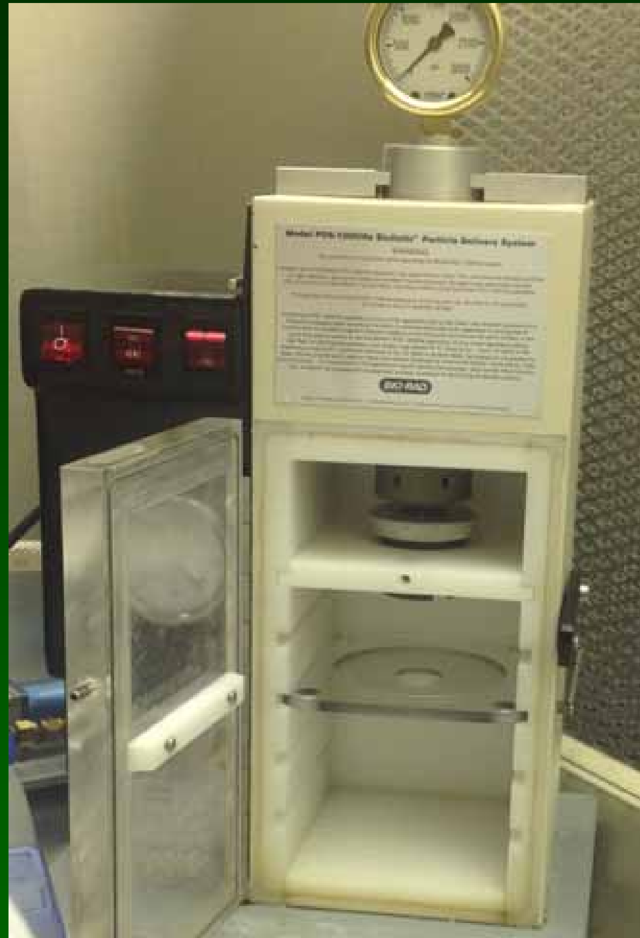
Microprojectile bombardment was developed by John Sanford and Ted Klein in the mid-1980's. Bombardment then proved as the protocol for the first achieved transformation of maize and other monocots resulting in fertile transformants. It was subsequently replaced by enhanced *Agrobacterium*- mediated protocols. However, this method is still important for several reasons and applications. First microprojectile bombardment, serves as a delivery method for transient expression assays. Second, this protocol has become increasingly important to the development of genome editing delivery.

Microprojectile Bombardment



Biolistic PDS-1000/ He
Particle Delivery System
Catalogue number 165-2257
BioRad

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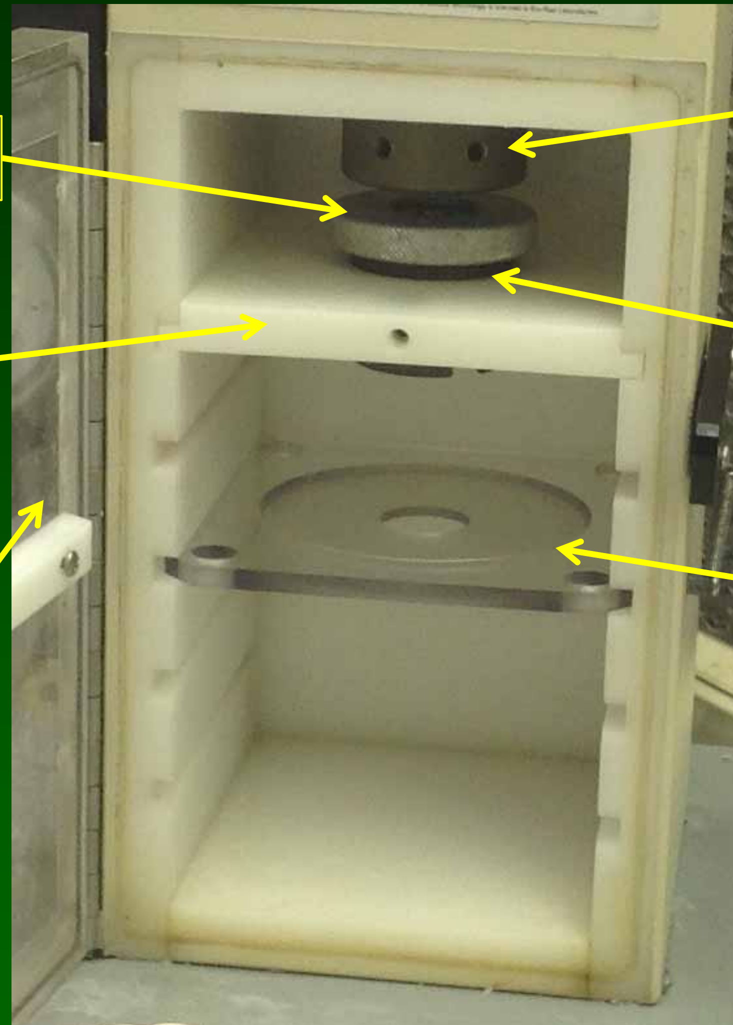
BioRad

Macrocarrier Cover Lid

Microcarrier Launch Assembly

Bombardment Chamber Door with gasket



Rupture disc retaining cap

Brass Assembly Nest

Target Plate Shelf

Biolistic PDS-1000/ He Particle Delivery System

3-way Helium Metering (Solenoid) Valve

Power cord

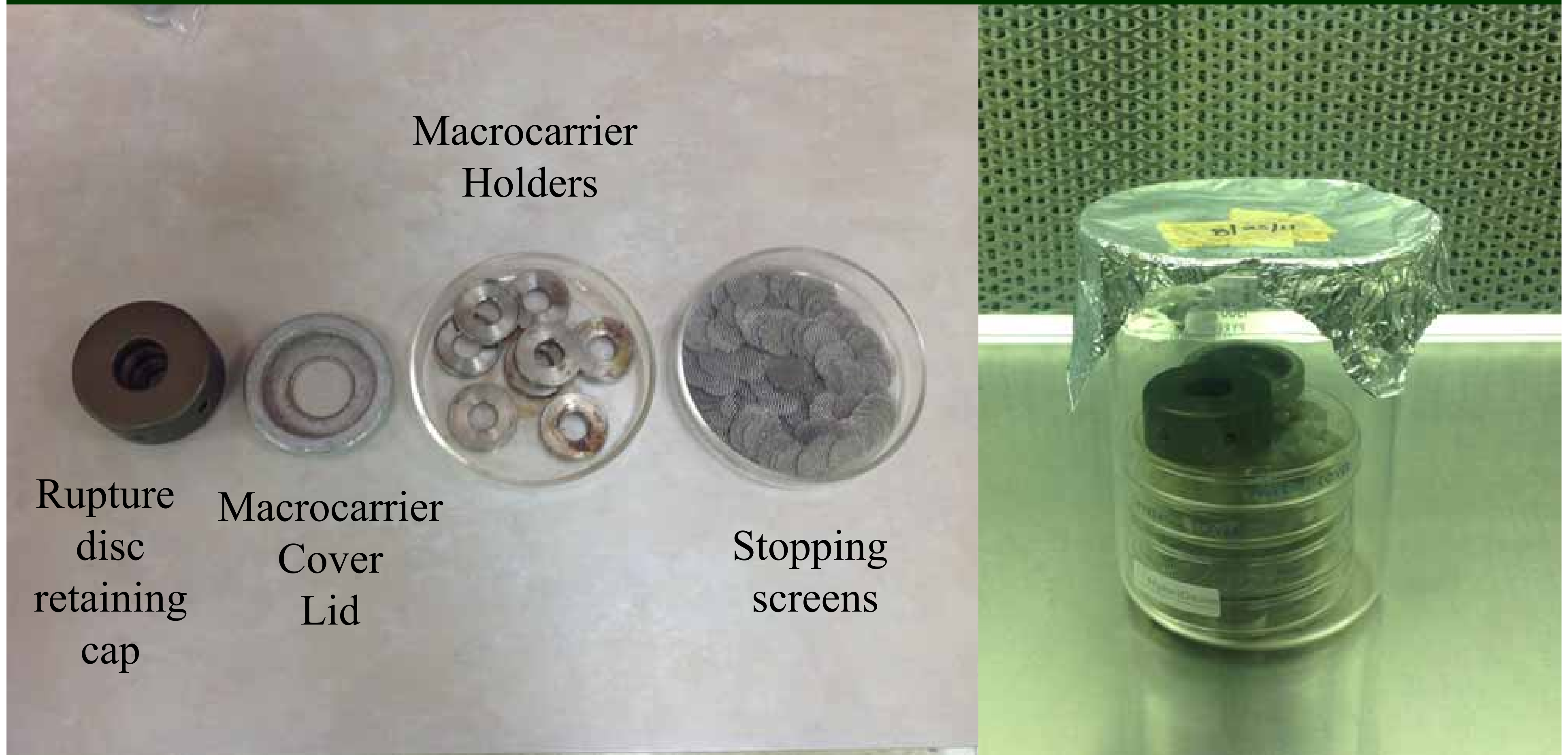
2.5 ft PEEK Tubing to top of bombardment device

6 ft PEEK Tubing to Helium tank

Reinforced Vacuum Tubing to Vacuum Pump

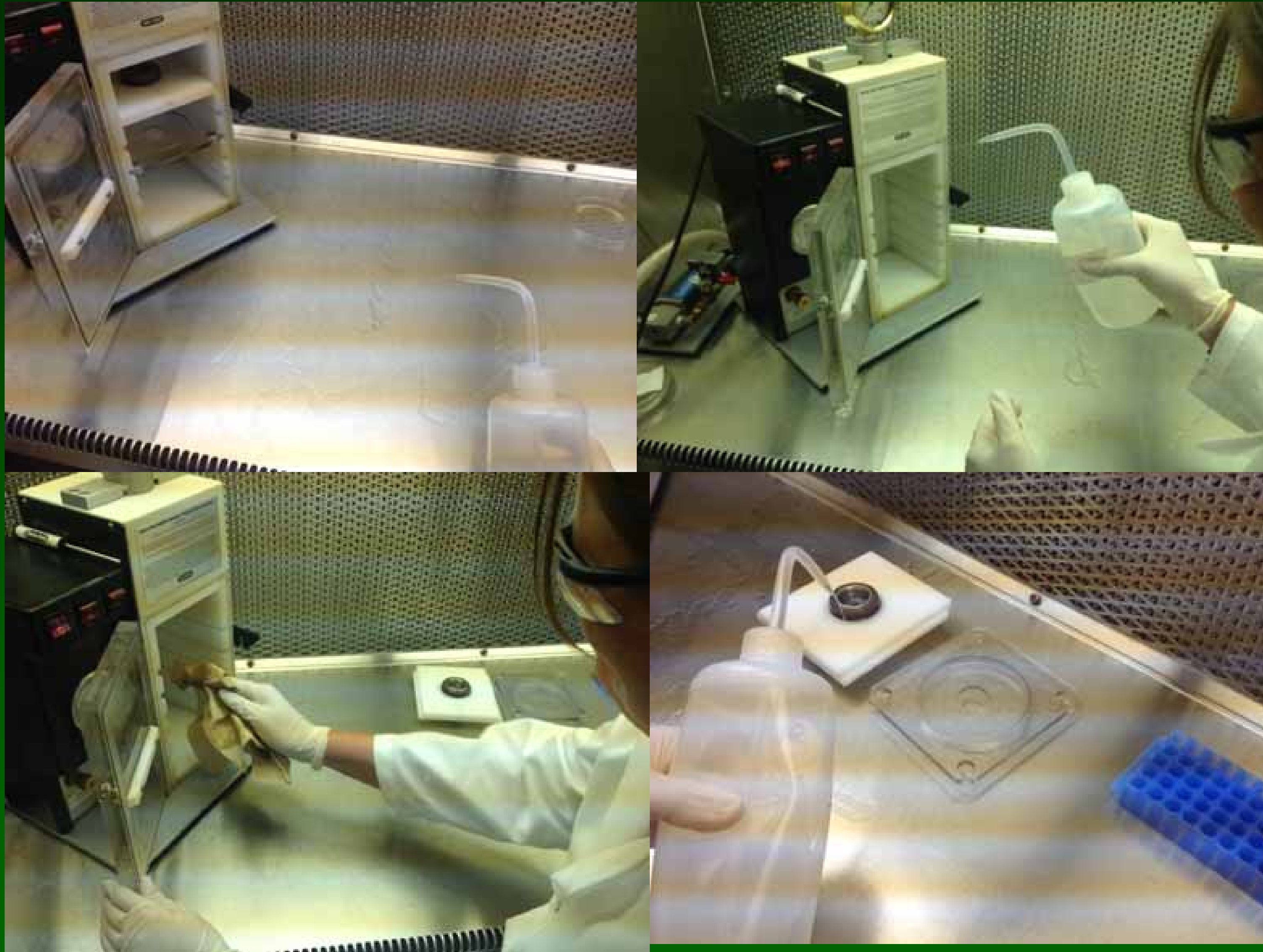
Microprojectile Bombardment Gun Components

Gun Preparation



Autoclave removable metal gun parts (as shown, right), stopping screens, macrocarrier holders and forceps. Sterilize macrocarriers (henceforth called Flyers) in 70% ETOH for 1 hour, and then dry in hood. *Ensure these and all gun parts are completely dry.*

Gun Preparation



Surface Sterilize the laminar flow hood working surfaces and the gene gun (inside and out) with 70% ETOH.

Microprojectile Bombardment Gun Components

Macro carriers
Of "Flyer"

Rupture Discs
These are purchased a with various psi rupture points.
The PSI rupture point is related to the acceleration of the particle

Microprojectile Bombardment Gun Components

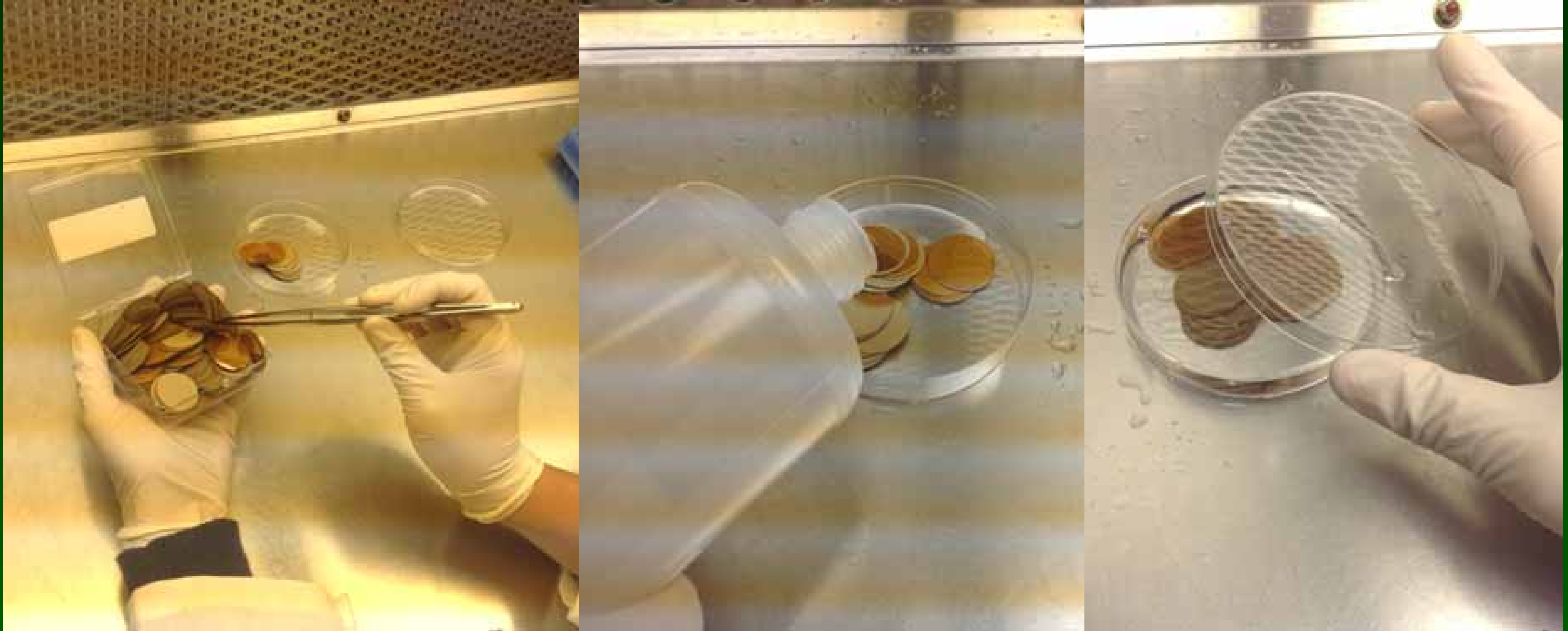
Various Gun Tools for making adjustments when required.

Gun Preparation

Surface sterilization of Flyers

Macrocarriers (also called “Flyers”)

Surface sterilization of Flyers



Sterilize macrocarriers (henceforth called flyers) in 70% ETOH for 1 hour

Surface sterilization of Flyers

Start surface sterilization in 70% ETOH for 1 hour.

Surface sterilization of Flyers

After surface sterilization in 70% ETOH for 1 hour, use sterile forceps to move the flyers to a sterile Petri dish placed toward the back of the hood to dry. Note: because they were sterilized in 70% ETOH the flyers may take 20-30 minutes to dry. The may be left in the bombardment hood until needed.

DNA Precipitation

Reagents: Prepared prior to procedure

2.5 M $CaCl_2$ $2H_2O$ (100ml):
Dissolve 36.76g in ~50ml ultrapure H_2o
Bring to a final volume of 100 ml
Filter sterilize or autoclave solution

DNA Precipitation

Reagents: Prepared prior to procedure

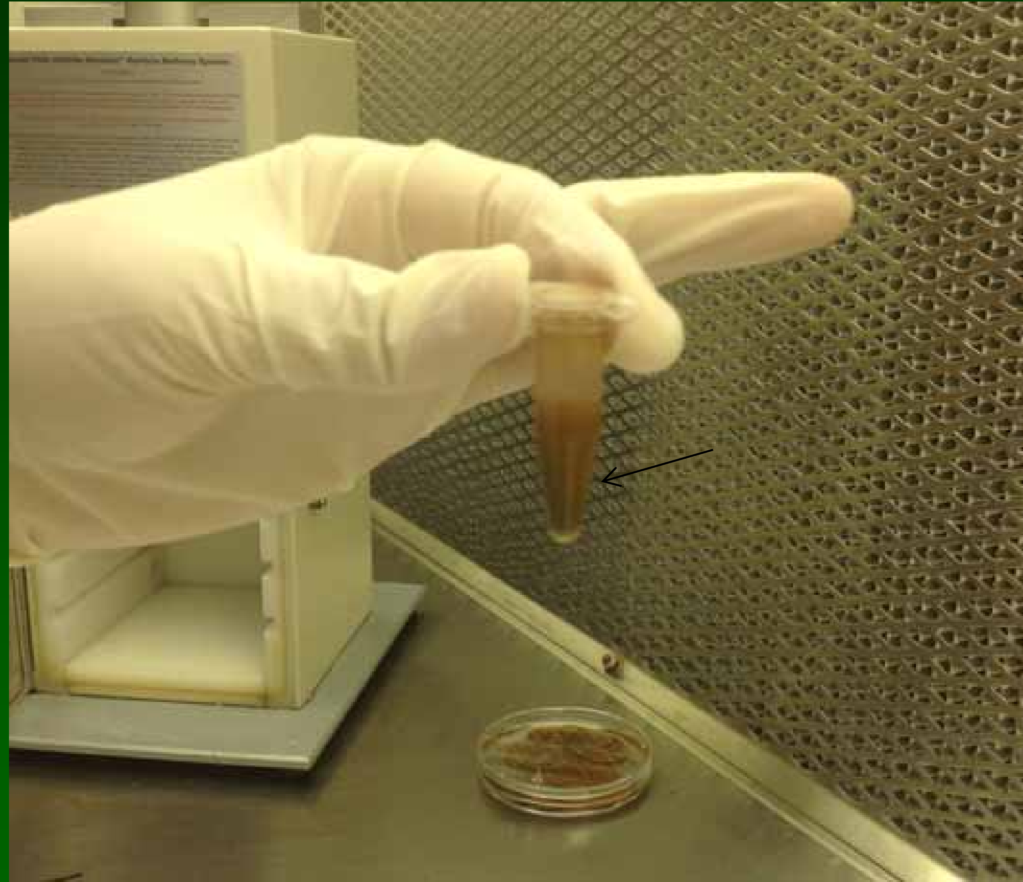
0.1 M Spermidine (1ml):

Mix 15.7 ul spermidine with 984.3ul ultra pure water

Filter sterilize *Note: Spermidine when properly stored (2-8°C) is a solid. Melt spermidine in 65°C water. Spermidine will oxidize and turn yellowish and should therefore be stored under N_2. In addition, keep bottle and a desiccant pack in a 50 ml Falcon tube. Mix a fresh 1 ml working solution each bombing campaign.*

DNA Precipitation

Particle Stock: Prepared prior to procedure



Prepare a gold stock solution. Transfer 60 mg of 0.6 µ gold to a 1.5 ml tube with 1 ml of 100% ETOH.

Note: 100% ethanol will hydrate quickly becoming 90-95%. So, Splurge and open a new bottle of ETOH. Using old ETOH is likely to cause clumping of gold particles and decrease the efficiency of the transformation.

DNA Precipitation

Prepare working solutions of gold particles and DNA. To maintain uniformity in bombardment parameters, only 3 bombardments should be performed per solution. Also ,keep experiments under six to eight tubes

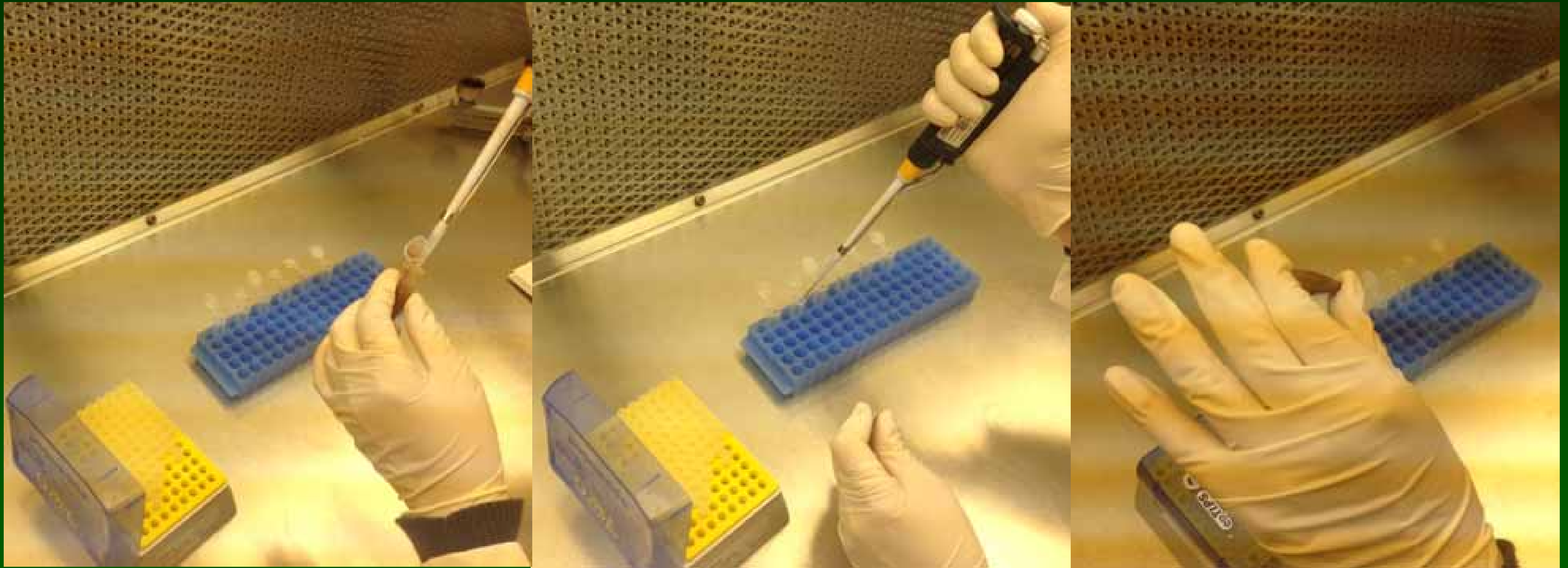
DNA Precipitation

Vortex the gold stock (60 mg/ml) for 1 min. to break up any gold aggregates.

DNA Precipitation

Sonicate the gold for 15-20 seconds. *Caution: Although it is important to find the "active spot" in the sonicator, leaving the gold in the sonicator for more than 20 seconds will overheat the gold particles*

DNA Precipitation

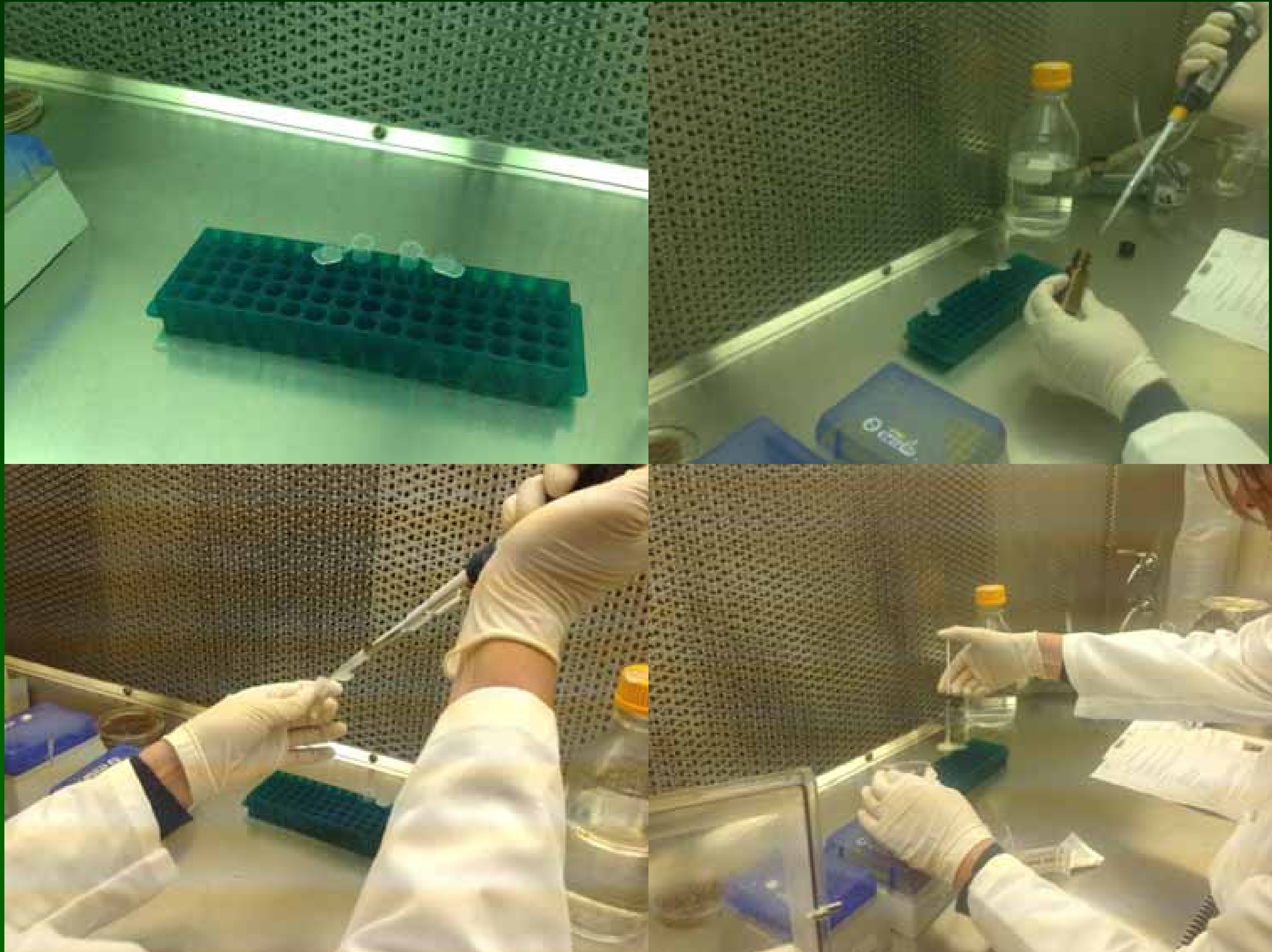


Invert the tube between sampling to prevent settling of gold particles,withdraw 35 µl of suspended gold from the middle of the 1.5 ml Eppendorf tube and dispense it to the bottom of a sterile 1.5 ml tube. Invert the tube between each sample to ensure a uniform sample concentration of the gold, remember gold particles will settle quickly

DNA Precipitation

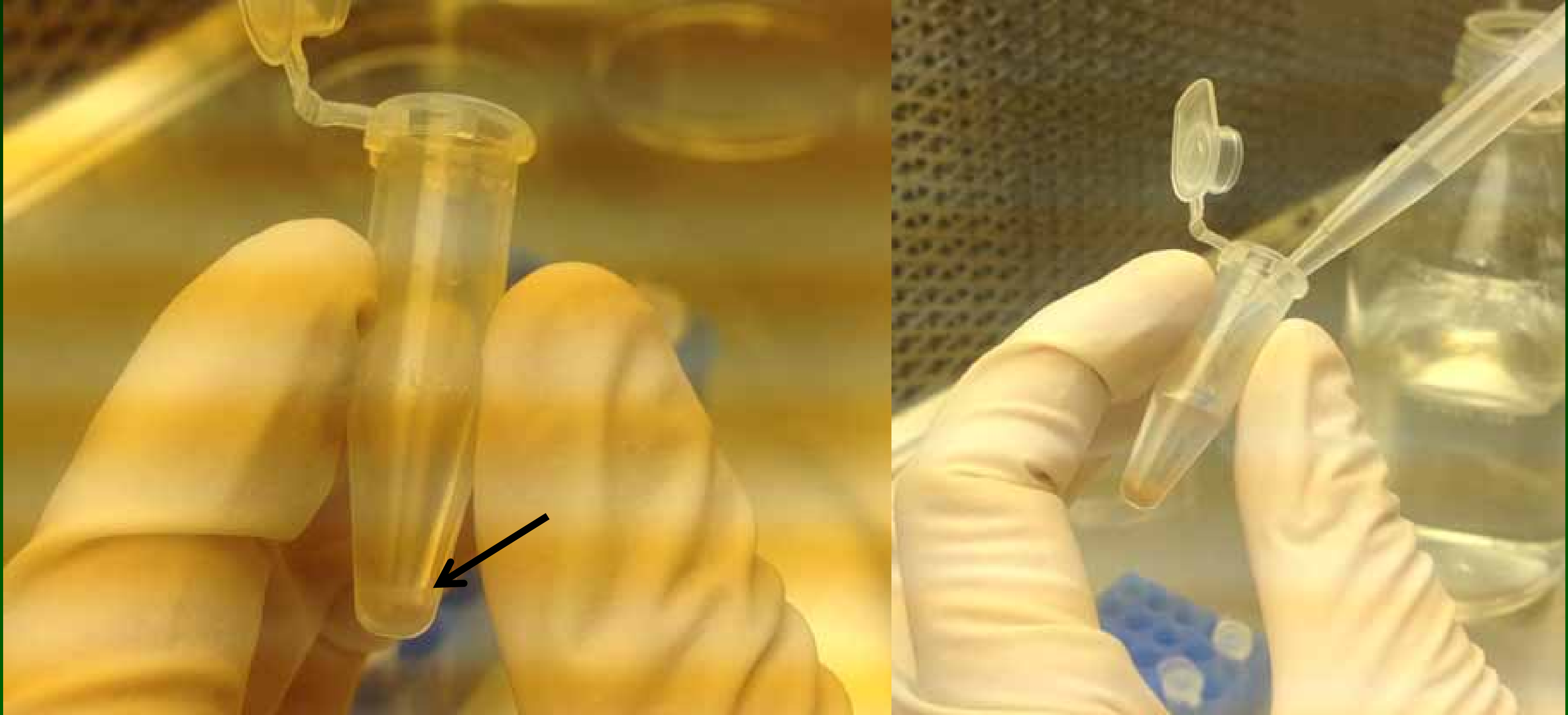
Using a centrifuge with a swinging bucket rotor, pellet the gold for 5 minutes at 500 RPM. (*This is a good time to prepare the spermidine.*)

DNA Precipitation



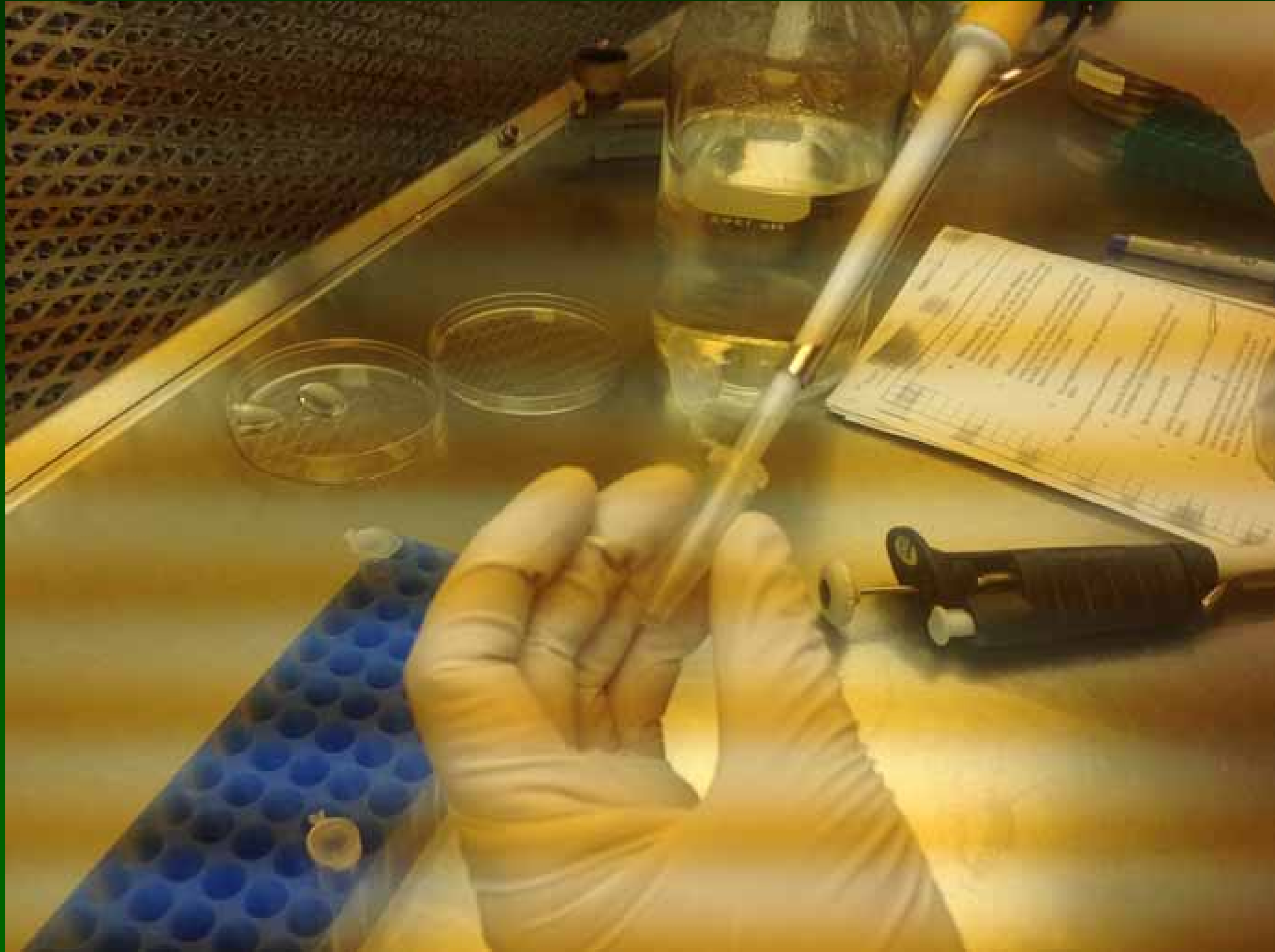
This is a good time to prepare the spermidine. Take an aliquot of meted spermidine from the stock and filter sterilize it into a sterile microfuge tube.

DNA Precipitation



Remove the ETOH and without disturbing the pellet (left), then carefully add 1 ml of sterile water without disturbing the pellet. If pellet is not disturbed proceed without an intermittent centrifugation step. Otherwise pellet the gold for 5 minutes at 500 RPM.

DNA Precipitation

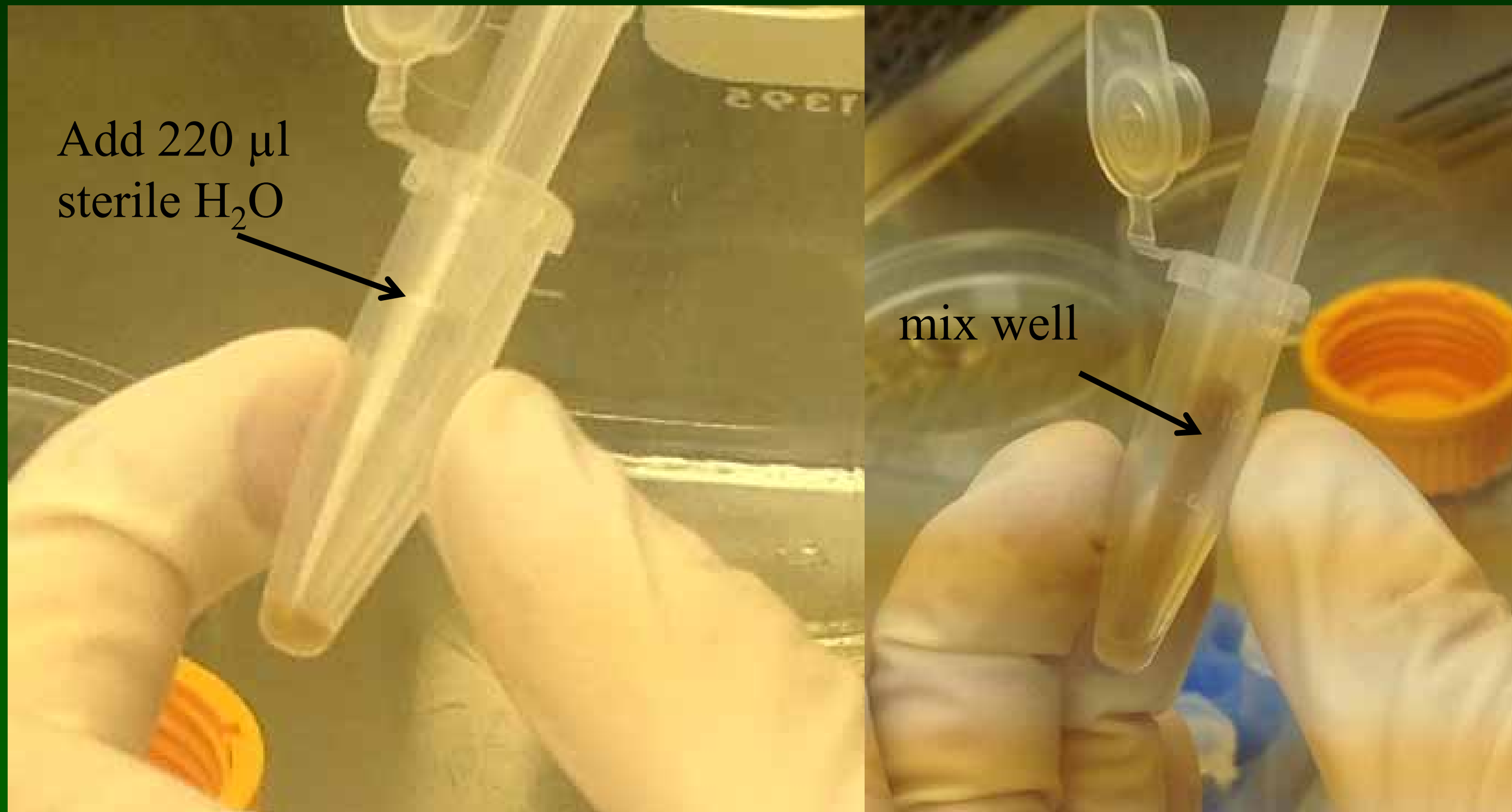


Carefully, without disturbing the pellet, remove the H_2O (as close to close to dryness as pipeting will allow).

DNA Precipitation

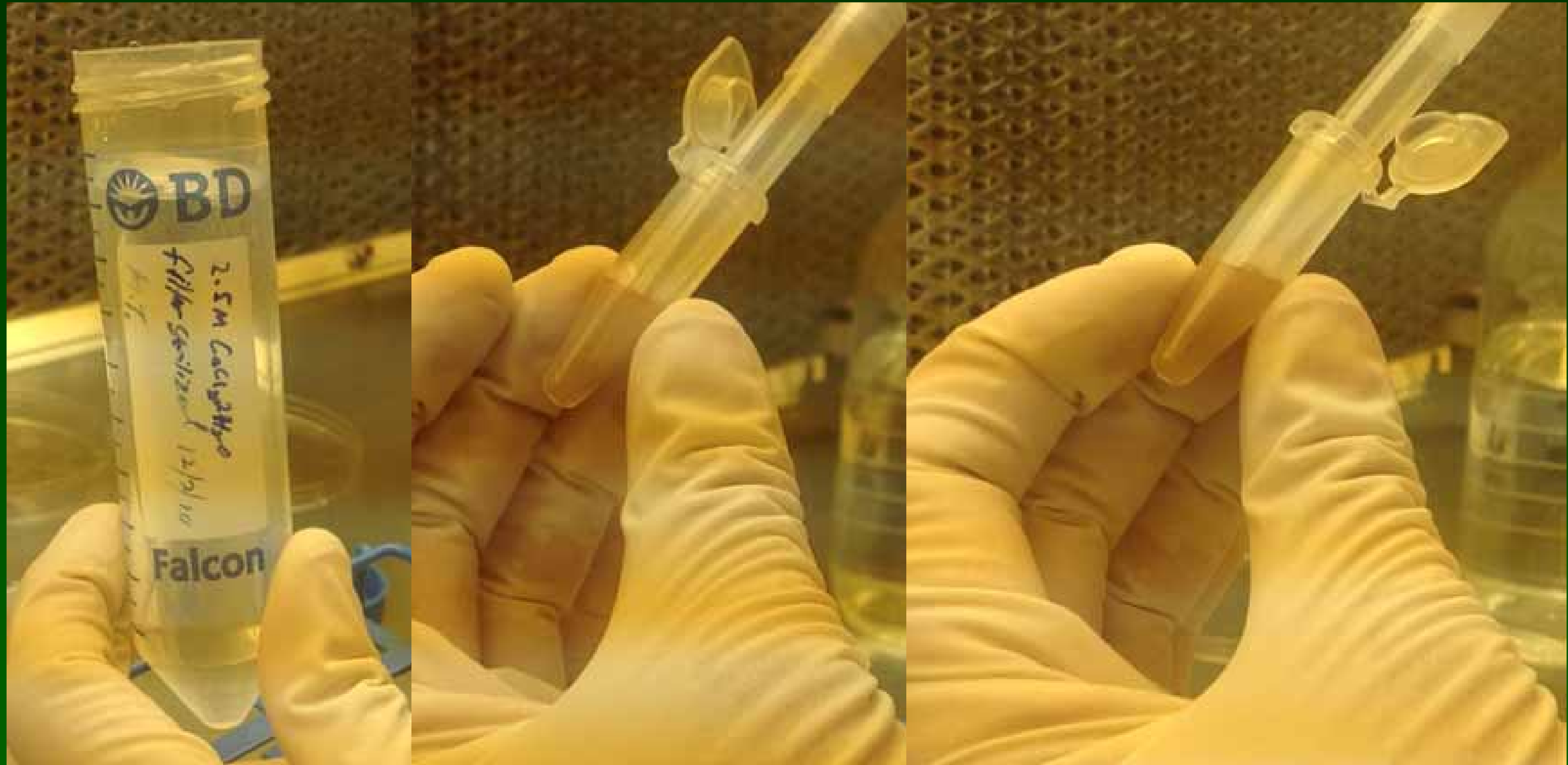
Resuspend the microprojectiles in 9µl of DNA solution [1µg/µl]. Pipette up and down to facilitate re-suspension. *The microprojectiles should go into solution readily and not be clumpy. Multiple constructs can be added. Co-transformation can occur up to 70% of the time.*

DNA Precipitation



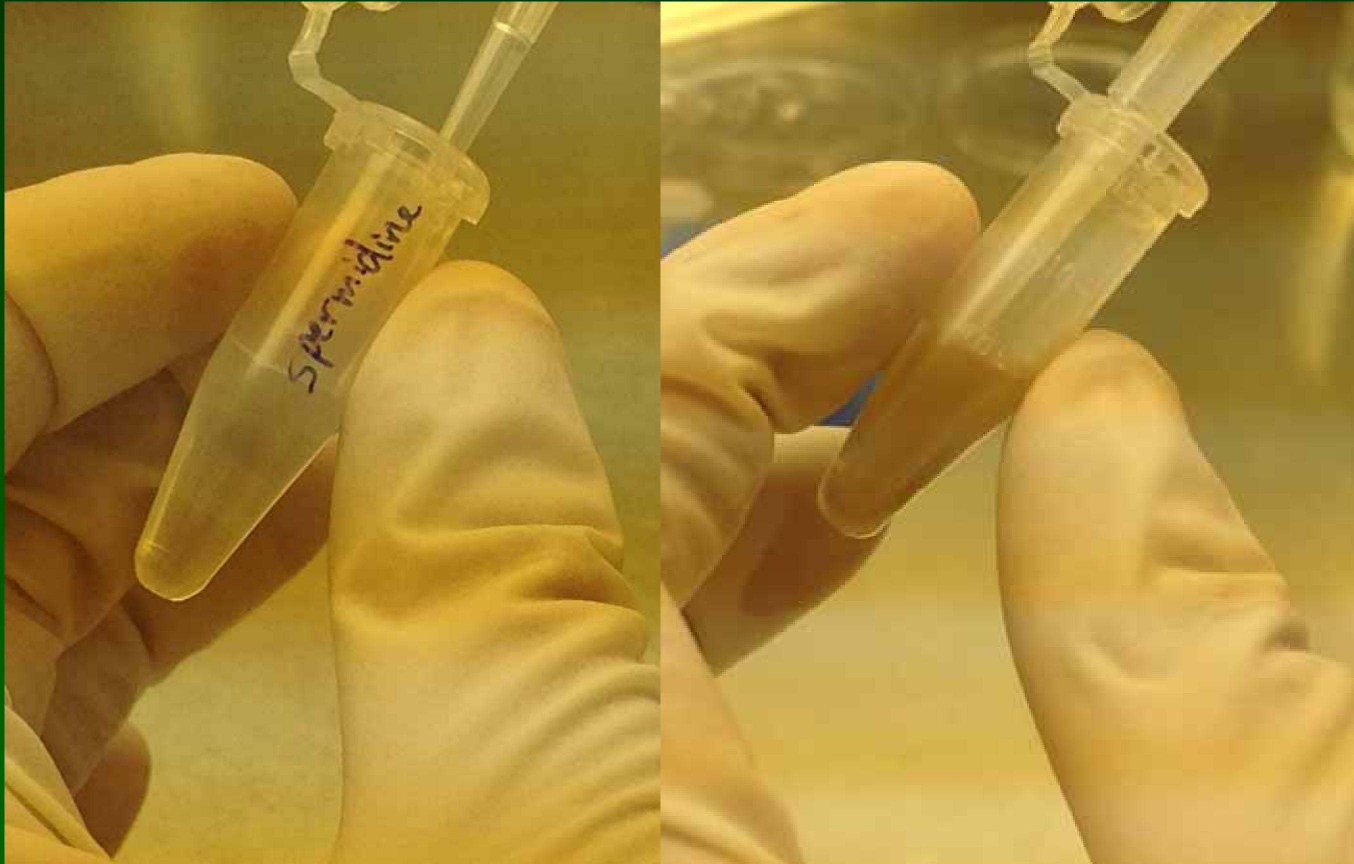
Add 220 µl of sterile H_2O, and mix well.

DNA Precipitation



Add 250 µl of sterile $CaCl_2$, and pipette up and down to mix well. *Calcium chloride salts the DNA out of solution.*

DNA Precipitation



Add 50 µl of sterile spermidine, mix well and place on ice. *Spermidine tends to stabilize the DNA.*

DNA Precipitation

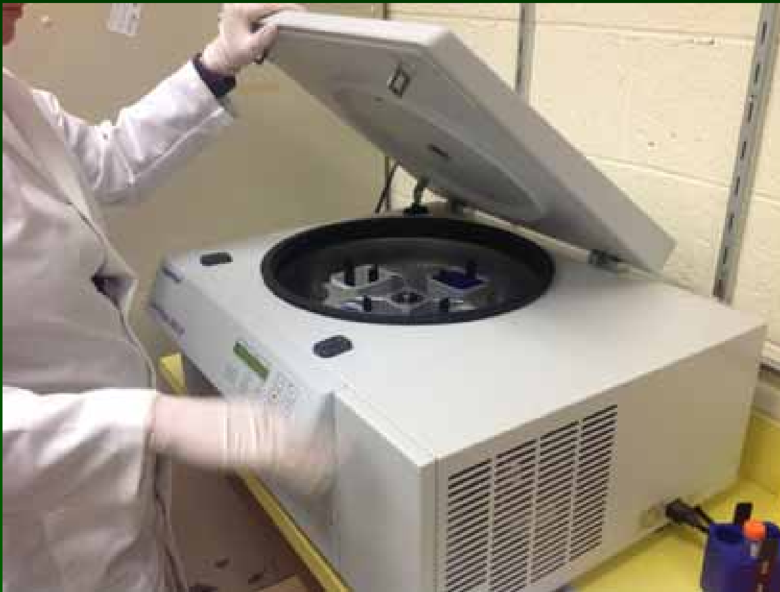
Using a Genie 2 vibrating mixing pad (left) mix this solution in refrigerator (4C) for 10 minutes. *A small amount of foam on the top of the solution indicates a good rate of vortexing. Note: This is a good time to start the flyers drying and start soaking the rupture discs*

Surface sterilization of Rupture discs

Note: This is a good time to start surface sterilization of the rupture discs in 70% ETOH. Place rupture discs in 70% ETOH for at least 20 minutes. The rupture discs should remain in 70% ETOH until used. They will be inserted into the gun rupture disc holder still wet with ETOH.

Soak rupture disks (1100psi) in 70% ETOH for at least 20 minutes prior to use.
Oversoaking may affect the integrity of the mylar material and rupture setpoint

DNA Precipitation



Centrifuge in a swinging bucket rotor for 5 minutes at 500 RPM in a refrigerated centrifuge *(if available, this may help to reduce DNase activity.)*

DNA Precipitation

Remove the supernatant from the DNA/gold precipitate (as much as possible) and carefully add 600 µl of 100% ETOH. Resuspend the pellet.. It may me necessary to break the pellet apart with a pipet tip.

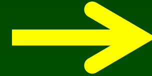
DNA Precipitation

Remove the supernatant from the DNA/gold precipitate (as much as possible) and carefully add 600 µl of 100% ETOH to each of the tubes. Recall that a freshly opened or properly stored bottle of 100% ETOH should be used. . Resuspend the pellet.. It may me necessary to break the pellet apart with a pipet tip.

DNA Precipitation

Spin for 5 minutes at 500 RPM in a refrigerated centrifuge, remove ETOH and re-suspend pellet in 38 μl of 100% ETOH. The precipitate should readily go into solution. The particles are now ready for bombardment

Microprojectile Bombardment



Open helium tank supply valve, and adjust line pressure to ~1200 psi (slightly higher than rupture disc setpoint.)

Microprojectile Bombardment

Turn on the vacuum pump

Microprojectile Bombardment

Place flyers in holders. The flyer must be insert underneath the ridge inside the holder. This may take some patience. *Make several of these at a time. (Multiples of 3- number of bombs per tube.)*

Microprojectile Bombardment

Resuspend DNA/gold by sonicating for 10 seconds

Microprojectile Bombardment

Particles on flyer

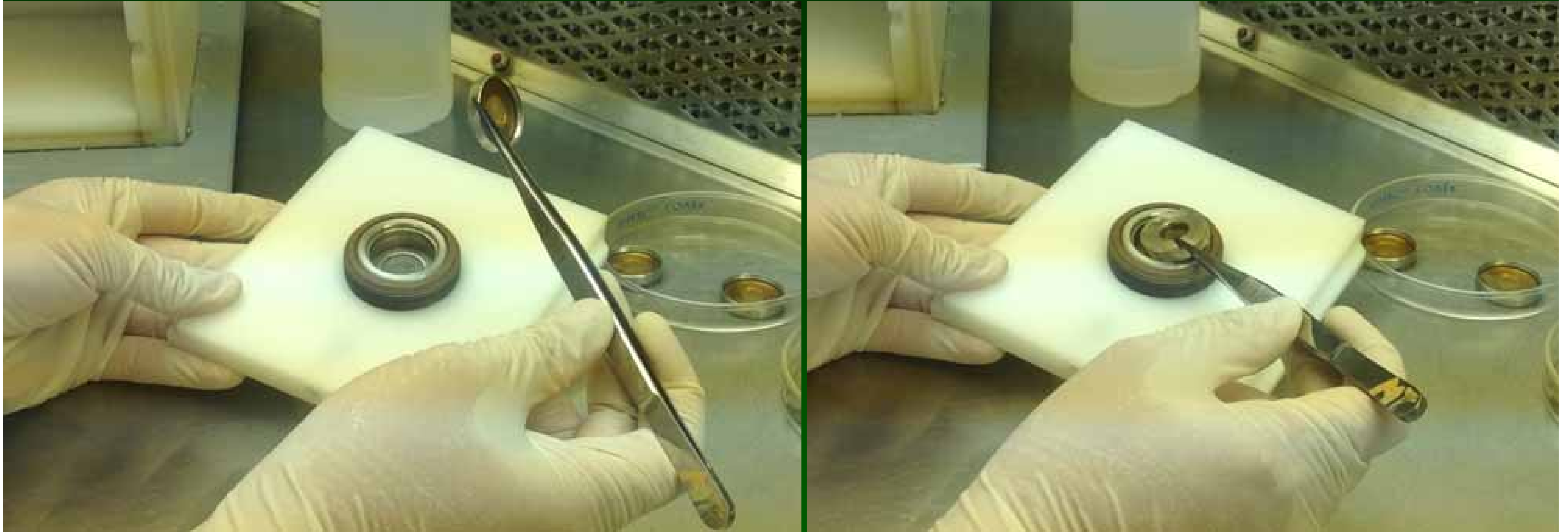
Using a micropipeter, place 10 µl of the sonicated particle solution on center of flyer. Allow the flyer to dry thoroughly.

Microprojectile Bombardment

Place stopping screen in the microcarrier launch assembly.

DO NOT FORGET TO THIS !!!!

Microprojectile Bombardment



Invert the flyer holder and insert into microcarrier launch body. hen screw flyer holder cap screw onto micrcarrier launch body. *Note: the flyer holder can be inserted in different positions pending the placement of spacers. Start with the middle location unless a different location has been optimized for.*

Microprojectile Bombardment

Screw flyer holder cap screw onto micrcarrier launch body. *Note: the flyer holder can be inserted in different positions pending the placement of spacers. Start with the middle location unless a different location has been optimized.*

Microprojectile Bombardment

Unscrew rupture disk retainer cap from inside of the gun, and insert a wet rupture disk which has been soaking in 70% ETOH until use. Rupture discs are available with different rupture setpoints, and can be used in pairs to achieve desired setpoints.

Microprojectile Bombardment

Hand screw retainer cap back on and then tighten firmly (but hand tight…do not over tighten!) .

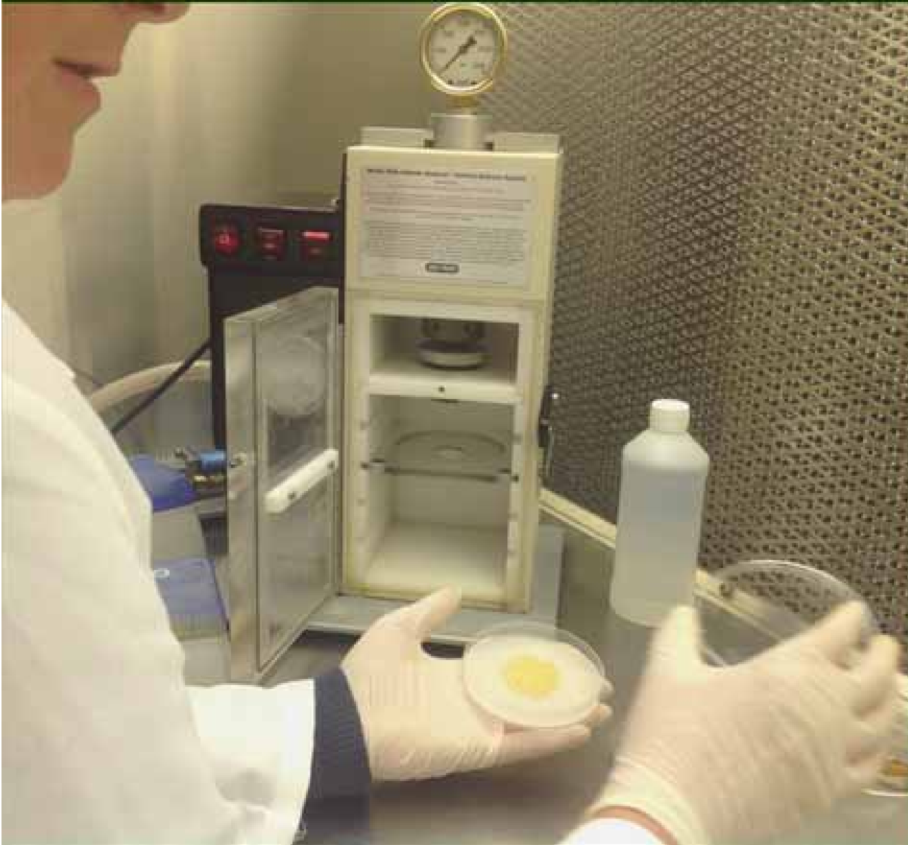
Microprojectile Bombardment

Inset loaded microcarrier launch body into the first slot from the top with the hole to the front (this distance may and is determined experimentally for each system)

Microprojectile Bombardment

Insert Petri dish platform into the third slot from the top

Microprojectile Bombardment

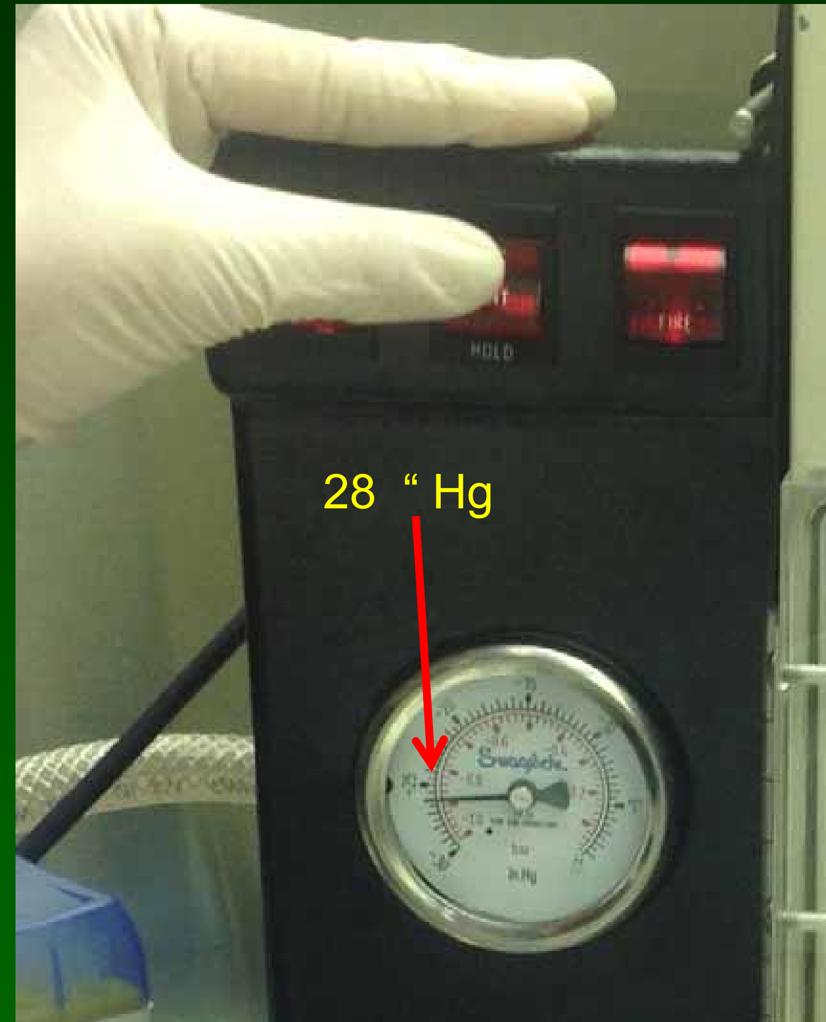


Insert Petri dish containing target tissue onto the platform

Microprojectile Bombardment

Close chamber door. *Operator should wear safety goggles.*

Microprojectile Bombardment



Begin drawing a vacuum by pressing upward on the vacuum button . Watch as the gauge goes up until it indicates 27-28"Hg.

Microprojectile Bombardment

Begin These valves control the Vacuum Flow Rate (left) and the vent Flow Rate (right)

Microprojectile Bombardment

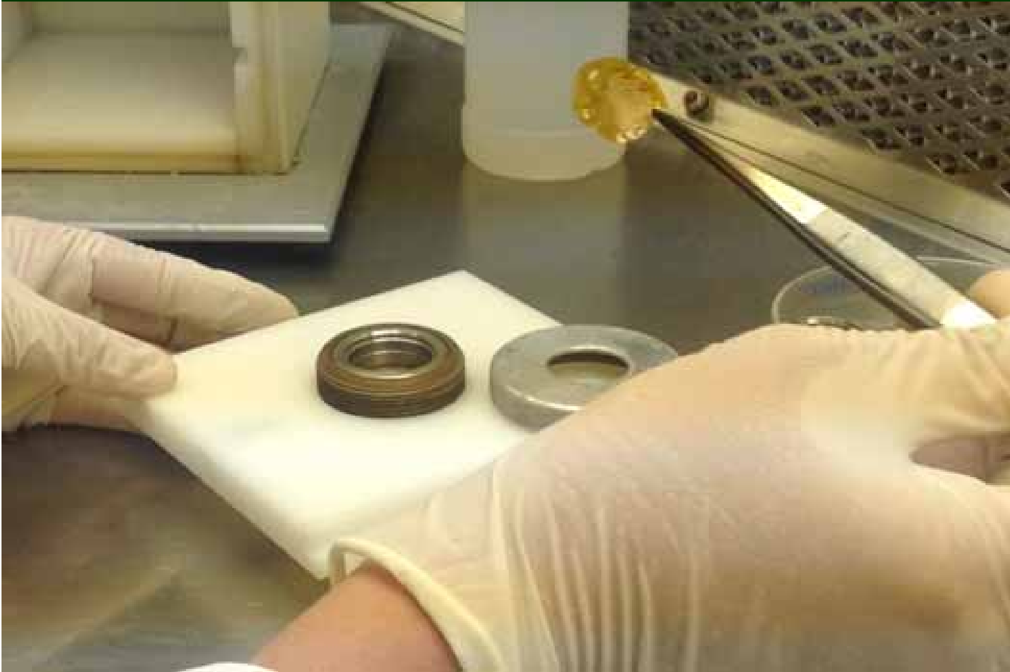
Rupture disc will burst at 1100 psi Hg

Press down the button rapidly to the ‘Hold” position . Press up on the “Fire position and monitor the gauge on top of the device. When the pressure exceeds rupture disk setpoint (in this case 1100 psi) the gun should fire. Note the actual fire pressure on the gauge located on the top of the gun *Operator should wear safety goggles.*

Microprojectile Bombardment

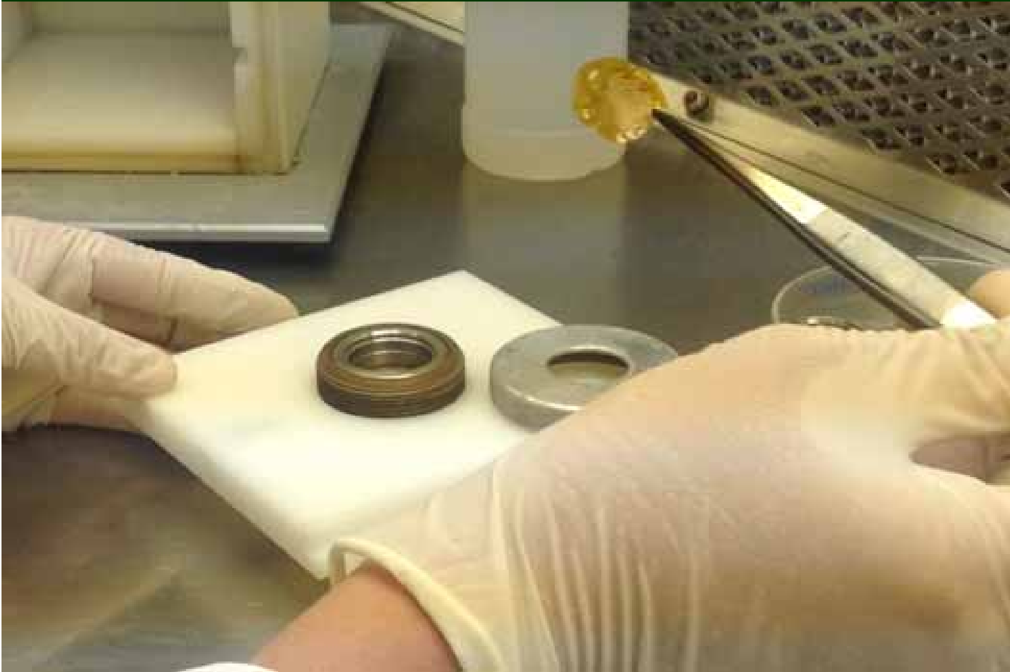
After gun fires, release the fire button and vent the chamber. When the vacuum gauge read zero the chamber door may be opened and the sample removed and covered.

Microprojectile Bombardment



Remove microcarrier launch body and place on a sterile surface. Unscrew cap and remove flyer holder. Unscrew rupture disk retainer cap and remove spent rupture disk. Return to step 4 for additional transformation attempts.

Microprojectile Bombardment



Remove microcarrier launch body and place on a sterile surface. Unscrew cap and remove flyer holder. Unscrew rupture disk retainer cap and remove spent rupture disk. Return to step 4 for additional transformation attempts.

Shutting down the Gun Apparatus

Lastly, after all the bombardments have been completed:

1. Shut off the valve to the Helium tank.

2. Draw a vacuum on the gun chamber by closing the chamber door, and pressing the vacuum button.

3. Next press the vent button to deplete the helium from the line. This procedure should be repeated until the pressure reaches zero.

4. Vent the chamber and open the door.

5. Turn off the vacuum pump last (*Caution : DO NOT leave the chamber under vacuum and then turn off the pump as the pump oil will be sucked into the chamber)*

Thank You