Plant Biotechnology

Bayer Russia Plant Biotechnology Conference

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Molecular Assays

DNA Extraction

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- PhD in Microbiology from North Carolina State University
- Over 25 years experience in Plant Biotechnology at Bayer Crop Science
- Project lead experience leading commercial development of Biotech crops
- Eight years of experience in developing and running molecular screening assays for Biotech crops
- Four years of experience as lead of the STL TaqMan Lab in Chesterfield, MO site

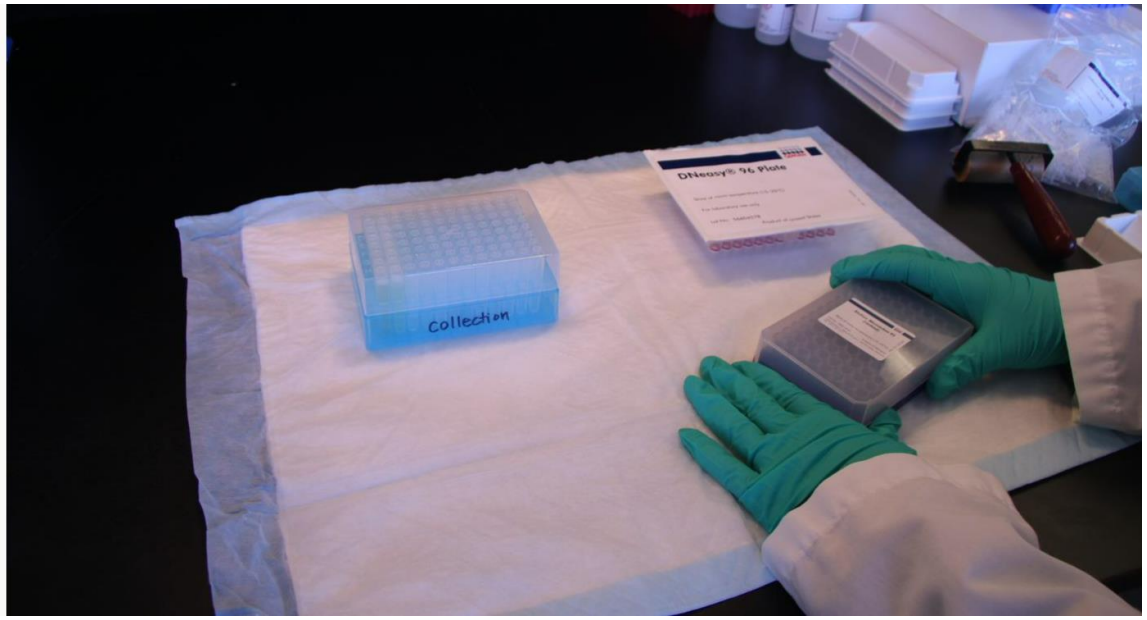
Assemble extraction kit materials

- This presentation shows how to extract DNA from corn or soy leaf samples for molecular analysis using the DNeasy® 96 plate method from Qiagen.
- Begin by assembling materials from the kit, including collection plate, elution plate, and filter plate.
- It is recommended to lyophilize leaf samples prior to DNA extraction.

Additional considerations for plant DNA extraction

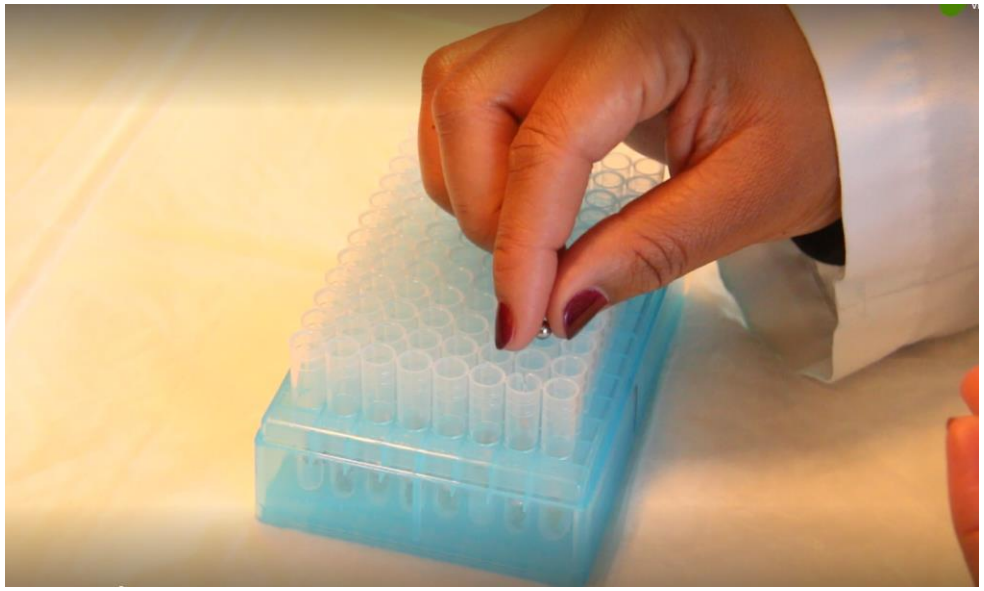
- There are many DNA extraction methods that may be suitable for molecular analyses, shown here is one example that is applicable to many plant species
- Inhibitors of PCR or other downstream molecular assays may be present and DNA extraction methods should be chosen that minimize inhibitors.
  - Inhibitors may vary by plant species, so one method may not be suitable for some plant species, and this may also vary by type of tissue (e.g. leaf, immature seed, mature seed)
- Freeze-thaw cycles in handling of leaf tissue should be avoided to minimize degradation of DNA and presence of inhibitors to downstream assays
- DNA extraction methods will shear DNA, and this can vary based on grinding and liquid handling methods. Where large fragment size of DNA is important, these variables also become more important considerations in the method

Prepare your work area



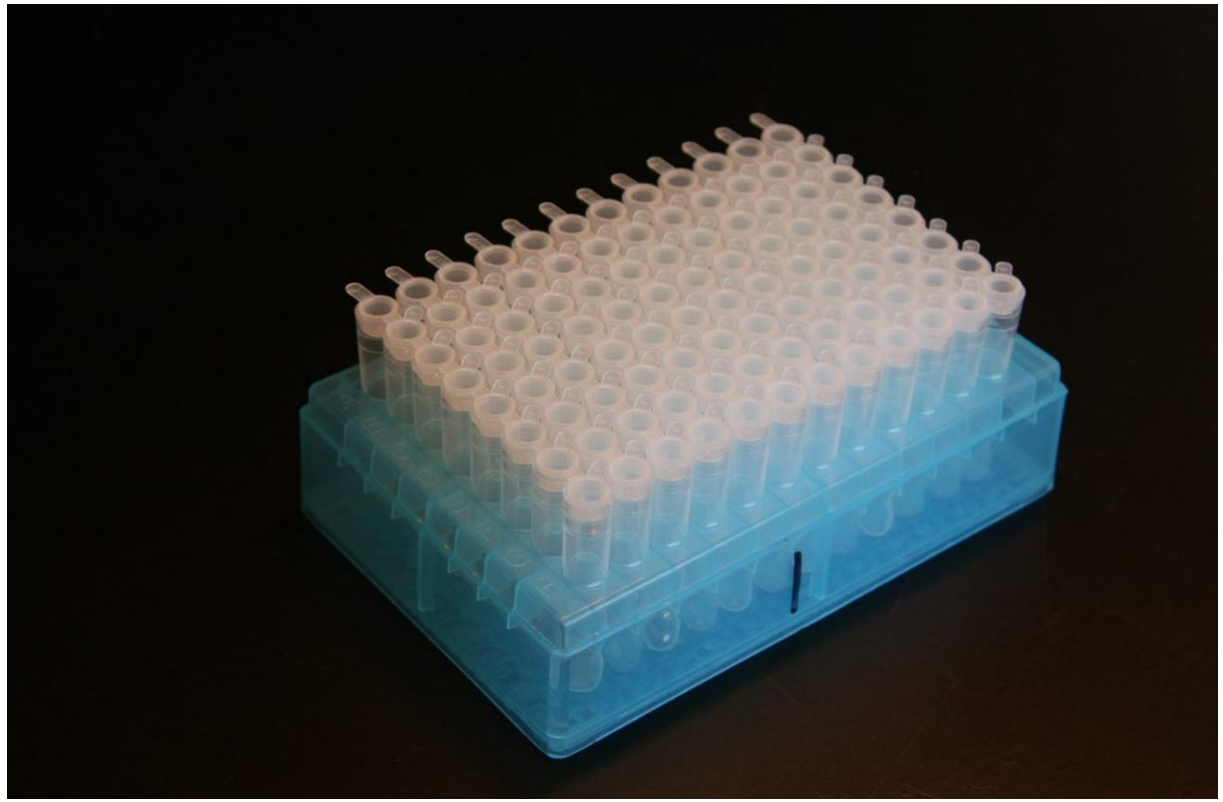
- Prepare a clean area to perform the DNA extraction.
- In preparation for extraction, pre-heat buffer AP1 to 65 degrees Celsius.
- Combine buffer AP1, RNase A, and Reagent DX to make a working lysis solution for the desired number of samples in volumes per sample:
  - 400 microliters of pre-warmed buffer AP1
  - one microliter RNaseA
  - one microliter Reagent DX.

Add beads to each lyophilized leaf sample and add caps to sample block



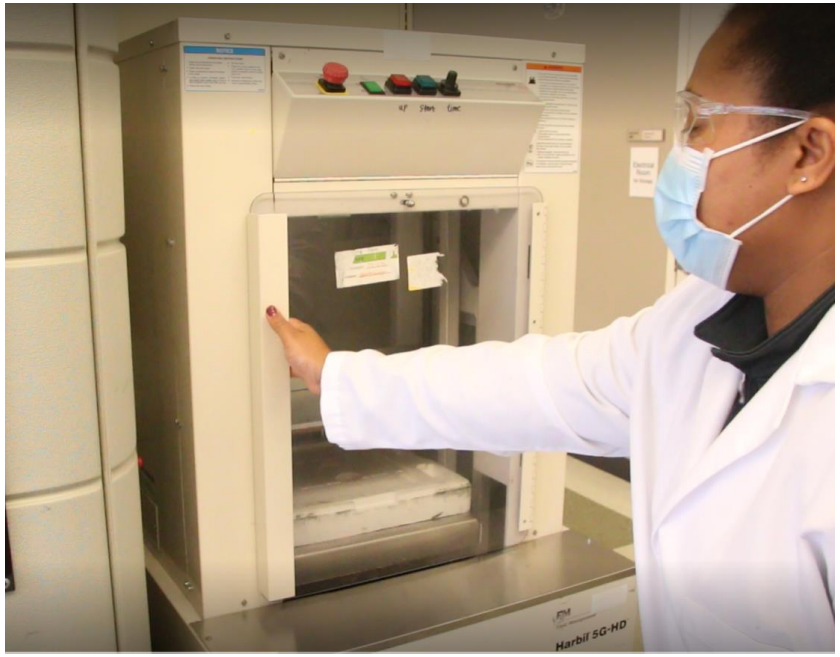
- Add a single steel bead to each sample.
- While these pictures show the 96-well leaf collection plate from the kit, other 96-well plates can be used for collection of leaf tissue and are suitable for the leaf grinding step.

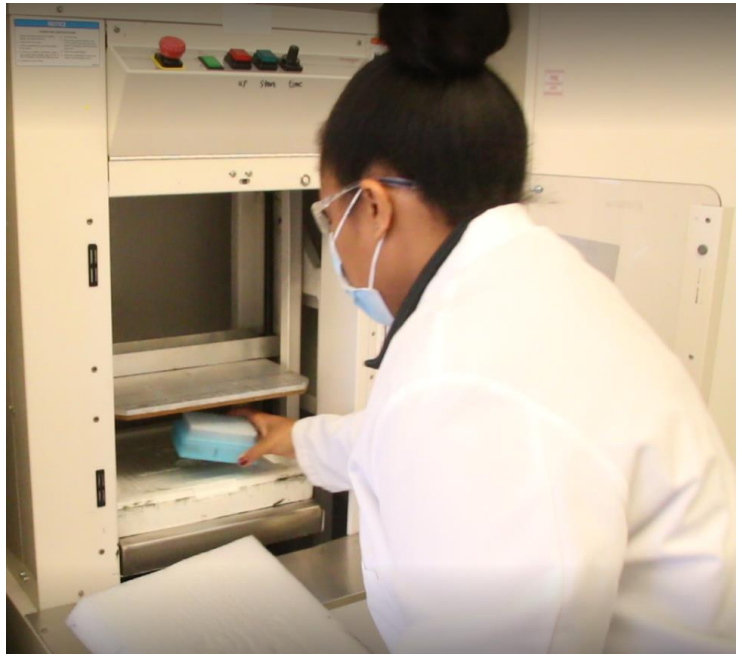
Capped block ready for grinding



- Add caps to wells to seal each well for grinding.
- If using a 96-well block, use a cap mat to seal the plate

Load shaker and shake for 4 minutes



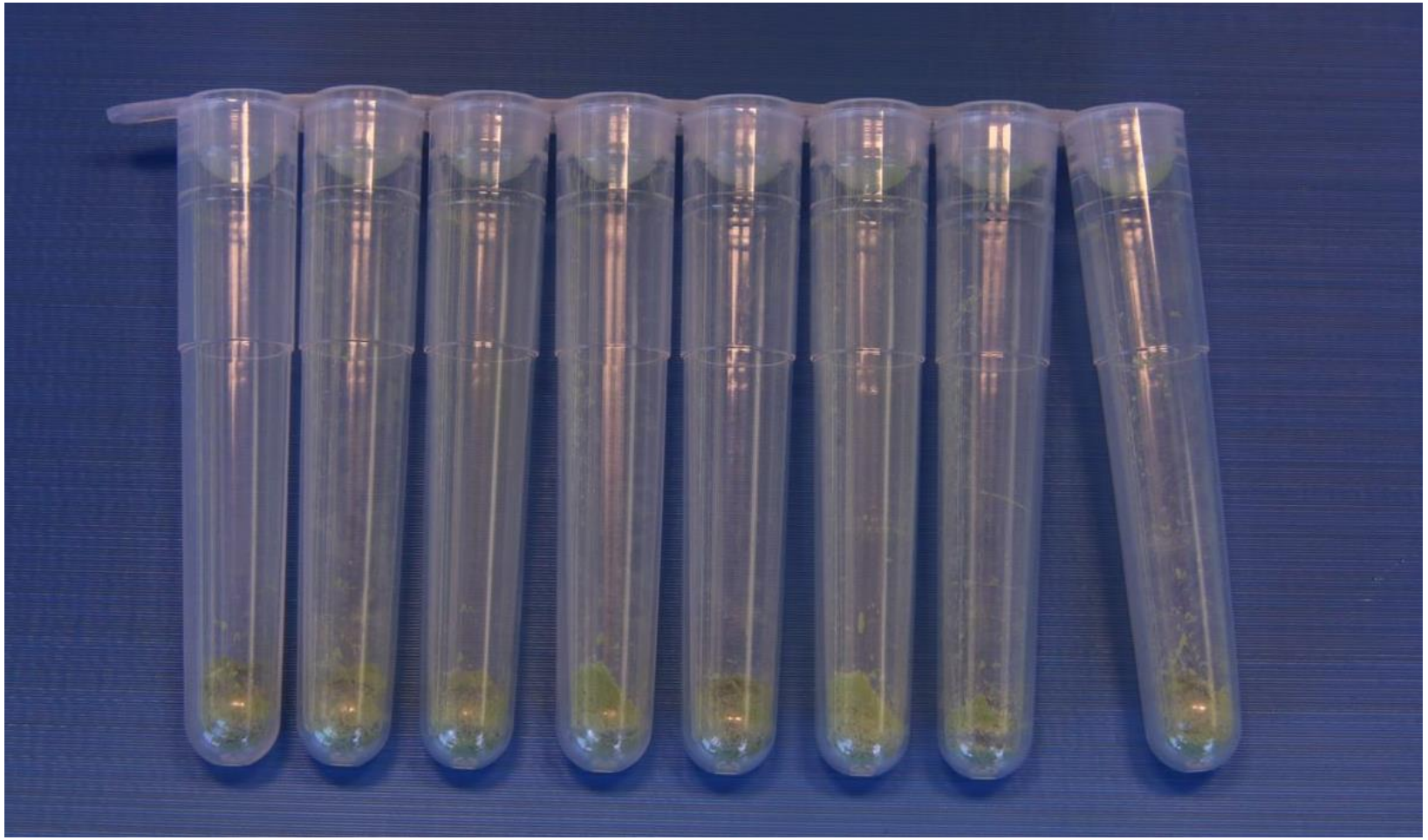


- Secure plate in Harbil Shaker and grind tissue by shaking for four minutes

Centrifuge ground tissue

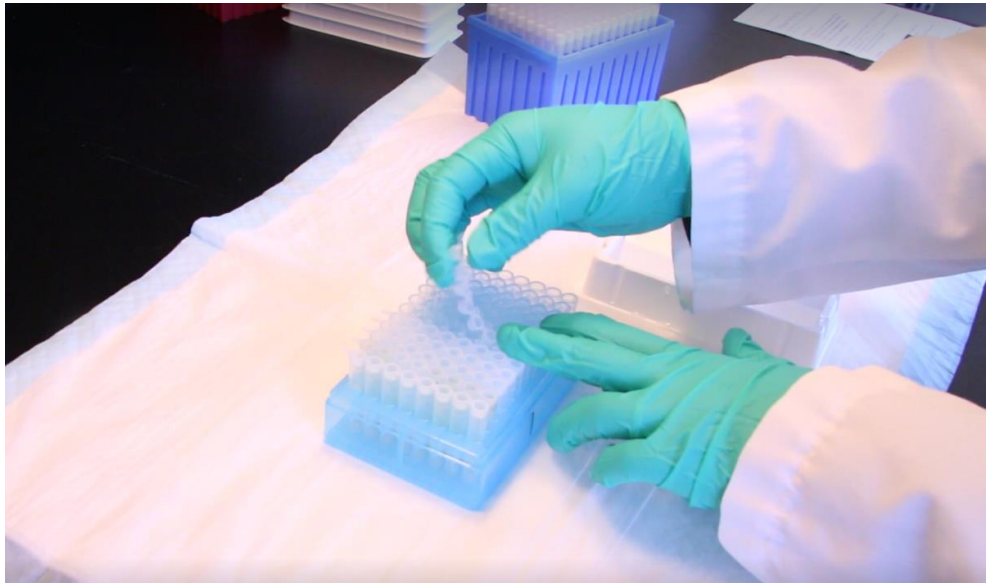
Centrifuge the plates with ground tissue at two thousand revolutions per minute for one minute to remove tissue powder from cap

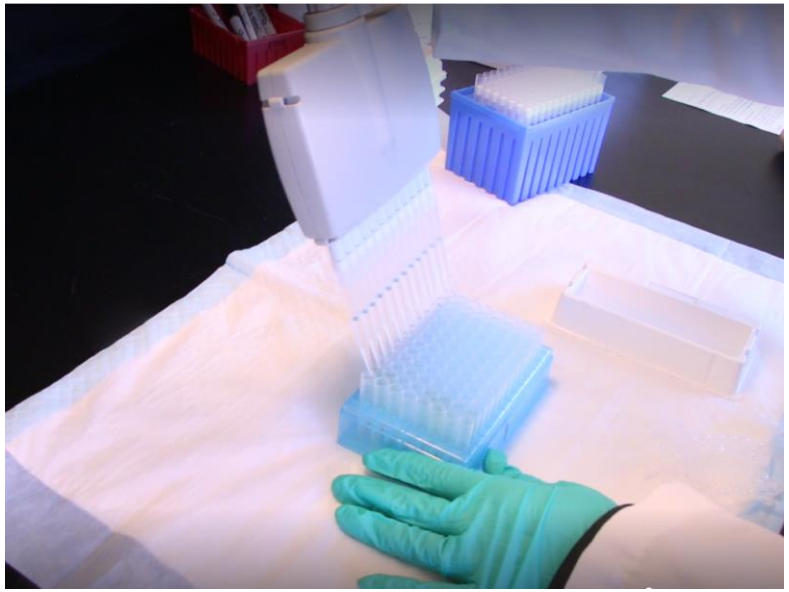
Example of lyophilized tissue after grinding



This is an example of how ground tissue should look after shaking

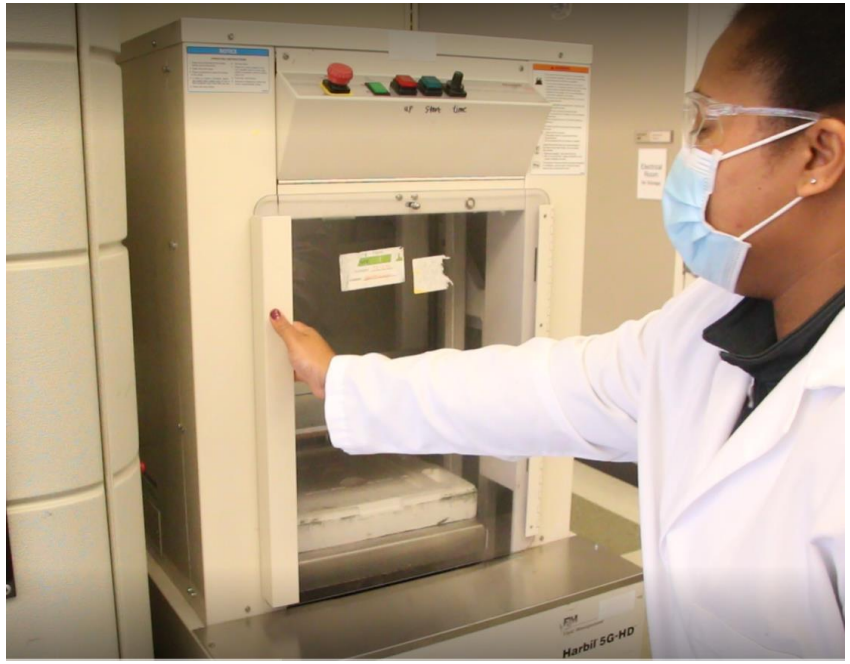
Uncap tube block and add lysis solution

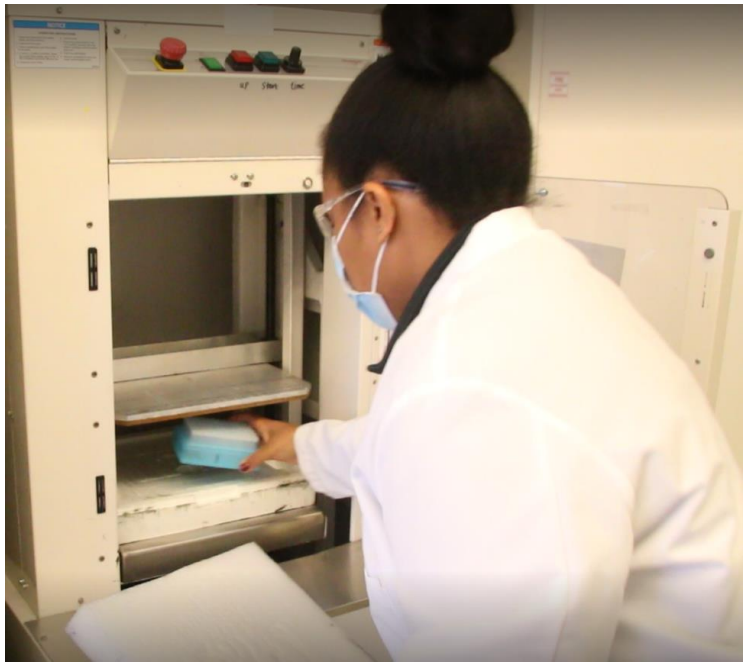




- Carefully remove the caps from the plate of ground tissue
- Pipette four hundred microliters of working lysis solution into each sample well
- Seal with caps or capmat.

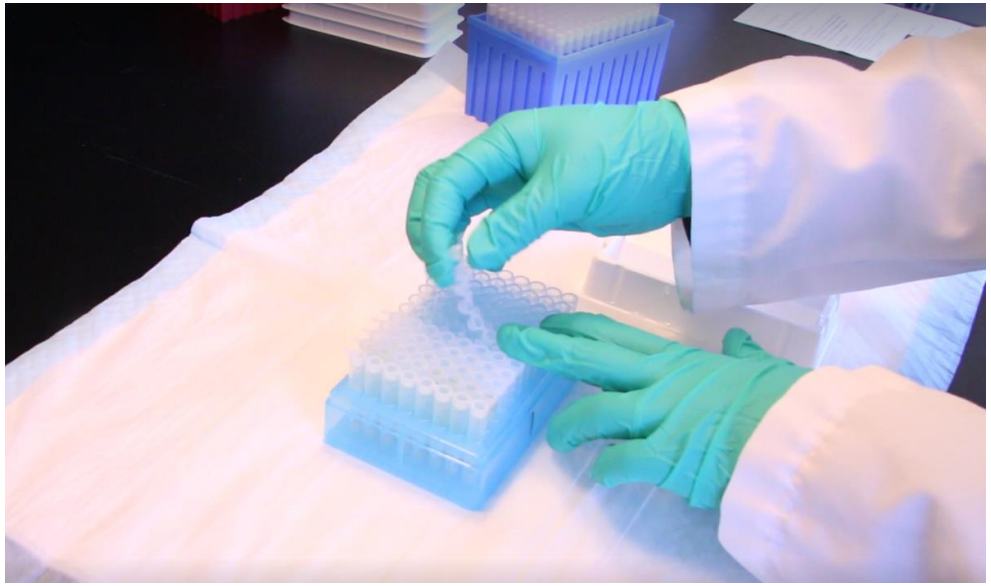
Load shaker and shake for 1 minute

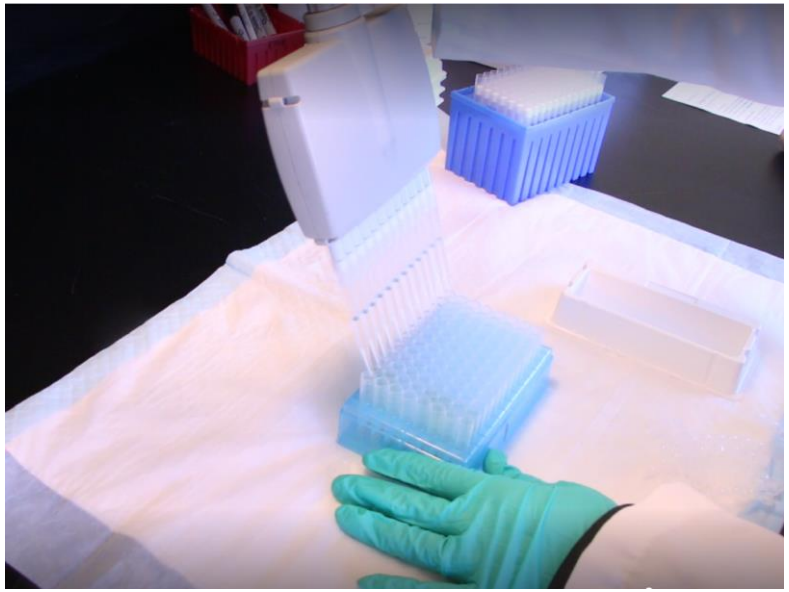




- Secure plate in Harbil Shaker
- Grind tissue by shaking for one minute
- After shaking, incubate plate in an oven at at 65 degrees Celsius for 15 minutes

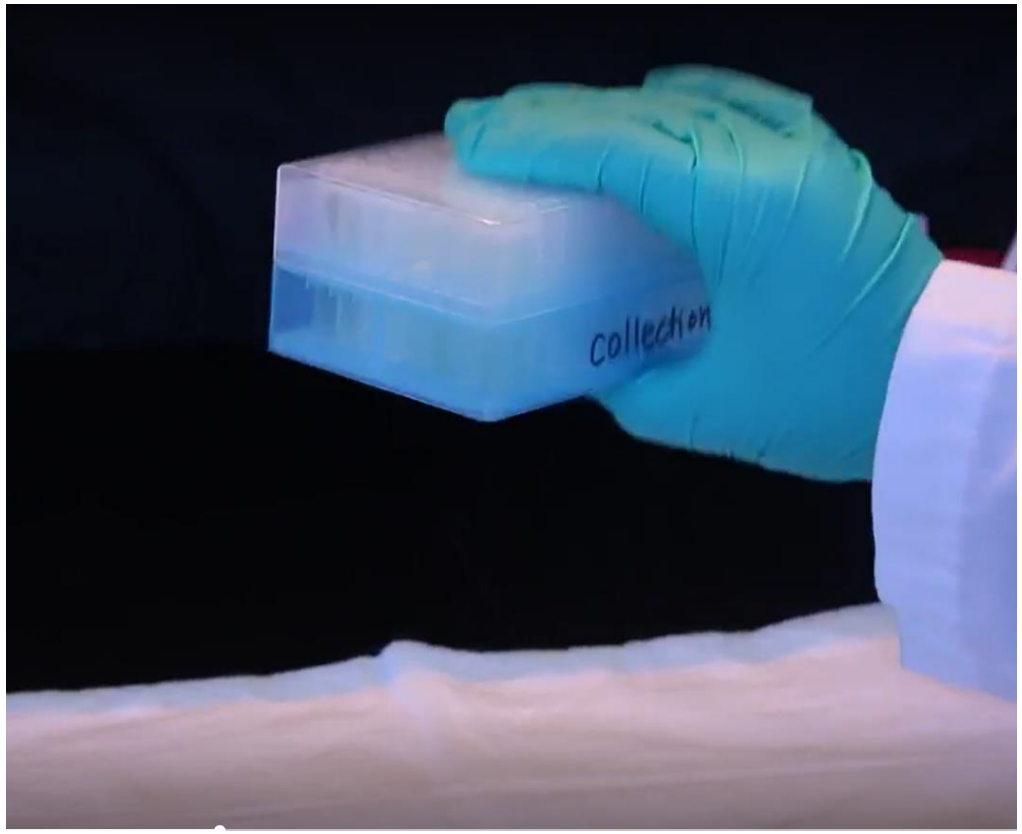
Uncap tube block and add P3 buffer solution

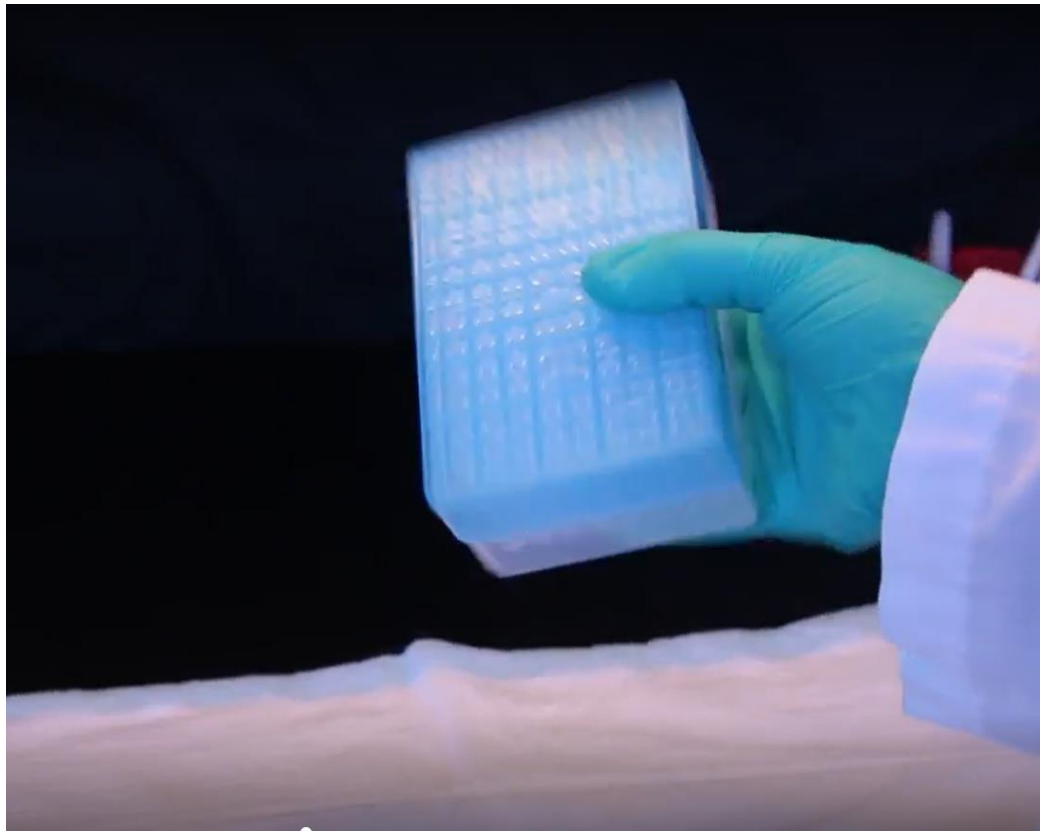




- Carefully remove the caps from the plate of ground tissue
- Pipette 130 microliters of P3 buffer into each sample well
- Seal with caps or capmat.

Shake collection block by hand



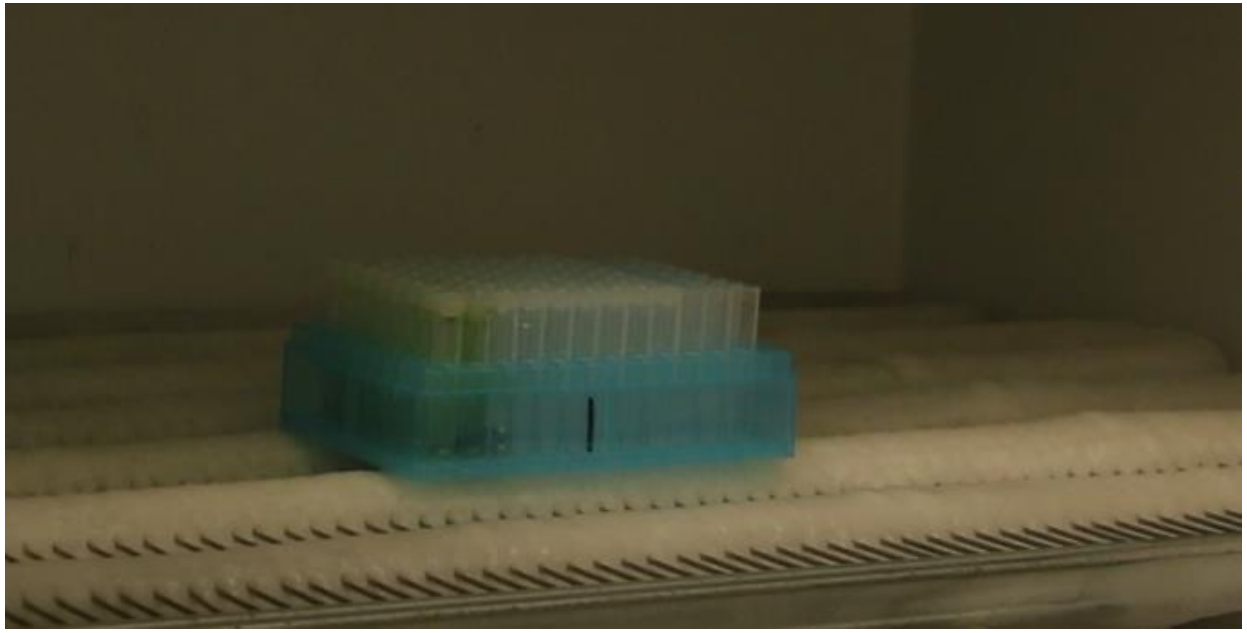


- Shake the block by hand for 15 seconds

Centrifuge samples with buffer

To collect any solution from the cap mat, centrifuge by allowing the centrifuge to reach three thousand revolutions per minute, then stop the centrifuge.

Sample block to -20C freezer

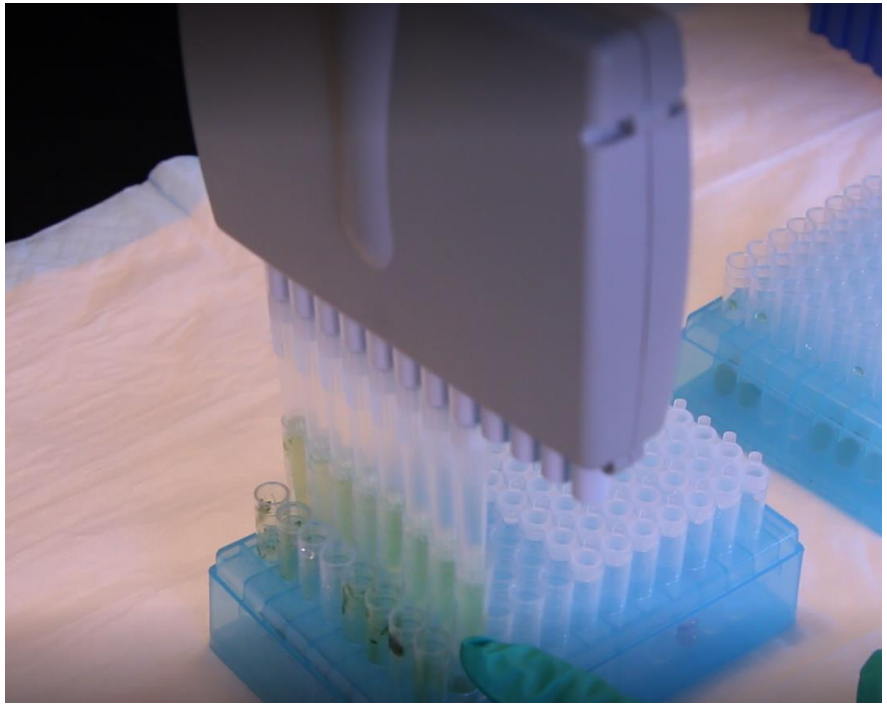


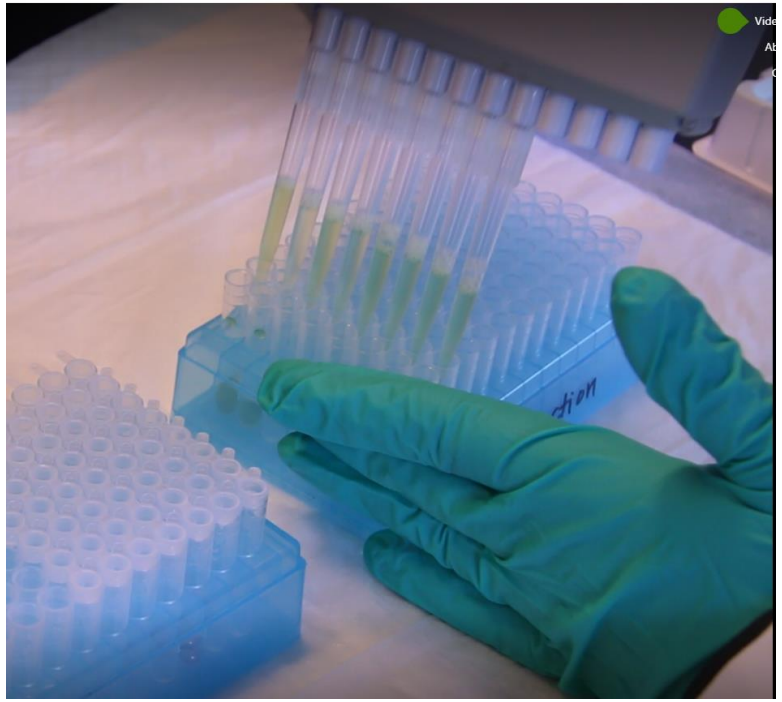
Incubate the block at minus 20 degrees Celsius for 10 minutes

Centrifuge lysed tissue

Centrifuge the lysed tissue plate at 6000 revolutions per minute for 5 minutes at 40C

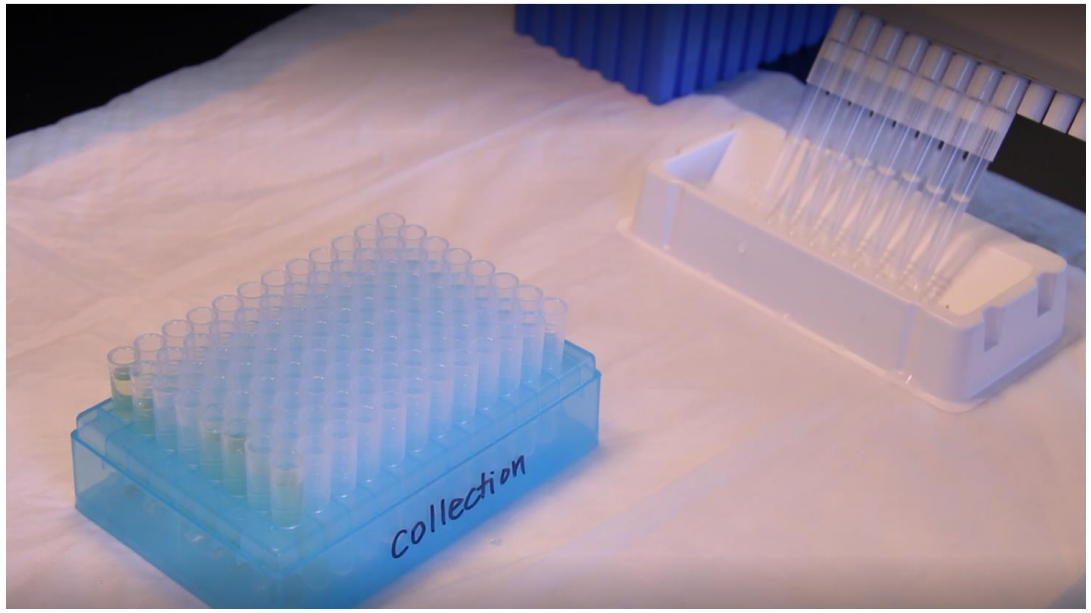
Sample Transfer to Collection Block

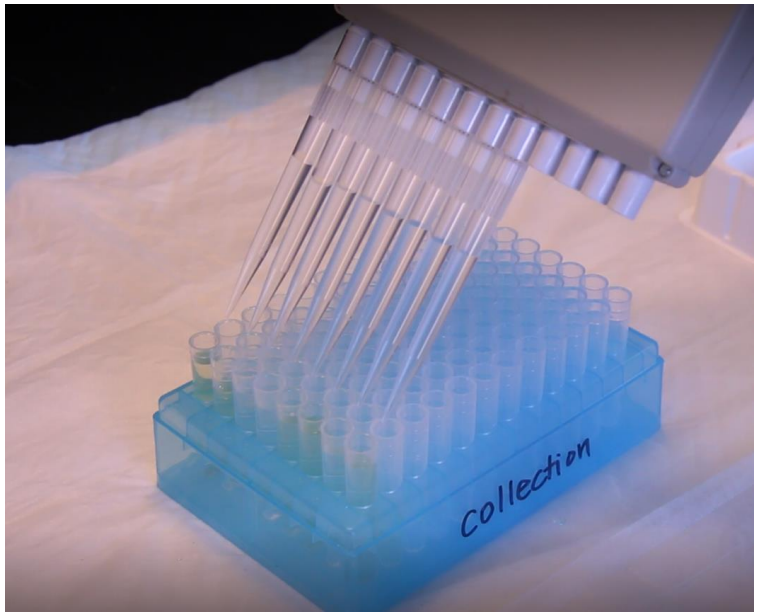




- Remove and discard caps or capmat
- Transfer 400 microliters of each supernatant to a new rack of collection microtubes

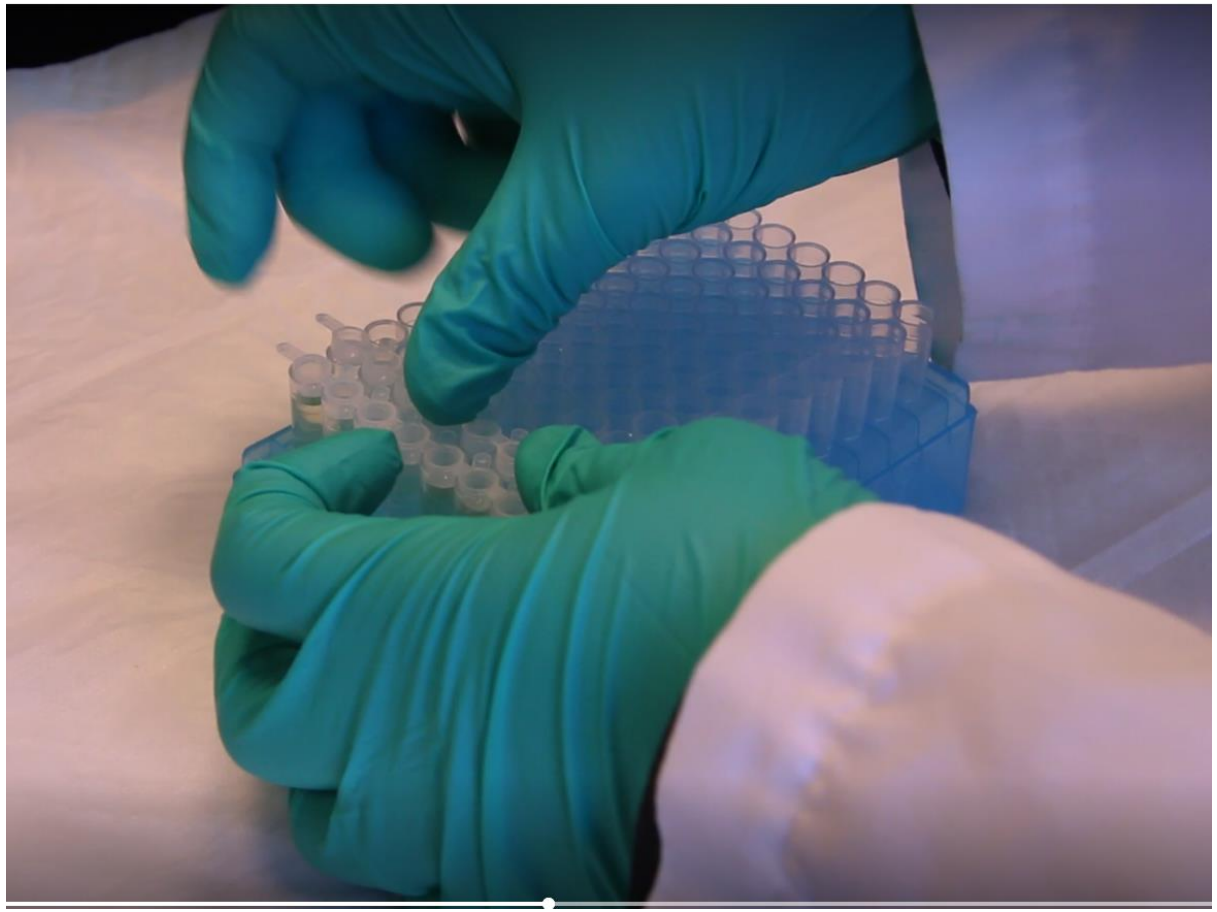
Buffer Addition to Collection Block





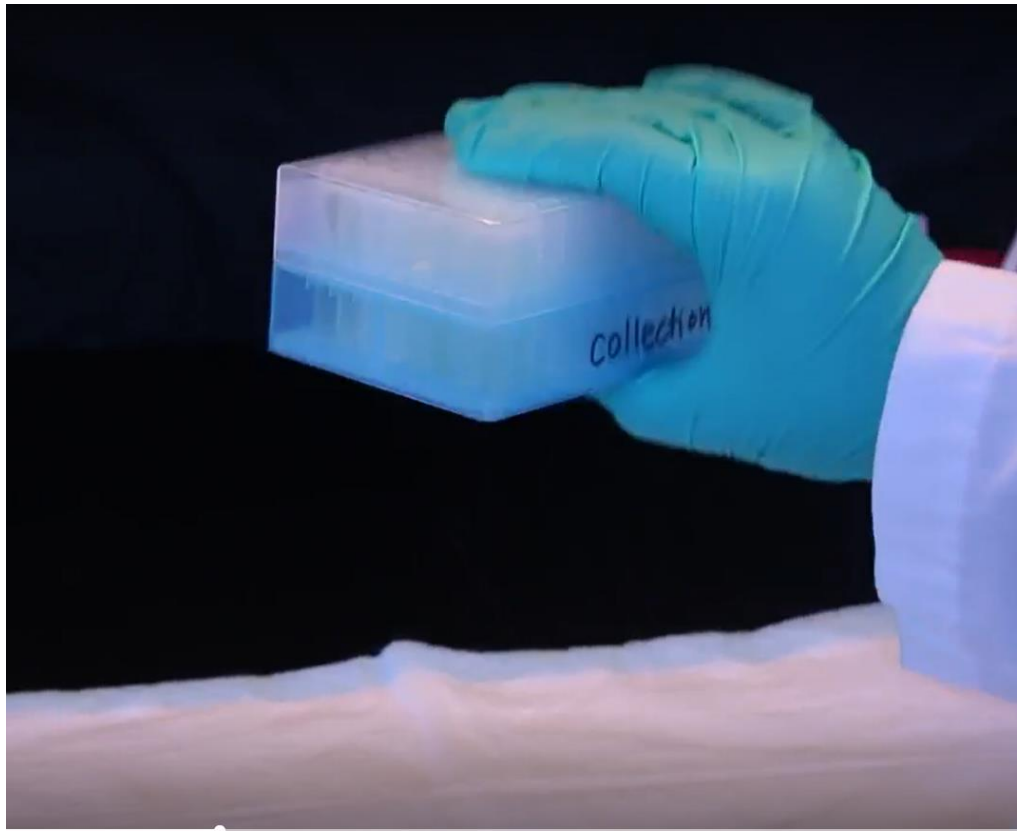
- Add 600 microliters of Buffer AW1 to each sample
- Ensure that ethanol has been added to Buffer AW1 prior to use.

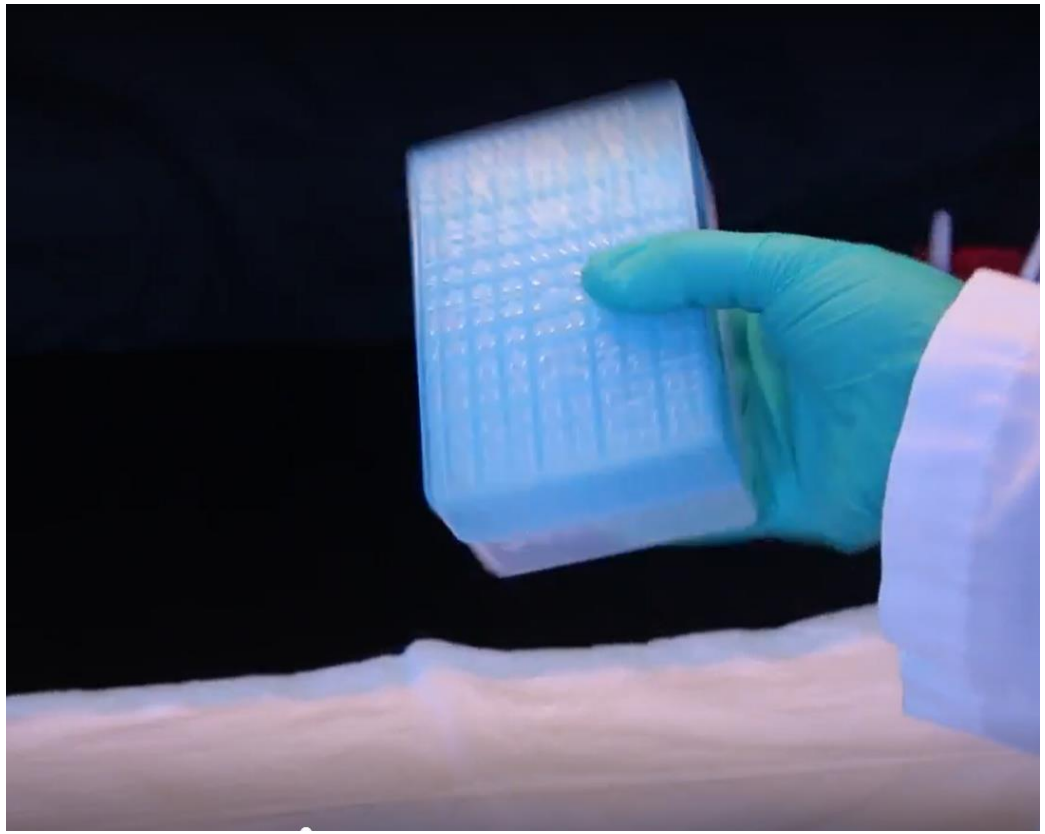
Capping Collection Block



Add caps to the collection box

Shake collection block by hand



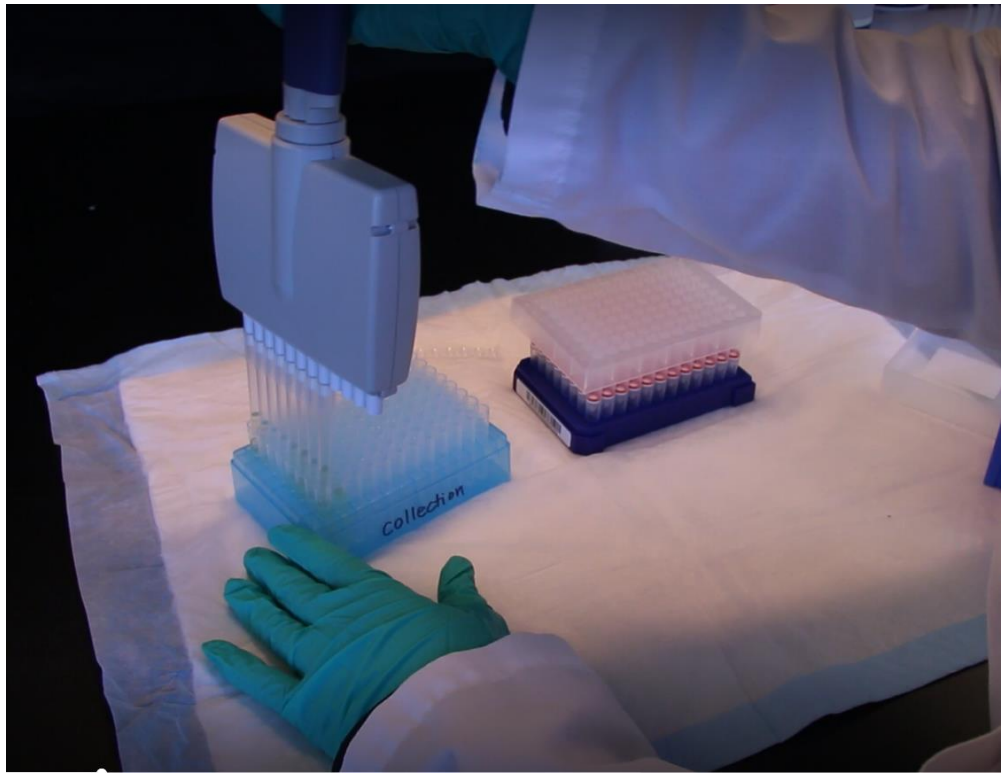


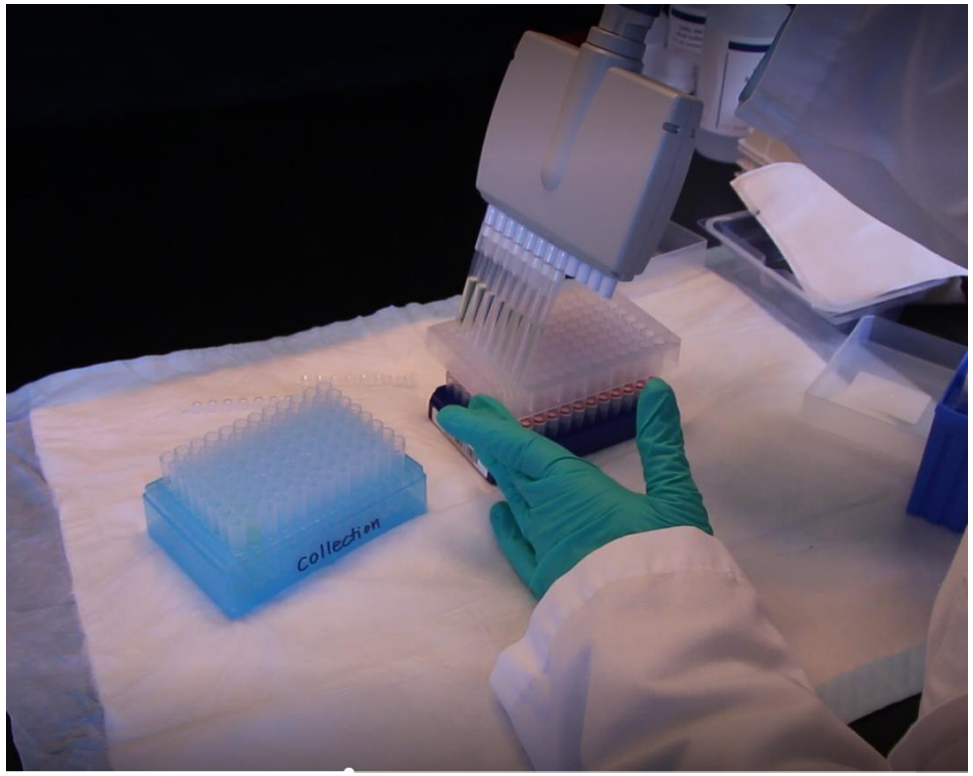
- Shake the block by hand for 15 seconds

Centrifuge Collection Block

To collect any solution from the cap mat, centrifuge by allowing the centrifuge to reach three thousand revolutions per minute, then stop the centrifuge.

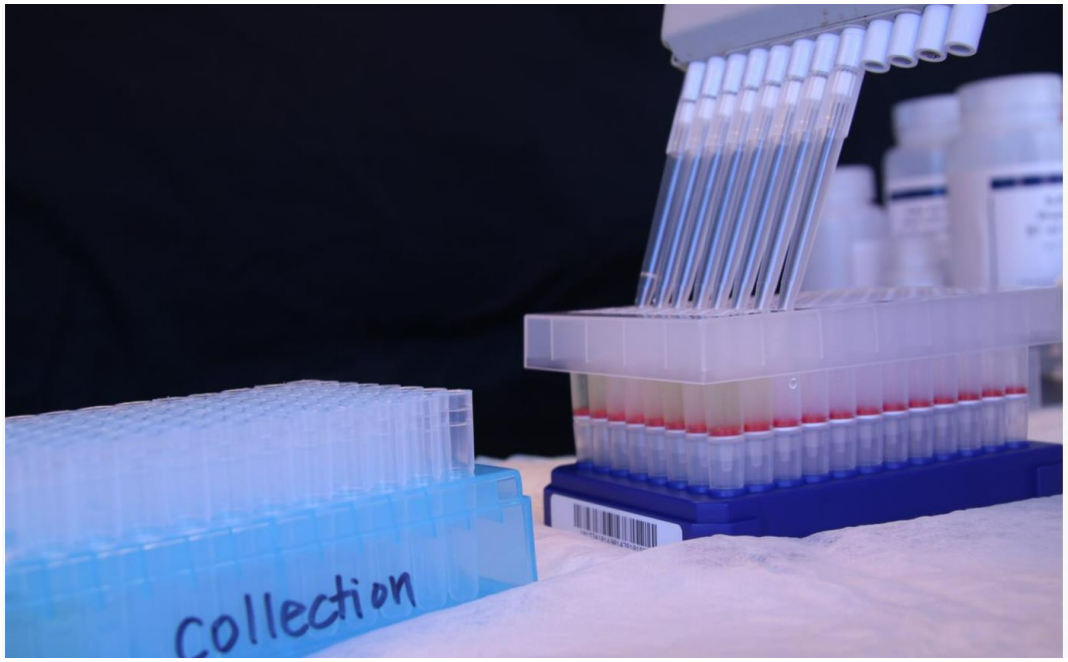
Transfer from Collection Block to Filter Block

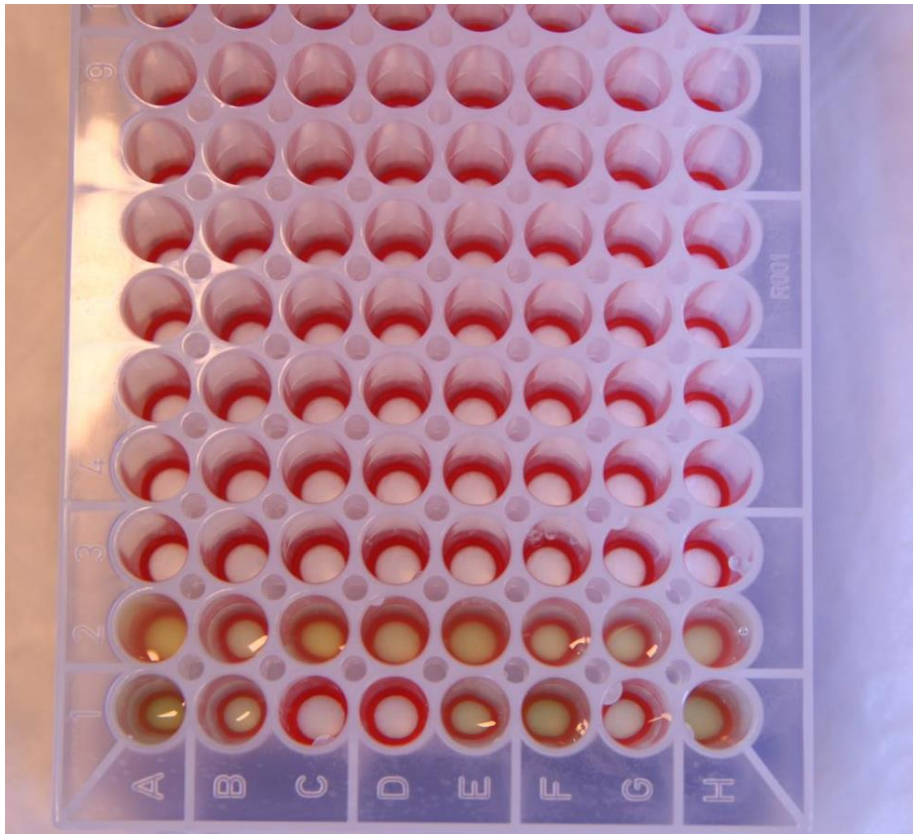




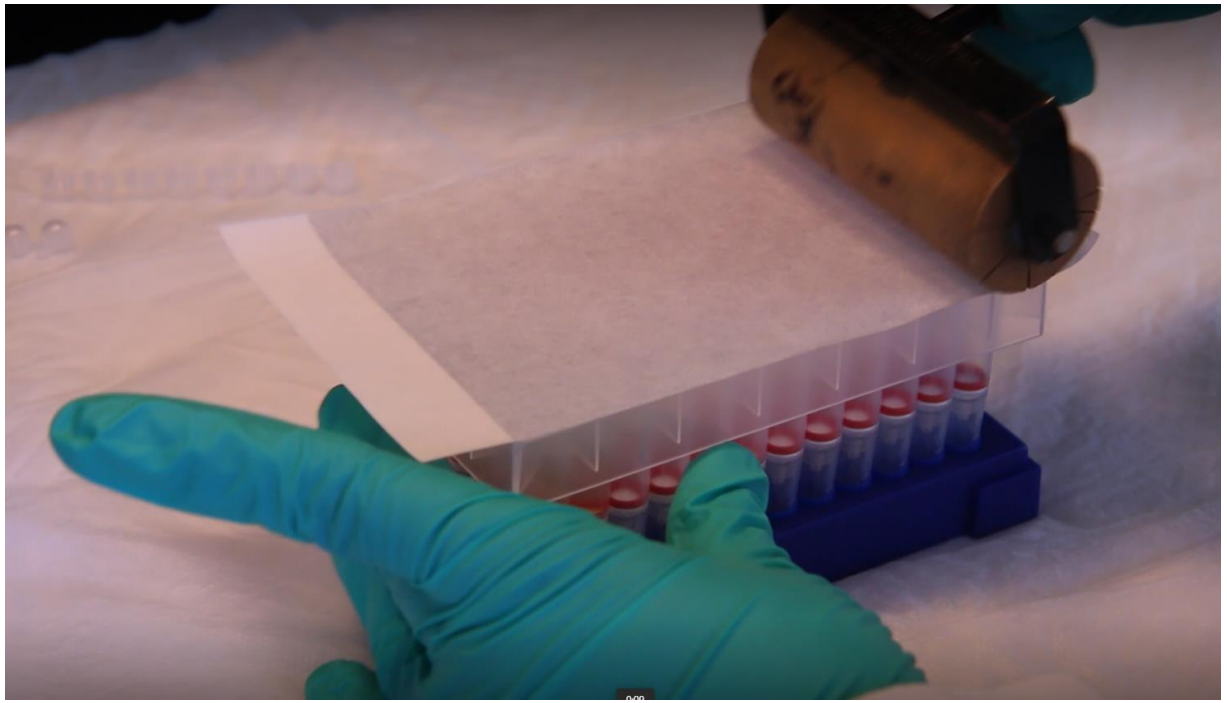
- Place a DNeasy 96 filter plate on top of an S-Block.
- Remove and discard the cap mat from the collection microtubes.
- Transfer 1 milliliter of each sample to each well of the DNeasy 96 filter plate.

Closeup pictures of the filter plate collection step





Seal Filter Block

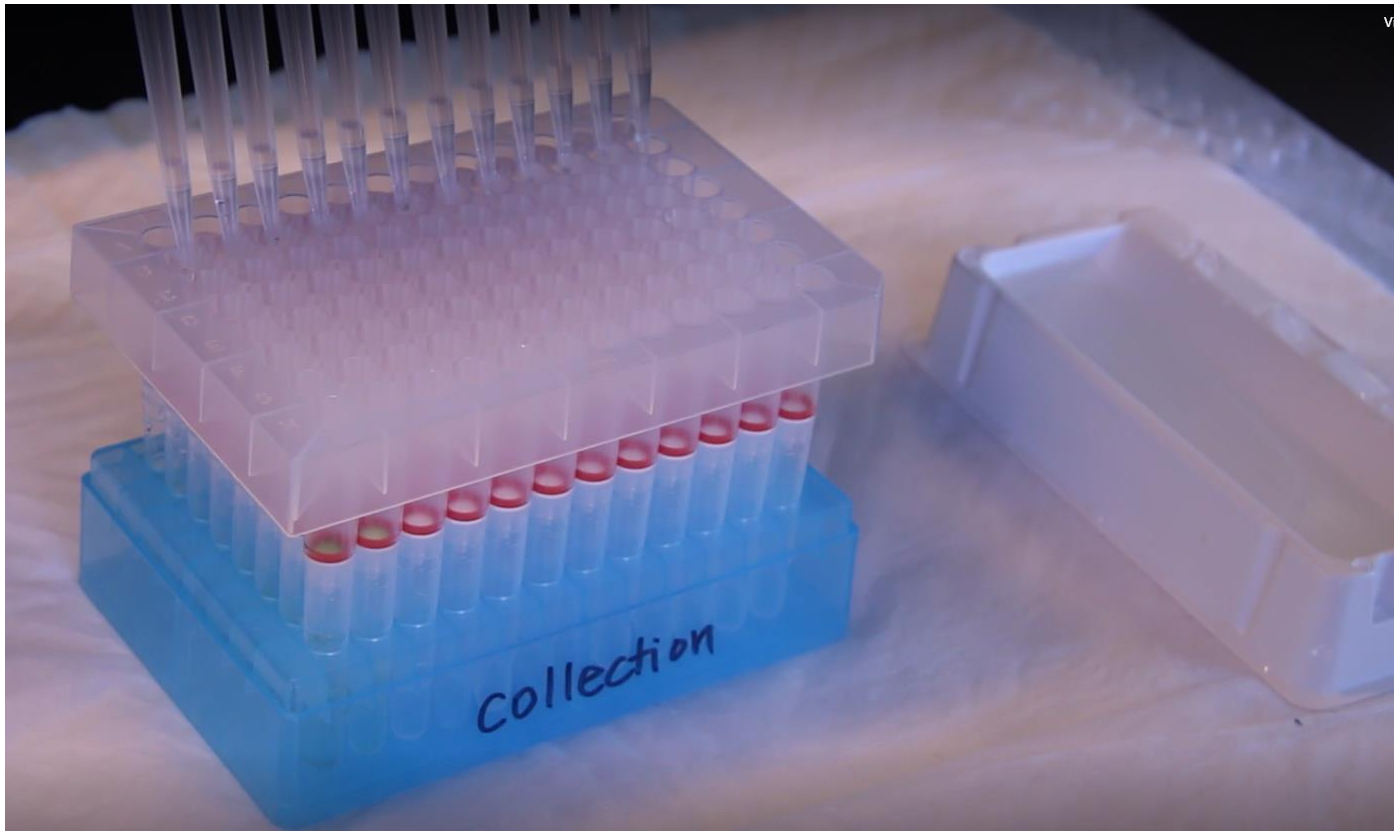


Seal the filter plate with an AirPore tape sheet provided in the kit

Load Filter Block into Centrifuge

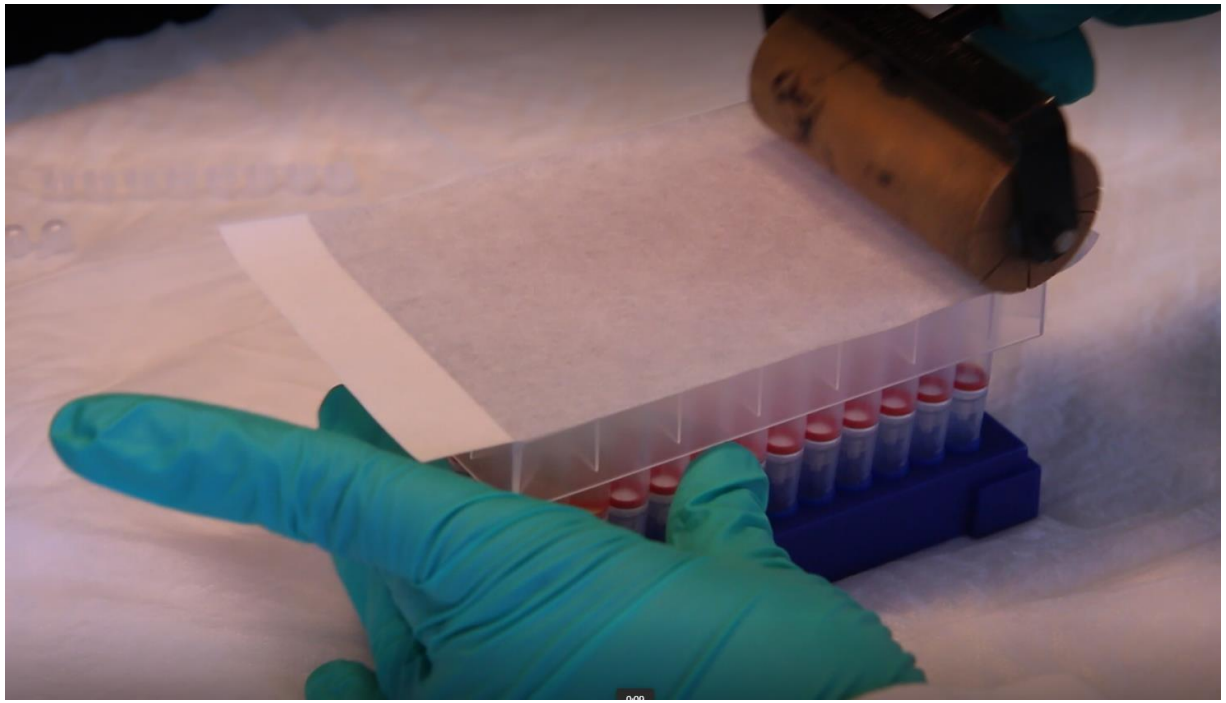
Centrifuge the collection plate at six thousand revolutions per minute for five minutes at forty degrees Celsius

Buffer Addition to Filter Block Over Tall Tubes



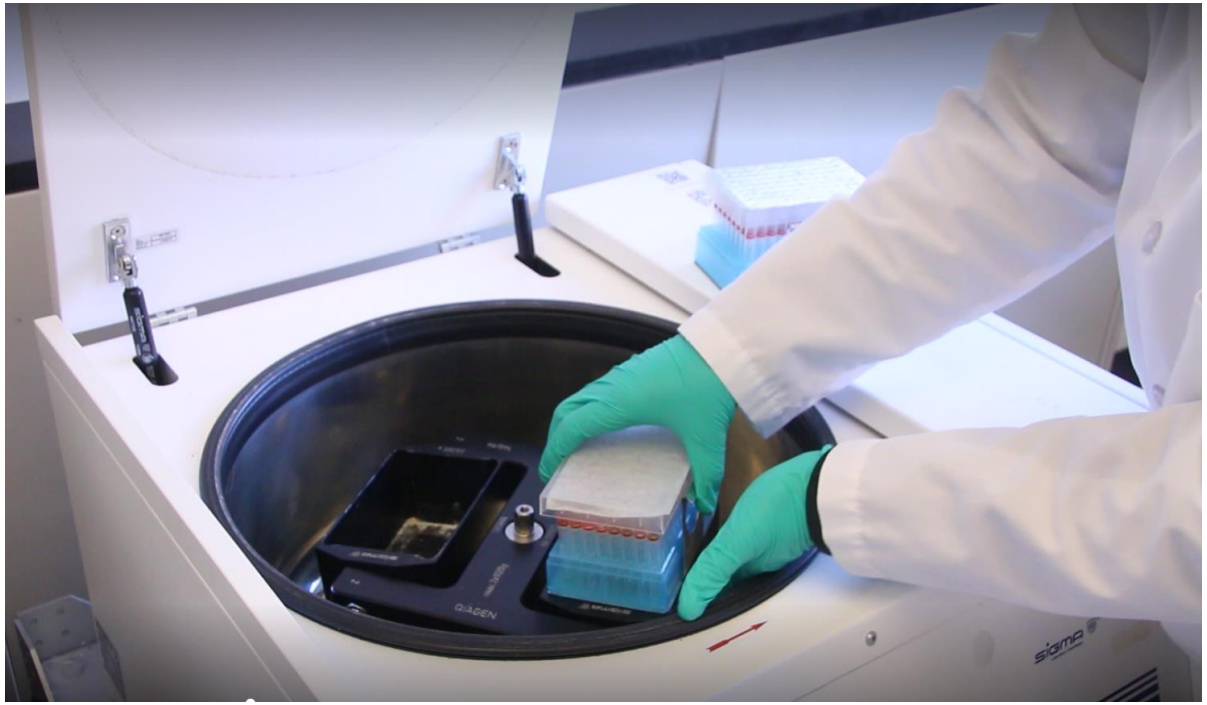
- Remove the seal and add eight hundred microliters of Buffer AW2 to each sample
- Ensure that ethanol has been added to Buffer AW2 prior to use.

Seal Filter Block



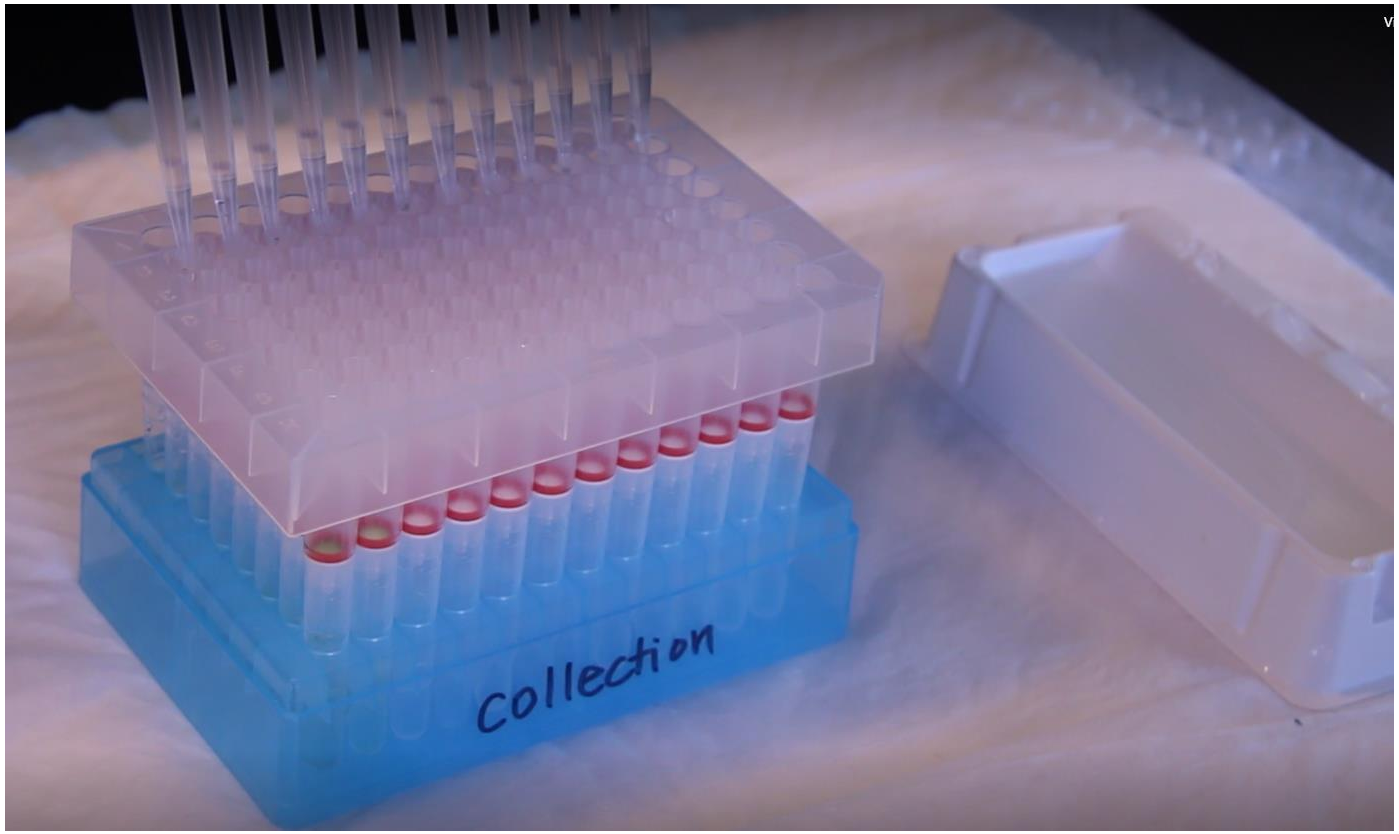
- Seal the filter plate with an AirPore tape sheet provided in the kit
- Sealing is optional for this step

Centrifuge Filter Block and Tall Tubes



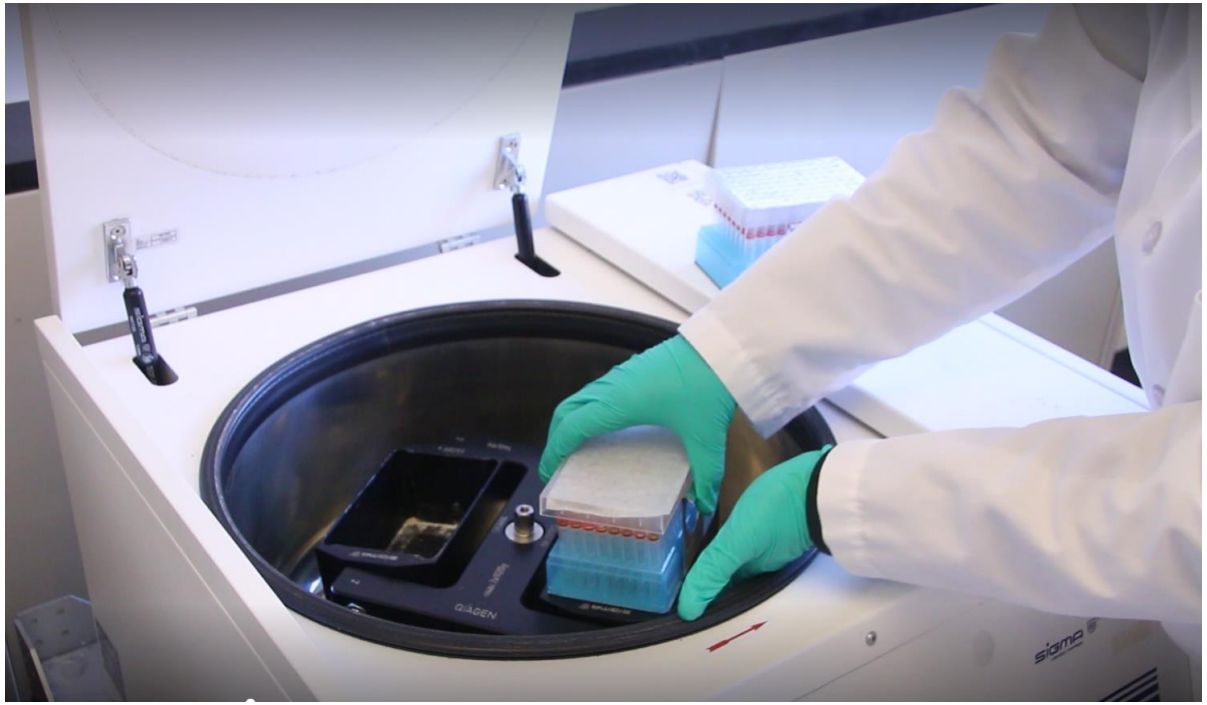
- Centrifuge the collection plate at six thousand revolutions per minute for two minutes at forty degrees Celsius.

Buffer Addition to Filter Block Over Tall Tubes



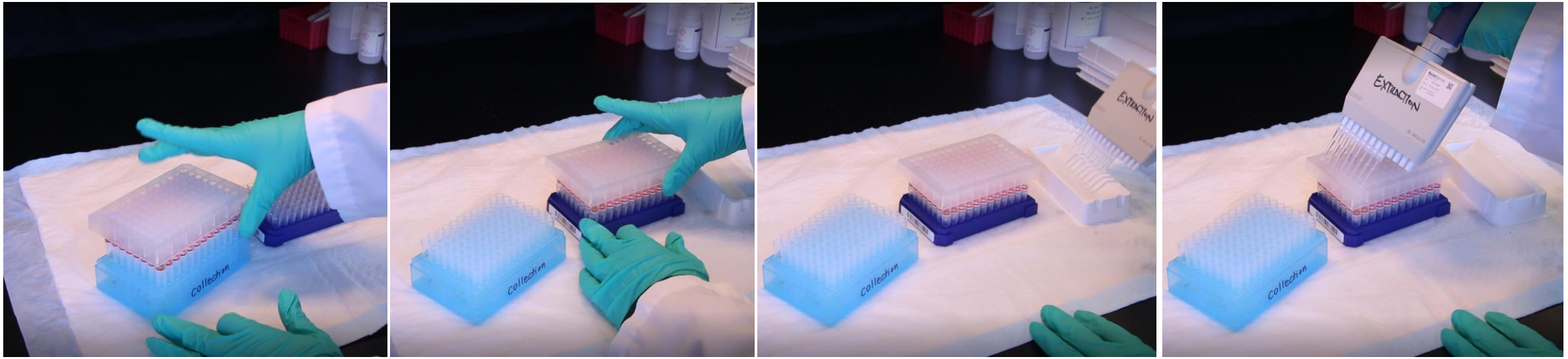
- Remove the seal and add eight hundred microliters of Buffer AW2 to each sample
- Ensure that ethanol has been added to Buffer AW2 prior to use.

Centrifuge Filter Block and Tall Tubes



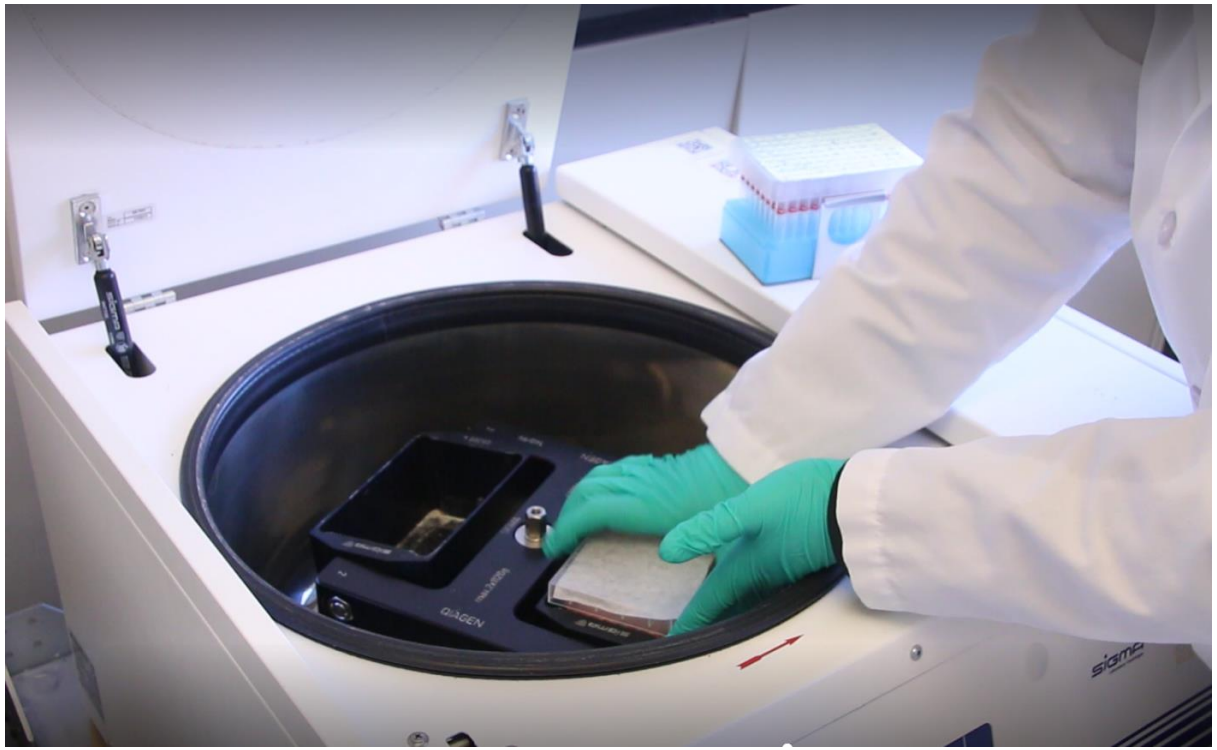
- Centrifuge the collection plate at six thousand revolutions per minute for twenty minutes at forty degrees Celsius.
- The membrane should be completely dry after centrifugation

Elution Buffer Addition to Filter Block over Short Tubes



- Remove the block to a rack of DNA collection tubes.
- Add fifty microliters of Buffer AE to each sample and seal the filter plate with an AirPore tape sheet.
- Incubate for one minute at room temperature.

Centrifuge Filter Block and Short Tubes



- Centrifuge the collection plate at six thousand revolutions per minute for two minutes at forty degrees Celsius.
- The elution step can be repeated a second time with another forty-five microliters of Buffer AE, with 1 minute incubation and repeated centrifugation.
- After centrifugation, seal the elution plate and store at minus twenty degrees Celsius until use.

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Any questions?