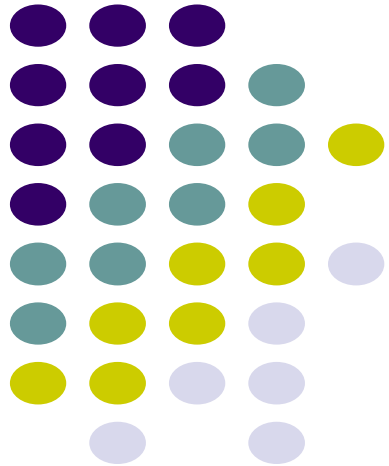
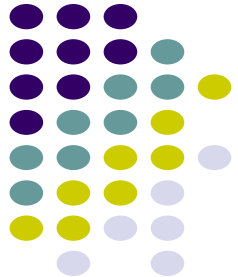
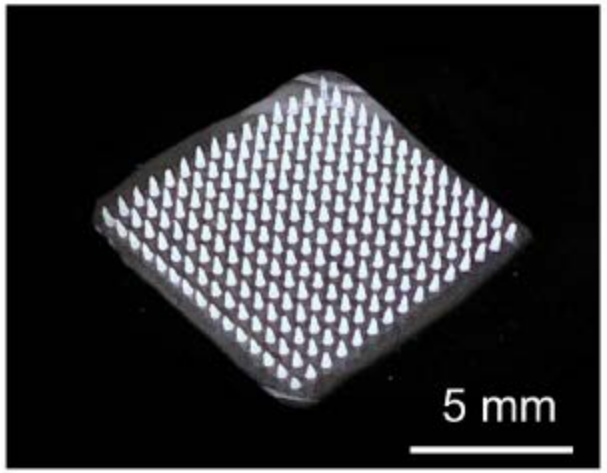
Microneedle based diagnosis

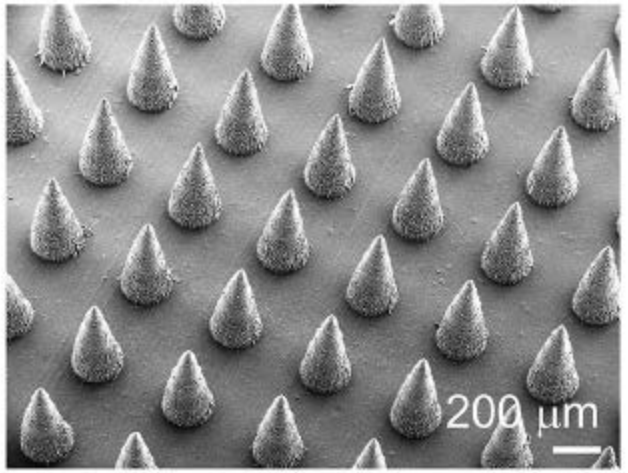




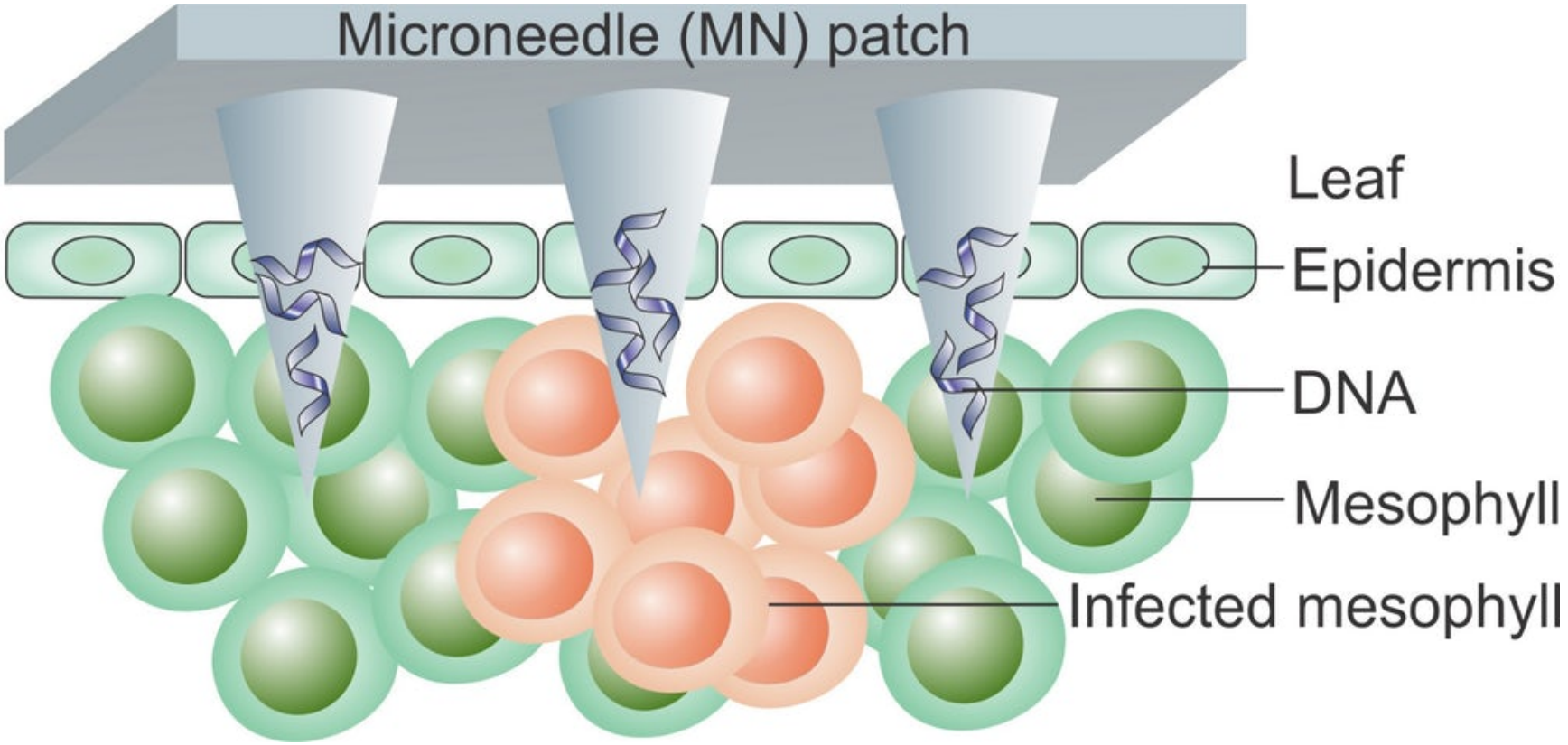
- In-field molecular diagnosis of plant diseases via nucleic acid amplification (NAA) is currently limited by cumbersome protocols for extracting and isolating pathogen DNA from plant tissues.
- To address this challenge, a rapid plant DNA extraction method has been developed using a disposable polymeric microneedle (MN) patch. By applying MN patches on plant leaves, amplification assay-ready DNA can be extracted within a minute from different plant species.
- MN-extracted DNA has been used for direct polymerase chain reaction (PCR) amplification of plant plastid DNA without purification. Furthermore, using this patch device, extraction of plant pathogen DNA (*Phytophthora infestans*) from both laboratory-inoculated and field-infected leaf samples was performed for detection of late blight disease in tomato.

- MN extraction achieved 100% detection rate of late blight infections for samples after 3 days of inoculation when compared to the conventional cetyl trimethyl ammonium bromide (CTAB-cetyl trimethyl ammonium bromide)-based DNA extraction method as a gold standard, and 100% detection rate for all blind field samples tested.
- This simple, cell lysis-free, and purification-free DNA extraction method could be a transformative approach to facilitate rapid sample preparation for molecular diagnosis of various plant diseases directly in the field.





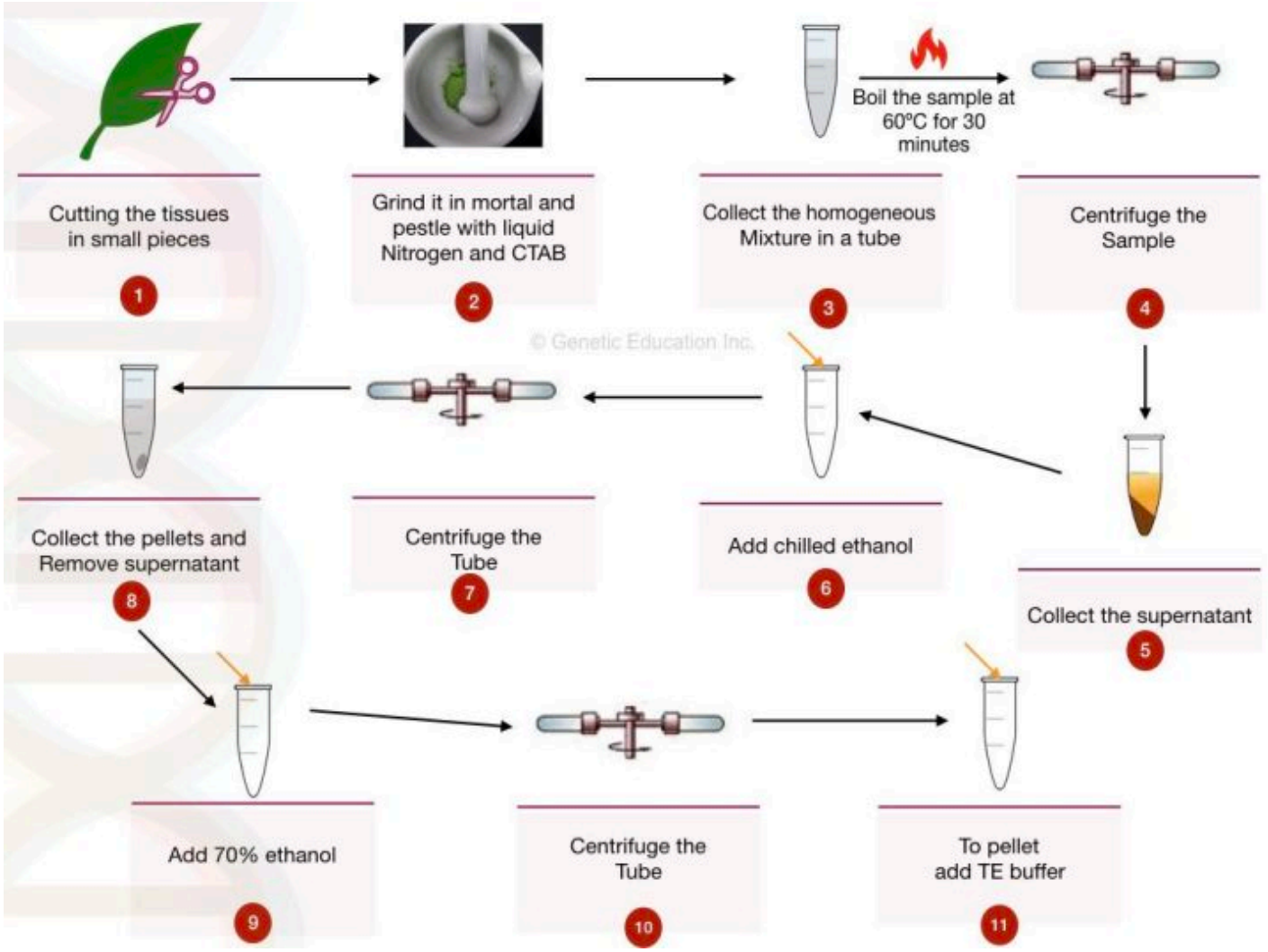
- The patch is only about the size of a postage stamp and is made of an inexpensive polymer.
- The surface on one side of the patch is made up of hundreds of needles that are only 0.8 millimeters long.



A diagram depicting how the microneedles gather DNA

- The MN-method has advantages over the CTAB method as:
  - DNA extraction by CTAB method requires a lot of equipment and
  - takes several hours.
  - Further, CTAB extraction is a multi-step process involving tissue grinding to organic solvents and centrifuges.
- By contrast, the new MN-based DNA extraction technique involves only few steps and requires only a microneedle patch and an aqueous buffer solution.

Brief outline of the CTAB method of DNA extraction



- A farmer or researcher can apply the microneedle patch to a plant they suspect is diseased, hold the patch in place for a few seconds, then peel it off. The patch is then rinsed with the buffer solution, washing genetic material off of the microneedles and into a sterile container. The entire process takes about a minute.
- The microneedle technique’s purity levels were comparable to other, validated laboratory methods of DNA extraction. Most importantly, the slight difference in purity levels between the microneedle and CTAB samples did not interfere with the ability to accurately test the samples by a PCR or LAMP assay

- Since, DNA extraction has been considered as a significant hurdle to the development of on-site testing tools, the MN-based technology has given a solution to the problem
- It is an integrated, low-cost, field-portable device that can perform every step of the process from taking the sample to identifying the pathogen and reporting the results of an assay.
- Further, LFD could be used to accurately detect the pathogen DNA extracted through the MN technology.