Quick Extraction of DNA Using Microneedle Patches

Microneedle patches are small pieces of water-soluble plastic designed for quick extraction of DNA from leaves via small needles on the patch's surface, which can puncture leaf cells (Fig. 1). A microneedle patch can be pressed into a leaf and then rinsed with buffer to extract DNA. The elution from this process can then be used for downstream applications such as PCR or LAMP.

Microneedle patches work best with soft, herbaceous leaves. Dry leaves or leaves with a waxy coating (e.g. Rhododendron) typically do not work as well with this extraction technique.

Note: Microneedle patches can dry out and become brittle if they are left exposed to air for too long. For long term storage, patches should be kept in a sealed container (Falcon tubes work well for this) in a refrigerator at 4°C. To check if a tube of patches has gone bad, take one patch and gently attempt to bend it. Patches should be pliable and flex easily.

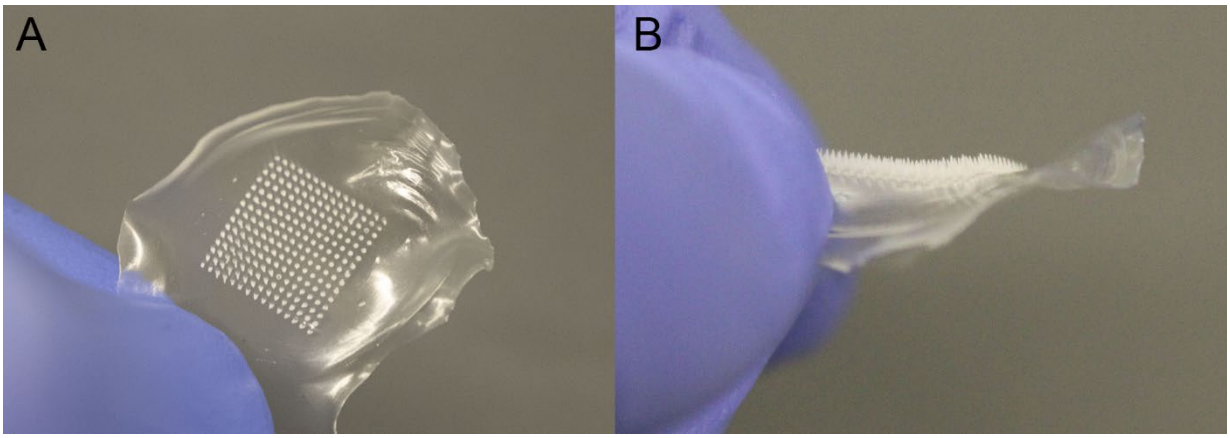

Fig. 1. Top (A) and Side (B) view of a microneedle patch.

Materials

- Leaf sample
- Microneedle patch
- 100 or 200µL pipette and tips
- 1.7mL tubes
- Buffer appropriate to your extraction
    - For DNA, any buffer suitable to your target and application (e.g. Tris buffer, TE, Te)
    - For RNA, use molecular grade water
- Petri dishes (optional – for providing a clean surface)

Procedure

1.) Select a location on the leaf to extract from. If extracting from a lesion, typically a region that is actively sporulating or near the edge of the lesion is best. Place the patch needles down onto the leaf (Fig 2A). Looking at the patch from the side can help with determining which side the needles are on.
2.) Press the patch into the leaf with your thumb or other blunt object. Apply firm pressure. Rolling your thumb or the blunt object may also be useful. Press for approximately ten seconds (Fig 2B).
3.) Remove the patch from the leaf and place face up on a clean surface (Fig 2C).
4.) Carefully apply approximately 60µL of your chosen buffer to the patch (Fig 2D). Rinse the patch by pipetting up and down onto the patch from 30 seconds to 1 minute. Remove the rinse and place in a 1.7mL tube and store on ice or in a freezer.
NOTE: The buffer rinse volume can be adjusted with the recognition that 1.) The needles will absorb a small amount of liquid, so you will have a slightly lower volume when you finish; 2.) The larger the volume, the more dilute the DNA will be.

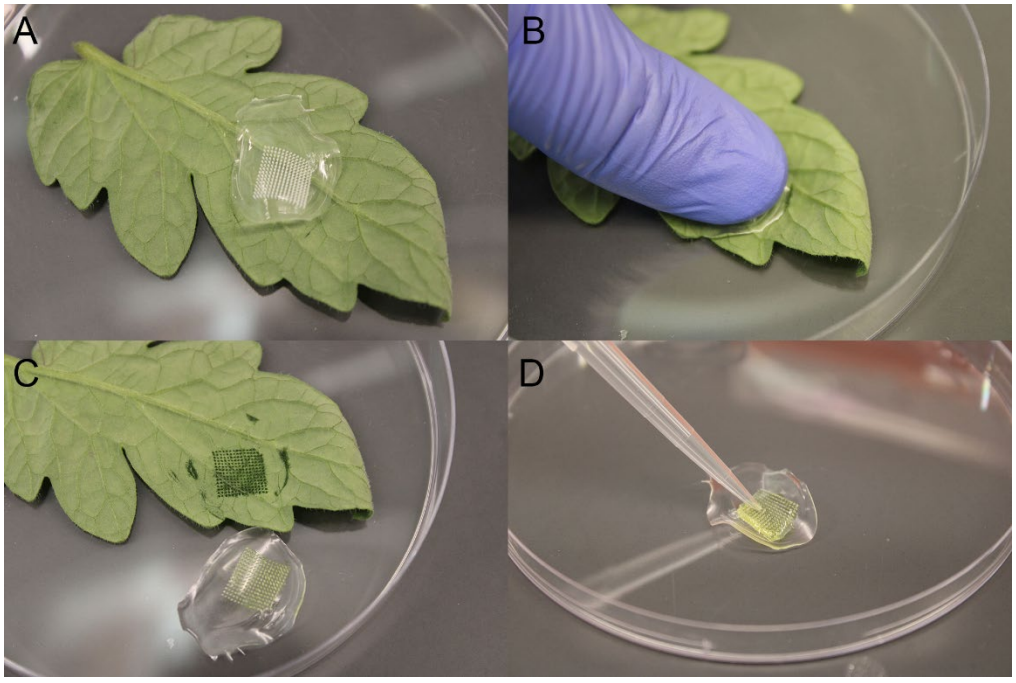

Fig 2. Microneedle extraction from a tomato leaf: A.) Placement of patch on leaf. B.) Pressing patch. C.) Removal of patch from leaf. D.) Rinsing patch with buffer.