DNA Extraction: Sodium Hydroxide (NaOH) Method

Reagents:

0.5N NaOH

Mix 2g NaOH pellets with 100ml dH_2O

100 mM Tris buffer, pH 8.0

Make a 1:10 dilution of 1M Tris-HCl (pH 8.0) stock

Te Buffer (pH 8.0): 100 ml

10 mM Tris	1 ml of 1 M Tris-HCl (pH 8.0)
0.1 mM EDTA	20 µl of 500 mM EDTA (pH 8.0)
ddH_2O	98.98 ml

For Leaves, Fruit, and Stems:

1.) Locate a lesion – you will want to sample from the edge of the lesion, where there is still healthy tissue.
2.) Using forceps or your fingers, remove a piece of leaf tissue roughly 2mm in diameter. There should be both healthy and diseased tissue. Place the sample in a clean 1.5ml tube.
3.) Add 90µl of 0.5N NaOH to the tube. Grind tissue using a sterile Konte pestle until sample is liquefied. You may still see bits of the tissue in suspension. This is okay.
4.) IMMEDIATELY transfer 3µl of the ground leaf tissue solution to a new tube containing 300µl of 100mM Tris buffer, pH 8.0. Flick or gently shake the tube until contents are well mixed.
5.) Place tube on ice.

For Potato Tubers:

1.) Locate a lesion – you want to sample from the edge of the lesion, where there is still healthy tissue.
2.) Using a scalpel, carefully remove a piece of tuber tissue roughly 2mm in diameter. There should be both healthy and diseased tissue. Place the sample in a clean 1.5ml tube.
3.) Add 100µl of Te buffer and crush the tissue sample using a sterile Konte pestle until liquefied. You may still see bits of tissue in the suspension. This is okay.
4.) Transfer 50µl of the liquefied sample to a new tube. Add 90µl of 0.5N NaOH and mix for approximately 30 seconds.
5.) IMMEDIATELY transfer 3µl of the ground tuber tissue to a new tube containing 300µl of 100mM Tris buffer, pH 8.0. Flick or gently shake the tube until contents are well mixed.
6.) Place tube on ice.

Notes:

--Make sure to use a clean Konte pestle and pipette tips for each sample.
--Tubes containing 300µl of 100mM Tris buffer, pH 8.0 can be prepared ahead of time to speed extraction process.
--It is important that the sample is not left in NaOH for very long. NaOH is corrosive and will break open the cells to release DNA, but will begin to degrade the DNA if left too long. When extracting from multiple samples, add the NaOH and grind each tube one at a time, instead of adding NaOH to all tubes and grinding sequentially.