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Seed Sterilization

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol details how to surface sterilize plant seeds for tissue culture regeneration/transformation or for other techniques requiring sterile seeds.

Guidelines

The ratios of bleach and HCl used here are very appoximate and also determined empirically. If you wish, you can calculate the volume of HCl necessary to fill the chamber with 6.1% chlorine gas (according to doi:10.3791/56587)

Materials

MATERIALS

Petri Dish **P212121 Catalog #**LI-PD01100

Hydrochloric Acid

Sodium Hypochlorite Solution

- You will also need a chamber to contain the gas for the sterilization. We find that an old dessication chamber works well. Note the exposed metal will rust in this environment, so it is best to use an older chamber you don't care about.
- None of the vendors for the materials are important.
- The sodium hypochlorite solution (bleach) will vary in concetration betwee 5 and 9% depending on the vendor. We do not find that the protocol is sensitive to these variations, so use, whichever you have on hand.

Safety warnings

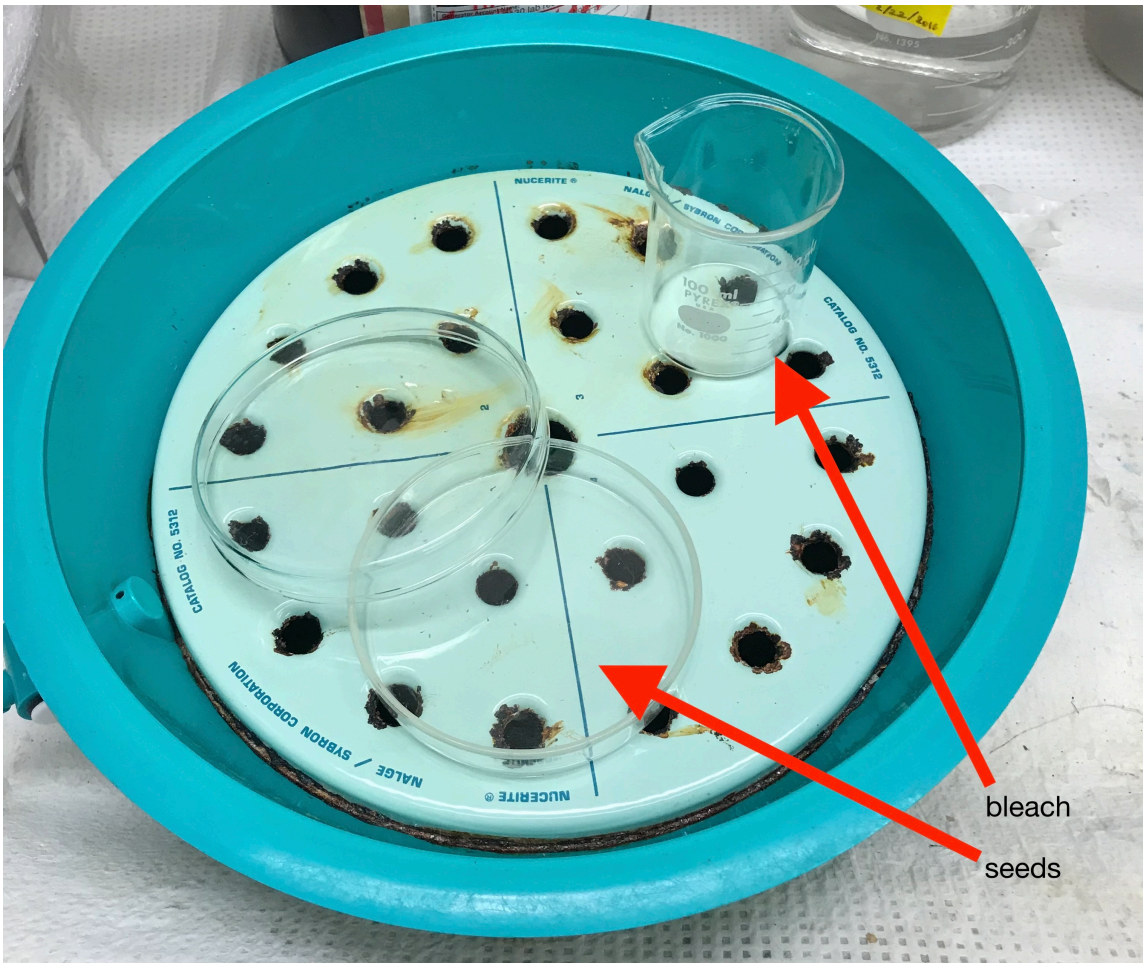
This protocol will create a chamber filled with chlorine gas, which is very toxic. It will also use concentrated HCl. Make sure that you have a proper fume hood to dispose of the gas and proper equiment to handle the HCl.

1 In a fume hood, place an empty dessication chamber with a petri dish of the seeds you wish to surface sterilize. Leave the lid of the petri dish halfway on, so that you can quickly close it later.

Note

- A dessication chamber per se is not necessary; you simply need some container to hold the gas for a few hours. It does not need to be thoroughly airtight, but it helps.
- If you plan to sterilize multiple genotypes of seeds, be aware that the gas produced will erase most markers. Pencil and paper labels work well though.

2 Place a 100mL beaker containing approximately 50 mL bleach in the chaber next to the petri dish of seeds.



3 Prepare approximately 3 mL HCl in a transfer pipet or similar.

4 The following steps must occur quickly to prevent the escape of the gas. Hold the lid to the chamber in one hand. With the other hand squirt the HCl into the beaker of bleach and quickly close the chamber to seal in the gas.

Note

The HCl and the bleach will react violently to produce the gas, do not be startled. The larger beaker should prevent any bubbles of bleach or HCl from contacting the rest of the set up.

5 Allow the seeds to be sterilized for approximately 3 hours. This time works for tomato, tobacco, and Arabidopsis seeds. The seeds may become whiter in color, this is also normal and does not seem to affect germination rates.

6 Once the time is up, quickly open the chamber and slide the lid completely onto the petri dish to seal it. The seeds can be stored like this for several weeks or transfered to a laminar flow hood for further use. Allow the chmaber to air out for an hour or so.

7 If the reaction of the bleach and HCl went to perfect completion, the beaker should only contain salt water. In reality it is likely acidic. Dispose of this according to your local regulations.