Colony PCR for *Bacillus subtilis*

1. Mark colony of interest on your agar plate.
2. Put one colony into 10 ul of weak Tris-HCl (I use the elution buffer of Qiagen kit, could be also be water).
3. Put the tube 5 min on ice.
4. 1 min in the microwave, full power
5. 30 sec on ice.
6. Repeat 3 and 4 twice (in total 3 times in the microwave).
7. 5 min on ice.
8. Take 1ul to PCR reaction.
9. Pick the positive colony from your agar plate for an o/n culture and finally a glycerol stock.

In the PCR reaction, keep the 10 min 95°C step, as it makes some of the DNAse that is released from the cells inactive.

In addition, in the end of the PCR do not let it stand at 4°C as Bacillus has thermostable DNAse that acts also after the PCR finishes.

What I do is add in the end of the reaction EDTA in final concentration of 50 mM. If you run it immediately after, then it is not necessary to add EDTA, but do not wait too long.

(This protocol was kindly provided by the lab of Prof. Ilana Kolodkin-Gal, Weizmann Institute, Israel. It was originally provided to them by Eyal Weinstock.)