TCA Protein Precipitation

The following procedure is used to precipitate proteins from the growth media of *Bacillus subtilis*.

Materials:

- 100% trichloroacetic acid (100%TCA, w/v).
- 0.1% trichloroacetic acid.
- Tris 1M.
- Ice cold Acetone.
- 1% SDS solution.

Procedure:

1. Add to the volume of the bacterial supernatant 100% TCA in order to reach a mixture of 20% TCA.
2. Incubate the mixture for 1 hour on ice.
3. Centrifuge the sample for 10 minutes at 20000xg and 4℃
4. Discard the supernatant.
5. **From this stage on make sure you don't resuspend the pellet.**
6. Add 0.1% trichloroacetic acid (enough to cover the pellet) and centrifuge for 2 minutes. Discard the supernatant.
7. Add acetone to the pellet and centrifuge for 2 minutes, discard the supernatant.
8. Repeat step 7 five times.
9. Add Tris 1M and centrifuge for 2 minutes, discard the supernatant.
10. Resuspend the pellet with 1% SDS.