Preparing Stock Cultures of *Sulfolobus* cells

1. Grow up 20-30 ml of culture from a single colony on a plate (either in tryptone or yeast/sucrose medium).

2. Let the culture grow well (good nose and turbid) (OD 600nm about 0.5). This can take over a week.

3. Transfer whole culture into 50ml screw-cap tube (purple or blue caps).

4. Spin for 10 minutes at 3000rpm in floor centrifuge (in Room 545, next to Jane's desk).

    a. Do not forget to balance the centrifuge and use the rubber inserts!

5. While centrifuging, label 2x 1.5 ml microcentrifuge tubes (screw-top if possible) with strain designation:

    a. If the strain is a new strain for the collection, find the next "S"-number in the strain book and write a description of the new strain.

6. Remove supernatant (save if you want to look for viruses, pour down the sink if not).

Move to sterile bench (laminar flow hood)

7. Resuspend the pellet in 1:10 of your culture volume of "Storage buffer" (See below*).

    (2ml if you started with 20ml in step 1, 3 ml if you started with 30 ml, etc. . .)
    I use the 5 ml micropipettor for this.

8. Split this suspension into 2 1.5 ml microcentrifuge tubes.

9. Freeze the cells at –80C in the proper freezer box (if new strains, in the green strain collection boxes).

*Storage buffer = *Sulfolobus* medium without carbon source and 20% glycerol, pH adjusted to 5.0.

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