

## Supplement 1

# **SBFSEM step-by-step protocol**

by Sebastian Büsse, Thomas Hörnschemeyer & Christian Fischer

All steps described in this protocol are realised using a rotary disc/plate and a fume cupboard. Always follow the recommended safety measures of the respective chemical. The precise concentrations and compositions of each chemical used can be found within the 'Material & Methods' section.

## Used Chemicals with hazard statements

Glutaraldehyde



– H302+H332-H314-H317-H334-H335-H410

Sodium cacodylate (rinsing buffer)



– H301+H331-H351-H410

Osmium tetroxide ( $\text{OsO}_4$ )



– H300-H310-H314-H330-H334

Potassium hexacyanoferrate

– H412

Thiocarbohydrazide (TCH)



– H300+H330-H311

Uranyl acetate



– H330+H300-H373-H411

Lead citrate



– H302+H332-H360Df-H373-H410

Ethanol ( $\text{EtOH}$ )



– H225-H319

Acetone



– H225-H319-H336

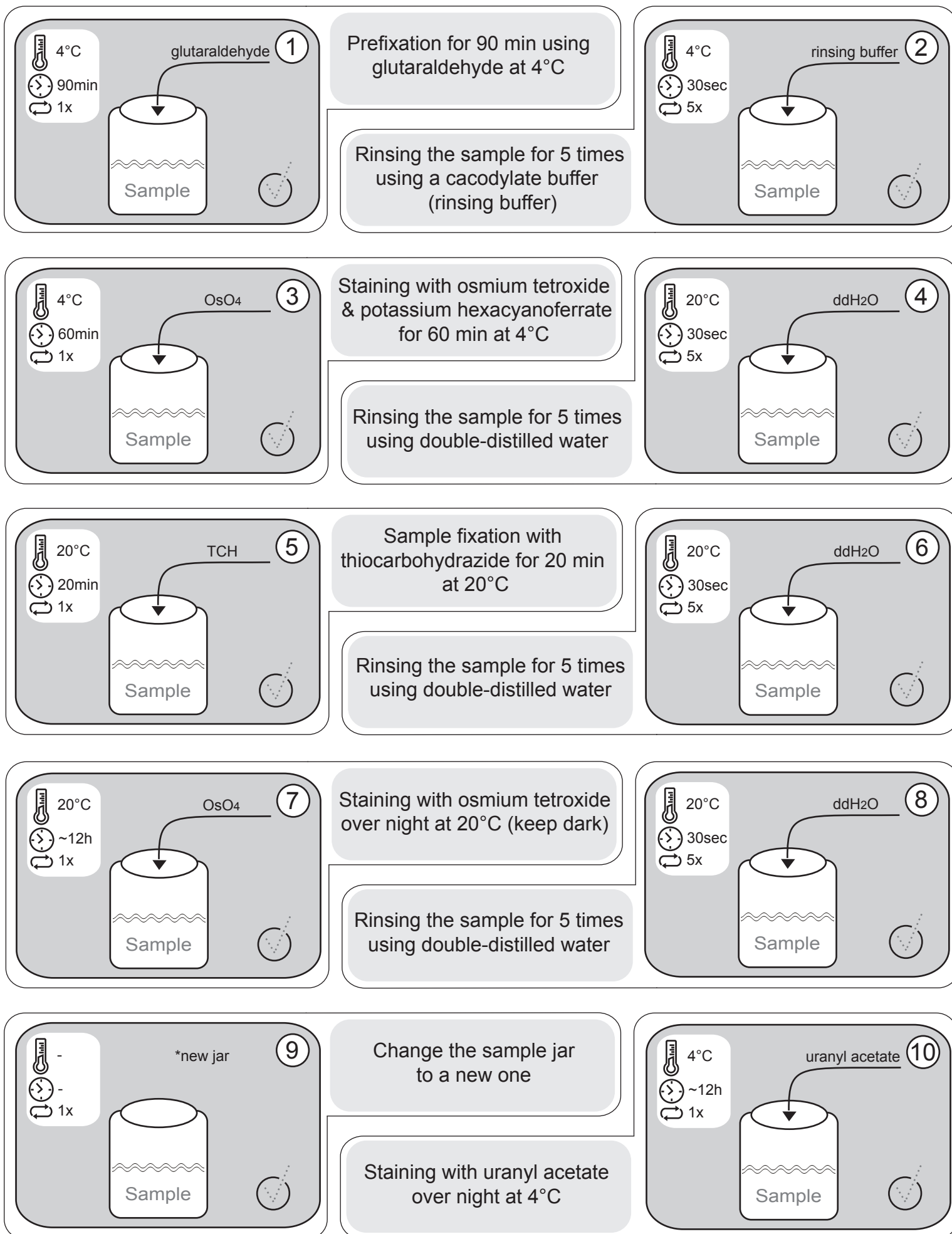
Durcupan

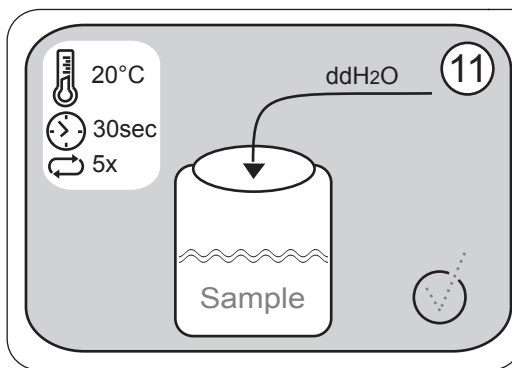


– H302-H315-H317-H319-H360Df-H400-H411

# SBFSEM step-by-step protocol

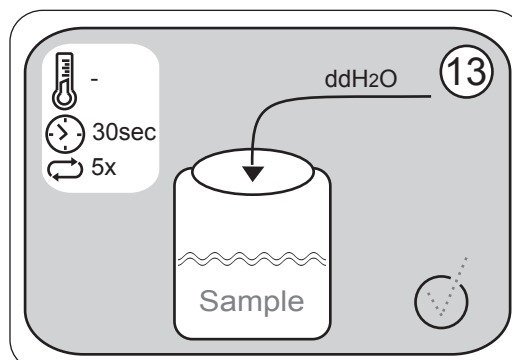
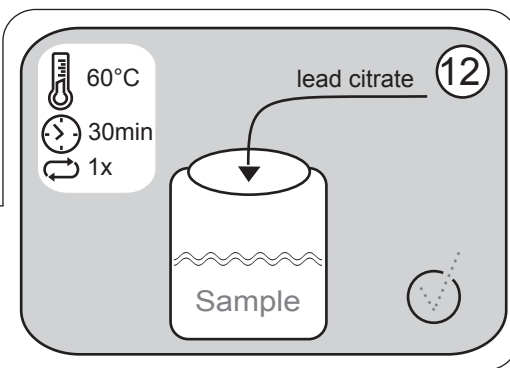
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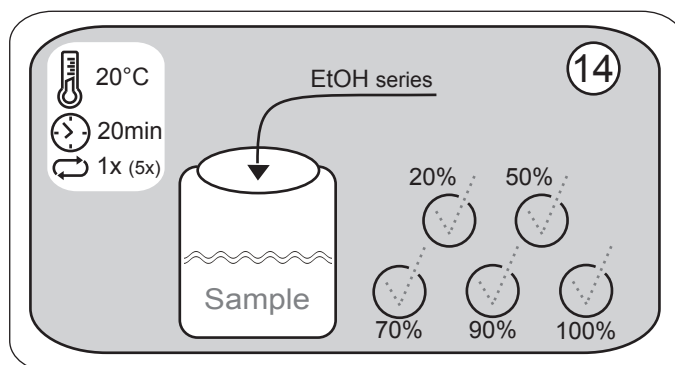


Rinsing the sample for 5 times  
using double-distilled water

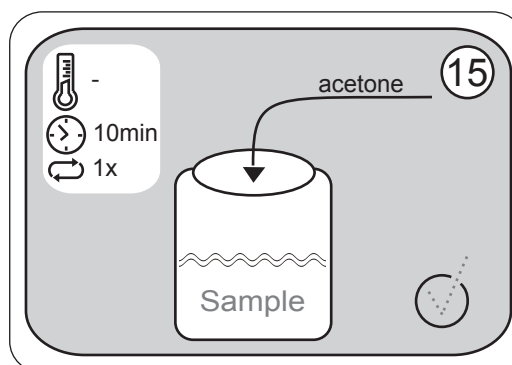
Staining with lead citrate  
for 30 min at 60°C



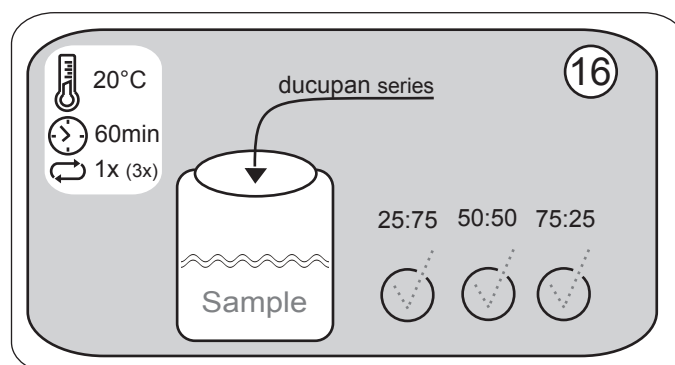
Rinsing the sample for 5 times  
using double-distilled water



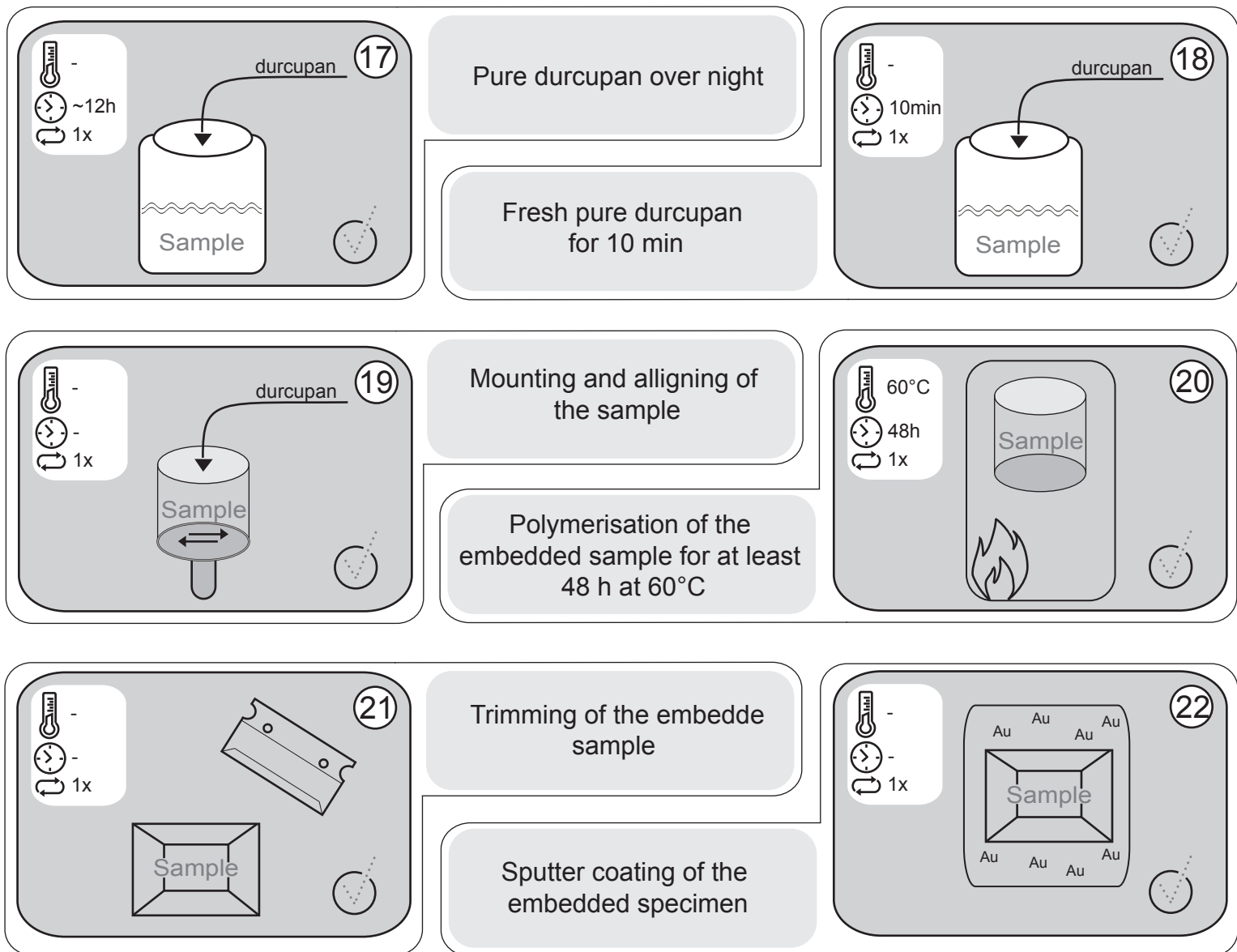
Series of graded ethanol concentrations  
from 20% to 50% to 70% to 90%  
to 99,99% for 20 min each



Rinsing for 10 min in acetone



Series of graded durcupan  
concentrations from 25:75 to 50:50 to  
75:50 for 60 min each at 20°C



... ready for SBFSEM!