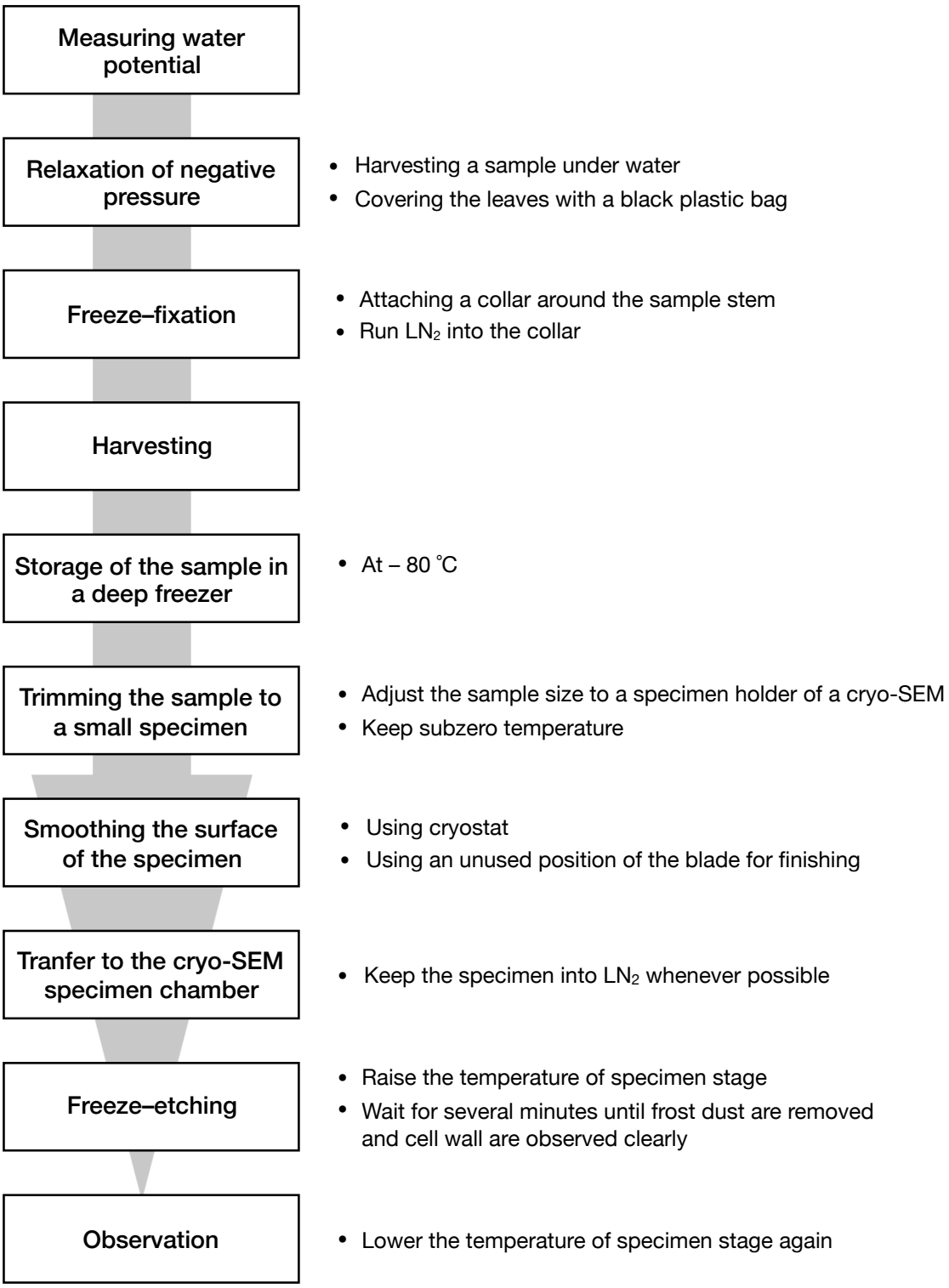
Measuring water potential
Relaxation of negative pressure
• Harvesting a sample under water
• Covering the leaves with a black plastic bag
Freeze–fixation
• Attaching a collar around the sample stem
• Run LN_2 into the collar
Harvesting
Storage of the sample in a deep freezer
• At – 80 ˚C
Trimming the sample to a small specimen
• Adjust the sample size to a specimen holder of a cryo-SEM
• Keep subzero temperature
Smoothing the surface of the specimen
• Using cryostat
• Using an unused position of the blade for finishing
Tranfer to the cryo-SEM specimen chamber
• Keep the specimen into LN_2 whenever possible
Freeze–etching
• Raise the temperature of specimen stage
• Wait for several minutes until frost dust are removed and cell wall are observed clearly
Observation
• Lower the temperature of specimen stage again