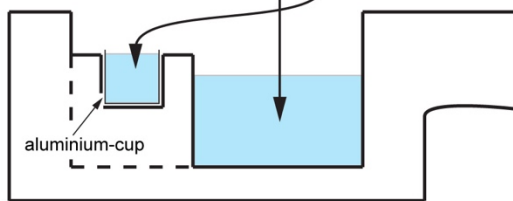


## STEPS

### Step 1

Cooling down of the **Chambers A and B** by filling up with LN2.

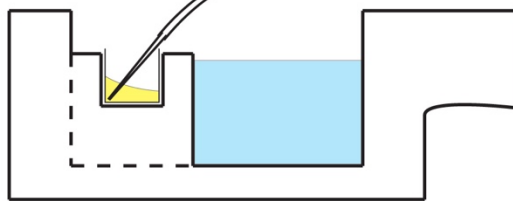


## NOTES

Prior to starting the cooling procedure, the aluminium-cup is placed in **Chamber B**.

### Step 2

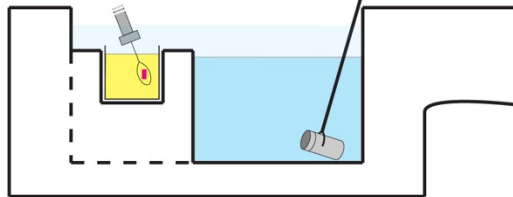
Condensation of **cryogen**.



Once the LN2 has evaporated from **Chamber B**, the cryogen outlet hose is placed such that it touches the bottom of the aluminium-cup in **Chamber B**. In addition, fresh LN2 is poured into **Chamber A**.

### Step 3

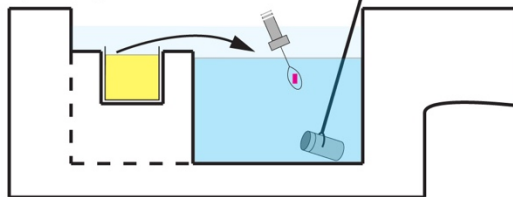
Flash-cooling of harvested crystal using a standard pin.



Prior to crystal harvesting, a first cryo-vial attached to the manipulator is pre-cooled in **Chamber A**.

### Step 4

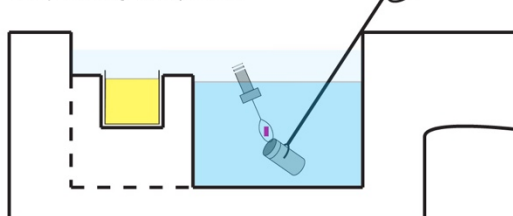
Transfer of flash-cooled specimen from liquid cryogen to LN2.



The transfer process has to be as fast as possible and within the cold gaseous N2 layer (illustrated in light blue) to prevent ice contamination or annealing effects.

### Step 5

Sample storage in cryo-vials.



For the experienced and highly trained user, sequential harvesting of 16 crystals and direct storage into cryo-pucks is feasible. In this case, a cryo-puck instead of cryo-vial attached to the manipulator is pre-cooled in Step 3

**S1 Fig. Schematic representation of the flash-cooling procedure using the new double-chamber device.** On the left-hand side, the flash-cooling Steps 1-5 described in the main text are illustrated and if deemed necessary additional notes are given on the right-hand side.