

## **Cryogenic Disruption Using Ball Mill Retsch PM 100**

### **Protocol**

The stainless steel grinding jars, the grinding balls and the storage tube with the noodles are immersed into liquid nitrogen using a styrofoam box. Pre-chill everything.

Adjust counterbalance weight depending on the weight of the grinding jar you will be using

Pre-cooling is finished when the vigorously boiling nitrogen bath calms down.

Always wear cryo-gloves

For 50 ml grinding jar, use 3 x 20 mm stainless steel bearings (large balls). For 125 ml jar, use 7

Transfer about 16 g of noodles into a 50 ml jar (Rule- use 1/3 of volume of jars.)

Be sure no liquid nitrogen is in the grinding jar prior to tightening to avoid explosion

Grinding is done in 9 cycles, 600 RPM, 2 minutes each

Between each grinding the jars are removed and cooled in liquid nitrogen

Remove powder with a spatula

Wait till it thaws slightly and remove more of it

Jars and balls can be cleaned with warm water and ethanol

Typically ~90% of yeast cells can be disrupted in such procedure. Frozen ground cells were stored at -80 °C.