

## Cryogenic Disruption of Yeast Cells (Retsch PM 100)

*Lysing of frozen yeast cells using a planetary ball mill.*

- 1) Always wear cryo-gloves.
- 2) Fill a rectangular ice bucket about  $\frac{1}{4}$  full with liquid nitrogen.
- 3) **Pre-chill everything.** Immerse the stainless steel grinding jars, the stainless steel lid, the grinding balls and the storage tube, with the frozen yeast noodles, in the liquid nitrogen.
- 4) Pre-cooling is finished when nitrogen bath is no longer bubbling vigorously.
- 5) Once everything is chilled pour the noodles into the grinding jar.
  - 20mL - 50mL of noodles: 125mL jar
  - 20mL and less of noodles: 50mL jar
- 6) Weigh the grinding jar with noodles and then adjust the counterbalance weight.
- 7) When using the 125 ml jar, use 7-11 of the 20mm stainless steel balls.
  - 50mL Falcon tube requires 7-9 balls
  - <50mL of noodles requires 9-11 balls
  - <25mL of noodles tube requires 11 balls
  - <15mL of noodles use 50mL grinding jar requires 3 balls
- 8) Be sure **no liquid nitrogen** is in the grinding jar prior to grinding to avoid an explosion.
- 9) Grinding is done in 8 cycles, each cycle is set in the following manner:
  - 400 RPM
  - 3 minutes
  - 1 minute reverse rotation with no breaks between rotations(NOTE: You **MUST** hear the balls rattling around in the jar! If there is no rattling then add/remove balls to the jar until you hear it rattle. It is not considered a grinding cycle unless there is rattling.)

When using the 50mL jar the grinding settings are as follows:

  - 500 RPM
  - 3 minutes
  - 1 minute reverse rotation with no breaks between rotations(NOTE: You **MUST** hear the balls rattling around in the jar! If there is no rattling then add/remove balls to the jar until you hear it rattle. It is not considered a grinding cycle unless there is rattling.)
- 10) Between each cycle the jars are removed and cooled in liquid nitrogen. **DO NOT REMOVE THE LID** (removal of lid may result in cell loss)! To ensure lid is chilled use an empty Falcon tube to pour liquid nitrogen over the top of the grinding jar while the bowl of the grinding jar cools in the liquid nitrogen bath. **DO NOT SUBMERGE THE JAR COMPLETELY** as this will allow liquid nitrogen into the grinding bowl and may also result in cell loss.

- 11) When 8 cycles are complete remove powder with a spatula – if there is powder stuck to the side of the jar repeat 1 grinding cycle at 350 RPM, 2 minutes, 1 minute reverse rotation no breaks between rotations.
- 12) Jars and balls can be cleaned with warm water and Windex.
- 13) Typically ~90% of yeast cells can be disrupted in such procedure. Frozen ground cells are stored at -80 °C.