Updated SSenyo, 3Feb09

Operation of PWM32-PS-R790 Spinner from Headway Research, Inc

1. Line spinner bowl with aluminum foil.
2. Turn on spinner.
3. Turn on vacuum pump.
4. Place Si chip on chuck (vacuum pressure pulls in chuck center).
5. Press recipe button on the spinner unit.
6. Select the desired recipe 0-9 and press enter when finished. The recipe number will be shown in the upper left-hand corner of the LCD display.
7. Depress and hold down the front portion of the speed control pedal (towards connect tubing) for approximately 3 seconds.
8. When the pedal is released the Si chip should begin to spin.
9. The vacuum is set to automatically engage at the chuck for each of the programs 0-9, once the spinner has been engaged. The speed and count-down timer will be shown on the LCD display. With the machine engaged the speed can be adjusted using the "step terminate" and "speed parameters" buttons to increase and decrease the speed respectively.
10. To disengage the spinner, depress the rear portion of the speed control pedal.
11. The vacuum is set to automatically disengage once the spinner has stopped and the Si chip can now be removed from the chuck.
12. Turn off the vacuum pump and the spinner when finished.
13. To edit or enter in an new program, see page 15-16 of the operation manual.

PWM32-PS-R790 Spinner

Placing Si chip on chuck in spinner bowl, lined with aluminum foil

Speed control pedal

Operation manual available next to spin cast for detailed information.

Programs Entered:

-Program – 0 – sperm in trehalose:
step 1 – speed 3500 rpm; acceleration 1000 rpm/sec; 999.9 secs.

Otherwise program/recipe number (#) reflects the rpm speed (# x 1000 e.g.

-Program – 1 –> speed 1000 rpm
-Program – 2 –> speed 2000 rpm
-Program – 3 –> speed 3000 rpm, etc.)

Recipe Review/Edit:

Spinner controller

1. Press the recipe button on the spin unit
2. Select the desired recipe 0-9 and press enter when finished. The recipe number will be shown in the upper left-hand corner of the LCD display. Each recipe has a number of steps shown in the second line of the upper left-hand corner preceded by a dash (e.g. -2 is 2 steps)
3. Press the step button on the spin unit
4. Select the desired step and press enter when finished. The step will be shown as part of the total number of steps in recipe (e.g. step 1 of two steps will apppear as 1-2)
5. Press the speed/ramp to toggle between speed (rpm) and ramp (ramp/sec)
6. Press the step button and then 0 to end review/programming mode and return to home page
7. (optional) Speed and ramp can be edited by typing a new value and pressing enter; follow with pressing the step button and 0 to return to the first page
8. (optional) Press step terminate button in review/programming mode to edit time. 999.9 sec is default
9. (optional) A new step can be added by pressing step button and then the next highest step number (e.g. with two steps, press step button then press 3) and proceed to add new values through instruction #7

Note: If 'Fatal Error' message appears – usually after ending a spin run – follow instructions on LCD display and press Start pedal to reset to the home page on LCD display

Notify NRIMS staff before proceeding to adjust recipe profile!

Use in Specific Experiments

Biggers ^{18}O Trehalose Sperm
Speed: 3500rpm. Accel: 1000rpm/sec. Step timer: 999.9 sec
Begin spin casting the control samples with no ^{18}O-trehalose. Then, spin cast sequentially control and experimental samples with the equivalent ^{18}O-trehalose concentration, starting with the 0.025M concentration, and stepwise proceed to the higher concentrations. (*see Biggers 18O Trehalose Sperm protocol for further details*).

Kleinfeld fatty acid studies

Spin drying adipocytes on Si substrates preserves the lipid droplets within the cell. After 2 days of growth, the silicon chips, with attached adipocytes, were removed from media and placed immediately on the vacuum chuck of a Headway spinner. The chuck with was set to spin at 2000 rpms for a period of 1 minute.

Waldbauer/Chisholm Prochlorococus experiments

Place Si chip on spinner, begin spinning @ 1000 rpm. Deposit ~5 ul of 1:10 dilution on chip; allow to spread & dry 30-60s. Stop spinner, then restart @ 3000 rpm to dry chip. Examine chip under scope w/ 100x objective; cells should appear as isolated dots with faint chlorophyll fluorescence (blue excitation/red emission) visible on CCD camera.

DNA Replication EXP1: REF and HFF 2-week thymidine labeling

Silicon chips removed from the culture dish at 40% density were fixed in FGP, post-fixed in OsO4 and spun at 3500 rpms for 2 minutes using PWM32 series spinner from Headway Research, Inc. (*see DNA Replication EXP1: REF and HFF 2 week 15N thymidine labeling protocol for further details*).

DNA Replication EXP2: HFF 24 hour Thymidine Pulse (label 0.1, 1.0, 10, 100 uM ^{15}N Thymidine)

Cells were fixed in FGP, post-fixed in OsO4 and Si chips were spun dry at 3500 rpm on the spin caster. (*see DNA Replication EXP2: HFF 24 hour Thymidine Pulse protocol for further details*).

HFF 13C Thymidine Protocol Methyl label vs universal

Cells were fixed in FGP, post-fixed in OsO4 and Si chips were spun dry at 3500 rpm on the spin caster. (*see HFF 13C Thymidine Protocol Methyl label vs universal protocol for further details*).