FACS of Candida - plate processing

Aleeza's variation of Mark's variation of a protocol from Darren Abbey, based on Sandy Johnson protocol

Solutions

50/50 TE (50mM Tris pH 8 / 50mM EDTA)

850mL dH20 + 50mL 1M TrisHCl pH8 + 100mL 0.5M EDTA.
Store extra at RT

RNAse A solution (1mg/ml RNAse A in 50/50 TE)

For 1 plate: 500uL RNAse A (10mg/mL) + 4.5mL 50/50 TE
Store extra at -20

Prot. K solution (5mg/ml proteinase K in 50/50 TE)

For 1 plate: 0.025g Prot K into 10mL 50/50 TE
Store extra at -20

Sybr solution (Sybr green diluted 1:100 into 50/50 TE)

1 tube = 500uL. Add 500uL to 50mL 50/50 TE.
Store extra wrapped in foil at -20

Notes:

-Centrifugation of microtiter plates done in plate centrifuge for 5 min. at 1000 x g.
-Resuspend by low speed vortexing or gentle mixing by hand
-After centrifugation, it should be fine to swiftly dump out liquid from plates without disrupting the pellet. Do this quickly and there should be very little (or no) spillover among wells. This should be done relatively soon after centrifugation (within half an hour or so) or the pellets may become loose (untested).

<u>Acquire culture</u>

<u>Option a:</u>

1) Streak cells from freezer to single colony on YPAD plate

2) Seed culture: Depending upon the experiment, pick 1 colony or pick from the thick area of the streak, into 500ul YPAD per well of a 96 well culture block. Grow in plate shaker /incubator at 30°, ~ 1 day, covering block with airpore sheet.

OR

<u>Option b:</u>

1) Inoculate from frozen into 500 ul YPAD per well of a 96 well culture block.

2) Grow in plate shaker /incubator at 30°, ~ 1 day, covering block with airpore sheet.

Acquire log phase culture

3) Subculture 10 ul into 500 ul of fresh YPAD in another culture block, 30°, 4-6 hrs. (to A600 of ~ 1). Tilt culture block in holder ~ 30° by wedging a wadded paper towel underneath to get better mixing. Make sure the cultures doesn't spill on to the airpore sheet as its mixing

Fix cells

4) Draw off 250 ul and transfer to a round bottom microtiter plate. Centrifuge, remove supernatant. If the pellet appears too small, transfer another 250 ul of culture to the wells and centrifuge again. In practice, there is likely plenty of cells even if only a small pellet (or even no pellet!) is present. Resuspend pellet in 20 ul 50/50 TE (vortex ~5s, on speed "2"), then add 180 ul of 95% ethanol, slowly, drop by drop. Seal in aluminum foil and store at -20° overnight.

Remove RNA

5) Centrifuge, remove supernatant. Add 50 ul 50/50 TE, vortex, then add 150 ul more TE.

6) Repeat step 5.

7) Centrifuge. Draw off supernatant and add 50 ul RNAse A solution, vortex. Incubate 3h - overnight, 37°.

Optional ProtK

8) Centrifuge, draw off supt. Add 50 ul Prot. K solution, vortex. Incubate 30 min., 37°

Stain

9) Centrifuge, draw off supernatant. Add 50ul 50/50 TE, vortex, add with 50 ul Sybr green solution. Incubate in dark, overnight at room temp.

Day of flow

10) Sonicate w/ 96 pin sonicator, 4 min., amplitude 40, 1sec/1sec on/off
11) Centrifuge, draw off supernatant, resuspend in 200ul 50/50 TE and transfer to FACS tube containing 0.5 ml of 50/50 TE (using Rainin moveable multichannel pipette)

11) FACS on FACScaliber/LSRII/etc.. Bring extra 50/50 TE and a pipettor to dilute the samples in case they should be too concentrated to accurately FACS.