

FACS -- SINGLE CELL PCR 1.2
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Do not bend caps.
Arrange tubes in covered flipper racks and leave on ice.
Prepare storage boxes

Lysis solution:

Keep tubes and solution cold
Add 4 μ l to the bottom of each tube

Do not prepare tubes too far in advance.

Extra Inhibitor

- for 125 rxns
25 μ l vial of RNasin (40 U/ μ l)

50 μ l 5'-3' RNase Inhibitor (1U/ μ l)

250 μ l PBS (BioWhit RNase free)

125 μ l Water (RNase free)

50 μ l 100mM DTT (Mo Bi Fresh!!! -- 15.4mg/1ml or from Taq kit)

*Per rxn
2 ~~ul~~
4 ~~ul~~*

Cover flipper racks
KEEP TUBES ON ICE

FACS

Prepare sheath fluid (Deficient RPMI, 10 mM Hepes, 0.02% NaN₃) with RNase A,
at .5ng/ml (make 2 liters)

Change filters. Avoid/remove any air bubbles.

Run 0.1M NaOH through the machine (~5 min.), including sample filters and sheath
tubing

Sterilize with 70% EtOH (~10 min.)

Connect to RNase-treated sheath fluid and run for ~5 min.

Sorting notes for FLASHER

Initialize with 4 color + time for recording data.

Set-up for VERTICAL SORT

Use cooling block

000 for 1 cell/well

coinc. in

Use J13 for left sort -- better vertical drop; J14 is for right sort

BULK SORT FIRST, REANALYZE, THEN DEPOSIT SINGLE CELLS

Spin tubes (2 or 4 at a time) and **freeze quickly!** on dry ice

Store samples at -70 °C. They are stable for at least one year.