Hybrid Capture protocol 2b

Solution hybridisation (Agilent SureSelect protocol)

This protocol is for hybridization of adapter-ligated or PCR-amplified library DNA, so must be performed after Hybrid Capture Protocol 1.

Sample preparation

Following SPRI bead cleanup (see Hybrid Capture Protocol 1), lyophilize 500 ng library + 7.5 µg C_0t1 DNA using a SpeedVac..

1. Add 3.4 µl of molecular biology grade water to each sample to rehydrate.

2. Vortex and spin down.

3. To each sample, add:

   SureSelect Block #1 2.5 µl
   SureSelect Block #2 2.5 µl
   SureSelect Block #3 0.6 µl

4. Prepare Hybridization Buffer as follows. Volume for 1 capture:

   SureSelect Hyb #1 25 µl
   SureSelect Hyb #2 1 µl
   SureSelect hyb #3 10 µl
   SureSelect Hyb #4 13 µl

   Note: Do NOT keep on ice.

5. Incubate 40 µl of Hybridization Buffer and library (from step 3.) at 95 °C for 5 minutes and 65 °C for at least 5 minutes. Keep at 65 °C until RNA baits are prepared (see below).

6. Dilute RNAse Block in 1:1 ratio with nuclease-free water and add 1 µl to 5 µl (500 ng) of RNA baits. Incubate for 2 min at 65 °C.

Hybridization

1. Mix 13 µl of hybridization buffer with RNA baits.

2. Add the 9 µl DNA library to the hyb buffer - RNA bait mixture.

3. Seal plate with the Greiner film and incubate for 24 hours at 65 °C with a heated lid at 105 °C.

Washing and elution

Selection with magnetic beads

1. Prepare magnetic beads by washing with SureSelect Binding buffer 3 times and resuspend in 200 µl of SureSelect Binding buffer. Incubate the hybrid-capture-bead solution on a Nutator for 30 minutes at room temperature (20 °C).

2. Mix hyb. mixture with beads and separate the beads by removing the supernatant.

3. Resuspend beads in SureSelect Wash Buffer #1.

4. Incubate the samples for 15 minutes at room temperature. Separate the beads and buffer on a Dynal magnetic separator and remove the supernatant.

5. Mix the beads in pre-warmed (65 °C) 500 µL SureSelect Wash Buffer #2. Incubate the samples for 10 minutes at 65 °C. Remove wash buffer and repeat these steps for 3 times.

6. Mix the beads in 50 µL SureSelect Elution Buffer. Incubate the samples for 10 minutes at room temperature. Separate the beads and buffer on a Dynal magnetic separator. Add 50 µL of SureSelect Neutralization Buffer.

7. Desalt the capture solution with a Qiagen MinElute PCR purification column, eluting in 34 µL buffer EB.

Proceed to the Post-hyb PCR (Hybrid Capture Protocol 3).