P. falciparum RT and amino-allyl incorporation

(06/01/10)

1. Prime the RNA

Need: OligodT/random primer (nonamer or hexamer) mix: **2 µg/µl each**

12 µg Total RNA in 22.3 µl water
Add 3.3 µl of oligodT/random primer mix

Incubate at 70 °C for 10 minutes, and then cool to 4 °C for 10 minutes.

2. RT Reaction

Need: 100mM DTT
Reverse Transcriptase
5X RT Buffer
50X aa-dUTP dNTPs: (2A:1C:1G:2U/T)

100 µl of 50X Stock:
30 µl dATP (100mM)
15 µl dCTP (100mM)
15 µl dGTP (100mM)
15 µl dTTP (100mM)
From Ambion (50mM) → 30 µl aa-dUTP (50mM)

Per reaction: 10.0 µl 5X RT Buffer
1.0 µl aa-dUTP dNTPs
5.0 µl 100mM DTT
2.0 µl Superscript II RTase (varies according to source)
6.4 µl DEPC•ddH_2O
(Make master mix for many RTs)

Add 24.4 ul RT mix and incubate at 42 °C for 2 hours. (For Superscript III, use 50°C)

3. Hydrolysis

Need: 0.1 N NaOH
0.1 N HCl

Add 25 µl 0.1N NaOH and incubate at 70°C for 10 minutes.

Neutralize w/ 25 µl 0.1N HCl

4. Cleanup using Zymo Research DNA Clean and Concentrator-5 (D4004)

- Set up one 1.5 ml eppendorf tube with 1.0 ml Binding Buffer per RT reaction.
- Add the RT reaction to the tube and mix well.
- Load a Zymo DNA Clean column with the sample and spin for 15 seconds.
- Re-load the Zymo column with the flow through and spin again 15 seconds.
- Aspirate off the flow through and reload the column with the rest. Spin 30 seconds.
- Re-load the Zymo column with the flow through and spin again 30 seconds.
- Add 200 µl Wash Buffer. Spin 15 seconds.
- Wash again with 200 µl Wash Buffer and spin 30 seconds.
- Spin dry 30 more seconds. (THIS IS CRUCIAL TO REMOVE ANY REMAINING ETHANOL!)
- Transfer column to a clean eppendorf tube and elute with 13.0 µl 0.1M sodium bicarbonate, pH 9.0 (FRESH!). Let sit a couple of minutes and then spin 30 seconds.

Run 2 µl on a gel and freeze the rest at –20 °C. If nothing is seen on the gel, it is likely that the microarray will not work very well.

NOTE:

It is safe to store cDNA samples containing amino-allyl dUTP for weeks to months at –20 C. It is actually advisable to have all samples for a given large experiment prepared before microarray hybridization so that all hybridizations can be carried out in a narrow timeframe, increasing consistency in results between related samples.