

P. falciparum RT and amino-allyl incorporation

(07/28/03)

1. Prime the RNA

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

12 µg Total RNA in 28.5 µl water

Add 2 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

10X RT Buffer

30 X aa-dUTP dNTPs: **100 µl of 30X Stock:** 30 µl dATP
(100mM)

15 µl dCTP (100mM)

15 µl dGTP (100mM)

15 µl dTTP (100mM)

15 µl aa-dUTP (100mM)

10 µl water

Per reaction: 4.5 µl 10X RT Buffer

1.5 µl aa-dUTP dNTPs

4.5 µl 100mM DTT

4.0 µl RTase (varies according to source)

(Make master mix for many RTs)

Add 14.5 ul RT mix and incubate at 42 °C for 2 hours.

3. Hydrolysis

Need: 0.5M EDTA

1.0N NaOH

Add 10 µl 0.5M EDTA and 10 µl 1.0N NaOH and incubate at 65 °C for 15 minutes.

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 column with 550 μ l of the mix per sample. Spin for 15 seconds.
- Aspirate off the flow through and reload the column with the rest. Spin 30 seconds.
- Add 200 μ l Wash Buffer. Spin 15 seconds.
- Wash again with 200 μ l 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 μ l 0.1M sodium bicarbonate, pH 9.0. Let sit a couple of minutes and then spin 30 seconds.

Run 2 μ l 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.