

Protocol A:

Harvest ~ 700 µg total RNA.

Perform the One-Cycle Target Labeling and Control Reagents Eukaryotic Target Preparation steps as described in [1]. This includes the Poly-A mRNA Isolation step. After purification, there is ~ 11 µg purified mRNA, of which 3-5 µg will be used in the following steps.

Disadvantages:

- time,
- money,
- since different mRNA's have different lengths of the poly A-tail, the purification step that filters on the mRNA poly-A tail could form a bias.

Advantages:

- rRNA and tRNA cannot interfere with cDNA synthesis.

Protocol B:

Harvest ~ 15 µg total RNA.

Perform the One-Cycle Target Labeling and Control Reagents Eukaryotic Target Preparation steps as described in [2]. However, do not perform the Poly-A mRNA Isolation step.

Disadvantages:

- rRNA and tRNA can interfere with cDNA synthesis.

Advantages:

- time,
- money,
- possible bias induced by filtering on the poly-A tails is circumvented.

[1] AffyMetrix, GeneChip Expression Analysis Technical Manual P/N 700219 rev 4, 2000

[2] AffyMetrix, GeneChip Expression Analysis Technical Manual P/N 701025 rev 5, 2000