1. PURPOSE

This procedure describes how to synthesize cDNA from RNA in 96 plate format using the ThermoScript RT-PCR Kit.

2. SCOPE

The instructions are for synthesizing cDNA from RNA samples to be used for QGE (Quantitative Gene Expression) application service.

3. REFERENCES

3.1. *ThermoScript™ RT-PCR System*, Part n. 11146.pps, Rev. date: 26 Sep 2003, *Invitrogen.*

3.2. Application note: *Multiplexed Gene Expression Analysis Using Competitive PCR and MassARRAY* (*Sequenom Inc*).

4. EQUIPMENT, REAGENTS, AND MATERIALS

4.1. Thermal Cycler

4.2. Vortexer

4.3. Centrifuge

4.4. ThermoScript™ RT-PCR System Kit, *Invitrogen*

4.5. 0.5 mL micro tubes

4.6. 96 PCR Plates

4.7. 200 uL pipette, 20 uL pipette, and 2.5 uL pipette

4.8. 200 uL and 10 uL Pipette tips

4.9. Trough

5. RESPONSIBILITIES

It is the responsibility of trained personnel synthesizing cDNA according to the guidelines described in this procedure.

6. PROCEDURES

6.1 cDNA Synthesis

6.1.1 In a 96 plate format, combine 50 ng/uL random primer, approximately 0.5 ug RNA, dNTP mix, and adjust volume to 12 uL with DEPC-treated water for each sample.

6.1.2 Vortex and centrifuge plates.

6.1.3 Denature RNA and primer contained in plate by incubating at 65◦C for 5 minutes.

Program ID: DENATURE

PCR Cycle	Cycling Conditions
Incubation	65°C for 5 minutes
Hold	4°C

6.1.4 Vortex the 5X cDNA Synthesis Buffer for 5 seconds just prior to use.

6.1.5 Prepare a master reaction mix using the values shown in Table below.

Reagents	Concentration	8 uL Conc.	1 Rxn	100 Rxns
5x cDNA Synthesis Buffer		**N/A**	4	400
DTT	0.1 M	12.5 mM	1	100
RNaseOUT	40 U/uL	40 U	1	100
DEPC-treated water		**N/A**	1	100
ThermoScript RT	15 U/uL	15 U	1	100
Total Volume	uL		**8 uL**	**800 uL**
Dead Volume	20%			

6.1.6 Vortex and centrifuge master reaction mix.

6.1.7 Add 8 uL of master reaction mix into each reaction in 96 plate.

6.1.8 Vortex and centrifuge plate.

6.1.9 Transfer the sample to a thermal cycler and incubate as shown below:

Program ID: RH

PCR Cycle	Cycling Conditions
Initial Incubation	25°C for 10 minutes
Secondary Incubation	50°C for 50 minutes
Terminal Incubation	85°C for 5 minutes
Hold	4°C

6.1.10 Add 1 uL of RNase H to each reaction in 96 plate.

6.1.11 Transfer reaction tubes to thermal cycler and incubate at 37◦C for 20 minutes.

Program ID: 37-20M

PCR Cycle	Cycling Conditions
Incubation	37°C for 20 minutes
Hold	4°C

6.1.12 Store cDNA synthesis reaction plate at -20◦C for daily use or at -80◦C for long term storage.