LSOP Title	Gene Expression Analysis by Real Time
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Version	1.1
Location	UQ Node/Centre-wide
Policy/Procedure Link	*UQ- Equipment* *UQ -waste* *OGTR*
Risk Assessments	
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1.0 Scope

This procedure covers the protocol for gene expression in real time and extends to reverse transcription and real time PCR including creating the plates for analysis.

This LSOP does not cover RNA extraction or how to analyse the gene expression results.

2.0 Definitions

RNA - Ribonucleic acid

PCR – polymerase chain reaction

cDNA – complementary deoxyribonucleic acid (DNA)

3.0 Material and Equipment

1. Calculator
2. PCR machine
3. Pipette & Pipette Tips
4. Biorad iScript RT supermix
5. PCR tubes
6. Water
7. Eppendorf Tubes

8. Reverse Primers
9. Forward Primers
10. Syber
11. 394 well plate
12. 394 well plate sticker
13. Centrifuge

4.0 Prescribed Actions

Real-time PCR:

Primer preparation

1. Calculate how many cDNA samples are to be tested and add 3 to this number
2. Make up a mastermix containing:
    a. (Number of samples + 3) x 1.5 ul primer mix (containing 1uM of both forward and reverse primers)
    b. (Number of samples + 3) x 3.5ul Syber mix

    NB: Be extremely careful not to contaminate the Syber mix as this will affect everyone who uses it in the future

    NB: Be conscientious about changing tips after adding the Syber mix and if there is any doubt, ANY DOUBT, discard the tip and use a new tip.

Realtime reaction in each well

1. 5uL Master mix: 3.5ul Biorad syber + 1.5ul 1uM primer
2. 5ul 0.5ng/ul cDNA

Plating out the 384 well plates

1. Using the 125ul multidispense pipette on 'Repeat dispense' protocol (see appendix or LSOP36) add 5 ul of syber+primer mastermix into the wells

from one side of the plate to the other (place the drop of mix at the bottom of each well)

2. Using the 125ul multidispense pipette on 'Repeat dispense' protocol pipette the 5ul of cDNA on to the side of the wells of the 384 well plate.

   NB: If the volume you need is bigger than the volume of the tips, change tip in between to avoid cross contamination.

   NB: the cDNA should be pipetted into the 384 well plate in a direction that is perpendicular to that of the SYBER mix

   NB: don't touch the liquid in the bottom of the wells with the pipette tips, only pipette onto the walls and use different side of wells for pipetting the cDNA then that used for the SYBER mix to avoid sample contamination.

3. Add a clear sticker to the top of the 384 well plate and use a ruler to rub across the surface of the plate to ensure that the sticker has good contact across the top of all the wells especially those on the edges. Identify the plate by adding notes on the edges of the sticker.

   NB: sticker has a darker appearance when good contact is made with the rim of the well

4. Spin the plate down at 2000 rpm for 1 minute to force all liquid to the bottom of the tube and to break most/all bubbles that may have formed in the wells

5. Leave in the fridge for at least 3 hours for better results. You can leave the plate in the fridge for up to 2 days.

5.0 Appendix

Repeat dispense (for dispensing a larger volume across multiple tubes)

This is useful for pipetting out the cDNA and SYBER mix into the 384 well plates

- From the main menu select 'repeat dispense' by moving your finger around the centre dial (like an iPod) and press the centre button to select
- Select 'edit'
    - Change 'dispense' volume to 5ul on the 125ul pipettor.

- Change ‘count’ to the desire number of dispenses you want to do + 2 extra ones.
- Select ‘▻’ to save

- Select the correct tip spacing for your needs by pressing ‘▻’ or ‘◅’ buttons
  - 4.5 for 384 well format
  - 8.5 for 8 well format
  - 14.0 for 1.5ml Eppendorf tube in standard racks