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qPCR (SYBR Green)

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Vanessa Howland[1], Yue Ma[1]

[1]Van Andel Institute

Y Yue Ma
VanAndelInstitute

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We use this protocol and it's working

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Abstract

qPCR protocol

Materials

- PowerUp™ SYBR™ Green Master Mix (2X)
- Forward and reverse primers (300-800nM)
- cDNA (1-10ng)
- H2O

Prepare the reagents

1 Swirl the PowerUp™ SYBR™ Green Master Mix to mix thoroughly.

2 Thaw the DNA samples and primers on ice, vortex to mix, then centrifuge briefly.

Set up the PCR reactions

3 Prepare the appropriate number of reactions, plus 10% overage.

4

	A	B	C
	Component	Volume (10 ul/well)	Volume (20 ul/well)
	PowerUp SYBR Green Master Mix (2X)	5	10
	Forward and reverse primers (300-800nM)		
	cDNA (1-10ng)		
	H20		
	Total	10	20

5 Mix the components thoroughly, then centrifuge briefly to spin down the contents and eliminate any air bubbles.

6 Transfer the appropriate volume of each reaction to each well of an optical plate.

7 Seal the plate with an optical adhesive cover, then centrifuge briefly to spin down the contents and eliminate any air bubbles.

7.1 PCR can be performed on the reaction plate up to 72 hours after completing the setup, when stored at room temperature. Protect the reaction plate from light if the PCR is not started immediately after the reactions are set up.

Set up and run the real-time PCR instrument

8 Place the reaction plate in the real-time PCR instrument.

9 Set the thermal cycling conditions using the default PCR thermal cycling conditions specified in the following tables according to the instrument cycling parameters and melting temperatures of the specific primers. (Easiest is to use the computer software for plate setup)

10

UDG activation	50°C	2 minutes	1
Activation (Dual-Lock™ DNA polymerase)	95°C	2 minutes	1
Denature	95°C	15 seconds	40
Anneal/extend	60°C	1 minute	

Standard cycling mode (primer Tm ≥60°C)

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	Step	Temperature	Time	Cycles
	UDG activation	50°C	2 minutes	1
	Activation (Dual-Lock‰ DNA polymerase)	95°C	2 minutes	1
	Denature	95°C	15 seconds	40

Anneal	55–60°C*	15 seconds	
Extend	72°C	1 minute	

Standard cycling mode (primer Tm <60°C), *Anneal temperature should be set to the melting point for your primers

12 Set the instrument to perform a default dissociation step.
A dissociation step can be performed up to 72 hours after the real-time PCR run if the plate is stored in the dark and up to 24 hours after the real-time PCR run if the plate is exposed to light.

13

Step	Ramp rate	Temperature	Time
1	1.6°C/second	95°C	15 seconds
2	1.6°C/second	60°C	1 minute
3[1]	0.15°C/second	95°C	15 seconds

Dissociation curve conditions (melt curve stage)

14 Use the following settings for Applied Biosystems™ instruments:
Experiment type: Standard curve or Relative Quantification
Reagent: SYBR™ Green reagents
Reporter: SYBR™
Quencher: None
Passive reference dye: ROX™
Ramp speed: Standard or fast (choose the same setting as in step 2)
Melt curve ramp increment: Continuous

14.1 Set the reaction volume appropriate for the type of plate being used for your PCR reaction.
Pay attention to whether you are using a 0.1 or 0.2 ml 96 well plate, the experiment files are not compatible between the two plate sizes.

15 Start the run.

16 When the run is finished, make sure to export the results to your USB.