qPCR

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10:46 AM

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qPCR

Materials:
RNase Free H_2O (Fisher BP5611)
SYBR Green PCR Master Mix (ABI 4309155)
96 Well PCR Optical plate (Eppendorf twin.tec skirted, cat. no. 951020401)
Optical adhesive cover (Eppendorf PCR film, cat. no. 951023019)
10 µM Forward Primer
10 µM Reverse Primer
cDNA Template

Method:
1. Make up master mix on ice according to the following formula
+ 10 µl 2X SYBR Green PCR Master Mix * # of wells
+ 1 µl 10 µM Forward Primer * # of wells
+ 1 µl 10 µM Reverse Primer * # of wells
+ 6 µl RNase free H_2O * # of wells
2. Pipette 18 µl of master mix into each well, keep plate cold
3. Add 2 µl cDNA Template made from 300 ng - 1 µg RNA
4. Put Optical adhesive cover on top the plate
5. Place plate in the thermocycler and run your program

Typical Program
1. 95C – 10 min
2. 95C – 15 sec
3. 60C – 1 min
5. Cycle to step 2 40 more times
6. Melting curve
7. Incubate at 4°C forever

Note: When generating standards use a high fidelity DNA polymerase

Typical Concentrations used for qPCR standards
Actin2
- For cDNA made with 500 ng RNA
15,625,000 copies/µl
3,125,000
625,000
125,000

IPP2
- For cDNA made with 300 ng RNA
125,000 copies/µl
25,000
5,000
1,000

200
qPCR

Math
To convert from ng/µl (obtained from spec) to copies/µl use the following formula

X ng/µl ÷ $1*10^{-9}$ng* ÷ molec wt of standard** g/mol * $6.022x10^{23}$ copies/mol ÷ 2*** = copies/µl

* This converts from ng/µl to g/µl
** To figure out the molecular weight of your PCR product you are going to use for a standard put your sequence into this website http://www.basic.northwestern.edu/biotools/oligocalc.html
- be sure to select dsDNA on the drop down box on the right (default is ssDNA)
- Do Not Use Vector NTI For This!, it only gives you the molecular weight of a single stand (it's meant for use with primers)
*** We divide by 2 since RNA is single stranded i.e. 1 strand not 2

Use 100x dilutions to get PCR product into range for standards needed (i.e. 2 µl PCR product into 198 µl dH_2O)