Laboratório de Genómica Funcional de Plantas

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cDNA synthesis protocol

Important: For qPCR you have to be sure that total RNA is not contaminated with gDNA.

Normally 2-5ug of total RNA is used for cDNA synthesis.

In grapevine normally we use 2.5ug of tRNA.

Reagents:

-Oligo dt primer (18 or 23 oligomer) / hexamers or decamers

-Buffer RT 5x

-Reverse transcriptase 200U/ul

-DNTPs (10mM)

-Ribolock RNase inhibitor 40 U/ul

Equipment:

-PCR

Protocol:

-total reaction volume: 20ul

-Add 1 ul of oligo dt primer to 2.5 ug of tRNA in a PCR tube and incubate 10min at 70ºC on a thermocycler.

-put the PCR tube on ice

-Add to the PCR tube the following:

-5x RT buffer ---------- 4 ul

-10mM DNTPs ---------- 2 ul

-Ribolock ---------- 0.5 ul

-Reverse Transcriptase -- 0.5 ul

-Optional: you may incubate the mix at 37 ºC during 5 min on a thermocycler prior to the addiction of the Reverse Transcriptase

-Incubate at 42 ºC for 60 min (up to 90 min)

-Stop the reaction by incubating at 70 ºC for 10 min

-Keep the cDNA at -20 ºC until further use

Check for cDNA on agarose gel

Run 1ul of the synthesized cDNA on 1-1.2% agarose gel. The cDNA should look like a smear on gel.