WCSB, Wageningen University	IBISBA-SOP-WU09
	Version 1.0

EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: cDNA synthesis

distribution list			
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Experimenter 1	Rita Volkers		14/3/2019

Instruction

cDNA synthesis

1. Introduction / Purpose

In this SOP, the synthesis of cDNA from template RNA is described. It doesn't describe how to isolate RNA.

Keywords: cDNA, RNA

2. Equipment and chemicals

2.1. Equipment

- PCR Machine

2.2. Chemicals

- Revertaid H Minus First Strand cDNA Synthesis Kit from Thermo Fisher Scientific

2.3 Other materials

- Template RNA (the RNA that needs to be converted to cDNA)

Special consumables:

- PCR tubes (RNAse free)
- Pipette tips (RNAse free)

3. Procedures

Put together into a PCR tube:

- 11 µl RNA of known concentration
- 1 µl Primer Oligo dT_{18}
- 4 µl 5x Reaction Buffer
- 1 µl RNAse inhibitor
- 2 µl dNTP mix
- 1 µl Reverse Transcriptase

Put tube in a PCR machine and run the following program:

- 42 °C for 60 minutes
- 70 °C for 5 minutes

Calculate amount of cDNA, assuming that all RNA was converted to cDNA

5. Remarks / troubleshooting

- Be sure to not contaminate your samples with RNAses!!
- The concentration of the RNA cannot be measured by spectrophotometer because the ingredients of the mixture will interfere with the measurement.

6. Biosafety

No biosafety issues were associated with this protocol.

7. Acknowledgements

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