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Reverse transcription using SuperScript IV V.3

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EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0205608

GUIDELINES

Use high quality RNA as the substrate.
Keep RNA on ice at all times.
Use RNase free water.

MATERIALS

NAME	CATALOG #	VENDOR
SuperScript™ IV Reverse Transcriptase	18090050	Thermo Fisher Scientific

1 Mix the following reagents from the kit. Please scale up if more RT reaction is desired.

Reagent	Amount (uL)
Primer (Random or dT)	0.5
dNTP (10mM)	1
RNA	11

2 Incubate the mixture at 72 °C for 00:02:00 . Then, incubate samples on ice for few minutes.

This step allows denaturation of RNA and proper priming for the downstream cDNA synthesis.

3 Mix the following reagents from the kit. Please scale up if more RT reaction is desired.

Reagent	Amount (uL)
RT Buffer (5x)	4
DTT(10mM)	1
RNase Inhibitor	1

Add the 6uL to the 12.5uL mix from Step 3.

4 Incubate the samples at 37 °C for 00:05:00 . Then, add 1.5uL SuperScript RT IV enzyme to the reaction and mix well.

5 Incubate the samples using the following incubation settings:

Temp (C)	Time (minutes)
25	5
45	40
55	10
75	10

5.1 Add 1uL RNase H to the cDNA samples and incubate at 37 °C for 00:20:00 .

5.2 Dilute the cDNA samples using Nuclease free water.