

**Supplementary Table 1.** Summary of RT-PCR thermocycling conditions, master mix, and primer sets used.

● **Primer Mix**

\*To prepare the **TUNI primer mix**, add primer stock solution so that there is 4: 1:5 ratio of Tuni12:Tuni12.4:Tuni13 primers. Sample calculation below:

Name	Sequence
Tuni 12	ACG CGT GAT <u>CAG CAA AAG CAG G</u>
Tuni 12.4	ACG CGT GAT <u>CAG CGA AAG CAG G</u>
Tuni 13	ACG CGT GAT <u>CAG TAG AAA CAA GG</u>

  

Primer	Volume
nuclease-free water	90 µl
Tuni 12 (100 µM)	4 µl
Tuni 12.4 (100 µM)	1 µl
Tuni 13 (100 µM)	5 µl
<b>TOTAL</b>	<b>100 µl</b>

\*To prepare the **OPTI primer mix**, add primer stock solution so that there is 0.35: 0.65:1 ratio of Opti F1:F2:R primers. Sample calculation below:

Name	Sequence
Opti F1	GTT ACG CGC CAG <u>CAA AAG CAG G</u>
Opti F2	GTT ACG CGC CAG <u>CGA AAG CAG G</u>
Opti R	GTT ACG CGC <u>CAG TAG AAA CAA GG</u>

  

Primer	Volume
nuclease-free water	80 µl
Opti F1 (100 µM)	3.5 µl
Opti F2 (100 µM)	6.5 µl
Opti R (100 µM)	10 µl
<b>TOTAL</b>	<b>100 µl</b>

● **Thermocycling conditions**

<b>SuperScript III Platinum with TUNI primers</b>			
42°C	60 min		
94°C	2 min		
94°C	30 sec		5 cycles
45°C	30 sec		
68°C	3 min		
94°C	30 sec		31 cycles
57°C	30 sec		
68°C	3 min		
4°C	hold		

<b>SuperScript III Platinum with OPTI primers</b>			
55°C	2 min		
42°C	90 min		
94°C	2 min		
94°C	15 sec		5 cycles
45°C	30 sec		
68°C	3.5 min		
94°C	15 sec		30 cycles
63°C	30 sec		
68°C	3.5 min		
68°C	10 min		
4°C	hold		

<b>SuperScript IV with TUNI primers</b>			
55°C	2 min		
42°C	90 min		
98°C	2 min		
98°C	10 sec		5 cycles
45°C	30 sec		
72°C	3.5 min		
98°C	10 sec		30 cycles
66°C	30 sec		
72°C	3.5 min		
72°C	10 min		
4°C	hold		

<b>SuperScript IV with OPTI primers</b>			
55°C	2 min		
42°C	90 min		
98°C	2 min		
98°C	10 sec		5 cycles
45°C	30 sec		
72°C	3.5 min		
98°C	10 sec		30 cycles
67°C	30 sec		
72°C	3.5 min		
72°C	10 min		
4°C	hold		