

Reverse transcription of the specific tRNA species

Reverse transcription of the specific tRNA species by means of specific primers into cDNA:

1. Set up reaction as follows:

Table 1: Representation of the composition I for the reverse transcription of specific tRNA

Component	reaction
ligation product of T4 RNA Ligase 2	1 µL
specific tRNA Primer (10pmol/µl)	0,5 µL
H2O	1 µL

2. Keep on 70°C for 5 min for melting of secondary structures
3. Keep on ice for 5 min for primer annealing
4. Set up reaction as follows:

Table 2: Representation of the composition for the reverse transcription of specific tRNA

Component	reaction
5xGoScript Buffer (Promega)	2 µL
25 mM MgCl ₂ (Promega)	0,6 µL
dNTPs (10mM each dNTP)	0,375 µl
H2O	4,025 µl
GoScript Reverse Transcriptase (Promega)	0,5 µl

5. Keep on 25°C for 5 min
6. Heat it to 42°C for 1h
7. Keep on 70°C for 15 min

RNase H digestion to eliminate all remaining RNA:

1. Set up reaction as follows:

Table 3: Representation of the composition of RNase H digestion

Component	reaction
cDNA from reverse transcription	total volume
RNase H (0,033 U/ µl; Thermo Fisher)	2 µl

2. Keep on 37°C for 20 min