TERRA RTq PCR - Protocol

1. RNA extraction

- Collect cell pellet by trypsinization (6 cm or 10 cm dish)
- Wash cell pellet once with PBS
- RNA extraction with RNeasy Mini Kit (Qiagen)
  - First step: Dissolve pellet in 600 µl RLT buffer + 0.6 µl Proteinase K (20 mg/ml, Genaxxon) + 6 µl β-Mercaptoethanol, pipet up and down several times, incubate at RT for 5 min -> continue with protocol according to manufacturer
  - Elute in 40 µl RNase-free H2O (add 1 µl Ribolock (RNase inhibitor) to collection tube first)
- Measure RNA concentration with Nanodrop

2. DNA digestion and Clean Up

- Digest with DNase for 30 min at 37 °C
  (e.g. for digestion of 15 µg RNA in 70 µl, add 18 µl DNase (Promega), 7 µl 10X DNase buffer (Promega), 2 µl Ribolock)
- RNA Clean Up with RNeasy Mini Kit (Qiagen) - Clean Up Protocol according to manufacturer
- Elute in 30 µl RNase-free H2O (add 1 µl Ribolock to collection tube first)
- Measure RNA concentration with Nanodrop
- Check RNA integrity on an RNA gel: 200 ng RNA in 50% Formamide, 5 min @65 °C, load on 4% E-Gel
- If not used immediately, store RNA at -80 °C

3. Reverse transcription

- Reverse transcription with Superscript III (Invitrogen) according to manufacturer's protocol
- 1 µg RNA per sample with 2 pmol gene-specific primer (β-actin RT and Telo RT)
- Include minus RT controls for each sample!
- RT reaction at 55 °C for 60 min, followed by inactivation at 70 °C for 15 min

- Add 0.5 µl RNaseH (10 U/µl) to 20 µl RT reaction and incubate for 30 min at 37 °C
- Dilute the RT reaction in 60 µl H2O (can be reduced to 40 µl if CT values are quite low)
- If not used immediately, store cDNA at -20 °C

4. RTq PCR

LightCycler 480 SYBR Green I Master Mix (Roche)

- Prepare a mastermix for each primer pair containing the following volumes per sample:

SYBR Green I Mastermix	5 µl
Primer Mix fwd + rev (both 5 µM)	1 µl
H2O	2 µl

- Load each sample in triplicates; include negative controls (only H2O) and minus RT controls for each primer pair
- Add 8 µl mastermix to a 96 well RTq PCR plate
- Add 2 µl cDNA sample per well
- Centrifuge plate for 2 min at 1000 g
- RTq PCR program:

95 °C – 10 min

95 °C – 10 s
58 °C – 10 s
72 °C – 10 s
] 40x

Primers for reverse transcription	
β-actin RT	AGT CCG CCT AGA AGC ATT TG
Telo RT	CCC TAA CCC TAA CCC TAA CCC TAA CCC TAA
Primers for RTq PCR	
β-actin	TCC CTG GAG AAG AGC TAC GA
	AGC ACT GTG TTG GCG TAC AG
1q-2q-10q-13q	GAA TCC TGC GCA CCG AGA T
	CTG CAC TTG AAC CCT GCA ATA C
15q	CAG CGA GAT TCT CCC AAG CTA AG
	AAC CCT AAC CAC ATG AGC AAC G
9p-15q-Xq-Yq	TTC CGC ACT GAA CCG CTC TAA
	GCA GCC ATG AAT AAT CAA GGT
5p	GAG TGC ATT AGC ATA CAG GTG
	TCC TAA TGC ACA CGT AAC AC
10p-18p	CCT TCT AAC TGG ACT CTG AC

List of Primers

	GCC ACA GCG ACG GTA AAT AA
TERRA total	CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT
	GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT