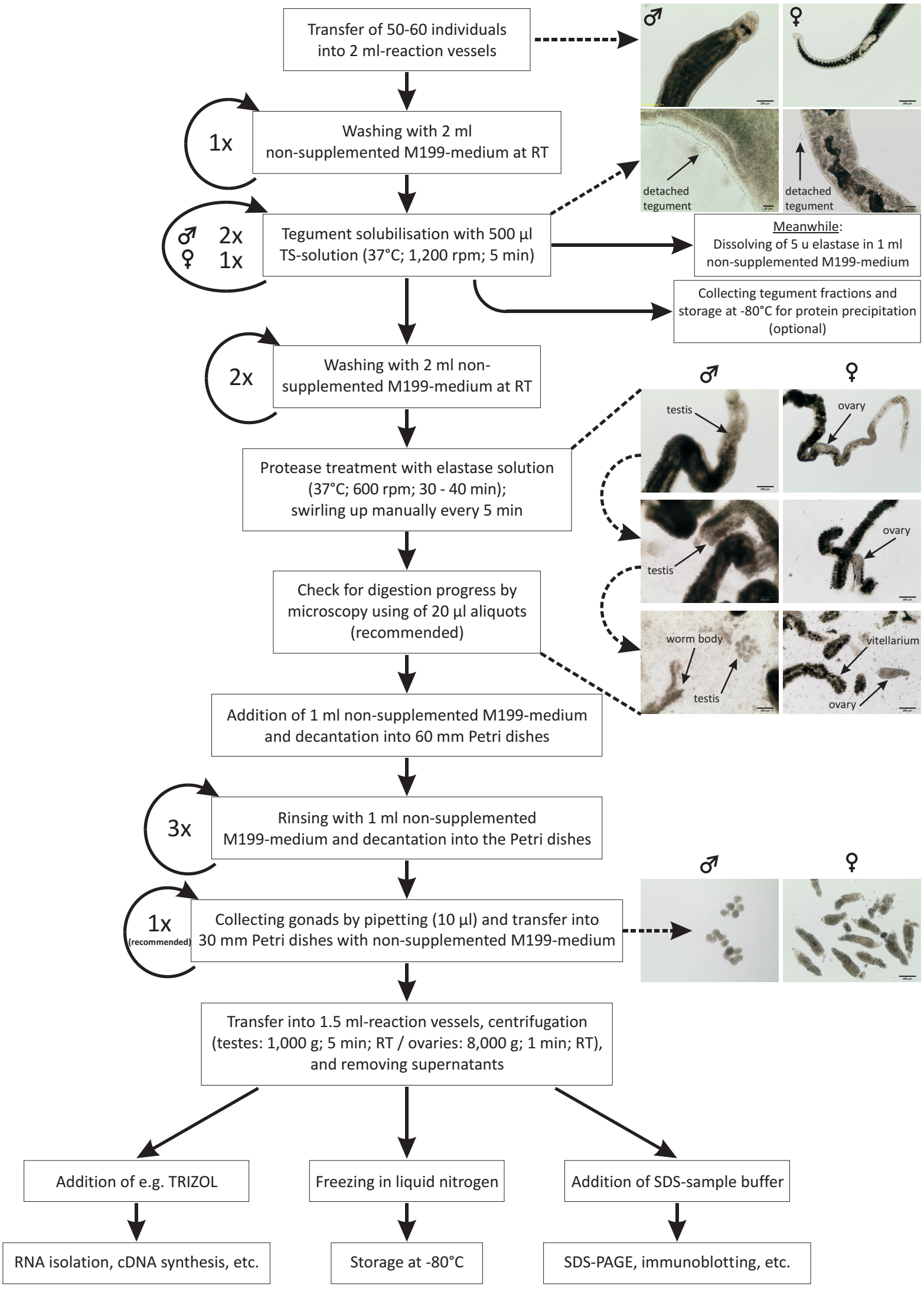
Transfer of 50-60 individuals into 2 ml-reaction vessels
♂
♀
1x
Washing with 2 ml non-supplemented M199-medium at RT
detached tegument
detached tegument
♂ 2x
♀ 1x
Tegument solubilisation with 500 µl TS-solution (37°C; 1,200 rpm; 5 min)
Meanwhile:
Dissolving of 5 u elastase in 1 ml non-supplemented M199-medium
Collecting tegument fractions and storage at -80°C for protein precipitation (optional)
2x
Washing with 2 ml non-supplemented M199-medium at RT
♂
♀
testis
ovary
Protease treatment with elastase solution (37°C; 600 rpm; 30 - 40 min); swirling up manually every 5 min
testis
ovary
Check for digestion progress by microscopy using of 20 µl aliquots (recommended)
worm body
testis
vitellarium
ovary
Addition of 1 ml non-supplemented M199-medium and decantation into 60 mm Petri dishes
3x
Rinsing with 1 ml non-supplemented M199-medium and decantation into the Petri dishes
♂
♀
1x
(recommended)
Collecting gonads by pipetting (10 µl) and transfer into 30 mm Petri dishes with non-supplemented M199-medium
Transfer into 1.5 ml-reaction vessels, centrifugation (testes: 1,000 g; 5 min; RT / ovaries: 8,000 g; 1 min; RT), and removing supernatants
Addition of e.g. TRIZOL
Freezing in liquid nitrogen
Addition of SDS-sample buffer
RNA isolation, cDNA synthesis, etc.
Storage at -80°C
SDS-PAGE, immunoblotting, etc.