Hybridization and ligation of tRNAs

Hybridization of an adapter to all tRNA species:

1. Set up reaction as follows:

Table 1: Representation of the composition for the hybridization of an adapter to tRNA

Component	reaction
RNA/DNA-adapter (20 pmol/µl)	1 µL
deacylated tRNA (50 ng/µl)	1 µL
H2O	13,5 µL

2. Heating at 90°C for 3 min for melting all secondary structures
3. Keep on 37°C for 20 min for the attachment of the adapter to the 3’ overhang of the tRNAs

Ligation of an adapter to all tRNA species:

1. Set up reaction as follows:

Table 2: Representation of the composition for ligation of an adapter to tRNA

Component	reaction
hybridization approach	total volume
10x T4 RNA Ligase 2 Reactionsbuffer (NEB)	2 µl
T4 RNA Ligase 2 (1 U/µl, NEB)	1,5 µl
T4 Polynucleotide Kinase (10U/µl, NEB)	1 µl

2. Keep on 37°C for 20 min
3. Store overnight at 4°