Protocol: Chemical Modification Quick Reference

I. Kethoxal

Compatible buffers: K-cacodylate, pH7.2 or Hepes pH 7.2
Stock conc of kethoxal: 37 mg/ml
Add 2.0 microL of stock kethoxal to final 25 microL reaction volume
Incubate at 37 C/10 min or Room temp/30 min or Ice/2 hours
Room temp/30min is best for A-site ASL protections
STOP: 12.5 microL of (0.3M) NaAc (pH6) + 12.5 microL of (0.5M) K-borate (pH7.0) + 300 microL of 100% ethanol
Always add k-borate to the sample otherwise the modifications are unstable during primer extension!

II. DMS

Compatible buffers: K-cacodylate, pH7.2 or Hepes pH 7.2
Dilute Aldrich stock solution 1:10 in 95% ethanol
Add 1.0 microL of 1:10 diluted to final 25 microL reaction volume
Incubate at 37 C/10 min or Room temp/30 min or Ice/2 hours
Room temp/30min is best for A-site ASL protections
STOP: 12.5 microL of (0.3M) NaAc (pH6) + 12.5 microL of fresh DMS stop + 300 microL of 100% ethanol
(DMS stop: 1M TrisCl pH 7.5, 0.1M EDTA, 1M BME)

III. CMCT

Compatible buffers: Only K-borate (pH 7.2, 8.1)
Make fresh 42mg/ml stock solution in K-borate binding buffer
Add equal volume of 42mg/ml to reaction mix (25 microL to 25 microL complex mix)
Incubate at 37 C/10 min or Room temp/30 min or Ice/2 hours
STOP: Add 50 microL of 0.3m NaAc (pH 6) to 50 microL of mods mix + 300 microL of 100% ethanol

Notes:
Avoid buffers containing polyamines. They react with the modification reagents.
Use ice-cold ethanol for precipitation. Precipitate on dry-ice/ethanol mix for 10 min only. Use only water saturated phenol for extractions of rRNA. Sometimes the samples do not extend due to excess salt. Remove salt by resuspending the samples in water to 100 microL final volume. Re-extract 2 times with chloroform. Repeat precipitation without any additional NaAc added to the sample. Try primer extension again.