4-thiouridine treatment crosslinking with 365 nm UV light

(a) Small RNA seq
(b) PAR-CLIP
(c) CLASH
(d) HITS-CLIP
(e) iCLIP

Cell lysis

RNA extraction

Size selection gel or kit extraction

Immunoprecipitation RNase treatment
Radioactive Phosphorylation

Gel purification
Proteinase K treatment

3′ adapter ligation

5′ adapter ligation

Adapter-flanked insert

3′ adapter ligation
PNK treatment

RNA-RNA hybrid

Gel purification
Proteinase K treatment

5′ adapter ligation
Reverse transcription
Circularization
Linearization
PCR amplification

Sequencing data analysis

cDNA synthesis
PCR amplification

Ni-NTA binding
Gu-HCl washing
PNK treatment

Intermolecular ligation

PNK treatment
3′ adapter ligation

Gel purification
Proteinase K treatment

Ni-NTA elution

PNK treatment

3′ adapter ligation

5′ adapter ligation

iCLIP