**Materials List for:**

# PAR-CliP - A Method to Identify Transcriptome-wide the Binding Sites of RNA Binding Proteins

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URL: https://www.jove.com/video/2034
DOI: doi:10.3791/2034

## Materials

### Buffers and reagents

**Growth medium HEK293 cells**

- DMEM
- 10% FBS
- 2 mM L-Glutamine
- 100 U/ml penicillin
- 100 U/ml streptomycin
- 100 µg/ml hygromycin
- 15 µg/ml blasticidin

**4-thiouridine stock solution (1 M)**

- 260.27 mg 4-thiouridine
- 1 ml DMSO

**Doxycyclin stock (10 mg/ml)**

- 10 mg doxycyclin
- 1 ml DMSO

**1x NP40 lysis buffer**

Prepare a stock of 5x buffer without DTT and protease inhibitors. Add DTT and protease inhibitor directly before the experiment.

- 50 mM HEPES, pH 7.5
- 150 mM KCl
- 2 mM EDTA
- 1 mM NaF
- 0.5% (v/v) NP40
- 0.5 mM DTT
- complete EDTA-free protease inhibitor cocktail (Roche)

**Citrate-Phosphate buffer**

- 4.7 g/l citric acid
- 9.2 g/l $Na_2HPO_4$
- pH 5.0

**IP-wash buffer**

- 50 mM HEPES-KOH, pH 7.5

- 300 mM KCl
- 0.05% (v/v) NP40
- 0.5 mM DTT (add directly before experiment)
- complete EDTA-free protease inhibitor cocktail (Roche) (add directly before experiment)

**High-salt wash buffer**

- 50 mM HEPES-KOH, pH 7.5
- 500 mM KCl
- 0.05% (v/v) NP40
- 0.5 mM DTT (add directly before the experiment)
- complete EDTA-free protease inhibitor cocktail (Roche) (add directly before the experiment)

**Dephosphorylation buffer**

- 50 mM Tris-HCl, pH 7.9
- 100 mM NaCl
- 10 mM $MgCl_2$
- 1 mM DTT

**Phosphatase wash buffer**

- 50 mM Tris-HCl, pH 7.5
- 20 mM EGTA
- 0.5% (v/v) NP40

**Polynucleotide kinase (PNK) buffer without DTT**

- 50 mM Tris-HCl, pH 7.5
- 50 mM NaCl
- 10 mM $MgCl_2$

**PNK buffer**

- 50 mM Tris-HCl, pH 7.5
- 50 mM NaCl
- 10 mM $MgCl_2$
- 5 mM DTT

**SDS PAGE loading buffer**

- 10% glycerol (v/v)
- 50 mM Tris-HCl, pH 6.8
- 2 mM EDTA
- 2% SDS (w/v)
- 100 mM DTT
- 0.1% bromophenol blue

**2x Proteinase K buffer**

- 100 mM Tris-HCl, pH 7.5
- 150 mM NaCl
- 12.5 mM EDTA
- 2% (w/v) SDS