

<b>Reaction</b>	<b>Original conditions</b>	<b>Optimised conditions</b>
Phosphatase reaction	2.4 U rSAP/ $\mu$ g RNA 2 hours, 37°C	2.4 U quickCIP/ $\mu$ g RNA 2 hours, 37°C
Decapping reaction	0.6 U CAPCLIP/ $\mu$ g RNA 2 hours, 37°C	1.2 U MDE/ $\mu$ g RNA 2 hours, 37°C
5' adaptor ligation	0% PEG Overnight, 16°C	5% PEG RNA + adaptor heat denatured 2 hours 25°C, overnight 16°C
3' adaptor ligation	10% PEG 2 hours 25°C, overnight 16°C	15% PEG RNA + adaptor heat denatured 2 hours 25°C, overnight 16°C

**Supplemental Table S1. Summary TSS-seq enzymatic reaction conditions.**

For the phosphatase reaction, rSAP was changed to quickCIP. For the decapping reaction, CAPCLIP was changed to MDE. A heat denaturation step was introduced before both adaptor ligation steps, and the concentration of PEG was also optimised. The 5' adaptor reaction conditions were also altered.