

## Supplementary Information

PRO-seq: precision nuclear run-on provides base-pair resolution mapping of active RNA polymerase genome-wide.

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**Supplementary Table 1.** Nuclear Run-On reaction mix (step 4)

	1× volume (μl)	Scaled volume (μl)
NRO master mix	28	
Biotin NTP mix (+rNTP/H <sub>2</sub> O)	20	
RNase inhibitor	2	
Sarkosyl (2%)	50	
<b>Total</b>	<b>100</b>	

**Supplementary Table 2.** RNA adaptor ligation mix (steps 52, 78)

	1× volume (μl)	Scaled volume (μl)
T4 RNA ligase buffer (10x)	1	
ATP (10 mM)	1	
50 % PEG	2	
RNase inhibitor	1	
T4 RNA ligase I	1	
<b>Total</b>	<b>6</b>	

**Supplementary Table 3.** 5'-phosphate-dependent exonuclease enzyme mix (Box 1, step 1, for PRO-cap only)

	1× volume (μl)	Scaled volume (μl)
DEPC H <sub>2</sub> O	2.5	
Buffer A (10x)	1	
RNase inhibitor	0.5	
5'-phosphate-dependent exonuclease	1	
<b>Total</b>	<b>5</b>	

**Supplementary Table 4.** Alkaline phosphatase enzyme mix (Box 1, step 14, for PRO-cap only)

	1× volume (μl)	Scaled volume (μl)
DEPC H <sub>2</sub> O	3	
Alkaline phosphatase buffer (10x)	1	
RNase inhibitor	0.5	
Alkaline phosphatase	0.5	
<b>Total</b>	<b>5</b>	

**Supplementary Table 5.** 5' cap repair enzyme mix (step 60)

	1× volume (μl)	Scaled volume (μl)
DEPC H <sub>2</sub> O	3/2.5	
TAP buffer/ThermoPol Reaction buffer (10x)	1	
RNase inhibitor	0.5	
TAP/RppH enzyme	0.5/1	
<b>Total</b>	<b>5</b>	

**Supplementary Table 6.** Polynucleotide kinase (PNK) enzyme mix (step 64, for PRO-seq only)

	<b>1× volume (μl)</b>	<b>Scaled volume (μl)</b>
DEPC H <sub>2</sub> O	65	
10× PNK buffer	10	
ATP (10 mM)	10	
RNase inhibitor	2.5	
T4 PNK enzyme	2.5	
<b>Total</b>	<b>90</b>	

**Supplementary Table 7.** Reverse transcription buffer mix (step 87)

	<b>1× volume (μl)</b>	<b>Scaled volume (μl)</b>
First strand buffer (5x)	4	
DTT (0.1 M)	1	
RNase inhibitor	1	
<b>Total</b>	<b>6</b>	

**Supplementary Table 8.** Test PCR amplification mix (step 94)

	<b>1× volume (μl)</b>	<b>Scaled volume (μl)</b>
H <sub>2</sub> O	5	
High Fidelity (HF) buffer (5x)	4	
Betaine (5 M)	4	
dNTP mix (12.5 mM each)	0.4	
RP1 primer (25 μM)	0.2	
RPI-1 primer (25 μM)	0.2	
Phusion DNA polymerase	0.	
<b>Total</b>	<b>14</b>	

**Supplementary Table 9.** Full-scale PCR amplification mix (step 102)

	<b>1× volume (μl)</b>	<b>Scaled volume (μl)</b>
H <sub>2</sub> O	3	
High Fidelity (HF) buffer (5x)	10	
Betaine (5 M)	10	
dNTP mix (12.5 mM each)	1	
RP1 primer (25 μM)	0.5	
Phusion DNA polymerase	1	
<b>Total</b>	<b>25</b>	