Supplementary material for

Transcription start site mapping using Super-Low Input Carrier-CAGE

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Supplementary Table 1. Primer sequences.

Primer name	Sequence	Step
RT primer	Phos TCTNNNNNN	2.1.
5'nAnT-iCAGE_01 N6*	CGACGCTCTTCCGATCTACCNNNNNN Phos	9.
5'nAnT-iCAGE_01 GN5*	CGACGCTCTTCCGATCTACCGN NNNN Phos	9.
5'nAnT-iCAGE_01 Dwn*	Phos GGTAGATCGGAAGAGCGTCG Phos	9.
3'nAnT-iCAGE N3 + AGA Up	NNNAGAUCGGAAGAGCGGUUCAGCAGGAA UGC CGAGACCGAUCUCGUAUGCCG UCUUCUGCUUG	10.
3'nAnT-iCAGE Dwn	CAAGCAGAAGACGGCATACGAGATCGGTCTC GGCATTCCTGCTGAACCGCTCTTCCGA	10.
nAnT-iCAGE 2nd primer	AATGATACGGCGACCACCGAGATCTACACTC TTTCCCTACACGACGCTCTTCCGATCT	13.1.

*barcoding is within the 5'linker. One barcode example is shown (ACC, underlined sequence). Other barcoding options are: CAC, AGT, GCG, ATG, TAC, ACG, GCT.

Supplementary Table 2. Annealing of 5’ and 3’ linkers

[a]Temperature / °C	Time/min
95	5
83	5
71	5
59	5
47	5
35	5
11	hold

[a]Speed of temperature change should be approximately 0.1 °C/s.

Note: linker annealing conditions are from[1] and linker sequences are from [2]

Supplementary Table 3. 5'linker annealing

component	concentration	volume	final concentration
	Annealing of 5' N6 linker		
5'nAnT-iCAGE_01 N6	1 mM	1 µl	100 µM
5'nAnT-iCAGE_01 Dwn	1 mM	1 µl	100 µM
NaCl	1 M	1 µl	0.1 M
0.1 x TE	1 mM Tris-Cl 7.5	7 µl	0.7 mM
	0.1 mM EDTA 8.0		0.07 mM
	Annealing of 5' GN6 linker		
5'nAnT-iCAGE_01 GN5	1 mM	1 µl	100 µM
5'nAnT-iCAGE_01 Dwn	1 mM	1 µl	100 µM
NaCl	1 M	1 µl	0.1 M
0.1 x TE	1 mM Tris-Cl 7.5	7 µl	0.7 mM
	0.1 mM EDTA 8.0		0.07 mM

Note: All single stranded linkers should be dissolved to be 1 mM in 0.1 x TE. Sequences of linkers are as in Supplementary Table 1 (and as in Murata et al 2014). Example of 5'linker 01 is shown (barcode ACC). Other barcoding options are shown below Supplementary Table 1. When annealing, use up (N6 and GN5) and down (Dwn) linkers with the same barcode. After mixing linkers according to Supplementary Table 3, run annealing program described in Supplementary Table 2. When annealing is done, mix the annealed linkers in 1:4 ratio (see Supplementary Table 4).

Supplementary Table 4. 5’linker mixing

component	concentration	volume	final concentration
5’nAnT-iCAGE_01 N6 annealed (N6 + Dwn)	100 µM	2.5 µl	20 µM
5’nAnT-iCAGE_01 GN5 annealed (GN5 + Dwn)	100 µM	10 µl	80 µM

Dilute the mixed 5’linkers to working concentration according to Supplementary Table 5. Store the rest of the linkers at -20 °C.

Supplementary Table 5. 5’linker dilution.

component	concentration	volume	final concentration
5’nAnT-iCAGE_01 (N6 and GN5)	100 µM	1 µl	10 µM
NaCl	0.1 M	9 µl	0.09 M

Store the diluted linkers at -20 °C.

Supplementary Table 6. 3’linker annealing

component	concentration	volume	final concentration
3’nAnT-iCAGE N3 + AGA Up	1 mM	1 µl	100 µM
3’nAnT-iCAGE Dwn	1 mM	1 µl	100 µM
NaCl	1 M	1 µl	0.1 M
0.1 x TE	1 mM Tris-Cl 7.5	7 µl	0.7 mM
	0.1 mM EDTA 8.0		0.07 mM

Note: All single stranded linkers should be dissolved to be 1 mM in 0.1 x TE. Sequences of linkers are as in Murata et al 2014. After mixing the single stranded linkers according to Supplementary Table 6, run the annealing program described in Supplementary Table 2. When annealing is done, dilute the 3’linker according to Supplementary Table 7.

Supplementary Table 7. 3’linker dilution.

component	concentration	volume	final concentration
3’nAnT-iCAGE	100 µM	2 µl	10 µM
NaCl	0.1 M	18 µl	0.09 M

Store the diluted linkers at -20 °C.

Supplementary Table 8. Preparation of standard serial dilutions.

Concentration /ng ml^{-1}	2 µg $µl^{-1}$ lambda DNA / µl	1 x TE / µl
0	0	100
10	1	99
20	2	98
40	4	96
60	6	94
80	8	92
100	10	90
200	20	80

Supplementary references

1 Takahashi, H., Lassmann, T., Murata, M. & Carninci, P. 5' end-centered expression profiling using cap-analysis gene expression and next-generation sequencing. *Nature Protocols.* **7** (3), 542-561, doi:10.1038/nprot.2012.005, (2012).

2 Murata, M. *et al.* Detecting expressed genes using CAGE. *Methods in Molecular Biology.* **1164** 67-85, doi:10.1007/978-1-4939-0805-9_7, (2014).