

**Supplementary Table 3. 5'linker annealing**

component	concentration	volume	final concentration
Annealing of 5' N6 linker			
5'nAnT-iCAGE_01 N6	1 mM	1 $\mu$ l	100 $\mu$ M
5'nAnT-iCAGE_01 Dwn	1 mM	1 $\mu$ l	100 $\mu$ M
NaCl	1 M	1 $\mu$ l	0.1 M
0.1 x TE	1 mM Tris-Cl 7.5	7 $\mu$ l	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 $\mu$ l	100 $\mu$ M
5'nAnT-iCAGE_01 Dwn	1 mM	1 $\mu$ l	100 $\mu$ M
NaCl	1 M	1 $\mu$ l	0.1 M
0.1 x TE	1 mM Tris-Cl 7.5	7 $\mu$ l	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).