

Supplementary Table 1. Details of a single guide RNA targeting the 5'UTR of the CENH3 gene

CRISPR RNA Name	Single Guide RNA Sequence
R026	^m C ^m A ^m <u>ACUCAUAAGAGGCAUGAAGUUUUAGAGCUAGAAAUAGCAAGUUA</u> AAAUAAAGGCUAGUCCGUUUCACUUGAAAAAGUGGCACCGAGUCGGUGC ^m U ^m U ^m UU

spacer sequence is underlined. O-Methyl RNA bases are indicated in superscript

Supplementary Table 2. ddPCR probes and primers used to quantify the frequency of mutation of *CENH3* alleles

Name	Type	Nucleotide sequence (5'-3')	Tm (°C)
Bn_R026_R035_F072_f2	Primer	ATGTGCTGAGCAAACCCTCT	63.4
Bn_R026_R035_F072_r1	Primer	AGATTATTTGTTTTCCCGCTTCTCC	63
Bn_R026_R035_abl_FAM	Probe ¹	FAM- ACCATTCATGCCTCTTATGAGTTGTAGTCTG- BHQ1	67
Bn_F072_HEX	Probe ²	HEX-TCCTTCTGCTTTCTTTACACCAACGC- BHQ1	68.3

¹ablation probe; ²control probe