**Supplementary information**

Generation of Transgenic Cloned Buffalo Embryos Harboring the EGFP Gene in the Y Chromosome Using CRISPR/Cas9-Mediated Targeted Integration

Running title: Optimization of Homologous Recombination in Transgenic Cloned Buffalo Embryos

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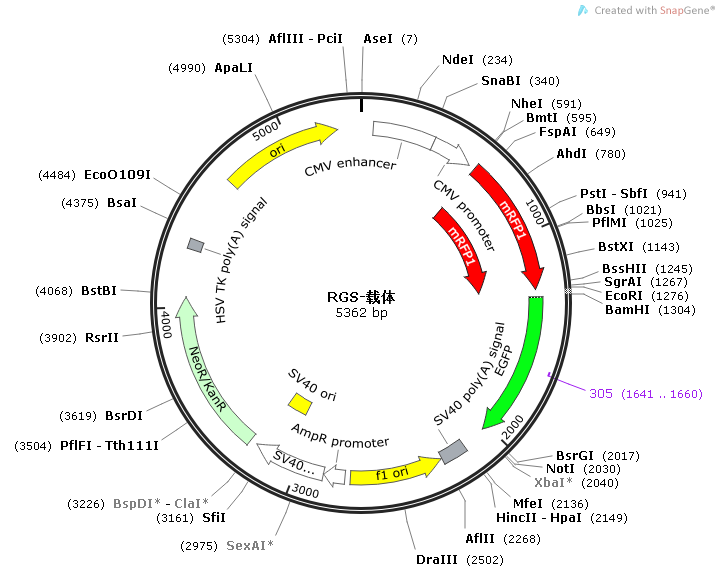
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**Figure S1.** Map of the RGS plasmid.

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**Figure S2.** The percentage of transfected cells, as determined by mCherry expression, was affected by voltage. C:\Users\Administrator\Desktop\图片5.tif

**Figure S3.** The percentage of transfected cells, as determined by mCherry expression, was affected by the number of pulses.



**Figure S4.** In vitro development of non-transgenic cloned embryos from buffalo.

|  |  |
| --- | --- |
| sgRNA | Sequence (5’-3’) |
| sgRNA1 | CACGAGCAAGCATCTGAAAG |
| sgRNA2 | GGTGAGTAAACACAGGGTAC |
| sgRNA3 | GCACTCATAAAGAATGCACA |

**Table S1.** The sequences of the sgRNAs targeting the Ddx3y gene from buffalo

|  |  |
| --- | --- |
| sgRNA | Sequence (5’-3’) |
| sgRNA1 | GATCCACATCTGCTGGAAGG |
| sgRNA2 | CGTCCACCGCAAATGCTTCT |
| sgRNA3 | GCAGATGTGGATCAGCAAGC |
| sgRNA4 | CCACCGCAAATGCTTCTAGG |

**Table S2.** The sequences of the sgRNAs targeting the Actin gene from buffalo

|  |  |
| --- | --- |
| Primer | Sequence (5’-3’) |
| 5’ OF | TCAACTCATTTTCATGTGGC |
| 5’ OR | GGCTATGAACTAATGACCC |
| 5’ IF | GTACAGTAGTAATGGCAGTG |
| 5’ IR | GGCTATGAACTAATGACCC |
| 3’ OF | CCCACTGTCCTTTCCTAA |
| 3’ OR | GGTGGTGCTGATATTGGT |
| 3’ IF | CCCACTGTCCTTTCCTAA |
| 3’ IR | GGATGTCACTTGTTGGTG |

**Table S3.** Primers used for amplification of 5’ and 3’ junctions

|  |  |  |  |
| --- | --- | --- | --- |
| Gene | Primer | Sequence (5’-3’) | Product size |
| DNMT1 | F | CTCAGAAGGGAGACGTGGAG | 138 bp |
|  | R | TAGTAGTCACAGTAGCTGAGGA |  |
| DNMT3a | F | GTGCTGTCTCTATTCGATGG | 188 bp |
|  | R | CCATTCCTGGATATGCTTCTG |  |
| HDAC1 | F | ATCGGTTAGGTTGCTTCAATCTG | 168 bp |
|  | R | TTGTATGGAAGCTCATTAGGGA |  |
| HDAC2 | F | ACAGGGTCATCCCATGAAAC | 115 bp |
|  | R | TTCTTCAGCAGTGGCTTTAT |  |
| HDAC3 | F | ATCCGGATGGAGCGTGAAGT | 137 bp |
|  | R | GTGGCTACACTGTCCGGAAT |  |
| GAPDH | F | TCAAGAAGGTGGTGAAGC | 122 bp |
|  | R | CCCAGCATCGAAGGTAGA |  |

**Table S4.** Primers used for amplification of epigenetic-related genes