Appendix A

1. Isolation and identification of bovine FST

FST gene was cloned from a bovine ovary by the Trizol protocol as described[12]. The primers (5′–3′) for FST were designed according to its sequence; forward primer, AACTGGAATTCTGCCCTCAGGATGGCCCGT and reverse primer, AACTGGGATCCTGAACATTGGTGGAGGGT. PCR reaction conditions: 95°C for 5 min, 95°C for 30 s, 58°C for 30 s, 72°C for 1 min 20 s (35 cycles), 72°C for 7 min, and then kept at 4°C. The full length FST is 1072 bp, as shown in Fig. S1, an obvious band at 1072 bp was obtained.

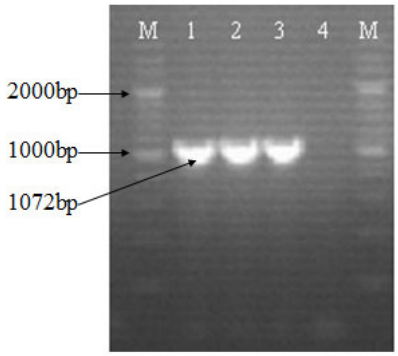


Fig. S1 Full-length PCR of FST. M, DL2000 Marker; 1–3, PCR of FST; 4, the control of ddH_2O.

2. Isolation and identification of bovine MAR

The primers were designed according to the published bovine MAR sequence, four restriction sites were available before the CAG promoter and the polyA signal: SacI, *Sal*I, *Pst*I and AflII. We introduced a MAR before the CAG promoter and following the polyA respectively. The primers (5′–3′) for MAR before the promoter; forward primer, AATCGTGATCAAATCGGAGCTCAAATTGTAACAATGTATAGA, introduced recognition sites for SacI (GAGCTC), and reverse primer, AATCG GTCGACTGAGTCATCCTTTCCTTG, introduced recognition site of *Sal*I (GTCGAC); The primers (5′–3′) of MAR after the PolyA signal; forward primer, AATCGCTGCAGAAATTGTAACAATGTATAGA, introduced recognition site for *Pst*I (CTGCAG), and reverse primer, AATCGTGATCAAATCGCTTAAGTGAGTCATCCTTTCCTTG, with introduced t recognition sites for AflII (CTTAAG). PCR reaction conditions: 95°C for 5 min, 95°C for 30 s, 58°C for 30 s, 72°C for 1 min 20 s (35 cycles), 72°C for 7 min, and then kept at 4°C; The full length MAR is 1203 bp. Following transformation into *E. coli* the sequences were analyzed. As shown in Fig. S2, there was an obvious band of 1203 bp. The content of AT of this MAR sequence was 62.3%. Bioinformatics analysis confirmed it contains a typical unwinding DNA sequences (AATATT), a potential T-box (TAATCAA) and a potential TATA-box (TATAAT) sequence structure.

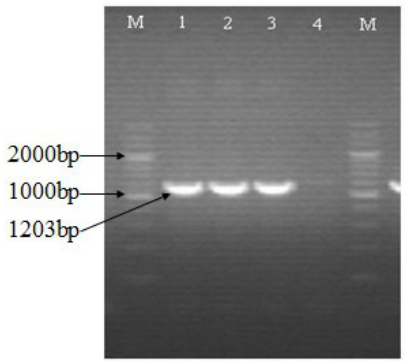
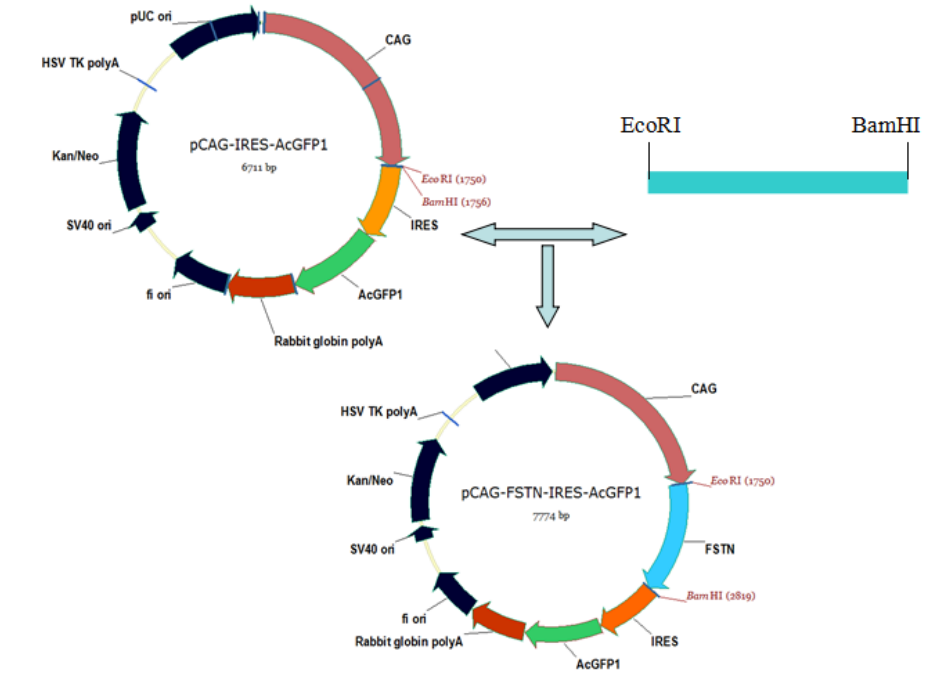


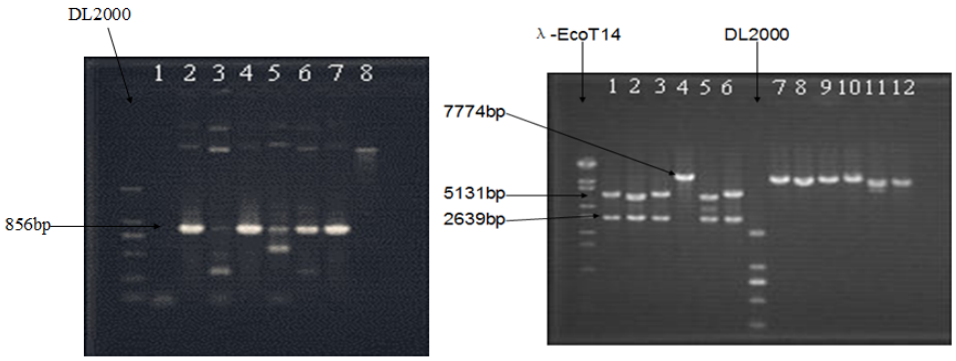
Fig. S2 Full-length PCR of MAR. M, DL2000 Marker; 1–3, PCR of MAR; 4, the control of ddH_2O.

3. Construction and identification of pCAG-FST-IRES-AcGFP1-polyA

The sequenced FST gene was conifmed to be correct and was inserted into the original vector pCAG-IRES-AcGFP1-polyA by *EcoR*I and *BamH*I restriction sites, to obtain co-expression vector for FST and AcGFP1 (pCAG-FST-IRES-AcGFP1-polyA) was obtained. Identification of the pCAG-FST-IRES-AcGFP1-polyA is performed by PCR and restriction. The results showed that the exogenous gene had been inserted correctly (Fig. S3).



(a)



(b) (c)

Fig . S3 Construction (a), PCR analysis (b) and restriction (c) analysis of the expression. (a) Vector pCAG-FST-IRES-AcGFP1-polyA; (b) 1, the control of ddH2O; 2–7, the plasmid of expression vector; 8, the vector without FST; (c) 1–6, the restriction result of *Nhe*I; 7–12, the restriction result of *Eco*RI.

4. Construction and Identification of pMAR-CAG-FST-IRES-AcGFP1-polyA-MAR vector

As shown in Fig. 4, there are two restriction sites respectively before CAG promoter and after the polyA signal in the pCAG-FST-IRES-AcGFP1-polyA. A MAR sequence was inserted before the CAG promoter and after the polyA signal by using the four restriction sites, to obtain the vector pMAR-CAG-FST-IRES-AcGFP1-polyA-MAR. Identification was performed by PCR and restriction enzyme analysis. The results showed the exogenous gene had been inserted correctly (Fig. S4). After double enzymatic digestion, two plasmid vectors, that is, pCAG-FST-IRES-AcGFP1-polyA and pMAR-CAG-FST-IRES-AcGFP1-polyA-MAR were obtained (Fig. S5).

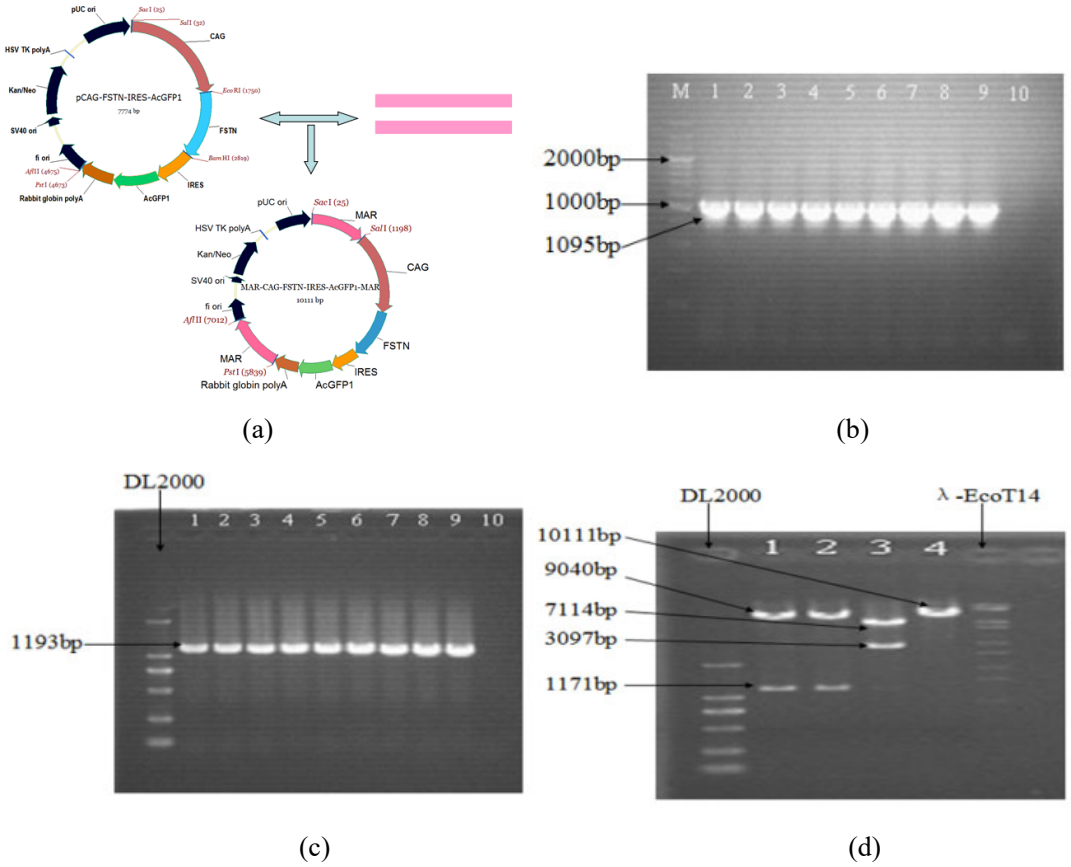


Fig. S4 Construction (a), PCR analysis (b, c) and restriction analysis (d) of the expression vectors. (a) pMAR-FST vector ; (b) The PCR analysis of pMAR-CAG-FST-IRES-AcGFP1-polyA. M, DL2000 Marker; 2–9, the plasmid of pMAR-CAG-FST-IRES2-AcGFP1-polyA; 10, the control of ddH_2O; (c) the PCR analysis of pMAR-FST. 1–9, the plasmid of pMAR-FST; 10, the control of ddH_2O; (d) the restriction analysis of pMAR-FST. 1, the restriction result of *Sac*I, *Sal*I; 2, the restriction result of *Pst*I, *Afl*I; 3, the restriction result of *Sac*I, *Afl*I; 4, the restriction result of *Sal*I.

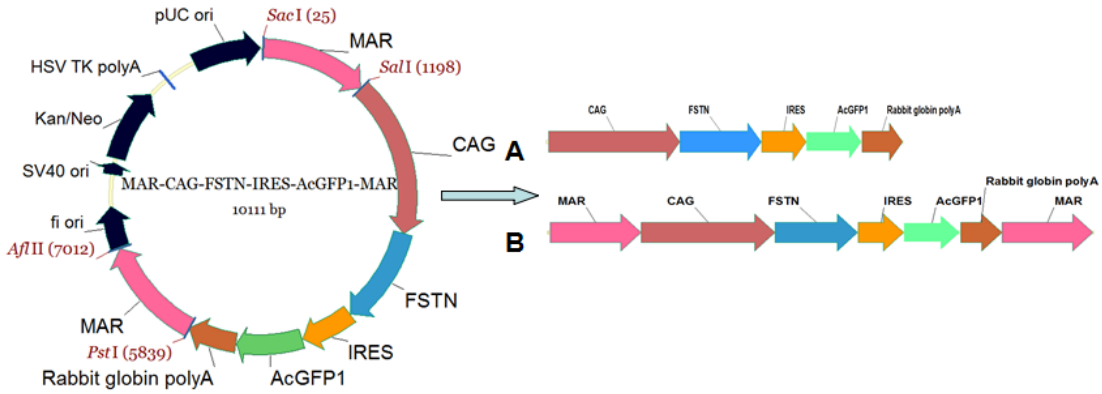


Fig. S5 Construction maps of the vectors. A, Bicistron gene transfer body; B, pMAR-FST.