**Co-expressing GroEL-GroES, Ssa1-Sis1 and Bip-PDI chaperones for enhanced intracellular production and partial-wall breaking improved stability of porcine growth hormone**

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**Hyg-Zeo-R (Kan- Zeo-R)**

**Hyg-Zeo-F (Kan- Zeo-F)**

**Ins1**

**Ins2**

**Ins2**

**Ins1**

**pGAPZA**

**Zeo**

**pGAPZA**

**Hyg (Kan)**

**Hyg (Kan)**

**Hyg (Kan)**

**pGAPK(H)A**

Zeo: Zeocin resistance gene

Hyg: Hygromycin resistance gene

Kan: G418 resistance gene

**Starting**

**plasmid**

**Chimeric**

**primers**

**Omega-PCR**

**Substitution mode**

**plasmid**

**First PCR**

**products**

**plasmid**

**Final**

**plasmid**

Fig. S1 Substitution omega PCR for pGAPK(H)A construction

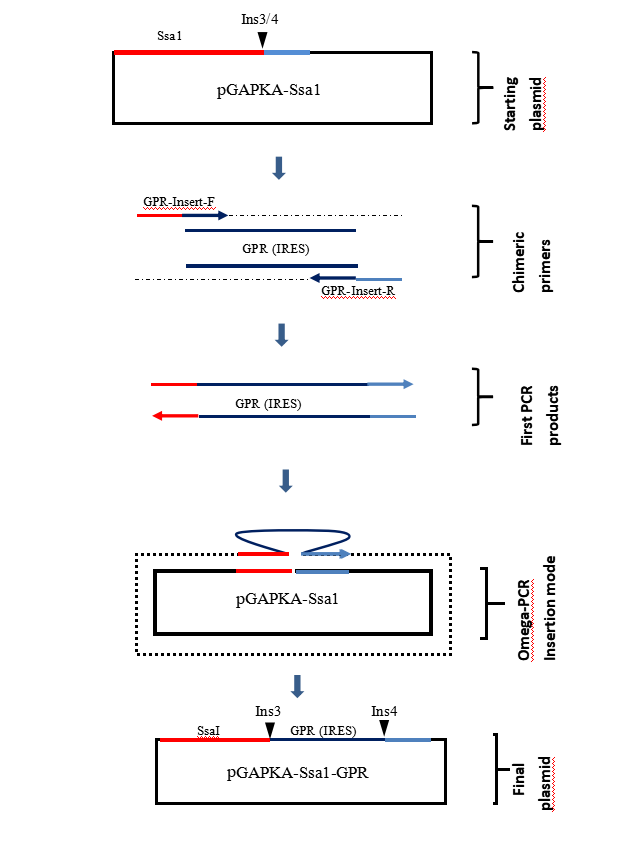
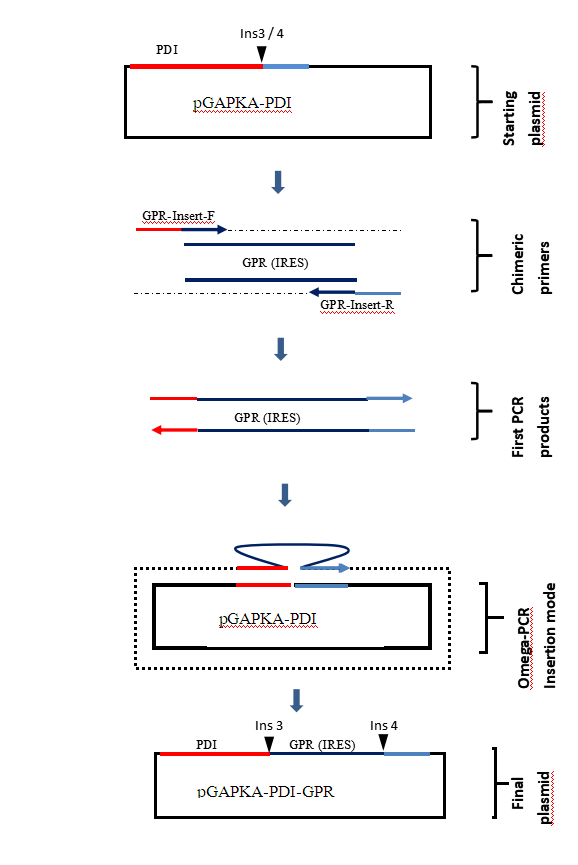


Fig. S2 Insertion omega PCR for pGAPKA-Ssa1-GPR construction



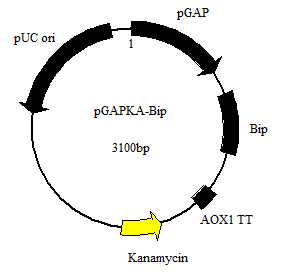
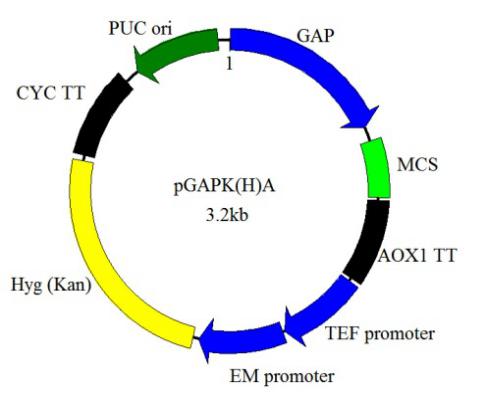
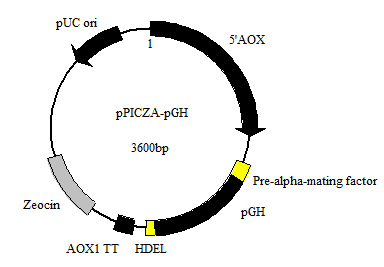
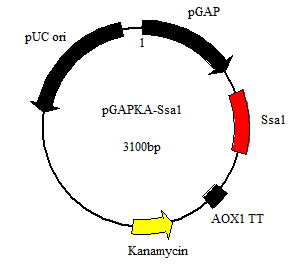
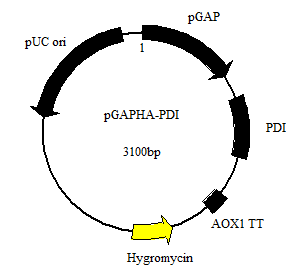
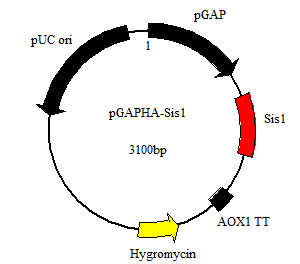
 

Fig. S3 Insertion omega PCR for pGAPKA-PDI-GPR construction

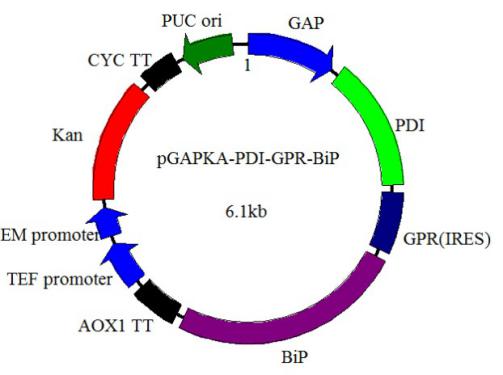
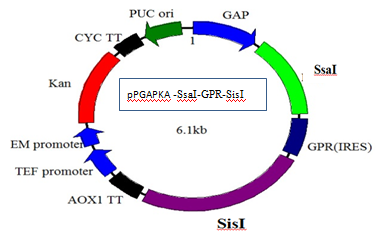
 

Fig. S4 The plasmids used in this study

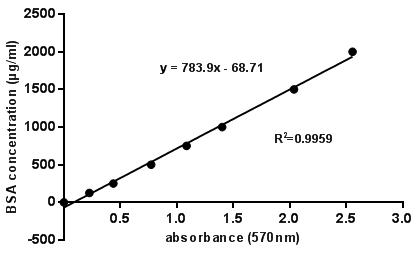


Fig. S5 Standard curve of BSA standard solution

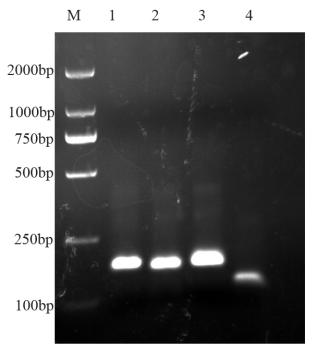
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Fig. S6 PCR splicing results

Lane M was DL2000 standard molecular weight Marker, Lane 1, the result of the first group P1-P4 PCR splicing, Lane 2, the result of the second group P5-P8 PCR splicing, Lane 3, the result of the third group P9-P12 PCR splicing, and Lane 4, the result of the fourth group P13-P15 PCR splicing.

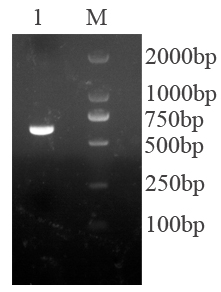


Fig. S7 PCR splicing results

Lane M, DL2000 standard molecular weight Marker, Lane 1, the result of PCR splicing of the target gene.

**Table S1 Primers for PCR reactions**

|  |  |
| --- | --- |
| Primer name | Sequencea |
| P1 | CCGGAATTCGCCACCATGTTTCCAGCTATGCCATTGTCTTCTTTGTTTGCTAACGCTG |
| P2 | GTATCAGCAGCCAATTGATGCAAATGTTGGGCTCTCAAGACAGCGTTAGCAAACAAAG |
| P3 | CAATTGGCTGCTGATACTTACAAGGAATTTGAAAGAGCTTACATTCCAGAAGGTCAAAG |
| P4 | CAGAGAAACAGAAAGCAGCTTGAGCGTTCTGAATAGAGTATCTTTGACCTTCTGGAATG |
| P5 | CAATTGGCTGCTGATACTTACAAGGAATTTGAAAGAGCTTACATTCCAGAAGGTCAAAG |
| P6 | CAACAAAGAGAATCTCAACAATTCAACATCAGATCTTTGTTGAGCTTCATCCTTACC |
| P7 | GTTGAGATTCTCTTTGTTGTTGATTCAATCTTGGTTGGGTCCAGTTCAATTCTTGTC |
| P8 | CAGAAGTACCAAAGACCAAAGAGTTAGTAAAGACTCTAGACAAGAATTGAACTGGAC |
| P9 | GGTCTTTGGTACTTCTGATAGAGTTTACGAGAAGTTGAAGGATTTGGAAGAAGGTATTC |
| P10 | GCTCTTGGAGAACCATCTTCCAATTCTCTCATCAAAGCTTGAATACCTTCTTCCAAATC |
| P11 | GATGGTTCTCCAAGAGCTGGTCAAATCTTGAAGCAAACTTACGATAAGTTTGATAC |
| P12 | CCGTAGTTCTTCAACAAAGCATCATCAGATCTCAAGTTAGTATCAAACTTATCGTAAG |
| P13 | CTTTGTTGAAGAACTACGGTTTGTTGTCTTGCTTCAAGAAGGATTTGCATAAGGCTG |
| P14 | GATTCAACAAATCTTCTACACTTCATAACTCTCAAGTAAGTTTCAGCCTTATGCAAATC |
| P15 | GTAGAAGATTTGTTGAATCTTCTTGTGCTTTCTAATAGCTCGAGCGG |
| PF | CCGGAATTCGCCACCATG |
| PR | CCGCTCGAGCTATTAGAAAG |
| GroEL-F | GGAATTCACCATGGCAGCTAAAGACGTAAAATTCGGTAACG |
| GroEL-R | ATAAGAATGCGGCCGCTTACATCATGCCGCCCATGCCACC |
| GroES-F | GGAATTCACCATGAATATTCGTCCATTGC |
| GroES-R | ATAAGAATGCGGCCGCTTACGCTTCAACAATTGCCAG |
| 5’AOX1 | GACTGGTTCCAATTGTTGACAAGC |
| 3’AOX1 | GCAAATGGCATTCTGACATCC |
| PGAP | GTCCCTATTTCAATCAATTGAA |
| Hsp70-F | ATGCCAGCTGTCGGTATTGATTTAGGAAC |
| Hsp70-R-Xba | GCTCTAGACTAATCGACTTCCTCAACAGTTGGTCCGT |
| Hsp70-F-Xho  Hsp40-F | CCGCTCGAGTTCAAACAAAATGCCAGCTGTCGGTATTGATTTAGGAAC  ATGGTGAAAGAACAAGGACTATACAAT |
| Hsp40-F-EcoR | CGGAATTCTTCAAACAAAATGGTGAAAGAACAAGGACTATACAAT |
| Hsp40-R-Not | CTTGCGGCCGCTTAAAACGCTTTGGAAATGGCATC |
| sig-F | ATGGCTGCTGGTCCAAGAACTTCAGTTTTATTGGCTTTCGCTTTGCTTTGTTTACCTTG |
| sig-R | AATGGCATTGCTGGAAACATAGCTCCAACTTCTTGAGTCCAAGGTAAACAAAGCAAAGC |
| sig-F/EcoR I | CGGAATTCTTCAAACAAAATGGCTGCTG |
| F-pre- alpha | ATGAGATTTCCTTCAATTTTTACTGCTGTTTTATTCGCAG |
| R-pre-alpha  R-HDEL-Not1 | TAATGGCATTGCTGGAAAAGCAGCTAATGCGGAGGATGCTGCGAATAAAACAGCAGT  CTTGCGGCCGCCTATTAAAGCTCGTCGTGGAAAGCGCAAGAGGATTCGAC |
| F-pre-alpha-EcoR1 | CGGAATTCTTCAAACAAAATGAGATTTCCTTCAAT |
| （wild）pgh-F | ATGTTGGGAGCCATGCCCTT |
| （wild）pgh-R | CTAGAAGGCACAGCTGCTC |
| phg-F-EcoRI | cggaattcaccATGTTGGGAGCCATGCCCTT |
| pgh-R-NotI | CTTGCGGCCGCCTAATGATGATGATGATGATGGAAGGCACAGCTGCTC |

**Table S2 The plasmids used in this study**

|  |  |
| --- | --- |
| Plasmids | Marker |
| pPICZA | Zeocin |
| pGAPZA | Zeocin |
| pPICZA-pGH（optimized）-HDEL | Zeocin |
| pPAO | Hygromycin |
| pPICZαA | Zeocin |
| pPICZA-pGH | Zeocin |
| pPICZαA-pGH | Zeocin |
| pPICZA-pGH（optimized ） | Zeocin |
| pGAPZA-GroEL-GroES | Zeocin |
| pGAPKA-Bip | Amp，Kan |
| pGAPHA-PDI | Hygromycin |
| pGAPKA-Ssa1 | Amp，Kan |
| pGAPHA-Sis1 | Hygromycin |

**Detailed procedure:**

**Native pGH cloing from cDNA:**

Full-length pGH gene splicing by overlapping PCR with optimized splicing primers P1-P15: using P1-P4 as the first group, P5-P8 as the second group, P9-P12 as the third group, and P13-P15 as the fourth group. These four groups were individually subjected to PCR with the following conditions:

20 μL reaction volume including 10 μL KOD-FX buffer, 0.2-0.4μL KOD-FX polymerase, 1~100 ng cDNA, 0.3 μM dNTP mix, 0.3 μM each forward and reverse primer. And the PCR cycling parameters were as follows: 94 ℃ for 2 min; 30 cycles of 98 ℃ for 10 s, 62 ℃ for 10 s, 72 ℃ for 15 s (1 kb/min) followed by a final 2 min extension.

**Optimized-pGH by splicing PCR:**

The four PCR products from above PCR were mixed, and primers PF/PR were used for the second round of splicing, the stitching was performed under condition as follows:

20 μL reaction volume including 10 μL KOD-FX buffer, 0.2-0.4μL KOD-FX polymerase, 1~100 ng cDNA, 0.3 μM dNTP mix, 0.3 μM each forward and reverse primer. And the PCR cycling parameters were as follows: 94 ℃ for 2 min; 30 cycles of 98 ℃ for 10 s, 53 ℃ for 10 s, 72 ℃ for 40 s (1 kb / min) followed by a final 2 min extension.

The results were shown in Fig. S6. The first to fourth groups of PCR products were added to the overlapping PCR reaction system, and splicing amplification was performed under splicing PCR conditions by primers PF/PR. The results were shown in Fig. S7.