**Insect High FiveTM cell line development using site-specific flipase recombination technology**

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# Supplementary Material

**Figure S1.** Genomic PCR analysis of Hi5-RMCE clones #11, #18, #38 and #44 - uncut gel image. L - Ladder. Target cassette - 5691 bp. Tagging cassette - 2951 bp.

**Figure S2.** Cell growth kinetics and protein expression of Hi5-RMCE master clone and producer cells. Cell growth and viability profiles of Hi5-RMCE master clone and producer cells **(A)**, and identification of eGFP **(B)** and iCherry **(C)** in culture samples collected over time by Western blot. Ctl (-): Negative control - Parental cells. L – Ladder. Expected MW of eGFP and iCherry protein is 27 kDa.

**Figure S3.** Identification of HA (**A**) and M1 (**B**) influenza proteins in culture supernatant samples of Hi5-RMCE producer cells by Western blot - uncut membrane image. Ctl (-): Negative control - Parental cells. L – Ladder. Expected MW of influenza HA and M1 proteins are 68 kDa and 28 kDa, respectively.

**Figure S1**

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**Figure S2**

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**Figure S3**



**Table S1:** Primers used to amplify the fragments for plasmid constructions and restriction enzymes used for vector digestion.

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| --- | --- | --- | --- | --- |
| **Final construct** | **Vector template** | **Primers used in vector amplification/ Restriction enzyme used for vector digestion** | **Insert template** | **Primers used for insert amplification** |
| **pTagging-iCherry-hyg (GenBank MW246024)** |
| pTagging-iCherry-hyg | pTagging-iCherry(GenScript, USA; GenBank MW246023) | NheI | *hygromycin* and SV40 polyA fragment from in-house pTag-OpIE2dsRed cassette | Fw: TTGCGAATTCGCTAGCCATGAAGAAACCTGAACTGACARv: TCTCACGTGAGCTAGCCCATACCACATTTGTAGAGGTTT |
| **phr5-AcIE1-iFlp (GenBank MW246025)** |
| phr5-IE1-iFlp | pIEx™-10 (Novagen, EMD Millipore) | BamHI/HindIII | *iflp* gene from OpIE2-iFlp-IEterm plasmid (GenBank MG051710) | Fw: ACAAGGTACCGGATCCGCTCCCATGGCTCCCAAGAAARv: CTGCGGCCGCAAGCTTTCTAGTCGACGATACGACGGTTGA |
| **pTarget-eGFP-neo (GenBank MW246027)** |
| pTarget-eGFP-neo | backbone containing F5 and Fwt from pTag-OpIE2dsRed cassette | Fw: CCATGGAGAAGTTCCTATTCCGARv: CGTGGGATCGATGCTCACTCGA | *egfp* with *OpIE2 polyA* fragment from pTarget-OpIE2eGFP cassette | Fw: CGCTCACTGACTCGCCGATCCCACGCGCTTGAAAGRv: AGCATCGATCCCACGACCATGGTGAGCAAGGGCGA |
| *neomycin* with *polyA* fragment from pTag-OpIE2dsRed cassette | Fw: GGAACTTCTCCATGGATGATTGAACAAGATGGATTGCACGRv: GCGAGTCAGTGAGCGAGGAA |
| **pTarget-eGFP-iFlp-neo (GenBank MW246026)** |
| pTarget-eGFP-iFlp-neo | pTarget-eGFP-neo | SfiI/PacI | *hr5-AcIE1* promoter, *iflp* gene and IE1 term fragment from phr5-AcIE1-iFlp vector | Fw: AGCGGAAGGCCCATGAACAATTTCACACAGGAAACAGCRv: TTTTTTGTGTTTAATGCATTTATCAGGGTTATTGTCTCA |
| **pTarget-iCherry-zeo (GenBank MW246028)** |
| pTarget-iCherry-neo | pTarget-eGFP-neo | NdeI/NotI | *iCherry* gene from pTagging-iCherry-hyg vector | Fw: TGAGAGTGCACCATATGTCTACCATGGTGTCCAAGGGTGRv: GCTAGCTTTGCGGCCGCGCCTTACTTGTACAGCTCGTCC |
| pTarget-iCherry-zeo | pTarget-iCherry-neo | Fw: CCATGGAGAAGTTCCTATTCCGAARv: GCGGGACTCTGGGGTTCGAAAT | *zeocin* gene from pIZT/V5-His vector | Fw: ACCCCAGAGTCCCGCCGTCGGTCAGTCCTGCTCCTRv: GGAACTTCTCCATGGATGGCCAAGTTGACCAGTGCCGT |
| **pFluTarget** |
| pIZT-M1 | pIZT/V5-His (Invitrogen) | SacI | *M1* gene (from Redbiotech bacmid) | Fw: GCTTGGTACCGAGCTATGAGCCTGCTGACCGAGGTRv: GGACTAGTGGATCCGGATCACTTGAACCGCTGCAT |
| pIZT-HA | pIZT/V5-His (Invitrogen) | NotI | *HA* gene (H3-4II, from Redbiotech bacmid) | Fw: agcacagtggcggccaaactcgtcaaagccaccatgaRv: TAGACTCGAGCGGCCGACGCCAGAAAGGGGATTAGATAC |
| pIZT-M1-HA | pIZT-M1 | Fw: caggaaagaacatgtgagcaaaagRv: TTGAGTGAGCATCGATCCCACG | OpIE2 promoter, *HA* gene, *polyA* fragment from pIZT-HA | Fw: tcgatgctcactcaagatcatgatgataaacaatgtatggRv: ACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTA |
| pFluTarget | pTarget-iCherry-zeo | NdeI/PacI | OpIE2 promoter, *M1* gene, *polyA*, OpIE2 promoter, *HA* gene, *polyA* fragment from pIZT-M1-HA | Fw: TGAGAGTGCACCATAaaacttgtttattgcagcttataatggttacaaataaagcaaRv: ggttttttgtgtttaTTGAGTGAGCATCGATCCCACG |