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

Mafalda M Dias1,2, João Vidigal1,2, Daniela P Sequeira1,2,§, Paula M Alves1,2, Ana P Teixeira1,2,#\*, António Roldão1,2,\*

1 IBET, Instituto de Biologia Experimental e Tecnológica, 2780-901 Oeiras, Portugal;

2 Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, 2780-901 Oeiras, Portugal;

§ Present address: Department of Molecular Life Sciences, University of Zurich, 8057 Zurich, Switzerland;

# Present address: ETH Zurich, Department of Biosystems Science and Engineering, Mattenstrasse 26, 4058 - Basel, Switzerland;

\* Corresponding authors: [aroldao@ibet.pt](mailto:aroldao@ibet.pt); anapa@ethz.ch

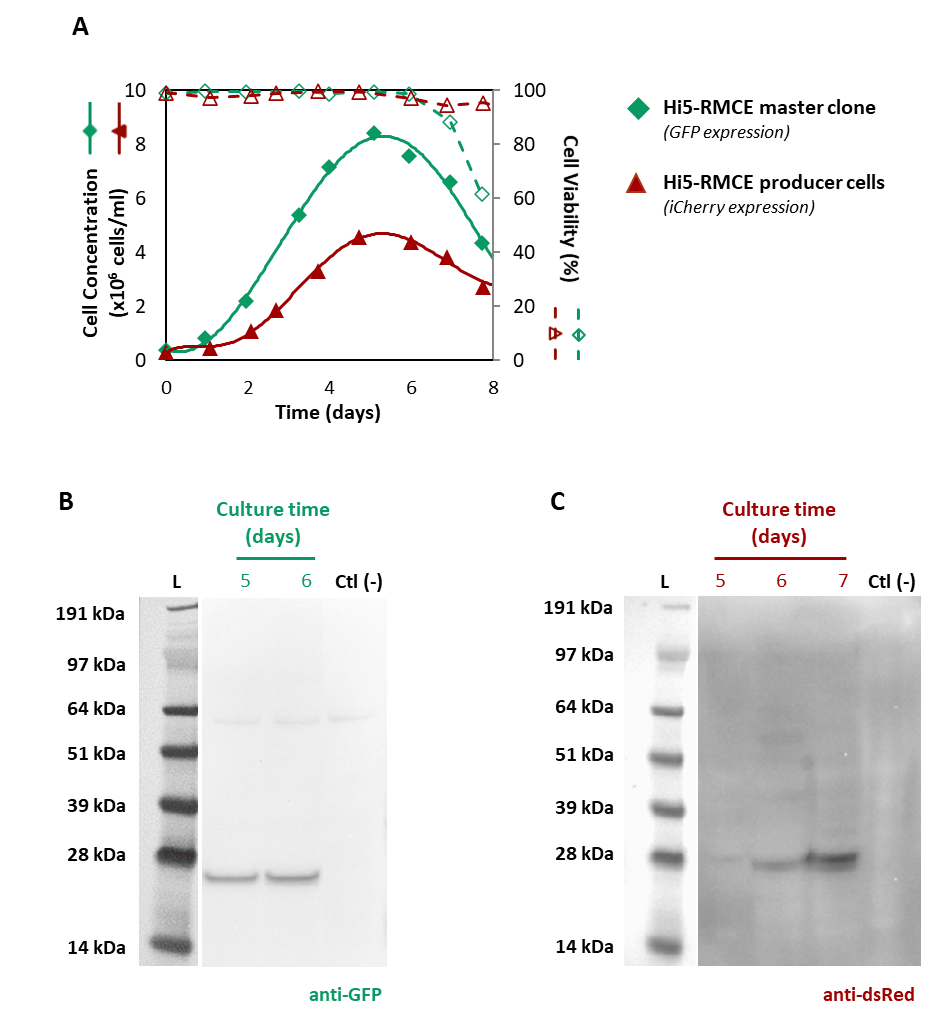
# Supplementary Material

**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.

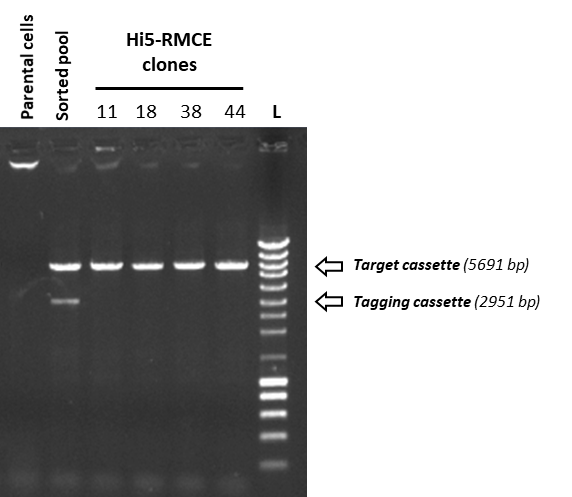
**Figure S2.** Cell growth kinetics and protein expression of Hi5-RMCE master clone and producer cells. Cell growth and viability profiles of Hi5-RMCE master clone and producer cells **(A)**, and identification of eGFP **(B)** and iCherry **(C)** in culture samples collected over time by Western blot. 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.

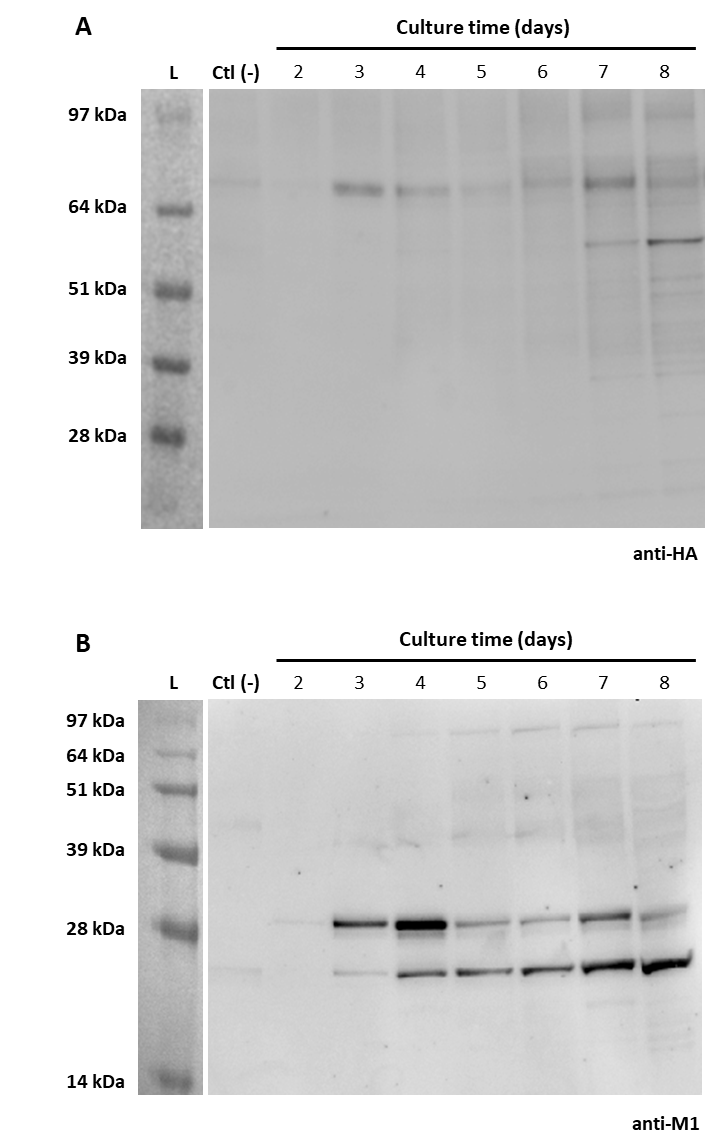
**Figure S1**

****

**Figure S2**

****

**Figure S3**



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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **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: TTGCGAATTCGCTAGCCATGAAGAAACCTGAACTGACA  Rv: 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: ACAAGGTACCGGATCCGCTCCCATGGCTCCCAAGAAA  Rv: CTGCGGCCGCAAGCTTTCTAGTCGACGATACGACGGTTGA |
| **pTarget-eGFP-neo (GenBank MW246027)** | | | | |
| pTarget-eGFP-neo | backbone containing F5 and Fwt from pTag-OpIE2dsRed cassette | Fw: CCATGGAGAAGTTCCTATTCCGA  Rv: CGTGGGATCGATGCTCACTCGA | *egfp* with *OpIE2 polyA* fragment from pTarget-OpIE2eGFP cassette | Fw: CGCTCACTGACTCGCCGATCCCACGCGCTTGAAAG  Rv: AGCATCGATCCCACGACCATGGTGAGCAAGGGCGA |
| *neomycin* with *polyA* fragment from pTag-OpIE2dsRed cassette | Fw: GGAACTTCTCCATGGATGATTGAACAAGATGGATTGCACG  Rv: 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: AGCGGAAGGCCCATGAACAATTTCACACAGGAAACAGC  Rv: TTTTTTGTGTTTAATGCATTTATCAGGGTTATTGTCTCA |
| **pTarget-iCherry-zeo (GenBank MW246028)** | | | | |
| pTarget-iCherry-neo | pTarget-eGFP-neo | NdeI/NotI | *iCherry* gene from pTagging-iCherry-hyg vector | Fw: TGAGAGTGCACCATATGTCTACCATGGTGTCCAAGGGTG  Rv: GCTAGCTTTGCGGCCGCGCCTTACTTGTACAGCTCGTCC |
| pTarget-iCherry-zeo | pTarget-iCherry-neo | Fw: CCATGGAGAAGTTCCTATTCCGAA  Rv: GCGGGACTCTGGGGTTCGAAAT | *zeocin* gene from pIZT/V5-His vector | Fw: ACCCCAGAGTCCCGCCGTCGGTCAGTCCTGCTCCT  Rv: GGAACTTCTCCATGGATGGCCAAGTTGACCAGTGCCGT |
| **pFluTarget** | | | | |
| pIZT-M1 | pIZT/V5-His (Invitrogen) | SacI | *M1* gene (from Redbiotech bacmid) | Fw: GCTTGGTACCGAGCTATGAGCCTGCTGACCGAGGT  Rv: GGACTAGTGGATCCGGATCACTTGAACCGCTGCAT |
| pIZT-HA | pIZT/V5-His (Invitrogen) | NotI | *HA* gene (H3-4II, from Redbiotech bacmid) | Fw: agcacagtggcggccaaactcgtcaaagccaccatga  Rv: TAGACTCGAGCGGCCGACGCCAGAAAGGGGATTAGATAC |
| pIZT-M1-HA | pIZT-M1 | Fw: caggaaagaacatgtgagcaaaag  Rv: TTGAGTGAGCATCGATCCCACG | OpIE2 promoter, *HA* gene, *polyA* fragment from pIZT-HA | Fw: tcgatgctcactcaagatcatgatgataaacaatgtatgg  Rv: ACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTA |
| pFluTarget | pTarget-iCherry-zeo | NdeI/PacI | OpIE2 promoter, *M1* gene, *polyA*, OpIE2 promoter, *HA* gene, *polyA* fragment from pIZT-M1-HA | Fw: TGAGAGTGCACCATAaaacttgtttattgcagcttataatggttacaaataaagcaa  Rv: ggttttttgtgtttaTTGAGTGAGCATCGATCCCACG |