Research Tools

Bioluminescent Tagging In Lactic Acid Bacteria

WARF: P230411US01

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The Invention

UW-Madison researchers have developed biomaterials useful for the luminescent-based quantification of recombinant protein production by lactic acid bacteria under a variety of stimuli, via a method that is more sensitive than commercially available immunoassays, based on the incorporation of the HiBiT tag into lactic acid bacteria (9 genera, 12 species). The inventors have demonstrated the use of the approach to detect microbes throughout the GI tract, and can use it to monitor in situ protein production; the latter only requires HiBiT as they discovered that the substrate can enter the cells. The list and description of the relevant plasmids is:

1. pVPL31599, pVPL31655: pJP028 (pVPL3583) → pJP028_SP_EFTu_Leptin (pVPL3585) → pJP028_noSP_EFTu_Leptin (pVPL3789) →pVPL31599 → pVPL31655

a. pJP028: pJP028 is a derivative of pNZ8048 in which we replaced the gene encoding chloramphenicol resistance with the gene encoding erythromycin resistance.

b. pJP028_SP_EFTu_Leptin (pVPL3585): pJP028_SP_EFTu_Leptin (Alexander et al. 2019) is derived from pJP028 by cloning a fusion of EFTu promoter (derived from L. reuteri 6475) and codon optimized murine leptin for expressing in L. reuteri.

c. pJP028_noSP_EFTu_Leptin (pVPL3789): pJP028_noSP_EFTu_Leptin (Alexander et al. 2019) is a derivative lacking the signal peptide gene from pVPL3585.

d. pJP028_noSP_EFTu_Leptin_HiBiT (pVPL31599): pJP028_noSP_EFTu_Leptin_HiBiT is a derivative of pVPL3589 in which we cloned gene encoding HiBiT (which is codon optimized to expressed in L. reuteri) directly upstream of the stop codon of murine leptin.

e. pJP028_noSP_EFTu_Leptin_HiBiT_EFTu_LgBiT (pVPL31655): pJP028_noSP_EFTu_Leptin_HiBiT_EFTu_LgBiT is a derivative of pVPL31599 in which we cloned a fusion of EFTu promoter (derived from L. reuteri 6475) and LgBiT.

2. pVPL31948, pPVL31954: pSIP411 (pVPL2005) → pSIP411_PlySs2_HiBiT (pVPL31659) → pSIP411_PlySs2_HiBiT_Anti-repressor (pVPL31780) → pSIP411_Leptin_HiBiT_Anti-repressor (pVPL31964) → pVPL31948 → pVPL31954

a. pSIP411_PlySs2_HiBiT (pVPL31659): This plasmid was constructed by cloning the codon optimized synthetic DNA fragment (PlySs2_HiBiT) into pSIP411.

b. pSIP411_PlySs2_HiBiT_Anti-repressor (pVPL31780): This plasmid is a derivative of pVPL31659 in which we cloned the anti-repressor gene which is derived from prophage 1 in L. reuteri 6475.

c. pSIP411_Leptin_HiBiT_Anti-repressor (pVPL31964): This plasmid was constructed by replacing the gene encoding PlySs2 with the gene encoding murine leptin which is amplified from pVPL3585.

d. pSIP411_Leptin_HiBiT (pVPL31948): This plasmid is a derivative lacking the anti-repressor gene from pVPL31964.

e. pSIP411_Leptin_HiBiT_EFTu_LgBiT (pVPL31954): This plasmid is constructed by cloning the fusion of EFTu promoter and LgBiT gene which was amplified from pVPL31655.

Additional Information

For More Information About the Inventors

- Jan Peter Van Pijkeren

Tech Fields

- Research Tools : DNA & RNA tools
- Research Tools : Microbial technologies
- Research Tools : Protein tools

For current licensing status, please contact Rafael Diaz at rdiaz@warf.org or 608-960-9847