Research Tools

Nanobody Expression Vectors

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The Invention

UW-Madison researchers have developed a set of vectors for expressing nanobodies. The researchers engineered a vector system for transferring nanobodies between bacterial and mammalian expression system. The expression system uses bacterial and mammalian expression vectors that have been modified to contain universal restriction sites to allow the gene constructs expressing the nanobodies to be easily transferred between the two. The bacterial vector has a novel expression tag included to enhance and stabilize the expression of the nanobodies. That vector also has a leucine zipper construct positioned to allow for expression of multiple nanobodies that dimerize upon expression. This vector can be used for creating multivalent nanobodies.

For bacterial expression, they utilized an improved T7 recognition site in the pET-28a vector. They also removed the His tag region after the *Xho1* restriction site. For mammalian expression, they modified a pcDNA3.1 by inserting a T7 promoter and T7 terminator. They added a WPRE site and an HSV poly tail. Both vectors are equipped with universal restriction sites, so it is easy to transfer the nanobody genetic construct between the two. The inventors also modified the bacterial vector to improve solubility of the expressed protein and created a novel signaling tag called LLAMA-T7 for improved expression of nanobodies.

The LLAMA-T7 tag contains a TIR-2 site (translation initiation region 2), two hydrophobic residues to stabilize translation of the protein, and a T7 tag. All of these components are known, but the combination hasn't been reported. The inventors believe that this tag is especially useful for nanobody production. The inventors also modified that vector to allow multiple nanobodies to be expressed and then dimerize via an expressed leucine zipper modified to contain cysteine residues that provide the means for disulfide bond formation. This creates a dimerized nanobody.

Additional Information

For More Information About the Inventors

- Aaron LeBeau

Tech Fields

- Research Tools : Protein tools

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