ABSTRACT

The efficient capture and functional analysis of genes and metabolic pathways is constrained by the choice of expression tools. A directed cloning system has been developed that uses a viral homologous recombination to seamlessly join PCR amplified genes with pre-processed plasmids, eliminating numerous time consuming and expensive reagents and steps. Unlike other cloning systems, no vector preparation, restriction or modifying enzymes, or purification steps are required. The desired insert is simply amplified with primers that include 18 bases of overlap with the ends of a small expression vector, and mixed directly with the plasmid. Amplified genes with pre-processed plasmids, eliminating numerous time consuming and expensive reagents and steps. Unlike other cloning systems, no vector preparation, restriction or modifying enzymes, or purification steps are required. The desired insert is simply amplified with primers that include 18 bases of overlap with the ends of a small expression vector, and mixed directly with the plasmid.

Figure 1. Expressioneering vectors pETite & pRham designed for expression and engineering in E. coli. Typically, >90% of randomly-selected colonies of each transformation plate; all contained inserts of correct size.

Primers contain 18 bases complementary to vector sequences flanking insertion site.

Figure 2. Five second enzyme-free expression cloning.

1. Amplify

2. Mix

3. Transform

Figure 3. Expressioneering large-scale cloning and expression case study.

Table 1. PCR products from 48 putative Fibrilobacter succinogenes ssp. hydrogenicus genes ranging from 1 to 3 kb. These PCR products were cloned into the pETite C-His vector. (B) Uninduced (1) and IPTG-induced (+) samples of H-Cont® (BL21(DE3)). Cells with 6 different genes cloned into the pETite C-His Vector. (C) Enhanced stability of SUMO-tagged 231 and 244 genes produced; total extract and soluble fractions are shown. (D) Removal of both SUMO-tag from purified SUMO-231 fusion protein by cold treatment; -prot: uncleaved SUMO-231 fusion protein; +prot: SUMO protease-treated fusion protein; C isolated 2011 protein after removal of both SUMO-fragment and SUMO protease by substrate MAC.

Figure 4. Amino acid composition of E. coli. Typically, >90% of randomly-selected colonies of each transformation plate; all contained inserts of correct size.

Figure 5. Novel linear phase derivative for large insert pathway expression and engineering in E. coli.

The pJAZZ vectors were derived from the linear phase n15 of E. coli by deletion of the structural and lytic genes (Gibot et al. 2010). Linear derived for cloning large or repetitive sequences in E. coli. Nuc. Acids Res. 38(8). The linear vectors provide critical features for cloning difficult DNAs:

1. The ends of the vectors are free to rotate during replication, eliminating torsional stress caused by supercoiling.
2. Transcriptional terminators at the cloning site minimize transcriptional interference between the insert and the vector, increasing insert stability.
3. The vectors are low-copy (3-5/col) to further promote stable propagation of inserts, and their copy number can be induced 5-20X for DNA preparation.
4. Dual selection ensures that recombinant clones contain both arms of the vector. The viral metagenomic DNA was sequenced and their copy number can be induced 5-20X for DNA preparation.

Figure 6. The MEP pathway (left) showing conversion of DMAD into isoprene by isoprene synthase. The MEP pathway encodes 9 genes and results in the product of isoprene, a valuable feedstock chemical that is capable of playing a central role in the future bio-economy. Efforts to clone a 9 kb synthetic MEP pathway were unsuccessful except in the linear vector pJAZZ behind a rhamnose promoter. A 96-well plate containing these clones was screened in University of Wisconsin Stevens Point scientists (Dr. Eric Singsaas, PI) for isoprene production (right panel). The last eight data points show that the best pJAZZ isoprene producing clones were ~10 fold better than previous constructs.

CONCLUSION

Two new tools for expression cloning of genes and pathways from genomes and metagenomes has accelerated our ability to ferment platform chemicals such as isoprene and access new enzymes for the breakdown of carbohydrates or the synthesis of nucleic acids. These tools, the Expresso® line of vectors and competent cells, and the pJAZZ™ linear cloning system, are available for research use exclusively from Lucigen Corp.