Large Protein Complex Production Using the SmartBac System---Strategies and Applications

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Recent revolution of cryo-electron microscopy has opened a new door to solve high-resolution structures of macromolecule complexes without crystallization while how to efficiently obtain homogenous macromolecule complex sample is therefore becoming a bottleneck. Here we report Smartbac, an easy and versatile system for constructing large-sized transfer plasmids used to generate recombinant baculoviruses that express large multiprotein complexes in insect cells. The Smartbac system integrates the univector plasmid-fusion system, Gibson assembly method and polyprotein strategy to construct the final transfer plasmids. There are four acceptor plasmids (4V1G, 4V1R, 5V1TG and 5V1TR) and two donor plasmids (4V2G and 4V2R) in the SmartBac system (Figure 1). The acceptors can recombine with the donors via Cre-LoxP site-specific recombination. To monitor the expression of target proteins, we added the most commonly used fluorescent proteins EGFP and tagRFP genes to the Smartbac vectors, so that observation can be easily performed with a basic fluorescence microscope. The acceptors harbor a p15A origin of replication that allows propagation in common cloning strains of E. coli at low copy number, which is better for the stability of large plasmids. The LacZ-alpha expression cassette on these vectors allows blue/white selection of recombinant clones.

Two example schemes for the expression of large multiprotein complexes using the Smartbac system are provided (Figure 2). If the molecular weight of the multiprotein complex is less than 600 kDa, we propose using Scheme 1 (Figure 2a). If the molecular weight of the multiprotein complex is greater than 600 kDa, the size of the final transfer plasmid constructed using Scheme 1 will be larger than 25kb. In this case Scheme 2 should be used. We mimicked the polyprotein production strategy of coronavirus to realize the expression of multiple subunits. Large numbers of gene fragments with overlapping sequences can be produced rapidly by PCR and
directly used for Gibson assembly with linearized vectors. Positive recombinants can be easily selected by blue-white screening and then donor and acceptor vectors carrying a long gene expression cassette can be combined by Cre-LoxP recombination to produce the final transfer plasmid.

![Figure 2. Schemes for the expression of large multiprotein complexes](image)

We have expressed six active human multiprotein complexes by SmartBac system (Figure 3). They are the exocyst complex (738 kDa, 8 subunits), dynactin complex (1.2 MDa, 23 subunits), CSN complex (343 kDa, 8 subunits), COPI complex (558 kDa, 7 subunits), cytoplasmic Dynein complex (1.4 MDa, 12 subunits), CSN complex (8 subunits, 343 kDa) and SCF complex (180 kDa, 5 subunits). Our results indicate that the SmartBac system can be used to express a wide range of large multiprotein complexes. More information (including related references) about SmartBac system can be found by this link: [https://www.biorxiv.org/content/early/2017/11/14/219246](https://www.biorxiv.org/content/early/2017/11/14/219246).