

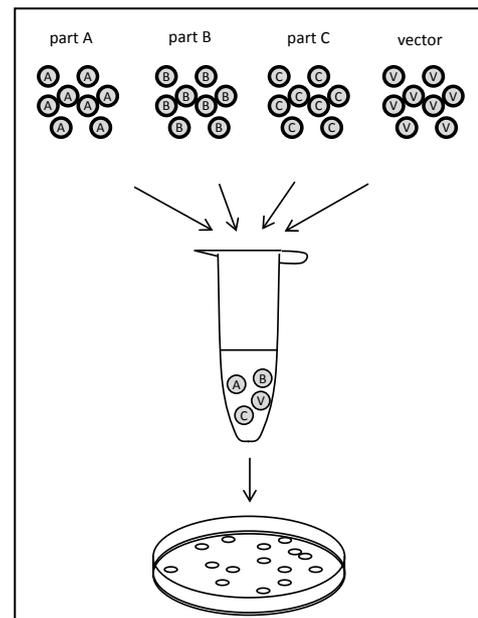
Low cost cloning

Problem to be solved

DNA assembly methods such as Gibson assembly (Gibson et al. 2009, Nature Methods 6, 343-345) and Golden Gate cloning (Engler et al. 2008, Plos ONE, 10.1371) are basic tools for synthetic biology. Both methods allow assembly of constructs from multiple DNA fragments in a one-pot one-step assembly reaction. Standardization of parts is another essential element of modern synthetic biology (Patron et al., 2015 New Phytologist 208: 13–19). Parts may include a promoter, a coding sequence or a terminator. Standardization of parts means that every part has structural features that fit a defined standard. This characteristic allows all equivalent parts of a standard (for example promoters) to be used interchangeably for assembly, allowing combinatorial assembly of parts to make a variety of constructs from a small number of parts. Due to their anticipated wide use for synthetic biology, the number of standard parts that fit any widely used standard is expected to grow exponentially. A limitation of current cloning protocols is the need to store DNA parts frozen at low temperature, with a cost that will be rising with the number of parts that need to be stored.

Novel approach

A new protocol has been developed to assemble constructs from DNA parts kept in a dry form. DNA fragments are stored in a dry form on a solid carrier in a ready-to-use format. Each aliquot of dry DNA sample contains a defined amount of DNA sufficient for a single cloning reaction. The assembly procedure takes place in a reaction vessel which contains the aliquots to be assembled, each on a separate carrier, and the buffer needed to perform the necessary reaction. The fragments are assembled directly and do not need to be put in solution separately. At the end of the reaction, the supernatant containing the assembled constructs can be transformed into competent cells and plated on selection medium.



Applications

Synthetic biology. Standardized products can be provided for cloning reactions. Storage and shipment would be less cost intensive and complex.

Patent situation

In case of interest, we will be pleased to inform you about the current status.