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Preview

Fragmid: A toolkit for rapid assembly and assessment of CRISPR technologies

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The CRISPR toolbox continues to expand at a rapid pace, leaving researchers scrambling to assess the latest tools in their systems of interest. McGee et al.[1] have developed a modular assembly platform with standardized and interchangeable components for rapid construction and deployment of novel CRISPR constructs.

Have you ever found yourself asking, "Is this stretch of sequence really important?" or "Is this the best promoter to use?" when trying to construct a new plasmid from bits and pieces of several other plasmids? These types of questions are muttered often in labs as researchers try to keep up with the fast-paced field of genome editing using CRISPR technology. In its simplest form, a CRISPR-Cas system is a two-component system composed of a Cas protein guided by an aptly named guide RNA to a specific site in the genome.[2] Although Cas proteins generally possess nuclease activity, their programmable homing capacity has led researchers to attach an assortment of different functional domains to a growing number of naturally occurring and engineered Cas proteins. This has enabled a wide variety of CRISPR applications, including transcriptional activation and inhibition, targeted chromatin modification, and direct DNA modification.[3,4] Although newly discovered or developed CRISPR systems have been a welcome addition to the genome-editing toolbox, implementation and comparison of these different tools are cumbersome because of the overwhelming number of options available to create a desired perturbation.

In this issue of *Cell Genomics,* McGee et al.[1] introduce Fragmid, a toolkit composed of modular fragments that allows users to create customized CRISPR plasmids in a matter of days. Approximately 200 Fragmid modules work together like building blocks to allow users to mix and match individual modules to create a desired targeting construct. Additionally, this toolkit offers a variety of destination vectors, which have the basic components of a plasmid along with the features necessary for a given delivery modality. For example, Fragmid contains destination vectors for delivery of CRISPR reagents via lentivirus, adeno-associated virus, Piggybac, or other nonviral methods. The CRISPR modules can be assembled into the destination vectors in an organized, single reaction using Golden Gate assembly. In brief, Golden Gate assembly exploits the ability of a type IIS restriction enzyme, in this case BbsI, to cleave DNA outside of its DNA recognition sequence.[5,6] This allows for the creation of unique and defined 4 base pair overhangs such that each module can only be cloned in the appropriate orientation and order (Figure 1). During Golden Gate assembly, the destination vector and selected Fragmid module plasmids are combined into a single tube with the BbsI enzyme and T4 ligase and cycled between 37°C and 16°C several times to allow for multiple rounds of digestion and ligation.

The Fragmid modules fall into six categories: (1) guide cassettes with RNA polymerase III promoters, (2) RNA polymerase II promoters to drive Cas protein expression, (3) N′-terminal domains, (4) Cas proteins, 5) C′-terminal domains, and (6) 2A-selection markers (Figure 1). Because some applications require the guide RNA and Cas protein components to be delivered separately, McGee et al. also developed a module that allows for the three Cas protein-related modules to be omitted to produce guide-only constructs. These categories allow researchers to create constructs appropriate for widely adopted genome-editing platforms, including CRISPR knockout, CRISPRi, CRISPRa, base editing, and prime editing. Moreover, the modules allow for each platform to be used with a variety of Cas orthologs and engineered variants. Impressively, the authors report a 100% success rate in obtaining the desired plasmid even when only two bacterial colonies were analyzed per construct.

Once the designed vector is assembled, users are able to add their guide sequence of interest using a routine cloning strategy. To expedite the process, McGee et al. equipped the Fragmid library with a suite of positive control guide modules targeting cell surface markers, which can be used to quickly assess the feasibility of a CRISPR platform in a new experimental setting using flow cytometry. The authors further showcased Fragmid's utility by using it to assemble and test vectors with improved lentiviral titers and newly reported Cas enzymes for use in all-in-one adeno-associated virus delivery.

The modularity of the Fragmid library was highlighted when assessing multiple CRISPR platforms at one time in a pooled setting. This was accomplished using a hybrid cloning strategy in which each Golden Gate assembly reaction was conducted in individual wells with the addition of new modules containing unique barcodes. These assembly reactions were then pooled and processed as one sample to create a lentiviral library of several hundred constructs, which was used to conduct a pooled CRISPR screen. The addition of the barcode modules allowed for the assessment of enriched and depleted constructs. This type of robust

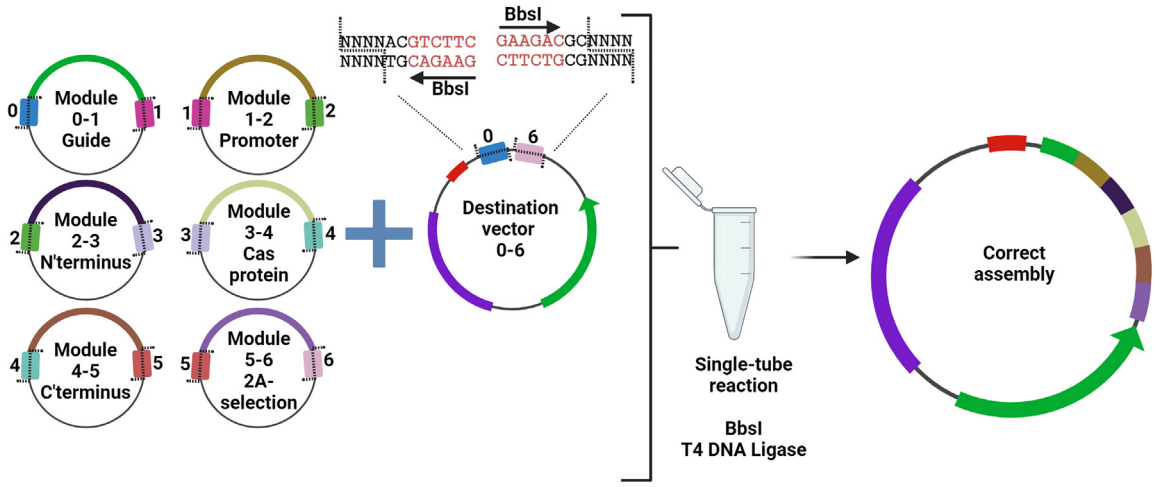

Figure 1. The Fragmid toolbox leverages Golden Gate assembly to rapidly create CRISPR constructs

Fragmid contains six main categories of fragments: (1) guide, (2) promoter, (3) N′ terminus, (4) Cas protein, (5) C′ terminus, and (6) 2A selection. These fragments, along with one of a number of destination vectors, can be combined into a single-tube Golden Gate reaction to create a desired CRISPR-targeting construct. Golden Gate assembly relies on type IIS restriction enzymes, in this case BbsI, which have recognition sites that are distinct from their cleavage sites. This allows for the creation of unique and programmed overhangs that can be stitched together in an organized manner and results in the construction of the desired CRISPR plasmid. In the Fragmid system, the unique overhangs of the six main fragment types are numbered 0–6, and each module has two unique overhangs. Overhangs with the same number will be stitched together by T4 DNA ligase during the Golden Gate reaction. Fragments and overhangs are not drawn to scale. This figure was created with BioRender.com.

cloning and analysis pipeline provides another option for individuals wanting to assess different CRISPR platforms in new cellular contexts.

The Fragmid platform also includes a helpful online tool to aid users in module and destination vector selection and provides an *in silico* plasmid map of the desired construct. The modular Fragmid toolkit, which currently consists of two 96-well plates of fragments, destination vectors, and positive controls, has been deposited with Addgene for distribution and is expected to be available in mid-2024. Although the Fragmid library already contains a diversity of modules for a variety of CRISPR modalities, the toolbox can easily be expanded, and the authors indicate that new fragments will be added and encourage others to add to the ecosystem by depositing new modules as well.

Overall, the Fragmid platform developed and reported by McGee et al. in this issue provides a plug-and-play system to create millions of different vectors for several CRISPR applications. Importantly, by standardizing the various components required for CRISPR editing, new technologies can quickly be assessed, adapted, and implemented to further accelerate scientific discovery.

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DECLARATION OF INTERESTS

The author declares no competing interests.

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