Case study

Molecular biology

Golden Gate assembly for enhanced high-throughput cloning for creation of bispecific antibodies: a case study

Company

The client was a biotech research lab working on commercialization of immunotherapies for cancer treatments.

Background

As a biotech research lab in immunotherapy, the client needed a more efficient way to develop bispecific antibodies (BsAbs) in high quantities for next-generation antibody therapy.

Over recent decades, immunotherapies including checkpoint inhibitors, adoptive cell transfer, monoclonal antibodies (mAbs), and vaccine treatments have become efficient and highly specific treatments for fighting cancer by boosting a patient's own immune system. Therapeutic mAbs have become the most widely used and approved form of immunotherapy in clinical practice [1]. However, mAbs have several limitations: patients may develop drug resistance or fail to respond to treatment. To improve the therapeutic utility of immunotherapy, BsAbs were introduced [2]. These work by binding to two different antigen sites and can provide more robust and tailored immunogenic targeting than is possible with natural antibodies [1]. Currently, BsAbs represent a key component in the next generation of antibody therapy.

Challenges

BsAbs usually do not appear in nature. They are primarily produced by three methods: (1) quadroma technology based on somatic fusion of two different hybridoma cell lines, (2) chemical conjugation, which involves chemical cross-linkers, and (3) genetic approaches utilizing recombinant DNA technology [2]. Development pf BsAbs often requires enormous amounts of time and resources.

Solutions

The Golden Gate assembly technique provides a seamless and orderly strategy to clone multiple DNA fragments into a mammalian expression vector that helps to produce BsAbs in high quantities and in a relatively short period of time (Figure 1).

This method utilizes type IIs restriction enzymes and T4 DNA ligase to allow simultaneous and directional assembly of multiple DNA fragments. This strategy enables high-throughput production of plasmids without scars, and with high fidelity; typically, a success rate of over 80% is achieved [4].

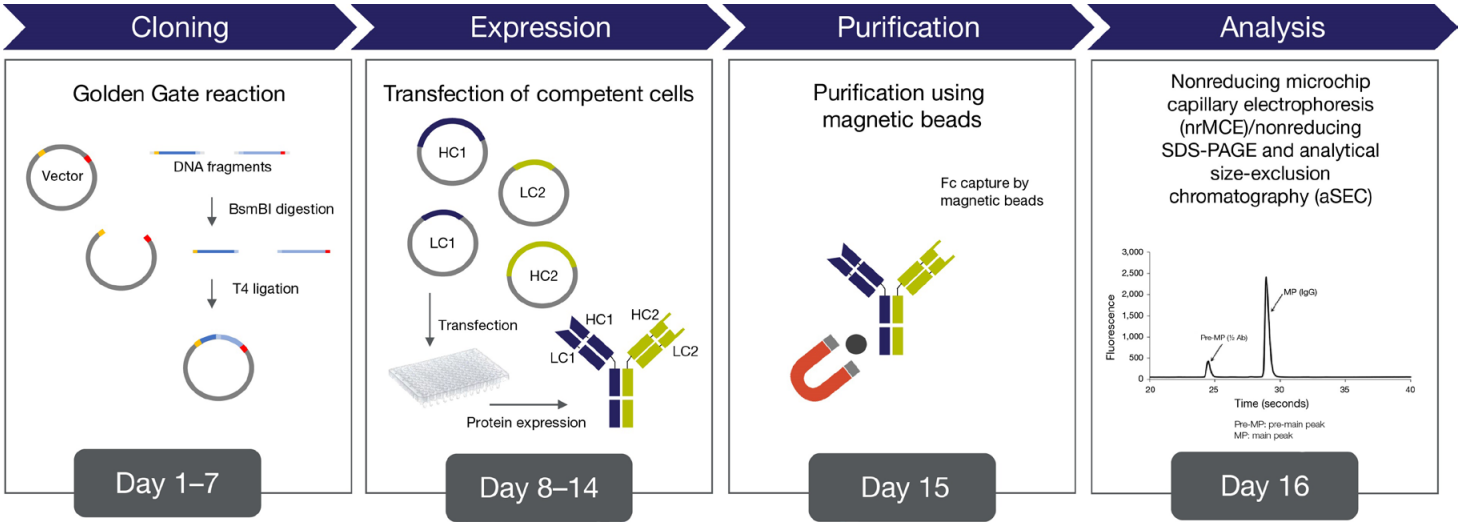

Figure 1. Workflow for creating bispecific antibodies. HC: heavy chain, LC: light chain.

Each step in the workflow is described in **Star protocols**.

Thermo Fisher Scientific offers a variety of products for performing Golden Gate cloning—Thermo Scientific™ FastDigest™ type IIs restriction enzymes, T4 DNA ligase, nuclease-free water, and Invitrogen™ One Shot™ *ccdB* Survival™ competent cells. (See ordering information.)

Summary

Bispecific antibodies are a powerful new class of therapeutics with huge potential in inflammatory, cancer, and autoimmune disorders. Thermo Fisher Scientific offers a variety of type IIs enzymes and other products for fast and convenient formation of scarless plasmids—a vital step for creation of BsAbs.

Partnering with Thermo Fisher Scientific has provided our customer with a novel solution. The type IIs restriction enzymes and other products enabled them to create BsAbs for targeted immunotherapies with speed, convenience, and reliability. Thus, the customer was able to advance their immunological research and have their needs met for faster commercialization of their immunotherapy products.

Ordering information

Product	Cat. No.
Type IIs restriction enzymes	
FastDigest Eam1104I	FD0234
FastDigest Eco31I	FD0293
FastDigest Esp3I	FD0454
FastDigest GsuI	FD0464
FastDigest MboII	FD0824
FastDigest BseGI	FD0874
FastDigest BseNI	FD0884
FastDigest Mva1269I	FD0964
FastDigest BpiI	FD1014
FastDigest MnlI	FD1074
FastDigest BseMI	FD1264
FastDigest SchI	FD1374
FastDigest BseXI	FD1454
FastDigest BveI	FD1744
FastDigest FaqI	FD1814
FastDigest CseI	FD1904
FastDigest LguI	FD1934
FastDigest Lsp1109I	FD2074
FastDigest FokI	FD2144
Ligase	
T4 DNA Ligase	EL0011/2/3/4
Competent cells	
One Shot *ccdB* Survival 2 T1^{R} Competent Cells	A10460
Water	
Water, nuclease-free	R0581/2

References

1. Wang Q et al. (2019) Design and production of bispecific antibodies. *Antibodies* 8(3):43. **doi.org/10.3390/antib8030043**
2. van Spriel A et al. (2000) Immunotherapeutic perspective for bispecific antibodies. *Immunol Today* 21(8):391–397. **sciencedirect.com/science/article/abs/pii/S0167569900016595**
3. Fan G et al. (2015) Bispecific antibodies and their applications. *Journal of Hematology & Oncology* 8:130. **https://link.springer.com/article/10.1186/s13045-015-0227-0**
4. Li D et al. (2022) Protocol for high-throughput cloning, expression, purification, and evaluation of bispecific antibodies. *STAR Protoc* 3:101428. **doi.org/10.1016/j.xpro.2022.101428**

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