Chee and Chin, Clon Transgen 2015, 4:2
DOI: 10.4172/2168-9849.1000138

Cloning & Transgenesis
ISSN: 2168-9849

Commentary Open Access

Gateway Cloning Technology: Advantages and Drawbacks

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Research studies in the heterologous expression of exogenous genes in living host cells are not new and have been ongoing since the first recombinant DNA molecule was created in the 1970's [1]. Conventionally, molecular cloning to produce recombinant DNA very often revolve around cleavage of chromosomal DNA or polymerase chain reaction (PCR)-amplified DNA molecules using restriction enzymes and insertion of the DNA fragments of interest with vector DNA, such as plasmids, bacmids or cosmids, with DNA ligase. The recombinant DNA that is constructed is then delivered or transformed into host cells for expression.

In recent years, many researchers are interested in the co-transformation of multiple DNA elements of interest for expression studies. Hence, the idea of incorporating more than one DNA element into a single vector for the ease of transformation is more eminent. Conventional cloning offers lower versatility in terms of cloning multiple DNA fragments because the selection of restriction sites can often be difficult, despite many commercial restriction enzymes come with standardized or optimized buffer conditions.

The requirement to clone multiple transgenes into one vector has led to the advancement of cloning strategies in recent years, which saw the invention of recombinational cloning technologies, such as the Gateway® Cloning System in the late 1990's. This cloning technology allows the simultaneous insertion of multiple DNA fragments into a single destination vector using site-specific recombinase, the Integrase enzyme, to produce the expression clone [2]. The Gateway system is advantageous as the throughput is high, and a wide collection of open reading frames is available. Many researchers around the world had produced their own Gateway-compatible vector sets and tested them for high throughput cloning and expression of genes in plants, bacteria, vertebra embryos and mammalian cell lines [3-5]. A variety of such vector sets were also incorporated with a range of promoters and terminators, as well as fluorescent protein tags and selectable markers, upstream and downstream of the open reading frames [6,7].

One of the major drawbacks of this system is that the cost is relatively high compared to conventional restriction-ligation cloning. The main reason is that Gateway system utilizes two enzyme mixes, commercially known as BP Clonase and LR Clonase, which are defined by the technology supplier. The BP Clonase is first needed for the cloning of DNA fragments of interest into the supplied donor vectors to generate entry clones. Subsequently, the LR Clonase is used for the sub cloning between the entry clones and the destination vector to generate the final expression clone [8]. On the other hand, conventional cloning utilizes only one or two restriction enzymes and a DNA ligase, depending on the nature of expression cassette and vector.

Originally, Gateway cloning requires two bacterial transformation steps before the final expression vector is obtained. One bacterial transformation step is done after the BP reaction and another step is done after the LR reaction, compared to just one step in other cloning methods. Nevertheless, it is now possible to perform both BP and LR reactions in a single reaction. By modifying the BP: LR ratio, the reaction product can be more entry clones oriented (higher BP) or expression clone oriented (higher LR) [9].

The BP and LR recombination reactions in Gateway cloning require the availability of *att* sites, in which *att*B-flanked PCR products recombine with *att*P donor vectors to make *att*L entry clones (BP reaction) and then the *att*L entry clones recombine with *att*R destination vector to produce the final expression clone (LR reaction). The recombination products are eventually left with "scar" *att* regions, which serve no other function in the expression cassette. Such characteristic is undesirable as they add extra amino acids to the expressed protein. To alleviate this drawback, cloning system like Golden Gate Cloning utilizes type IIS restriction enzymes that cut outside their recognition regions. As such, with proper design and incorporation of the recognition regions into the transgene, the additional sites can be removed after restriction [10] (Figure 1).

In short, the Gateway Cloning system provides a versatile platform for the study of multigene expression with the availability of high throughput vector sets and a wide range of open reading frames in choice. Aside from the aforementioned drawbacks, it is arguable that the cloning efficiency can still be affected by the complexity and folding structures of the transgene of interest. As with other cloning strategies, bigger DNA size can potentially decrease the efficiency. Many factors have to be considered, from the incorporation of elements into the expression cassette to the availability of the desired vectors, when deciding on the suitable cloning technologies.

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Received April 20, 2015; **Accepted** June 04, 2015; **Published** June 06, 2015

Citation: Chee JY, Chin CF (2015) Gateway Cloning Technology: Advantages and Drawbacks. Clon Transgen 4: 138. doi: 10.4172/2168-9849.1000138

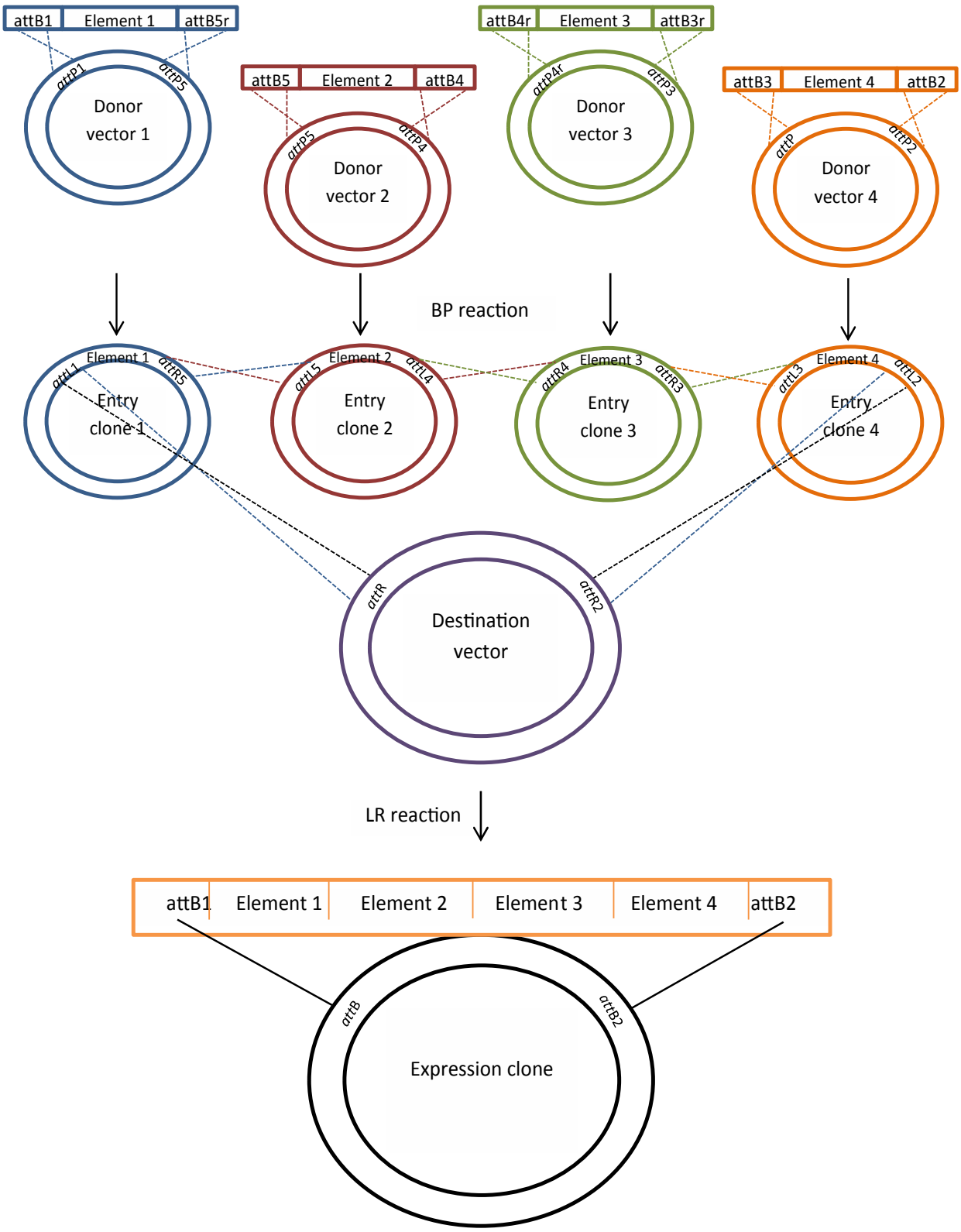

Figure 1: Diagram above shows the recombinational cloning process of four DNA elements into a destination vector to produce the final expression clone. The DNA elements amplified by polymerase chain reaction (PCR) are cloned into plasmids, designated as donor vectors 1, 2, 3 and 4, through BP reaction using BP clonase. PCR products and donor vectors contain specific *att* recombination regions that are recognised by BP clonase. Recombination occurs with the crossing of respective *att*B and *att*P to produce *att*L and crossing of respective *att*Br and *att*Pr to produce *att*R. Recombination event is represented by dashed lines. The resulting plasmids are known as entry clones. The entry clones are then mixed with the destination vector and undergo LR reaction using LR clonase. The recombination of respective *att*L and *att*R produces *att*B. As a result, the recombinant destination vector, known as expression clone, will be added with the four DNA elements.

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