

BBF RFC 69: A New Standard to Connect BioBrick Parts for Precise Extraction of an Enzyme Digestion Product

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1. Purpose

This BioBricks Foundation Request for Comments (BBF RFC) introduces a new standard to connect BioBrick parts using BglI site.

2. Relation to other BBF RFCs

BBF RFC 69 does not update or replace any earlier BBF RFC.

3. Copyright Notice

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4. Description

Usually, BioBrick part is digested by using EX cut or SP cut as a vector and ES cut or XP cut as an insert. However, there are two problems in this standard.

First of all, plasmids digested by EcoRI and XbaI are hard to differentiate because the fragments are almost same in size. This is because EcoRI site and XbaI site are close to each other.

Secondly, a small insert would be needed in order to connect the short parts. However, if the fragment is too small, it would be hard to extract from agarose gel after electrophoresis because the band would be too thin.

By using BglI site, the problems written above were solved. BglI site is located at ampicillin resistance coding region. Since BglI site is away from EcoRI site, there is no need to cut out thin band from agarose gel, thus making it much easier to connect short parts.

Also, the location BglI site is coded is very preferable. As mentioned before, BglI site was found on ampicillin resistance coding region. Thus, *E. coli* with unexpected ligation product would die out under ampicillin, making it easier to determine which colony has the target ligation product.

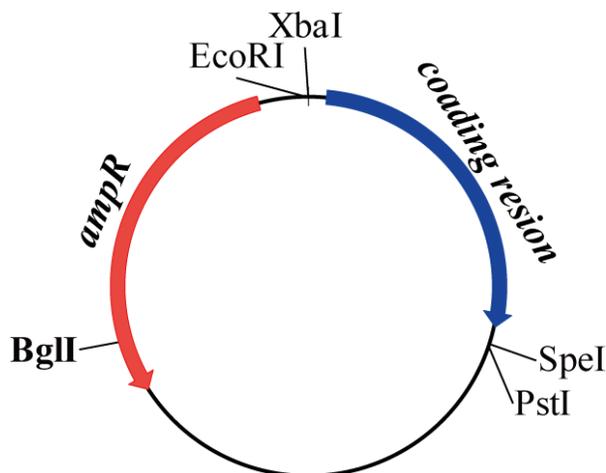


Fig.1 It shows the location of EcoRI, XbaI, SpeI, PstI, and BglII on the plasmid. As you can see, EcoRI and XbaI, or SpeI and PstI are too close to each other to connect them.

The standard used by iGEM now is not a very useful method if one wants to connect the short parts. In order to get a more precise product, we propose to arrange EX and SP more distantly. We believe that BglII site would be a very good alternative site.

5. How we found this out

When we tried to insert MS2 in the plasmid, it did not work properly. This was because MS2 had multiple E sites. Therefore, plasmid digestion did not work stably. So, we tried to find other restriction enzyme site. As a result, we found BglII site which is located on upstream of the E site we originally wanted to cut. Moreover, this site was coded only once on the vector we wanted to use. This BS, BP cut made us possible to insert MS2 in the plasmid.

6. Acknowledgment

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