Cloning in Asaia

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Introduction

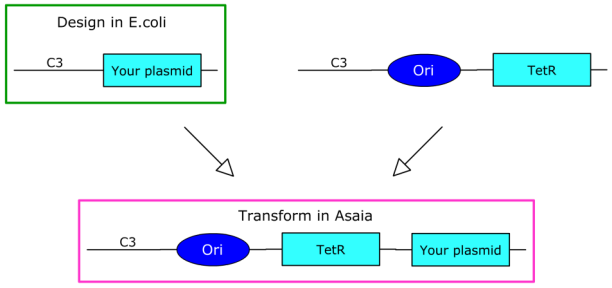
You can't do cloning with Asaia like you would in E. coli. You have to follow some specific guidelines. You can find those here.

This document features the indispensable plasmid you need to work with Asaia. You will also find all the plasmids we have created until now.

We also listed what we tried to do but without success, so maybe you will manage to triumph in those tasks, good luck!

Working with Asaia

We recommend you to work with E. coli when possible, as its doubling time is twice as short. Typically, design and create your plasmid in E. coli and then cut out the part you are interested in and ligate it into a plasmid containing the Asaia Origin. You can order C3+Asaia origin+TetR or C3+Asaia origin+KanR in the parts registry.



Once the cloning is done you can transform it into Asaia and E. coli and proceed with your experiments. We recommend you to make GLY stock with E. coli so, in the case you have to do a liquid culture for a mini-prep, it will take only one day and not 2 days.

Note that the Asaia origin is compatible with E. coli (though the inverse is not true (!)) You can therefore design a plasmid with the Asaia origin and work with it in E. coli.

Acquisition of those parts

If you want any of the parts mentioned below, you can just order it from the web site : http://partsregistry.org

Parts we created

This section contains all the parts we designed and created, based on pSB1C3, followed by a short description. Details can be obtained by following the links associated with each plasmid description.

Each parts description is structured this way :

[BioBricks ID] // [sequence in block]

[Image of the plasmid]

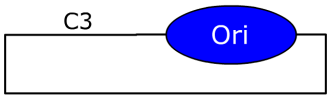
[Description of the plasmid]

[link to the registry]

Here are the abbreviations we used:

Abbreviation	Full name
C3	pSB1C3
ori	Asaia origin
Strong	Strong promoter
KanR	Kanamaycin Resistance
AmpR	Ampicillin Resistance
TetR	Tetracyclin Resistance
Immuno	Immunotoxin expression
p25	sequence expressing p25 protein
p28	sequence expressing p28 protein

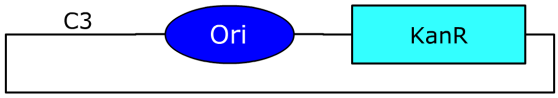
BBa_K320000 // C3+ori



This plasmid only contains Asaia origin, you can use it to just cut out this origin.

[link to the registry]

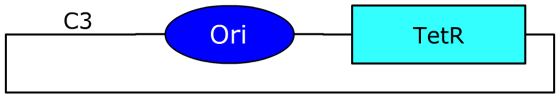
BBa_K320004 // C3+ori+KanR



This part is very useful when you want to adapt your plasmid for work in Asaia. Just cut this plasmid with SpeI and PstI and your plasmid with XbaI and PstI and ligate them to create your Asaia compatible plasmid.

[link to the registry]

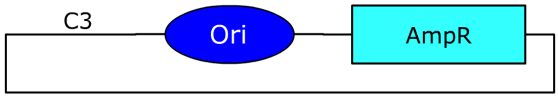
BBa_K320003 // C3+ori+TetR



This part is very useful when you want to adapt your plasmid for work in Asaia. Just cut this plasmid with SpeI and PstI and your plasmid with XbaI and PstI and ligate them to create your Asaia compatible plasmid.

[link to the registry]

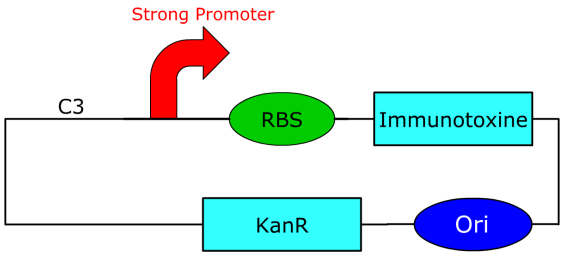
BBa_K320011 // C3+ori+AmpR



This part is very useful when you want to adapt your plasmid for Asaia. Just cut this plasmid with SpeI and PstI and your plasmid with XbaI and PstI and ligate them to create your Asaia compatible plasmid. Caution : read the "Growing Asaia" sheet to see that Asaia is naturally resistant to Ampicillin

[link to the registry]

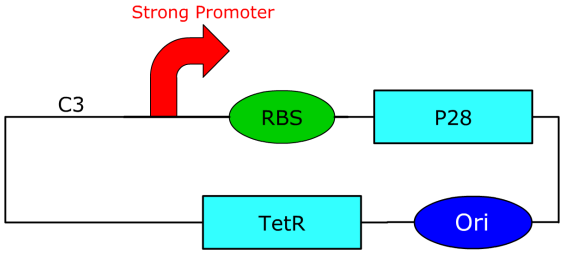
BBa_K320008 // C3+Strong+Immuno+ori+KanR



This plasmid is an Asaia-compatible plasmid expressing the immunotoxin. It was a major aim of our project. You will find more information on the web site : http://2010.igem.org/Team:EPF_Lausanne

[link to the registry]

BBa_K320009 // C3+Strong+P28+ori+TetR



This plasmid is an Asaia-compatible plasmid expressing the p28 protein. It was another aim of our project. You will find more information on the web site : http://2010.igem.org/Team:EPF_Lausanne

[link to the registry]