

Cloning in Asaia



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Introduction

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

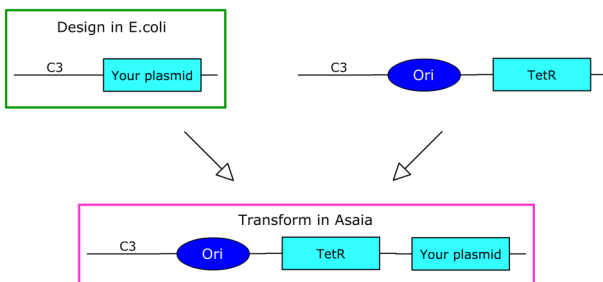
This document contains the indispensable plasmid you need to work with Asaia. You will also find all the plasmids we have created until now.

We have given what we tried to do without success, so maybe you will manage to triumph in those tasks, good luck.

HOW TO WORK WITH ASAIA

As you know yet, the doubling time of Asaia is a big disadvantage. Therefore the best way to clone in Asaia is first to do all stuffs in E.coli and at the end just cut out our plasmid and ligate it into a plasmid containing the Asaia origin. You can procure yourself C3+Asaia origin+TetR or C3+Asaia origin+KanR in the parts registry.

When you have done this cloning, transform it into Asaia



but also into E.coli. We recommend you to make GLY stock with E.coli so, in the case you have to do a liquid culture to do mini-prep, it will take only one day and not 2 days.

One thing very important to say about Asaia origin is that it works into E.coli. So you can design a complete plasmid without E.coli origin and always work 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>

PLASMID WE CREATED

Here you can find all plasmids we have designed and created with a little description. For more details, just follow the link associated with each plasmid description. We have based all our parts on pSB1C3.

Each part's description is structured this way :

[BIOBRICKS ID] // [SEQUENCE IN BLOCK]

[Image of the plasmid]

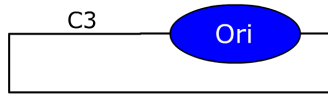
[Description of the plasmid]

[link to the registry]

Here are the abbreviations we used

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

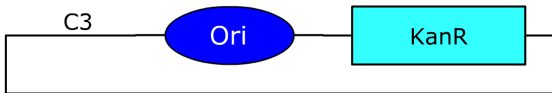
BBA_K320000 // C3+ORI



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

[\[link to the registry\]](#)

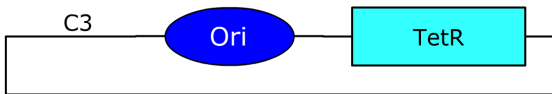
BBA_K320004 // C3+ORI+KANR



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

[\[link to the registry\]](#)

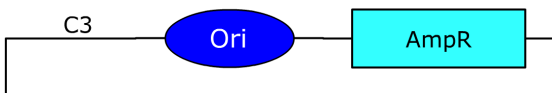
BBA_K320003 // C3+ORI+TETR



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

[\[link to the registry\]](#)

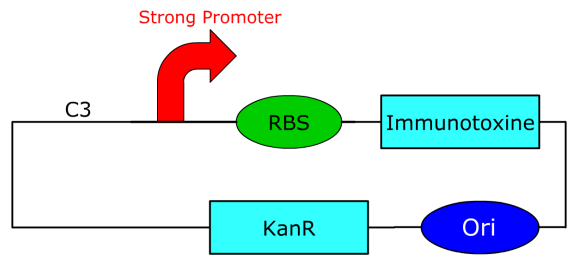
BBA_K320011 // C3+ORI+AMPR



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 YbaI 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

[\[link to the registry\]](#)

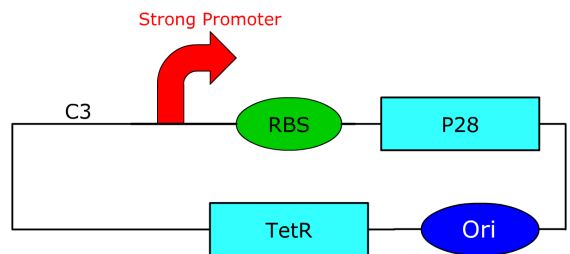
BBA_K320008 // C3+STRONG+IMMUNO+ORI+KANR



This plasmid is an Asaia compatible plasmid expressing the immunotoxine. It was one aim of our project. You will found 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 found more information on the web site : http://2010.igem.org/Team:EPF_Lausanne

[\[link to the registry\]](#)