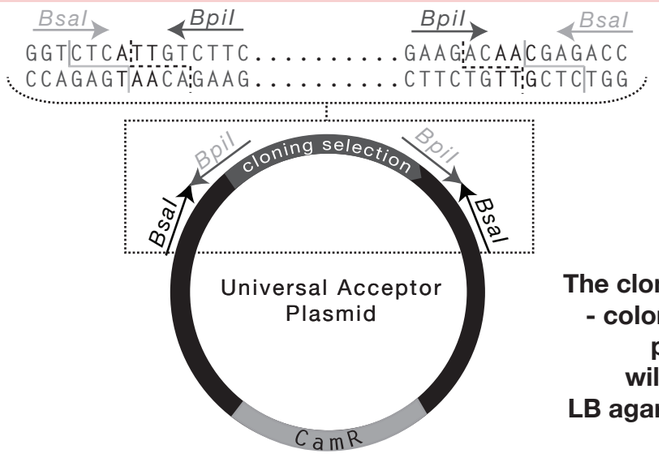
MAKING NEW PARTS IN THE UNIVERSAL ACCEPTOR pUAP1:

The easiest way of making new parts is to use a Universal Acceptor Plasmid such as pUAP 1 (Addgene Plasmid #63674)

NB The vector **pUPD2**, based on the same backbone as pUAP1, is equivalvent to pUAP1 but uses BsmBI in place of BpiI (see https://gbcloning.upv.es/tools/domestication/for instructions)

pUAP1 looks like this:



The cloning selection is RFP - colonies with the intact pUAP plasmid will appear pink on LB agar + chloramphenicol.

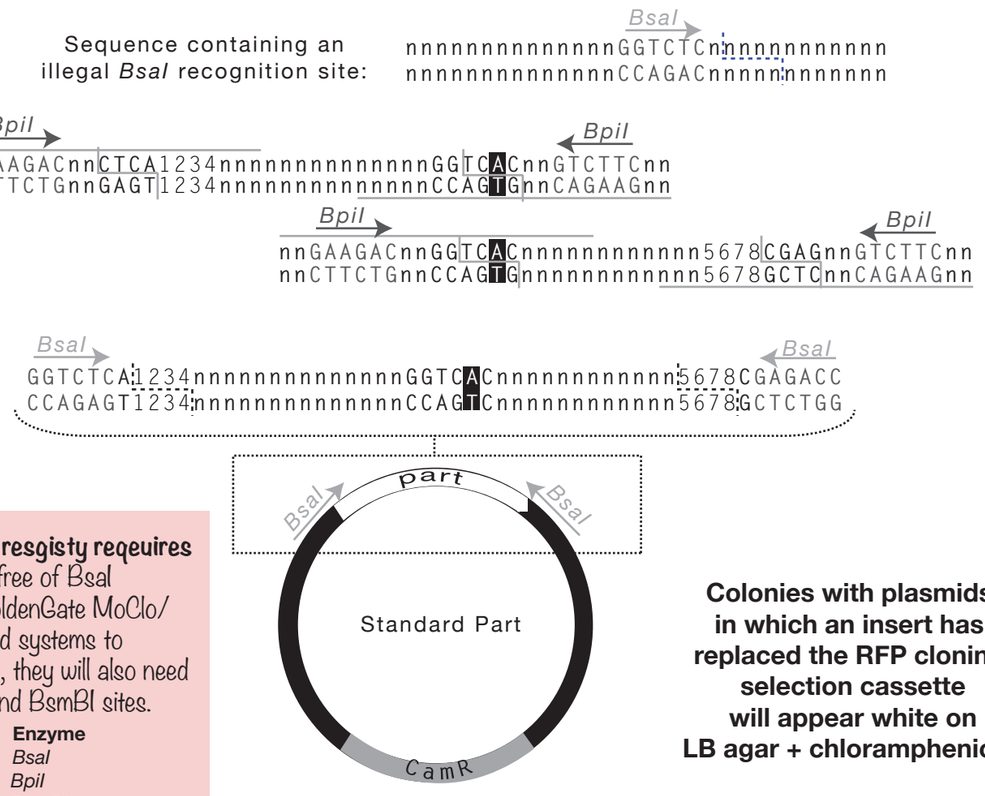
How to use the - Universal Acceptor Plasmids:

Sequences containing no BsaI or BpiI* sites can be amplified with oligonucleotide primers with 5' overhangs that
(i) Add BpiI recognition sequences and fusion sites to allow one step digestion-ligation into the universal acceptor and
(ii) Add the desired fusion sites (1234 and 5678) that will flank the part when re-released from the pUAP backbone with BsaI.

e.g. FWD primer: nnGAAGACnnCTCA**1234**+18-30bp 5' end of your new part
e.g. REV primer: nnGAAGACnnCTCG**8765**+18-30bp 3' end (rev-comp) of your new part

LEARN MORE ABOUT PRIMER DESIGN HERE

Sequences containing illegal BsaI recognition sequences (see example below) can be synthesized free of these sites or can be amplified in two (or more) fragments using oligonucleotide primers with 5' overhangs that **(i)** introduce a mutation to destroy the illegal site
(ii) adds BpiI recognition sequences and fusion sites to allow one step digestion-ligation into the universal acceptor and
(iii) add the desired fusion sites that will flank the part when re-released from the pUAP backbone with BsaI:



Colonies with plasmids in which an insert has replaced the RFP cloning selection cassette will appear white on LB agar + chloramphenicol

***Remember! The resgisty reqeuires** PhytoBricks to be free of BsaI sites. To use the GoldenGate MoClo/ GoldenBraid plasmid systems to assembleyour parts, they will also need to be free of BpiI and BsmBI sites.

Sequence	Type	Enzyme
gttctc	Illegal	*BsaI*
gaaga	Avoid	*BpiI*
cgtctc	Avoid	*BsmBI*

If your sequence is non-coding (e.g. promoter, untranslated region, terminator) you can follow the instructions on the left without worrying about how to keep things in frame. Just substiture 1234 and 5678 with the appropriate 4 base pair overhangs rom the standard syntax (see Cheat Sheet 1) and 18-30bp to anneal to your part.

Keeping coding parts in frame -

Parts that start **AATG**:
The last three bases will make the ATG start codon (Met) so your sequence should begin at the 4th base pair (i.e. do not include the native ATG as well). Your forward primer will be nnGAAGACnnCTCA**AATG** + 18-30bp starting with the first base pair after the native ATG

Parts starting **AGCC/CGGT** and **TTCG** follow a similar rule. The last 3 bases of theese overhangs also encode an amino acid so the sequence of the part can be kept in frame by beginning with the first base pair of the first codon.

Parts that start **CCAT**:
The last two bases make the **AT** of the **ATG** start codon (**Met**) so your sequence MUST begin with a **G**. Your forward primer will be: nnGAAGACnnCTCA**CCATg** + 18-30bp starting at the first position of the next codon to keep the frame.

Parts ending **GCTT**:
The native stop codon should be included before the 3' **GCTT** overhang.
Your reverse primer will be nnGAAGACnnCTCG**AAGC**+18-30bp (rev-comp) starting with the STOP codon.

Parts ending **TTCG**:
You must not include a stop codon at the end of this part. The last three bases (**TCG**) of the overhang will encode a **Ser** residue. The **T** in the first position of the **TTCG** overhang will therefore be the third position of the last codon of the part. There are two ways to do this:

a. Include two additional base-bairs to make a new codon of which the last position is the first T from the TTCG overhang, thus making a two-codon linker. Typically you would include GG, TC or AG as these will make Gly(GGU), Ser(UCU)or Ser(AGU), which are small amino acids that are less likely to interfere with function. To make a Gly, y our reverse primer will be nnGAAGACnnCTCG**CGAAcc**+18-30bp (rev-comp) starting with the last codon before the stop codon.

b. Remove the last base pair of the last codon before the stop codon and allow it to be replaced with the first T from the TTCG overhang. Pay attention to what amino acid this codon will make as introducing a structural/charged base may interfere with folding or function. Your reverse primer will be nnGAAGACnnCTCG**CGAA**+18-30bp (rev-comp), starting with position 2 of the last codon and not including the stop codon.

These two options can also be applied to parts ending **AATG** or **AGCC/AGGT**