

Description of pGULIG

oriV R6K - this is the origin of replication of the R6K plasmid. Note that it is only about 0.1 kb. It cannot encode either the Pir protein or RepA (if that's what you want to call it). In either case, Pir or RepA are essential proteins for initiation of replication of DNA at the oriV. Therefore, unless Pir or RepA are provided by the host strain, this plasmid will not be a functional replicon and will serve as a suicide plasmid.

oriT RK2 - This is the origin of transfer of plasmid DNA for conjugation of the RK2 plasmid. A site-specific nuclease acts at this site so that a single strand of linearized plasmid may be transferred to the recipient cell through a pilus. This site also is too small to encode any proteins, hence all of the trans-active conjugation functions (about 30 kb) must be provided by the host cell. If these genes/functions are provided, the plasmid can be transferred by conjugation.

f1 ori - This is the origin of replication of the filamentous phage f1. This locus is too small to encode a protein, hence the f1 products that act at this origin to initiate DNA replication must be provided by the host strain. If these genes are provided, then a single stranded DNA molecule will be packaged by the capsid protein (encoded by the host), and the single strand DNA can be transduced into a recipient cell, as long as the cell is expressing an F plasmid. Note that no phage will replicate in the recipient, because the packaged product will contain no phage DNA, other than the origin of replication. All of this is useful because only a single strand of one of the two strands of plasmid DNA will always be packaged. This single stranded DNA can be used for DNA sequencing, site-directed mutagenesis, use as a probe, etc.

MCS - The multiple cloning site contains a phage T7 promoter and therefore requires the T7 RNA polymerase to be provided by the host cell. This would usually be done in a regulated manner since the T7 promoter is so strong. I also said (erroneously) that there was a wild-type *lac* promoter present. That being said, you should note that the *lac* promoter is inducible by IPTG or lactose, as long as the LacI protein is present. The MCS is too small to encode the *lacI* gene, hence, for regulation to occur, the *lacI* gene must be provided by the host. Since the wild-type *lac* promoter is under catabolite repression, glucose should not be present in the medium (even if LacI is not present). I'm not too concerned about confusion that might have occurred with two promoters driving the expression from the MCS. The T7 would probably win, if the T7 RNA polymerase is present.

The LacZ-alpha fragment is capable of working with the LacZ-omega fragment to form functional beta-galactosidase. The omega fragment must be provided by the host. If the alpha fragment is interrupted by a cloned fragment in the MCS, when the *E. coli* are plated on XGAL + IPTG, the colonies will be white instead of blue.

There is not a good ribosome binding site upstream of the **ATG start codon**. You need something like AGGAGG, which is complementary to the rRNA. The ATG start codon is within the NcoI site to enable easy cloning in frame, but this requires that the beginning of the second codon start with a G. An NdeI site would be a better choice since this places no restrictions on the second codon.

rrnBT1T2 - transcriptional terminators from the ribosomal RNA operon. These are very strong terminators to prevent read through transcription from the very strong promoters driving your favorite gene in the MCS.

asd gene - Encodes aspartate semialdehyde dehydrogenase required for production of DAP, which is essential for cross-linking peptidoglycan and cell structure. If the host strain is *Asd⁻*, then the bacteria will either require DAP to be provided in the medium or they require complementation with the *asd* gene. This provides a selection for pGULIG without the use of antibiotics. However, the regulation of the *asd* gene is very important.

pBAD - the promoter for the arabinose operon. pBAD is considered a model for positive regulation in that the AraC protein in the presence of arabinose, can increase the transcription of the pBAD promoter. So AraC must be provided by the host (there is no room for it on pGULIG) and arabinose must be provided in the medium. Furthermore, like the lac promoter, pBAD is under catabolite repression, so the medium should not contain glucose. If AraC is not present, if arabinose is not provided, and if glucose is present, expression of *asd* will not be good and the bacteria will require DAP, or they will die.

Lambda phage loci -

cos - the nucleotide sequence used by lambda to circularize the linear phage genome after infection of a host cell because the cos site is a sticky ended site. If cos is placed on a plasmid, such as pGULIG, then the plasmid can be packaged into lambda phage heads and tails either in vivo, if helper phage are provided by the host, or in vitro, if plasmid DNA is mixed with phage heads and tails. Note that packageable product will occur only if the total length of the DNA is around 45 kb, the size of the lambda genome, which packages by the headful method. pGULIG would therefore be classified as a cosmid. This is useful because you can force the cloning of large sizes of DNA and you can move the DNA around by using lambda phage as a vector, under certain conditions.

attP - the phage attachment site which recombines with the bacterial component attB, which is located between the gal and bio genes. If the site-specific integrase, Int, is expressed, this will cause pGULIG to integrate into the host genome. Integrating your favorite gene into the host genome/chromosome is useful because it ensures single copy and stability.

int - the integrase described above that causes the site-specific recombination between attP and attB

pL - the left promoter of phage lambda, which normally causes the expression of lytic genes. It is normally repressed by the CI repressor. However, the CI repressor is not encoded by pGULIG, so unless CI is encoded by the host, pL will be constitutive, Int will be expressed constitutively, and pGULIG will be driven into the host chromosome by the Int protein. Hence, pGULIG is really aimed at integrating recombinant genes into the host chromosome. This is why having the defective R6K oriV is important. If Pir is not provided in trans, the plasmid is dead. But having Int expressed will enable the plasmid to integrate into the host genome. If the host is *Asd⁻*, and the bacteria are grown in arabinose without glucose, they will be genotypically and phenotypically *Asd⁺*.

Questions:

1.
 - a. Transduction using phage lambda, if the size of the plasmid is around 45 kb. The recipient cells should be grown in maltose to cause the induction of the LamB outer membrane receptor for the phage.
 - b. Transduction using the f1 origin to package single stranded DNA. The recipient cells should express the F pilus, which is the receptor for f1.
 - c. conjugation using the RK2 oriT, as long as trans-active factors are provided by the host cell.
 - d. Of course, electroporation or artificial transformation are also useful.

2. If *asd* is not expressed, DAP will not be made, peptidoglycan will not cross-link, and the bacterial will lyse, as long as they are growing. This is very similar to the beta-lactam antibiotics or vancomycin, which each prevent transpeptidation or cross linking in very different ways.

3. At *oriV*, DnaA will bind to open up the DNA, DnaB is a helicase that will unwind the dsDNA helped by DnaC. ssDNA-binding protein will stabilize the unwound DNA, DnaG will lay down an RNA primer for DNA initiation, and either DNA Pol III or Pol I will carry out the polymerase activity. DNA gyrase will add supercoils, and DNA ligase will repair nicks.

4. Assuming that the host is providing the Pir or RepA proteins, there will NOT be an immediate effect. This is because rifampin inhibits initiation of transcription by binding to the RNA polymerase subunit B. Active transcription will continue. Hence the level of Pir will be maintained for at least a little while. There will be a delayed effect on plasmid replication when the Pir protein is either titrated away or is degraded.

5. As noted above CI represses the pL promoter driving *int*. At the permissive temperature, CI will repress pL and *Int* will not be produced. At the non-permissive temperature, the CI protein will change conformation and no longer act as a repressor. *Int* will be expressed, and assuming that the host has the *attB* site, pGULIG will integrate into the chromosome.