

# DARPA BioComp Plasmid Distribution 1.00 of Standard Biobrick Components

Tom Knight (tk@ai.mit.edu)

MIT Artificial Intelligence Laboratory

May 22, 2002

This distribution consists of 300 ng aliquots of plasmid DNA for each of twelve components and compound constructs utilizing our idempotent assembly strategy. The dried DNA should be stable at room temperature for many weeks, but long term storage at -20 or -80 in the resealable foil pouch with desiccant is recommended. This document and much additional information, protocols, and detailed sequences is or will be available from the site <http://ks.ai.mit.edu>

Well number 1 is marked with a red marker. The foil top can be pierced with a pipet tip to add DI water for resuspending the sample. We recommend resuspending in 100  $\mu$ l water, and transferring the resuspended sample to another labelled tube for more permanent storage. Standard heat shock or electroporation transformation techniques can be used to introduce the plasmid into E. coli cell lines. Samples were dried down from 10  $\mu$ l of TE 10:0.1, so the solution will contain small amounts of Tris and EDTA. For electroporation, it might be necessary to further dilute or dialyze the samples to avoid arcing. Our standard strain for cloning is the Invitrogen library efficiency chemically competent DH5 $\alpha$  strain, catalog number 18263-012. All constructs are based on a pUC18 backbone, and are high copy number and ampicillin resistant. We grow strains in Difco LB Lennox broth or agar with 100  $\mu$ g/l of ampicillin (Sigma A0166).

Expression of the lacZ $\alpha$  gene can best be seen on Sigma S-gal plates, which provide a marked improvement over X-gal plates.

Note that the DH5 $\alpha$  strain does not express LacI. Controlled expression of LacI should be done with a construct providing LacI or in a lacI<sup>q</sup> strain such as XL1-Blue.

Much of the power of these assembly techniques arise from a consistent, widely available set of components. We strongly encourage others who develop components in this form to contact us with complete information and samples, if possible. We volunteer to act as a community coordination and distribution point for these components as they are developed. If you have components which you believe are useful but not in this form, we may, in many cases, be interested in doing the work necessary to cast them into standard component form.

We are working to develop licenses which would encourage widespread free distribution of DNA components, sequences, and documentation and intend those licenses, as developed, to apply to these components and assembly strategies.

## Standard Components and Assemblies, May, 2002 Distribution

1. pSB103  
Base plasmid for the Biobrick idempotent assembly technique.
2. pSB103-Plac  
Plasmid containing the Plac promoter. CAP and LacI repressor binding sites. Wild type sequence.
3. pSB103-lacZ $\alpha$   
Plasmid containing the lacZ $\alpha$  coding sequence and an upstream ribosomal binding site. This is the alpha complementation portion of the wild type gene, missing the multiple cloning site introduced by Messing.
4. pSB103-ECFP  
Plasmid containing the Clontech ECFP gene and an upstream ribosomal binding site.
5. pSB103-EYFP  
Plasmid containing the Clontech EYFP gene and an upstream ribosomal binding site. A silent mutation to eliminate the PstI cut site within the coding sequence has been introduced to conform with standard biobrick assembly standards.
6. pSB103-T1  
Plasmid containing the rrnB-T1 transcriptional terminator.
7. pSB103-Plac-ECFP  
Plasmid expressing the ECFP gene from the Plac promoter.
8. pSB103-Plac-EYFP  
Plasmid expressing the EYFP gene from the Plac promoter.
9. pSB103-Plac-ECFP-EYFP  
Plasmid expressing both the ECFP and EYFP genes from the Plac promoter.
10. pSB103-Plac-EYFP-ECFP  
Plasmid expressing both the EYFP and ECFP genes (opposite order) from the Plac promoter.
11. pSB103-Plac-ECFP-T1-EYFP  
Plasmid expressing ECFP with dramatically reduced EYFP expression due to the T1 terminator.
12. pSB103-Plac-EYFP-T1-ECFP  
Plasmid expressing EYFP with dramatically reduced ECFP expression due to the T1 terminator.

This work is supported by the DARPA/ONR contract "Computing with Synthetic Biology."