Chapter 6 - Final Discussion

6.1 Conclusions

DNA Ligases are essential repair enzymes. Consequently, they are required to quickly locate and seal a nicked site in the DNA backbone. The aim of the work presented in this thesis was to determine if *Eco*LigA could adopt a facilitated diffusion mechanism to locate a nicked site in various DNA substrates. Facilitated diffusion is important during cellular processes such as restriction and repair. Enzymes required in these processes, including the restriction enzyme EcoRV (Stanford *et al.* 2000, Bonnet *et al*. 2008) have been observed adopting a hopping motion along DNA to locate a target site. It is possible that Ligase, as repair rather than a restriction enzyme, could also adopt a similar method to locate a target site. In this thesis three key sets of experiments were carried out. The first (Chapter 3) concluded the *Eco*LigA reaction fitted the double displacement (ping-pong) mechanism to locate a nick. This was an important mechanism to establish at the outset, since the enzyme was proven to be adenylated first, and the nick ligation step second. The second set of experiments (Chapter 4) showed that it was possible for *Eco*LigA to use DNA flanking a target site as a guide to locating a nick. The third main result (Chapter 5) showed that *Eco*LigA is a weakly processive enzyme, but in the presence of Beta Clamp and clamp-loader molecules, the processivity was greatly increased. These are discussed in turn below.

In Chapter 3, initial experiments revealed that during the Ligase preparation, 12.4% of the protein was pre-adenylated. This was important to know as it would affect the results when trying to calculate initial rates. When pre-adenylated Ligase was added to a nicked DNA timecourse, 12.4% of the reaction would be completed almost instantly. This is known as burst kinetics. So to minimise the effect this had on reaction rate

experiments it was decided to use a limiting concentration of Ligase, set at 0.2 nM throughout the entire thesis. This meant that at time zero, 12.4% of 0.2 nM (0.025 nM) of *Eco*LigA was pre-adenylated. Therefore only 0.025 nM DNA was sealed immediately, a concentration so small that it can not affect the results when calculating the much larger initial rates of reaction.

It is widely accepted that *Eco*LigA requires NAD^+ to perform its nick-sealing function (Gellert, *et al*. 1967). The next part of Chapter 3 investigated whether Ligase could also use other cofactors just as efficiently. The results concluded that NAD^+ was the most efficient cofactor used. However, when the concentration was increased to from 25 to 100 µM, NADH was just as efficient, giving an initial reaction similar to that of NAD^+ at 1.4 $nMmin^{-1}$. It is possible that oxidation of NADH to NAD^+ was responsible from this observation, or whether NADH in its own right participated in the reaction. The order of preference for cofactors tested was NAD^+>NADH>$NADP^+$>>NADPH=ATP.

The main aim of Chapter 3 was to determine if Ligase had an obligatory off-step from DNA once a nick has been sealed. That is, does Ligase have to first react with free NAD^+ and release nicotinamide adenine mononucleotide (NMN) before then locating and sealing a nick in DNA? The results confirm those from previous work by the Lehman group (1974). Ligase first binds NAD^+, reacts and then must release NMN before it can locate a DNA molecule. On a 40 bp duplex, the V_{max} of Ligase for NAD^+ increased from 0.8 to 4.4 $nMmin^{-1}$. A similar value was achieved by Lehman (1974) at saturating NAD^+ concentrations of 4.0 $nMmin^{-1}$.

More recent studies on DNA Ligases have revealed Ligase undergoes conformational changes during the enzymatic reaction. Before adenylation, Ligase is in an open conformation (Popov *et al.* 2012, Mills *et al.* 2012). After reacting with its corresponding cofactor, the adenylated-Ligase adopts a more closed conformation (Suh

et al. 2009). It is now thought that the binding of the AMP group to Ligase drives the conformational change, allowing a more specific tertiary fold for interaction with a DNA substrate (Samai and Shuman 2011). This is supported by the kinetic results in Chapter 3, in which Ligase appears to first bind and react with cofactor before releasing product. The adenylated-Ligase then locates a nicked site and reacts, releasing free enzyme and nick-sealed DNA. One aspect that remained unknown despite this double displacement mechanism is whether the Ligase has to depart from a sealed DNA nick into free solution for re-adenylation, before searching for another nick.

The main aim in Chapter 4 was to answer the question above and determine if Ligase could locate a nicked site in DNA using the flanking DNA lengths as a guide. This was achieved by conducting length-dependency competition experiments. Previous studies on EcoRI (Jack *et al.* 1982) showed that when two different DNA lengths are within the same reaction mix, the enzyme, given the 'choice', would locate the restriction site on the longer DNA length faster than that on the shorter DNA length. In this chapter, the results showed that when two different DNA lengths were in the same reaction mix, the initial reaction rate of *Eco*LigA were nearly always faster for longer DNA lengths. For example, for the 100 vs 301 bp competition reaction (Figure 4.7A), the rate at which Ligase locate the nicked site on the 301 bp substrate was 0.55 $nMmin^{-1}$ (average result), which was 1.77 times faster than the initial rate for the 100 bp substrate.

Figure 4.7C shows a graph of all the ratios of initial rates against the ratios of DNA lengths. A total of 15 different length ratios were investigated, with 8 being repeated. A simple line of best fit showed a clear positive correlation between the ratio or lengths and the ratio of rates. Essentially, it can be seen that Ligase locates a nicked site far faster for longer DNA lengths in a given length competition. The results suggest that Ligase is indeed able to use flanking DNA as a guide to locate a nicked site. This is a

definite example of a protein (that is not a restriction enzyme) undergoing facilitated diffusion along a DNA chain. If Ligase wasn't associating with flanking DNA, we would expect to see initial rates for both DNA substrates in a competition to be nearly the same, according to random diffusion. Further competition experiments would be interesting using DNA lengths of 5000 bp +. This would also help determine which method of facilitated diffusion Ligase adopts, whether 1D sliding or 3D hopping. On very long lengths of DNA it may be an efficient search strategy for a DNA repair enzyme to adopt a 3D hopping mechanism (Halford and Marko 2004).

The main aim of Chapter 5 was to determine if Ligase was a processive enzyme. That is, was it able to move between two well defined nicks without leaving the DNA molecule. This was achieved using a series of 471 bp DNA substrates, each containing two nicked sites separated by 21 to 75 bases. The first experiments conducted used DNA substrates containing two nicks on the same DNA strand (directly-repeated nicks). Results showed that Ligase was a very weakly processive enzyme, showing about 32% processivity. However, the fraction of processive reactions (*fp*) increased slightly as the intersite distance between nicks increased (ranging overall from 16-39% processivity).

Using inverted nick sites, that is one nick on each strand of the same DNA molecule, the initial association rate of Ligase was slower than on the directly-repeated nicked DNA substrates. This suggested that Ligase adopts a 1D sliding motion to locate a nick, with the protein remaining on the same DNA strand, unable to hop to the other strand. However no conclusive answers can be obtained from the inverted repeat experiments, since no intermediate singly-sealed species was separable on polyacrylamide gels. That is, the singly-sealed species (471 b) were the same length as the doubly-sealed species (471 bp). For example, for the 24inv DNA substrate with one nick in the top stand and one nick in the bottom strand, the top strand fragments a (268 b) and b (203 b) produced

a 471 b product when the nick was sealed. Bottom strand fragments C (244b) and D (227 b) also produced a 471 b DNA intermediate. Figure 5.15 showed an alternative strategy to the above, where either the top or the bottom DNA strand was 5'-radiolabelled instead of labelling throughout the DNA molecule. This allowed each intermediate in the reaction to be visualised individually. However, this method does not show the formation of the final DNA product (471 bp) and therefore also can not be fitted to a processivity calculation. An experimental design to overcome the problem of distinguishing intermediate and final product species has been devised subsequently (discussions with Prof. Steve Halford, Bristol University). In this new strategy, the use of a hairpin at one end of the double stranded substrate is introduced, and this links fragment b with fragment D. This converts what is a 2-body problem into a 3-body problem and processivity can be fully measured. This would allow all DNA fragments and products to be seen on a denaturing polyacrylamide gel and therefore a processivity calculation could be made to determine the *fp* for both the directly and inverted nick repeats. This work will be carried out using hairpin molecules in the near future.

The final section of work in Chapter 5 concerned the interaction of *E. coli* beta sliding-clamp (beta-clamp) and gamma clamp-loading complex (gamma-loader) with Ligase and doubly-nicked DNA. Beta-clamp and gamma-loader were kindly provided by Professor Mike O'Donnell (Rockefeller Institute, New York). In combination, these proteins increase the processivity rates of enzymes at the replication fork in bacteria, playing a leading role in lagging and leading strand synthesis (Lopez and O'Donnell 2001).

In Chapter 5, it was determined that beta-clamp, once loaded onto a 471 bp doubly-nicked DNA clearly increased the processivity of Ligase. The reaction was conducted at 0.2, 5, 20 and 200 nM of the gamma-loader, with 5 nM beta-clamp present in all reactions. The initial reaction rate appeared to be lower than that previously observed

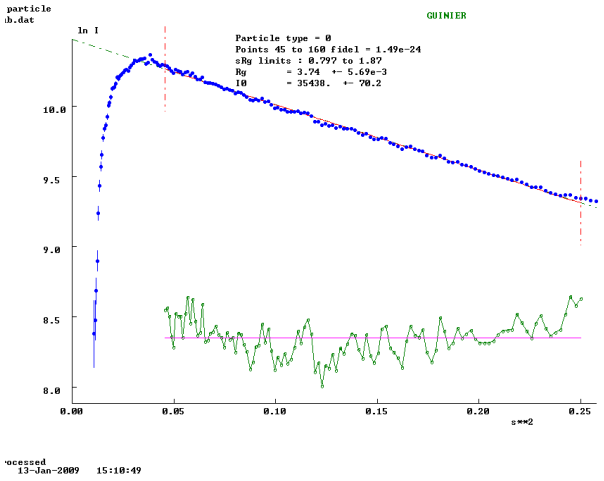
for Ligase alone (Figure 5.6), and also when in solution with beta-clamp but with no gamma-loader present; the reaction rate being about one quarter of the reaction of Ligase alone in solution. The reason for this drop in Ligase activity was unknown. It could be that Ligase associates with the beta-clamp in free solution and was unable to 'get onto' DNA without the presence of gamma-loader. Further experiments are required to determine the actual effect. However, at high gamma-loader concentrations (200 nM) the reaction was notably faster; processivity increased from 0.06% at 0.2 nM gamma-loader to 34% at 200 nM gamma-loader.

6.2 Future work

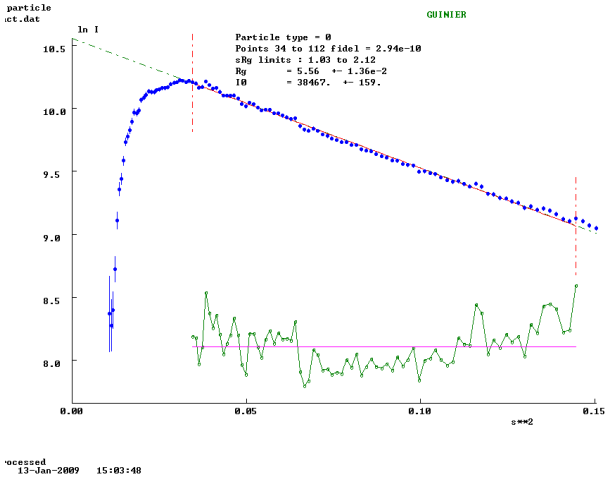
The results in the first part of Chapter 5 indicated that when alone in solution, Ligase is weakly processive. However, preliminary results showed that in the presence of beta-clamp and gamma-loader, processivity was increased by a factor of nearly 500. Further work needs to be conducted before any firm conclusions can be made. Firstly, the initial experiments conducted here using Ligase with beta-clamp and gamma-loader must be repeated. Secondly, the intersite distance between nicks should be varied more widely. Results in Chapter 5 showed an increase in processivity as the distance between nicks increased. If Ligase – beta-clamp proves to be a highly processive enzyme complex, the intersite distance could be increased to 100's and also 1000's of base pairs. This would allow us to answer the question: does processivity increase when intersite distance increases? Chapter 5 did however support there being a distinct interaction between Ligase and sliding clamp.

Samples of Ligase and ligase:clamp were kindly taken by Dr. John McGeehan (Portsmouth University) to the ESRF, Grenoble to be analysed by small-angle X-ray scattering (SAXS). Figure 6.1 below shows the preliminary data obtained, and this indicates that both proteins do interact with each other. Further work to establish the

A **Ligase alone**



B **Ligase+β-clamp**



C **Ligase + β-clamp complex**

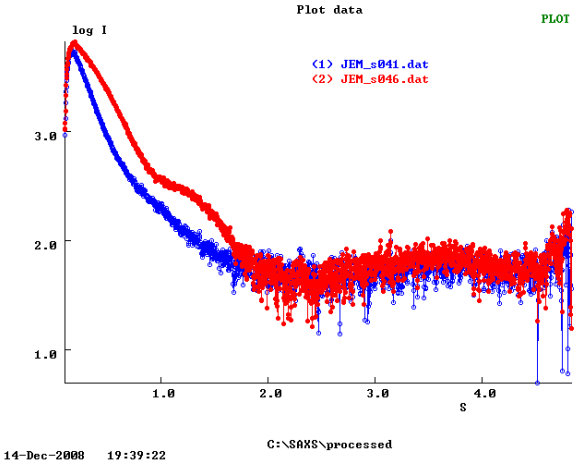


Figure 6.1. Preliminary shape (SAXS) work on ligase, clamp and ligase-clamp complex.

nature of the interacting components (Ligase enzyme, NAD^+, Mg^{2+}, nicked-DNA, beta-clamp and clamp-loader) is under way.

Results in this thesis suggest that Ligase does bind non-specifically to DNA and uses the flanking sequences as an antenna to locate a nick site. As described in the section above, we have devised a new strategy using hairpin DNA molecules that will allow unambiguous determination of 1D vs 3D routes using the same processivity approach as Stanford *et al*. 2000. Another experimental approach that would help deduce the mechanism is that of DNA catenane studies as have previously been used to show that a restriction enzyme adopts a 3D mechanism to locate a specific sequence (Gowers and Halford, 2003). Briefly, catenanes comprise two interlinked DNA rings, one much larger than the other (The smaller circle is termed a minicircle). This is shown in Figure 6.2 below. If ligase is only able to adopt a 1D sliding motion, then once associated with the larger DNA circle, a nick site in the minicircle would rarely be found by sliding alone. However, 3D hopping motion would be revealed by rapid sealing of the minicircle. One further method is by direct visualisation of the Ligase on stretched DNA molecules as has been observed for some restriction enzymes (Bonnet *et al*, 2008).

6.2.1 Future publications

Other than one protein:protein gel shift by the O'Donnell group (Lopez 2001) there have been no other papers about NAD^+ ligase associating with beta-clamp. It is well known that in eukaryotic cells, ATP ligase associates with the PCNA sliding clamp (Wei *et al*, 2009). Therefore the work in this thesis will be published. Data to be included will be from Chapter 4 (length dependency studies). Figure 4.7A and C will be included to show the competition experiments. These results showed that Ligase is able to use flanking DNA to locate a nick; rates were faster than if Ligase locates a nick by

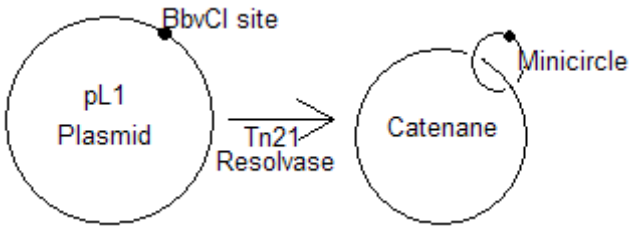


Figure 6.2 Making catenanes

The plasmid pL1 contains the recognition site for *BbvCI* and two sites for the Tn*21* resolvase. Recombination by Tn*21* resolvase produces a catenane with two interlinked rings. The BbvCI recognition site is located on the minicircle.

random diffusion alone. Figures 5.7 and 5.16 will also be included. These figures show that Ligase is weakly processive without the presence of beta-clamp. After further analysis and repeated experiments, Figure 6.1 will also be included as this gives a visual of ligase:beta clamp interaction. Additionally, the hairpin experiments discussed above will be conducted and prepared for publication after a conclusion can be drawn about whether 1D or 3D pathways operate.

References

Berg, O.G. and von Hippel, P.H. (1985). Diffusion-controlled macromolecular interactions. Annu. *Rev. Biophys. Chem*. **14**, 131-160

Biebricher, A., Wende, W., Escude, C., Pingoud, A. and Desbiolles, P. (2009). Tracking of single quantum dot labeled EcoRV sliding along DNA manipulated by double optical tweezers. *Biophys. J*. **96**, 50-52

Billen, D., Hellermann, G. R. & Stallions, D. R. (1975). Role for deoxyribonucleic acid ligase in deoxyribonucleic acid polymerase i-dependent repair synthesis in toluene-treated escherichia coli. *J Bacteriol* **124**, 585-588

Birnboim, H.C. & Doly, J. (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nuc. Acids Res*., **7**, 1513-1523

Bonnet, I., Biebricher, A., Porté, P.L., Loverdo, C., Bénichou, O., Voituriez, R., Escudé, C., Wende, W., Pingoud, A.,and Desbiolles, P. (2008). Sliding and jumping of single EcoRV restriction enzymes on non-cognate DNA. *Nuc. Acid. Res*. **36**, 4118-4127

Brotz-Oesterhelt, H., Knezevic, I., Bartel, S., Lampe, T., Warnecke-Eberz, U., Ziegelbauer, K., Habich, D. & Labischinski, H. (2003). Specific and potent inhibition of NAD+-dependent DNA ligase by pyridochromanones. *J Biol Chem* **278**, 39435-39442.

Cheng, C., Shuman, S. (1997). Characterization of an ATP-dependent DNA ligase encoded by Haemophilus influenza. *Nuc. Acid Res*. **25**, 1369-1374

Cherepanov, A.V. and de Vries, S. (2002). Dynamic mechanism of nick recognition by DNA ligase. *Eur. J. Biochem*. **269**, 5993–5999

Ciarrocchi, G., MacPhee, D. G., Deady, L. W. & Tilley, L. (1999). Specific inhibition of the eubacterial DNA ligase by arylamino compounds. *Antimicrob Agents Chemother* **4**3, 2766-2772.

Cong, P. and Shuman, S. (1993). Covalent catalysis in nucleotidyl transfer. A KTDG motif essential for enzyme-GMP complex formation by mRNA capping enzyme is conserved at the active sites of RNA and DNA ligases. *JBC*. **268**, 7256-7260

DeSantis, M.C., Li, J.L. and Wang, Y.M. (2011). Protein sliding and hopping kinetics on DNA. *Phys Rev E Stat Nonlin Soft Matter Phys*. **83**

Doherty, A.J. and Suh, S.W. (2000). Structural and mechanistic conservation in DNA ligases. *Nuc. Acid Res*. **28**, 4051-4058

Dwivedi, N., Dube, D., Pandey, J.,Singh, B., Kukshal, V., Ramachandran, R. and Tripathi, R.P. (2008). NAD(+)-dependent DNA ligase: a novel target waiting for the right inhibitor. *Med. Res. Rev*. **28**, 545-568

Fabre, F. and Roman, H. (1979). Evidence that a single DNA ligase is involved in replication and recombination in yeast. . *PNAS*. **76**, 4586-4588

Fausnaugh, J. and Shatkin, A.J. (1990). Active site localization in a viral mRNA capping enzyme. *JBC*. **265**, 7669-7672

Feng, H., Parker, J. M., Lu, J. & Cao, W. (2004). Effects of deletion and site-directed mutations on ligation steps of NAD+-dependent DNA ligase: a biochemical analysis of BRCA1 C-terminal domain. *Biochemistry*. **43**, 12648-12659.

Florescu, A.M. and Joyeux, M. (2010). Comparison of kinetic and dynamic models of DNA-protein interaction and facilitated diffusion. *J. Phys. Chem. A*. **16**, 9662-9672

Fried, M.G. and Crothers, D.M. (1984). Kinetics and mechanism in the reaction of gene regulatory proteins. *J. Mol. Biol.* **17**2, 263-282

Gefter, M. L., Becker, A. and Hurwitz, J. (1967). The enzymatic repair of DNA. I. Formation of circular Lamda-DNA. *Proc. Nat. Acad. Sci*. **58**, 240-247.

a Gellert, M. (1967). Formation of covalent circles of lamda DNA by E.coli extracts. *Proc. Nat. Acad. Sci*. **57**, 148-155

Georlette, D., Blaise, V., Dohmen, C., Bouillenne, F., Damien, B., Depiereux, E., Gerday, C., Uversky, V. N. & Feller, G. (2003). Cofactor binding modulates the conformational stabilities and unfolding patterns of NAD(+)-dependent DNA ligases from Escherichia coli and Thermus scotoductus. *J Biol Chem* **278**, 49945-49953

Georlette, D., Blaise, V., Bouillenne, F., Damien, B., Thorbjarnardottir, S. H., Depiereux, E., Gerday, C., Uversky, V. N. & Feller, G. (2004). Adenylation-dependent conformation and unfolding pathways of the NAD+-dependent DNA ligase from the thermophile Thermus scotoductus. *Biophys J.* **86**, 1089-1104.

Gottesman, M. M., Hicks, M. L. and Gellert, M. (1973). Genetics and function of DNA ligase in Escherichia coli. *J. Mol. Biol*. **15**, 531-547

Gowers, D. M., Halford, S. E., (2003). Protein motion form non-specific to specific DNA by three-dimensional routes aided by supercoiling. *EMBO* **22**, 1410-1418.

Gowers, D. M., Wilson, G. G., Halford, S. E., (2005). Measurement of the contributions of 1D and 3D pathways to the translocation of a protein along DNA. *PNAS*. **102**, 15883-15888

Halford, S. E. & Marko, J. F. (2004). How do site-specific DNA-binding proteins find their targets? *Nucleic Acids Res.* **32**, 3040-52

Hannon, R., Richards, E. G. & Gould, H. J. (1986). Facilitated diffusion of a DNA binding protein on chromatin. *EMBO J*. **5**, 3313-9.

Hu, T. and Shklovskii, B.I. (2007). How a protein searches for its specific site on DNA: the role of intersegment transfer. *Phys. Rev. E. Stat*. **76**, 051909

Ishino, Y., Shinagawa, H., Makino, K., Tsunasawa, S., Sakiyama, F. & Nakata, A. (1986). Nucleotide sequence of the lig gene and primary structure of DNA ligase of Escherichia coli. *Mol Gen Genet*. **204**, 1-7

Jack, W. E., Terry, B. J. and Modrich, P. (1982). Involvement of outside DNA sequences in the major kinetic path by which EcoRI endonuclease locates and leaves its recognition sequence. *Proc. Natl. Acad. Sci. USA.* **79**, 4010-4014

Jackson, B.R., Noble, C., Lavesa-Curto, M., Bond, P.L., Bowater, R.P. (2007). Characterization of an ATP-dependent DNA ligase from the acidophilic achaeon "Ferroplasma acidarmanus" Fer1. *Extremophiles*. **11**, 315-327

Kaczmarek, F. S., Zaniewski, R. P., Gootz, T. D., Danley, D. E., Mansour, M. N., Griffor, M., Kamath, A. V., Cronan, M., Mueller, J., Sun, D., Martin, P. K., Benton, B., McDowell, L., Biek, D. & Schmid, M. B. (2001). Cloning and functional characterization of an NAD(+)-dependent DNA ligase from Staphylococcus aureus. *J Bacterio.l* **183**, 3016-24

Kampmann, M. (2004). Obstacle bypass in protein motion along DNA by two-dimensional rather than one-dimensional sliding. *J. Biol. Chem.* **279**, 38715-20.

Kampmann, M. (2005). Facilitated diffusion in chromatin lattices: mechanistic diversity and regulatory potential. *Mol Microbiol* **57**, 889-99.

Kolomeisky, A.B. (2011). Physics of protein-DNA interactions: mechanisms of facilitated target search. *Phys Chem Chem Phys*. **13**, 2088-2095

Lahiri, S. D., Gu, R. F., Gao, N., Karantzeni I, Walkup G. K., Mills, S. D. (2012). Structure guided understanding of NAD+ recognition in bacterial DNA ligases. *ACS Chem Biol.* **7**, 571-580

Lavesa-Curto, M., Sayer, H., Bullard, D., MacDonald, A., Wilkinson, A., Smith, A., Bowater, L., Hemmings, A. & Bowater, R. P. (2004). Characterization of a temperature-sensitive DNA ligase from Escherichia coli. *Microbiology.* **150**, 4171-80

Lee, J. Y., Chang, C., Song, H. K., Moon, J., Yang, J. K., Kim, H. K., Kwon, S. T. & Suh, S. W. (2000). Crystal structure of NAD(+)-dependent DNA ligase: modular architecture and functional implications. *EMBO J*. **19**, 1119-1129

Lehman, I. R. (1974). DNA ligase: structure, mechanism, and function. *Science.* **186**, 790-797

Little, J. W., Zimmerman, S. B., Oshinsky, C. K. and Gellert, M. (1967). Enzymatic joining of DNA strands, II. An enzyme-adenylate intermediate in the dpn-dependent DNA ligase reaction. *Proc. Natl. Acad. Sci. USA*. **58**, 2004-2011

López de Saro, F.J. and O'Donnell, M. (2001). Interaction of the beta sliding clamp with MutS, ligase, and DNA polymerase I. *PNAS*. **98**, 8376-8380.

Montecucco, A., Pedrali-Noy, G., Spadari, S. & Ciarrocchi, G. (1988). Multiple roles of DNA ligase at the replication fork. *Biochim Biophys Acta* **951**, 330-334.

Mossi, R., Ferrari, E., and Hübscher, U. (1998). DNA ligase I selectively affects DNA synthesis by DNA polymerases delta and epsilon suggesting differential functions in DNA replication and repair. *JBC.* **273**, 14322-14330

Murugan, R., (2004). DNA-protein interactions under random jump conditions. *Phys. Ref.* **69**, 011911

Murzin, A. G. (1993). OB (oligonucleotide/oligosaccharide binding)-fold: common structural and functional solution for non-homologous sequences. *Embo J*. **12**, 861-867

Nandakumar, J., Nair,P.A. and Shuman, S. (2007). Last stop on road to repair: structure of E. coli DNA ligase bound to nicked DNA-adenylate. *Mol. Cell*. **26**, 257-271

Odell, M., Sriskanda, V., Shuman , S., Nikolov, D.B. (2000) Crystal structure of eukaryotic DNA ligase-adenylate illuminates the mechanism of nick sensing and strand joining. *Mol Cell.* **6**, 1183-1193

a Olivera, B. M., and Lehman, I. R. (1967). Linkage of polynucleotides through phosphodiester bonds by an enzyme from *Escherichia coli*. *Proc. Nat. Acad. Sci*. **57**, 1426-1433

b Olivera, B. M. and Lehman, I. R. (1967). Diphosphopyridine nucleotide: a cofactor for the polynucleotide-joining enzyme from Escherichia coli. *Proc. Natl. Acad. Sci. USA*. **57**, 1700-1704

Olivera, B. M., Hall, Z. W., Anraku, Y., Chien, J. R. and Lehman, I. R. (1968). On the mechanism of the polynucleotide joining reaction. *Cold Spring Harb. Symp. Quant. Biol*. **33**, 27-31

Pascal, J.M., O'Brien, P.J., Tomkinson, A.E., and Ellenberger, T. (2004). Human DNA ligase I completely encircles and partially unwindsnicked DNA. *Nature*. **432**, 473–478

Petrova, T., Bezsudnova, E.Y., Boyko, K.M., Mardanov, A.V., Polyakov, K.M., Volkov, V.V., Kozin, M., Ravin, N.V., Shabalin, I.G., Skryabin, K..G, Stekhanova, T.N, Kovalchuk, M.V., Popov, V.O. ATP-dependent DNA ligase from Thermococcus sp. 1519 displays a new arrangement of the

OB-fold domain. (2012). *Acta Crystallogr Sect F Struct Biol Cryst Commun*. **68**, 1440-1447

Porecha, R.H. and Stivers, J.T. (2008) Uracil DNA glycosylase uses DNA hopping and short-range sliding to trap extrahelical uracils. *PNAS*. **105**, 10791-10796

Rau, D.C. and Sidorova, N.Y. (2010). Diffusion on the restriction enzyme EcoRI along DNA. *J. Mol. Biol*. **395**, 408-416

Samai P**.** and Shuman S. (2011). Functional dissection of the DNA interface of the nucleotidyltransferase domain of chlorella virus DNA ligase. *J Biol Chem.* **286**, 13314-13326

Sambrook, J.& Russsell, D.W. (2001) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Lab. Press, Woodbury, NY).

Shimamoto, N. (1999). One dimensional diffusion of proteins along DNA. Its biological and chemical significance revealed by single-molecule measurements. *J. Biol. Chem*. **274**, 15293-15296

Shuman, S. & Lima, C. D. (2004). The polynucleotide ligase and RNA capping enzyme superfamily of covalent nucleotidyltransferases. *Curr. Opin. Struct. Biol*. **14**, 757-764

Shuman, S., and Benarroch, D. (2006). Characterization of mimivirus NAD+-dependent DNA ligase. *Virology*. **353**, 133-143

Singleton, M. R., Hakansson, K., Timson, D. J. & Wigley, D. B. (1999). Structure of the adenylation domain of an NAD+-dependent DNA ligase. *Structure.* **7**, 35-42

a Sriskanda, V. & Shuman, S. (2002). Conserved residues in domain Ia are required for the reaction of Escherichia coli DNA ligase with NAD+. *J Biol Chem* **277**, 9695-9700.

b Sriskanda, V. & Shuman, S. (2002). Role of nucleotidyl transferase motif V in strand joining by chlorella virus DNA ligase. *J Biol Chem* **277**, 9661-9667.

Söderhäll, S. (1975). Properties of a DNA-adenylate complex formed in the reaction between mammalian DNA ligase I and DNA containing single-strand breaks. *Eur. J. Biochem*. **57**, 129-136

Stanford, N.P., Szczelkun, M.D., Marko, J.F. and Halford, S.E. (2000). One- and three-dimensional pathways for proteins to reach specific DNA sites. *EMBO*. **19**, 6546-6557

Terry, B J., Jack, W.E. and Modrich, P. (1985). Facilitated diffusion during catalysis by EcoRI endonuclease. Nonspecific interactions in EcoRI catalysis. *J Biol Chem.* **260**,13130-13137.

Wei, Y.F., Robins, P., Carter, K., Caldecott, K., Pappin, D.J., Yu, G.L., Wang, R.P., Shell, B.K., Nash, R.A., Schär, P., Barnes, D.E., Haseltine, W.A., and Lindahl, T. (1995). Molecular cloning and expression of human cDNAs encoding a novel DNA ligase IV and DNA ligase III, and enzyme active in DNA repair and recombination. *Mol. Cell. Biol*. **15**, 3206-2316

Song, W., Pascal, J.M., Ellenberger, T. and Tomkinson, A.E. (2009). The DNA binding domain of human DNA ligase I interacts with both nicked DNA and the DNA sliding clamps, PCNA and hRad9-hRad1-hHus1. *DNA Repair*. **8**, 912–919

Wilkinson, A., Sayer, H., Bullard, D., Smith, A., Day, J., Kieser, T. & Bowater, R. (2003). NAD+-dependent DNA ligases of Mycobacterium tuberculosis and Streptomyces coelicolor. Proteins **51**, 321-326.

Winter, R. B., Berg, O. G. & von Hippel, P. H. (1981). Diffusion-driven mechanisms of protein translocation on nucleic acids. 3. The Escherichia coli lac repressor--operator interaction: kinetic measurements and conclusions. *Biochemistry* **20**, 6961-77

Wilkinson, A., Smith, A., Bullard, D., Lavesa-Curto, M., Sayer, H., Bonner, A., Hemmings, A. & Bowater, R. (2005). Analysis of ligation and DNA binding by Escherichia coli DNA ligase (LigA). *Biochim Biophys Acta* **1749**, 113-122

von Hippel, P. H. & Berg, O. G. (1989). Facilitated target location in biological systems. *J Biol Chem* **264**, 675-8

Zimmerman, S. B., Little, J. W., Oshinsky, C. K., and Gellert, M. (1967). Enzymatic joining of DNA strands: A novel reaction of diphosphopyridine nucleotide. *PNAS*. **57**, 1841-1848.

Zhu, H. & Shuman, S. (2005). Structure-guided mutational analysis of the nucleotidyltransferase domain of Escherichia coli NAD+-dependent DNA ligase (LigA). *J. Biol. Chem*. **280**, 12137-12144

Appendices

Appendix I

DNA sequence of plasmid pRB20 (pET-16b + EcLigA) for LigaseA expression in *E.coli*. From Dr Richard Bowater, UEA. Cloned and sequenced in 2000.

```
TTCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCT
TAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATT
CAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTAT
GAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCAC
CCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGG
ATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAA
AGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACAC
TATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAA
GAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGG
AGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAA
CCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGT
TGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGC
GGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGA
GCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAG
TTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTC
ACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCAT
TTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGT
TTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCG
CGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTA
CCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGC
CGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACC
AGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAG
GCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAAC
TGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCC
GGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTAT
AGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCC
TATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTT
CTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGC
CGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTC
TCCTTACGCATCTGTGCGGTATTTCACACCGCATATATGGTGCACTCTCAGTACAATCTGCTCTGATGCC
GCATAGTTAAGCCAGTATACACTCCGCTATCGCTACGTGACTGGGTCATGGCTGCGCCCCGACACCCGCC
AACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTC
TCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGAGGCAGCTGCGGTAAAGCT
CATCAGCGTGGTCGTGAAGCGATTCACAGATGTCTGCCTGTTCATCCGCGTCCAGCTCGTTGAGTTTCTC
CAGAAGCGTTAATGTCTGGCTTCTGATAAAGCGGGCCATGTTAAGGGCGGTTTTTTCCTGTTTGGTCACT
GATGCCTCCGTGTAAGGGGGATTTCTGTTCATGGGGGTAATGATACCGATGAAACGAGAGAGGATGCTCA
CGATACGGGTTACTGATGATGAACATGCCCGGTTACTGGAACGTTGTGAGGGTAAACAACTGGCGGTATG
GATGCGGCGGGACCAGAGAAAAATCACTCAGGGTCAATGCCAGCGCTTCGTTAATACAGATGTAGGTGTT
CCACAGGGTAGCCAGCAGCATCCTGCGATGCAGATCCGGAACATAATGGTGCAGGGCGCTGACTTCCGCG
TTTCCAGACTTTACGAAACACGGAAACCGAAGACCATTCATGTTGTTGCTCAGGTCGCAGACGTTTTGCA
GCAGCAGTCGCTTCACGTTCGCTCGCGTATCGGTGATTCATTCTGCTAACCAGTAAGGCAACCCCGCCAG
CCTAGCCGGGTCCTCAACGACAGGAGCACGATCATGCGCACCCGTGGCCAGGACCCAACGCTGCCCGAGA
TGCGCCGCGTGCGGCTGCTGGAGATGGCGGACGCGATGGATATGTTCTGCCAAGGGTTGGTTTGCGCATT
CACAGTTCTCCGCAAGAATTGATTGGCTCCAATTCTTGGAGTGGTGAATCCGTTAGCGAGGTGCCGCCGG
CTTCCATTCAGGTCGAGGTGGCCCGGCTCCATGCACCGCGACGCAACGCGGGGAGGCAGACAAGGTATAG
GGCGGCGCCTACAATCCATGCCAACCCGTTCCATGTGCTCGCCGAGGCGGCATAAATCGCCGTGACGATC
AGCGGTCCAGTGATCGAAGTTAGGCTGGTAAGAGCCGCGAGCGATCCTTGAAGCTGTCCCTGATGGTCGT
CATCTACCTGCCTGGACAGCATGGCCTGCAACGCGGGCATCCCGATGCCGCCGGAAGCGAGAAGAATCAT
AATGGGGAAGGCCATCCAGCCTCGCGTCGCGAACGCCAGCAAGACGTAGCCCAGCGCGTCGGCCGCCATG
CCGGCGATAATGGCCTGCTTCTCGCCGAAACGTTTGGTGGCGGGACCAGTGACGAAGGCTTGAGCGAGGG
```

CGTGCAAGATTCCGAATACCGCAAGCGACAGGCCGATCATCGTCGCGCTCCAGCGAAAGCGGTCCTCGCC
GAAAATGACCCAGAGCGCTGCCGGCACCTGTCCTACGAGTTGCATGATAAAGAAGACAGTCATAAGTGCG
GCGACGATAGTCATGCCCCGCGCCCACCGGAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGGCATCGGTC
GAGATCCCGGTGCCTAATGAGTGAGCTAACTTACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTC
GGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGG
CGCCAGGGTGGTTTTTCTTTTCACCAGTGAGACGGGCAACAGCTGATTGCCCTTCACCGCCTGGCCCTGA
GAGAGTTGCAGCAAGCGGTCCACGCTGGTTTGCCCCAGCAGGCGAAAATCCTGTTTGATGGTGGTTAACG
GCGGGATATAACATGAGCTGTCTTCGGTATCGTCGTATCCCACTACCGAGATATCCGCACCAACGCGCAG
CCCGGACTCGGTAATGGCGCGCATTGCGCCCAGCGCCATCTGATCGTTGGCAACCAGCATCGCAGTGGGA
ACGATGCCCTCATTCAGCATTTGCATGGTTTGTTGAAAACCGGACATGGCACTCCAGTCGCCTTCCCGTT
CCGCTATCGGCTGAATTTGATTGCGAGTGAGATATTTATGCCAGCCAGCCAGACGCAGACGCGCCGAGAC
AGAACTTAATGGGCCCGCTAACAGCGCGATTTGCTGGTGACCCAATGCGACCAGATGCTCCACGCCCAGT
CGCGTACCGTCTTCATGGGAGAAAATAATACTGTTGATGGGTGTCTGGTCAGAGACATCAAGAAATAACG
CCGGAACATTAGTGCAGGCAGCTTCCACAGCAATGGCATCCTGGTCATCCAGCGGATAGTTAATGATCAG
CCCACTGACGCGTTGCGCGAGAAGATTGTGCACCGCCGCTTTACAGGCTTCGACGCCGCTTCGTTCTACC
ATCGACACCACCACGCTGGCACCCAGTTGATCGGCGCGAGATTTAATCGCCGCGACAATTTGCGACGGCG
CGTGCAGGGCCAGACTGGAGGTGGCAACGCCAATCAGCAACGACTGTTTGCCCGCCAGTTGTTGTGCCAC
GCGGTTGGGAATGTAATTCAGCTCCGCCATCGCCGCTTCCACTTTTTCCCGCGTTTTCGCAGAAACGTGG
CTGGCCTGGTTCACCACGCGGGAAACGGTCTGATAAGAGACACCGGCATACTCTGCGACATCGTATAACG
TTACTGGTTTCACATTCACCACCCTGAATTGACTCTCTTCCGGGCGCTATCATGCCATACCGCGAAAGGT
TTTGCGCCATTCGATGGTGTCCGGGATCTCGACGCTCTCCCTTATGCGACTCCTGCATTAGGAAGCAGCC
CAGTAGTAGGTTGAGGCCGTTGAGCACCGCCGCCGCAAGGAATGGTGCATGCAAGGAGATGGCGCCCAAC
AGTCCCCCGGCCACGGGGCCTGCCACCATACCCACGCCGAAACAAGCGCTCATGAGCCCGAAGTGGCGAG
CCCGATCTTCCCCATCGGTGATGTCGGCGATATAGGCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCC
GGCCACGATGCGTCCGGCGTAGAGGATCGAGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGG
AATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGG
GCCATCATCATCATCATCATCATCATCATCACAGCAGCGGCCATATCGAAGGTCGTCATATGGAATCAAT
CGAACAACAACTGACAGAACTGCGAACGACGCTTCGCCATCATGAATATCTTTATCATGTGATGGATGCG
CCGGAAATTCCCGACGCTGAATACGACAGGCTGATGCGCGAACTGCGCGAGCTGGAAACCAAACATCCAG
AACTGATTACGCCTGATTCGCCTACTCAACGTGTAGGCGCTGCGCCGCTGGCGGCTTTCAGCCAGATACG
CCATGAAGTACCAATGCTGTCACTGGATAACGTTTTTGATGAAGAAAGCTTTCTTGCTTTCAACAAACGT
GTGCAGGACCGTCTGAAAAACAACGAGAAAGTCACCTGGTGCTGTGAGCTGAAGCTGGATGGTCTTGCCG
TCAGTATTCTGTATGAAAATGGCGTTTTAGTCAGTGCCGCGACCCGTGGCGATGGCACCACCGGGGAAGA
TATCACGTCTAATGTGCGTACTATTCGCGCCATTCCGCTGAAGCTGCACGGAGAGAATATCCCGGCGCGT
CTGGAAGTGCGTGGTGAAGTGTTCCTGCCGCAGGCGGGGTTCGAAAAGATTAACGAAGATGCGCGACGCA
CGGGCGGGAAAGTGTTTGCTAACCCACGTAATGCGGCAGCTGGTTCACTGCGTCAGCTTGATCCGCGTAT
TACAGCGAAGCGACCGCTCACTTTTTTCTGCTATGGCGTTGGTGTTCTGGAAGGTGGCGAGCTGCCGGAT
ACTCATCTTGGCCGTTTACTGCAATTTAAAAAGTGGGGGTTGCCGGTCAGCGATCGGGTAACGCTTTGTG
AATCGGCGGAAGAAGTGCTGGCGTTCTATCACAAAGTGGAAGAAGACCGCCCGACGCTGGGCTTTGATAT
CGACGGCGTGGTGATTAAGGTCAACTCACTGGCACAGCAGGAGCAGCTTGGCTTTGTCGCGCGTGCCCCG
CGCTGGGCGGTAGCGTTTAAATTCCCGGCGCAGGAGCAGATGACCTTTGTGCGTGACGTCGAGTTTCAGG
TTGGGCGTACTGGCGCGATTACGCCTGTTGCGCGTCTGGAACCTGTCCATGTTGCAGGCGTGCTGGTGAG
TAACGCAACCTTACACAATGCGGATGAAATCGAACGTCTTGGTTTACGCATTGGCGATAAAGTGGTGATT
CGCCGCGCTGGCGACGTGATCCCGCAGGTGGTTAACGTCGTGCTTTCTGAACGCCCGGAAGATACCCGTG
AGGTTGTATTCCCGACGCATTGTCCGGTATGTGGTTCTGACGTTGAGCGTGTGGAAGGTGAAGCGGTTGC
CCGCTGTACCGGTGGCCTGATTTGCGGTGCGCAGCGTAAAGAGTCGCTGAAACACTTTGTTTCCCGCCGT
GCGATGGATGTTGACGGAATGGGCGACAAAATCATCGATCAGCTGGTTGAAAAAGAATATGTCCATACTC
CGGCAGATCTGTTCAAACTCACCGCAGGCAAACTGACCGGACTGGAGCGTATGGGGCCAAAATCGGCACA
AAACGTGGTTAACGCGCTGGAAAAAGCGAAAGAAACCACCTTTGCTCGCTTCCTCTATGCACTTGGCATC
CGTGAAGTCGGCGAGGCCACCGCAGCAGGTCTGGCGGCATATTTCGGCACGCTGGAAGCGCTGGAAGCCG
CTTCGATTGAAGAGCTGCAAAAGGTGCCTGATGTTGGCATTGTCGTTGCATCCCACGTTCACAACTTCTT
TGCCGAAGAAAGCAACCGCAATGTCATCAGCGAGCTGTTGGCGGAAGGTGTTCACTGGCCTGCGCCGATC
GTTATCAACGCGGAAGAGATTGACAGCCCGTTTGCTGGTAAAACCGTGGTGCTTACGGGCAGCTTAAGCC
AGATGTCGCGTGATGACGCTAAAGCTCGACTGGTCGAACTGGGCGCGAAAGTCGCGGGCAGCGTGTCGAA
GAAAACCGATCTGGTGATAGCGGGTGAAGCTGCAGGATCTAAACTGGCGAAGGCGCAGGAACTGGGCATT
GAAGTCATCGACGAAGCGGAAATGCTGCGTTTGCTGGGTAGCTGAGGATCCGGCTGCTAACAAAGCCCGA
AAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGG
TCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGATATCCCGCAAGAGGCCCGGCAGTACCGGC
ATAACCAAGCCTATGCCTACAGCATCCAGGGTGACGGTGCCGAGGATGACGATGAGCGCATTGTTAGATT
TCATACACGGTGCCTGACTGCGTTAGCAATTTAACTGTGATAAACTACCGCATTAAAGCTTATCGATGAT
AAGCTGTCAAACATGAGAA

Purple -	**ribosome binding site (rbs)**
Orange -	**ATG starting methionine codon**
Red -	**deca-histidine seq**
Blue -	**IEGR Factor Xa site**
Green -	**LigA seq (2016 nucleotides)**
Pink -	**TGA stop codon (opal)**

Appendix II

Plasmid pL1

Only the top strand is shown, starting at the 5'-end.
The single BbvCI site is shown underlined in blue.
Length of supercoiled circular plasmid = 2707 bp.

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCT
GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGG
CTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGAT
GCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATC
GGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTA
ACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTTGCAT**CCTCAGC**TGACTAAT
TCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCGTAATCATGGTCAT
AGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTG
TAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAG
TCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTG
GGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGC
TCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAA
GGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTG
ACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGC
GTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCC
TTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCG
TTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTA
TCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGC
AGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGA
CAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGG
CAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGA
TCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGA
TTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATC
AATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCA
GCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGG
GCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGC
AATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCT
ATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTG
CTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAG
GCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGA
AGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCAT
CCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACC
GAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATC
ATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAAC
CCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGG
AAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTT
CAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAA
ATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTAT
CATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTC

Plasmid pL2

Only the top strand is shown, starting at the 5'-end.

The two BbvCI sites are shown underlined in blue.
Length of supercoiled circular plasmid = 2728 bp.

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCT
GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGG
CTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGAT
GCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATC
GGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTA
ACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTTGCAT**CCTCAGC**TGACTAAC
GTGCAT**CCTCAGC**TGACTAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAA
GCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACAT
ACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTG
CGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGG
GGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCG
GCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCA
GGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTT
TCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGAC
AGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCG
CTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGT
ATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCG
CTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCA
GCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTA
ACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAG
AGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAG
ATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGA
ACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAA
TTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTA
ATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGT
AGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTC
ACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACT
TTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTT
TGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAG
CTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTC
GGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATA
ATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTG
AGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGC
AGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGT
TGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGT
TTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGA
ATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACA
TATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGA
CGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTC

Plasmid pL4

Only the top strand is shown, starting at the 5’-end.
The two BbvCI sites are shown underlined in blue.
Length of supercoiled circular plasmid = 2731 bp.

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCT
GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGG
CTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGAT
GCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATC
GGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTA
ACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTTGCAT**CCTCAGC**TGACTAAT
TCGAGCTACGTGCAT**CCTCAGC**TGACTAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATG
CAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAA
CATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCG
TTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCG
CGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGT
TCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAAC
GCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGT

TTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCC
GACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTG
CCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTA
GGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGA
CCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCA
GCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGC
CTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAA
AAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAG
CAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGT
GGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTT
AAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGC
TTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCG
TGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACG
CTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCA
ACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATA
GTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATT
CAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCC
TTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGC
ATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATT
CTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACAT
AGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGC
TGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAG
CGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGT
TGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGAT
ACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACC
TGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGT
C

Plasmid pL5

Only the top strand is shown, starting at the 5’-end.
The two BbvCI sites are shown underlined in blue.
Length of supercoiled circular plasmid = 2731 bp.

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCT
GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGG
CTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGAT
GCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATC
GGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTA
ACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTTGCAT**CCTCAGC**TGACTAAT
TCGAGCTAGTCA**GCTGAGG**ATGCACGTAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATG
CAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAA
CATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCG
TTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCG
CGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGT
TCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAAC
GCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGT
TTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCC
GACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTG
CCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTA
GGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGA
CCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCA
GCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGC
CTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAA
AAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAG
CAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGT
GGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTT
AAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGC
TTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCG
TGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACG
CTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCA
ACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATA

GTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATT
CAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCC
TTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGC
ATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATT
CTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACAT
AGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGC
TGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAG
CGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGT
TGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGAT
ACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACC
TGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGT
C

Plasmid pL6

Only the top strand is shown, starting at the 5’-end.
The two BbvCI sites are shown underlined in blue.
Length of supercoiled circular plasmid = 2731 bp.

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCT
GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGG
CTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGAT
GCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATC
GGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTA
ACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTTGCAT**CCTCAGC**TGACTAAT
TCGAGCTCGGTACACGTGCAT**CCTCAGC**TGACTGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATG
CAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAA
CATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCG
TTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCG
CGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGT
TCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAAC
GCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGT
TTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCC
GACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTG
CCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTA
GGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGA
CCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCA
GCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGC
CTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAA
AAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAG
CAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGT
GGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTT
AAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGC
TTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCG
TGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACG
CTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCA
ACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATA
GTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATT
CAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCC
TTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGC
ATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATT
CTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACAT
AGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGC
TGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAG
CGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGT
TGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGAT
ACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACC
TGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGT
C

Plasmid pL7

Only the top strand is shown, starting at the 5′-end.
The two BbvCI sites are shown underlined in blue.
Length of supercoiled circular plasmid = 2731 bp.

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCT
GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGG
CTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGAT
GCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATC
GGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTA
ACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTTGCAT**CCTCAGC**TGACTAAT
TCGAGCTCGGTACAGTCA**GCTGAGG**ATGCACGTGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATG
CAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAA
CATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCG
TTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCG
CGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGT
TCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAAC
GCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGT
TTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCC
GACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTG
CCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTA
GGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGA
CCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCA
GCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGC
CTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAA
AAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAG
CAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGT
GGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTT
AAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGC
TTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCG
TGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACG
CTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCA
ACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATA
GTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATT
CAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCC
TTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGC
ATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATT
CTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACAT
AGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGC
TGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAG
CGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGT
TGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGAT
ACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACC
TGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGT
C

Plasmid pL8

Only the top strand is shown, starting at the 5′-end.
The two BbvCI sites are shown underlined in blue.
Length of supercoiled circular plasmid = 2731 bp.

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCT
GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGG
CTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGAT
GCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATC
GGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTA
ACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTTGCAT**CCTCAGC**TGACTAAT
TCGAGCTCGGTACCCGGACGTGCAT**CCTCAGC**TGACTCCGGGGATCCTCTAGAGTCGACCTGCAGGCATG
CAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAA
CATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCG
TTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCG

CGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGT
TCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAAC
GCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGT
TTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCC
GACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTG
CCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTA
GGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGA
CCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCA
GCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGC
CTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAA
AAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAG
CAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGT
GGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTT
AAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGC
TTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCG
TGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACG
CTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCA
ACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATA
GTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATT
CAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCC
TTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGC
ATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATT
CTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACAT
AGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGC
TGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAG
CGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGT
TGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGAT
ACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACC
TGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGT
C

Plasmid pL9

Only the top strand is shown, starting at the 5′-end.
The two BbvCI sites are shown underlined in blue.
Length of supercoiled circular plasmid = 2731 bp.

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCT
GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGG
CTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGAT
GCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATC
GGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTA
ACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTTGCAT**CCTCAGC**TGACTAAT
TCGAGCTCGGTACCCGGAGTCA**GCTGAGG**ATGCACGTCCGGGGATCCTCTAGAGTCGACCTGCAGGCATG
CAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAA
CATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCG
TTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCG
CGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGT
TCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAAC
GCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGT
TTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCC
GACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTG
CCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTA
GGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGA
CCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCA
GCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGC
CTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAA
AAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAG
CAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGT
GGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTT
AAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGC
TTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCG

TGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACG
CTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCA
ACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATA
GTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATT
CAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCC
TTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGC
ATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATT
CTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACAT
AGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGC
TGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAG
CGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGT
TGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGAT
ACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACC
TGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGT
C

Plasmid pL10

Only the top strand is shown, starting at the 5’-end.
The two BbvCI sites are shown underlined in blue.
Length of supercoiled circular plasmid = 2731 bp.

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCT
GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGG
CTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGAT
GCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATC
GGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTA
ACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTTGCAT**CCTCAGC**TGACTAAT
TCGAGCTCGGTACCCGGGGATCACGTGCAT**CCTCAGC**TGACTGATCCTCTAGAGTCGACCTGCAGGCATG
CAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAA
CATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCG
TTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCG
CGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGT
TCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAAC
GCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGT
TTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCC
GACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTG
CCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTA
GGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGA
CCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCA
GCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGC
CTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAA
AAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAG
CAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGT
GGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTT
AAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGC
TTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCG
TGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACG
CTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCA
ACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATA
GTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATT
CAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCC
TTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGC
ATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATT
CTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACAT
AGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGC
TGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAG
CGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGT
TGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGAT
ACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACC
TGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGT
C

Plasmid pL11

Only the top strand is shown, starting at the 5’-end.
The two BbvCI sites are shown underlined in blue.
Length of supercoiled circular plasmid = 2731 bp.

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCT
GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGG
CTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGAT
GCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATC
GGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTA
ACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTTGCAT**CCTCAGC**TGACTAAT
TCGAGCTCGGTACCCGGGGATCAGTCA**GCTGAGG**ATGCACGTGATCCTCTAGAGTCGACCTGCAGGCATG
CAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAA
CATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCG
TTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCG
CGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGT
TCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAAC
GCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGT
TTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCC
GACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTG
CCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTA
GGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGA
CCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCA
GCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGC
CTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAA
AAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAG
CAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGT
GGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTT
AAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGC
TTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCG
TGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACG
CTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCA
ACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATA
GTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATT
CAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCC
TTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGC
ATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATT
CTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACAT
AGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGC
TGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAG
CGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGT
TGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGAT
ACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACC
TGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGT
C

Plasmid pL12

Only the top strand is shown, starting at the 5’-end.
The two BbvCI sites are shown underlined in blue.
Length of supercoiled circular plasmid = 2731 bp.

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCT
GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGG
CTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGAT
GCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATC
GGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTA
ACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTTGCAT**CCTCAGC**TGACTAAT
TCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTACGTGCAT**CCTCAGC**TGA
CTAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAA

CATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCG
TTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCG
CGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGT
TCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAAC
GCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGT
TTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCC
GACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTG
CCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTA
GGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGA
CCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCA
GCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGC
CTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAA
AAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAG
CAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGT
GGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTT
AAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGC
TTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCG
TGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACG
CTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCA
ACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATA
GTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATT
CAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCC
TTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGC
ATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATT
CTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACAT
AGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGC
TGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAG
CGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGT
TGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGAT
ACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACC
TGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGT
C

Plasmid pL13

Only the top strand is shown, starting at the 5’-end.
The two BbvCI sites are shown underlined in blue.
Length of supercoiled circular plasmid = 2731 bp.

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCT
GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGG
CTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGAT
GCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATC
GGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTA
ACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTTGCAT**CCTCAGC**TGACTAAT
TCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTAGTCA**GCTGAGG**ATGCAC
GTAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAA
CATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCG
TTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCG
CGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGT
TCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAAC
GCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGT
TTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCC
GACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTG
CCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTA
GGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGA
CCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCA
GCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGC
CTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAA
AAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAG
CAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGT
GGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTT

AAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGC
TTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCG
TGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACG
CTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCA
ACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATA
GTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATT
CAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCC
TTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGC
ATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATT
CTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACAT
AGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGC
TGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAG
CGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGT
TGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGAT
ACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACC
TGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGT
C