University of Helsinki: Doctoral Programme In Biomedicine

Practical Molecular Biology and Genetic Engineering Course (Practical Part)

Project 3: Creation of DNA ladder (page 1 of 2)

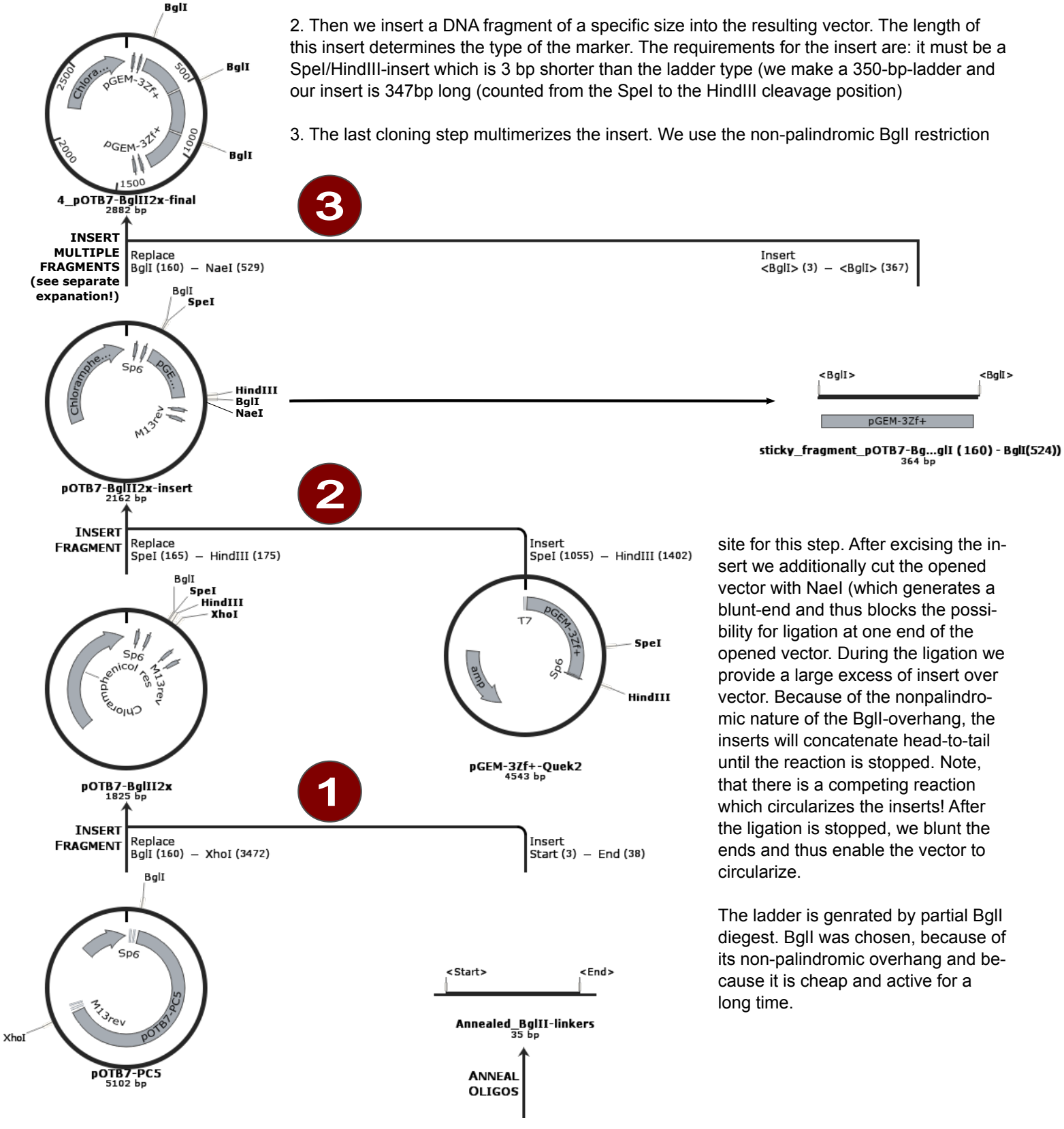
In this project, we want to generate a plasmid, from which we can produce a DNA ladder. The idea is to replace commercial DNA ladders and thus to save reagent costs. Different approaches for the generation of DNA ladders were contemplated (PCR-based-generation versus complete restriction enzyme digest versus partial restriction enzyme digest), and the partial enzyme digest of a designed plasmid was chosen for execution due to its low costs and the possibility to generate multiple types of ladders (100-bp-ladder, 1-kb-ladder, etc.). During this course we implemented a 350-bp-ladder with the fragment sizes 350bp, 700bp, 1050bp, etc. The cloning plan consists of 3 steps:

1. We modify the multiple cloning site of a vector by inserting a short, synthetic linker (made by annealing two oligonucleotides)

2. Then we insert a DNA fragment of a specific size into the resulting vector. The length of this insert determines the type of the marker. The requirements for the insert are: it must be a SpeI/HindIII-insert which is 3 bp shorter than the ladder type (we make a 350-bp-ladder and our insert is 347bp long (counted from the SpeI to the HindIII cleavage position)

3. The last cloning step multimerizes the insert. We use the non-palindromic BglI restriction site for this step. After excising the insert we additionally cut the opened vector with NaeI (which generates a blunt-end and thus blocks the possibility for ligation at one end of the opened vector. During the ligation we provide a large excess of insert over vector. Because of the nonpalindromic nature of the BglI-overhang, the inserts will concatenate head-to-tail until the reaction is stopped. Note, that there is a competing reaction which circularizes the inserts! After the ligation is stopped, we blunt the ends and thus enable the vector to circularize.

The ladder is genrated by partial BglI diegest. BglI was chosen, because of its non-palindromic overhang and because it is cheap and active for a long time.



3rd cloning step: forced concatenation of multiple inserts during ligation