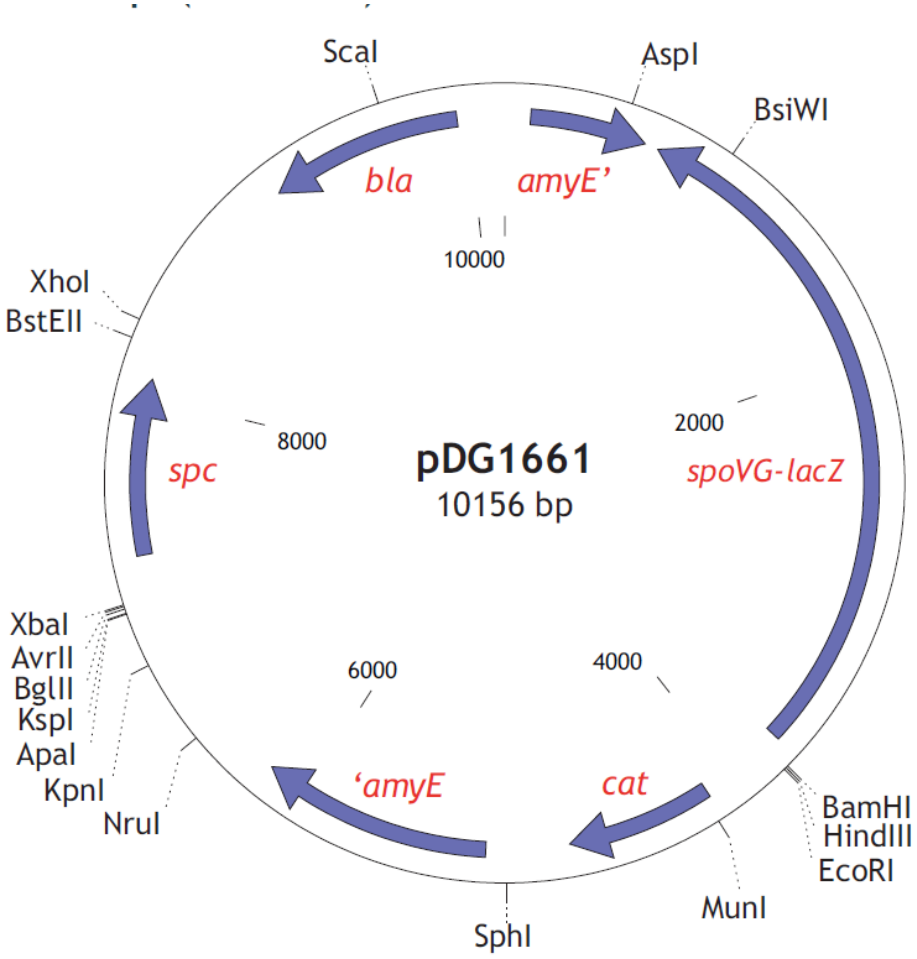
Assembly:

To fully constructed our engineered B. Hercules and execute anti-colon tumor function, parts from different modules need to be assembled.

To assembly the three modules together and transform our construct into B. subtilis, an integration plasmid, pDG1661, from BGSC was employed. There are several reasons why we choose to use this vector.

1. Plasmid instability in B. subtilis: Plasmid instability is a phenomenon observed with recombinant DNA in Gram-positive hosts. Frequently occurred structure alterations and loss of plasmids prompt the study in integration vectors. In our project, we utilize an integration plasmid. The whole construct are inserted into its multiple cloning site and integrated into the B. subtilis genome through homologous recombination. This integration can help stabilize the recombinant DNA and generate relatively genetically stable bacteria.
2. Biosafety: In consideration of possible horizontal gene transfer between bacteria within normal flora in gut, integration plasmid is decided to use in order to reduce the chance of plasmid loss and antibiotic resistant gene spreading in gut.



pDG1661, the integration vector we used in our project facilitate the integration through the 5' and 3' segment of B. subtilis amyE gene franking at the two end of multiple cloning site. A cat gene encoding chloramphenicol acetyl tranferase is located within the integration area for B. subtilis transformation selection. Containing only a replication origin for E coli, this vector needs

to be replicated in E coli first and selected through ampicillin resistance obtaining from the bla gene on this vector. After replication in E coli, the recombinant DNA plasmicd is transformed into B. subtilis and only the one with plasmid integration can survived under chloramphenicol selection, forming colony. The integration can further verified through the starch test. As shown in figure (…), the one without pDG1661 integrated can use starch and surrounded by a white halo after the overnight incubation on LB plate supplemented with starch when gram iodine is adding the next morning. However, since the integration will split amyE gene, the defective B. subtilis can no longer use starch, no white halo can be observed after iodine adding.

The final recombinant DNA plasmid we get is shown in figure (……). Driven by pVeg promoter, RPMrel peptide can be highly expressed and displayed on cell wall after fusing with lytC displaying system. Two protein coding genes, bmp2 and toxin ydcE is under the control of xylose inducible promoter so that after binding, the induction of xylose can trigger the expression toxin and anti-tumor chemokine at the same time. pTms with antitoxin ydcD is then inserted into the same vector as well in order to provide a threshold BMP2 production through its counteracting with toxin ydcE.

Since pDG1661 is not a standard bio-brick, which contains one xbaI cutting site and three pstI cutting site outside its MCS, we utilize vector pBluescript II KS+ to add BamHI cutting site after pstI as shown in figure (…) and inserting all our construct through EcoRI and BamHI site.