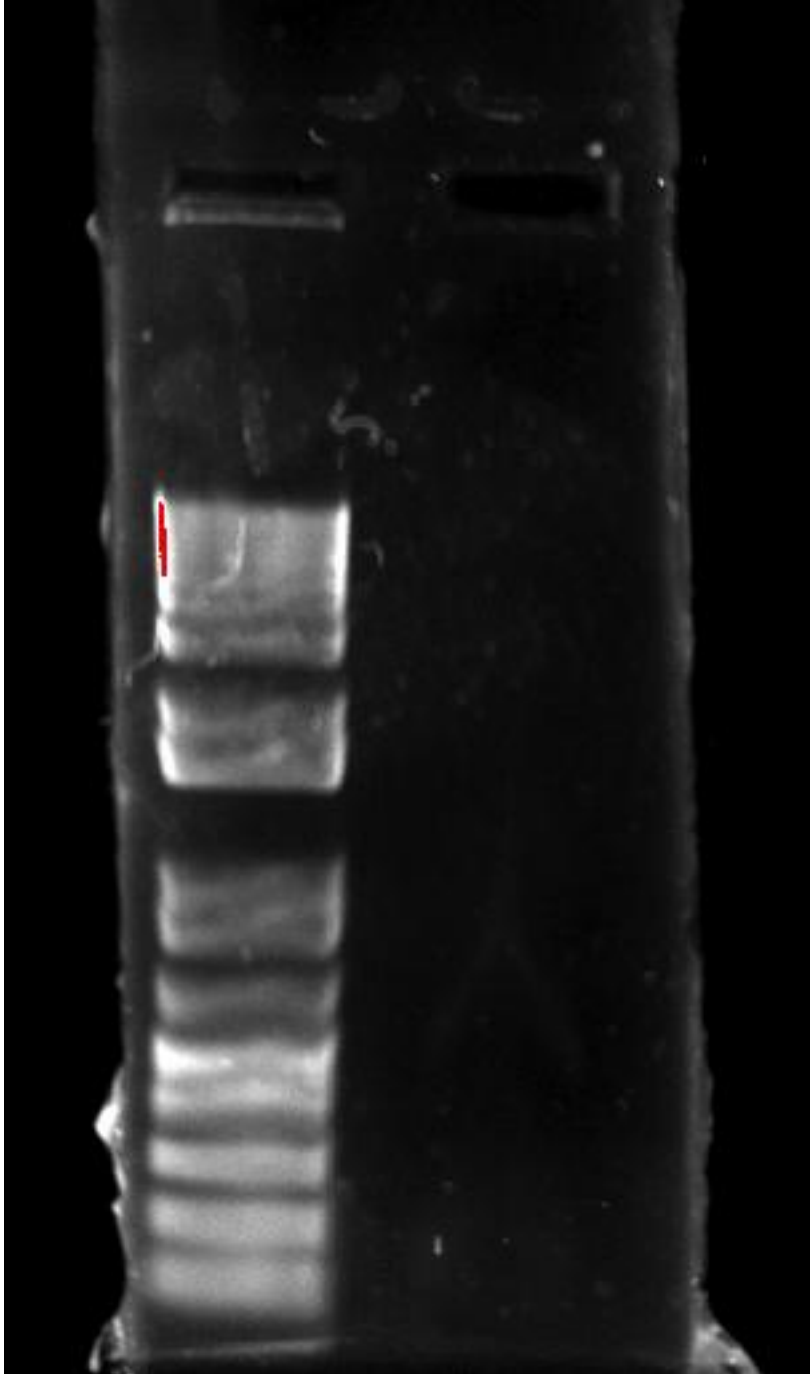


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- This might be why colony PCR using colonies from previous 2 tries might not work:

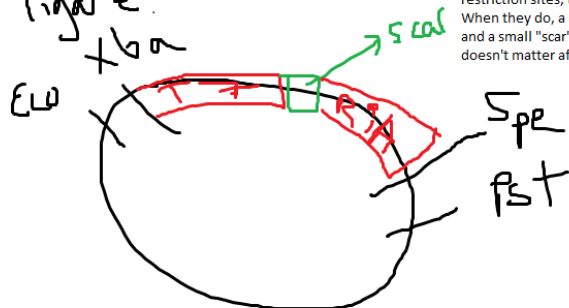


Analysis: lack of clear band at 600bp indicates insufficient amount of insert.  
Also, these instructions might have been incorrect:



Legend:  
 - Yellow is where you make a cut  
 - Green is scar  
 - Red is the "part"  
 - The linear RiA is from PCR of a plasmid following DpnI digestion  
 - Xba and Spe can stick together even though they are different. When they do this eliminates the restriction site (unlike when Pst and Pst stick together, giving you Pst restriction site).

after ligate:



Even though Xba and Spe are different restriction sites, they still can "stick together". When they do, a restriction site is eliminated and a small "scar" sequence results. But this doesn't matter after a promoter.

- Analysis of all possible digest sites for everything relevant –see T7 file