COLONY PCR pfuX7

Sample preparation:

In the PCR tubes:

- 10µl MilliQ Water
- a small amount of the colony from the plate

After mixing the small amount of colony in the water, close the tubes and put them in liquid nitrogen for a few seconds. Immediately transfer them to a 37°C water bath to make a heat shock for 5 minutes. If no liquid nitrogen is available, put in PCR machine at 98C for 10 minutes.

Preparation of the Master Mix:

PCR mix (on ice!)	1 x PCR mix (µl)
5x GC Buffer with $MgCl_2$	4
2mM PCR Nucleotides	2
10µM Primer forward	1
10µM Primer reverse	1
2u/µl pfu X7	0.5
DMSO	1
MgCl	0.5

Add 10µl of the Mix in each PCR tube and place tubes in the PCR machine. Launch the appropriate program.

98° C	1'	
98° C	20''	35 Cycles
52° C	45''	
72° C	2'30'	
72° C	5'	
Hold 12 ° C		

Spin down and use only the supernatant.