Colony PCR

Goal: PCR Amplification directly from a colony rather than a DNA sample can be used to verify an insertion of a sizeable chunk of DNA in a vector by using primers that bind on either side of the insertion. This allows users to get an answer back right away (2-3 hours) and avoid time-cost of miniprepping and sequencing.

Materials:
*Colony-PCR mix
*Primers
*Ice
*Ultra-pure water

Protocol:

Component	volume (1reaction)
RCR Mix (X2)	10 µl
DNA Template	Single colony
ForWarD Primer	2 µl
REVerse Primer	2 µl
Ultra-pure water	Up to 20 µl
Total	20 µl

1. prepare a mix with all the components besides the colony (for X reactions). for each reaction: use a tip to pick a colony. Before placing the colony into the tube, back up colony by slightly touching it on the new plat.
2. Gently mix the tubes and spin down.
3.Insert tube in thermocycler and run the program:

Cycler Protocol:
94ºC for 3 minutes
30x [94ºC for 30 sec, 60ºC for 30 sec, 72ºC for 1 kb/min]
72ºC for 10 min
Hold at 10ºC