Testing bacterial colonies by colony PCR

The following method can be used to screen bacterial colonies that have grown overnight on an LB+antibiotic plate after ligation, transformation, PCR cloning, etc. This allows identification of correct colonies the day after plating, without the additional time and labor of setting up multiple overnight cultures, doing minipreps, and testing potential plasmids by restriction digest. Screening by colony PCR allows you to select only correct colonies for minipreps. For best results, choose a pair of oligos that will only produce a band of a particular size if the recombinant plasmid/BAC is the correct product (for example, use one primer in the plasmid backbone and the other in your desired insert).

Procedure:

1. Choose well-isolated colonies from your ligation plate for PCR. Mark each colony with a dot on the underside of the LB plate; clearly number each colony so that you can return to the correct colony later to set up a miniprep culture.
2. Make PCR master mix(es) for each set of oligos (see recipe below). Adjust volumes accordingly: you will need 25ul per colony. Always make at least 1x more volume than you will use (i.e. make a 6x MM for testing 5 colonies).
3. Pipet 25ul of master mix into labeled 0.2ml PCR strip tubes. Be sure that the label on the tube matches the labeled colony on the LB plate!
4. Touch a P200 pipet tip to the colony, then agitate the tip in the PCR mix in the corresponding 0.2ml tube. **NOTE: you do not need to pick up the entire colony; a few cells are sufficient. Make sure you leave some of the colony behind to inoculate the miniprep culture later.**
5. Run the PCR and check the resulting products on an agarose gel.
6. For colonies that produce the expected size PCR product, return to the LB plate and pick some of the remaining cells to inoculate an overnight LB+antibiotic miniprep culture.

Example PCR conditions (for a 1-kb PCR product):

component	**1x volume**
dH_2O	13.9 ul
10x Taq Buffer	2.5 ul
dNTP (10mM)	2.5 ul
oligo 1 (10uM)	2.5 ul
oligo 2 (10uM)	2.5 ul
Taq DNA polymerase	0.1 ul
	25 ul

cycling conditions:	
1. 95°C	10:00
2. 95°C	0:30
3. 55°C	0:30
4. 72°C	1:00
5. repeat steps 2-4	24x
6. 4°C	hold