Colony PCR Screen for Bacterial Cloning

Use: To quickly test for the presence of a desired DNA fragment in E. coli without need for growth and mini-prep of several candidate colonies.

1. From an LB + antibiotic plate that has allowed transformed cells to grow for 12-16 hours at 37^{o}C, pick single, isolated colonies and resuspend them in 50ul 1x TE. Label the tubes to establish a nomenclature for the experiment.
   Note: Scraping the single colony onto the side of a P200 pipette tip and then smearing the colony onto the side of a microfuge tube allows for easy transfer and resuspension of the cells.
2. Create a Master Mix to dispense into PCR tubes labeled for each colony to be tested as follows: (multiply each ingredient by (# colonies to test +1))
   - 26.5ul dH_2O
   - 5ul 10x Herculase Buffer
   - 5ul 10mM dNTP
   - 4ul Forward Primer (1pmol/ul)
   - 4ul Reverse Primer (1pmol/ul)
3. Aliquot 44.5ul of Master Mix per PCR reaction tube.
4. Add 5ul of the resuspended bacterial colony in 1xTE to a PCR reaction tube. Add 0.5ul Herculase enzyme.
5. Run PCR reaction in a ThermoCycler ~25 cycles at appropriate times and temperatures for the primers and PCR product expected.
   95^{o}C, 10 min
   25x (95^{o}C, 30 sec; primer lower Tm – 5^{o}C, 30 sec; 72^{o}C, 1 min/kb PCR product)
   72^{o}C, 10 min
   4^{o}C, Hold
6. Run PCR reactions with appropriate standards on a gel to determine positive colonies.
7. Grow up positive colonies, mini/maxi-prep, and sequence for confirmation.