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Standard Colony PCR protocol

Make a master mix as follows – for example here's making enough for 40 screens:

x45	each	cpnt
270	6	GoTaq Green
22.5	0.5	10uM Forward Primer
22.5	0.5	10uM Reverse Primer
225	5	H2O
540	12	uL total

0) Make fresh LB-Carb – final 75ug/ml, The working stock is good for a few months at 4°C
1) Fill LB-Carb plate 100uL each well
2) To the PCR plate, 12ul/well master mix
3) For each colony, pick up with a toothpick or pipet tip, dip first into LB-Carb well and wiggle/bang aruond, then put into the PCR plate and leave it there until you're done
4) Run the PCR – 25 cycles for standard amplicons, 30 for hard ones. 52°C annealing temperature, extension time is 30s/kb up to 2kb and then 1min/kb for longer PCRs
   a. (<1kb is recommended, also best primer pairs are one in insert, one in vector)
5) While the PCR and gel are running, put the LB plate in the 37°C incubator
6) Pour gel – I use a slab gel with 50-well combs and load with a multichannel pipet. Ethidium bromide (5mg/ml stock) is added at 5ul/100ml gel, and I usually pour a 1% gel unless the products are <200bp, at which point a 2% gel has better resolution.
7) For positive colonies, add 50uL of the LB to a 5ml overnight culture. Make sure you pipet the 50ul up and down in the well a number of times, to transfer settled bacteria.