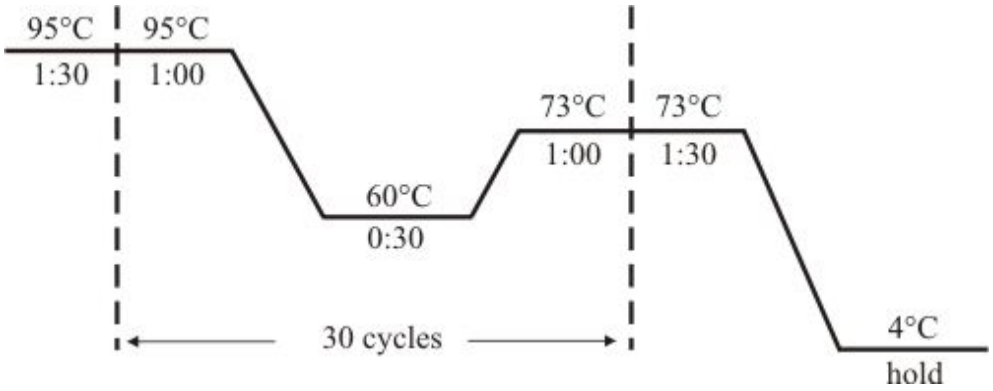
Screening for inserts with colony PCR

→ Colony PCR is a nice alternative to e.g. an enzymatic digestion of vectors. You save expensive restriction enzymes and avoid the purification of plasmids potentially not containing the required insert after the ligation step. The two primers should anneal to the vector part of the plasmid – especially if you are screening for an *E. coli* protein to be over-expressed in *E. coli*. In this case, protein specific primers will also anneal to the chromosomes of *E. coli* and will give you false positives (sounds trivial, right...).

- for 5 reactions use (adjust for your needs):

2	µL	forward primer	(100 $^{pmol}/_{\mu L}$)
2	µL	reversed primer	(100 $^{pmol}/_{\mu L}$)
2	µL	dNTP mix	(10 mM each)
10	µL	10x polymerase buffer	
83	µL	ddH_2O, mix well	
1	µL	*Taq* DNA polymerase (Q·BIOgene, EPTQA025, 250u, 5u/µL), mix well	
100	µL		

- dispense into 5 0.2mL thin walled PCR tubes (20 µL each)
- fill 5 culture tubes with LB medium and the appropriate antibiotic
- pick each colony with a sterile pipet tip, swirl in PCR tube to get some cells into the solution and eject tip into culture tube with same numbering (!)
- shake culture tubes at 37°C and perform PCR reaction as follows:



- adjust annealing time to your specific primer if necessary

- adjust elongation (and final extension) time to your transcript length if necessary (rule of thumb: 1:00 for 1000bases, minimum 0:45!)

- resolve PCR mixture on 1.5-2% agarose gel to identify amplified inserts
- let corresponding colonies grow overnight in the culture tubes, isolate plasmids and check by sequencing