Colony PCR

According to instruction provided by NEB (below) from Onetaq polymerase kit.
Colonies diluted from water, used as template.

NEB OneTaq Hot Start PCR (M0481) (PCR)

Introduction

Note: When using OneTaq for colony PCR, an excess of cells will inhibit the reaction. Take a sterile loop and *touch* it to a colony, then mix this colony in ~1 mL nuclease-free water and from this dilution take 1µl for your PCR reaction**.**

Materials

Reaction Component Table

Component	25µL reaction	50µL reaction	Final Concentration
5X OneTaq Standard Reaction Buffer*	5µl	10µL	1X
10mM dNTPs (#N0447)	0.5µL	1µL	200µM
10µM Forward Primer	0.5µL	1µL	0.2µM
10µM Reverse Primer	0.5µL	1µL	0.2µM
OneTaq Hot Start DNA Polymerase	0.125µL	0.25µL	1.25 units/50µL PCR**
Template DNA	Variable	Variable	< 1000 ng
Nuclease-free water	To 25µL	To 50µL	-

*OneTaq GC Reaction Buffer and High GC Enhancer can be used for difficult amplicons.
**For amplicons between 3–6kb, use 2.5–5 units/50µl reaction

Templates

Use of high quality, purified DNA templates greatly enhances the success of PCR. Recommended amounts of DNA template for a 50µl reaction are as follows:

- Genomic DNA: 1ng-1µg
- Plasmid or viral DNA: 1 pg–1 ng

Equipment

- Thermocycler
- PCR tubes

Procedure

Reaction Setup:

Due to the presence of the inhibitor, reactions can be assembled on the bench at room temperature and transferred to a thermocycler. No separate activation step is required to release the inhibitor from the enzyme.

1. Preheat a thermocycler to the denaturation temperature (94°C)
2. Mix and centrifuge all components prior to use.
3. Assemble all reaction components. Gently mix the reaction, collecting all liquid to the bottom of the tube by a quick spin if necessary.
    - Note: Overlay the sample with mineral oil if using a PCR machine without a heated lid.
1. Quickly transfer PCR tubes from ice to PCR machine previously preheated to 98°C and begin thermocycling.

Thermocycling conditions

Step		**Temperature**	**Time**
Initial Denaturation		94°C	30 seconds
30 cycles	Denaturation	94°C	15-20 seconds
	Annealing	45-68°C	15-60 seconds
	Extension	68°C	1 min/kb
Final Extension		68°C	5 min
Hold		4-10°C	

Acknowledgements

This protocol was sourced from NEB:
https://international.neb.com/protocols/2012/09/05/one-taq-hot-start-dna-polymerase-m0481