PCR reaction - Phusion and Colony PCR

Introduction

PCR reaction for Phusion polymerase and DreamTaq screening.
Inspiration was taken from the protocol for extending primers with Taq-polymerase from iGEM: https://openwetware.org/wiki/Knight:Annealing_and_primer_extension_with_Taq_polymerase
And from the guide for using Phusion polymerase from Thermo Fisher: https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2FMAN0012393_Phusion_HighFidelity_DNAPolymerase_UG.pdf&title=VXNlciBHdWlkZTogUGh1c2lvbiBIaWdoLUZpZGVsaXR5IEROQSBQb2x5bWVyYXNl

Materials

- Phusion (50/25 uL) (Final concentration)
    - HF buffer 5x 10/5 uL
    - dNTP 10mM 1/0.5 uL (200uM)
    - primer1 10uM 2.5/1.25 uL (0.5uM recommended, can be 0.2-1uM)
    - primer2 10uM 2.5/1.25 uL (0.5uM recommended, can be 0.2-1uM)
    - Phusion 0.5/0.25 uL
    - Template 0.1-1/0.05-0.5 ug (genomic) or ng (plasmidic)
    - Sterile dH2O up to 50/25 uL
- DreamTaq screening (25uL) (Colony PCR)
    - Dreamtaq buffer x10 2.5 uL
    - dNTP 10mM 0.5 uL
    - primer1 10uM 2.5/1.25 uL (0.5uM recommended, can be 0.2-1uM)
    - primer2 10uM 2.5/1.25 uL (0.5uM recommended, can be 0.2-1uM)
    - dreamtaq 0.25 uL
    - Template (cell susp)/from Miniprep 1 uL/10 pg-1 ug
    - Sterile dH20 18.25 uL

Procedure

Cycle

1. Initial denaturation 98ºC 30s - 1min (95ºC for DreamTaq)
2. Denaturation 98ºC 10s (95ºC for DreamTaq)
3. Annealing Tm 30s
4. Extension 72ºC, 1min*kb approx (see notes)

5. Go to step 2, 34X (25-35X)
6. Final extension 72ºC, 5-10min
7. Final 4ºC, Forever

Notes

- Use NEB (BioLabs) Tm calculator for annealing temperature
- Phusion High-Fidelity DNA pol (F530S) extension time: 15-30s/kb, do not exceed 1min/kb
- DreamTaq DNA pol (EP0703) extension time: 1min up to 2kb, then +1min/kb if over 2kb
- Do not go above 95ºC for DreamTaq