Supplemental Protocol 1: Generation of DNA fragments by PCR assembly of pooled oligos (NEB #M0689)

Overview

Each lab will have their own preference for how to produce genes of interest that can be further processed by Authenticase® for error correction. If you desire to take a “DIY” approach and choose to design oligos on your own followed by PCR to amplify the gene of interest, the following protocol may be helpful. Steps 1 and 2 are suggestions to generate PCR fragments less than 1 kb from your oligonucleotide design (each ≤ 60-mer) (Figure 6).

1. **Convert gene of interest into oligonucleotides less than 60 nt.**
    1. Encode gene of interest in DNA manipulation software and break up into oligos of 60 nt or less.
    2. Order oligos from your preferred vendor or synthesize them in-house.
    3. Adjust each oligo to 10 µM to facilitate downstream processes.
2. **Prepare gene-specific oligo pool.**
    1. Transfer 5 µl of each oligo (10 µM) to a low bind microcentrifuge tube to form a pool of oligos encoding the gene of interest. Add nuclease-free water to a final volume of 500 µl (100 fmol/µl).

REAGENT	REACTION	FINAL RXN CONC.
Oligo 1.1 (10 µM stock)	5 µl	
Oligo 1.2 (10 µM stock)	5 µl	
…		
Oligo 1.x (10 µM stock)	5 µl	
Nuclease-free water	to 500 µl	100 nM of each oligo

    2. Setup PCR reaction:

REAGENT	REACTION	FINAL RXN CONC.
Q5 Hot Start High-Fidelity 2X Master Mix	25 µl	1X

REAGENT	REACTION	FINAL RXN CONC.
10 µM Forward Primer	2.5 µl	0.5 µM
10 µM Reverse Primer	2.5 µl	0.5 µM
Template DNA (pooled oligos from 2.1)	5 µl	500 fmol of each oligo
Nuclease-free water	to 50 µl	

3. Setup PCR reaction conditions:

CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	98°C	2 minutes	
Denaturation	98°C	10 seconds	36
Annealing	60–64°C*	10 seconds	
Extension (for 500–700 bp)	72°C	40 seconds	
Final Extension	72°C	5 minutes	
Hold	4–10°C		

* Please visit tmcalculator.neb.com to determine correct annealing temperature.