

In vitro Mutagenesis with dut ung single stranded DNA

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Phosphorylation of oligonucleotide

0.5 microgram oligonucleotide (30-mer)
2 microliters 10X Kinase Buffer
1 microliter 10 mM ATP
H₂O to a final volume of 20 microliters
10 Units T4 Kinase
Incubate 45 min at 37°C.
Freeze at -20 deg. to store kinased oligo

10x Kinase Buffer

0.5 M Tris pH 7.6
0.1 M MgCl₂
50 mM DTT

In vitro mutagenesis

(Also do a control reaction with no oligonucleotide added):

In an 0.5 ml microfuge tube add:

0.2 microgram single stranded dut ung DNA
1.3 microliter kinased oligo from above (33 ng)
1 microliter 10X annealing Buffer
H₂O to a final vol of 10 microliters

heat to 90 deg. , 3 min, then reduce temp to 70 deg and let cool slowly to 30 deg. over about 30 min using PCR machine or Boiling H₂O Bath (reducing temp from boiling to 30 deg).

Put on ice. As soon as possible, add:

1 microliter 10X synthesis Buffer
0.3 microliter Ligase (NEB)
0.3 microliter (3 units) Unit T7 DNA polymerase (NEB)
(The above buffer and enzymes can all be mixed just before addition to rxn if desired)

Incubate 5 min at 0 deg.
Incubate 5 min at room temp.
Incubate 40 min at 37 deg..
Freeze at -20 deg. to stop rxn

For electroporation to very competent E. coli, use 0.5 microliter of reaction for electroporation. Transform to wild-type E. coli (DUT UNG F+) and plate 100 microliters and 20 microliters of cells to two separate plates. Screen colonies for desired mutation. Expect about 60% mutagenesis frequency when all works well. The control mutagenesis reaction with no oligonucleotide added should have ~3-5 times less E. coli transformants.

Annealing Buffer (10X) store -20 deg

200 mM Tris 7.4

20 mM MgCl₂

500 mM NaCl

Synthesis Buffer (10X) store -20 deg

4 mM each dNTP

7.5 mM ATP

175 mM Tris 7.4

37.5 mM MgCl₂

15 mM DTT