Quickchange Mutagenesis with KOD Extreme

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Quickchange Mutagenesis with KOD extreme DNA Polymerase
This is much faster than using Pfu Polymerase and typically gives a high frequency of the desired mutation. Works for point mutations, small insertions, and deletions. For large insertions, we use PCR products rather than oligonucleotides (see below)

Each reaction contains:
25 microliters 2X KOD extreme buffer
30 ng plasmid
0.2 micrograms ea oligonucleotide (both top and bottom strands ~50-80 bases long depending on mutation)
10 microliters 2 mM dNTP mix
H2O to 49 microliters total
1 microliter KOD extreme (Novagen)

1. 98 deg 2 min
2. 98 deg 1 sec
3. 55 deg 10 sec
4. 68 deg 1 min/KB

repeat steps 2-4 for a total of 18 times.

5. Hold at 4 deg

(The mutagenesis reaction can probably be scaled down to 25 microliters volume but we haven't yet tried this)

Ethanol precipitate the mutagenesis reaction and resuspend DNA in 30 microliters H2O.

Digest the mutagenesis reaction with Dpn1 to remove the starting plasmid DNA:

10 microliters of PCR reaction from above
16 microliters H2O
3 microliters NEB buffer 4
1 microliter Dpn1 enzyme

Digest 3 hrs at 37 deg (can do for 2 hr if pressed for time)

Transform 1 microliter of Dpn1 digested DNA to E. coli and select for transformants.

Typically get >50% mutagenesis efficiency.

Generating large insertions with Quickchange:

For large insertions, we use PCR products instead of top and bottom oligonucleotides. Design the oligos for PCR amplification with ~35 nucleotides at the 5' ends that are identical to the template sequence flanking the insertion point.

Do the PCR amplification in a 50 microliter reaction. For the mutagenesis to work, the PCR reaction must work very well (a very prominent band on a gel when ~5% of the total reaction is run on an agarose gel). A minor band or two is OK - no need to purify the PCR product.

Ethanol precipitate and resuspend the amplified DNA in 30 microliters H2O.

Perform quickchange with KOD extreme as above, except use 13 microliters of the PCR product that was resuspended in H2O instead of oligonucleotides.

Ethanol precipitate, and digest with Dpn1.

Typically get >50% insertions.