

KLD cloning

KLD stands for **K**inase, **L**igase, **D**pnl.

This kit can be used to modify bases, insert and delete.

For this to work the PCR needs to have blunt ends, so use NEB Q5 as the polymerase (not KAPA)

1. Prepare a 10 μ l reaction as follows:

| COMPONENTS | VOLUME |
|--------------------------|------------|
| PCR Product | 1 μ l |
| KLD Reaction Buffer (2X) | 5 μ l |
| KLD Enzyme Mix (10X) | 1 μ l |
| Nuclease-free Water | 3 μ l |
| Total Volume | 10 μ l |

2. Mix well by pipetting up and down. Incubate at room temperature (25°C) for 5 minutes. Place on ice or store at -20°C.
3. Transformation: Add 5 μ l of the KLD reaction to 50 μ l of competent E. coli.