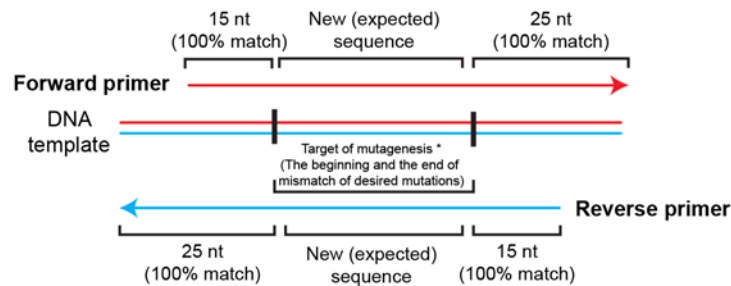


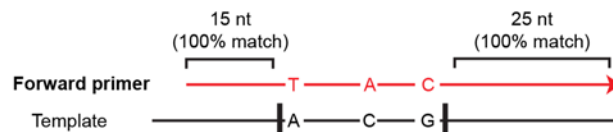
Mutagenesis protocol (Jennifer Doudna lab)

1. The protocol is able to make site-directed mutagenesis for single or multiple nucleotides, introducing new sequences, and deleting a chunk of sequences.
2. Design the primers following the rules below.



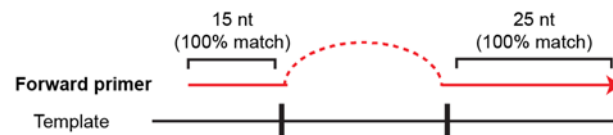
Example:

Site-directed mutagenesis



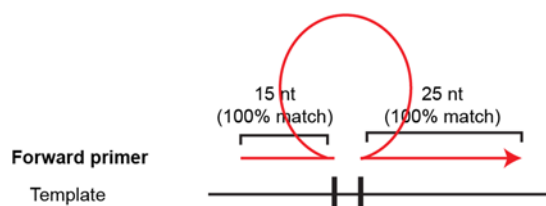
Example:

Deletion



Example:

Addition



3. Synthesize primers using standard desalting method (no extra purification needed)
4. Perform long-range PCR with PfuTurbo (Agilent # 600250), PfuUltra (Agilent # 600380) or PfuUltra II (Agilent # 600670).

5. Condition for PfuTurbo:

**Optimization Parameters and Suggested Reaction Conditions
(50 µl reaction volume)**

Parameter	Targets: <10 kb (vector DNA) or <6 kb (genomic DNA)	Targets: >10 kb (vector DNA) or >6 kb (genomic DNA)
Extension time	1 min per kb	2 min per kb
<i>PfuTurbo</i> DNA polymerase	2.5 U	5.0 U
Input template	50–100 ng genomic DNA ^a	200–250 ng genomic DNA ^a
Primers (each)	100–200 ng (0.2–0.5 µM)	200 ng (0.5 µM)
dNTP concentration	100–250 µM each dNTP (0.4–1.0 mM total)	500 µM each dNTP (2 mM total)
Final reaction buffer concentration	1.0×	1.5× (genomic DNA targets) 1.0× (vector DNA targets)
Denaturing temperature	95°C	92°C
Extension temperature	72°C	68°C

**Reaction Mixture for a Typical Single-Copy Chromosomal Locus
PCR Amplification**

Component	Amount per reaction
Distilled water (dH ₂ O)	40.6 µl
10× cloned <i>Pfu</i> reaction buffer ^a	5.0 µl
dNTPs (25 mM each dNTP)	0.4 µl
DNA template (100 ng/µl)	1.0 µl ^b
Primer #1 (100 ng/µl)	1.0 µl ^c
Primer #2 (100 ng/µl)	1.0 µl ^c
<i>PfuTurbo</i> DNA polymerase (2.5 U/µl)	1.0 µl (2.5 U) ^d
Total reaction volume	50 µl

A. Targets <10 kb (vector DNA) or <6 kb (genomic DNA)

Segment	Number of cycles	Temperature	Duration
1	1	95°C ^b	2 minutes
2	30	95°C	30 seconds
		Primer $T_m - 5^\circ\text{C}^c$	30 seconds
		72°C	1 minute for targets ≤1 kb 1 minute per kb for targets >1 kb
3	1	72°C	10 minutes

B. Targets >10 kb (vector DNA) or >6 kb (genomic DNA)

Segment	Number of cycles	Temperature	Duration
1	1	92°C	2 minutes
2	10	92°C	10 seconds
		Primer $T_m - 5^\circ\text{C}^d$	30 seconds
		68°C	2 minutes per kb
3	20	92°C	10 seconds
		Primer $T_m - 5^\circ\text{C}^d$	30 seconds
		68°C	2 minutes per kb plus 10 seconds/cycle