

USER ligation

Used for transforming a gene fragment with USER overhangs into a linearized vector (see protocol for linearizing USER cassette).

Protocol

1. Mix reagents in the listed order.
2. Run the PCR program

Component	10 uL reaction
Cutsmart buffer	0.5 uL
Linearized vector	1.0 uL
Insert 1	1.0 uL
Insert 2	1.0 uL
USER enzyme	0.5 uL

USER program

Set the PCR machine to the following program:

1. 37 °C 35 minutes
2. 25 °C 20 minutes
3. 20 °C 10 minutes
4. 15 °C 10 minutes
5. 10 °C until you can put it on ice and transform.

Transformation

Immediately following the USER ligation, perform the transformation. Use mix and go protocol for transforming in Mach1 cells.