Gateway cloning

1. Multisite 3-fragment gateway cloning

Entry clones (5 fmole/µl)	5' entry clone	1 µl
	middle entry clone	1 µl
	3' entry clone	1 µl
Destination vector (10 fmole/ µl)		1 µl
1x TE buffer		2 µl
LR Clonase II Plus		1 µl
	Total	5 µl

→ 16 hr at 25 °C.

→ add 0.5 µl of proteinase K and incubate for 10 min at 37 °C.

→ Use 5 µl for transformation

2. BP recombination reaction

Purified PCR product (100 fmol)	0.5	µl
pDONR vector (150 ng/µl)	0.5	µl
Tris buffer	3	µl
BP Clonase II	1	µl
Total	5	µl

→ 1 hr at 25 °C. (N.B. for large PCR products (> 5 kb), 16 hr at 25 °C.

→ add 0.5 µl of proteinase K and incubate for 10 min at 37 °C.

→ Use 5 µl for transformation

3. LR recombination reaction

Entry clone (50-150 ng)	0.5	µl
Destination vector (150 ng/µl)	0.5	µl
Tris buffer	3	µl
LR Clonase II (or Plus)	1	µl
Total	5	µl

→ 1 hr at 25 °C. (N.B. for large entry clone (> 5 kb), 16 hr at 25 °C.

→ add 0.5 µl of proteinase K and incubate for 10 min at 37 °C.

→ Use 5 µl for transformation

To convert µg to pmole:

$$\text{pmole} = \mu g \times \frac{10^6 \text{ pg}}{1 \mu g} \times \frac{\text{pmole}}{660 \text{ pg}} \times \frac{1}{N}$$

$$= \mu g \text{ of plasmid} \times 10^6 / (660 \times N)$$

where N is the number of nucleotides and 660 pg/pmole is the average MW of a nucleotide pair.