Restriction enzyme digestion

1. Prepare a restriction mixture for the vector that is used for cloning and for the inserts.
E.g. a 10µL restriction mixture (according to https://tools.thermofisher.com/content/sfs/manuals/MAN0012413_Fast_Digestion_DNA_UG.pdf):

10X FastDigest buffer (ThermoFisher Scientific)	1.0µL
DNA	up to 1µg
FastDigest enzyme (NdeI)	0.5µL
FastDigest enzyme (XhoI)	0.5µL
Nuclease-free H_2O	up to 10µL
Total	10µL

2. Incubate the mixtures at +37°C for ~1 hour. (According to the manual 5min is sufficient with FastDigest enzymes.)

3. Perform heat-inactivation according to the manufacturer's manuals or proceed directly to the purification of the vector (DNA extraction from an agarose gel) and the inserts (PCR clean-up).

DNA ligation

1. Choose the amount of vector to be used (e.g. 50ng) and the molar ratio of the insert and the vector (e.g. 3:1 or 5:1).

2. Calculate the amount of insert that is needed with the following formula:
Insert (ng) = vector (ng) * insert (bp)vector (bp) x 5 (here: 5:1 molar ratio).

3. Prepare the ligation mixture with the restricted vector & insert, and a negative control mixture with the restricted vector only.
E.g. a 10µL mixture:

T4 DNA ligase buffer	1.0µL
vector	e.g. 50ng
insert	according to calculations
T4 DNA ligase	1.0µL
H_2O	up to 10µL
Total	10µL

NOTE: Remember to vortex the buffer carefully in order to dissolve the DTT completely.

4. Incubate the mixture at RT overnight (or according to the manufacturer's manual).