Blunt end ligation

- For cloning blunt-end PCR products generated by proofreading DNA polymerases, such as Pfu DNA polymerase. (If the DNA end structure of the PCR product is not specified by the supplier of the DNA polymerase, follow the Sticky-End Cloning Protocol).
- For cloning of blunt-end DNA fragments generated by restriction enzyme digestion, gelpurify the DNA fragment prior to ligation and use in a 3:1 molar ratio with pJET1.2/blunt (see table below).
- Set up the ligation reaction (20 µL) on ice:

Component	**Volume**
2x Reaction Buffer	10 µL
Non-purifies PCR product	1 µL
or	
purifies PCR product/ other blunt-end DNA fragment	0.15 pmol ends
pJET1.2/blunt Cloning Vector (50 ng/µL)	1 µL (0.05 pmol ends)
T4 DNA Ligase	1 µL
Water (nuclease free)	up to 19 µL

- Vortex briefly and centrifuge for 3 – 5 seconds.
- Incubate the ligation mixture at room temperature (22 °C) for 5 minutes.
- Use the ligation mixture directly for transformation.

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