

DNA Ligation Reaction: Cloning

Basic concepts: Review of analytical techniques. Cloning of desired DNA is made possible by ligation into plasmids.

T4 DNA ligase- fuses DNA strands along S-P backbone.

Specific example reviewed in video: HIV promoter + firefly luciferase gene into pBLU plasmid. **Diagram:**

p Blu (*Jimbo I**) HIV promoter (*Eco RI**)(*Eco RI*) firefly (*Jimbo I*) p Blu

Staggered cut permits alignment of new ligation candidates. A specific orientation is ensured by EN* (shown above in italics) with specific sticky ends.

Using EN to give fragments that can only bind in a single manner results in a forced ligation.

DNA ligation reaction:	5 X ligation buffer	4 ul
	Plasmid DNA (40 ng/ul)	5 ul
	Insert DNA (6 ng/ul)	10 ul
	DI H2O as needed	
	T4 DNA Ligase (1 U/ul)	1 ul

Ligation buffer has: ATP, dithiothreitol, BSA and Mg⁺⁺ and buffer

Add components: Add in order as noted above.

Incubate @ 14 °C: Low temperature is required for adequate annealing. If too high, no annealing, if too low, not enough enzyme activity.

Results: Overview of the ligation reaction: New plasmids were transformed, selection, grow out, plasmid miniprep, EN digest, and electrophoresis with predicated versus observed results.

Optimizing ligation reactions

Compatible sites: Sometimes different EN will give similar overhangs and so can ligate. Further more, if this is the case, the ligation product may lack site for either original EN.

5' & 3' protruding ends: Works on either as long as compatible.

Blunt end ligation: Requires lots more ligase than in staggered ends (as there is no over hang to bind fragments which can prime reaction.)

Linkers: Blunt end ligation permits inserting of a linker. Linker is a piece of DNA of about 10 bP that has a RE site (and therefore allows for new EN site to be inserted into genome/plasmid.)

For example: *CCC/GGG CCC CG/AATTCCG GGG*
GGG/CCC GGG /GCTTAA/GGC CCC
SMA 1 Eco R 1

Many linkers are commercially available.

Intra and intermolecular ligation:

- Intra: Same molecule will ligate (as in linear molecule into a plasmid.)
- Inter: Between separate molecules
- Most ligation reactions involve both intra and inter molecular linking.
- Inter: High concentration of ends (i.e. lots of fragments) of DNA favors this.
- Intra: Low concentration of ends of DNA favor this (as the other end is the only thing present in any significant amount.)

If there is an appropriate balance, will get both inter and intra bonding and create an novel plasmid construct.

Shorter DNA fragments more prone to intra-molecular bonding (since DNA ends are closer together.)

Ratio of plasmids to insert. Equa-molar concentration of plasmid and insert DNA is desired.

Note: Equa-molar does not mean same weight or concentration. Molar amounts reveal the true number of possible bonding sites.

These ratios are often specific to a given reaction and are usually cited in the relevant literature.