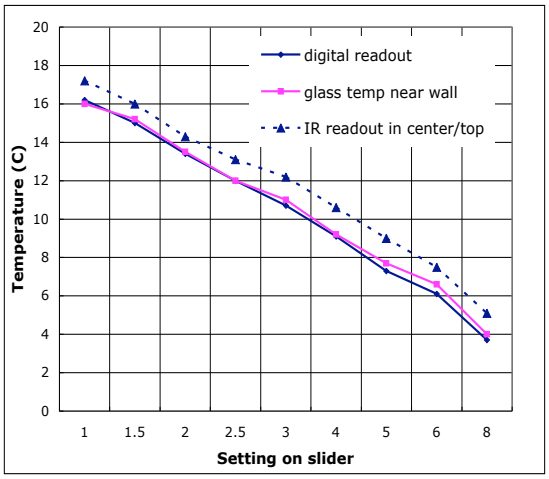
# Use of the microcooler for ligations

1. This was purchased for ligations, although other applications are possible.
2. It takes <10 minutes to cool down; turn on just before use, turn off when done.
3. Put your tubes in the black rack (don't fill the chamber with water).
4. The temperature in the top/middle of the rack is slightly higher than the digital read-out, which measures the temperature at the chamber wall (see below). The temperature in the middle of the chamber is probably in between. So for 14°C or 16°C ligations, aim for about 13 or 15°C on the readout (slider positions 1.5 and 2, respectively). The exact temperature is not critical.

Recorded temperatures (°C)
*( may vary slightly depending on room temperature?)*

| Setting (slider) | Instrument readout | Thermometer (top at wall)* | IR gauge (rack center/top)* |
|---|---|---|---|
| 1 | 16.2 | 16.0 | 17.2 |
| 1.5 | 15.0 | 15.2 | 16.0 |
| 1.75 | 14.4 | 14.8 | 14.9 |
| 2 | 13.4 | 13.5 | 14.3 |
| 2.5 | 12.0 | 12.0 | 13.1 |
| 3 | 10.7 | 11.0 | 12.2 |
| 4 | 9.1 | 9.2 | 10.6 |
| 5 | 7.3 | 7.7 | 9.0 |
| 6 | 6.1 | 6.6 | 7.5 |
| 8 | 3.7 | 4.0 | 5.1 |

*readings may not be 100% accurate.




Supplementary information: Most experiments use T4 DNA Ligase, which is most active at 25°C. However, to perform efficient ligations with cohesive-ended fragments ("sticky ends"), the optimal enzyme temperature is balanced with the melting temperature of the ends. All ligations work fine at low temperatures (4°C), but go faster at higher temperatures, as long as the $T_m$ is not exceeded.

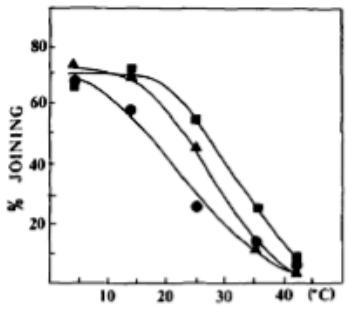



Figure 2. Temperature dependence of the joining of DNA termini produced by EcoRI (▲), PstI (■) and HaeIII (●).

*Eco*RI sticky ends are 100% AT*; Pst*I sticky ends are 50% AT; *Hae*III ends are blunt. Hence *Eco*RI ends ligate most efficiently at the lowest temperature, *Hae*III at the highest, *Pst*I is intermediate. From Ferretti, 1981.