

TOPO-cloning of PCR products

1. Perform PCR:
 - if Taq was used alone, continue the rest of this protocol within the same day as the PCR was performed; if the PCR was performed more than 1 day earlier, add Taq again and incubate at 72°C for 20 min followed by 4°C for 5 min before proceeding with the rest of this protocol
 - if Pfu or another proofreading polymerase was used (alone or in combination with Taq), allow reaction to sit at room temperature (RT) or at 4°C overnight, then add Taq and incubate at 72°C for 20 min followed by 4°C for 5 min before proceeding with the rest of this protocol
2. Mix the following into an eppi tube:
 - 2 μ L PCR
 - 1 μ L TOPO salt solution
 - 0.25 μ L TOPO vector
 - 2.75 μ L sterile water
3. Incubate at RT 20-30 min
 - Thaw competent XL1-blue bacteria (or other bacterial strain suitable for blue/white screening) on ice during this incubation step
4. Chill TOPO ligation reaction on ice for 5 min
5. Transform competent XL1-blue with 3 μ L of TOPO ligation reaction; spread transformation onto agar LB + ampicillin

Depending upon the competency of the bacteria and the efficiency of the ligation reaction, hundreds of colonies may be obtained. Therefore, after completion of the transformation, spread 90% of the bacteria onto 1 LB+amp plate and 10% of the bacteria onto another LB+amp plate.