DNA ligation & transformation

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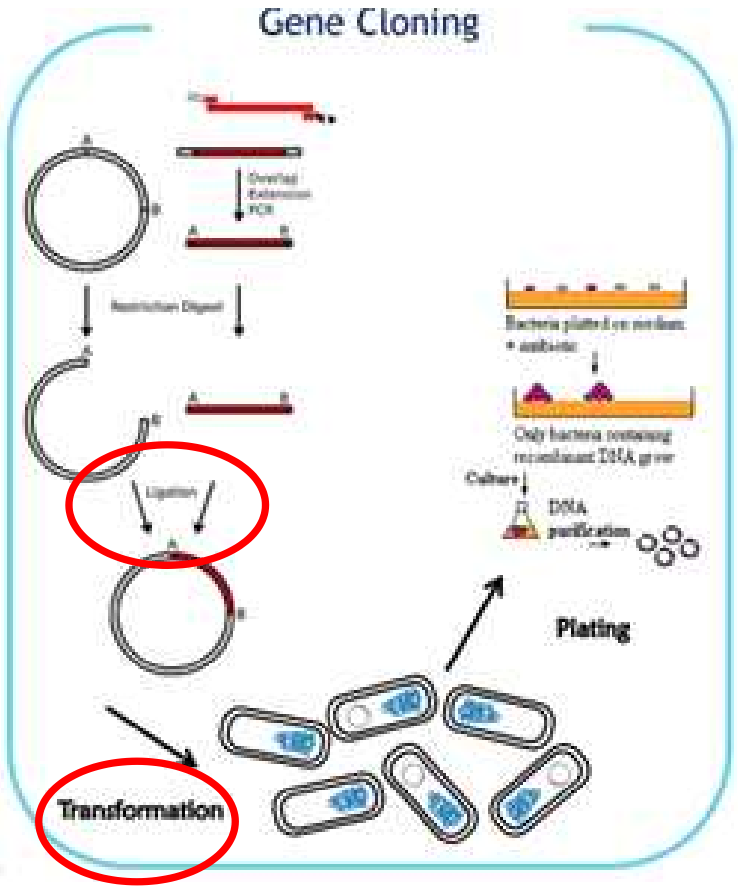
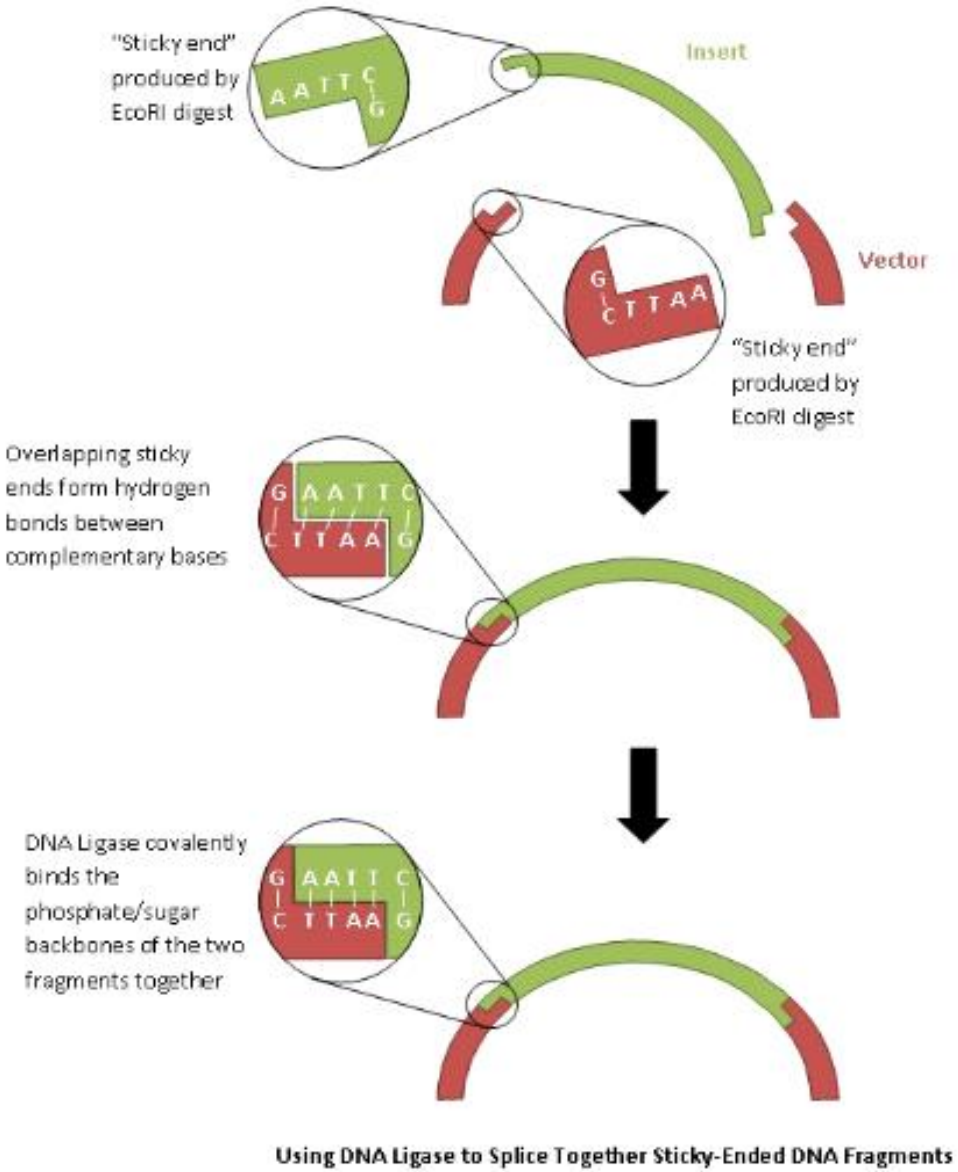
What we will do?

PCR Amplification of DNA of interest (DOI)
Purification of amplified POI
Restriction of amplified POI and plasmid
Purification of restricted DNAs
Ligation of POI and plasmid
Transformation of ligated DNA
DNA sequencing
Overexpression
purification



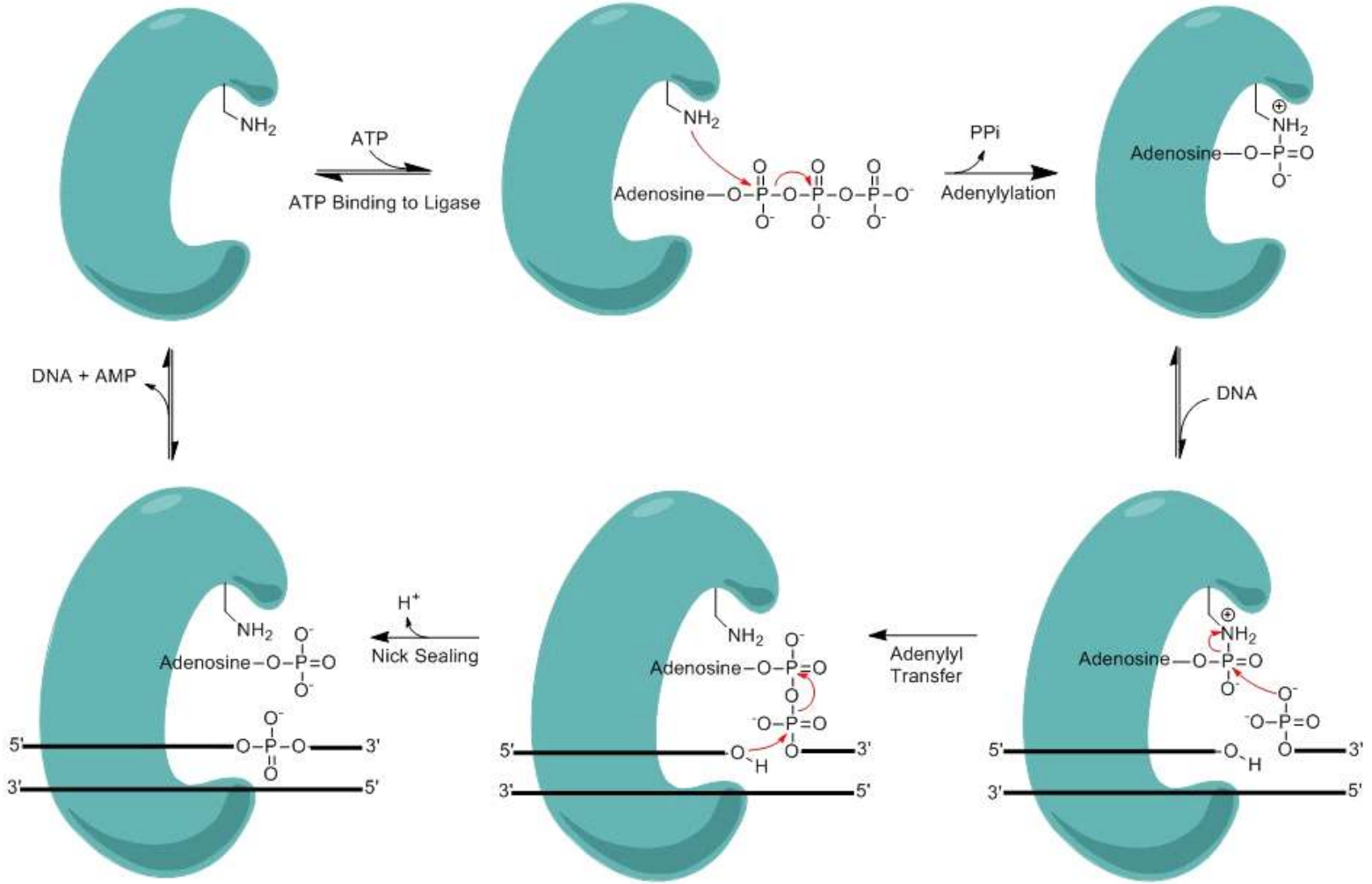
DNA ligation



Using DNA Ligase to Splice Together Sticky-Ended DNA Fragments

http://www.di.uq.edu.au/sparqligation

DNA ligation



https://en.wikipedia.org/wiki/LIG1#/media/File:Ligase_nick_repair_mecanism.jpg

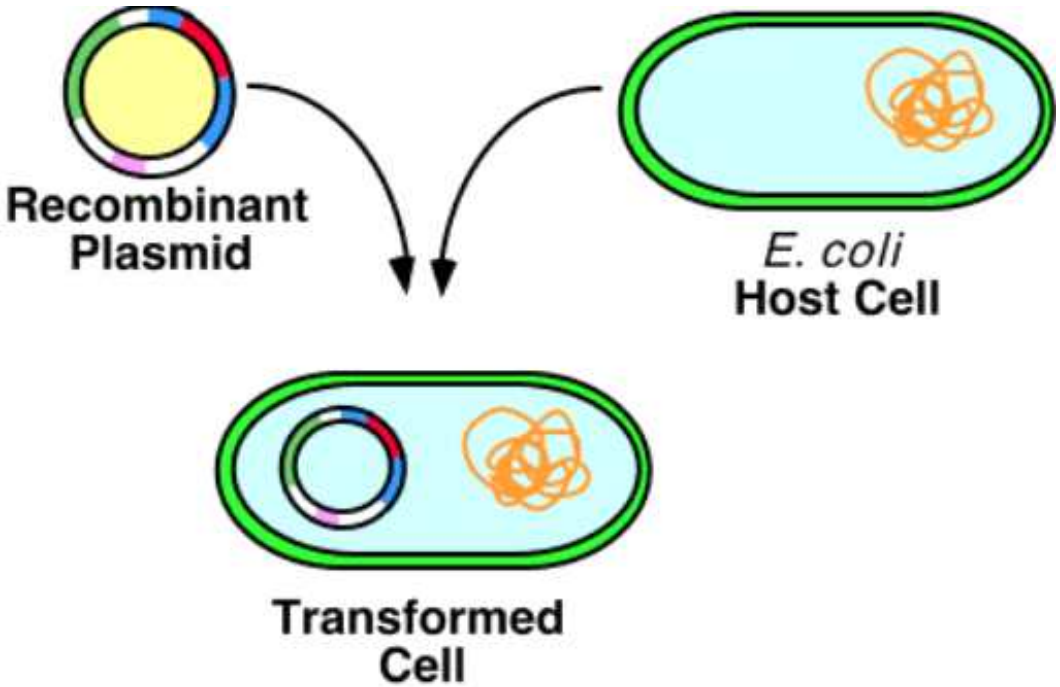
DNA ligation

Materials

DDW, T4 ligase ligation buffer, T4 ligase, restricted DNAs (target of interest and plasmid)

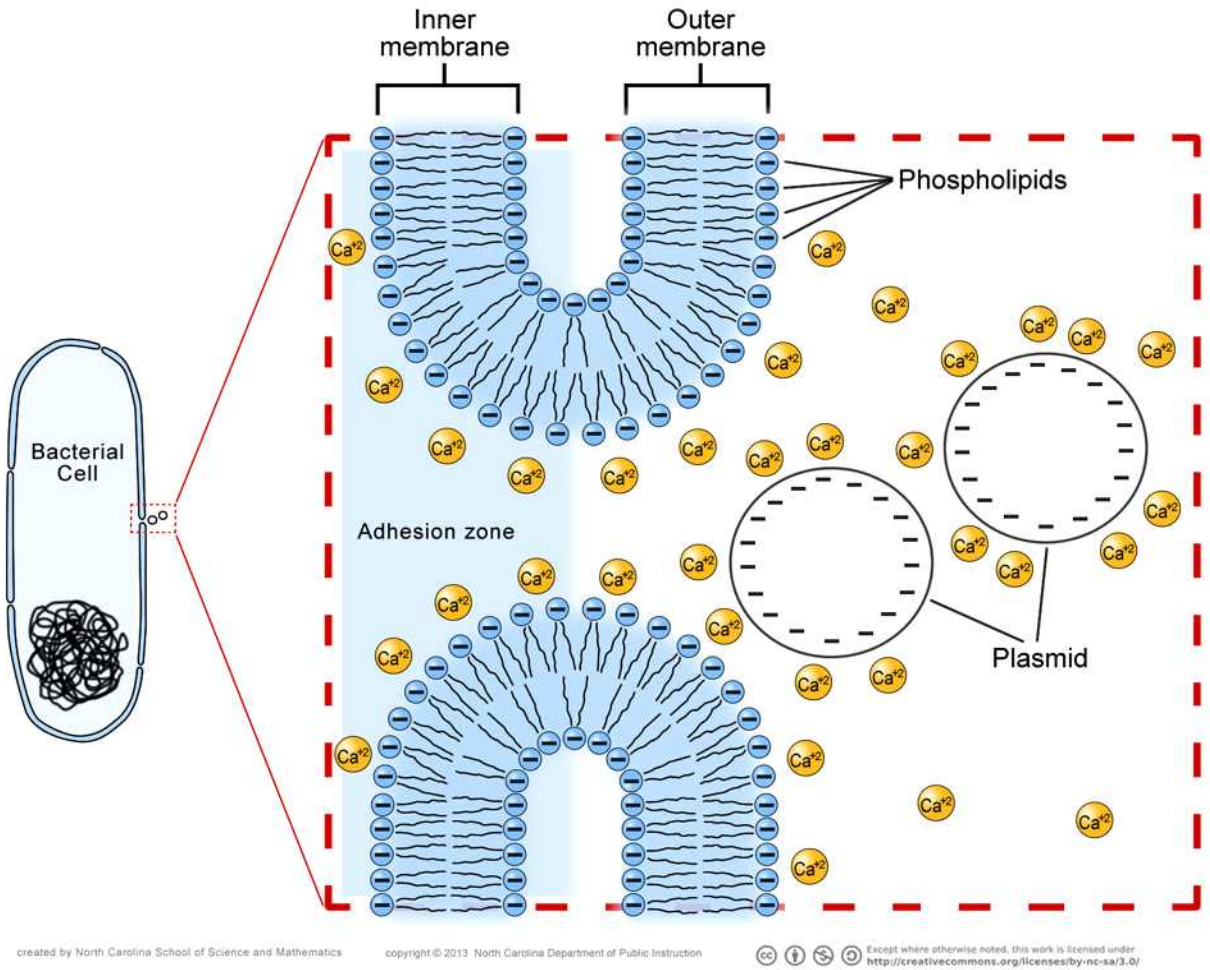
1. Thaw digested (restricted) DNA of interest (DOI) and plasmid and T4 ligase ligation buffer.
2. Prepare two new 1.7 ml micro-tubes.
3. Data from last week, calculate relative amount of DOI to plasmid.
4. Add DOI and plasmid into a micro-tube → insert ligation sample (Ratio of DOI and plasmid should be 3:1 ~ 5:1)
   Add DOI only into a micro-tube and add DDW instead of plasmid → self ligation sample
5. Add 1 ul of T4 ligase ligation buffer.
6. Add DDW up to 9 ul.
7. Add 1 ul of T4 ligase (total volume should be 10 ul).
8. Close lid of micro-tube and centrifuge, vortex and centrifuge.
9. Incubate for 2h at 37℃.

Transformation



https://www.flickr.com/photos/biotechnology/2978049157

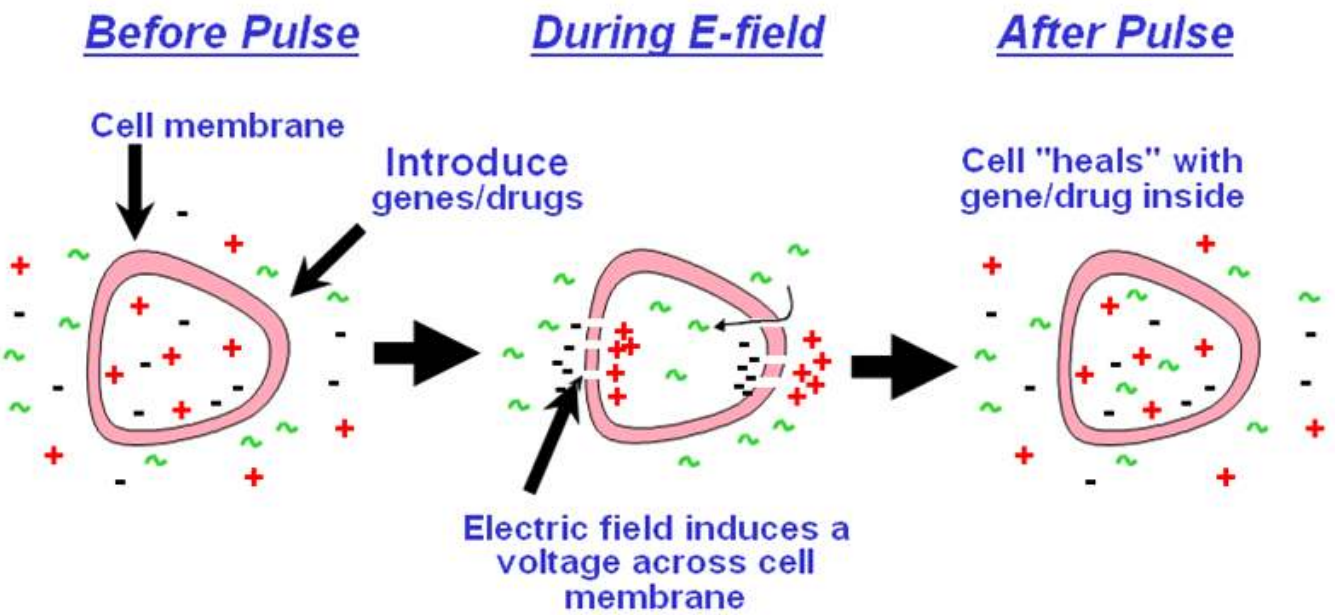
Transformation



Heat-shock transformation

http://www.dlt.ncssm.edu/tiger/diagrams/BiologyStills/E-Coli_transformation.jpg

Transformation



electroporation transformation

http://www.btxonline.com/pages/FAQ.html

Heat-shock Transformation

Materials

competent cell (DH5a), ligated DNA, 42℃ heat-block, LB

1. Thaw competent cell in ice.
2. Prepare two new 1.7 ml micro-tubes.
3. Add 20 ul of competent cell into micro-tubes.
4. Add 2 ul of insert ligation sample and self ligation sample to each tube. (Generally, the ratio between cell and gene is 10 : 1.)
5. Tapping and incubate for 10 min in ice.
6. Move the micro-tube to 42℃ heat-block and incubate for 30 sec.
7. After incubation, move the micro-tube to ice and incubate for 1 min.
8. Add 800 ul of LB medium.
9. Incubate for 30 min at 37℃.
10. Prepare LB-agar plate that contains appropriate antibiotics.
11. Spread the cell+gene solution in the LB-agar plate with spreader.
12. Incubate for 12~18h at 37℃.