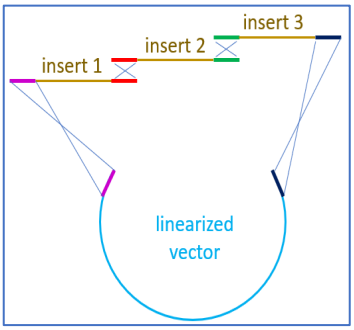
McGill Biochemistry Department | Département de biochimie

BIOC 462/491 Internship Position in Industry

Winter 2025

Company:

Paraza Pharma Inc.
2525 Marie-Curie
Montreal, QC
Canada H4S2E1
https://parazapharma.com/



Project Title: Selecting a cloning technology for a modern laboratory

Project Description:

Background/context

The evolution of cloning over time has seen the development of methods no longer relying on restriction endonucleases. Newer technologies, using homologous DNA ends, allow for simultaneous plasmid assembly from multiple fragments.

Aim/hypothesis

The project aims at comparing different technologies to select the approach providing the best cloning efficiency. The selected method will be used to assemble multiple plasmids for protein expression in mammalian cells. The goal will be to assemble up to 4 fragments.

Methodology to be used by the student

The student will first take two genes and clone each into corresponding vectors. This system will be used as a model system to compare cloning efficiency of the following five methods:

1. iVEC, *in-vivo* cloning (Kostylev et al., 2015; Jacobus & Gross, 2015)
2. SLiCE, cloning using JM109 cell lysate (Motohashi, 2017)
3. sLIC T4, sequence and ligation independent cloning, using T4 DNA polymerase (Li & Elledge 2007; Stevenson et al., 2013)
4. sLIC T5, sequence and ligation independent cloning, using T5 exonuclease (Yu et al., 2023)
5. CPEC, overlap extension-based cloning (Quan & Tian, 2009; Cao et al., 2014)

After selecting the most efficient approach, the student will construct plasmids for expression of three model proteins – rhodopsin, bRAF and rabbit Fc (fragment crystallizable). These plasmids can then be used in a follow-up research project to optimize mammalian protein expression.

Relevance of the project

Many laboratories either rely on inefficient cloning technologies, which slows down research progress, or rely on outsourcing of cloning to third parties, which leads to additional costs. This project aims to create a budget-friendly and ultraefficient cloning setup.

Contact Information:

Prof. Jose Teodoro, PhD, BIOC 491 Course Coordinator
jose.teodoro@mail.mcgill.ca

References:

- Cao P, Wang L, Zhou G, Wang Y, Chen Y. Rapid assembly of multiple DNA fragments through direct transformation of PCR products into E. coli and Lactobacillus. Plasmid. 2014 Nov:76:40-6.
- Jacobus AP, Gross J. Optimal cloning of PCR fragments by homologous recombination in *Escherichia coli*. PLoS One. 2015 Mar 16;10(3):e0119221.
- Kostylev M, Otwell AE, Richardson RE, Suzuki Y. Cloning Should Be Simple: *Escherichia coli* DH5α-Mediated Assembly of Multiple DNA Fragments with Short End Homologies. PLoS One. 2015 Sep 8;10(9):e0137466.
- Li MZ, Elledge SJ. Harnessing homologous recombination in vitro to generate recombinant DNA via SLIC. Nat Methods. 2007 Mar;4(3):251-6.
- Motohashi K. Evaluation of the efficiency and utility of recombinant enzyme-free seamless DNA cloning methods. Biochem Biophys Rep. 2017 Jan 26:9:310-315.
- Quan J, Tian J. Circular polymerase extension cloning of complex gene libraries and pathways. PLoS One. 2009 Jul 30;4(7):e6441.
- Stevenson J, Krycer JR, Phan L, Brown AJ. A practical comparison of ligation-independent cloning techniques. PLoS One. 2013 Dec 23;8(12):e83888.
- Yu F, Li X, Wang F, Liu Y, Zhai C, Li W, Ma L, Chen W. TLTC, a T5 exonuclease-mediated low-temperature DNA cloning method. Front Bioeng Biotechnol. 2023 Aug 11;11:1167534.