

Background

Pain is a vital biological process that serves as a warning to prevent further bodily harm and helps in the healing process by protecting the damaged or injured part of the body. When this pain persists and does not subside after the injury or disease heals for 3 months, that is referred to as chronic pain¹.

Chronic pain affects 18.9% of Canadians and has a huge economic burden²⁻⁴. Yet, to date, there has been no long-term effective treatments for chronic pain^{5,6}. Opioids are prescribed most, and while they have short-term efficacy, tolerance builds up, and efficacy drops⁷⁻⁹.

Recent studies have implicated the ErbB4 receptor with pain management^{10,11}. This poster discusses the summer project I carried out and its future direction. It also reviews the possibility of small molecule inhibitors of ErbB4 receptors serving as a pharmacological intervention for chronic pain relief.

Methods

Cell Culture: HEK-293 cells cultured in 100 mm x 15 mm petri dishes. These cells were maintained in the University of Toronto Mississauga Cell Culture Facility in an incubator at 37 degrees Celsius and 5% CO₂. The cells were grown in a solution of DMEM containing 10% FBS to promote rapid growth and 1% P/S mix.

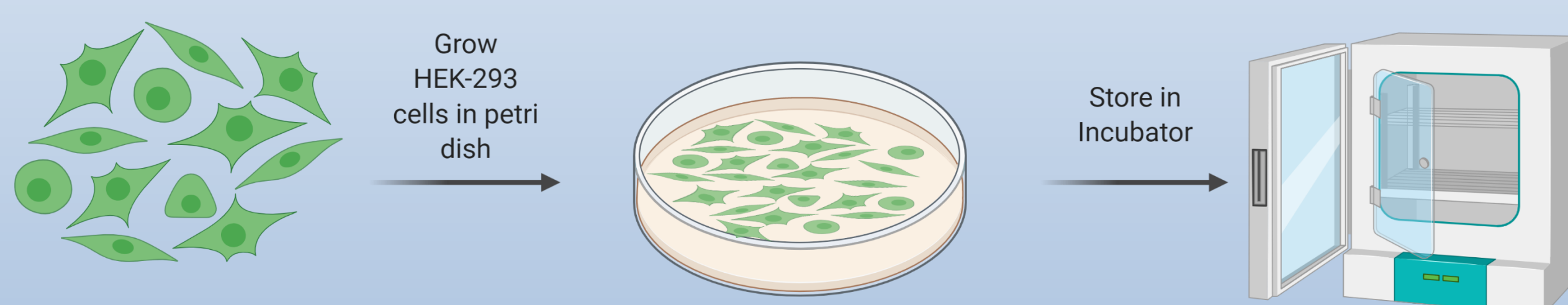


Figure 1 showing the process of cell culture starting from culturing HEK-293 cells to incubating them.

Methods

Transfections: DNA transfections are then carried out with the help of expression vectors. These expression vectors contain the ErbB4 cDNA and will express the receptor. An ErbB4 sandwich ELISA kit can then be used to quantitatively determine how many ErbB4 receptors are found in the cell culture, coupled with immunofluorescence staining and imaging, and western blots.

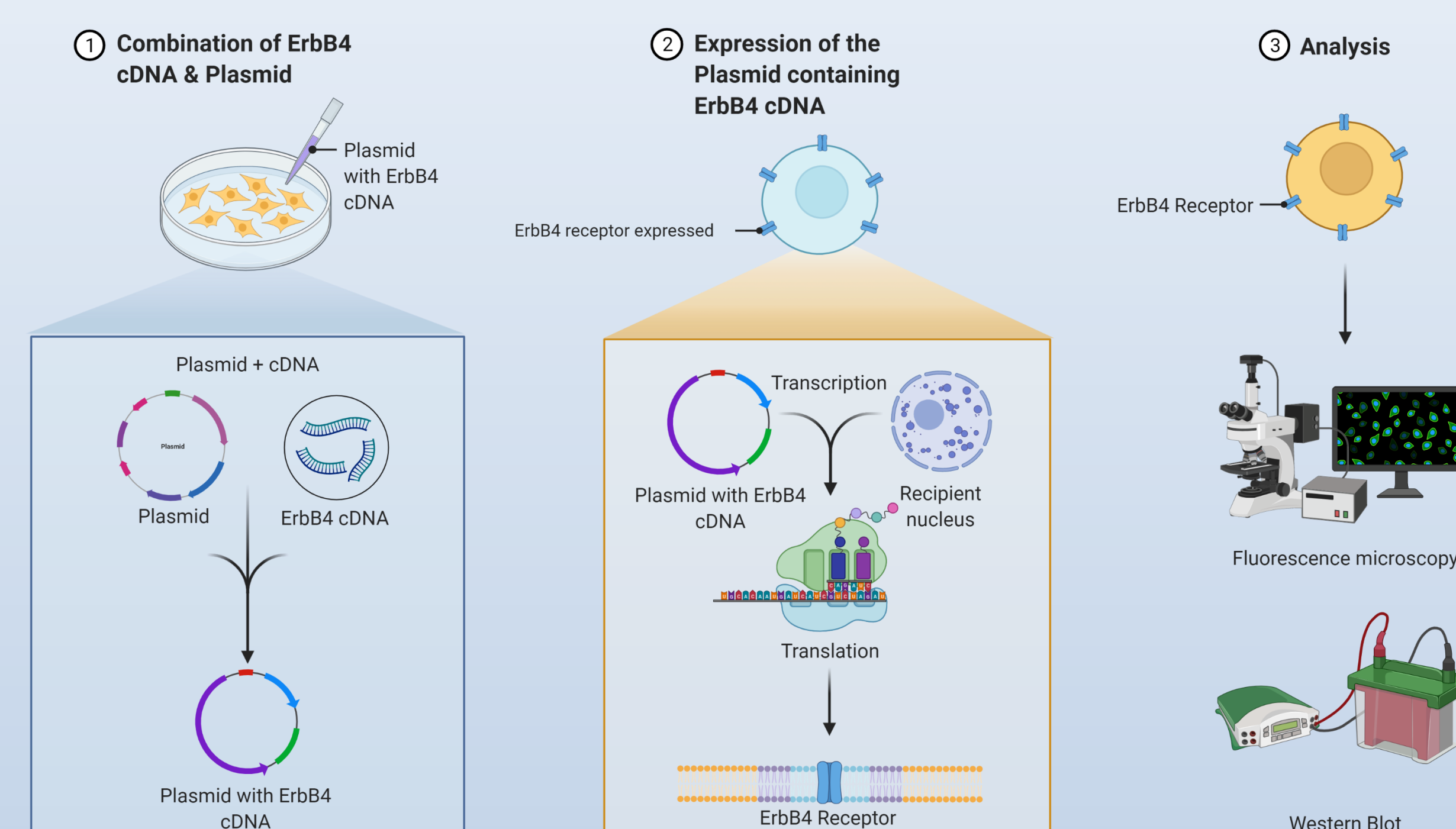


Figure 2 showing the breakdown of transfection and analyzing the efficiency of the transfection and quantity of ErbB4 receptors

cAMP Production Assay: A cAMP assay kit is used to assess binding affinity of the inhibitors. The cells are treated with varying concentration of the inhibitors for 30 minutes and then undergoing a challenge with the agonist (NRG1). cAMP levels can then be assessed through its luminescence about 10 minutes after treating with the agonist through a cell imaging machine. Through this experiment, a serial dose curve can be constructed for each inhibitor to give values for EC₅₀, IC₅₀, K_d, and K_i. Furthermore, a Schild plot can then be created to assess the affinity of the inhibitors for the receptor.

Future Directions

Determine the downstream effects of the inhibition of ErbB4 receptors as they are implicated with various development, homeostasis and cell processes.

Test the affinity of promising inhibitors towards other ErbB receptors to make sure only inhibitors that are specific towards ErbB4 are selected, since general inhibition of ErbB receptors would be detrimental.

In vivo testing of the inhibitors needs to be carried out to test their effectiveness in a complex living system. While various drugs are successful *in vitro*, the transfer of these drugs to animals is a complex matter and needs to be tested accurately to consider moving to clinical trials.

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