Automated Directed Evolution Protocol

This protocol automates a directed evolution experiment, involving PCR library preparation with varying dNTP and manganese levels, followed by SELEX with increasing selection pressure.

Materials:

- Opentrons OT-2 robot
- 20µl pipette tips
- 300µl pipette tips
- 12-well reservoir
- 96-well plates
- Thermocycler module
- Magnetic module
- Master mix solutions (A, T, C, G)
- Manganese solution
- Initial aptamer library
- Milk powder
- Streptavidin magnetic beads previously incubated with biotinylated target protein (Magnetic Beads)
- Ethanol
- Water

Procedure:

This protocol is broken down into three parts which can be run consecutively.

1. **Robot Setup:**
    - Load the OT-2 with necessary labware and reagents as described in the code comments.
2. **PCR Library Preparation:**
    - Each part of the protocol performs two rounds of PCR. For each round:
        - Transfer a small amount of the initial aptamer library (or the product of the previous PCR round) to a column of wells in a 96-well plate within the thermocycler.
        - Add extra manganese to half of the wells and water to others to create varying reaction conditions.
        - Add different master mix solutions to each well to create libraries with taq polymerase, manganese and different dNTP ratios.
        - Run the PCR program in the thermocycler.
        - Pool the PCR products from each well into a single well.
3. **SELEX (Systematic Evolution of Ligands by Exponential Enrichment):**
    - For each PCR round, perform four rounds of SELEX:
        - Add increasing amounts of milk powder to the aptamer pool.
        - Add magnetic beads to bind the aptamers.
        - Incubate to allow binding.

- Transfer the mixture to the magnetic module to separate the beads (and bound aptamers) from the solution.
- Aspirate the supernatant to remove unbound molecules.
- Wash the beads with ethanol to destabilise the remaining interactions and then transfer the beads to the thermocycler and heat to release the bound aptamers.
- Add water to elute the aptamers from the beads.
- Use the magnetic module again to separate the beads from the solution containing the selected aptamers.
- Transfer the selected aptamers to a new well for the next round of SELEX or for storage.
- Last round of SELEX is set to feedback into the epPCR.

Notes:

- The protocol includes specific volumes, timings, and temperatures for each step. Refer to the code for these details.
- The SELEX process involves increasing the stringency of selection by adding increasing amounts of milk powder in each round.
- The protocol includes steps to avoid overflowing wells when transferring liquids.
- The thermocycler is used for both PCR and heating during the SELEX process.

This automated protocol allows for efficient and reproducible directed evolution experiments, enabling the selection of aptamers with desired binding properties.