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Avidity Recommended Electroporation Protocol

This protocol uses the BioRad Gene Pulser Electroporation device

Materials needed:

- Sterile 0.2 cm electroporation cuvettes
- Sterile culture tubes (two, minimum)
- Electro-competent *E. coli* (e.g. Avidity EVB strain)
- Rich media of choice (e.g. LB, SOC, etc.)
- Plasmid DNA (e.g. Avidity pAN or pAC vectors)
- 37°C incubator with shaking capabilities
- Antibiotic (e.g. ampicillin for Avidity pAN or pAC vectors)
- Sterile ddH_2O

Protocol:

1. Prepare one 10-mL culture tube with 1 mL of culture media (without antibiotics) per electroporation sample. Keep at room temperature.
2. Thaw and keep on ice at least two tubes of electro-competent cells (50 µL per tube).
3. Thaw the plasmid DNA and keep on ice until ready to be transformed in to the cells.
4. To one set of electro-competent cells add 10 µg of plasmid DNA. To the second set of cells add an equal volume of sterile dd H_2O (as a negative control). Mix the suspensions well by pipetting gently up and down. Keep all cells on ice.
5. Pipette the samples into 0.2 cm, pre-chilled, electro-cuvettes. Place on ice.
6. Set the BioRad Gene Pulser to the following settings:
   - Pulse Controller 200 Ohms
   - Capacitance Extender 250 µF
   - Capacitance 25 µF
   - Voltage 2.50 kV
7. Place one of the cuvettes into the electroporation chamber and electroporate according to the manufacturer's instructions.
8. Immediately pipette into the cuvette 1 mL of the awaiting media from the 10-mL culture tubes. Mix well, then pipette back into the culture tube.
9. Repeat with all the samples to be electroporated.
10. Place the 1 mL cultures at 37°C for one hour with gentle shaking.
11. Transfer cultures to awaiting expression media (**with** appropriate antibiotics) or prepare a dilution series for plating out on agar media plates with appropriate antibiotics. *

***Note:** If using an Avidity electro-competent strain (EVB99 or EVB101) in conjunction with an Avidity plasmid vector (pAN or pAC vectors), **TWO antibiotics** will be necessary in the final step: Chloramphenicol for maintenance of the BirA-overproducing plasmid in the EVB strain and Ampicillin for maintenance of the pAN or pAC plasmid vector. If using the EVB100 strain with the stable chromosomal integration of the BirA gene, only Ampicillin will be necessary to maintain the pAN or pAC vector.