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ATG3 construct cloning

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ABSTRACT

ATG3 cloning

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Protocol status: Working
We use this protocol and it's working

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Ligation Independent Cloning

1 Amplify gene using PCR with Q5 polymerase. Design primers with overhangs compatible with 1GFP and 2BT vectors. For mutants, design overlapping primers in opposing direction as if using around the horn. Use these to perform 2-step PCR, verifying each step via gel.

2 Gel extract correct size band and measure concentration

3 Digest vector with SspI at 37 C for 1 hour. After 1 hour add CIAP to vector digest and incubate at 37 for another 30 minutes

4 Gel extract vector digest. PCR clean up insert digest. Measure concentrations of both.

5 Mix vector and insert at 1:4 molar ratio for ligation with 1 µL NEBuffer 2.1 and 1 µL 100 mM DTT to a final volume of 10 µL. Add 0.3 µL of NEB T4 DNA polymerase, and immediately incubate for 30 seconds at 50°C

6 Transform 4uL of reaction into DH5alpha cells. Plate everything. Sequence colonies to ensure correct mutation.