**ApoB-NanoLuc genotyping protocol:**

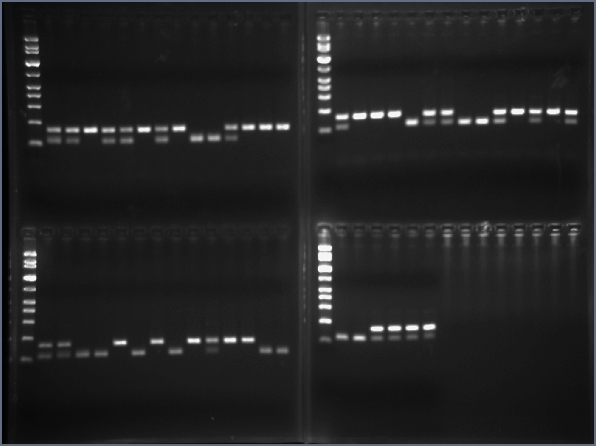
JHT

09/25/2018

**Purpose:**

PCR genotyping assay for genotyping the ApoB-NanoLuc locus

**Background:**

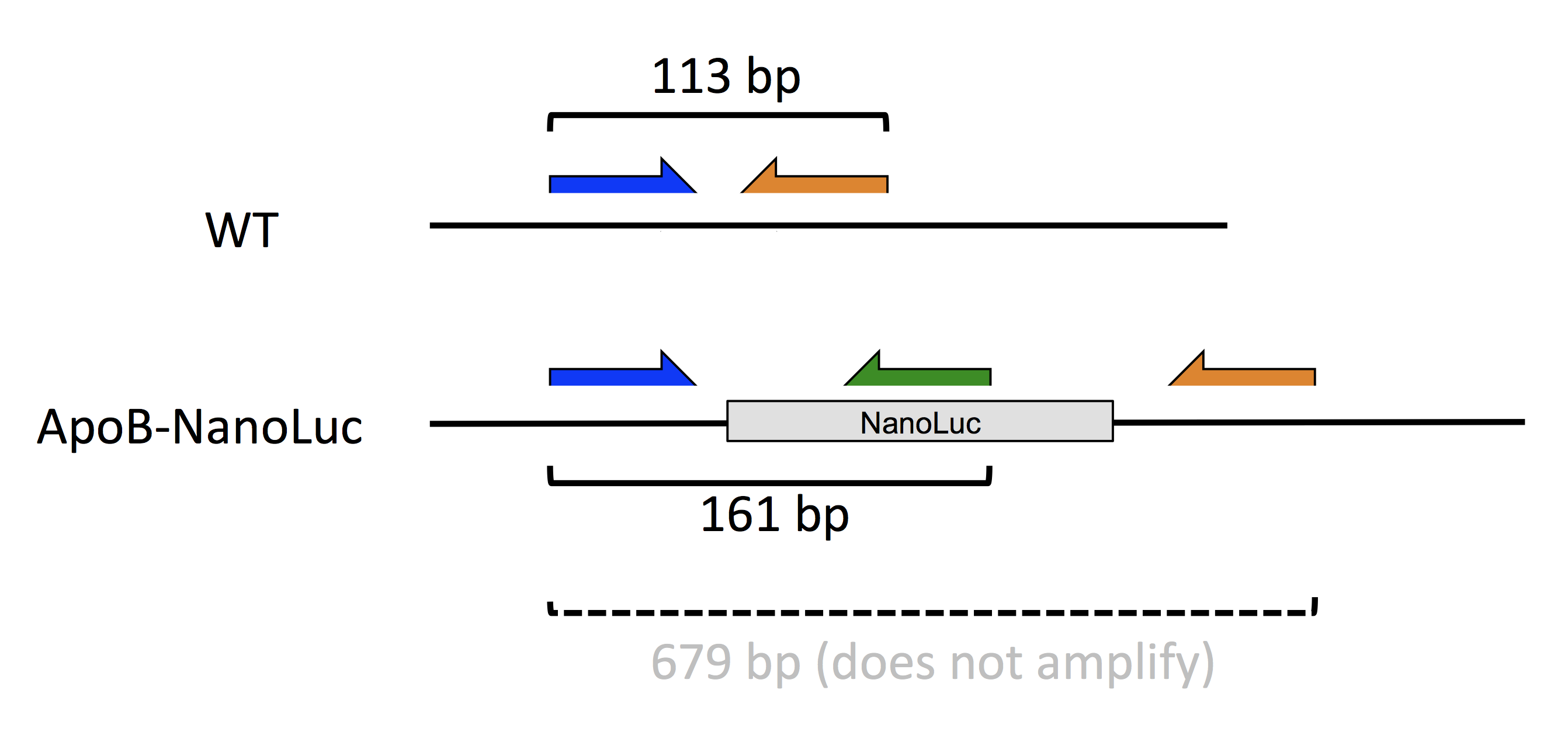
The “NanoLuc” luciferase reporter was integrated into the endogenous *apoBb.1* locus of the zebrafish genome as a carboxy-terminal in-frame fusion. This PCR protocol is used to identify fish that are wild-type, heterozygous, or homozygous for the NanoLuc reporter at the ApoB locus. 3 primers are used, one forward primer (SF-JHT-435), one reverse primer that anneals to the wild-type 3’ UTR (SF-JHT-437), and an additional reverse primer that anneals to the NanoLuc coding sequence (SF-JHT-382). These primers will generate a ~113 bp band if the wild-type stop codon is present, and a ~161 bp band if the NanoLuc fusion allele is present, or both bands if the fish is heterozygous.

Primer sequences:

SF-JHT-435: **GCTTCCTCTCCCATTTTTCC**

SF-JHT-437: **AAGTGTCCATTGGCTTCGAT**

SF-JHT-382: **CCCCGAGATTCTGAAACAAAC**



**Protocol:**

Prepare a standard GoTaq reaction, supplemented with 1 uM shared forward primer, and a total of 1 uM reverse primer but a 4:1 ratio of primer 437 to 382 as shown below (.8 uM 437 and .2 uM 382).

|  |  |  |
| --- | --- | --- |
|  | **25 uL** | **50 uL** |
| H2O | 14 | 28 |
| Green | 5 | 10 |
| MgCl2 | 1.5 | 3 |
| dNTPs | 1 | 2 |
| 435 | 2.5 | 5 |
| 437 | 2 | 4 |
| 382 | .5 | 1 |
| Temp | 2 | 3 |
| Taq | 0.3 | 0.3 |

|  |  |  |
| --- | --- | --- |
|  | Time | Loop |
| 95 | 3’ |  |
| 95 | 30’’ | 35x |
| 57 | 30’’ |  |
| 72 | 20’’ |  |
| 72 | 2’ |  |
| 12 | hold |  |

Run the PCR cycle shown at right and separate bands on a 2% agarose gel. See sequence information on the following page.

Sequence of region of interest, with primer binding sites color coded and Nanoluc insertion underlined

>WT-ApoB region

GAAATGCTGAGGAATTTTGGTAACAACTTGGAGGTATACTTCTCTCCCATCATGGATAACTTTAATACAATTCAATTGACATTTCCCAATGGAAAGGTCATGACAGTAGCTGAGTTCCAACAAAATGTGCAGAGTATCCTGAGAAGCAACCTAGTTATGATAGCAGATGCAATGAAGCAAATCGAAAGTCCTGATGTAGTTCTTAAGAAACTTGGACAAACTTTACAAGAGGTTGTTGAAAAAGGACAAGAGTTTGTGGACAAAATGAATTTAAGTTTATTAGAAGATATTGCCGCGGCCATTAACACGTTCTACCTGGAGCTCATGAAGATCATAGAAGACATTAGTGAGGCAGTCATGTTTGGTTTTTCAATCCCTGCAATTAAAATATATGAAATGTCTCAAAACATAGAGAAAGTGTTGAACGCAAATAATGGAATACATCAATTTGA**GCTTCCTCTCCCATTTTTCC**AGTAATATGGTCATTCTGAACAGAAAGTAAACTAAGTAAACACTACCATATTTTGAAATGAACATTCTTGGAC**ATCGAAGCCAATGGACACTT**CACTGAAGTATAGTACAAACATGTATATAAAGCAATTACTGACTCAAAACATGTCTCATTGTTATGCTGCTCTATTATTCTATTTGTATAAATGAGGATTGAAATACTAAGCTAGTTATCAAAAATGCCACCTGCAACCTATTGTCATCCTTGTTCTGTATCCAACATTAATGGCATGATGGATGGGGATCAATAAAGAACTTTTATTATGCAACACGACCCAATCTTGTCATTTCTATATTCTAGAAGTGTTTTACACTAGTTTGGACCATTCAATAATATAATATAATTGAATATACAGGTGTAATAACATGCAACAAAATGTAAAATAAGCAGTGGTACATGGGTACATTTTAGTTTTTTATGCAAACTACTGGTGACTTTAAAAAAAAAATTACAAATAAAATATTTTATTCTATTT

>ApoB-NanoLuc-fusion-region

GAAATGCTGAGGAATTTTGGTAACAACTTGGAGGTATACTTCTCTCCCATCATGGATAACTTTAATACAATTCAATTGACATTTCCCAATGGAAAGGTCATGACAGTAGCTGAGTTCCAACAAAATGTGCAGAGTATCCTGAGAAGCAACCTAGTTATGATAGCAGATGCAATGAAGCAAATCGAAAGTCCTGATGTAGTTCTTAAGAAACTTGGACAAACTTTACAAGAGGTTGTTGAAAAAGGACAAGAGTTTGTGGACAAAATGAATTTAAGTTTATTAGAAGATATTGCCGCGGCCATTAACACGTTCTACCTGGAGCTCATGAAGATCATAGAAGACATTAGTGAGGCAGTCATGTTTGGTTTTTCAATCCCTGCAATTAAAATATATGAAATGTCTCAAAACATAGAGAAAGTGTTGAACGCAAATAATGGAATACATCAATTTGA**GCTTCCTCTCCCATTTTTCC**AGACAAGTTTGTACAAAAAAGCAGGCTTGATGGTCTTCACACTCGAAGATTTCGTTGGGGACTGGCGACAGACAGCCGGCTACAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCA**GTTTGTTTCAGAATCTCGGGG**TGTCCGTAACTCCGATCCAAAGGATTGTCCTGAGCGGTGAAAATGGGCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGGCGACCAAATGGGCCAGATCGAAAAAATTTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTGATCCTGCACTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGATCGACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTGTAACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGATCAACCCCGACGGCTCCCTGCTGTTCCGAGTAACCATCAACGGAGTGACCGGCTGGCGGCTGTGCGAACGCATTCTGGCGTAAACCCAGCTTTCTTGTACAAAGTGGAATATGGTCATTCTGAACAGAAAGTAAACTAAGTAAACACTACCATATTTTGAAATGAACATTCTTGGAC**ATCGAAGCCAATGGACACTT**CACTGAAGTATAGTACAAACATGTATATAAAGCAATTACTGACTCAAAACATGTCTCATTGTTATGCTGCTCTATTATTCTATTTGTATAAATGAGGATTGAAATACTAAGCTAGTTATCAAAAATGCCACCTGCAACCTATTGTCATCCTTGTTCTGTATCCAACATTAATGGCATGATGGATGGGGATCAATAAAGAACTTTTATTATGCAACACGACCCAATCTTGTCATTTCTATATTCTAGAAGTGTTTTACACTAGTTTGGACCATTCAATAATATAATATAATTGAATATACAGGTGTAATAACATGCAACAAAATGTAAAATAAGCAGTGGTACATGGGTACATTTTAGTTTTTTATGCAAACTACTGGTGACTTTAAAAAAAAAATTACAAATAAAATATTTTATTCTATTT