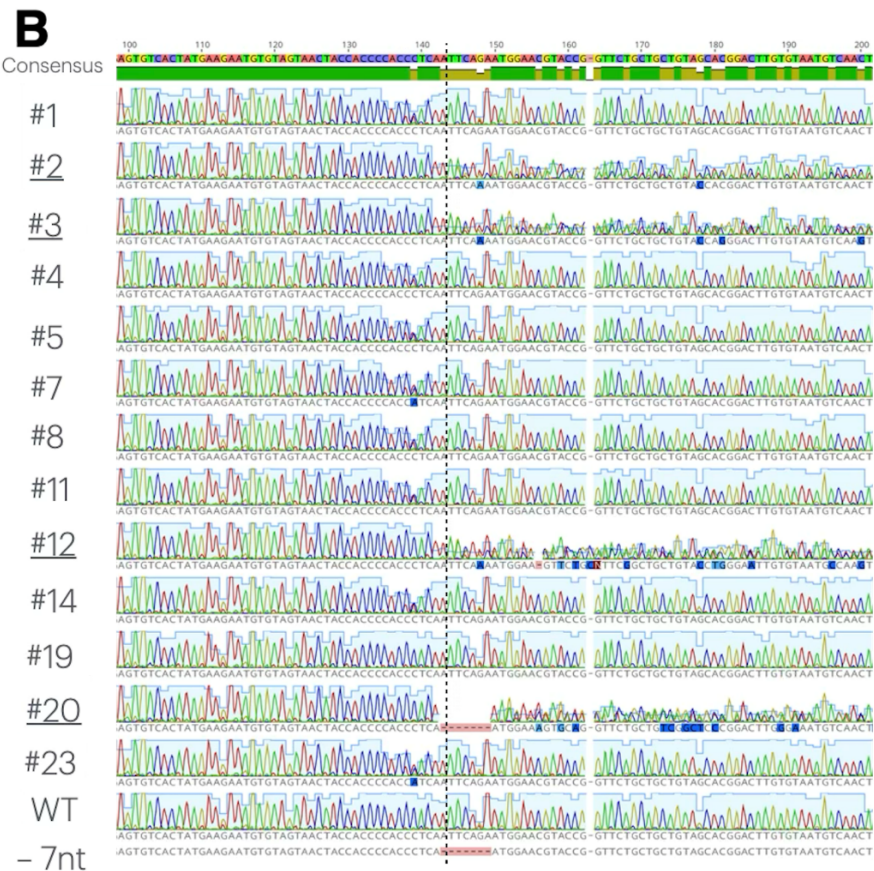
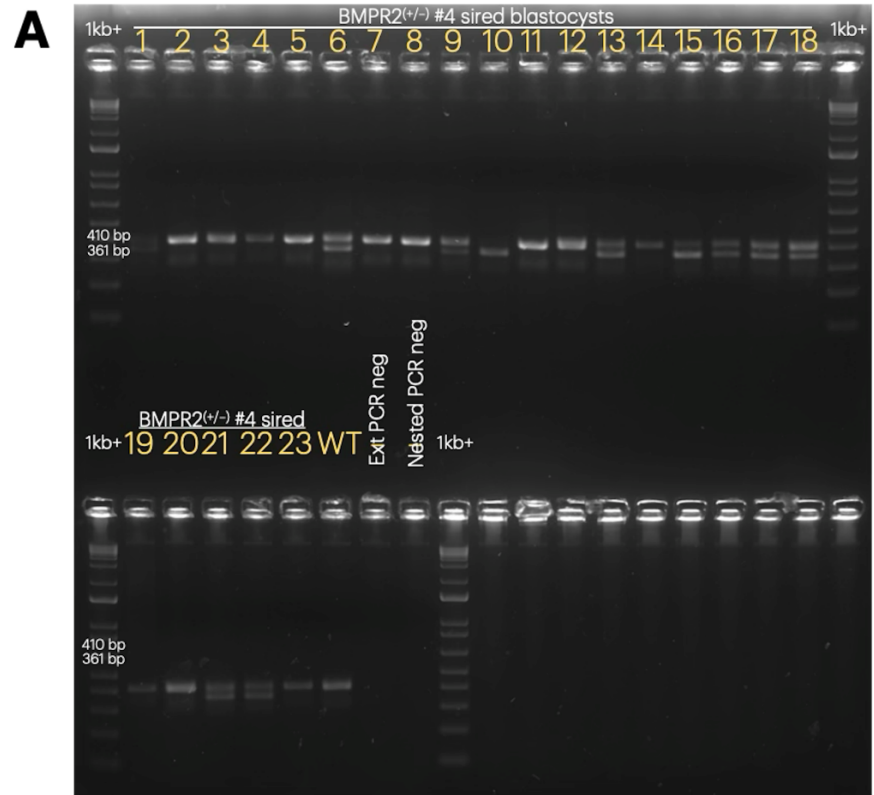


## Supplementary Figures and Tables



**Supplementary Figure 1.** Radiographs of Lamb #6 taken on day of life 1. (A) Arthrogryposis of both forelimbs, with hyperextension of the metacarpal phalangeal joint and hyperflexion of the distal interphalangeal joints; (B) mandibular brachygnathia.

**Supplementary Figure 2.** Analysis of 23 sheep blastocysts produced through in vitro production with Lamb #4 sperm and oocytes derived from slaughterhouse-collected wild type (WT) ovaries. (A) Nested PCR targeting *BMPR2* was performed to identify 11/23 (47.8%) embryos with the -49 bp deletion. (B) Embryos with only the WT-sized band were Sanger sequenced to identify the 4/23 (17.4%) embryos (underlined number) with both WT and the 7 bp deletion alleles. Vertical dashed line indicates canonical cut site of Cas9 with single guide RNA 1.



**Supplementary Table 1.** Predicted nuclei acid sequences of edited and wild type (WT) *BMPR2* alleles. Snapgene software (www.snapgene.com) predicted the amino acid sequences of *BMPR2* edits described in Table 1 and Figure 2. \*indicates stop codon, in-frame insertions are bolded, frame-shifted amino acids are underlined, in-frame deletion locations indicated by /.

|                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|----------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>WT and WT (CAT)</b>                       | MTSSPRRPRRVPSLLWTVLLVSAAAAAQNQERLCAFKDPYQQDLGIGESRISHENGITILCSKGSTC<br>YGLWEKSKGDINLVKQGCWSHIGDPQECHYECCVVTTPPSIQNGTYRFCCCSTDLCNVNFTENFP<br>PPDTPPLSPPHSFNRDETI I IALASVSVLAVLIVALCFGYRMLTGDRKQGLHSMNMMEAAASEPSL<br>DLNKLLELIGRGYGA VYKGS LDERP VAVKVFSXANRQNFINEKN IYRVPLMEHDNIARFIVGD<br>ERV TADGRMEYLLVMEYYPNGSLCKYLSLHTSDWVSSCRLAHSVTRGLAYLHTELPRGDHYKPAIS<br>HRDLNSRNVLVKNDGTCVISDFGLSMKLTGNXLVLRPGEEDNAAISEVGTIRYMAPEVLEGA VNL RD<br>CESALKQVDMYALGLIYWEIFMRCTDLFPGXSVPEYQMAFQTEVGNHPTFEDMQVLVSR EKQRPKF<br>PEAWKENS LA VRSLKETIEDCWD XDAEARLTAQCAEERMAELMMI WERNKSVSPTV NPMSTAMQNE<br>RNL SHNRVPKIGPYPDYSSSSYIEDSIHHTDSIVKNISSEHSMSSSTPLTIG EKNRNSIN YERQQA<br>QARIPSPETS VTSLS T NTTTTNTTGLTPSTGMTTISEVPYPDETS LHATNVSQPVGPTPVCLQLTE<br>EDLETNKLDPK EVDKNLKESSDENLMEHSLKQFSGPDPLSSTSSSLPYPLIKLAVEVTGQQDFTQA<br>ANGQACLI PDVPPTQIYPLPKQQLPKRPTSLPLNTKNSTKEPRLKFGSKHKS NLKQVETGVAKMN<br>TINAAEPHIVTVMNGVAGR NHSVNSHTATTQYANGVVP SGQTANTVAHRAQ EMLQNQF IGEDTRL<br>NINSSPDEHEP LLRREQQAGHDEGVLDRLVDRRERPLEGGRTNSNNNNSNPCSEQEVPTQGV PSTV<br>ADPGPSKPRRAQRPN SLDLSATNVLDGSSLQLGDSTQDGKSGSGEKIKKRVKTPYSLKRWRPSTWV<br>ISTEPLDCEVNNNGKDRAVH SKSSTTVYLADGGTATTMVSKDIGMNCL*           |
| <b>#1 Allele 2 (+21)</b>                     | MTSSPRRPRRVPSLLWTVLLVSAAAAAQNQERLCAFKDPYQQDLGIGESRISHENGITILCSKGSTC<br>YGLWEKSKGDINLVKQGCWSHIGDPQECHYECCVVTTPPS <b>SPCTPPSI</b> QNGTYRFCCCSTDLCNV<br>NFTENFPPDTPPLSPPHSFNRDETI I IALASVSVLAVLIVALCFGYRMLTGDRKQGLHSMNMMEA<br>AASEPSLDL NKLLELIGRGYGA VYKGS LDERP VAVKVFSXANRQNFINEKN IYRVPLMEHDNI<br>ARFIVGDERVTADGRMEYLLVMEYYPNGSLCKYLSLHTSDWVSSCRLAHSVTRGLAYLHTELPRGD<br>HYKPAISHRDLNSRNVLVKNDGTCVISDFGLSMKLTGNXLVLRPGEEDNAAISEVGTIRYMAPEVLE<br>GAVNLRDCESALKQVDMYALGLIYWEIFMRCTDLFPGXSVPEYQMAFQTEVGNHPTFEDMQVLVSR<br>EKQRPKFPEAWKENS LA VRSLKETIEDCWD XDAEARLTAQCAEERMAELMMI WERNKSVSPTV NPM<br>STAMQNERNL SHNRVPKIGPYPDYSSSSYIEDSIHHTDSIVKNISSEHSMSSSTPLTIG EKNRNSI<br>NYERQQAQARIPSPETS VTSLS T NTTTTNTTGLTPSTGMTTISEVPYPDETS LHATNVSQPVGPTP<br>VCLQLTEEDLETNKLDPK EVDKNLKESSDENLMEHSLKQFSGPDPLSSTSSSLPYPLIKLAVEVTG<br>QQDFTQAANGQACLI PDVPPTQIYPLPKQQLPKRPTSLPLNTKNSTKEPRLKFGSKHKS NLKQVE<br>TGVAKMNTINAAEPHIVTVMNGVAGR NHSVNSHTATTQYANGVVP SGQTANTVAHRAQ EMLQNQF<br>IGEDTRLNINSSPDEHEP LLRREQQAGHDEGVLDRLVDRRERPLEGGRTNSNNNNSNPCSEQEVPT<br>QGV PSTVADPGPSKPRRAQRPN SLDLSATNVLDGSSLQLGDSTQDGKSGSGEKIKKRVKTPYSLKR<br>WRPSTWVISTEPLDCEVNNNGKDRAVH SKSSTTVYLADGGTATTMVSKDIGMNCL* |
| <b>#2 Allele 2 (-4,+3)</b>                   | MTSSPRRPRRVPSLLWTVLLVSAAAAAQNQERLCAFKDPYQQDLGIGESRISHENGITILCSKGSTC<br>YGLWEKSKGDINLVKQGCWSHIGDPQECHYECCVVTTPPTFRMERTGSAAVARTCVMSTLLRIFH<br><u>LQTQHHSVHLIHLTEM RQ*</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>#3 Allele 3 (+1)</b>                      | MTSSPRRPRRVPSLLWTVLLVSAAAAAQNQERLCAFKDPYQQDLGIGESRISHENGITILCSKGSTC<br>YGLWEKSKGDINLVKQGCWSHIGDPQECHYECCVVTTPPSNSEWNVPVLLL*                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <b>#4 Allele 2 (-49)</b>                     | MTSSPRRPRRVPSLLWTVLLVSAAAAAQNQERLCAFKDPYQQDLGIGESRISHENGITILCSKGSTC<br>YGLWEKSKGDINLVKQGCWSHIGDPQECHYECCVVTTPPSMSTLLRIFHLQTQHHSVHLIHLTEM<br>RQ*                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <b>#4 Allele 3 (-7) and #6 Allele 2 (-7)</b> | MTSSPRRPRRVPSLLWTVLLVSAAAAAQNQERLCAFKDPYQQDLGIGESRISHENGITILCSKGSTC<br>YGLWEKSKGDINLVKQGCWSHIGDPQECHYECCVVTTPPSMERTGSAAVARTCVMSTLLRIFHLQ<br><u>TQHHSVHLIHLTEM RQ*</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>#5 Allele 2 (-9,+1)</b>                   | MTSSPRRPRRVPSLLWTVLLVSAAAAAQNQERLCAFKDPYQQDLGIGESRISHENGITILCSKGSTC<br>YGLWEKSKGDINLVKQGCWSHIGDPQECHYECCVVTTPPSTNVPVLLL*                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>#5 Allele 2 (-15)</b>                     | MTSSPRRPRRVPSLLWTVLLVSAAAAAQNQERLCAFKDPYQQDLGIGESRISHENGITILCSKGSTC<br>YGLWEKSKGDINLVKQGCWSHIGDPQECHYECCVVT / IQNGTYRFCCCSTDLCNVNFTENFPPDTP<br>TPLSPPHSFNRDETI I IALASVSVLAVLIVALCFGYRMLTGDRKQGLHSMNMMEAAASEPSLDL N<br>LKLELIGRGYGA VYKGS LDERP VAVKVFSXANRQNFINEKN IYRVPLMEHDNIARFIVGDERVT<br>ADGRMEYLLVMEYYPNGSLCKYLSLHTSDWVSSCRLAHSVTRGLAYLHTELPRGDHYKPAISHRDL                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |

|                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                              | NSRNVLVKNDGTCVISDFGLSMKLTGNXLVRPGEEDNAAISEVGTIRYMAPEVLEGAVNLRDCESA<br>LKQVDMYALGLIYWEIFMRCTDLFPGXSVPEYQMAFQTEVGNHPTFEDMQVLVSREKQRPKFPEAW<br>KENSLAVRSLKETIEDCWDXDAEARLTAQCAEERMAELMMIWERNKSVSPTVNPMTAMQNERNLS<br>HNRRVPKIGPYPDYSSSSYIEDSIHHTDSIVKNISSEHSMSSTPLTIGEKNRNSINYERQQAQARI<br>PSPETSVTSLSTNTTTTNTTGLTPSTGMTTISEVPYPDETS LHATNVSQPVGPTPVCLQLTEEDLE<br>TNKLDPKVEVDKNLKESSDENLMEHSLKQFSGPDPLSSTSSSLPYPLIKLAVEVTGQQDFTQAANGQ<br>ACLIPDVPPTQIYPLPKQONLPKRPTSLPLNTKNSTKEPRLKFGSKHKSNLKQVETGVAKMNTINA<br>AEPHIVTVTMNGVAGRNVNSHTATTQYANGVVPSTANTVAHRAQEMLQNQFIGEDTRLNINS<br>SPDEHEPLLRRQQAGHDEGVLDRLVDRRERPLEGGRTNSNNNSNPCSEQEVPTQGVPTVADPG<br>PSKPRRAQRPNSLDLSATNVLDGSSLQLGDSTQDGKSGSGEKKRVKTPYSLKRWRPSTWVISTE<br>PLDCEVNNNGKDRVHSHKSSSTTVYLADGGTATTMVSKDIGMNCL* |
| <b>#8 Allele 2<br/>(+94)</b> | MTSSPRRPRRVPSLLWTVLLVSAAAASQNQERLCAFKDPYQQDLGIGESRISHENGITLCSKGSTC<br>YGLWEKSKGDINLVKQGCWSHIGDPQECHYECCVVTTPPSIQNGTYRFCCCSTDLCNVNYEEMCS<br>NYHPTINSEWNVPVLLL*                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |

**Supplementary Table 2.** PCR primers, reaction conditions, cycling conditions, expected wild type (WT) product size and quantitative PCR amplification efficiency (E). Primers were 10  $\mu$ M except where indicated. GoTaq Green, Promega. KAPAG2 Robust, Roche. Q5 High-Fidelity, New England Biolabs. Apex Taq RED, Genesee Scientific.

| Purpose                                           | Primer                                                                                                                                                                                                                                                                                                                                                | Reagents/rxn                                                                                                                                                 | Rxn conditions                                                           | WT Amplicon Size (bp)   | E (%)                  |
|---------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|-------------------------|------------------------|
| Nested_1 for Blastocysts Sanger Sequencing/ TIDER | Intron2-F: ACAGCAGAAGGACTTAGCCA<br>Intron3-R: TCTTGAAATCCTGGTGTACCTT                                                                                                                                                                                                                                                                                  | 1 $\mu$ L Primer Mix<br>10 $\mu$ L KAPAG2 Robust ReadyMix<br>4 $\mu$ L Water<br>5 $\mu$ L Lysis product                                                      | 95°C 5 mins<br>[95°C 15 s<br>58°C 30 s<br>72°C 1 min] x35<br>72°C 5 mins | 838                     | N/A                    |
| Nested_2 for Blastocysts Sanger Sequencing/ TIDER | Intron2-F2: TTGTTTGCAATTGTTTGTGTTTTCC<br>Intron3-R2: TCATAATGCTGGAACCTCTCTCTT                                                                                                                                                                                                                                                                         | 1 $\mu$ L Primer Mix<br>10 $\mu$ L KAPAG2 Robust ReadyMix<br>9 $\mu$ L Water<br>5 $\mu$ L Nested 1 Product                                                   | 95°C 3 mins<br>[95°C 15 s<br>60°C 30 s<br>72°C 1 min] x35<br>72°C 5 mins | 376                     | N/A                    |
| Genotype Tissues/ Blood                           | Intron2-F2: TTGTTTGCAATTGTTTGTGTTTTCC<br>Intron3-R: TCTTGAAATCCTGGTGTACCTT                                                                                                                                                                                                                                                                            | 1 $\mu$ L F Primer<br>1 $\mu$ L R Primer<br>12.5 $\mu$ L GoTaq Green<br>10.5 $\mu$ L Water<br>1 $\mu$ L DNA                                                  | 95°C 5 mins<br>[95°C 15 s<br>60°C 30 s<br>72°C 45 s] x35<br>72°C 5 mins  | 431                     | N/A                    |
| Rare/mosaic allele identification (Lambs 1-7)     | Intron2-F2: TTGTTTGCAATTGTTTGTGTTTTCC<br>Intron3-R: TCTTGAAATCCTGGTGTACCTT                                                                                                                                                                                                                                                                            | 500 nM ea. primer<br>Q5 High-Fidelity Master Mix                                                                                                             | 98°C 30 s<br>[98°C 10 s<br>60°C 30 s<br>72°C 30 s] x35<br>72°C 5 mins    | 431                     | N/A                    |
| Rare/mosaic allele identification (Lamb 8)        | Exon3-F: GGTGATCCCCAGGAGTGTC<br>7DelIntron3_R: TCTTTGGAGAAAGGAATTTTCAGGA                                                                                                                                                                                                                                                                              | 500 nM ea. primer<br>Q5 High-Fidelity Master Mix                                                                                                             | 98°C 2 mins<br>[98°C 10 s<br>60°C 30 s<br>72°C 25 s] x40<br>72°C 10 mins | 201                     | N/A                    |
| Semi-Quantitative RT-PCR of BMPR2 (Lambs 4, 8)    | cDNAEx2_F: CGTCTCTGTGCATTTAAAGATCCA<br>cDNAEx4_R: TGTCAGCATCTATATCCAAAGCA                                                                                                                                                                                                                                                                             | 400 nM ea primer<br>Apex Taq RED                                                                                                                             | 95°C 2 mins<br>[95°C 15 s<br>60°C 30 s<br>72°C 45 s] x37<br>72°C 5 mins  | 435                     | N/A                    |
| Semi-Quantitative RT-PCR of ACTB (Lambs 4, 8)     | ACTB_F3: GACACCGCAACCAGTTCG<br>ACTB_R3: CCCACCATCACGCCCTG                                                                                                                                                                                                                                                                                             | 400 nM ea primer<br>Apex Taq RED                                                                                                                             | 95°C 2 mins<br>[95°C 15 s<br>60°C 30 s<br>72°C 30 s] x30<br>72°C 5 mins  | 157                     | N/A                    |
| Quantitative PCR                                  | ACTB_qPCR_F: TCCTGGGCATGGAATCCTG<br>ACTB_qPCR_R: GGGGCGCGATGATCTTGAT<br>ACTB Ex5Probe: 6-FAM/CGGGACCAC/ZEN/CATGTACCCTGGCATCG/IA BkFQ<br>BMPR2cDNA_F4: TCCCCAGGAGTGTCACTATGAA<br>BMPR2qPCR R3-2: ATGATTATTGTCTCATCTCGGTTAAAT<br>BMPR2_Ex3_WTC_Probe: SUN/CACC+CTC+AAT+TCA+GAATGG/IABkFQ<br>BMPR2_Ex3_WTA_LNA_Probe: SUN/CACCAT+CAA+TTC+AGA+ATGG/IABkFQ | 5 $\mu$ L TaqMan Fast Advanced (ABI)<br>900 nM ea. primer<br>150 nM ea. BMPR2 probe<br>250 nM ACTB probe<br>1 $\mu$ L cDNA<br>Water to 10 $\mu$ L final vol. | 50°C 2 mins<br>95°C 20 s<br>[95°C 5 s<br>60°C 20 s] x40                  | ACTB: 201<br>BMPR2: 189 | ACTB: 104<br>BMPR2: 97 |
| Nested_1 for Germline Transmission                | Intron2-F: ACAGCAGAAGGACTTAGCCA<br>Intron3-R: TCTTGAAATCCTGGTGTACCTT                                                                                                                                                                                                                                                                                  | 0.4 $\mu$ L F Primer<br>0.4 $\mu$ L R Primer<br>10 $\mu$ L GoTaq Green<br>4.2 $\mu$ L Water<br>5 $\mu$ L Lysis product                                       | 95°C 5 mins<br>[95°C 15 s<br>55°C 30 s<br>72°C 90 s] x40<br>72°C 5 mins  | 842                     | N/A                    |
| Nested_2 for Germline Transmission                | oBPMR2-Ex3-NestR2_F: TGTA CTCCATCACATTGTTTGCA<br>oBPMR2-Ex3-NestR2_R: GCCATTAGCTAGATGTACTCTCA                                                                                                                                                                                                                                                         | 0.4 $\mu$ L F Primer<br>0.4 $\mu$ L R Primer<br>10 $\mu$ L GoTaq Green<br>7.2 $\mu$ L Water<br>2 $\mu$ L Nested 1 product                                    | 95°C 2 mins<br>[95°C 15 s<br>62°C 30 s<br>72°C 1 min] x35<br>72°C 5 mins | 410                     | N/A                    |