Central Manchester University Hospitals NHS
NHS Foundation Trust

# A complex NF2 mutation detected by custom Next Generation Sequencing (NGS) analysis

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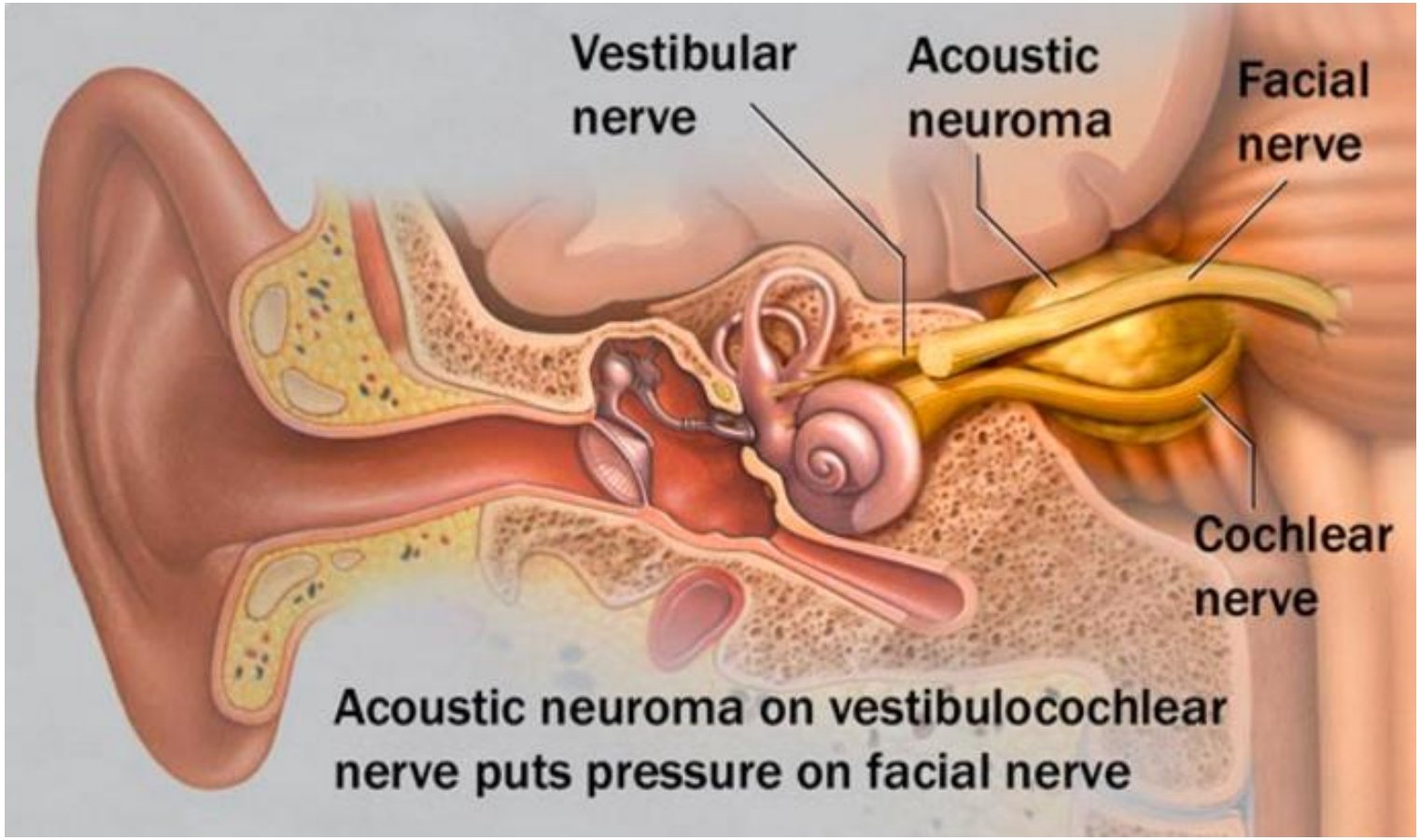
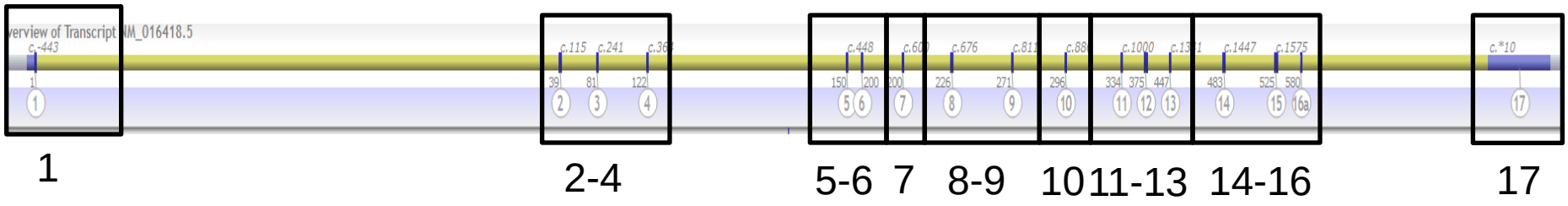
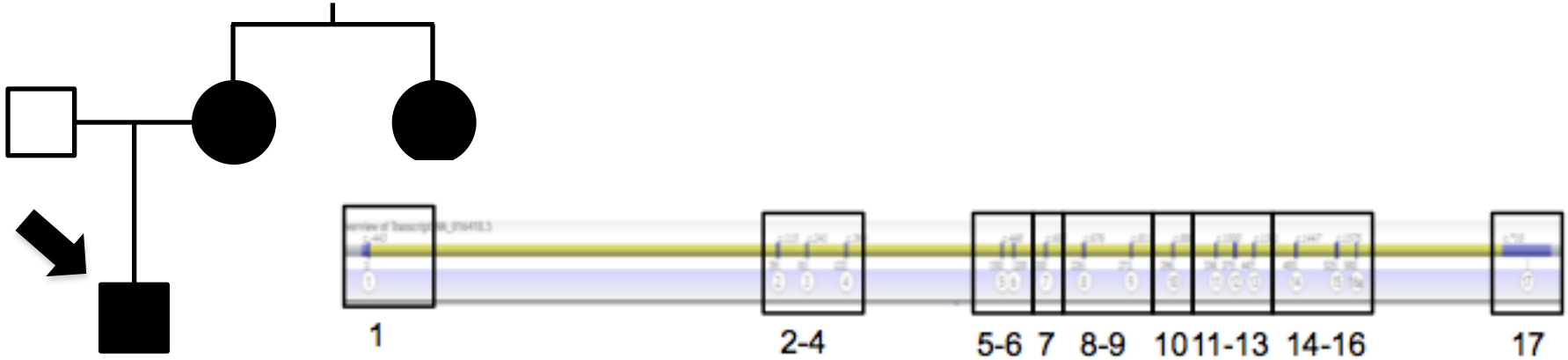
NF2 is an autosomal dominant cancer syndrome characterized by multiple benign nervous system tumours, most commonly bilateral vestibular schwannomas (BVS)



Incidence of NF2 1 in 33,000
Caused by mutations in the schwannomin/merlin gene
NF2 analysis is complex and challenging - a high degree of mosaicism in blood
NF2 is screened by LR-PCR NGS and MLPA
The whole coding region and flanking sequences +/- 15bp is analysed by NGS at high coverage depth (minimum >1000x)



Patient SG diagnosed with BVS at 24 years
Additional strong family history



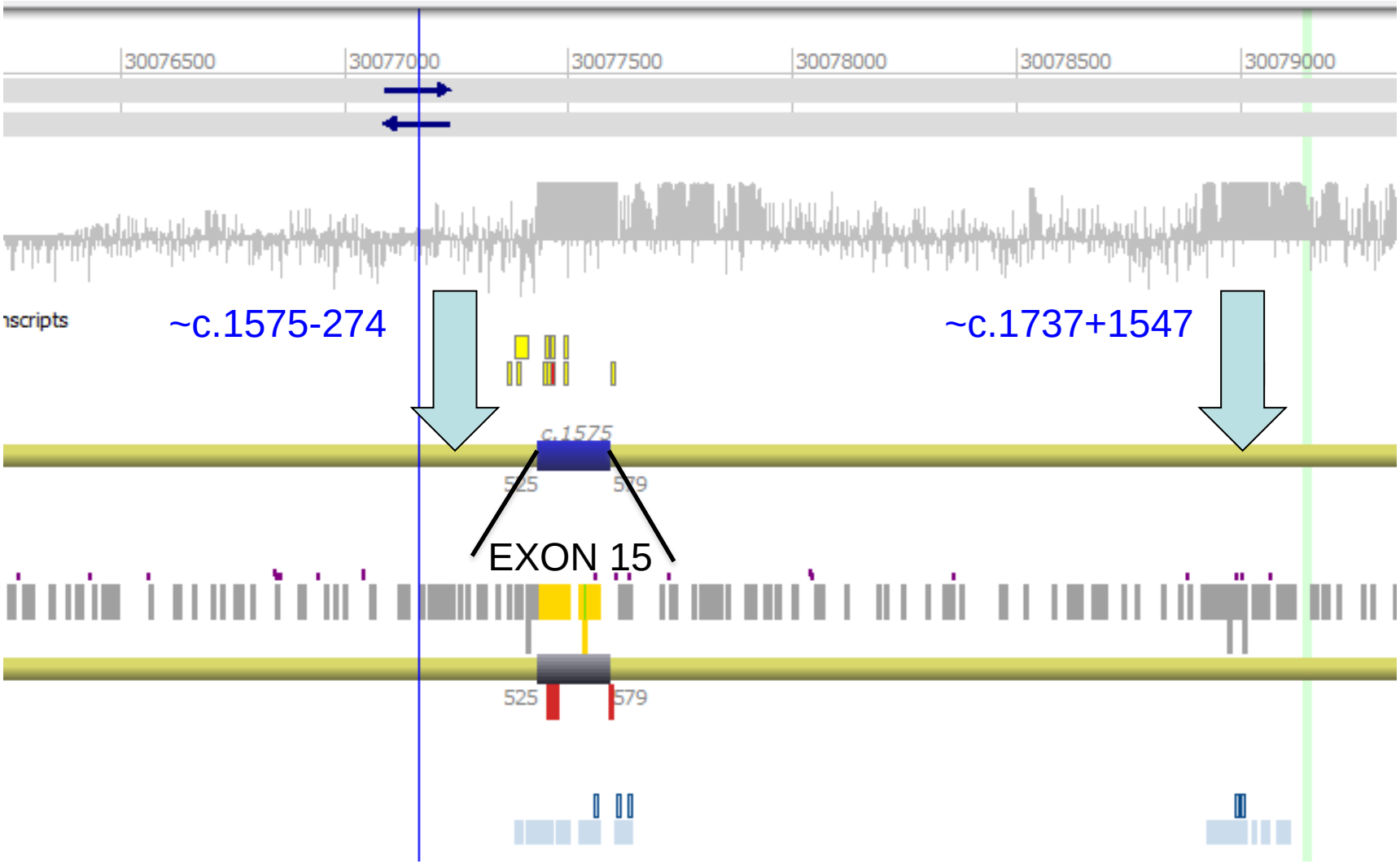

No mutation identified!
Likelihood that a mutation having been missed by standard analysis
Requested a “custom” bioinformatics analysis – the report includes all variants within the NF2 gene region

| | A | B | C | D | E | F | G | H | I | J | K | L | M |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Chr | St | End | Gene | Transcrip | HGVS_cDI | HGVS_pr | RunFreq | Depth | Ratio(%) | MeanRati | Allele1_C | Allele2_C |
| 2 | 22 | 30072163 | 30072163 | 4771;NF2 | NM_00026 | c.1446+12 | - | 16/16 | 5070 | 18.01 | 16.68 | 3502 | 769 |
| 3 | 22 | 30091089 | 30091090 | 4771;NF2 | NM_00026 | c.*298_*29 | - | 16/16 | 5051 | 4.11 | 3.71 | 4786 | 205 |
| 4 | 22 | 30001097 | 30001097 | 4771;NF2 | NM_00026 | c.114+996 | - | 15/16 | 3566 | 4.38 | 4.58 | 3343 | 153 |
| 5 | 22 | 30001122 | 30001122 | 4771;NF2 | NM_00026 | c.114+102 | - | 10/16 | 3678 | 6.34 | 6.89 | 3337 | 226 |
| 6 | 22 | 30001166 | 30001166 | 4771;NF2 | NM_00026 | c.114+106 | - | 5/16 | 3627 | 6.36 | 7.38 | 3223 | 219 |
| 7 | 22 | 30001184 | 30001184 | 4771;NF2 | NM_00026 | c.114+108 | - | 16/16 | 3475 | 12.06 | 13.94 | 2967 | 407 |
| 8 | 22 | 30001227 | 30001227 | 4771;NF2 | NM_00026 | c.114+112 | - | 12/16 | 3480 | 4.57 | 4.3 | 3280 | 157 |
| 9 | 22 | 30001231 | 30001231 | 4771;NF2 | NM_00026 | c.114+113 | - | 14/16 | 3354 | 4.23 | 4.09 | 3144 | 139 |
| 10 | 22 | 30001235 | 30001235 | 4771;NF2 | NM_00026 | c.114+113 | - | 16/16 | 3380 | 10.17 | 10.41 | 2940 | 333 |
| 11 | 22 | 30035780 | 30035780 | 4771;NF2 | NM_00026 | c.363+579 | - | 16/16 | 4515 | 4.19 | 4.41 | 4298 | 188 |
| 12 | 22 | 30035795 | 30035795 | 4771;NF2 | NM_00026 | c.363+594 | - | 15/16 | 4819 | 4.21 | 4.53 | 4389 | 193 |
| 13 | 22 | 30035801 | 30035801 | 4771;NF2 | NM_00026 | c.363+600 | - | 16/16 | 4907 | 5.35 | 4.72 | 4549 | 257 |
| 14 | 22 | 30035808 | 30035808 | 4771;NF2 | NM_00026 | c.363+607 | - | 16/16 | 4943 | 5.73 | 5.97 | 4524 | 275 |
| 15 | 22 | 30035814 | 30035814 | 4771;NF2 | NM_00026 | c.363+613 | - | 16/16 | 4821 | 11.27 | 10.8 | 4143 | 526 |
| 16 | 22 | 30035824 | 30035824 | 4771;NF2 | NM_00026 | c.363+623 | - | 7/16 | 5027 | 6.48 | 6.43 | 4317 | 299 |
| 17 | 22 | 30035833 | 30035833 | 4771;NF2 | NM_00026 | c.363+632 | - | 15/16 | 4860 | 5.47 | 5.6 | 4252 | 246 |
| 18 | 22 | 30035862 | 30035862 | 4771;NF2 | NM_00026 | c.363+661 | - | 16/16 | 5033 | 5.73 | 6.12 | 4472 | 272 |
| 19 | 22 | 30035867 | 30035867 | 4771;NF2 | NM_00026 | c.363+666 | - | 16/16 | 4948 | 6.3 | 6.98 | 4298 | 289 |
| 20 | 22 | 30035898 | 30035898 | 4771;NF2 | NM_00026 | c.363+697 | - | 16/16 | 4734 | 5.77 | 6.08 | 4183 | 256 |
| 21 | 22 | 30035899 | 30035899 | 4771;NF2 | NM_00026 | c.363+698 | - | 15/16 | 4728 | 7.47 | 8.48 | 3900 | 315 |
| 22 | 22 | 30035902 | 30035902 | 4771;NF2 | NM_00026 | c.363+701 | - | 16/16 | 4796 | 9.73 | 10 | 3989 | 430 |
| 23 | 22 | 30035915 | 30035915 | 4771;NF2 | NM_00026 | c.363+714 | - | 16/16 | 4979 | 16.75 | 16.48 | 3931 | 791 |
| 24 | 22 | 30036121 | 30036121 | 4771;NF2 | NM_00026 | c.363+920 | - | 14/16 | 5190 | 5.16 | 4.65 | 4830 | 263 |
| 25 | 22 | 30036759 | 30036759 | 4771;NF2 | NM_00026 | c.364-143 | - | 16/16 | 3864 | 7.89 | 6.42 | 2137 | 183 |
| 26 | 22 | 30036779 | 30036779 | 4771;NF2 | NM_00026 | c.364-141 | - | 16/16 | 4273 | 5.18 | 4.77 | 2950 | 161 |
| 27 | 22 | 30036924 | 30036924 | 4771;NF2 | NM_00026 | c.364-126 | - | 6/16 | 4417 | 4.12 | 4.45 | 4185 | 180 |
| 28 | 22 | 30038477 | 30038477 | 4771;NF2 | NM_00026 | c.447+203 | - | 16/16 | 5119 | 4.79 | 4.26 | 4791 | 241 |
| 29 | 22 | 30052431 | 30052431 | 4771;NF2 | NM_00026 | c.599+766 | - | 12/16 | 5411 | 5.44 | 5.11 | 5036 | 290 |
| 30 | 22 | 30052433 | 30052433 | 4771;NF2 | NM_00026 | c.599+768 | - | 16/16 | 5418 | 12.26 | 11.7 | 4574 | 639 |
| 31 | 22 | 30052435 | 30052435 | 4771;NF2 | NM_00026 | c.599+770 | - | 14/16 | 5371 | 6.91 | 6.51 | 4906 | 364 |
| 32 | 22 | 30052438 | 30052438 | 4771;NF2 | NM_00026 | c.599+773 | - | 16/16 | 5316 | 4.96 | 5.28 | 4869 | 254 |
| 33 | 22 | 30052441 | 30052441 | 4771;NF2 | NM_00026 | c.599+776 | - | 16/16 | 5391 | 14.24 | 14.49 | 4416 | 733 |
| 34 | 22 | 30052448 | 30052448 | 4771;NF2 | NM_00026 | c.599+783 | - | 16/16 | 5575 | 5.88 | 6.34 | 5038 | 315 |
| 35 | 22 | 30052453 | 30052453 | 4771;NF2 | NM_00026 | c.599+788 | - | 16/16 | 5678 | 4.45 | 5.21 | 5330 | 248 |
| 36 | 22 | 30052454 | 30052454 | 4771;NF2 | NM_00026 | c.599+789 | - | 10/16 | 5653 | 7.67 | 8.17 | 5150 | 428 |
| 37 | 22 | 30052455 | 30052455 | 4771;NF2 | NM_00026 | c.599+790 | - | 13/16 | 5609 | 5.33 | 5.67 | 5201 | 293 |
| 38 | 22 | 30052457 | 30052457 | 4771;NF2 | NM_00026 | c.599+792 | - | 16/16 | 5520 | 4.64 | 5.19 | 4846 | 236 |
| 39 | 22 | 30052458 | 30052458 | 4771;NF2 | NM_00026 | c.599+793 | - | 16/16 | 5517 | 4.14 | 4.96 | 5183 | 224 |
| 40 | 22 | 30052459 | 30052459 | 4771;NF2 | NM_00026 | c.599+794 | - | 16/16 | 5502 | 11.17 | 10.87 | 4813 | 605 |
| 41 | 22 | 30052466 | 30052466 | 4771;NF2 | NM_00026 | c.599+801 | - | 16/16 | 5581 | 7.46 | 9.03 | 4789 | 386 |
| 42 | 22 | 30052468 | 30052468 | 4771;NF2 | NM_00026 | c.599+803 | - | 15/16 | 5626 | 8.32 | 9.13 | 5082 | 461 |
| 43 | 22 | 30052469 | 30052469 | 4771;NF2 | NM_00026 | c.599+804 | - | 16/16 | 5612 | 5.38 | 5.6 | 5220 | 297 |
| 44 | 22 | 30052474 | 30052474 | 4771;NF2 | NM_00026 | c.599+809 | - | 14/16 | 5663 | 10.15 | 10.39 | 5009 | 566 |
| 45 | 22 | 30052476 | 30052476 | 4771;NF2 | NM_00026 | c.599+811 | - | 16/16 | 5736 | 14.13 | 14.69 | 4729 | 778 |
| 46 | 22 | 30052478 | 30052478 | 4771;NF2 | NM_00026 | c.599+813 | - | 15/16 | 5822 | 4.21 | 4.37 | 5394 | 237 |
| 47 | 22 | 30052481 | 30052481 | 4771;NF2 | NM_00026 | c.599+816 | - | 16/16 | 5849 | 6.45 | 6.6 | 5107 | 352 |
| 48 | 22 | 30052482 | 30052482 | 4771;NF2 | NM_00026 | c.599+817 | - | 16/16 | 5853 | 4.18 | 4.02 | 5485 | 239 |
| 49 | 22 | 30052483 | 30052483 | 4771;NF2 | NM_00026 | c.599+818 | - | 16/16 | 5868 | 5.09 | 5.3 | 5501 | 295 |
| 50 | 22 | 30052484 | 30052484 | 4771;NF2 | NM_00026 | c.599+819 | - | 16/16 | 5903 | 11.12 | 11.56 | 5169 | 647 |
| 51 | 22 | 30052490 | 30052490 | 4771;NF2 | NM_00026 | c.599+825 | - | 16/16 | 5884 | 14.1 | 14.44 | 4983 | 818 |
| 52 | 22 | 30052496 | 30052496 | 4771;NF2 | NM_00026 | c.599+831 | - | 3/16 | 6022 | 7.01 | 6.69 | 5528 | 417 |
| 53 | 22 | 30052500 | 30052500 | 4771;NF2 | NM_00026 | c.599+835 | - | 16/16 | 5871 | 10.19 | 9.79 | 5139 | 583 |
| 54 | 22 | 30052502 | 30052502 | 4771;NF2 | NM_00026 | c.599+837 | - | 16/16 | 5893 | 5.68 | 5.94 | 5463 | 329 |
| 55 | 22 | 30052509 | 30052509 | 4771;NF2 | NM_00026 | c.599+844 | - | 16/16 | 5943 | 7.74 | 7.92 | 5422 | 455 |
| 56 | 22 | 30052512 | 30052512 | 4771;NF2 | NM_00026 | c.599+847 | - | 14/16 | 5818 | 4.69 | 4.9 | 5406 | 266 |
| 57 | 22 | 30052513 | 30052513 | 4771;NF2 | NM_00026 | c.599+848 | - | 16/16 | 5851 | 4.02 | 4.25 | 5440 | 228 |
| 58 | 22 | 30052516 | 30052516 | 4771;NF2 | NM_00026 | c.599+851 | - | 16/16 | 5825 | 6.67 | 7.22 | 5355 | 383 |
| 59 | 22 | 30052519 | 30052519 | 4771;NF2 | NM_00026 | c.599+854 | - | 16/16 | 5626 | 4.21 | 4.56 | 5276 | 232 |
| 60 | 22 | 30052522 | 30052522 | 4771;NF2 | NM_00026 | c.599+857 | - | 16/16 | 5599 | 8.13 | 8.57 | 5072 | 449 |
| 61 | 22 | 30052527 | 30052527 | 4771;NF2 | NM_00026 | c.599+862 | - | 16/16 | 5557 | 4.08 | 4.43 | 5119 | 218 |
| 62 | 22 | 30052836 | 30052836 | 4771;NF2 | NM_00026 | c.599+117 | - | 16/16 | 2806 | 7.25 | 8.27 | 2289 | 179 |
| 63 | 22 | 30052837 | 30052837 | 4771;NF2 | NM_00026 | c.599+117 | - | 15/16 | 2759 | 5.63 | 5.54 | 2313 | 138 |
| 64 | 22 | 30052838 | 30052838 | 4771;NF2 | NM_00026 | c.599+117 | - | 16/16 | 2734 | 6.06 | 6.31 | 2248 | 145 |
| 65 | 22 | 30052839 | 30052839 | 4771;NF2 | NM_00026 | c.599+117 | - | 15/16 | 2719 | 5.32 | 5.25 | 2260 | 127 |
| 66 | 22 | 30052840 | 30052840 | 4771;NF2 | NM_00026 | c.599+117 | - | 13/16 | 2674 | 5.87 | 5.59 | 2165 | 135 |
| 67 | 22 | 30052841 | 30052841 | 4771;NF2 | NM_00026 | c.599+117 | - | 16/16 | 2671 | 5.45 | 5.51 | 2203 | 127 |
| 68 | 22 | 30052842 | 30052842 | 4771;NF2 | NM_00026 | c.599+117 | - | 16/16 | 2593 | 7.07 | 6.53 | 2091 | 159 |

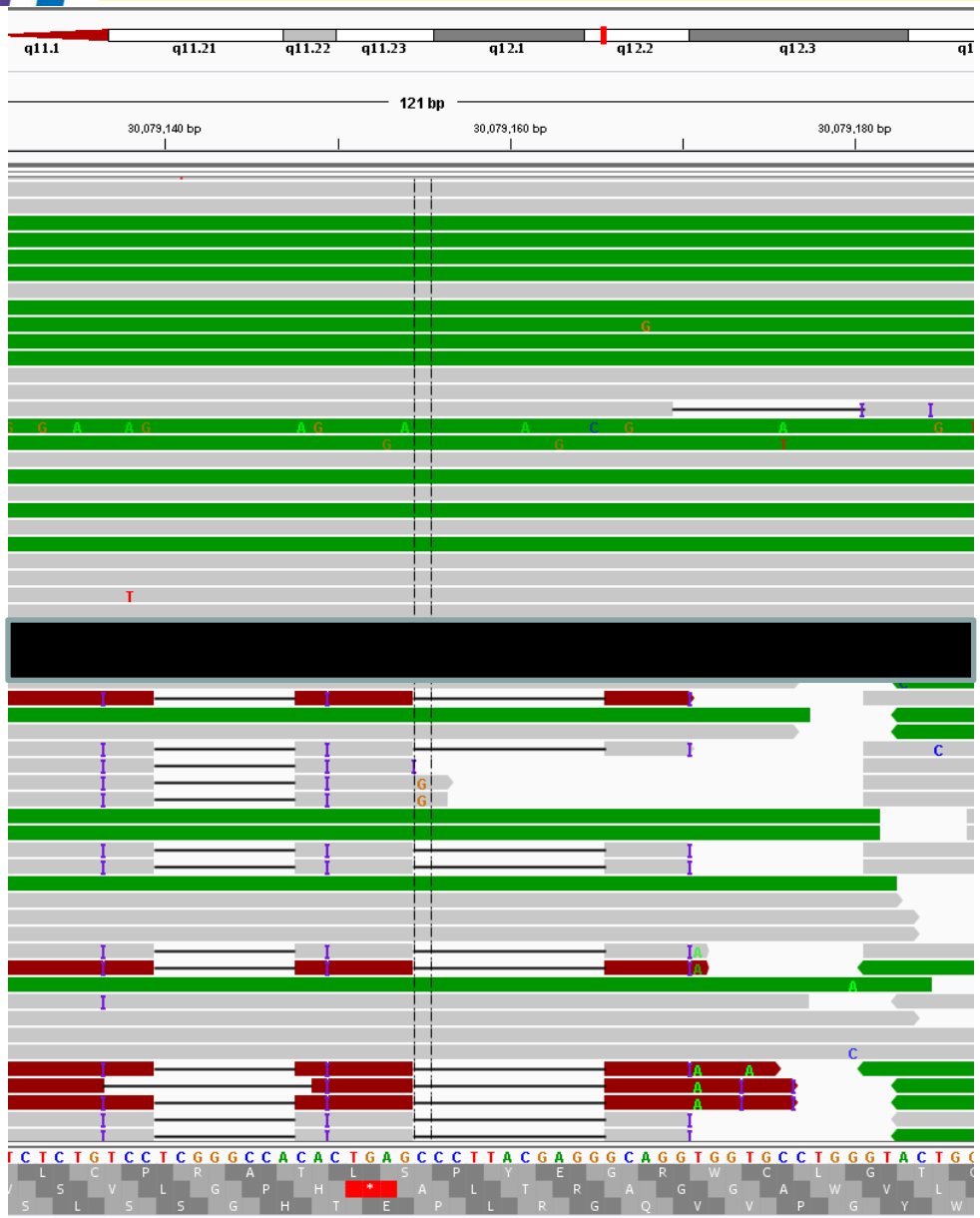
295 extra calls!

| 1 | Chr | St | End | Gene | Transc | RunFr | HGVS_cDNA |
|---|---|---|---|---|---|---|---|
| 80 | 22 | 30077119 | 30077120 | 4771;NF2 | NM_0002 | 1/16 | c.1575-309_1575-308delAT |
| 81 | 22 | 30077129 | 30077130 | 4771;NF2 | NM_0002 | 1/16 | c.1575-299_1575-298insCTCGTAAGGGC |
| 82 | 22 | 30077134 | 30077152 | 4771;NF2 | NM_0002 | 1/16 | c.1575-294_1575-276delCCACCCAAAGTACTGGGAT |
| 83 | 22 | 30077153 | 30077154 | 4771;NF2 | NM_0002 | 1/16 | c.1575-275_1575-274insCAGAG |
| 84 | 22 | 30077201 | 30077203 | 4771;NF2 | NM_0002 | 1/16 | c.1575-227_1575-225delCTA |
| 85 | 22 | 30079122 | 30079123 | 4771;NF2 | NM_0002 | 1/16 | c.1737+1532_1737+1533insGCCAGGCACGGTGGCTCCA |
| 86 | 22 | 30079124 | 30079133 | 4771;NF2 | NM_0002 | 1/16 | c.1737+1534_1737+1543delTTGCCTGTCT |
| 87 | 22 | 30079128 | 30079129 | 4771;NF2 | NM_0002 | 1/16 | c.1737+1538_1737+1539insAGGCACGG |
| 88 | 22 | 30079134 | 30079135 | 4771;NF2 | NM_0002 | 1/16 | c.1737+1544_1737+1545insCACC |
| 89 | 22 | 30079136 | 30079137 | 4771;NF2 | NM_0002 | 1/16 | c.1737+1546_1737+1547insAA |
| 90 | 22 | 30079140 | 30079147 | 4771;NF2 | NM_0002 | 1/16 | c.1737+1550_1737+1557delTCGGGCCA |
| 91 | 22 | 30079149 | 30079150 | 4771;NF2 | NM_0002 | 1/16 | c.1737+1559_1737+1560insGTACTTTGGGTGG |
| 92 | 22 | 30079155 | 30079165 | 4771;NF2 | NM_0002 | 1/16 | c.1737+1565_1737+1575delCCCTTACGAGG |
| 108 | | | | | | | |

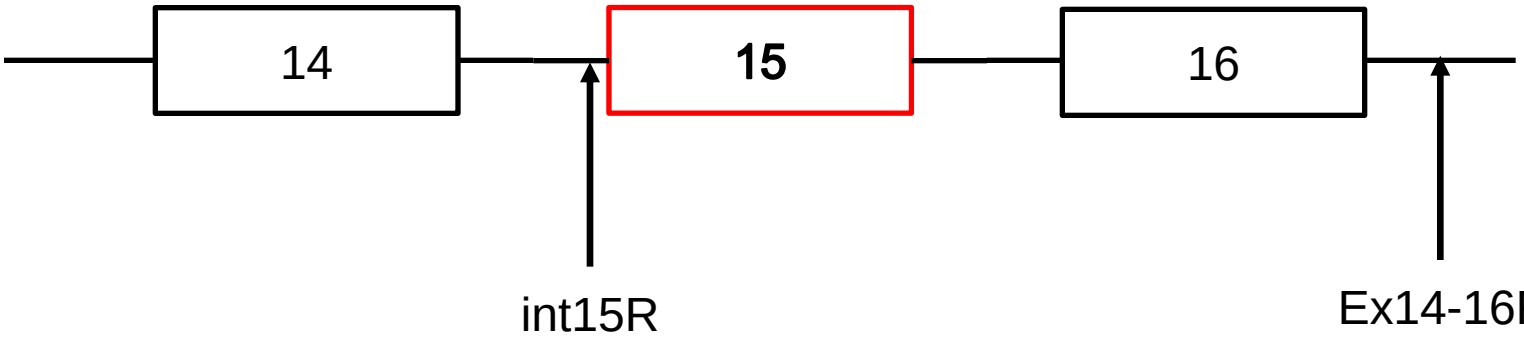
Two potential regions of interest flanking NF2 Ex15 where a significant number of ‘broken’ reads were identified by Pindel


30076500
30077000
30077500
30078000
30078500
30079000
~c.1575-274
~c.1737+1547
c.1575
525
579
EXON 15
525
579

SG

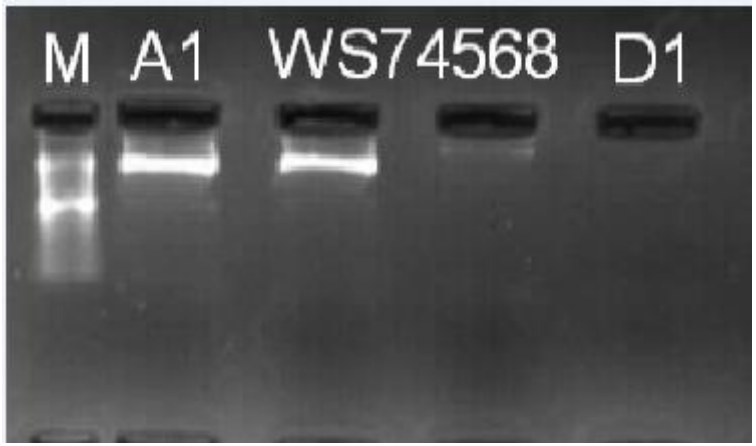

q11.1
q11.21
q11.22
q11.23
q12.1
q12.2
q12.3
121 bp
30,079,140 bp
30,079,160 bp
30,079,180 bp

Hypothesis?



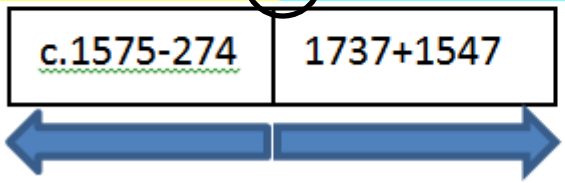

LR-PCR with two reverse primers

A product would only be generated by an inverted sequence




Product Sanger sequenced

CAAAATACAAGAAAGAGACCCTGGGTACCTTTTTAATGGTATTGTGCTTGCTGCTGCCACCCCTGTCGGAGTTC
TCATTGTGCAGAATATCCAGAGCTGTCTCCCTCTCTTTCAGTTTCAAGGCCTCGATTTCTGTCTTGAGTTCATTG
AGCTGCTCCTGCAGATGCTTGCTCTTTTCCATGTATTCCACTCTGCCGGCAAGAGGGTATCATGCATCAGGGCT
TGGGCAGACAGTGAGACACGGCTCAGGTGCTCTGTGGCCCACAAGCTGCTTGGTGTGCGATCTAGGGTTTGA
GGCCTGGGGTTCACGTCTCTACTTGGCCACTCACAGGCCATGGGAGCTTGGGCTGGTCACTTAAATGCTATGA
ATGTTTCCTCATCATTAAAATTGGGATAATGATCACAGGGCCGTGGTTAGGATTAAATAAAACAGTGTAGGAG
GCCAGGCACGGTGGCTCCACCTGTCCTCGGGCCACACTGAGCCCTTACGAGGGCAGGTGGTGCCTGGGTACT




Intron 15 Exon 15 Intron 14 Intron 15

Defined this as a complex inversion mutation – c.[1575-274_1737+1546inv;1737+1547del]
This is an intragenic inversion of the whole of NF2 exon 15 is predicted to disrupt normal expression of the NF2 gene and is therefore considered pathogenic
One other unrelated patient has this same mutation and 2 other patients have possible mutations (TBC)

**Conclusions**

Patients with a strong indication of NF2 where no mutation has been identified are routinely subjected to extended bioinformatic analysis.
The use of custom NGS reports has proved to be a useful tool to detect novel NF2 mutations