SUPPLEMENTARY MATERIAL

Supplementary Figure 1:

Regulatory elements and coding regions of mRNAs encoding putative AtPop1p variants

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                         10        20        30        40        50        60        70        80        90       100
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Promoter                                         T-Box
Gene            -------------------------------------Promoter region------------------------------------------------
NC003071.7      CCATTGAGGGTGGGCCTTAACAATTCTATTTGACAAAGTTTGATATTGGGCTAGAAGCCCCATTAAGTAAACCTTTTATTATATTGGAGAAGTAAGAAAA


                        110       120       130       140       150       160       170       180       190       200
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Promoter                                LFY          SORLIP2                                   SORLIP2         T-BOX
Gene            -------------------------------------Promoter region------------------------------------------------
NC003071.7      CCCATTAAGTAGACCTCTTTATTACATTGGGCTATAAGGCCCATTAATCTCATTAGTTTGAGCAAATACTGTAAATATAGGCCCCTTTTGTTCTTCTCAA


                        210       220       230       240       250       260       270       280       290       300
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Promoter        T-Box                      ■         ••  • •                   •    •    ■     •  •  •         •
Gene            --------------------------------------------5'UTR---------------------------------------------------
NC003071.7      AGTACACCATAAGTAAAAAAAAAAAAAACATGCGTATATGAATCGAAAAACCTTAATTTTCTGATTTACAAAAATTCCGACCAAATCTCTGTTTCCCTCT
AT2G47300.2     --------------------------------------TGAATCGAAAAACCTTAATTTTCTGATTTACAAAAATTCCGACCAAATCTCTGTTTCCCTCT
AT2G47300.3     --------------------------------------TGAATCGAAAAACCTTAATTTTCTGATTTACAAAAATTCCGACCAAATCTCTGTTTCCCTCT
FN673552        ---------------------------ACATGCGTATATGAATCGAAAAACCTTAATTTTCTGATTTACAAAAATTCCGACCAAATCTCTGTTTCCCTCT


                        310       320       330       340       350       360       370       380       390       400
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Promoter                                                       LFY
Promoter          GATA     •RAV1-A     ••                      DPBF1+2  •           GATA
Gene            --------------------------------------------5'UTR ---------------------------------------------------
NC003071.7      CTTTATCTCCTTTGTTGCGTACTGATTCTCTAAGCCGTGACGGTGTGCCAGTGTCTGTCCTTGTCTTATTATCACTGCCGCAGTTCCTATTCGTGCCCGC
AT2G47300.2     CTTTATCTCCTTTGTTGCGTACTGATTCTCTAAGCCGTGACGGTGTGCCAGTGTCTGTCCTTGTCTTATTATCACTGCCGCAGTTCCTATTCGTGCCCGC
AT2G47300.3     CTTTATCTCCTTTGTTGCGTACTGATTCTCTAAGCCGTGACGGTGTGCCAGTGTCTGTCCTTGTCTTATTATCACTGCCGCAGTTCCTATTCGTGCCCGC
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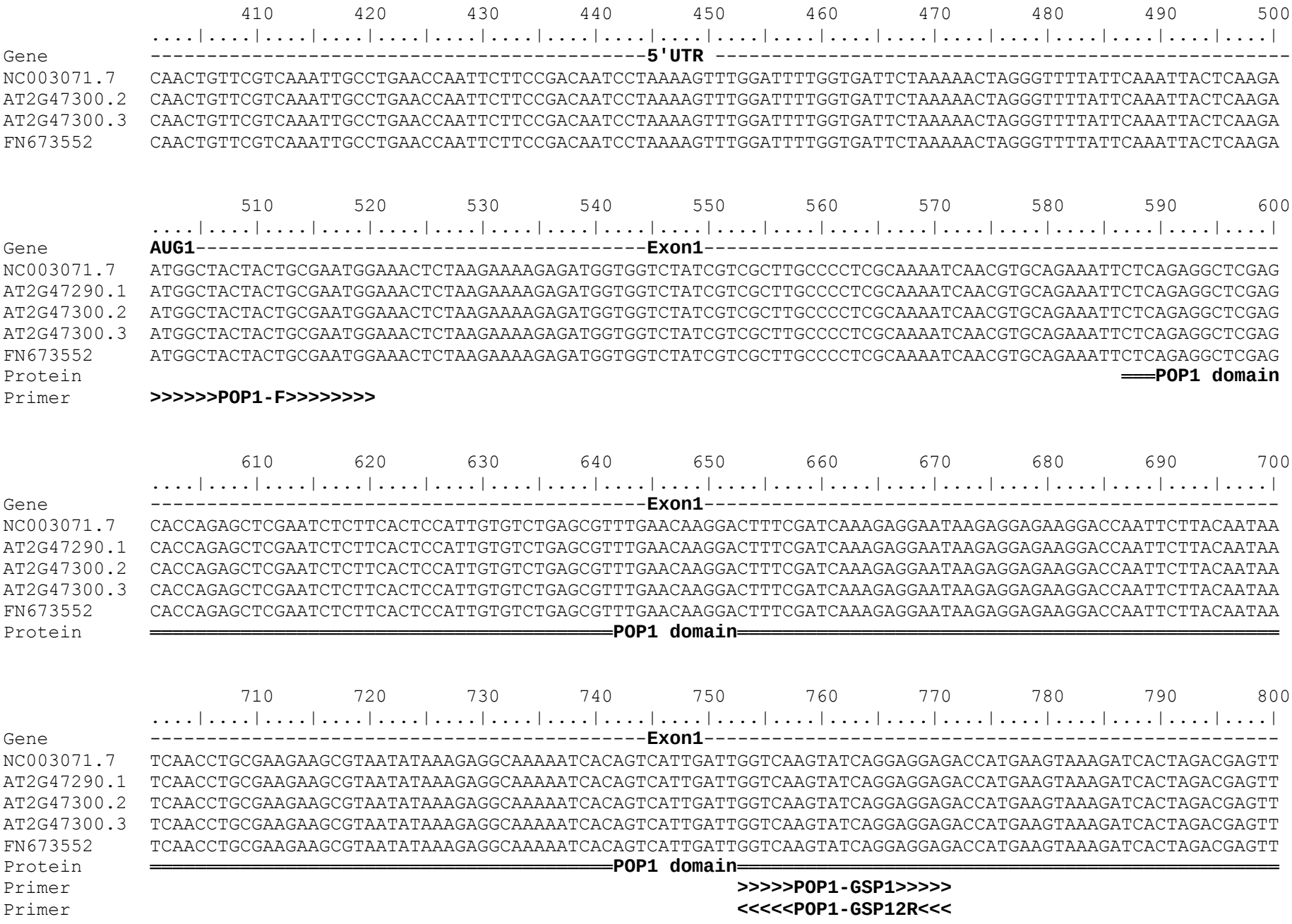
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Gene 5'UTR
NC003071.7 CAACTGTTCGTCAAATTGCCTGAACCAATTCTTCCGACAATCCTAAAAGTTTGGATTTTGGTGATTCTAAAAACTAGGGTTTTATTCAAATTACTCAAGA
AT2G47300.2 CAACTGTTCGTCAAATTGCCTGAACCAATTCTTCCGACAATCCTAAAAGTTTGGATTTTGGTGATTCTAAAAACTAGGGTTTTATTCAAATTACTCAAGA
AT2G47300.3 CAACTGTTCGTCAAATTGCCTGAACCAATTCTTCCGACAATCCTAAAAGTTTGGATTTTGGTGATTCTAAAAACTAGGGTTTTATTCAAATTACTCAAGA
FN673552 CAACTGTTCGTCAAATTGCCTGAACCAATTCTTCCGACAATCCTAAAAGTTTGGATTTTGGTGATTCTAAAAACTAGGGTTTTATTCAAATTACTCAAGA
510 520 530 540 550 560 570 580 590 600
Gene AUG1 Exon1
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AT2G47290.1 ATGGCTACTACTGCGAATGGAAACTCTAAGAAAAGAGATGGTGGTCTATCGTCGCTTGCCCCTCGCAAAATCAACGTGCAGAAATTCTCAGAGGCTCGAG
AT2G47300.2 ATGGCTACTACTGCGAATGGAAACTCTAAGAAAAGAGATGGTGGTCTATCGTCGCTTGCCCCTCGCAAAATCAACGTGCAGAAATTCTCAGAGGCTCGAG
AT2G47300.3 ATGGCTACTACTGCGAATGGAAACTCTAAGAAAAGAGATGGTGGTCTATCGTCGCTTGCCCCTCGCAAAATCAACGTGCAGAAATTCTCAGAGGCTCGAG
FN673552 ATGGCTACTACTGCGAATGGAAACTCTAAGAAAAGAGATGGTGGTCTATCGTCGCTTGCCCCTCGCAAAATCAACGTGCAGAAATTCTCAGAGGCTCGAG
Protein POP1 domain
Primer >>>>>>POP1-F>>>>>>>>
610 620 630 640 650 660 670 680 690 700
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AT2G47290.1 CACCAGAGCTCGAATCTCTTCACTCCATTGTGTCTGAGCGTTTGAACAAGGACTTTCGATCAAAGAGGAATAAGAGGAGAAGGACCAATTCTTACAATAA
AT2G47300.2 CACCAGAGCTCGAATCTCTTCACTCCATTGTGTCTGAGCGTTTGAACAAGGACTTTCGATCAAAGAGGAATAAGAGGAGAAGGACCAATTCTTACAATAA
AT2G47300.3 CACCAGAGCTCGAATCTCTTCACTCCATTGTGTCTGAGCGTTTGAACAAGGACTTTCGATCAAAGAGGAATAAGAGGAGAAGGACCAATTCTTACAATAA
FN673552 CACCAGAGCTCGAATCTCTTCACTCCATTGTGTCTGAGCGTTTGAACAAGGACTTTCGATCAAAGAGGAATAAGAGGAGAAGGACCAATTCTTACAATAA
Protein POP1 domain
710 720 730 740 750 760 770 780 790 800
Gene Exon1
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AT2G47300.2 TCAACCTGCGAAGAAGCGTAATATAAAGAGGCAAAAATCACAGTCATTGATTGGTCAAGTATCAGGAGGAGACCATGAAGTAAAGATCACTAGACGAGTT
AT2G47300.3 TCAACCTGCGAAGAAGCGTAATATAAAGAGGCAAAAATCACAGTCATTGATTGGTCAAGTATCAGGAGGAGACCATGAAGTAAAGATCACTAGACGAGTT
FN673552 TCAACCTGCGAAGAAGCGTAATATAAAGAGGCAAAAATCACAGTCATTGATTGGTCAAGTATCAGGAGGAGACCATGAAGTAAAGATCACTAGACGAGTT
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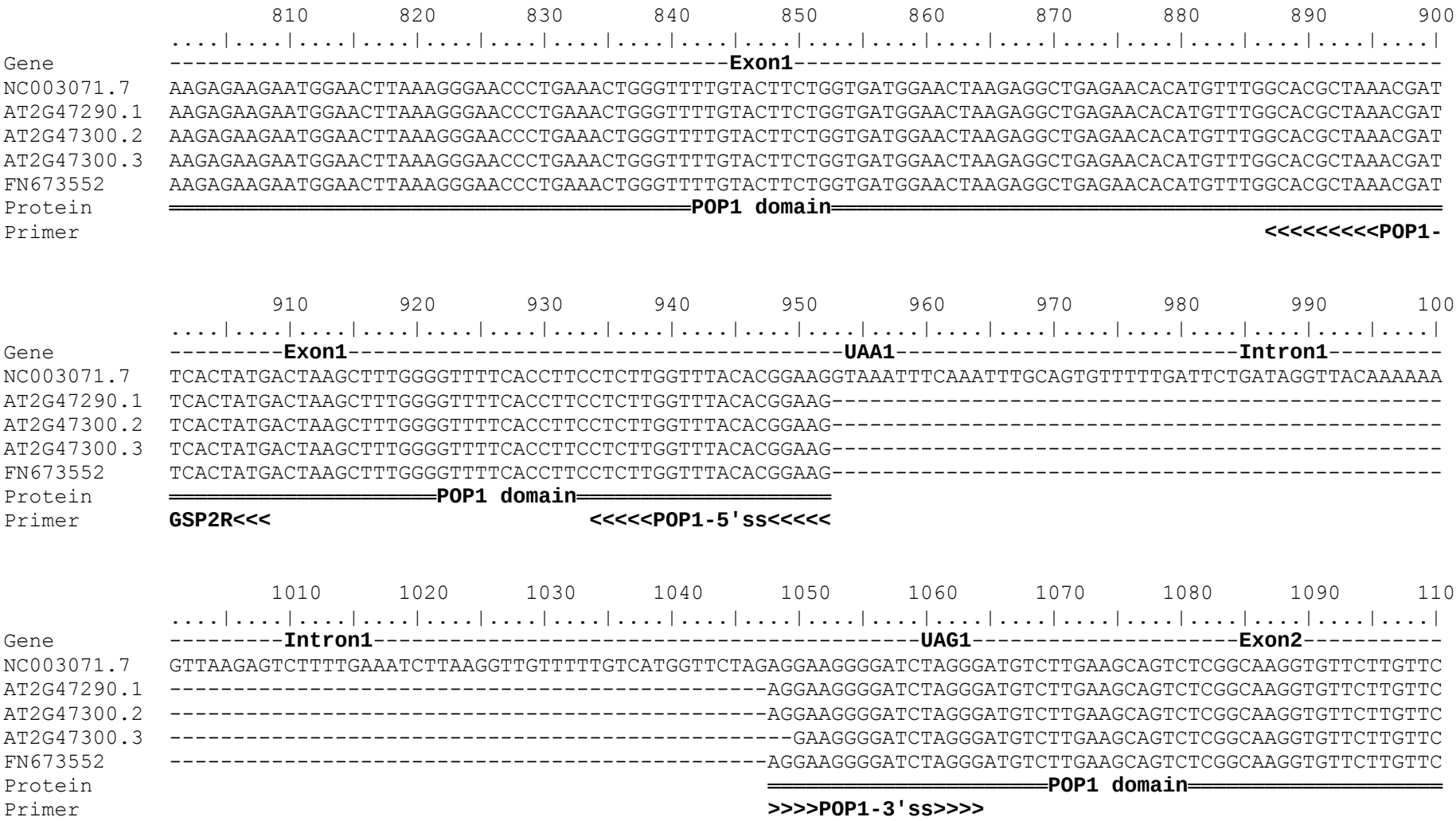
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Gene
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AT2G47290.1 AAGAGAAGAATGGAACTTAAAGGGAACCCTGAAACTGGGTTTTGTACTTCTGGTGATGGAACTAAGAGGCTGAGAACACATGTTTGGCACGCTAAACGAT
AT2G47300.2 AAGAGAAGAATGGAACTTAAAGGGAACCCTGAAACTGGGTTTTGTACTTCTGGTGATGGAACTAAGAGGCTGAGAACACATGTTTGGCACGCTAAACGAT
AT2G47300.3 AAGAGAAGAATGGAACTTAAAGGGAACCCTGAAACTGGGTTTTGTACTTCTGGTGATGGAACTAAGAGGCTGAGAACACATGTTTGGCACGCTAAACGAT
FN673552 AAGAGAAGAATGGAACTTAAAGGGAACCCTGAAACTGGGTTTTGTACTTCTGGTGATGGAACTAAGAGGCTGAGAACACATGTTTGGCACGCTAAACGAT
Protein
POP1 domain
Primer
<<<<<<<<<POP1-
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Gene
Exon1
UAA1
Intron1
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AT2G47290.1 TCACTATGACTAAGCTTTGGGGTTTTCACCTTCCTCTTGGTTTACACGGAAG
AT2G47300.2 TCACTATGACTAAGCTTTGGGGTTTTCACCTTCCTCTTGGTTTACACGGAAG
AT2G47300.3 TCACTATGACTAAGCTTTGGGGTTTTCACCTTCCTCTTGGTTTACACGGAAG
FN673552 TCACTATGACTAAGCTTTGGGGTTTTCACCTTCCTCTTGGTTTACACGGAAG
Protein
POP1 domain
Primer
GSP2R<<<
<<<<<POP1-5'ss<<<<<
1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
Gene
Intron1
UAG1
Exon2
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AT2G47290.1 AGGAAGGGGATCTAGGGATGTCTTGAAGCAGTCTCGGCAAGGTGTTCTTGTTC
AT2G47300.2 AGGAAGGGGATCTAGGGATGTCTTGAAGCAGTCTCGGCAAGGTGTTCTTGTTC
AT2G47300.3 GAAGGGGATCTAGGGATGTCTTGAAGCAGTCTCGGCAAGGTGTTCTTGTTC
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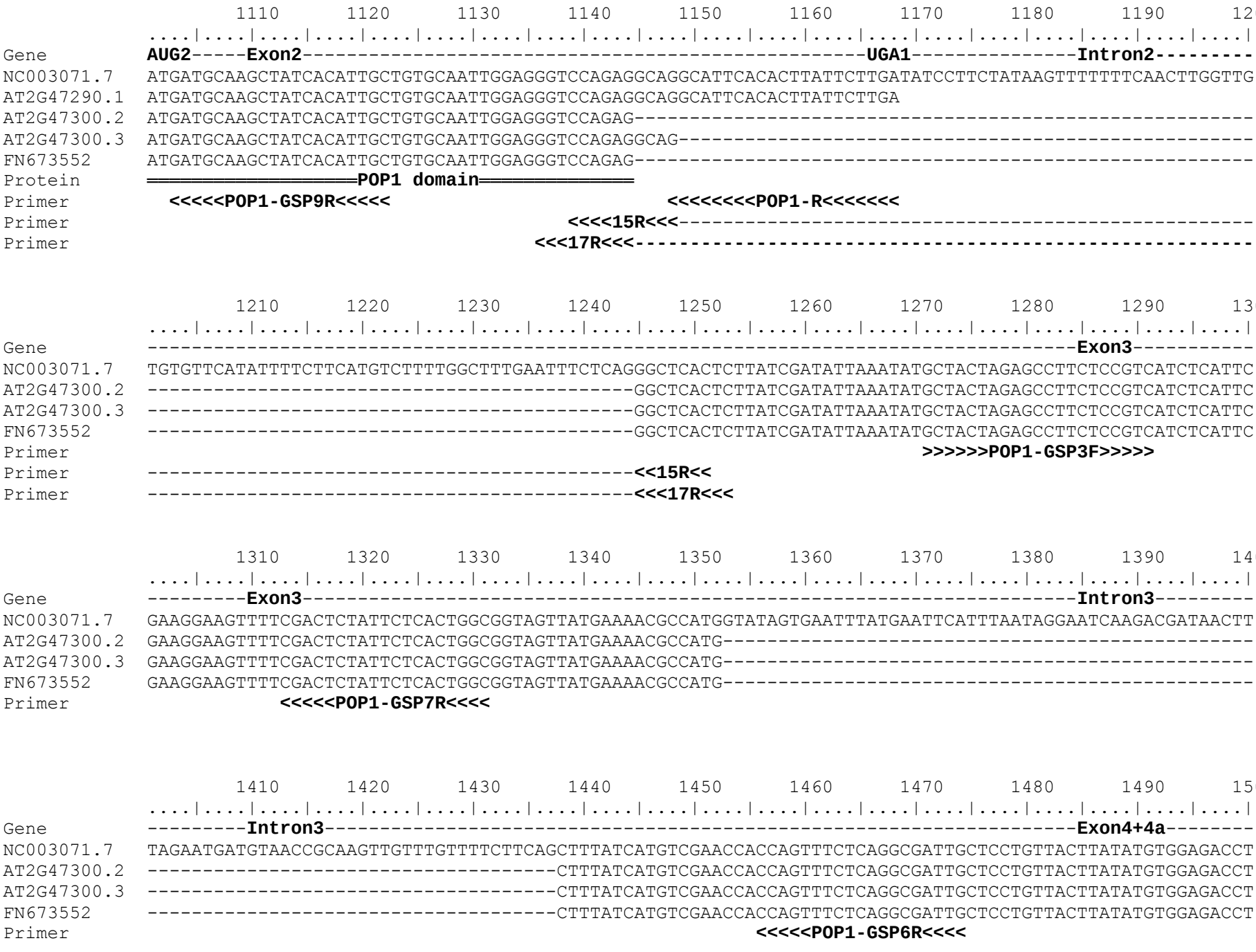
Protein
POP1 domain
Primer
>>>>POP1-3'ss>>>>>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AT2G47290.1 ATGATGCAAGCTATCACATTGCTGTGCAATTGGAGGGTCCAGAGGCAGGCATTCACACTTATTCTTGA
AT2G47300.2 ATGATGCAAGCTATCACATTGCTGTGCAATTGGAGGGTCCAGAG----------
AT2G47300.3 ATGATGCAAGCTATCACATTGCTGTGCAATTGGAGGGTCCAGAGGCAG----------
FN673552 ATGATGCAAGCTATCACATTGCTGTGCAATTGGAGGGTCCAGAG----------
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Primer <<<<<POP1-GSP9R<<<<<
Primer <<<<<<<<POP1-R<<<<<<<
Primer <<<<15R<<<----------
Primer <<<17R<<<----------
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Gene ----------Exon3-----------
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AT2G47300.2 ----------GGCTCACTCTTATCGATATTAAATATGCTACTAGAGCCTTCTCCGTCATCTCATTC
AT2G47300.3 ----------GGCTCACTCTTATCGATATTAAATATGCTACTAGAGCCTTCTCCGTCATCTCATTC
FN673552 ----------GGCTCACTCTTATCGATATTAAATATGCTACTAGAGCCTTCTCCGTCATCTCATTC
Primer >>>>>>POP1-GSP3F>>>>>
Primer ----------<<15R<<
Primer ----------<<<17R<<<
1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
Gene ---------Exon3----------Intron3---------
NC003071.7 GAAGGAAGTTTTCGACTCTATTCTCACTGGCGGTAGTTATGAAAACGCCATGGTATAGTGAATTTATGAATTCATTTAATAGGAATCAAGACGATAACTT
AT2G47300.2 GAAGGAAGTTTTCGACTCTATTCTCACTGGCGGTAGTTATGAAAACGCCATG----------
AT2G47300.3 GAAGGAAGTTTTCGACTCTATTCTCACTGGCGGTAGTTATGAAAACGCCATG----------
FN673552 GAAGGAAGTTTTCGACTCTATTCTCACTGGCGGTAGTTATGAAAACGCCATG----------
Primer <<<<<POP1-GSP7R<<<<
1410 1420 1430 1440 1450 1460 1470 1480 1490 1500
Gene ---------Intron3----------Exon4+4a--------
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AT2G47300.2 ----------CTTTATCATGTCGAACCACCAGTTTCTCAGGCGATTGCTCCTGTTACTTATATGTGGAGACCT
AT2G47300.3 ----------CTTTATCATGTCGAACCACCAGTTTCTCAGGCGATTGCTCCTGTTACTTATATGTGGAGACCT
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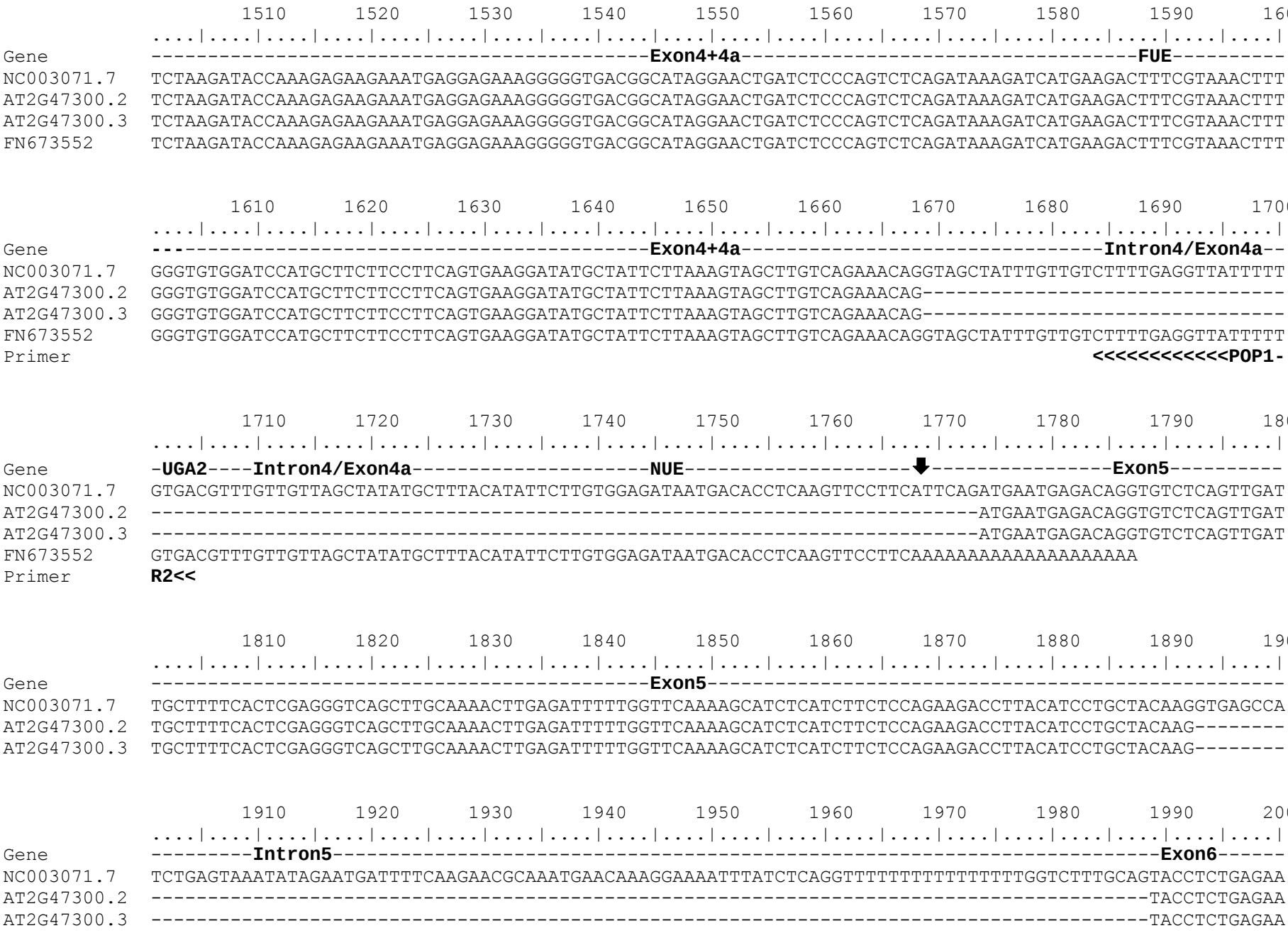
1510 1520 1530 1540 1550 1560 1570 1580 1590 1600
Gene Exon4+4a FUE
NC003071.7 TCTAAGATACCAAAGAGAAGAAATGAGGAGAAAGGGGGGTGACGGCATAGGAACTGATCTCCCAGTCTCAGATAAAGATCATGAAGACTTTCGTAAACTTT
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AT2G47300.3 TCTAAGATACCAAAGAGAAGAAATGAGGAGAAAGGGGGGTGACGGCATAGGAACTGATCTCCCAGTCTCAGATAAAGATCATGAAGACTTTCGTAAACTTT
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1610 1620 1630 1640 1650 1660 1670 1680 1690 1700
Gene Exon4+4a Intron4/Exon4a
NC003071.7 GGGTGTGGATCCATGCTTCTTCCTTCAGTGAAGGATATGCTATTCTTAAAGTAGCTTGTCAGAAACAGGTAGCTATTTGTTGTCTTTTGAGGTTATTTTT
AT2G47300.2 GGGTGTGGATCCATGCTTCTTCCTTCAGTGAAGGATATGCTATTCTTAAAGTAGCTTGTCAGAAACAG
AT2G47300.3 GGGTGTGGATCCATGCTTCTTCCTTCAGTGAAGGATATGCTATTCTTAAAGTAGCTTGTCAGAAACAG
FN673552 GGGTGTGGATCCATGCTTCTTCCTTCAGTGAAGGATATGCTATTCTTAAAGTAGCTTGTCAGAAACAGGTAGCTATTTGTTGTCTTTTGAGGTTATTTTT
Primer <<<<<<<<<<<POP1-
1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
Gene -UGA2 Intron4/Exon4a NUE Exon5
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AT2G47300.2 ATGAATGAGACAGGTGTCTCAGTTGAT
AT2G47300.3 ATGAATGAGACAGGTGTCTCAGTTGAT
FN673552 GTGACGTTTGTTGTTAGCTATATGCTTTACATATTCTTGTGGAGATAATGACACCTCAAGTTCCTTCAAAAAAAAAAAAAAAAAAAA
Primer R2<<
1810 1820 1830 1840 1850 1860 1870 1880 1890 1900
Gene Exon5
NC003071.7 TGCTTTTCACTCGAGGGTCAGCTTGCAAAACTTGAGATTTTTGGTTCAAAAGCATCTCATCTTCTCCAGAAGACCTTACATCCTGCTACAAGGTGAGCCA
AT2G47300.2 TGCTTTTCACTCGAGGGTCAGCTTGCAAAACTTGAGATTTTTGGTTCAAAAGCATCTCATCTTCTCCAGAAGACCTTACATCCTGCTACAAG
AT2G47300.3 TGCTTTTCACTCGAGGGTCAGCTTGCAAAACTTGAGATTTTTGGTTCAAAAGCATCTCATCTTCTCCAGAAGACCTTACATCCTGCTACAAG
1910 1920 1930 1940 1950 1960 1970 1980 1990 2000
Gene Intron5 Exon6
NC003071.7 TCTGAGTAAATATAGAATGATTTTCAAGAACGCAAATGAACAAAGGAAAATTTATCTCAGGTTTTTTTTTTTTTTTGGTCTTTGCAGTACCTCTGAGAA
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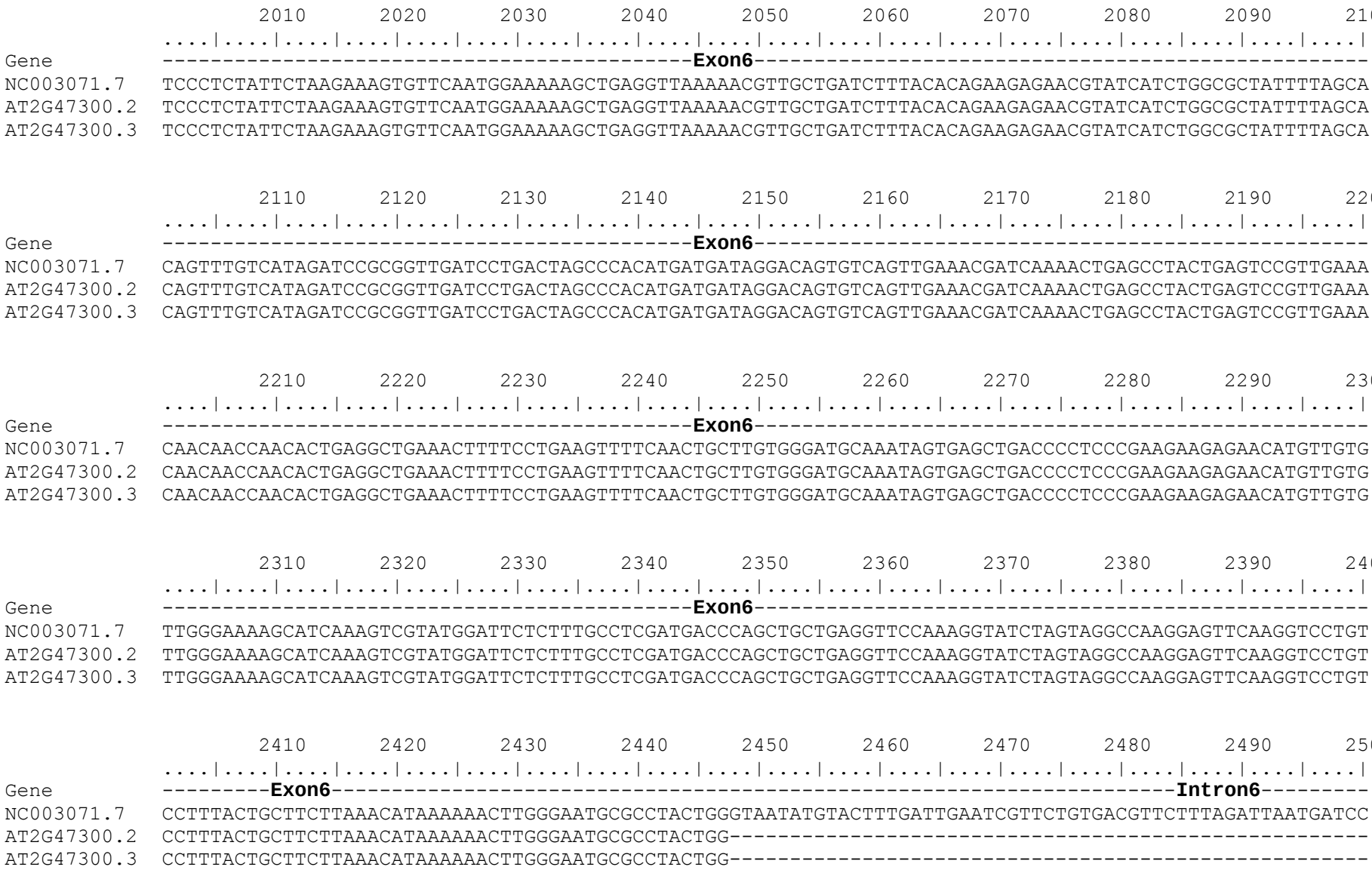
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Gene Exon6
NC003071.7 TCCCTCTATTCTAAGAAAGTGTTCAATGGAAAAAGCTGAGGTTAAAAACGTTGCTGATCTTTACACAGAAGAGAACGTATCATCTGGCGCTATTTTAGCA
AT2G47300.2 TCCCTCTATTCTAAGAAAGTGTTCAATGGAAAAAGCTGAGGTTAAAAACGTTGCTGATCTTTACACAGAAGAGAACGTATCATCTGGCGCTATTTTAGCA
AT2G47300.3 TCCCTCTATTCTAAGAAAGTGTTCAATGGAAAAAGCTGAGGTTAAAAACGTTGCTGATCTTTACACAGAAGAGAACGTATCATCTGGCGCTATTTTAGCA
2110 2120 2130 2140 2150 2160 2170 2180 2190 2200
Gene Exon6
NC003071.7 CAGTTTGTCATAGATCCGCGGTTGATCCTGACTAGCCCACATGATGATAGGACAGTGTCAGTTGAAACGATCAAAACTGAGCCTACTGAGTCCGTTGAAA
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AT2G47300.3 CAGTTTGTCATAGATCCGCGGTTGATCCTGACTAGCCCACATGATGATAGGACAGTGTCAGTTGAAACGATCAAAACTGAGCCTACTGAGTCCGTTGAAA
2210 2220 2230 2240 2250 2260 2270 2280 2290 2300
Gene Exon6
NC003071.7 CAACAACCAACACTGAGGCTGAAACTTTTCCTGAAGTTTTCAACTGCTTGTGGGATGCAAATAGTGAGCTGACCCCTCCCGAAGAAGAGAACATGTTGTG
AT2G47300.2 CAACAACCAACACTGAGGCTGAAACTTTTCCTGAAGTTTTCAACTGCTTGTGGGATGCAAATAGTGAGCTGACCCCTCCCGAAGAAGAGAACATGTTGTG
AT2G47300.3 CAACAACCAACACTGAGGCTGAAACTTTTCCTGAAGTTTTCAACTGCTTGTGGGATGCAAATAGTGAGCTGACCCCTCCCGAAGAAGAGAACATGTTGTG
2310 2320 2330 2340 2350 2360 2370 2380 2390 2400
Gene Exon6
NC003071.7 TTGGGAAAAGCATCAAAGTCGTATGGATTCTCTTTGCCTCGATGACCCAGCTGCTGAGGTTCCAAAGGTATCTAGTAGGCCAAGGAGTTCAAGGTCCTGT
AT2G47300.2 TTGGGAAAAGCATCAAAGTCGTATGGATTCTCTTTGCCTCGATGACCCAGCTGCTGAGGTTCCAAAGGTATCTAGTAGGCCAAGGAGTTCAAGGTCCTGT
AT2G47300.3 TTGGGAAAAGCATCAAAGTCGTATGGATTCTCTTTGCCTCGATGACCCAGCTGCTGAGGTTCCAAAGGTATCTAGTAGGCCAAGGAGTTCAAGGTCCTGT
2410 2420 2430 2440 2450 2460 2470 2480 2490 2500
Gene Exon6 Intron6
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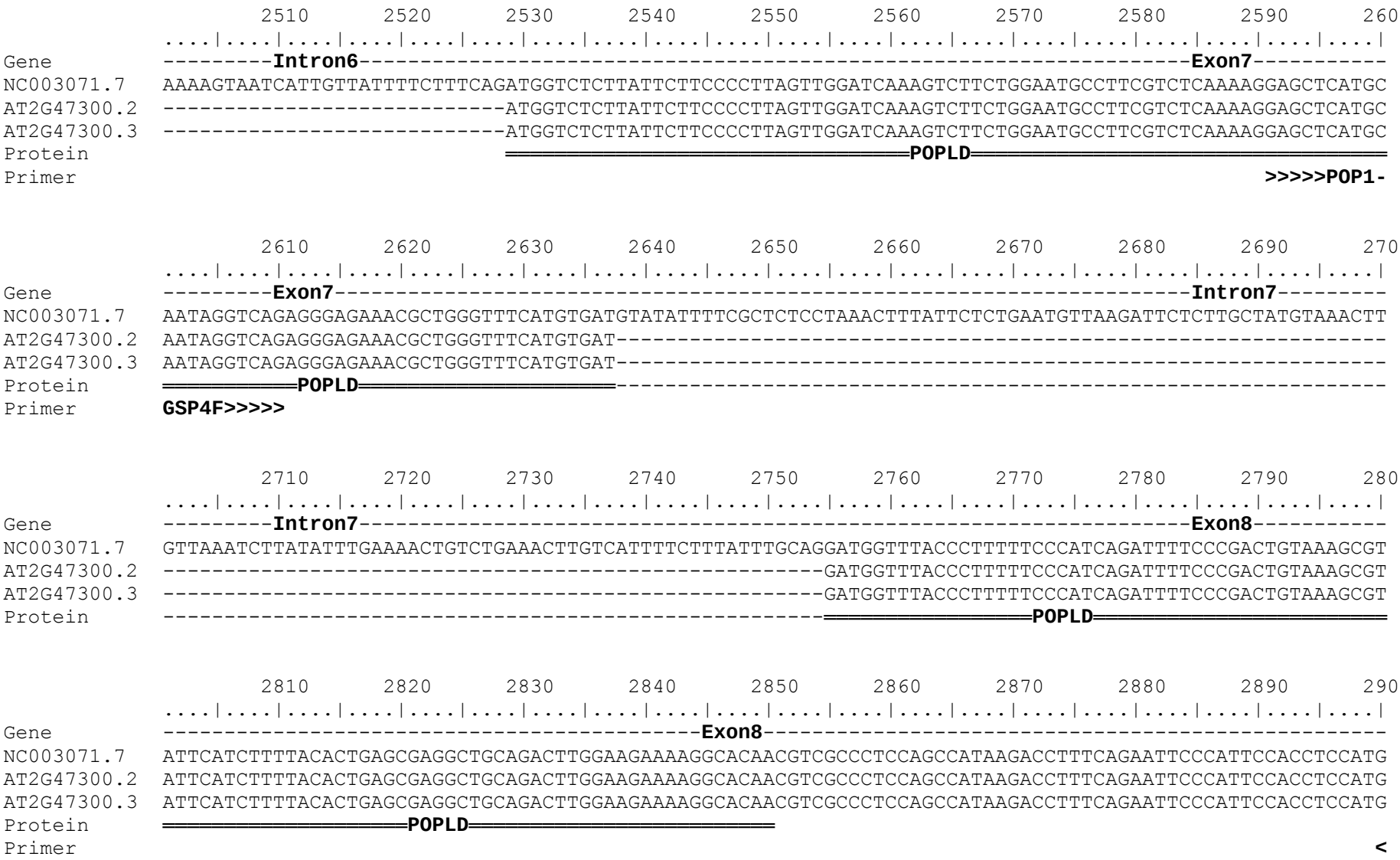
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Gene ---------Intron6----------Exon7----------
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AT2G47300.2 ----------ATGGTCTCTTATTCTTCCCCTTAGTTGGATCAAAGTCTTCTGGAATGCCTTCGTCTCAAAAGGAGCTCATGC
AT2G47300.3 ----------ATGGTCTCTTATTCTTCCCCTTAGTTGGATCAAAGTCTTCTGGAATGCCTTCGTCTCAAAAGGAGCTCATGC
Protein ==========POPLD==========
Primer >>>>>POP1-
2610 2620 2630 2640 2650 2660 2670 2680 2690 2700
Gene ---------Exon7----------Intron7---------
NC003071.7 AATAGGTCAGAGGGAGAAACGCTGGGTTTCATGTGATGTATATTTTCGCTCTCCTAAACTTTATTCTCTGAATGTTAAGATTCTCTTGCTATGTAAACTT
AT2G47300.2 AATAGGTCAGAGGGAGAAACGCTGGGTTTCATGTGAT----------
AT2G47300.3 AATAGGTCAGAGGGAGAAACGCTGGGTTTCATGTGAT----------
Protein ==========POPLD==========----------
Primer GSP4F>>>>>
2710 2720 2730 2740 2750 2760 2770 2780 2790 2800
Gene ---------Intron7----------Exon8----------
NC003071.7 GTTAAATCTTATATTTGAAAACTGTCTGAAACTTGTCATTTTCTTTATTTGCAGGATGGTTTACCCTTTTTCCCATCAGATTTTCCCGACTGTAAAGCGT
AT2G47300.2 ----------GATGGTTTACCCTTTTTCCCATCAGATTTTCCCGACTGTAAAGCGT
AT2G47300.3 ----------GATGGTTTACCCTTTTTCCCATCAGATTTTCCCGACTGTAAAGCGT
Protein ----------==========POPLD==========
2810 2820 2830 2840 2850 2860 2870 2880 2890 2900
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AT2G47300.2 ATTCATCTTTTACACTGAGCGAGGCTGCAGACTTGGAAGAAAAGGCACAACGTCGCCCTCCAGCCATAAGACCTTTCAGAATTCCCATTCCACCTCCATG
AT2G47300.3 ATTCATCTTTTACACTGAGCGAGGCTGCAGACTTGGAAGAAAAGGCACAACGTCGCCCTCCAGCCATAAGACCTTTCAGAATTCCCATTCCACCTCCATG
Protein ==========POPLD==========
Primer <

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                2910      2920      2930      2940      2950      2960      2970      2980      2990      3000
            ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Gene        ----------------------------------------------Exon8-----------------------------------------------
NC003071.7  GAATAGTATCCATGTCACCCGTTCTATTGGAGAGGGTTCCAATCAAAAGTTTTCAAGCAATGGGAGGAGTGTTGTGGAAATTTCCAGTTATGGTGGTAAT
AT2G47300.2 GAATAGTATCCATGTCACCCGTTCTATTGGAGAGGGTTCCAATCAAAAGTTTTCAAGCAATGGGAGGAGTGTTGTGGAAATTTCCAGTTATGGTGGTAAT
AT2G47300.3 GAATAGTATCCATGTCACCCGTTCTATTGGAGAGGGTTCCAATCAAAAGTTTTCAAGCAATGGGAGGAGTGTTGTGGAAATTTCCAGTTATGGTGGTAAT
Primer      <<<<POP1-GSP5R<<<<<

                3010      3020      3030      3040      3050      3060      3070      3080      3090      3100
            ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Gene        ----------------------------------------------Exon8-----------------------------------------------
NC003071.7  CTGTTTGATGGTATCGTGGCAAGAACATCAGATTCGCTAACTACTTTTCTCCAAACATTCACAAGTGACAACATGCTTTTATTTCCTCACAACACTTCAA
AT2G47300.2 CTGTTTGATGGTATCGTGGCAAGAACATCAGATTCGCTAACTACTTTTCTCCAAACATTCACAAGTGACAACATGCTTTTATTTCCTCACAACACTTCAA
AT2G47300.3 CTGTTTGATGGTATCGTGGCAAGAACATCAGATTCGCTAACTACTTTTCTCCAAACATTCACAAGTGACAACATGCTTTTATTTCCTCACAACACTTCAA

                3110      3120      3130      3140      3150      3160      3170      3180      3190      3200
            ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Gene        ----------------------------------------------Exon8-----------------------------------------------
NC003071.7  AACCAAGCACAGACCTTATGATGACGCTTCAAGAAGATGATAAAAAAGTAAGAGCTCAGATCCATCAGAGTAGTAACAAACTGTGCCTCGTCAGAGTTCT
AT2G47300.2 AACCAAGCACAGACCTTATGATGACGCTTCAAGAAGATGATAAAAAAGTAAGAGCTCAGATCCATCAGAGTAGTAACAAACTGTGCCTCGTCAGAGTTCT
AT2G47300.3 AACCAAGCACAGACCTTATGATGACGCTTCAAGAAGATGATAAAAAAGTAAGAGCTCAGATCCATCAGAGTAGTAACAAACTGTGCCTCGTCAGAGTTCT

                3210      3220      3230      3240      3250      3260      3270      3280      3290      3300
            ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Gene        ---------Exon8-------------------------------------------------------------------------Intron8----
NC003071.7  TCTACATGCTTTCAAGGAAGGTTCTTTCGAAGAAGGCGCGGTTGTATGTGCACCAACTCTTGCAGACATATCATTGCTAAAATCCAGGTAACATCTCCAA
AT2G47300.2 TCTACATGCTTTCAAGGAAGGTTCTTTCGAAGAAGGCGCGGTTGTATGTGCACCAACTCTTGCAGACATATCATTGCTAAAATCCAG-------------
AT2G47300.3 TCTACATGCTTTCAAGGAAGGTTCTTTCGAAGAAGGCGCGGTTGTATGTGCACCAACTCTTGCAGACATATCATTGCTAAAATCCAG-------------

                3310      3320      3330      3340      3350      3360      3370      3380      3390      3400
            ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Gene        ---------Intron8-------------------------------------------------------------------------Exon9------
NC003071.7  GAATCCATGTTCAACAAATTGTGATCCATTGGAACTATTTCATTTTGGTATCACATTCTTATACATTATGTTTTACAGCTGCAGTGAAGGAGAAGATGGG
AT2G47300.2 ----------------------------------------------------------------------------CTGCAGTGAAGGAGAAGATGGG
AT2G47300.3 ----------------------------------------------------------------------------CTGCAGTGAAGGAGAAGATGGG
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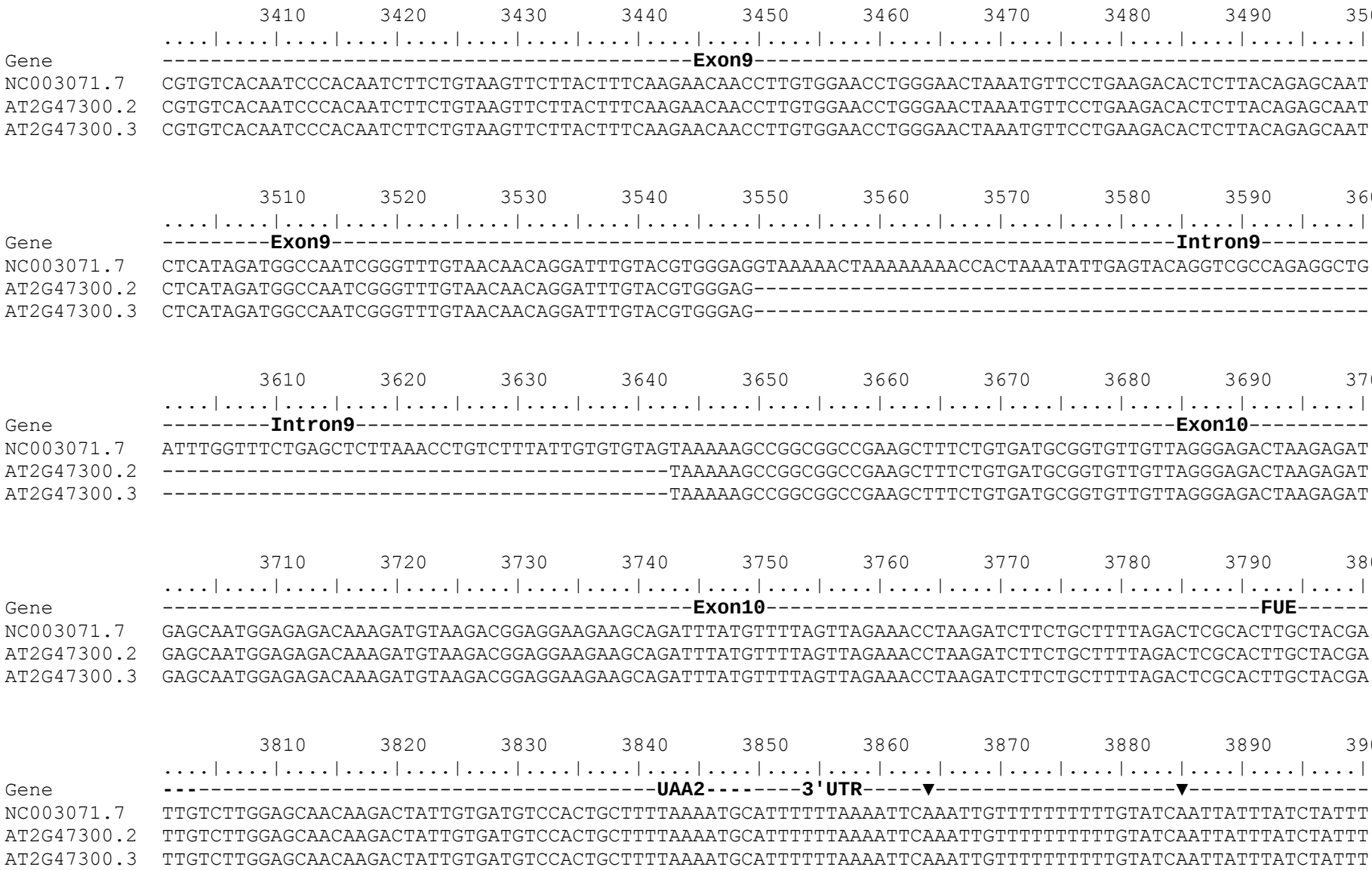
3410 3420 3430 3440 3450 3460 3470 3480 3490 3500
Gene ----------Exon9----------
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AT2G47300.2 CGTGTCACAATCCCACAATCTTCTGTAAGTTCTTACTTTCAAGAACAACCTTGTGGAACCTGGGAACTAAATGTTCCTGAAGACACTCTTACAGAGCAAT
AT2G47300.3 CGTGTCACAATCCCACAATCTTCTGTAAGTTCTTACTTTCAAGAACAACCTTGTGGAACCTGGGAACTAAATGTTCCTGAAGACACTCTTACAGAGCAAT
3510 3520 3530 3540 3550 3560 3570 3580 3590 3600
Gene ---------Exon9----------Intron9---------
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AT2G47300.2 CTCATAGATGGCCAATCGGGTTTGTAACAACAGGATTTGTACGTGGGAG----------
AT2G47300.3 CTCATAGATGGCCAATCGGGTTTGTAACAACAGGATTTGTACGTGGGAG----------
3610 3620 3630 3640 3650 3660 3670 3680 3690 3700
Gene ---------Intron9----------Exon10----------
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AT2G47300.2 ----------TAAAAAGCCGGCGGCCGAAGCTTTCTGTGATGCGGTGTTGTTAGGGAGACTAAGAGAT
AT2G47300.3 ----------TAAAAAGCCGGCGGCCGAAGCTTTCTGTGATGCGGTGTTGTTAGGGAGACTAAGAGAT
3710 3720 3730 3740 3750 3760 3770 3780 3790 3800
Gene ----------Exon10----------FUE------
NC003071.7 GAGCAATGGAGAGACAAAGATGTAAGACGGAGGAAGAAGCAGATTTATGTTTTAGTTAGAAACCTAAGATCTTCTGCTTTTAGACTCGCACTTGCTACGA
AT2G47300.2 GAGCAATGGAGAGACAAAGATGTAAGACGGAGGAAGAAGCAGATTTATGTTTTAGTTAGAAACCTAAGATCTTCTGCTTTTAGACTCGCACTTGCTACGA
AT2G47300.3 GAGCAATGGAGAGACAAAGATGTAAGACGGAGGAAGAAGCAGATTTATGTTTTAGTTAGAAACCTAAGATCTTCTGCTTTTAGACTCGCACTTGCTACGA
3810 3820 3830 3840 3850 3860 3870 3880 3890 3900
Gene ---UAA2--------3'UTR-----▼----------▼----------
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AT2G47300.2 TTGTCTTGGAGCAACAAGACTATTGTGATGTCCACTGCTTTTAAAATGCATTTTTTAAAATTCAAATTGTTTTTTTTTTGTATCAATTATTTATCTATTT
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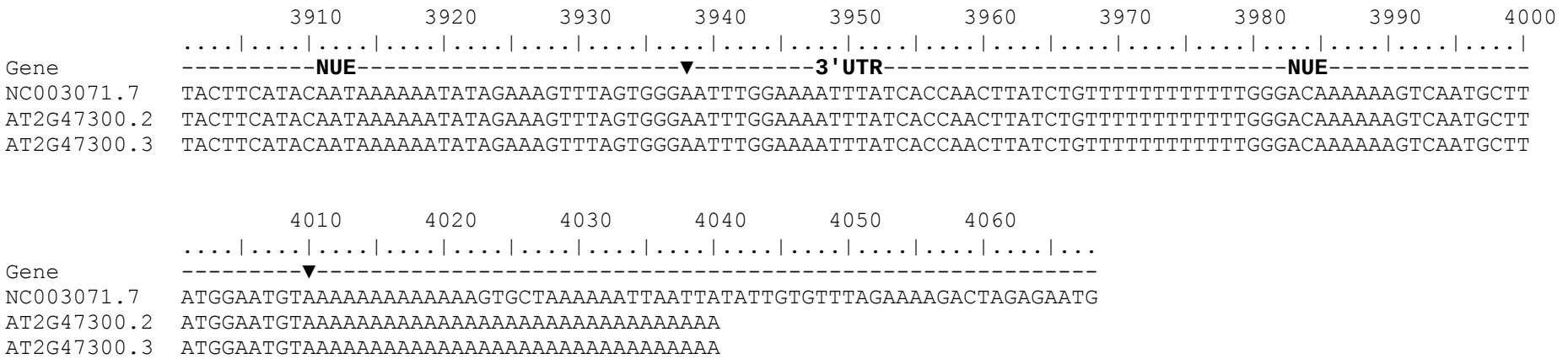


Legend to Supplementary Fig. 1:

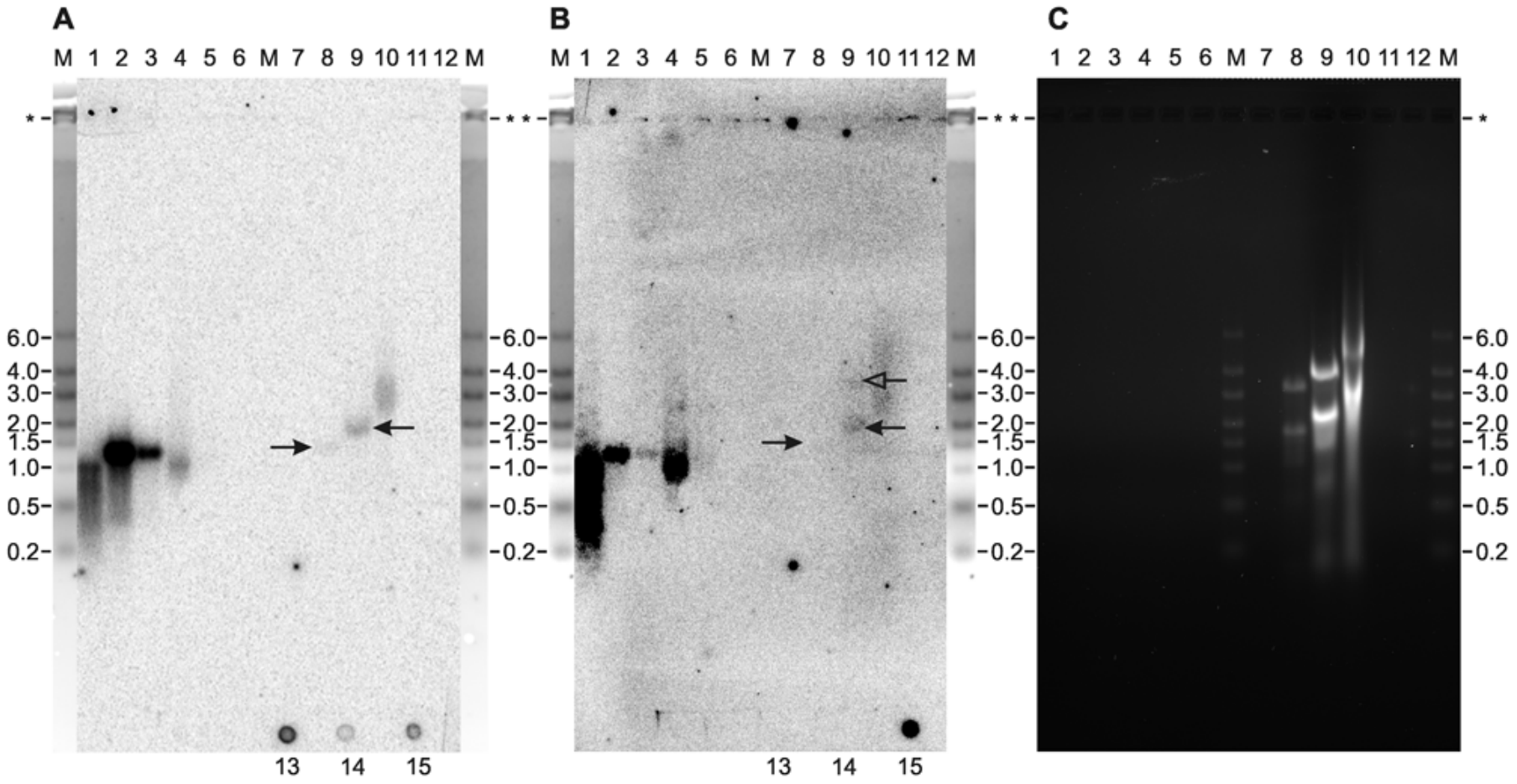
Promoter: possible transcription factor binding sites. **Gene:** gene structure (mRNA processing pattern). **Protein:** protein domains (POP1 domain [pfam06978] and POP-like domain [POPLD; NUC188 domain; pfam08170]); coding regions are indicated by double lines. **Primer:** position of primers used for RACE and Northern analysis; primer orientation is indicated by carets. For graphical clarity, the prefix "AT" is omitted from primer names; 15R/17R are AtPOP1-GSP15R and AtPOP1-GSP17R, respectively.

Symbol characters: • transcription start sites from published ESTs and ■ from this study; ▼ cleavage and polyadenylation sites from published annotations and ESTs and ⬇ experimentally determined (this study). RNAs are shown as cDNA sequences; intron positions are depicted as dashed lines. Primers and protein domains pertaining to two exons (or being subject to a line break) are split and connected by dashed lines. FUE, NUE and CS: far and near upstream elements and cleavage/polyadenylation sites, respectively.

Sequences: NC003071.7: *A. thaliana* Chr.2, genomic region encoding Pop1p (19418233-19422300); AT2G47290.1: 573 nt mRNA (NCBI) encoding a 190 aa ORF (variant 1; EBI-Accession: AAB63829.1). Due to the in-frame UAA1 stop codon at the beginning of intron 1, the unspliced RNA encodes a 151 aa long truncated version of AtPop1p which still contains most of the conserved POP1 domain and was used for antigen production and RNA binding studies. AT2G47300.2: 2908 nt mRNA (TAIR) encoding a 826 aa protein (variant 2a); AT2G47300.3: 2910 nt mRNA (TAIR) encoding a putative 659 aa protein (variant 2b). The main polyadenylation signals for these two mRNAs are located between positions 3792-3979 (FUE), 3983-3990 (NUE) and 4010 (annotated CS); additional upstream cleavage sites identified in ESTs and a putative corresponding NUE are also shown.

FN673552: experimentally determined novel 1259 nt mRNA encoding a 307 aa protein (variant 3; this study). The putative polyadenylation signals for this mRNA are located between positions 1588-1717 (FUE), 1745-1750 (NUE) and at 1768 (CS).

Supplementary Figure 2: Identification of AtPop1 mRNAs by Northern blot hybridization



Electrophoresis samples: Lane 1: Pop1 mRNA transcript 1 (crude, 20 ng); 2 and 3: transcription template, 1112 bp (20 and 2 ng, respectively); 4 and 5: gel purified AtPop1 transcript, 1095 nt (10 and 1 ng, corresponding to 28 and 2.8 fmoles); 8-10: *Arabidopsis* total RNA (1, 10 and 25 µg, respectively); Lanes 6, 7, 11 and 12, sample buffer. M, size marker (Fermentas RiboRuler High Range; length in kb). -*-, position of gel slots.
Dot Blot hybridization controls: 13 and 14: ATPOP1-GSP15R-target representing the alternative splice site (0.1 and 0.02 ng corresponding to 7 and 1.4 fmoles, respectively); the target oligonucleotide contains two binding sites for the probe. 15: POP1 transcript 1 representing the wild-type splice site (1 ng, corresponding to about 2.8 fmoles).

Panel A: Hybridization with AtPOP1-GSP15R recognizing the alternatively spliced intron 2 of the annotated mRNA 2b (encoding the putative 659 aa protein; At2G47300.3); **B:** Hybridization with AtPOP1-GSP17R recognizing the "wild type" intron 2 splice sites leading to the annotated mRNA 2a (encoding the 826 aa protein; At2G47300.3) and the novel mRNA3 encoding a 307 aa protein (FN673552). **C:** EtBr stained gel prior to blotting. To facilitate RNA size estimation, the right marker lane from (C) has been copied to the edges of both blots.

Methods:

To generate a hybridization control and size marker, a transcription template covering the coding region of mRNA variant 3 was constructed. The 5' part of the sequence was amplified from pT7AtPOP1ΔI using T7Promseq and AtPOP1-GSP9R; the 3' part was amplified from the 3' RACE product of variant 3, using AtPOP1-3'ss and AtPOP1-R2. The purified products were used as a combined template for amplification with T7Promseq and AtPOP1-R2, cloned into pCRII-TOPO and sequenced. Transcription was performed from the PCR product and the transcript was DNase I-treated before electrophoresis; the 1095 nt long transcript includes the vector-coded region and ends at UGA2 (see suppl. Fig. 1). The transcripts, PCR products and total *Arabidopsis* RNA preparations were subjected to agarose gel electrophoresis on a 1 % agarose / formaldehyde gel, followed by Northern blotting onto ZetaProbe membrane (Biorad). Additional hybridization controls were spotted onto the dry membrane as indicated. After immobilization by baking, the filter was hybridized successively with different ^{32}P-labeled oligonucleotide probes at 3 °C below calculated T_m and stripped between reprobing.

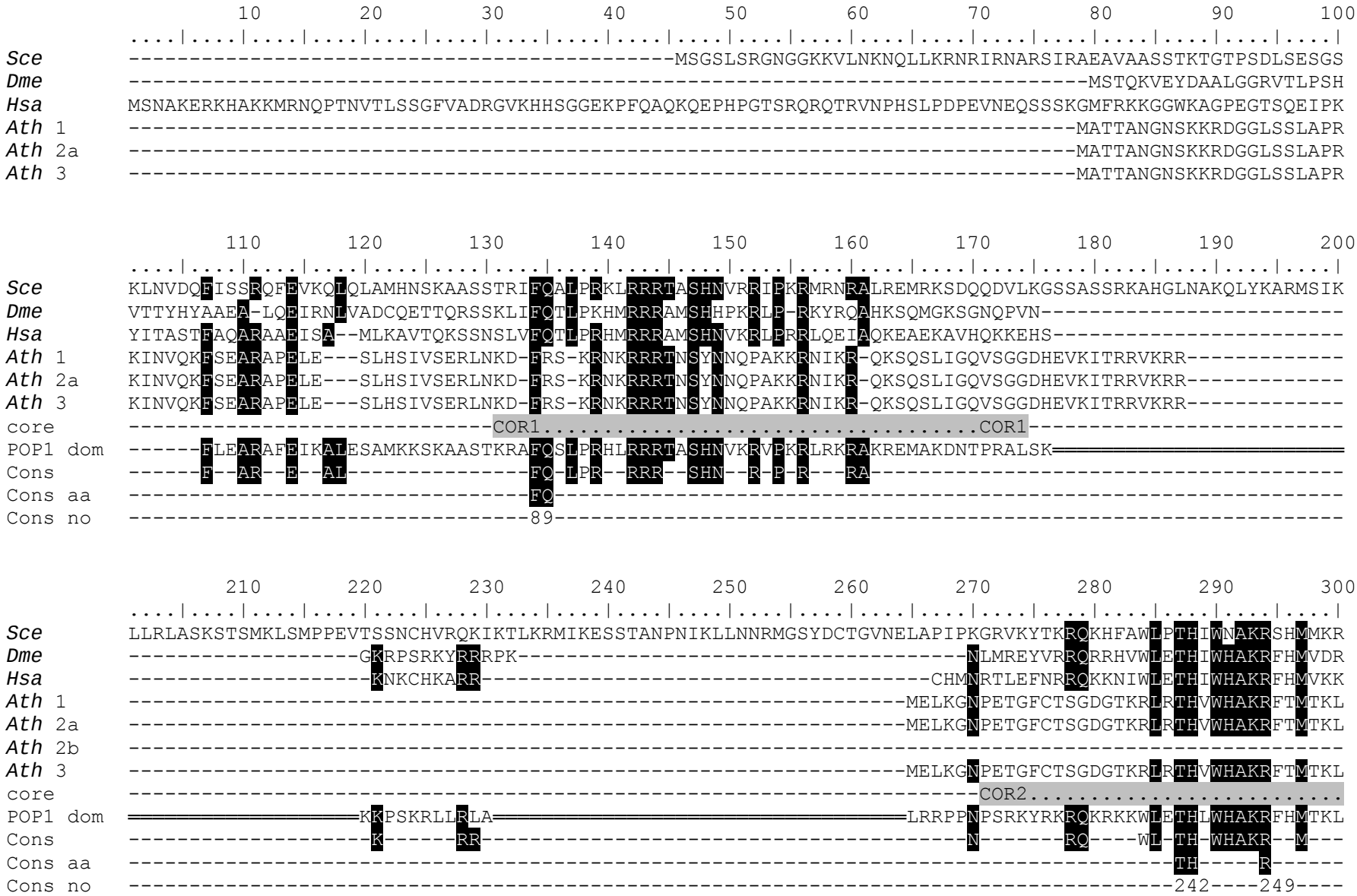
Oligonucleotides used in this experiment and not mentioned in the main text: AtPOP1-GSP15R: GTGAGCCCTGCCTCTGG; AtPOP1-GSP17R: GAGTGAGCCCTCTGGACC; AtPOP1-GSP15R-target: ATCCAGAGGCAGGGCTCACGATTCTCATCCAGAGGCAGGGCTCACAT; T7Promseq: TAATACGACTCACTATAGGG; AtPOP1-R2: TCACAAAAATAACCTCAAAAG.

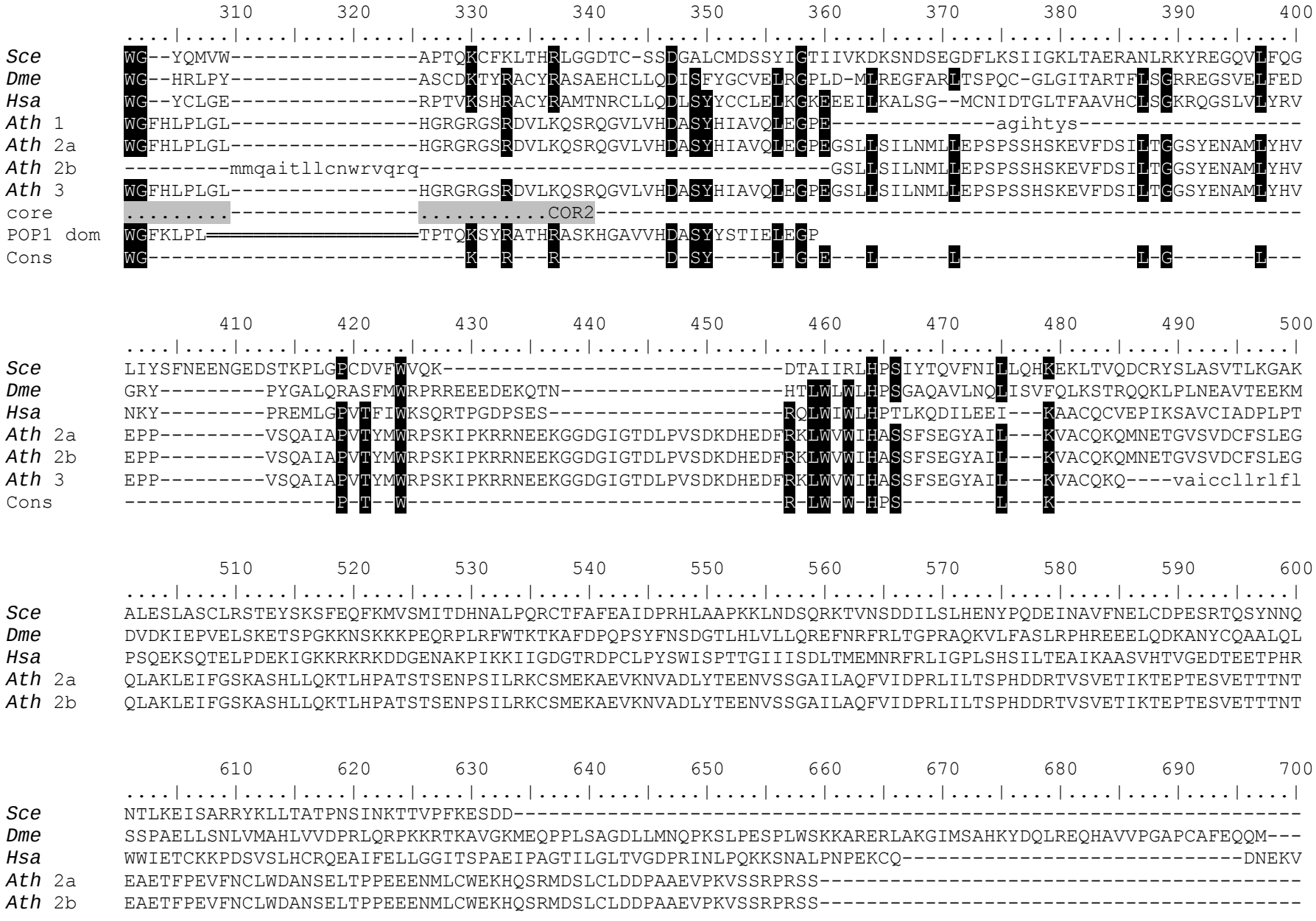
Results:

The two hybridization experiments were performed with primers discriminating between differently spliced intron 2 boundaries (see suppl. Fig. 1) leading to frameshift events and thus to different putative translation products. The probe recognizing the "wild type" splice sites of intron 2 (present in mRNA variant 2a and 3) detected two RNA populations (panel B): one of about 1200-1500 nt, possibly representing the mRNA 3 encoding the 307 aa protein (FN673552; mRNA length: 1260 nt including 5' UTR, but without poly(A) tail). The other RNA population has a length of about 3000 nt and is thus in accordance with the mRNA 2 a encoding the 826 aa protein (At2G47300.3; length of annotated mRNA: 2910 nt). In contrast, the probe recognizing the "alternative" splice sites of mRNA 2b reveals only a single RNA population (panel A), which migrates on the same position as the shorter RNAs in (B). Careful examination of RNA positions on gel and blots (taking into consideration the effects due to gel overloading in samples 9 and 10) shows that the position of the signals is close, but not identical to the rRNAs. The observation that the probe AtPOP1-GSP15R recognizes variant 2b and - though significantly weaker - the wild type splice sites, but AtPOP1-GSP17R recognizes only the wild type pattern (see dot blot samples 13-15), makes a quantification of the RNA populations difficult. The results show clearly that only the wild type splice pattern is present in the long mRNAs which encode the 826 aa protein; the shorter mRNA population is very likely a mixture of both splicing isoforms, including the mRNA encoding the 307 aa protein.

Supplementary Figure 3:

Similarity of AtPop1p variants to the homologous protein from yeast, man and fly, and position of conserved protein domains

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Dme WG--HRLPY----------ASCDKTYRACYRASAEHCLLQDISFYGCVELRGPLD-MLREGFARLTSPQC-GLGITARTFLSGRREGSVELFED
Hsa WG--YCLGE----------RPTVKSHRACYRAMTNRCLLQDLSYYCCLELKGKEEEILKALSG--MCNIDTGLTFAAVHCLSGKRQGSLVLYRV
Ath 1 WGFHLPLGL----------HGRGRGSRDVLKQSRQGVLVHDASYHIAVQLEGPE----------agihtys----------
Ath 2a WGFHLPLGL----------HGRGRGSRDVLKQSRQGVLVHDASYHIAVQLEGPEGSLLSILNMLLEPSPSSHSKEVFDSILTGGSYENAMLYHV
Ath 2b ---------mmqaitllcnwrvqrq----------GSLLSILNMLLEPSPSSHSKEVFDSILTGGSYENAMLYHV
Ath 3 WGFHLPLGL----------HGRGRGSRDVLKQSRQGVLVHDASYHIAVQLEGPEGSLLSILNMLLEPSPSSHSKEVFDSILTGGSYENAMLYHV
core .....----------.....COR2----------
POP1 dom WGFKLPL==========TPTQKSYRATHRASKHGAVVHDASYYSTIELEGP
Cons WG----------K--R---R---------D-SY-----L-G-E---L------L----------L-G-------L---
410 420 430 440 450 460 470 480 490 500
Sce LIYSFNEENGEDSTKPLGPCDVFWVQK----------DTAIIRLHPSIYTQVFNILLQHKEKLTVQDCRYSLASVTLKGAK
Dme GRY---------PYGALQRASFMWRPRREEEDEKQTN----------HTLWLWLHPSGAQAVLNQLISVFQLKSTRQQKLPLNEAVTEEKM
Hsa NKY---------PREMLGPVTFIWKSQRTPGDPSES----------RQLWIWLHPTLKQDILEEI---KAACQCVEPIKSAVCIADPLPT
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Ath 2b EPP---------VSQAIAPVTYMWRPSKIPKRRNEEKGGDGIGTDLPVSDKDHEDFRKLWVWIHASSFSEGYAIL---KVACQKQMNETGVSVDCFSLEG
Ath 3 EPP---------VSQAIAPVTYMWRPSKIPKRRNEEKGGDGIGTDLPVSDKDHEDFRKLWVWIHASSFSEGYAIL---KVACQKQ----vaiccllrlfl
Cons ----------P-T--W----------R-LW-W-HPS--------L---K----------
510 520 530 540 550 560 570 580 590 600
Sce ALESLASCLRSTEYSKSFEQFKMVSMITDHNALPQRCTFAFEAIDPRHLAAPKKLNDSQRKTVNSDDILSLHENYPQDEINAVFNELCDPESRTQSYNNQ
Dme DVDKIEPVELSKETSPGKKNSKKKPEQRPLRFWTKTKAFDPQPSYFNSDGTLHLVLLQREFNRFRLTGPRAQKVLFASLRPHREEELQDKANYCQAALQL
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Ath 2b QLAKLEIFGSKASHLLQKTLHPATSTSENPSILRKCSMEKAEVKNVADLYTEENVSSGAILAQFVIDPRLILTSPHDDRTVSVETIKTEPTESVETTTNT
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Dme SSPAELLSNLVMAHLVVDPRLQRPKKRTKAVGKMEQPPLSAGDLLMNQPKSLPESPLWSKKARERLAKGIMSAHKYDQLREQHAVVPGAPCAFEQQM---
Hsa WWIETCKKPDSVSLHCRQEAIFELLGGITSPAEIPAGTILGLTVGDPRINLPQKKSNALPNPEKCQ----------DNEKV
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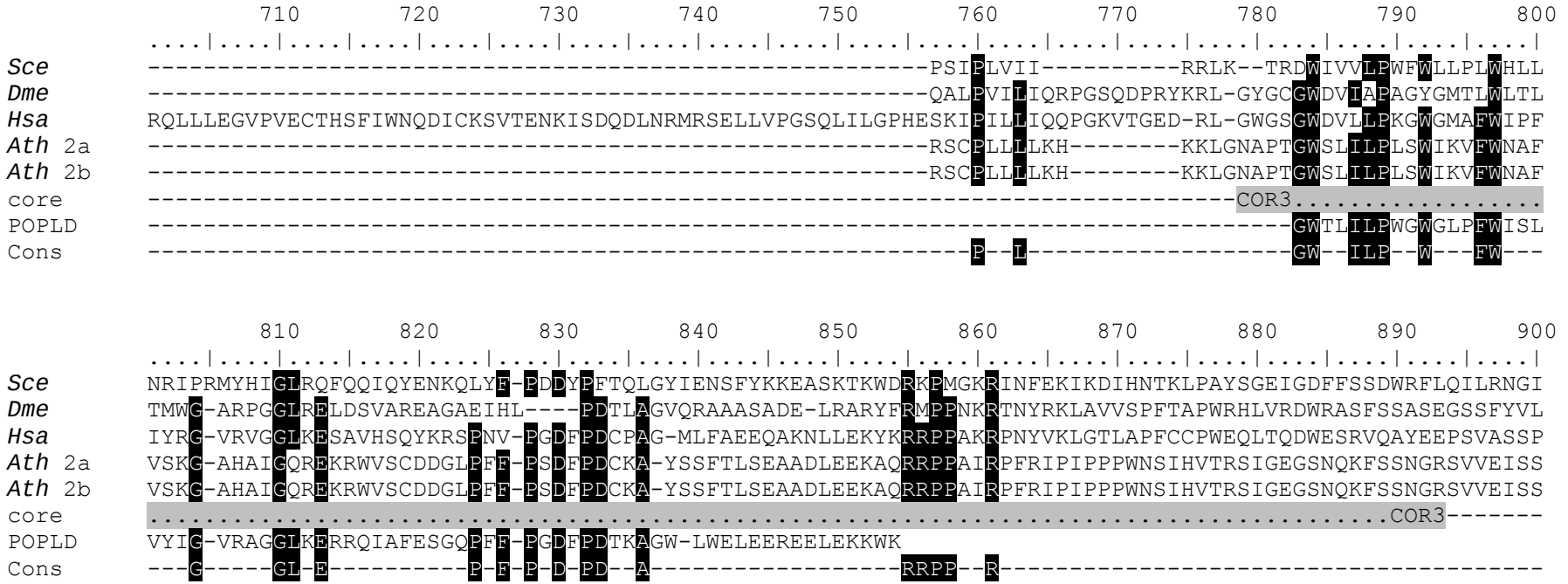
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Hsa
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Ath 2b
core
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Cons
COR3
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Sce
Dme
Hsa
Ath 2a
Ath 2b
core
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Cons
COR3
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Sce
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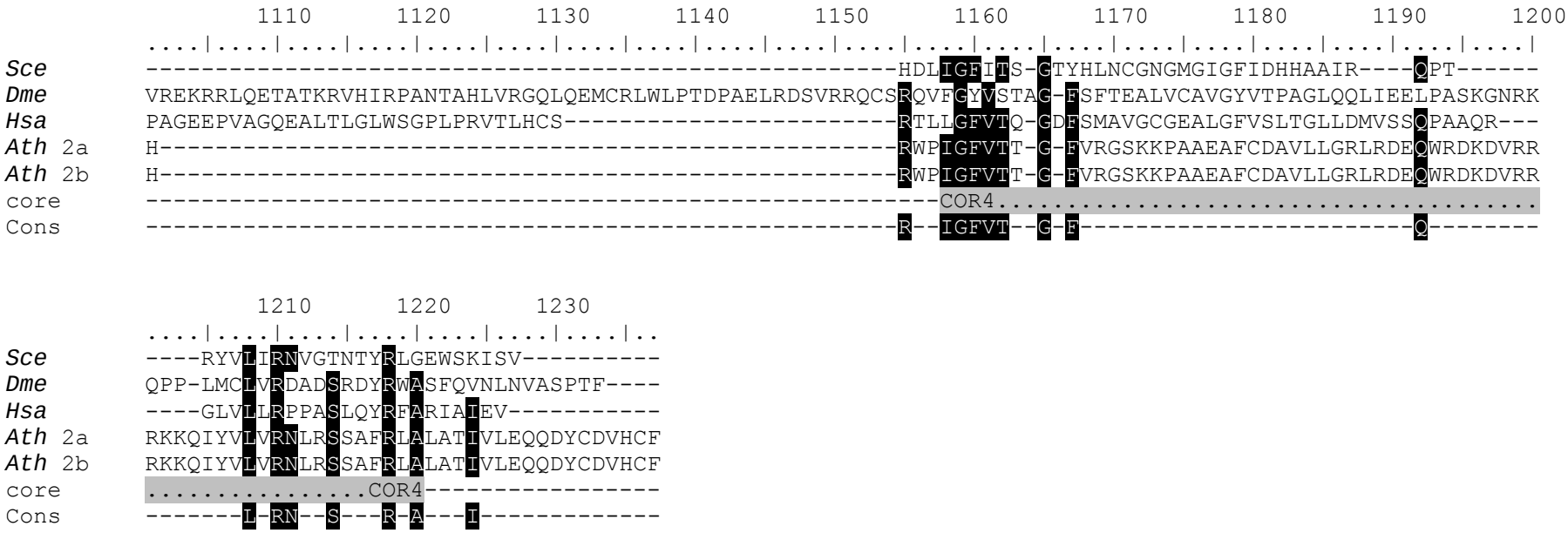


Alignment method:

A consensus sequence was obtained by aligning the Pop1p sequences from *M. musculus*, *S. cerevisiae*, *D. melanogaster*, *H. sapiens*, *A. thaliana*, *O. sativa*, *A. fumigatus*, *V. vinifera*, *S. pombe* and *D. discoideum* with ClustalW, followed by manual refinement according to the conserved regions. Amino acid positions with over 60 % identity in this original alignment are highlighted in black. The alignment shown here is reduced to the sequences from *S. cerevisiae* (*Sce*), *D. melanogaster (Dme*), *H. sapiens* (*Hsa*) and the four annotations from *Arabidopsis* (*Ath*).

Ath 1, originally annotated 190 aa *A. thaliana* protein (At2g47290.1; AAB63829.1); *Ath* 2a, annotated 826 aa protein (NP_001078072; DQ069804); *Ath* 2b, annotated 659 aa protein (NP_001078073; DQ069805): *Ath* 3, 307 aa protein (FN673552; this study). The truncated variant 1-u (151 aa) used for antibody production has been omitted from this alignment; its C-terminus is at position 328.

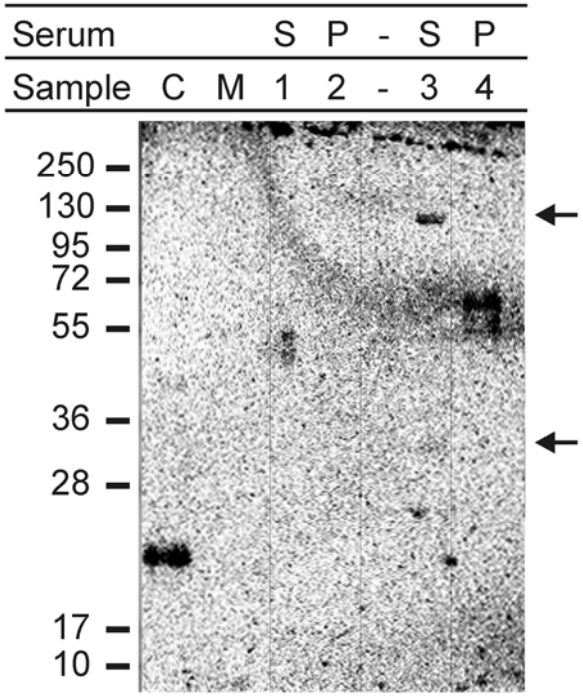
Core, the conserved regions COR1-COR4 described by (27). They are highlighted in grey; their termini are connected by dots and indicated by the respective names. **POP1 dom / POPLD**: the conserved POP and POP-like domains; the isolated stretches of the POP1 domain are connected by a double line. **Cons**, amino acids with >60 % identity between all tested organisms. **Cons aa/Cons no**: identity and position of conserved amino acids which have been tested by mutation analysis in yeast (27). Sequences in minuscules arose from frameshift events in the mRNAs and were placed outside of conserved regions.

Supplementary Figure 4: Assessment of antibody specificity for AtPop1-related proteins by Western analysis

Methods:

Arabidopsis protein extracts were obtained by grinding young leaves in dry ice, followed by suspension of the tissue powder in a buffer containing 20 mM Tris-Cl pH8, 0.5% SDS; 5% β-Mercaptoethanol, 1 mM PMSF, 2 µM Leupeptin and 1 µM Pepstatin, and homogenisation with an Ultra-Turrax. After centrifugation at 16000 g for 30 min, the supernatant (S16) was further concentrated by methanol:aceton precipitation. The dry sediment was resuspended in a buffer containing 20 mM Tris-Cl pH8, 5 mM $MgCl_2$, 1 mM PMSF, 2 µM Leupeptin and 1µM Pepstatin.

AtPop1p-specific serum or preimmune serum, respectively was coupled to two aliquots of Protein A/G Agarose (10 mg each; Pierce Crosslink IP Kit) according to the manufacturer's instructions. 750 µg of the above extract was loaded onto the columns. After washing, bound proteins were eluted in three fractions and again precipitated as above. Following solubilisation in sample buffer containing 2 % SDS and 2 M urea, the proteins were separated by SDS PAGE (12 % PAA) and transferred to PVDF membrane by semidry electroblotting. After blocking for 16 h with 3 % nonfat dry milk in PBS + 0.1 % Tween 20, the membrane was incubated for 16 h with AtPop1p-specific antiserum depleted of *E. coli* specific antibodies (32). The blot was washed 5 times with PBS + 0.3 % Tween 20 and incubated for 1 h with HRP-coupled Goat-anti-rabbit antibodies (dil. 1: 10000; Sigma) in PBS containing 3 % nonfat milk powder + 0.05 % Tween 20. After washing 5 times with PBS + 0.05 % Tween 20, the signals were developed with ECL Plus substrate at 20 °C and recorded for 60 min on an AlphaImager.



Detection of AtPop1-related proteins by IP-Western with AtPop1p-specific antibodies.
Serum specificity: S, eluates from the column coupled with AtPop1p-specific antiserum; P, eluates from the column with preimmune serum.
Samples: Lanes 1 and 2 contain the first eluate, lanes 3 and 4 the combined eluates 2 and 3 from each column, respectively. C, 22.5 ng recombinant AtPop1p antigen (expressed from pRSET-AtPOP1; 187 aa, 21.4 kDa). M: Page Ruler Plus Prestained Protein Ladder, Fermentas. The size and position of the marker proteins visible on the membrane directly after blotting is shown to the left. The two signals of about 35 and 100 kDa detected in the eluate from the AtPop1p-specific column (lane 3) are indicated with arrows.

Results and Discussion:

Prior to performing immunoprecipitation experiments, the specificity of the antisera created against the recombinant *Arabidopsis* POP1 domain (AtPop1p, variant 1-u) was examined by Western blotting. No specific bands could be recognized in crude extracts or S23 preparations from *Arabidopsis*, possibly due to low abundance of the target protein (not shown). Using proteins enriched by immunoprecipitation with the same antiserum, a clear signal at approximately 100 kDa and a weak band at about 35 kDa were detected. They most likely correspond to the 826 aa (92.5 kDa) and 307 aa (34.6 kDa) ORFs evident from mRNA analysis, respectively. The signal at about 70 kDa visible in lane 4 is due to partially denatured IgG leaking from the column.

The presence of two specific signals corroborates the translation of mRNA variant 2a and of the novel mRNA variant 3 into the 92.5 and 34.6 kDa proteins, with the smaller protein present in a significantly lower amount. The putative 73.8 kDa ORF encoded by mRNA variant 2b lacks the POP1 domain and thus can not be detected by this antiserum.

Supplementary Figure 5: Complex formation between AtMRP1 RNA and the POP1 domain of AtPop1p.

Methods:

The transcription clone for AtMRP RNA1 was constructed by amplification of the coding region from genomic DNA with the primers 5'MRP-2 and 3'MRPanti-2 and ligation into SmaI-cleaved pUC19. The T7 promoter and appropriate restriction sites were introduced by amplification with 5'MRPEcoT7 and 3'MRPFokHind, cleavage with EcoRI and HindIII and ligation into appropriately cut pUC19 to obtain pT7ATMRP1, ready for runoff transcription after cleavage with FokI.
Oligonucleotides used for this construct: 5'MRP-2: CAATTGTCACTGGACG; 3'MRPanti-2: GTTGCTTGTCATTGAAAC; 5'MRP-EcoT7: CGGAATTCTAATACGACTCACTATAGGGACAATTGTCACTG; 3'MRP-FokHind: CGCAAGCTTGGATGTCCGGATCCAAAAGTTGCTTGTCATTGAAAC.

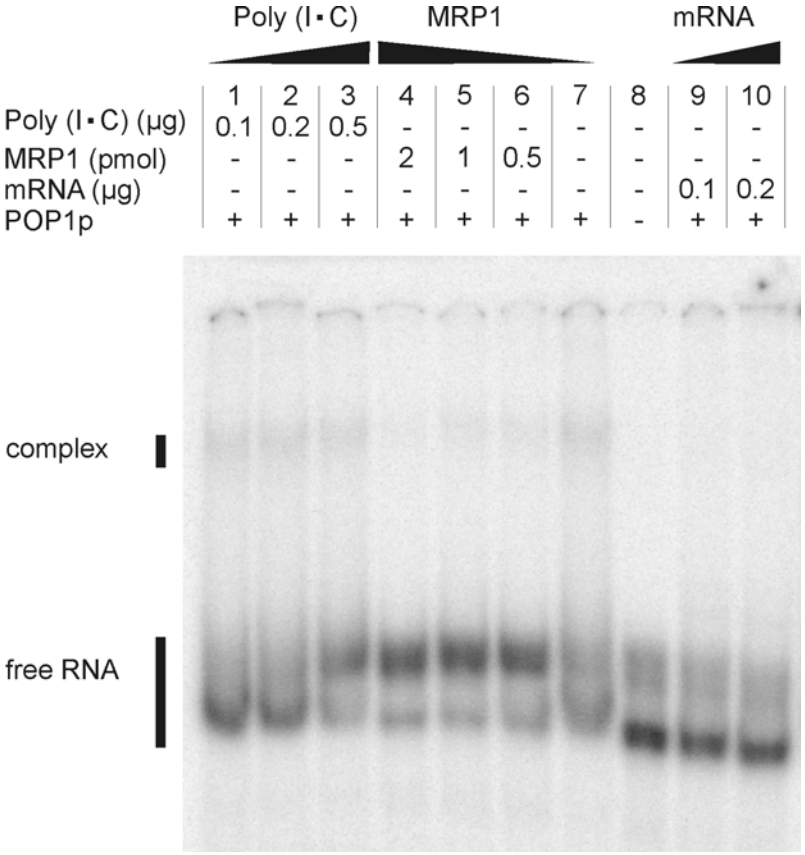
Construction of expression plasmids for the *Arabidopsis* POP1 domain was achieved by amplification of the pAtPOP1 insert with AtPOP1-Bam-F and AtPOP1-Sma-R, and cloning as an in-frame fusion into the BamHI and XmaI sites of pQE30 (Qiagen). The original starting ATG of the ORF was converted into GTG by PCR directed mutagenesis using the primers POP1-mutF and POP1-mutR to give pQE-AtPOP1. A second expression vector (pBlue-AtPOP1) was constructed by introducing a synthetic T7 terminator composed of oligonucleotides T7Term1 and T7Term2 into the HindIII site of pBluescribe M13$^-$, and ligating the EcoRI-XmaI fragment of pQE-AtPOP1 into this plasmid to give pBlue-AtPOP1. The final expression plasmid was created by ligating the BamHI fragment of pBlue-AtPOP1 into pRSETa (Invitrogen) to yield pRSET-AtPOP1. The 187 aa long recombinant AtPop1p obtained from this construct contains an N-terminal His-tag attached to the 151 aa long truncated POP1 domain.
Oligonucleotides used for this construct: AtPOP1-Bam-F: CGCGGATCCATGGCTACTACTGCG; AtPOP1-Sma-R: GCACCCGGGTCAAGAATAAGTGTG; POP1-mutF: CCATCACGGATCCGTGGCTACTACTGCGAATGG; POP1-mutR: CCATTCGCAGTAGTAGCCACGGATCCGTGATGG; T7Term1: AGCTTAATTAGCTGAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTA;
T7Term2: AGCTTAAAAAACCCCTCAAGACCCGTTTAGAGGCCCCAAGGGGTTCAGCTAATTA.

For formation of RNA-protein complexes, recombinant AtPop1p was dialysed against buffer F-GP. MRP RNA transcripts dissolved in TE plus 2 mM $MgCl_2$ were carried through a folding cycle by heating to 75 °C for 5 min followed by cooling to 25 °C. After addition of binding buffer (final concentrations: 10 mM Tris-HCl pH 8, 50 mM NaCl, 5 mM $MgCl_2$, 0.1 % (v/v) Triton X-100, 1 mM DTT; 0.4 u RNasin/assay), assays were set up with 1 pmol protein and 50 fmoles of radiolabelled, renatured MRPR1 in a total volume of 15 µl. Competition assays were supplemented with different unlabeled RNAs (see figure caption). Binding was initiated by addition of protein to the refolded RNA. After 30 min incubation at 22 °C with gentle agitation (Eppendorf Thermomixer, 350 rpm), 1 vol. of EMSA sample buffer (50 % v/v glycerol, 0.03 % (w/v) Bromophenol blue and Xylene cyanol FF) was added. The samples were immediately loaded onto native gels (5.6 % (w/v) Acrylamide-bisacrylamide (29:1) in 0.5 x TG (12.5 mM Tris, 96 mM Glycine, pH 8.3) and separated at 4 °C. Gels were dried before exposure to a Phosphoimager and densitometric analysis.

Results:

The POP domain of AtPop1p can form RNA-protein complexes with AtMRP RNA

Under the conditions used for optimal complex formation, AtMRP1 is present in at least two interconvertible conformational populations, visible as distinct bands in the lower part of the gel. MRP1 RNA and the POP1 domain can form a complex with retarded gel mobility; the radioactive transcript can be competed out by an excess of unlabeled MRP RNA (lanes 4-7). Binding specificity was tested by competition with unrelated RNAs. The double-stranded copolymer poly(I•C) did not exert a detectable influence on complex formation (lanes 1-3). A preparation of total poly(A^+)-RNA led to almost complete inhibition of MRP1 binding by AtPop1p (lanes 9-10), suggesting a preference for single-stranded RNA. This is in contrast to the finding made by Aspinall et al. (24), where a preference for double stranded RNA was deduced from pull-down assays. The weak binding observed in our study may possibly be attributed to the lack of the POPLD region in this truncated protein. Due to the diffuse bands and differences between individual protein preparations, an estimation of binding parameters is unreliable and gives estimated K_d values between 30 and 130 nM.



Supplementary Figure 5:
Electrophoretic Mobility Shift Assay of complex formation between AtMRP1 RNA and the POP1 domain of AtPop1p.
Complexes were formed from 1 pmol AtPop1p and 50 fmoles radiolabelled MRPR1; amounts of competing RNAs are given in the caption. Samples 1-3: competition with double-stranded poly(I•C); 4-7: homologous competition with unlabeled MRP1; 8: MRP1 under binding conditions; 9-10: competition with total mRNA. Positions of free RNA and complex are indicated to the left of the gel.