## S3 File. DNA and protein alignments built to determine intron landscapes.

Alignments were performed using Muscle as described in the materials and methods section. Exonic sequences from *Passalora* species were aligned to the previous DNA alignment using Muscle and new alignment was manually edited in GeneDoc. All names are as described in Figure 3 and S4 File.

DNA alignments: introns are highlighted in yellow. Four-level shade indicates nucleotide conservation over 90%, 80%, 60%, and under 60%. In D, two-level shade indicates nucleotide conservation over and lower than 65%. In B, insertion of a single nucleotide in the sequence of *Lentfl* makes it a pseudogene.

Protein alignments: positions of RSIs, previously identified ILEs and novel ILEs are indicated in yellow, orange and red, respectively. For novel ILEs, the position is indicated within the sequence of the two most closely related fungal species. Numbers indicate phase 0, 1 or 2. Numbering of intron positions in Figure 3 corresponds to the position in the corresponding alignment below, minus the number of upstream intron positions. Four-level shade indicates amino acid identity of 100%, over 80%, over 60%, and under 60%.

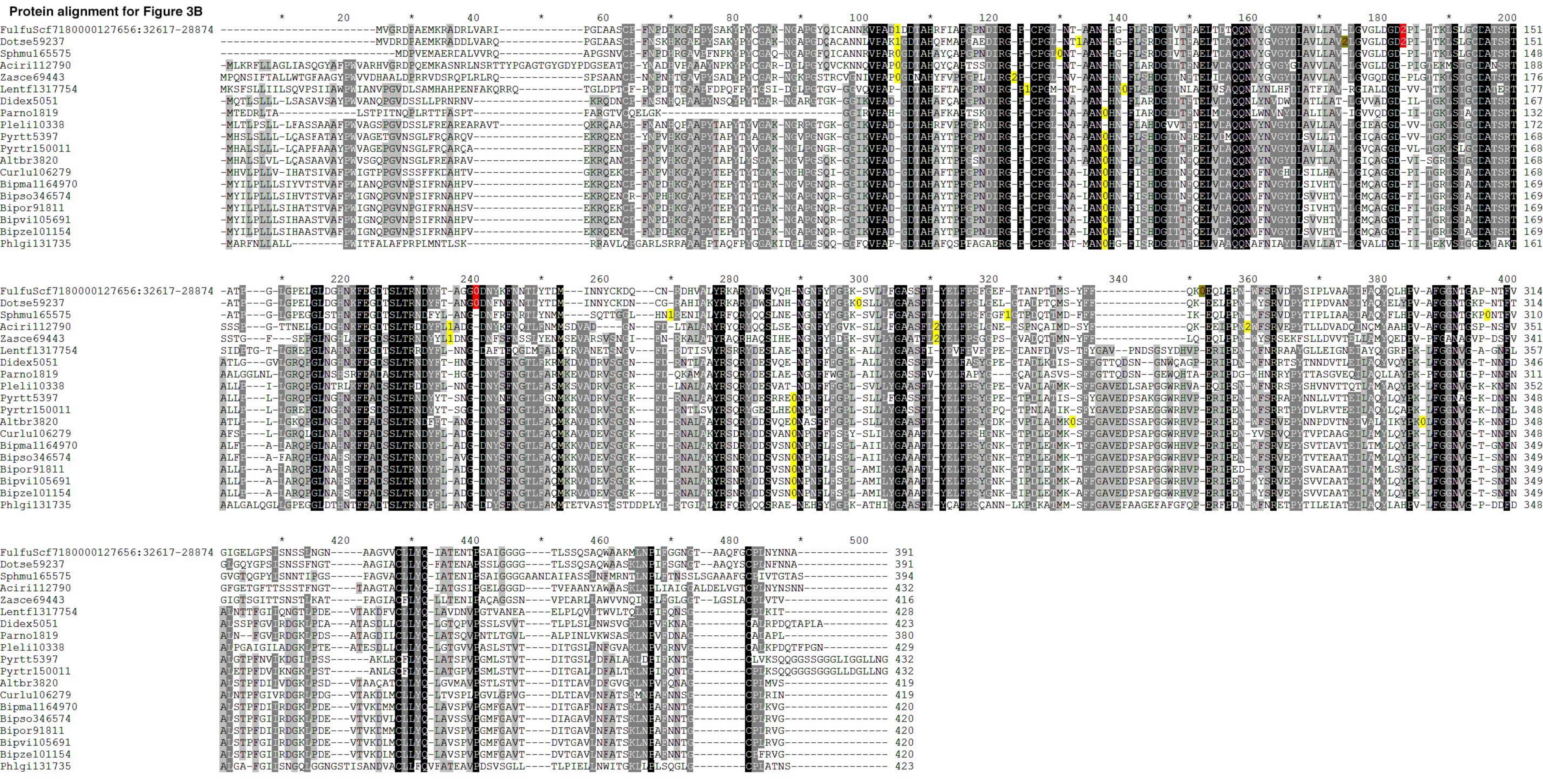


Protein alignment for Figure 3A VLSLVWFAVQSWTGGLCVQNILAAIFPSYQHMDSVFFA
ILLSLVWFSVQSWTGGLCVQNILASMFPSYQHMGNHFFA
ILLSLVWFSVQSWTGGLCVQNILASMFPSYQHMGNHFFA
IVLSLVWFSVQSWTGGLCVQNILAAIFPSYQYMPNHF ASANMNAKQ<mark>1</mark>FIGWVIFNILIVPLLYIRPEKLKWV 194 FASANMNTKQOFIGWVVENVLMVEILYVKPEKMQWV 194
FASANMNTKQOFIGWVVENVLMVEILYVKPEKMQWV 194
SASADMTTKQ-FIGWIIFNLLMIFILYIKPERIQWV 194
PESAKMCAKQ-FVGWIIFNVLMIFILYVRPEKMKWI 194 Cerzm42490 MAFRDILDFVRLPSDSRSVTNVWINEDIRPLPPHRRTWTRWAFASFWAINQLTLSNWQLGASLVSAGLSVWQAIIA-TVIGKVIIAAVAVGNG-YVGAE-WHIGFPVASRYIW-GMYG-HYLALIQRIV
Psefi36375 MGLGRVLEIIRLPTNSRSVVSFWINEDIRPLPPSRRTWTRWAYISFWAINQICLSNFQLGASLVSAGLAVWQAILA-VIIGKFIIAAVAIANG-YVGAE-WHIGFPVVSRYIW1GMYG-SYLAIVQRII
Zymtr50149 MFVGSFLESIRLPSNDRSVANVWINEDIRPLPPNRRTWTRWAYVSFWAINQIALGNFQIGASLVSTGLSVWQAVIA-VLVGKIIIAVVAVANGOTVGAE-WHIGFPVVSRYIW-GIYG-HYTALIQRII I<mark>LSLVWF</mark>SVQSWTGGL HMDTRQ-FIGWIIFNLIMIPILWVRPEKMKWV 194 LLSLVWFSVQSWTGGL --MESGLRFLQVHSSGDE-TSVWINDDIRPLPPSRRTWDKLAFISFWAINQIALSNWQTGSSLVAVGLSVWQTMIA-VIVAKIIISLVAIYNG-YVGAE-Glotr82366 Fulful93200 vlemnivtaitilsiviwvlsaaggggplitopaettisselemaivhgvtviggiavgltnomdysrfarrpgdovf-gomesiivegaimeslgofsasasogvygeai-wnpenlvokwldtdynpksrsaaffaggglitopaettisselemaivhgvtviggiavgltnomdysrfarrpgdovf-gomesiivegaimeslgofsasasogvygeai-wnpenlvokwldtdynpksrsaaffaggglitopaettisselemaivhgvtviggiavgltnomdysrfarrpgdovf-gomesiivegvimealgoiasasogvygeai-wnpenlvokwldtdynaksraaaffaggglitopaettisselemaivhgvtviggiavgltnomdysrfarrpgdovm-gomesiivegvimealgoiasasogvygeai-wnpenlvokwldtdynsrsraagffaggglitopaettisselemaivhgvtviggiavgltnomdysrfarrpgdovm-gomesiivlgvimealgoissasogvygeai-wnpenlvokwldtdynsrsraagffaggglitopaettisselemaiss Zymtr50149 VLWMNAVSFVTLLAMTIWLLAEAGGAGPLLKQPATVKSSSA Glotr82366 FIVMNVISFFTLISMMIWALSQAHGGGPLL<mark>S</mark>QFAAVSSSSELGWAIVS<mark>GITTVIGSIAVGL</mark>TNQFDYSRFARKPGDQI -LYYLAFPLGFTVSFTVYWCLNKVWTLDGFGVKDEIDYYGTFTEEFAAKLGVAMMETIEGEVRDGVSVEEEMGKDEKFVAGSMNVTAV 512 Fulfu193200 SVLSAY<mark>1</mark>SVFLGFMVGIMVCDYWTLRHRII Dotse126132 SVLSAY<mark>1</mark>SVFLGFMVGIMVCDYWILRHRVVKLSDLY

LPGFAHAVTPKVLVPEACTH-LYYLAFPLGFAVSFTVYWSLNKIWTFAGFGVKDDIDYYGTFTEEEAAKLGVAMTETIEGEARERVSIEQDMHKDDKFAAGLTDIRAV 563 LPGFAHAVTPSIKVPEACTN-LYYLAFPLGFAVSFSVHFCINTAFPPVGLQEIDDVDYFGTFTETEAAKMGVAVSETIESEDGGSPPNELDKGK----AGLTQVRSV 558 SVFLGPMVGIMICDY<mark>FI</mark>LRKRRVKLSDLY Sphmu82757 SVFLGFMVGIMICDYFILRKRRVKLSDLYH LPGFAHAVTPIVKVPEACTN-LYYLAFPLGFAVSFSVHFCINTAFPPRGLQEIDDVDYFGTFTETEATRMGVAVPETIESEDGGSPPDELDKGK----AGWTQVRRV 558 Sphpo39262 SVLSAY-SVFLGFMVGIMICDYWVLRKRRVKLSDLYHF SVLSAY-SVFLGFMVGIMTCDYWILRKRKVKLSDLYNF Cerzm42490 Psefi36375 Zymtr50149

Glotr82366

DNA alignment for Figure 3B  Pasbra-cf0203-14-7-3 FulfuScf7180000127656:32617-28874 Dotse59237 Sphmu165575 Aciri112790 Zasce69443 Lentf1317754 Didex5051 Parno1819 Pleli10338 Pyrtt5397 Pyrtr150011 Altbr3820 Curlu106279 Bipma1164970 Bipso346574 Bipor1811 Bipvi105691 Bipze101154	ATGTTGAAAAGGTTTCTCCT CGCTGGCTTGATA-CATCACAAGGTTATGCCTTC CATGG TACCTCGACATGTG GGGAGAGA CCGCAGGAAATGAAAGGTTATGCCTTC CATGG TACCTCGACATGTG GGGAGAGA CCGCAGGAAATGAAAGATCCTTCTACCTTTTGAT TATTTTGTCCCAAA TGCCATCCATCATAGAATGG CATGGATTTCCCAAATGTCCAAG TGTAGACCT	GATC  AGGCTTCAAACAGGCTCAATTCACGAACCACCTATCCGGGAGCTCGTACGGGTTA GCGCGTAGACAGCCGGCAGCCAT TGAGACTACCTCA GTCGGCCATGCACGCACATCCGGAGAACTTTGCCAAACAGCGTCCCCA GTCCCCCGTAACCGCA	
Pasbra-cf0203-14-7-3 FulfuScf7180000127656:32617-28874 Dotse59237 Sphmu165575 Aciri112790 Zasce69443 Lentf1317754 Didex5051 Parno1819 Pleli10338 Pyrtt5397 Pyrtr150011 Altbr3820 Curlu106279 Bipma1164970 Bipso346574 Bipor1811 Bipvi105691 Bipze101154  Pasbra-cf0203-14-7-3 FulfuScf7180000127656:32617-28874	TCAATCCTGATCATAAGGGCGCTCCTCCATACACAGAGCCTTACACCTACACAGGAGCAAAGAATGGCGTTCCTGGTAACCAGA——GAGGTGGTATTAAAGTTCCACTCATCACACAGAGGCGCTCCTACACACAGAGCCTACACAGGAGCAAAGAATGGCGCTCCTGGTAACCAGA——GAGGCGGTATCAAAGTTCCACTCATCACACACAGAGCCTTACACACAGAGCCTACACAGGAATGGCGCTCCCGGTAACCAGA——GAGGCGGTATCAAAGTTCCACTCATCATCATAAAGGCGCCCCCCCC	GCTAGGGTATGAATATGTGTGTACGTGAGGATGGATTGCAGCAGAAGCTGACTGTGACCAGAACCAGTGAGTTATTTTTTTT	TAGTTCCAACAGG       24         AAGCCATGGCAGG       36         TAACATGTCCAGG       31         27       24
Dotse59237 Sphmu165575 Aciri112790 Zasce69443 Lentf1317754 Didex5051 Parno1819 Ple1i10338 Pyrtt5397 Pyrtr150011 Altbr3820 Curlu106279 Bipma1164970 Bipso346574 Bipor1811 Bipvi105691 Bipze101154  Pasbra-cf0203-14-7-3 FulfuScf71800000127656:32617-28874	ATGAACGTTGACACGTGTCTAGGCCAACACTGCCCACCACTTCATGGCCCCAACACGCGAACACACGCCGAACACTGCTGGAACCACCACCACCACCACCACCACCACCACCACCACCA	C	* 780 * 800  * 780 * 800  * 780 * 800  * 780 * 800
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Aciri112790 Zasce69443 Lentf1317754 Didex5051 Parno1819 Pleli10338 Pyrtt5397 Pyrtr150011 Altbr3820 Curlu106279 Bipma1164970 Bipso346574 Bipor1811 Bipvi105691 Bipze101154  Pasbra-cf0203-14-7-3 FulfuScf7180000127656:32617-28874 Dotse59237 Sphmu165575 Aciri112790	CAGCAAAC  CTGCAAATGTAAGTACTAAGACA  CTGCAAATGTAAGTACTAAGACA  CTGCAAATGTAAGTACTAAGACA  CTGCAAATGTAAGCACACAAC  CTGCAAATGTAAGCACACACACACACACACACACACACAC	GATAAACACCAGCACAAT	TTCCTTGCCCACGATGCAGTCGTG 36TTCCTCTCCCATGACGCAATCACC 41TTCCTCTCCCATGACGCAATCACC 41TTCCTTTCCCCATGACGCAATCACC 40TTCATTTCTCACGATGCAATCACA 40TTTATTTCTCACGATGCAATCACA 41TTTATTTCTCACGATGCAATCACA 41TTTATTTCTCACGATGCAATCACA 42TTTATTTCTCACGATGCAATCACA 42TTTATTTCTCACGATGCAATCACA 41TTTATTTCTCACGATGCAATCACA 41TTTATTTCTCACGATGCAATCACA 41TTTATTTCTCACGATGCAATCACA 41TTTATTTCTCACGATGCAATCACA 41
Zasce69443 Lentf1317754 Didex5051 Parno1819 Pleli10338 Pyrtt5397 Pyrtr150011 Altbr3820 Curlu106279 Bipma1164970 Bipso346574 Bipor1811 Bipvi105691 Bipze101154  Pasbra-cf0203-14-7-3 FulfuScf7180000127656:32617-28874 Dotse59237 Sphmu165575 Aciri112790 Zasce69443 Lentf1317754	ACCTTCACTAGCTCGTCGTGTGTCAACAGAACTTGTAATATGTTTACTGGGACCTTTGTTACCGTCTTTCGGACCCTCACGACCCTCATGGTTTACCGTCCTTTGTTACCGACCTCATGGTTTACCGACCTCATGGTTTACCGTCCTTTGTTACCGACCTCATGGTTTACCGTCCTTTTACCGACCTCATGGTTTACCGTCCTTTTACCGACCTTTTACCGACCTTTTACCGACCTTTTACCGACCTTTTACCTTTACCGACCACCACCACACACA	GACCTTGGTCTCGAC GCCACAACAAGTTCGAGGTGACACTTCCCTTACCCGCA GAACTTGGTCTTGACGGACACAACAAGTTCGAGGGTGACACTTCTCTCACCCGCA GAAGGTGGTCTGAGCACGCACAACAAGTTTGAGGGAGACACTTCTTTGACCAGAA GAGTTAGGCCTAGA <mark>T</mark> GGCCACAATAAGTTTGAAGGAGA <mark>T</mark> ACTTCCCTGACTCGTG GAACCTGGGCTCAA <mark>T</mark> GGCCACTCAAGTTCGAAGGAGACTCATCGCTCACACGCA	CGGTG 43CGGAG 37CGGAA 45CGGAA 49CGGAA 49CGGTA 49
Didex5051 Parno1819 Ple1i10338 Pyrtt5397 Pyrtr150011 Altbr3820 Curlu106279 Bipma1164970 Bipso346574 Bipor1811 Bipvi105691 Bipze101154  Pasbra-cf0203-14-7-3	TCGTTGCC AT GGTGA CATTCT	CAGCCCGGACTGAACGACACAACAAGTTCGAGGCGACTCATCGCTGACCCGAAGAGCCTGGCTTGAACAGCAGATTCGAGGCAGATTCGAGCCTGACCCGAAGAGCCTGGACTGAACAGCAGATTCGAGGCAGATTCGTTGAACAGGAAGCAGATTCGAAGCCTGAAACAAGTTTGAAGCCGAAGCCTGAAACAACAAGTTTGAAGCCGAAGCCTGACAGTTCGTTGACCCGAACAACAAGTTTGAAGCGGATTCTTCGCTTACGCGCAACAACAAGTTTGAATCGATTCGTTGACCCGCAACAACAAGTTTGAATCGATTCGTCGCTTACGCGCAACAACAAGTTTGAGGCCTGATTCGTCGCTTACGCGCAACAACAAGTTTGAGGCCTGACTCGTCGCTTACACGCAACAACAAGTTTGAGGCCTGACTCGTCGCTAACACCAACAAGTTTGAGGCCTGACTCGTCACTCAC	# 1580 * 1600  *** 1600  *** 1600 * 1600  ** 1600 * 1600  *** 1600 * 1600  *** 1600 * 1600  *** 1600 * 1600
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Pyrtt5397 Pyrtr150011 Altbr3820 Curlu106279 Bipma1164970 Bipso346574 Bipor1811 Bipvi105691 Bipze101154  Pasbra-cf0203-14-7-3 FulfuScf7180000127656:32617-28874 Dotse59237 Sphmu165575 Aciri112790 Zasce69443 Lentf1317754 Didex5051 Parno1819 Pleli10338 Pyrtt5397		ACCCARACTTCTTCTTCAGCCCTTAC	* 1980 * 2000  ATCTTT GGCGAGTTC
Pyrtr150011 Altbr3820 Curlu106279 Bipma1164970 Bipso346574 Bipor1811 Bipvi105691 Bipze101154  Pasbra-cf0203-14-7-3 FulfuScf7180000127656:32617-28874 Dotse59237 Sphmu165575 Aciri112790 Zasce69443 Lentf1317754 Didex5051 Parno1819 Pleli10338 Pyrtt5397 Pyrtr150011 Altbr3820	TGACTACTGCTTTATGCTGCCGCATCCTTTATCTTTATGCTGCTGCATCCTTTATCTCTCTATATGCTTGCT	TACTTCTTC TTCTTCTTC TTCTTCTTC TTCTTCTTC TTCTTC	-CAAACGGAGAATGGCAACATACAGCC
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DNA alignment for Pasmiu-cf0203-AA61 Basce60808 Berzm65622 Bymtr43851 Bopma596116 Byrtr157321 Burlu123898 Bipso218368 Bipso218368 Bipso218368 Bipso218457 Bipvi84790 Bipze101753 Beng1219443 Baspve124547 Baspve124547 Benox5982 Benbi454008 Benbr34900 Benca423940 Bench69724 Bolgr10528 Bolgl1931734 Bolfi1350467 Melva566401	ATGG CTC		* 160 * 180 * 200
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Pasmiu-cf0203-AA61 Zasce60808 Cerzm65622 Zymtr43851 Copma596116 Pyrtr157321 Curlu123898 Sipma1034928 Sipso218368 Sipor28457 Sipvi84790 Sipze101753 Peng1219443 Aspve124547 Aspwe173916 Penox5982 Penbi454008 Penca423940 Penca423940 Pench69724 Colgr10528 Colgl1931734 Colfi1350467 Melva566401	CATAC TARCCAACAATGGAGGT TTCGGCAGATGAGCTG	-CAAGATG GCGCCCGATGGTAGACGAAGCAGACG ACAGCACGATGATGATGAAGCAGACG ACAGCAGATGATGATGAAGCAGACG ACAGCAGATGATGATGAAGCA	TTCCGAATCTACAACACCGAAGGCACCAAAGIG 32TTCGAATTTTCAATGTTGATGCTAAGAAGIT 27TTCGAGATCTACAATACCGATGCAACATGGTA 26TTCCGCATCTACAATACCGATGCCAACATGGTA 26TTCCGCATCTTTAATTCGGAAGGCCAAGATGGTG 26TTCCGTATCTTTAATTCGGAAGGAAAGAAGATGGTC 26TTCCGTATCTTTAATTCGGAAGGAAAAAAGGTC 26TTCCGTATCTTTAATTCGGATGCCAAAATGGTC 26TTCCGTATCTTTAATTCGGATGCCAAAATGGTC 26TTCCGTATCTTTAATTCGGATGCCAAAATGGTC 26TTCCGTATCTTTAATTCGGATGCCAAAATGGTC 26TTCCGTATCTTTAATTCGGATGCCAAAATGGTC 26TTCCGTATCTTTAATTCGGATGCCAAAATGGTC 26TTCCGTATCTTTAATTCGGATGCCAAAATGGTT 26TTCCGTATCTTTAATTCGGATGCCAAAATGGTT 26TTCCGTATCTTTAATTCGGATGCCAAAATGGTT 26TTCCGTATCTTTAACACTGGATGCCAAAATGGTT 26TTCCGTATCTTTAACACTGGATGCCAAAATGGTT 26
Pasmiu-cf0203-AA61 Basce60808 Cerzm65622 Eymtr43851 Copma596116 Pyrtr157321 Curlu123898 Bipma1034928 Bipso218368 Bipor28457 Bipvi84790 Bipze101753 Peng1219443 Aspve124547 Aspwe173916 Penox5982 Penfe431082 Penbi454008 Penca423940 Penca423940 Penca423940 Pench69724 Colgr10528 Colgl1931734 Colfi1350467 Melva566401	* 620 * 640 * 660 * 660 * 680  AACCATALACTATACTTGAAAAGCAGGAGTATGCTGCA AACCATALACTGAAAAGCAGGAGAAAGTACCATGCA AACCAGGTTACACTCAAGGGAGGAAAGTACCATGCA GGTGAGATTCCGTTGAAAAGCAGAAGTATCCAAGCA AATCAAAATCCGTTGAAAGAACAAAATACCAAGCA AATCAAAATCCACGCTTGCCAAGAGAAATATCCAAGC AACCAGAATCCATTGCTCTACAAAGAAATATCCAAGCC AACCAAAATTCACTTGCATACAAAAAAAATATCCAAGCCAAACAAA	- GACAGAATCATGT ACCACCG	
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