**Data S4.**

Alignment analysis of *Bradyrhizobium elkanii* USDA61 NopL and its homologs among bradyrhizobia and sinorhizobia (**A**); sinorhizobia (**B**); *B. diazoefficiens*/*B. japonicum* (**C**); and several selected rhizobia (**D**). The alignment was done using Clustal Omega algorithm. The “\*” indicates perfect alignment, the “:” and “.” indicate sites belonging to the group exhibiting strong and weak similarity, respectively. In (**A**), the 62 N-terminal amino acid residues are shown (bordered). In (**A**–**C**), nuclear localization signal (NLS) motifs (grey) are highlighted. No NLS motif is predicted in NopLs of *B. diazoefficiens* USDA110, *B. japonicum* USDA6, and *Sinorhizobium fredii* HH103. The computationally predicted phosphorylation residues (Serine, S; Threonine, T; and Tyrosine, Y) in NopLs of USDA61 (249 residues), USDA110 (167 residues), and *S. fredii* NGR234 (338 residues) are blue. In (**B**), the four phosphorylated residues (S89, S139, S148, and S198) biochemically verified in NGR234 NopL are yellow highlighted; and the other S residues essential for NGR234 NopL functions [1] are blue highlighted. The seven Serine-Proline (SP) of USDA61 NopL are double underlined. The two SP-containing repeats and three tandem repeat motifs (3xSQAGP) of USDA61 NopL are underlined by blue and green, respectively. The two tandem repeats previously identified in NGR234 NopL is underlined with black and red, respectively [2]. The three putative catalytic domains of NGR234 NopL [2] are orange highlighted in (**A**) and (**B**), with the catalytic residue candidates are bordered.

(**A**) **USDA61 NopL, bradyrhizobial and sinorhizobial NopLs**

**NopL\_BeUSDA61** MAFVELWLEPNFQRR**S**RMDFN**S**I**S**P**T**N**TSPQPDS**PSAPAGPAGFEHQLREVED**S**ALPPAA 60

**NopL\_BdUSDA110** -----------------MDFNAVAPAN**TSPEPDT**AR**T**A**T**DA**T**EFERQL**S**G**S**EAPASAQGV 43

BAL13259\_BjUSDA6 -----------------MDFNAVAPANT**SPEPD**TARTATDATEFERQLSGSEAPASAQGV 43

**NopL\_SfNGR234** -----------------MDIN**S**T**S**PLNA**SPQPDS**PPP-ANASAFAHQLSGFQY**S**-PPHAA 41

CCE98803\_SfHH103 -----------------MDINSTRPLNA**SPQPD**SPPP-ANESAFAHQLSGFQYS-PPHAA 41

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**NopL\_BeUSDA61** G**S**PVQQGKAYSPYLDARHPY**S**QYLE**S**GHP**Y**S**S**LLDREDDL**Y**------------------- 101

**NopL\_BdUSDA110** AHPVLQGEAYSP**Y**LDAGHPY**S**PYLE**T**GHLYPPYPDLAHPLGPD----------------- 86

BAL13259\_BjUSDA6 AHPVLQGEAYSPYLDAGHPYSPYLETGHLYPPYPDLAHPLGPD----------------- 86

**NopL\_SfNGR234** DSLLPQVEAD**S**PY**L**DTRHPY**S**Q**Y**LD**S**AYPYP**S**PCEWQHDL**YT**RTRER**S**PHP**S**EQRPHARV 101

CCE98803\_SfHH103 DSLLPQVEADSPYLDTGHPYSQYLDSAYPYPSPCEWQHDLYTRTRERSPHPSEQRPHARV 101

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**NopL\_BeUSDA61** ------------------------------------------------------------ 101

**NopL\_BdUSDA110** ------------------------------------------------------------ 86

BAL13259\_BjUSDA6 ------------------------------------------------------------ 86

**NopL\_SfNGR234** LQGAPEHDQDQHLEAAGPREG**S**WQVGP**S**R**S**GP**S**QAGL**S**P**S**ATPLNP**S**PPPHA**T**DLE**T**KHP 161

CCE98803\_SfHH103 LQDAPEHDQDQHVEAAGPRAGSWQVGPSRSGPSQAGPSSSATPLNASPPPHATDLETEHP 161

**NopL\_BeUSDA61** --------------------APAAP**S**PGPLVAARE**SSPQPGS**QQPIAQAIAELPEFDPDL 141

**NopL\_BdUSDA110** ------------SGWQDNL**Y**AAPAAVAAP---EPDNGQ**Q**HL**S**PQAIAQAIEEHPGFDQDV 131

BAL13259\_BjUSDA6 ------------SGWQDNLYAAPAAVAAP---EPDNGQLHL**S**PQAIAQAIEEHPGFDQDV 131

**NopL\_SfNGR234** Y**S**QYLDWANPSLLDWQQDLH**T**RA**T**A**S**PAPLTAERGR**SPQPS**EQQPHARA-LQVPEYDQDL 220

CCE98803\_SfHH103 YSQYLDWANPSLLDWQHDLHTRATASPAPLTAERGK**SPQP**SEQQPHARA-LQVPEYDQDL 220

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**NopL\_BeUSDA61** IWQNVEAGS**SQAGP----SQAGPSQAG----------PSS**SAGAALSELTNFIPEDERFI 187

**NopL\_BdUSDA110** IWQ**T**LDVGP**S**E**A**E**P**RHGEP**Q**A**GTS**HA**G**P**S**RTAPF**S**G------------------------ 167

BAL13259\_BjUSDA6 IWQTLDVGPSE**A**E**P**RHGEP**Q**A**G**T**S**HA**G**PSRTAPFSG------------------------ 167

**NopL\_SfNGR234** IWQRVDAAGPQ**A**G**P**----W**Q**V**G**P**S**HS**G**P**S**QARP**S**HAWPS**S**SAGAEPAEL**S**DFVM**D**SGVRA 276

CCE98803\_SfHH103 IWQRVDAAGPQ**A**G**P**----W**Q**V**G**P**S**HS**G**PSQARPSHAWPSSSAGAEPTELSDFVMDSGVRA 276

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**NopL\_BeUSDA61** ADHWVFCPHTASDAQINILRRAGLLP**S**NN**S**RT**TS**FTMLGMPHTAEFRQEGFVRIKP**S**MDA 247

**NopL\_BdUSDA110** ------------------------------------------------------------ 167

BAL13259\_BjUSDA6 ------------------------------------------------------------ 167

**NopL\_SfNGR234** WDHWFLAPHMASEDQM**S**MLRA**T**GLMPTAEVPTTTFLMMGMRHVAEFRGEGVIRIRP**S**VDF 336

CCE98803\_SfHH103 WDHWFLAPHMASEDQMSMLRATGLMPTAEVPTTTFLMMGMPHVAEFRGEGVIRIRPSLDF 336

**NopL\_BeUSDA61** GL 249

**NopL\_BdUSDA110** -- 167

BAL13259\_BjUSDA6 -- 167

**NopL\_SfNGR234** DI 338

CCE98803\_SfHH103 DI 338

(**B**) **USDA61 NopL and sinorhizobial NopLs**

**NopL\_BeUSDA61** MAFVELWLEPNFQRR**S**RMDFN**S**I**S**P**T**N**TSPQPDS**PSAPAGPAGFEHQLREVED**S**ALPPAA 60

**NopL\_SfNGR234** -----------------MDIN**S**T**S**PLNA**SPQPDS**PPP-ANASAFAHQLSGFQY**S**-PPHAA 41

CCE98803\_SfHH103 -----------------MDINSTRPLNA**SPQPDS**PPP-ANESAFAHQLSGFQYS-PPHAA 41

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**NopL\_BeUSDA61** G**S**PVQQGKAYSPYLDARHPY**S**QYLE**S**GHP**Y**S**S**LLDREDDL**Y**------------------- 101

**NopL\_SfNGR234** DSLLPQVEAD**S**P**Y**LDTRHPY**S**Q**Y**LD**S**AYPYP**S**PCEWQHDL**YT**RTRER**S**PHP**S**EQRPHARV 101

CCE98803\_SfHH103 DSLLPQVEADSPYLDTGHPYSQYLDSAYPYPSPCEWQHDLYTRTRERSPHPSEQRPHARV 101

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**NopL\_BeUSDA61** ------------------------------------------------------------ 101

**NopL\_SfNGR234** LQGAPEHDQDQHLEAAGPREG**S**WQVGP**S**R**S**GP**S**QAGL**S**P**S**A**T**PLNP**S**PPPHA**T**DLE**T**KHP 161

CCE98803\_SfHH103 LQDAPEHDQDQHVEAAGPRAGSWQVGPSRSGPSQAGPSSSATPLNASPPPHATDLETEHP 161

**NopL\_BeUSDA61** --------------------APAAP**S**PGPLVAARE**SSPQPGS**QQPIAQAIAELPEFDPDL 141

**NopL\_SfNGR234** Y**S**QYLDWANPSLLDWQQDLH**T**RA**T**A**S**PAPLTAERGR**SPQPS**EQQPHARA-LQVPEYDQDL 220

CCE98803\_SfHH103 YSQYLDWANPSLLDWQHDLHTRATASPAPLTAERGK**SPQP**SEQQPHARA-LQVPEYDQDL 220

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**NopL\_BeUSDA61** IWQNVEAGS**SQAGPSQAGPSQAG----------PSS**SAGAALSELTNFIPEDERFIADHW 191

**NopL\_SfNGR234** IWQRVDAAGP**QAGP**W**Q**V**GPS**HS**G**P**S**QARP**S**HAW**P**S**S**SAGAEPAEL**S**DFVM**D**SGVRAWDHW 280

CCE98803\_SfHH103 IWQRVDAAGP**QAGP**W**Q**V**GPS**HS**G**PSQARPSHAW**P**SSSAGAEPTELSDFVMDSGVRAWDHW 280

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**NopL\_BeUSDA61** VFCPHTASDAQINILRRAGLLP**S**NN**S**RT**TS**FTMLGMPHTAEFRQEGFVRIKP**S**MDAGL 249

**NopL\_SfNGR234** FLAPHMASEDQM**S**MLRA**T**GLMPTAEVPTTTFLMMGMRHVAEFRGEGVIRIRP**S**VDFDI 338

CCE98803\_SfHH103 FLAPHMASEDQMSMLRATGLMPTAEVPTTTFLMMGMPHVAEFRGEGVIRIRPSLDFDI 338

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(**C**) **USDA61 NopL and bradyrhizobial NopLs**

**NopL\_BeUSDA61** MAFVELWLEPNFQRR**S**RMDFN**S**I**S**P**T**N**TSPQPDS**PSAPAGPAGFEHQLREVED**S**ALPPAA 60

**NopL\_BdUSDA110** -----------------MDFNAVAPAN**TSP**E**PDT**AR**T**A**T**DA**T**EFERQL**S**G**S**EAPASAQGV 43

BAL13259\_BjUSDA6 -----------------MDFNAVAPANT**SP**E**PD**TARTATDATEFERQLSGSEAPASAQGV 43

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**NopL\_BeUSDA61** G**S**PVQQGKAYSPYLDARHPY**S**QYLE**S**GHP**Y**S**S**LLDRE----------DDL**Y**APAAP**S**PGP 110

**NopL\_BdUSDA110** AHPVLQGEAYSP**Y**LDAGHPY**S**PYLE**T**GHLYPPYPDLAHPLGPDSGWQDNL**Y**AAPAAVAAP 103

BAL13259\_BjUSDA6 AHPVLQGEAYSPYLDAGHPYSPYLETGHLYPPYPDLAHPLGPDSGWQDNLYAAPAAVAAP 103

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**NopL\_BeUSDA61** LVAARE**SSPQPGS**QQPIAQAIAELPEFDPDLIWQNVEAGS**SQAGPSQ----AGPSQAGPS** 166

**NopL\_BdUSDA110** ---EPDNGQ**Q**HL**S**PQAIAQAIEEHPGFDQDVIWQ**T**LDVGP**S**E**A**E**P**RHGEPQ**AGTS**H**AGPS** 160

BAL13259\_BjUSDA6 ---EPDNGQLHL**S**PQAIAQAIEEHPGFDQDVIWQTLDVGP**S**E**A**E**P**RHGEPQ**AG**T**S**H**AGP**S 160

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**NopL\_BeUSDA61** **S**SAGAALSELTNFIPEDERFIADHWVFCPHTASDAQINILRRAGLLP**S**NN**S**RT**TS**FTMLG 226

**NopL\_BdUSDA110** RTAPF**S**G----------------------------------------------------- 167

BAL13259\_BjUSDA6 RTAPFSG----------------------------------------------------- 167

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**NopL\_BeUSDA61** MPHTAEFRQEGFVRIKP**S**MDAGL 249

**NopL\_BdUSDA110** ----------------------- 167

BAL13259\_BjUSDA6 ----------------------- 167

(**D**) **USDA61 NopL and several selected rhizobial NopLs**

**NopL\_BeUSDA61** MAFVELWLEPNFQRRSRMDFNSISPTNT**SPQPDS**PSAPAGPAGFEHQLREVEDSALPPAA 60

GEC57378\_BeNBRC14791 -----------------MDFNSISPTNT**SPQPDS**PSAPAGPAGFEHQLREVEDSALPPAA 43

WP\_141713659\_BeBLY3-8 -----------------MDFNAIGPANTR**P**E**PD**LPEPSMDATGFEQRLSGAQAGALPQHA 43

WP\_137479484\_BeSemia938 -----------------MDFNAIGSANAG**P**E**P**EPPEPFTDAPSFEQRLSGAHASAPPQHA 43

WP\_155255696\_BeWSM2783 ------------------------------------------------------------ 0

NopL\_SfNGR234 -----------------MDINSTSPLNA**SPQPDS**PP-PANASAFAHQLSGFQYS-PPHAA 41

CCE98803\_SfHH103 -----------------MDINSTRPLNA**SPQPDS**PP-PANESAFAHQLSGFQYS-PPHAA 41

WP\_136510575\_EspMPMI2T -----------------MDINSTSPLNA**SPQPDS**PP-PANESAFAHQLSGFQYS-PPHAA 41

WP\_146207671\_BspSUTN9-2 ------------------------------------------------------------ 0

SPP98400\_BspORS3257 ------------------------------------------------------------ 0

**NopL\_BeUSDA61** GSPVQQGKAYSPYLDARHPYSQYLESGHPYSSLLDRED---------------------- 98

GEC57378\_BeNBRC14791 GSPVQQGKAYSPYLDARHPYSQYLESGHPYSSLLDRED---------------------- 81

WP\_141713659\_BeBLY3-8 APPVLQGGAYSPYLDAGHPYSPYLDAGHPYSPYLDLAHPWP------------------- 84

WP\_137479484\_BeSemia938 APPVLQGDAYSPYLGAGHPYSPYLDAGHPYSPYLDLAHPST------------------- 84

WP\_155255696\_BeWSM2783 ---MPQGEAYSPYLEAGHPYSS-------------------------------------- 19

NopL\_SfNGR234 DSLLPQVEADSPYLDTRHPYSQYLDSAYPYPSPCEWQHDLYTRTRERSPHPSEQRPHARV 101

CCE98803\_SfHH103 DSLLPQVEADSPYLDTGHPYSQYLDSAYPYPSPCEWQHDLYTRTRERSPHPSEQRPHARV 101

WP\_136510575\_EspMPMI2T DSLLPQVEAYSPYLDTGHPYSQYLDSANPYSSSFEWQDDPYTRARERSPHPSEQQPHARV 101

WP\_146207671\_BspSUTN9-2 ------------------------------------------------------------ 0

SPP98400\_BspORS3257 ------------------------------------------------------------ 0

**NopL\_BeUSDA61** ------------------------------------------------------------ 98

GEC57378\_BeNBRC14791 ------------------------------------------------------------ 81

WP\_141713659\_BeBLY3-8 ------------------------------------------------------------ 84

WP\_137479484\_BeSemia938 ------------------------------------------------------------ 84

WP\_155255696\_BeWSM2783 ------------------------------------------------------------ 19

NopL\_SfNGR234 LQGAPEHDQDQHLEAAGPREGSWQVGPSRSGPSQAGLSPSATPLN--------------- 146

CCE98803\_SfHH103 LQDAPEHDQDQHVEAAGPRAGSWQVGPSRSGPSQAGPSSSATPLN--------------- 146

WP\_136510575\_EspMPMI2T LQQVPEPDQDQHVEAAGTQAGSWQVGPSRSGPSQAGPSSSANPLNERPQPADERPFAQQL 161

WP\_146207671\_BspSUTN9-2 ------------------------------------------------------------ 0

SPP98400\_BspORS3257 ------------------------------------------------------------ 0

**NopL\_BeUSDA61** ------------------------------------DLYAPAAPSPGPLVAARES**SPQPG** 122

GEC57378\_BeNBRC14791 ------------------------------------DLYAPAAPSPGPLVAARES**SPQP**G 105

WP\_141713659\_BeBLY3-8 ------------------------------DLDRENDPFTP---SAEHVAPEPES-**PQ**YL 110

WP\_137479484\_BeSemia938 ------------------------------DLGWEDNSHTP---TADHIAPELESF**PQP**L 111

WP\_155255696\_BeWSM2783 ------------------------------HLNWQDDLHTPAAVSPEPLPATGESFQ**QP**S 49

NopL\_SfNGR234 ----PSPPPHATDLETKHPYSQYLDWANPSLLDWQQDLHTRATASPAPLTAERGR**SPQP**S 202

CCE98803\_SfHH103 ----ASPPPHATDLETEHPYSQYLDWANPSLLDWQHDLHTRATASPAPLTAERGK**SPQP**S 202

WP\_136510575\_EspMPMI2T SGFQDSPPPHATDLGTEHPYSQYLDWANPSSLDWRHDLYTRATASPSPLAAERER**SPQP**G 221

WP\_146207671\_BspSUTN9-2 -------------------------------MDF--NALSRADNSPASHP--SSSPAG**P**R 25

SPP98400\_BspORS3257 ------------------------------------------------------------ 0

**NopL\_BeUSDA61** **S**QQPIAQAIAELPEFDPDLIWQNVEAGS**SQAG--PSQAG----------P**---------- 160

GEC57378\_BeNBRC14791 SQQPIAQAIAELPEFDPDLIWQNVEAGS**S**QAG--P**S**QAG----------P---------- 143

WP\_141713659\_BeBLY3-8 PQQTIAQAIEAHPGVDQDLIWQNVAG-P**S**HAG--P**S**YAGPLLEGPSHAGP---------- 157

WP\_137479484\_BeSemia938 SQQTIAQAIEAHPDIDQDLIWQNVDAGP**S**GAGPSP**S**HAGPSYAGLVQARP---------- 161

WP\_155255696\_BeWSM2783 SQQAIAEPLAKAAYFDQDLIWQELESG-LQAG--P**S**QAGPSSFALEGPQRPISQQPLAQS 106

NopL\_SfNGR234 EQQPHARAL-QVPEYDQDLIWQRVDAAGPQAG--PWQVG----------P---------- 239

CCE98803\_SfHH103 EQQPHARAL-QVPEYDQDLIWQRVDAAGPQAG--PWQVG----------P---------- 239

WP\_136510575\_EspMPMI2T EQQPHARAL-QVPEHDQDLIWQHVDAAGPQAG--P**S**HAG----------R---------- 258

WP\_146207671\_BspSUTN9-2 EFERELMELHEIPEYSQDLIWQELGGRS**S**RVEPSL**S**G----------GGRSNSQQPIAQH 75

SPP98400\_BspORS3257 --------MHKIPEYSQDLIWQELGAGS**S**RVEPSL**S**G----------GERTNSQQPIAQH 42

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**NopL\_BeUSDA61** --------------**SQAG-----------PS**S--SAGAALSELTNFIPEDERFIAD---- 189

GEC57378\_BeNBRC14791 --------------**S**QAG-----------**PS**S--SAGAALSELTNFIPEDERFIAD---- 172

WP\_141713659\_BeBLY3-8 --------------**S**HAGP-THARPLQAG**PS**QAGPSETAPPELSEFRMLNGRLAKD---- 198

WP\_137479484\_BeSemia938 --------------**S**HAGP-SHARPLQAG**PS**EAGPSEATPPELSGFRMVEGRLAKD---- 202

WP\_155255696\_BeWSM2783 LAEAPDFDKDPVWQELDPG-LQAGPSQAG**PS**S--SAGAAPRELGDFVMNNGRRASE---- 159

NopL\_SfNGR234 --------------**S**HSGP-SQARPSHAW**PS**S--SAGAEPAELSDFVMDSGVRAWD---- 278

CCE98803\_SfHH103 --------------**S**HSGP-SQARPSHAW**PS**S--SAGAEPTELSDFVMDSGVRAWD---- 278

WP\_136510575\_EspMPMI2T --------------**S**EAG-----------**PS**S--SAGAEPAELNDFVMDSGVRAWD---- 287

WP\_146207671\_BspSUTN9-2 RADASDFGNDLVWQDLEPWLMWAGSLHAG**PS**MLHAGVA-SSEIGDFAMANGRRAKD---- 130

SPP98400\_BspORS3257 RVDASEFGNDLVWQDPG-------HLNAG**PS**QADSRARTGCSAGTWRFRHGERPSRQGRV 95

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**NopL\_BeUSDA61** --HWVFCPHTASDAQINILRRAGLLPSNNSRTTSFTMLGMPHTAEFRQEGFVRIK**PS**MDA 247

GEC57378\_BeNBRC14791 --HWVFCPHTASDAQINILRRAGLLPSNNSRTTSFTMLGMPHTAEFRQEGFVRIK**PS**MDA 230

WP\_141713659\_BeBLY3-8 --YWVFTGQTATAAQIDMLERSGVKPSKDHPTKVFTFLGVPHTAEWREEGFIRLT**PS**LDQ 256

WP\_137479484\_BeSemia938 --YWVFTGQTATDAEIDMLERSGVKPSREQPSKVFTLLGVPHTAEWRDEGFIRLT**PS**LDP 260

WP\_155255696\_BeWSM2783 --YWVFFPQTASNAQMNMLRSVGLMPSRDSPT-TFSIIGVPHTAEWRDEDFVRIN**PS**LDP 216

NopL\_SfNGR234 --HWFLAPHMASEDQMSMLRATGLMPTAEVPTTTFLMMGMRHVAEFRGEGVIRIR**PS**VDF 336

CCE98803\_SfHH103 --HWFLAPHMASEDQMSMLRATGLMPTAEVPTTTFLMMGMPHVAEFRGEGVIRIR**PS**LDF 336

WP\_136510575\_EspMPMI2T --HWVLAPHLASEDEMSMLRAKGLMPTAEVPTTTFLMMGMPHIAEFRGEGLIRIR**PS**LDL 345

WP\_146207671\_BspSUTN9-2 --DWVFTGQTATPAQIEMLRSRDLKPTRDVRTTTFTILGVPHTAEWREEGFIRLK**PS**LGP 188

SPP98400\_BspORS3257 GLHWPNR--YACS--DRNAKKPQSHTDQGSQTITFTNLGVPVRAEWREEGSIRIK**PS**LNS 151

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**NopL\_BeUSDA61** GL----------------------- 249

GEC57378\_BeNBRC14791 GL----------------------- 232

WP\_141713659\_BeBLY3-8 TLWPEEPAENRSLDSDSEEDPRIR- 280

WP\_137479484\_BeSemia938 TLWPEEPAEDRPADSEEDPPAR--- 282

WP\_155255696\_BeWSM2783 SLWPGYSGEDSPTG----------- 230

NopL\_SfNGR234 DI----------------------- 338

CCE98803\_SfHH103 DI----------------------- 338

WP\_136510575\_EspMPMI2T GI----------------------- 347

WP\_146207671\_BspSUTN9-2 SLWLEVRANTRQAAKHDDMKNAERS 213

SPP98400\_BspORS3257 ALWPVGSSEHSPSG----------- 165

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