

**MISKOLCI EGYETEM**

**ANYAG- ÉS VEGYÉSZMÉRNÖKI KAR**



**MIKROORGANIZMUSOK ALKALMAZÁSA POLIURETÁN  
BIOLÓGIAI LEBONTÁSÁBAN**

**APPLICATION OF MICROORGANISMS IN THE BIODEGRADATION  
OF POLYURETHANE**

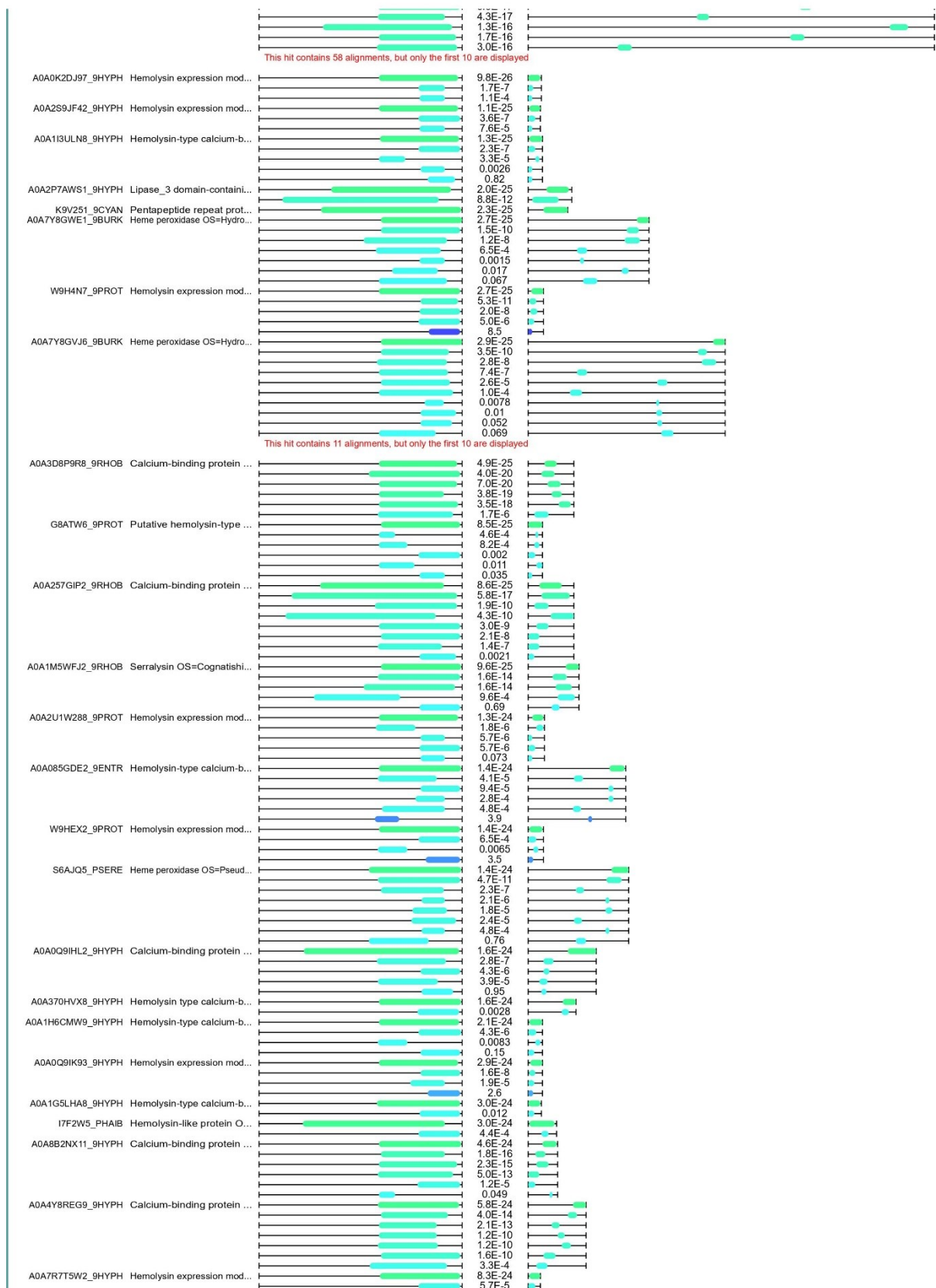
**SUPPLEMENTARY INFORMATION**

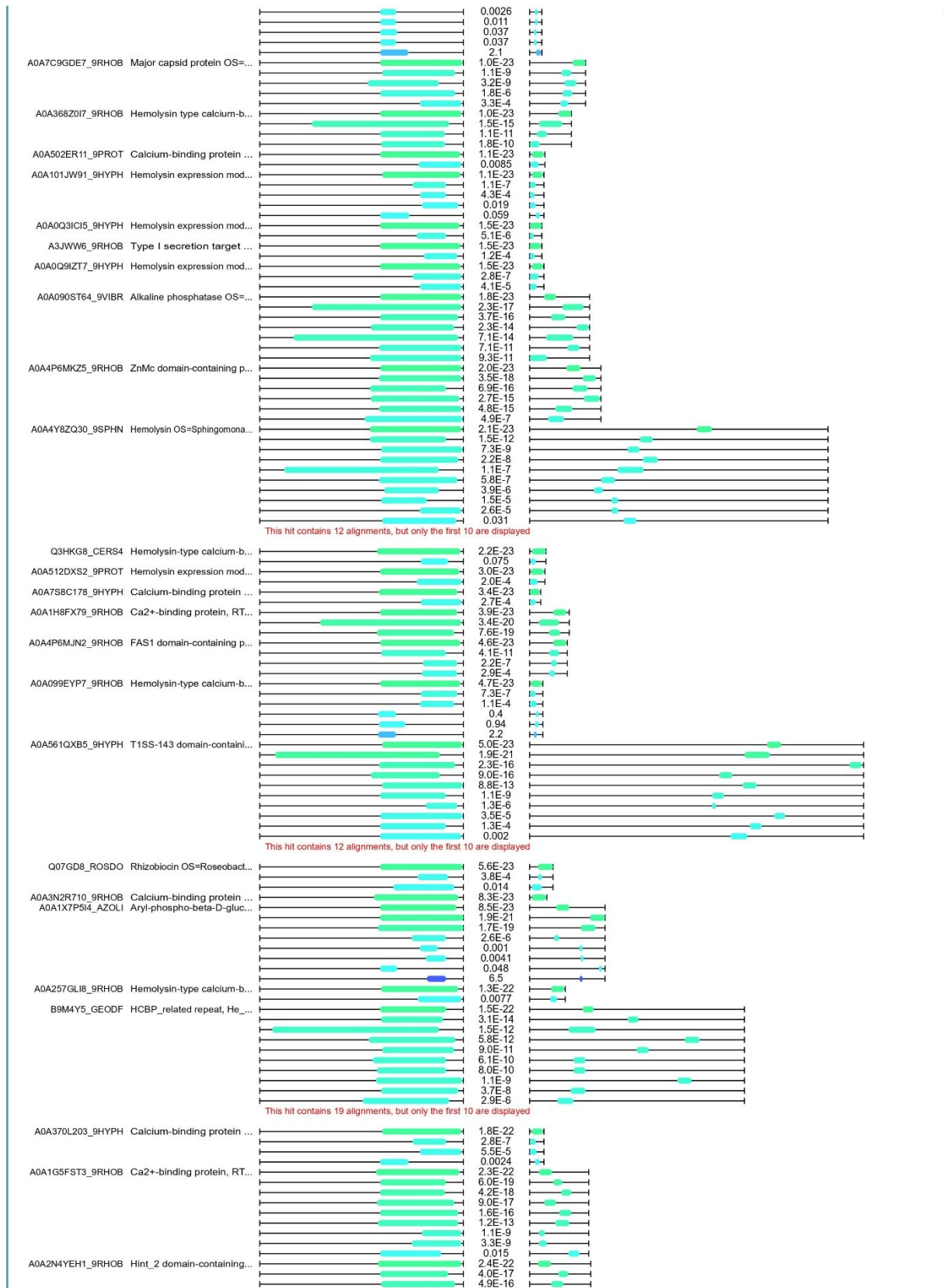
**Jordán Anikó  
Tokaji György Marcell**

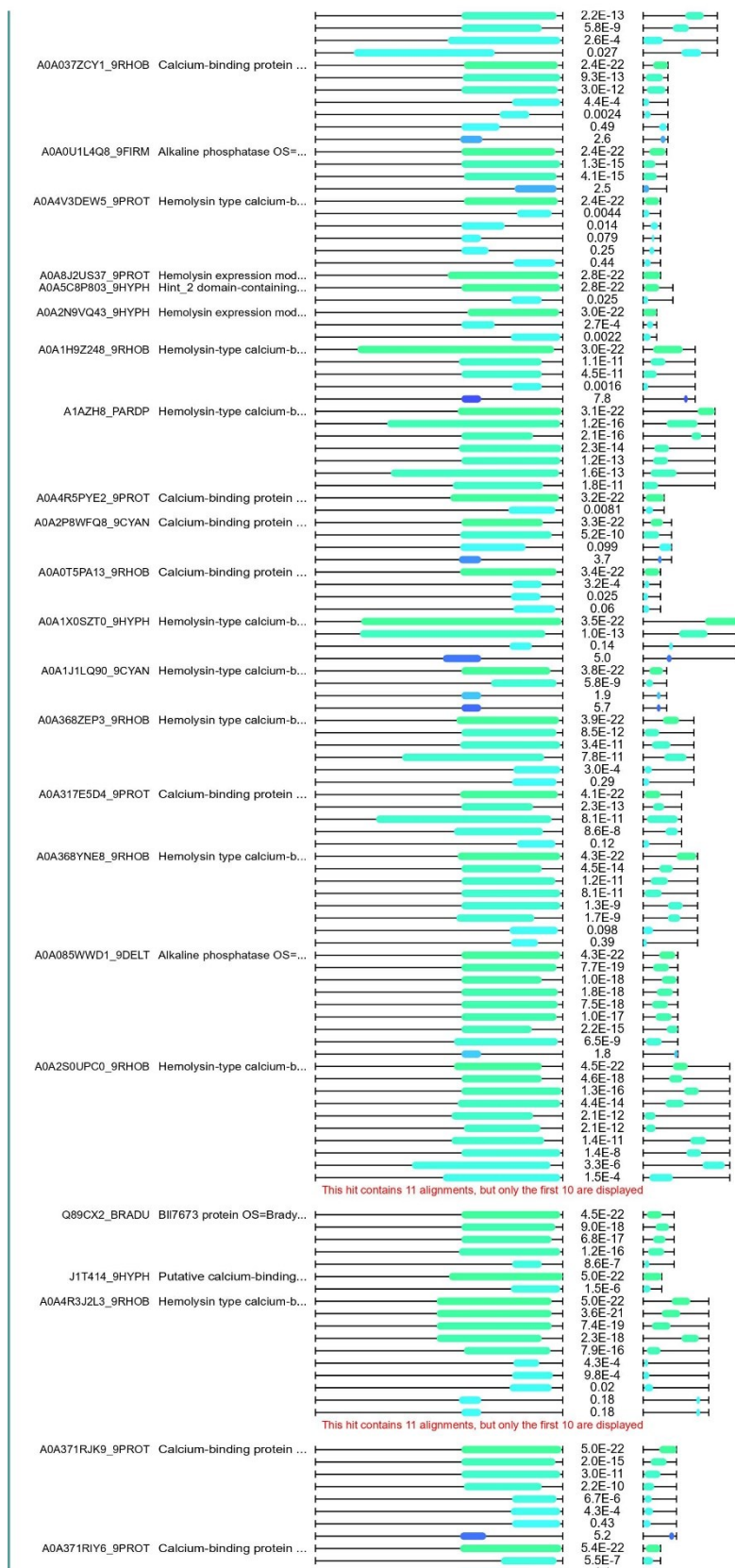
Konzulensek: Dr. Szőri-Dorogházi Emma  
Dr. Fiser Béla

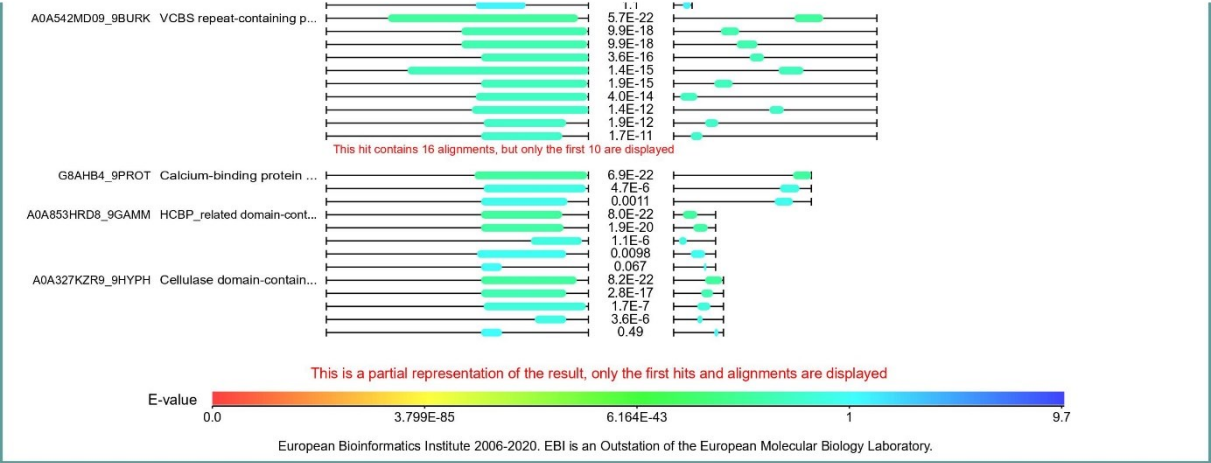
## Annex 1 E-values of BLAST in case of PueA







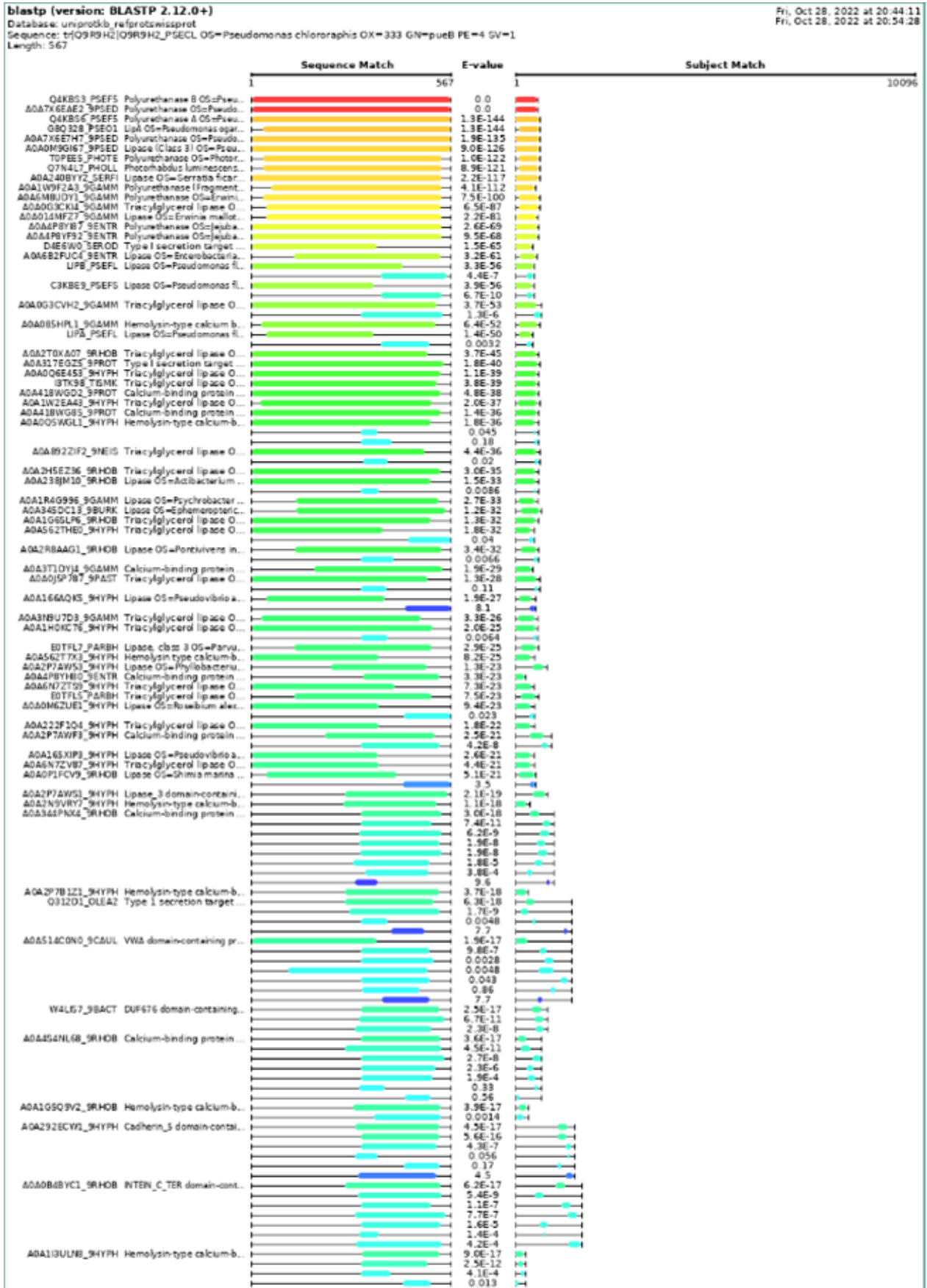


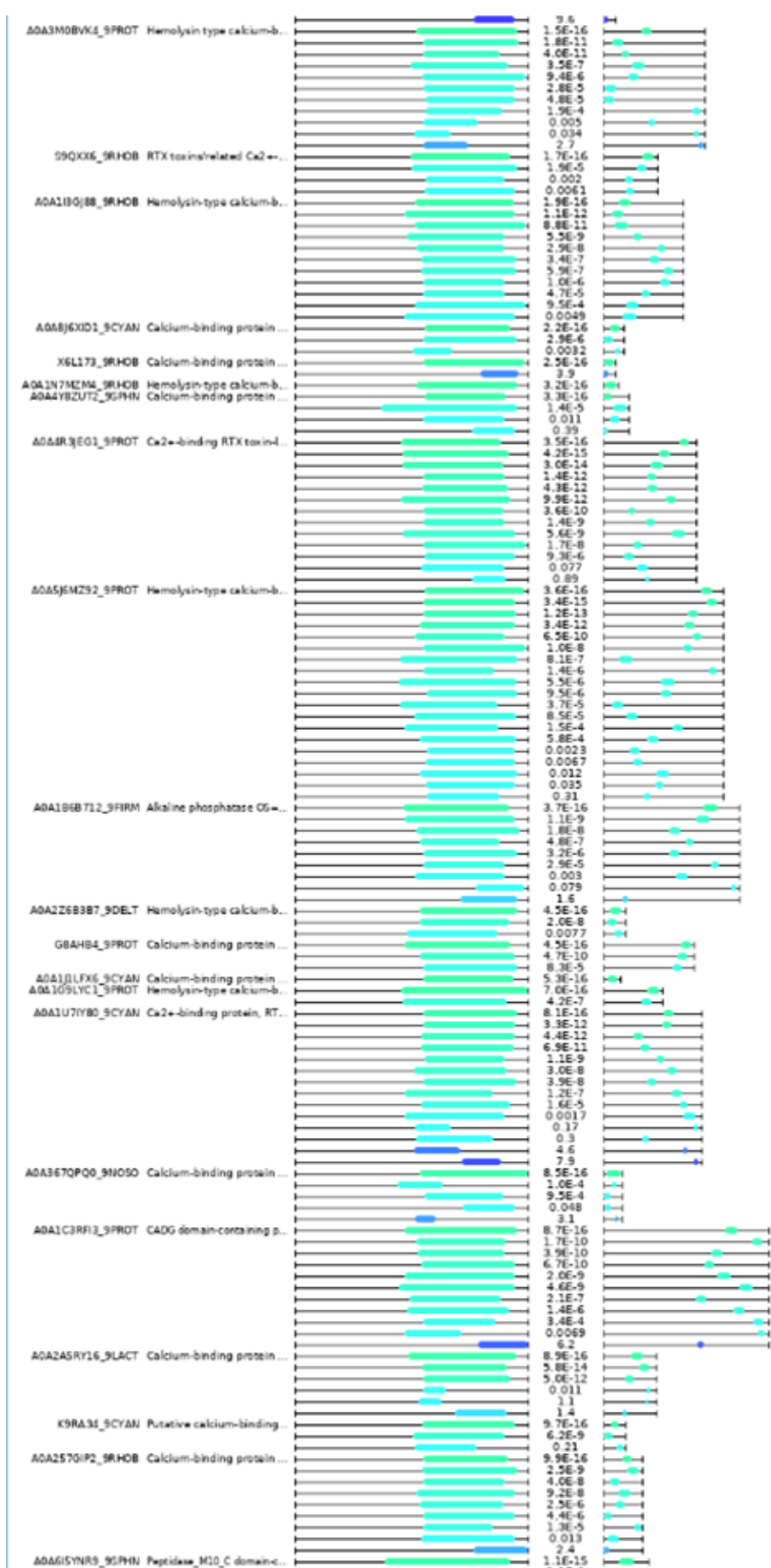


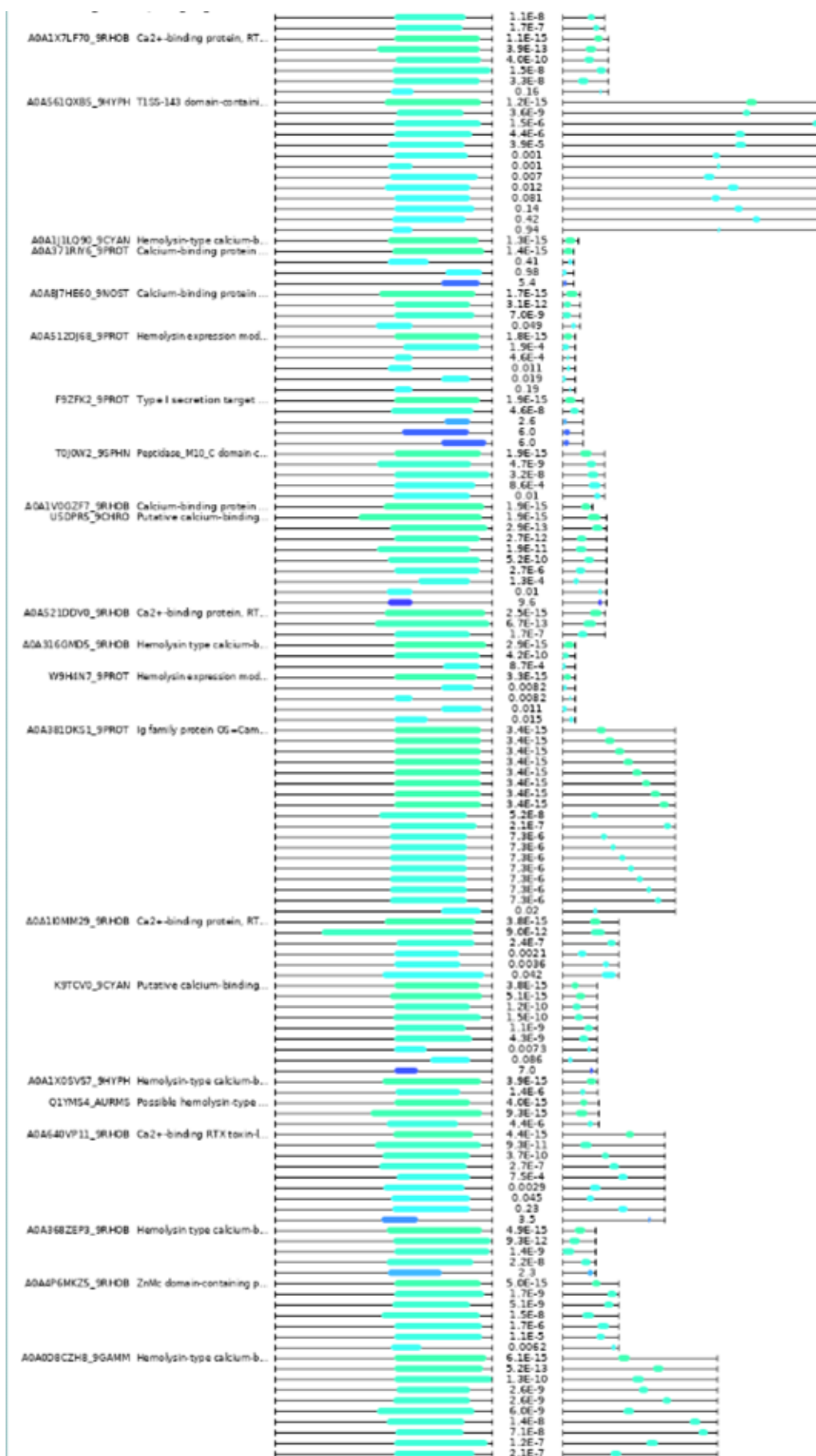
**Annex 2** Table of selected homolog proteins by PueA

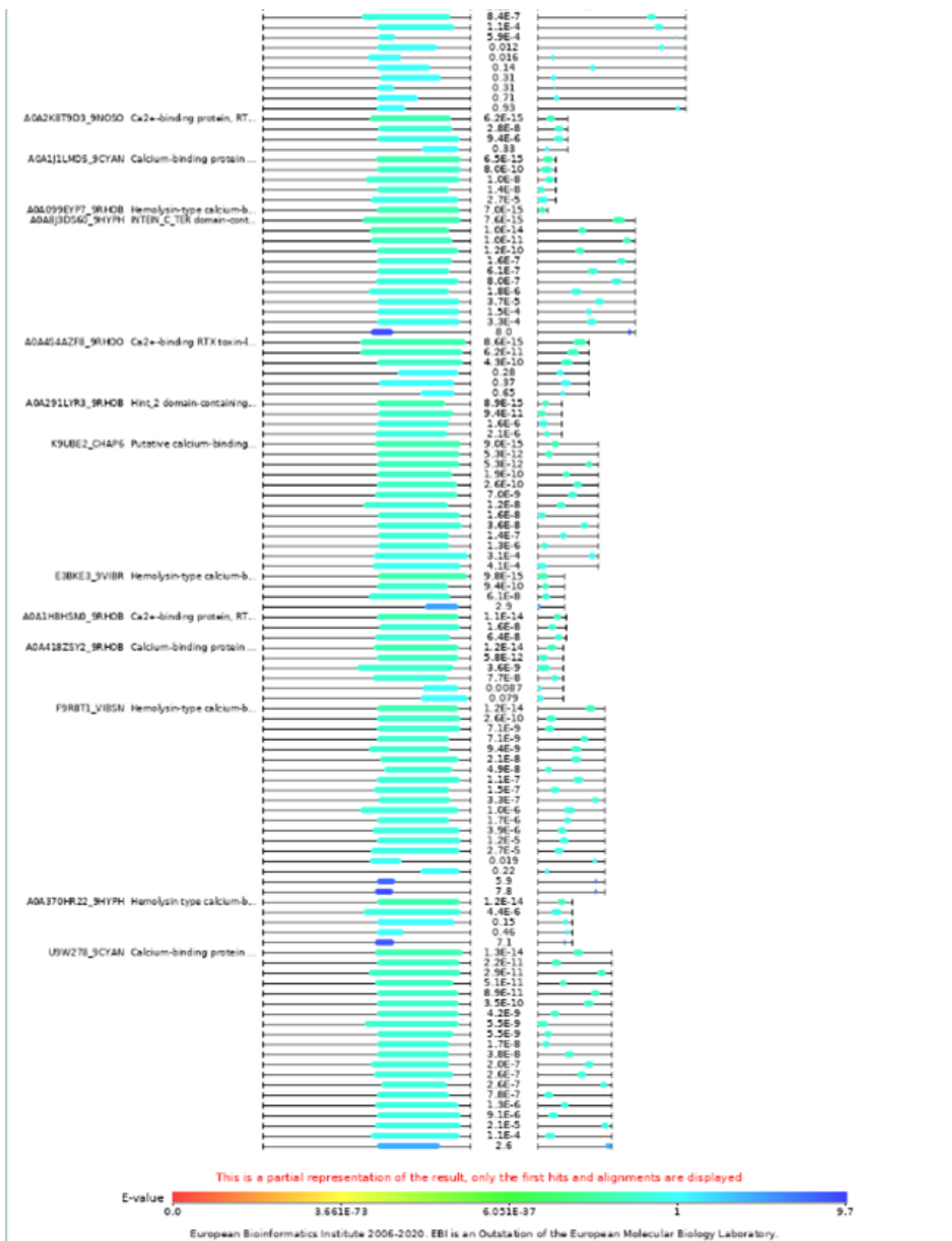
| Entry      | Entry Name       | Protein names                                                                  | Gene Names         | Organism                                                                         | Length | E-value |
|------------|------------------|--------------------------------------------------------------------------------|--------------------|----------------------------------------------------------------------------------|--------|---------|
| A0A0G3CKI4 | A0A0G3CKI4_9GAMM | Triacylglycerol lipase                                                         | QQ39_10940         | Pragia fontium                                                                   | 607    | 0       |
| A0A0M9GI67 | A0A0M9GI67_9PSED | Lipase (Class 3)                                                               | PF66_01458         | Pseudomonas fuscovaginae                                                         | 612    | 0       |
| A0A1W9F2A3 | A0A1W9F2A3_9GAMM | Polyurethanase                                                                 | AK51_25165         | Serratia nematodiphila DZ0503SBS1                                                | 512    | 0       |
| A0A240BYY2 | A0A240BYY2_SERFI | Lipase (EC 3.1.1.3)                                                            | SAMEA4384070_02275 | Serratia ficaria                                                                 | 614    | 0       |
| A0A6M8UDY1 | A0A6M8UDY1_9GAMM | Polyurethanase                                                                 | PMPD1_3864         | Erwiniaceae bacterium PD-1                                                       | 612    | 0       |
| A0A7X6E7H7 | A0A7X6E7H7_9PSED | Polyurethanase                                                                 | HER21_01725        | Pseudomonas sp. BG5                                                              | 617    | 0       |
| A0A7X6EAE2 | A0A7X6EAE2_9PSED | Polyurethanase                                                                 | HER21_01730        | Pseudomonas sp. BG5                                                              | 562    | 0       |
| C3KBE9     | C3KBE9_PSEFS     | Lipase (EC 3.1.1.3)                                                            | lipB PFLU_3141     | Pseudomonas fluorescens (strain SBW25)                                           | 469    | 0       |
| D4E6W0     | D4E6W0_SEROD     | Type I secretion target<br>GGXGXDXXX repeat (2 copies)                         | HMPREF0758_3910    | Serratia odorifera DSM 4582                                                      | 444    | 0       |
| G8Q328     | G8Q328_PSEO1     | LipA (EC 3.1.1.3)                                                              | lipA PSF113_2944   | Pseudomonas ogarae (strain DSM 112162 / CECT 30235 / F113)                       | 617    | 0       |
| P41773     | LIPB_PSEFL       | Lipase (EC 3.1.1.3) (Triacylglycerol lipase)                                   |                    | Pseudomonas fluorescens                                                          | 476    | 0       |
| Q4KBS3     | Q4KBS3_PSEF5     | Polyurethanase B (EC 3.1.-.-)                                                  | pueB PFL_3205      | Pseudomonas fluorescens (strain ATCC BAA-477 / NRRL B-23932 / Pf-5)              | 561    | 0       |
| Q4KBS6     | Q4KBS6_PSEF5     | Polyurethanase A (EC 3.1.-.-)                                                  | pueA PFL_3202      | Pseudomonas fluorescens (strain ATCC BAA-477 / NRRL B-23932 / Pf-5)              | 617    | 0       |
| Q7N4L7     | Q7N4L7_PHOLL     | Phototrhhabdus luminescens subsp. laumondii TTO1 complete genome; segment 8/17 | plu2313            | Phototrhhabdus laumondii subsp. laumondii (strain DSM 15139 / CIP 105565 / TT01) | 617    | 0       |
| T0PEE5     | T0PEE5_PHOTE     | Polyurethanase                                                                 | B738_06459         | Phototrhhabdus temperata subsp. temperata M1021                                  | 617    | 0       |
| Q9X3C0     | Q9X3C0_PSECL     | Polyurethanase esterase A                                                      | pueA               | Pseudomonas chlororaphis                                                         | 617    | 0       |

# Annex 3 E-values of BLAST in case of PueB









**Annex 4** Table of selected homolog proteins by PueB

| Entry      | Entry Name       | Protein names                                                | Gene Names      | Organism                                                                                | Length | E-score   |
|------------|------------------|--------------------------------------------------------------|-----------------|-----------------------------------------------------------------------------------------|--------|-----------|
| A0A014MFZ7 | A0A014MFZ7_9GAMM | Lipase                                                       | BG55_01520      | <i>Erwinia<br/>mallotivora</i>                                                          | 575    | 0         |
| A0A085HPL1 | A0A085HPL1_9GAMM | Hemolysin-type<br>calcium binding<br>protein                 | GLGR_0142       | <i>Leminorella<br/>grimontii</i> ATCC<br>33999 = DSM<br>5078                            | 613    | 0         |
| A0A0G3CKI4 | A0A0G3CKI4_9GAMM | Triacylglycerol<br>lipase                                    | QQ39_10940      | <i>Pragia fontium</i>                                                                   | 607    | 1,00E-144 |
| A0A0G3CVH2 | A0A0G3CVH2_9GAMM | Triacylglycerol<br>lipase                                    | QQ39_14395      | <i>Pragia fontium</i>                                                                   | 680    | 1,00E-144 |
| A0A4P8YF92 | A0A4P8YF92_9ENTR | Polyurethanase                                               | FEM41_06345     | <i>Jejubacter<br/>calystegiae</i>                                                       | 582    | 2,00E-135 |
| A0A4P8YI87 | A0A4P8YI87_9ENTR | Polyurethanase                                               | FEM41_06340     | <i>Jejubacter<br/>calystegiae</i>                                                       | 585    | 9,00E-126 |
| A0A6B2FUC4 | A0A6B2FUC4_9ENTR | Lipase                                                       | GWD52_19900     | <i>Enterobacteriaceae<br/>bacterium</i><br>4M9                                          | 555    | 1,00E-122 |
| D4E6W0     | D4E6W0_SEROD     | Type I secretion<br>target<br>GGXGXDXXX<br>repeat (2 copies) | HMPREF0758_3910 | <i>Serratia<br/>odorifera</i> DSM<br>4582                                               | 444    | 9,00E-121 |
| P26504     | LIPA_PSEFL       | Lipase (EC 3.1.1.3)<br>(Triacylglycerol<br>lipase)           |                 | <i>Pseudomonas<br/>fluorescens</i>                                                      | 449    | 2,00E-117 |
| Q4KBS3     | Q4KBS3_PSEF5     | Polyurethanase B<br>(EC 3.1.-.-)                             | pueB PFL_3205   | <i>Pseudomonas<br/>fluorescens</i><br>(strain ATCC<br>BAA-477 / NRRL<br>B-23932 / Pf-5) | 561    | 4,00E-112 |
| Q9R9H2     | Q9R9H2_PSECL     | Polyurethanase B<br>(Polyurethanase<br>esterase B)           | pueB            | <i>Pseudomonas<br/>chlororaphis</i><br>( <i>Pseudomonas<br/>aureofaciens</i> )          | 567    | 7,00E-100 |
| Q9X3C0     | Q9X3C0_PSECL     | Polyurethanase<br>esterase A                                 | pueA            | <i>Pseudomonas<br/>chlororaphis</i><br>( <i>Pseudomonas<br/>aureofaciens</i> )          | 617    | 6,00E-87  |

**Annex 5** MSA results of PueA with serin hydrolase regions (red), other conserved regions (green).

|                                |                                       |             |                                     |                     |      |     |
|--------------------------------|---------------------------------------|-------------|-------------------------------------|---------------------|------|-----|
| tr A0A0G3CKI4 A0A0G3CKI4_9GAMM | QYESAGNVINVGYENDPVFRVL                | SGKQLEL     | SLFIHDADK                           | SATNNIVNFNDYYAS     | FWKF | 299 |
| tr A0A7X6EAE2 A0A7X6EAE2_9PSED | QSSTGTNVINIGYENDPVFRVL                | STTFTS      | ASMGKHDKPH                          | STTDNIIVNFNDNYAS    | QAQL | 246 |
| tr Q4KBS3 Q4KBS3_PSEF5         | QSANSSQVLINIGYENDPVFRAL               | STHFNAS     | SLGTHDKPQ                           | SATNNIVSFNDHYAS     | LGKL | 242 |
| tr A0A6M8UDY1 A0A6M8UDY1_9GAMM | EYEVGQKVLINIGFENDPVFRTL               | STDFKL      | STLLTHDKNY                          | STTDNIIVSFNDYYAS    | QVNL | 300 |
| tr A0A0M9GI67 A0A0M9GI67_9PSED | QSGAGTHVLINIGYENDPVFRAL               | GSSSLT      | SLTGVHDGKY                          | SATNNIVNFNDHYAS     | AWNA | 300 |
| sp P41773 LIPB_PSEFL           | QSA-GDKVLINIGYENDPVFRAL               | GSSFNLS     | SLGVHDKPH                           | STTDNIIVSFNDHYAS    | LWNV | 299 |
| tr C3KBE9 C3KBE9_PSEF5         | QSA-GDKVLINIGYENDPVFRAL               | GSSFNLS     | SLGVHDKPH                           | STTDNIIVSFNDHYAS    | LWNV | 299 |
| tr G8Q328 G8Q328_PSE01         | QSS-TDKVLINIGYENDPVFRAL               | GSTFNL      | SLGVHDGQ                            | SATNNIVSFNDHYAS     | AWN  | 299 |
| tr Q4KBS6 Q4KBS6_PSEF5         | QSA-SDKVLINIGYENDPVFRAL               | GSTFKL      | SLGVHDAHQ                           | SATNNIVNFNDHYAS     | LWNV | 299 |
| tr Q9X3C0 Q9X3C0_PSECL         | QSA-SDKVLINIGYENDPVFRAL               | GSTFNL      | SLGVHDAHQ                           | SATNNIVNFNDHYAS     | LWNV | 299 |
| tr A0A7X6E7H7 A0A7X6E7H7_9PSED | QSS-TDKVLINVGYENDPVFRAL               | GSTFTG      | SLGVHDAPK                           | SATDNIVSFNDHYAS     | AWN  | 299 |
| tr T0PEE5 T0PEE5_PHOTE         | QYEKGKVLINVGYENDPVFRVL                | GTSTTA      | SLGVHDAPK                           | SATNNIVNFNDHYAS     | AWN  | 300 |
| tr Q7N4L7 Q7N4L7_PHOLL         | QYEKGKVLINVGYENDPVFRVL                | GTSVDS      | SPGVHDVPR                           | SATNNIVNFNDHYAS     | AWN  | 300 |
| tr D4E6W0 D4E6W0_SEROD         | QFTADNRVANIIGYENDPVFRAL               | GSDLTAS     | SLGVHDTAK                           | SATNNIVNFNDHYAS     | AWN  | 300 |
| tr A0A240BYY2 A0A240BYY2_SERFI | QYETGGKVNIIGYENDPVFRAL                | GTTTLP      | SLGVHDAPO                           | SATNNIVNFNDHYAS     | AWN  | 300 |
| tr A0A1W9F2A3 A0A1W9F2A3_9GAMM | QYENGKGVNIIGYENDPVFRAL                | GTSLTG      | SLGVHDAPH                           | SATNNIVNFNDHYAS     | AWN  | 198 |
|                                | :                                     | ..*         | ..*                                 | ..*                 | :    |     |
| tr A0A0G3CKI4 A0A0G3CKI4_9GAMM | IPTSILNLFWSWISHMPYAYDEGMQRIVESQWYNL   | TEKDS       | TVVVAL                              | SDNMQEKTVVEDLN      |      | 359 |
| tr A0A7X6EAE2 A0A7X6EAE2_9PSED | VPFSIANPLNWSAHSLSGYADGLNRIASKFYGLT    | HKDS        | TIIVSN                              | LEQASRGKTWVEDLG     |      | 306 |
| tr Q4KBS3 Q4KBS3_PSEF5         | IPQSI LNPQSWSAHSAYDYAGGLNRLINSDFYDL   | SRDST       | VVISN                               | LESGKRDQVWVVDLN     |      | 302 |
| tr A0A6M8UDY1 A0A6M8UDY1_9GAMM | LPFLGLFNLPSWLTHLPFLYEGGVTSIME SAFYPLT | TEKDS       | TVIVSA                              | LSDAKRGSWVVDLN      |      | 360 |
| tr A0A0M9GI67 A0A0M9GI67_9PSED | MPFSIGNIATWLSHLPTAYGNGMQRIVDSFYDLT    | GRNST       | IIVSN                               | LSDPARDKTHVVDLN     |      | 360 |
| sp P41773 LIPB_PSEFL           | LPFSIVNLPTWVSHLPTAYGDMTRILESGFYDQNT   | TRDST       | VIVAN                               | LSDPARANTHWVDLN     |      | 359 |
| tr C3KBE9 C3KBE9_PSEF5         | LPFSIANLPTWLSHLPTGYGDMTRIVE SGFYEQMS  | SRDAT       | VIVAN                               | LSDPARANTHWVDLN     |      | 359 |
| tr G8Q328 G8Q328_PSE01         | LPFSILNIPTWLSHLPTGYGDMGRIMGSFAFYDL    | TEKDS       | TIIVAN                              | LSDPARANTHWVDLN     |      | 359 |
| tr Q4KBS6 Q4KBS6_PSEF5         | LPFSILNIPTWLSHLPTGYGDLTRVLESKFYDFT    | SKDS        | TIIVAN                              | LSDPARASTHWVDLN     |      | 359 |
| tr Q9X3C0 Q9X3C0_PSECL         | LPSSI LNIPTWLSHLPTGYGDLGRVLESKFYDFT   | SKDS        | TIIVAN                              | LSDPARASTHWVDLN     |      | 359 |
| tr A0A7X6E7H7 A0A7X6E7H7_9PSED | LPFSI LNIPTWLSHLPTAYGDMNRVIESKFYDLT   | SKDS        | TIIVAN                              | LSDPARANTHWVDLN     |      | 359 |
| tr T0PEE5 T0PEE5_PHOTE         | LPFSIANLSTWVSHVTPFYQSGMQRVMDSTFYDL    | HKDS        | TIIVSN                              | LSNVTRGNTWVEDLN     |      | 360 |
| tr Q7N4L7 Q7N4L7_PHOLL         | LPFSIANPSTWVSHVTPFYQSGMQRIMDSTFYDL    | HKDS        | TIIVSN                              | LSNVTRGNTWVEDLN     |      | 360 |
| tr D4E6W0 D4E6W0_SEROD         | LPFSILNVPTWLSHLPPFYQDGLRLVNSEFYPLT    | QDST        | TVIVSN                              | LSNVTRDNTWVDVN      |      | 360 |
| tr A0A240BYY2 A0A240BYY2_SERFI | LPFSILNVPTWLSHLPPFYQDGLMRVNSEFYSLT    | TEKDS       | TIIVSN                              | LSNVTRGNTWVEDLN     |      | 360 |
| tr A0A1W9F2A3 A0A1W9F2A3_9GAMM | LPFSILNIPTWLSHLPPFYQDGLMRVNSEFYSLT    | TEKDS       | TIIVSN                              | LSNVTRGNTWVEDLN     |      | 258 |
|                                | :                                     | ..*         | ..*                                 | ..*                 | :    |     |
| tr A0A0G3CKI4 A0A0G3CKI4_9GAMM | RYAQPHVGTTFIIGSD                      | NDLIRTKS    | GINYLDGAGGDDIFS                     | IDKGFNIYGGTGQNTLELH |      | 419 |
| tr A0A7X6EAE2 A0A7X6EAE2_9PSED | RSGEPTHTGTFIIGTD                      | GDWLKGGAGND | FLEGLGGDRFRDDGGFNILLGGQGHNTFELQ     |                     |      | 366 |
| tr Q4KBS3 Q4KBS3_PSEF5         | LYAEKHTGSTFIIGTQ                      | NDL LHGGK   | NDYLDGGAGDDRFRDDGGYNIILHGGQGHNTLELQ |                     |      | 362 |
| tr A0A6M8UDY1 A0A6M8UDY1_9GAMM | RDAQKHGHTGTFIIGSD                     | DDL IKGGK   | NDYLEGRGGNDIFRDEGGFNILLGGQGHNTLDLQ  |                     |      | 420 |
| tr A0A0M9GI67 A0A0M9GI67_9PSED | RNAEAHSGTTFIIGSE                      | SDLLKGGK    | NDYLEGGGGDDTFRDDGGYNIILGGQGHNTLDLQ  |                     |      | 420 |
| sp P41773 LIPB_PSEFL           | RNAEPHKGHTTFIIGSD                     | NDL IQGGK   | NDYFIEGGK GNDTIRDN                  |                     |      | 401 |
| tr C3KBE9 C3KBE9_PSEF5         | RNAEPHKGHTTFIIGSD                     | DDL IQGGK   | NDYFIEGGK GNDTIRDN                  |                     |      | 401 |
| tr G8Q328 G8Q328_PSE01         | RNAETHGHTTFIIGSD                      | NDL IQGGK   | NDYLEGRAGNDTFRDGGGYNIILGGQGSNTLDLQ  |                     |      | 419 |
| tr Q4KBS6 Q4KBS6_PSEF5         | RNAETHKGSTTFIIGSD                     | NDL IQGGK   | NDYLEGRAGNDTFRDGGGYNIILGGQGSNTLDLQ  |                     |      | 419 |
| tr Q9X3C0 Q9X3C0_PSECL         | RNAETHKGSTTFIIGSD                     | NDL IQGGK   | NDYLEGRAGNDTFRDGGGYNIILGGQGSNTLDLQ  |                     |      | 419 |
| tr A0A7X6E7H7 A0A7X6E7H7_9PSED | RNAETHKGSTTFIIGSD                     | NDL IQGGK   | NDYLEGRAGNDTFRDGGGYNIILGGQGSNTLELQ  |                     |      | 419 |
| tr T0PEE5 T0PEE5_PHOTE         | RNAEKHTGSTFIIGTN                      | NDL IKGGK   | NDYLEGLDGNIDFRDDGGFNIIAGGKGHNTFDLQ  |                     |      | 420 |
| tr Q7N4L7 Q7N4L7_PHOLL         | RNAEKHTGSTFIIGTD                      | NDL IKGGK   | NDYLEGRDGNIDFRDDGGFNIIAGGKGHNTFDLQ  |                     |      | 420 |
| tr D4E6W0 D4E6W0_SEROD         | RNAQPHSGTTFIIGSD                      | NDL IKGGK   | NDYLEGRAGDDTFRDDGGFNIIAGGKGHNTFDLQ  |                     |      | 420 |
| tr A0A240BYY2 A0A240BYY2_SERFI | RNAETHSGTTFIIGSD                      | NDRIKGGT    | NDYLEGRDGNIDFRDGGGYNIIVSGGKGNVLDTR  |                     |      | 420 |
| tr A0A1W9F2A3 A0A1W9F2A3_9GAMM | RNAETHSGTTFIIGSD                      | NDL IKGGK   | NDYLEGRDGNIDFRDAGGYNIIAGGKGHNTFDLQ  |                     |      | 318 |
|                                | :                                     | ..*         | ..*                                 | ..*                 | :    |     |

An \* (asterisk) indicates positions which have a single, fully conserved residue. A : (colon) indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix. A . (period) indicates conservation between groups of weakly similar properties - scoring <= 0.5 in the Gonnet PAM 250 matrix.



### Annex 7 Binding affinity results of polyurethan esterase A

|                  | Affinity (kcal/mol) |             |             |             |             |             |             |
|------------------|---------------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Modes/AminoAcid  | SER93               | SER242      | SER294      | SER301      | SER582      | SER207      | SER270      |
| <u>1</u>         | <b>-4.3</b>         | <b>-4.8</b> | <b>-4.2</b> | <b>-4.7</b> | <b>-4.5</b> | <b>-5.2</b> | <b>-4.7</b> |
| <u>2</u>         | -4,2                | -4,5        | -4,1        | -4,5        | -4,4        | -5,2        | -4,5        |
| <u>3</u>         | -4,1                | -4,4        | -4,1        | -4,3        | -4,3        | -5,1        | -4,4        |
| <u>4</u>         | -4                  | -4,3        | -4,1        | -4,1        | -4,3        | -5,1        | -4,2        |
| <u>5</u>         | -3,9                | -4,3        | -4          | -4          | -4,2        | -5,1        | -4,2        |
| <u>6</u>         | -3,8                | -4,3        | -4          | -3,9        | -4,1        | -4,9        | -4,1        |
| <u>7</u>         | -3,8                | -4,2        | -3,9        | -3,9        | -4,1        | -4,9        | -4,1        |
| <u>8</u>         | -3,7                | -4,2        | -3,9        | -3,9        | -4,1        | -4,9        | -4,1        |
| <u>9</u>         | -3,7                | -4,2        | -3,9        | -3,8        | -4,1        | -4,8        | -4          |
| <b>Blind (U)</b> |                     |             |             |             |             |             |             |
| <u>1</u>         | <b>-4.6</b>         |             |             |             |             |             |             |

|                 | Affinity (kcal/mol) |        |        |        |        |        |        |
|-----------------|---------------------|--------|--------|--------|--------|--------|--------|
| Modes/AminoAcid | SER93               | SER242 | SER294 | SER301 | SER582 | SER207 | SER270 |
| <u>1</u>        | -4.5                | -5     | -5.3   | -6     | -5.4   | -6.7   | -5.6   |
| <u>2</u>        | -4.5                | -4.9   | -5.2   | -5.6   | -5.1   | -6.7   | -5.1   |
| <u>3</u>        | -4.3                | -4.9   | -5.2   | -5.5   | -5     | -6.6   | -5     |
| <u>4</u>        | -4.3                | -4.8   | -5.1   | -5.5   | -5     | -6.6   | -4.8   |
| <u>5</u>        | -4.2                | -4.8   | -4.5   | -5.4   | -4.9   | -6.4   | -4.7   |
| <u>6</u>        | -4.1                | -4.7   | -4.5   | -5.4   | -4.7   | -6.4   | -4.6   |
| <u>7</u>        | -4                  | -4.6   | -4.4   | -5.1   | -4.7   | -6.3   | -4.6   |
| <u>8</u>        | -4                  | -4.5   | -4.3   | -5     | -4.7   | -6.3   | -4.6   |
| <u>9</u>        | -4                  | -4.5   | -4.3   | -4.9   | -4.6   | -6.2   | -4.6   |
| Blind (U)       |                     |        |        |        |        |        |        |
| <u>1</u>        | -4.5                |        |        |        |        |        |        |

|                  | Affinity (kcal/mol) |             |             |             |             |             |             |
|------------------|---------------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Modes/AminoAcid  | SER93               | SER242      | SER294      | SER301      | SER582      | SER207      | SER270      |
| <u>1</u>         | <u>-4,7</u>         | <u>-5,1</u> | <u>-4,7</u> | <u>-6,5</u> | <u>-4,6</u> | <u>-7,1</u> | <u>-4,8</u> |
| <u>2</u>         | -4,5                | -4,7        | -4,4        | -6,2        | -4,4        | -6,5        | -4,8        |
| <u>3</u>         | -4,5                | -4,6        | -4,3        | -6,1        | -4,4        | -6,3        | -4,7        |
| <u>4</u>         | -4,2                | -4,4        | -4,3        | -5,9        | -4,4        | -6,3        | -4,7        |
| <u>5</u>         | -4,2                | -4,4        | -4,2        | -5,8        | -4,3        | -6,2        | -4,7        |
| <u>6</u>         | -4,1                | -4,3        | -4,2        | -5,7        | -4,3        | -6,2        | -4,5        |
| <u>7</u>         | -4,1                | -4,3        | -4,1        | -5,5        | -4,2        | -6,2        | -4,4        |
| <u>8</u>         | -3,9                | -4,2        | -4,1        | -5,4        | -4,1        | -6,2        | -4,4        |
| <u>9</u>         | -3,9                | -4,2        | -4          | -5,4        | -4,1        | -6,1        | -4,3        |
| <b>Blind (U)</b> |                     |             |             |             |             |             |             |
| <u>1</u>         | <u>-4,1</u>         |             |             |             |             |             |             |

| Affinity (kcal/mol) |             |             |             |             |             |             |             |
|---------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Modes/AminoAcid     | SER93       | SER242      | SER294      | SER301      | SER582      | SER207      | SER270      |
| <u>1</u>            | <u>-4.3</u> | <u>-5.2</u> | <u>-5.1</u> | <u>-5.7</u> | <u>-4.6</u> | <u>-6.7</u> | <u>-4.9</u> |
| <u>2</u>            | -4.1        | -5          | -5          | -5.5        | -4.6        | -6.5        | -4.7        |
| <u>3</u>            | -3.9        | -4.9        | -5          | -5.4        | -4.5        | -6.3        | -4.6        |
| <u>4</u>            | -3.8        | -4.8        | -4.7        | -5.4        | -4.4        | -6.2        | -4.5        |
| <u>5</u>            | -3.8        | -4.8        | -4.6        | -5.3        | -4.1        | -6.1        | -4.5        |
| <u>6</u>            | -3.7        | -4.7        | -4.5        | -5.1        | -3.8        | -6.1        | -4.3        |
| <u>7</u>            | -3.7        | -4.6        | -4.2        | -5.1        | -3.7        | -6          | -4          |
| <u>8</u>            | -3.7        | -4.4        | -4.2        | -5          | -3.6        | -5.9        | -4          |
| <u>9</u>            | -3.6        | -4.3        | -4.1        | -5          | -3.5        | -5.9        | -4          |
| <b>Blind (U)</b>    |             |             |             |             |             |             |             |
| <u>1</u>            | <u>-5.4</u> |             |             |             |             |             |             |

| Affinity (kcal/mol) |             |             |             |           |             |             |             |
|---------------------|-------------|-------------|-------------|-----------|-------------|-------------|-------------|
| Modes/AminoAcid     | SER93       | SER242      | SER294      | SER301    | SER582      | SER207      | SER270      |
| <u>1</u>            | <u>-4,7</u> | <u>-5,1</u> | <u>-5,6</u> | <u>-6</u> | <u>-5,6</u> | <u>-7,6</u> | <u>-7,1</u> |
| <u>2</u>            | -4,6        | -4,9        | -5,5        | -6        | -5,5        | -7,2        | -6,2        |
| <u>3</u>            | -4,1        | -4,9        | -5,3        | -6        | -4,9        | -6,7        | -5,9        |
| <u>4</u>            | -4,1        | -4,7        | -5,3        | -6        | -4,9        | -6,4        | -5,7        |
| <u>5</u>            | -4          | -4,7        | -5,3        | -5,9      | -4,7        | -6,3        | -5,6        |
| <u>6</u>            | -4          | -4,6        | -5,3        | -5,9      | -4,7        | -6,2        | -5,3        |
| <u>7</u>            | -3,9        | -4,5        | -5,2        | -5,5      | -4,6        | -6,2        | -5,1        |
| <u>8</u>            | -3,9        | -4,5        | -5,2        | -5,5      | -4,5        | -6,1        | -4,9        |
| <u>9</u>            | -3,8        | -4,4        | -5,2        | -5,4      | -4,5        | -6,1        | -4,8        |
| <b>Blind (U)</b>    |             |             |             |           |             |             |             |
| <b>1</b>            | <b>-4,8</b> |             |             |           |             |             |             |

Annex 8 Binding affinity results of polyurethan esterase B

| Table 1. Binding affinity results of PU-HDI-PueB |             |             |             |             |             |             |           |
|--------------------------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-----------|
| Affinity (kcal/mol)                              |             |             |             |             |             |             |           |
| Modes/AminoAcid                                  | SER212      | SER253      | SER401      | SER416      | SER554      | SER83       | SER58     |
| <u>1</u>                                         | <u>-4,4</u> | <u>-4,4</u> | <u>-3,9</u> | <u>-4,1</u> | <u>-3,9</u> | <u>-4,4</u> | <u>-4</u> |
| 2                                                | -4,3        | -4,1        | -3,9        | -4          | -3,7        | -4          | -3,6      |
| 3                                                | -4          | -4          | -3,8        | -3,9        | -3,5        | -4          | -3,5      |
| 4                                                | -4          | -4          | -3,7        | -3,6        | -3,5        | -3,5        | -3,5      |
| 5                                                | -3,9        | -3,9        | -3,6        | -3,5        | -3,5        | -3,4        | -3,4      |
| 6                                                | -3,9        | -3,9        | -3,6        | -3,5        | -3,3        | -3,4        | -3,3      |
| 7                                                | -3,6        | -3,7        | -3,6        | -3,4        | -3,2        | -3,3        | -3,2      |
| 8                                                | -3,6        | -3,7        | -3,5        | -3,4        | -3,2        | -3,2        | -3,2      |
| 9                                                | -3,6        | -3,7        | -3,5        | -3,3        | -3,1        | -3,2        | -3,2      |
| Blind (U)                                        |             |             |             |             |             |             |           |
| <u>1</u>                                         | <u>-4</u>   |             |             |             |             |             |           |

| Table 2. Binding affinity results of PU-HMDI-PueB |             |             |             |             |             |             |             |
|---------------------------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Affinity (kcal/mol)                               |             |             |             |             |             |             |             |
| Modes/AminoAcid                                   | SER212      | SER253      | SER401      | SER416      | SER554      | SER83       | SER58       |
| <u>1</u>                                          | <u>-5.1</u> | <u>-4.6</u> | <u>-5.5</u> | <u>-4.6</u> | <u>-4.3</u> | <u>-3.8</u> | <u>-3.8</u> |
| 2                                                 | -4.8        | -4.5        | -5.3        | -4.5        | -4          | -3.7        | -3.7        |
| <u>3</u>                                          | <u>-4.8</u> | <u>-4.3</u> | <u>-5.2</u> | <u>-4.2</u> | <u>-3.9</u> | <u>-3.5</u> | <u>-3.7</u> |
| 4                                                 | -4.7        | -4.3        | -5          | -4.2        | -3.9        | -3.5        | -3.7        |
| <u>5</u>                                          | <u>-4.7</u> | <u>-4.2</u> | <u>-5</u>   | <u>-4.2</u> | <u>-3.8</u> | <u>-3.4</u> | <u>-3.7</u> |
| 6                                                 | -4.6        | -4.2        | -4.9        | -4.1        | -3.8        | -3.4        | -3.6        |
| 7                                                 | -4.6        | -4.2        | -4.9        | -4.1        | -3.8        | -3.3        | -3.6        |
| 8                                                 | -4.6        | -4.1        | -4.9        | -4          | -3.6        | -3.3        | -3.5        |
| 9                                                 | -4.6        | -4.1        | -4.8        | -3.9        | -3.6        | -3.3        | -3.4        |
| Blind (U)                                         |             |             |             |             |             |             |             |
| <u>1</u>                                          | <u>-4.6</u> |             |             |             |             |             |             |

| Table 3. Binding affinity results of PU-MDI-PueB |             |             |             |           |             |             |             |
|--------------------------------------------------|-------------|-------------|-------------|-----------|-------------|-------------|-------------|
| Affinity (kcal/mol)                              |             |             |             |           |             |             |             |
| Modes/AminoAcid                                  | SER212      | SER253      | SER401      | SER416    | SER554      | SER83       | SER58       |
| <u>1</u>                                         | <u>-4,9</u> | <u>-4,1</u> | <u>-4,7</u> | <u>-5</u> | <u>-4,4</u> | <u>-4,8</u> | <u>-4,6</u> |
| 2                                                | -4,8        | -3,8        | -4,5        | -4,7      | -4,3        | -4,1        | -4,5        |
| 3                                                | -4,8        | -3,7        | -4,4        | -4,4      | -4,2        | -4,1        | -4,3        |
| 4                                                | -4,8        | -3,7        | -4,4        | -4,3      | -4          | -3,9        | -4,3        |
| 5                                                | -4,7        | -3,7        | -4,4        | -4,3      | -4          | -3,9        | -4,2        |
| 6                                                | -4,7        | -3,7        | -4,3        | -4,2      | -4          | -3,9        | -4,1        |
| 7                                                | -4,6        | -3,6        | -4,3        | -4,2      | -3,9        | -3,9        | -4,1        |
| 8                                                | -4,6        | -3,6        | -4,2        | -4        | -3,9        | -3,8        | -4,1        |
| 9                                                | -4,5        | -3,6        | -4,2        | -4        | -3,9        | -3,8        | -4          |
| Blind (U)                                        |             |             |             |           |             |             |             |
| <u>1</u>                                         | <u>-5,1</u> |             |             |           |             |             |             |

| Table 4. Binding affinity results of PU-TDI24-PueB |             |           |             |           |             |             |             |
|----------------------------------------------------|-------------|-----------|-------------|-----------|-------------|-------------|-------------|
| Affinity (kcal/mol)                                |             |           |             |           |             |             |             |
| Modes/AminoAcid                                    | SER212      | SER253    | SER401      | SER416    | SER554      | SER83       | SER58       |
| <u>1</u>                                           | <u>-4,8</u> | <u>-5</u> | <u>-4,8</u> | <u>-4</u> | <u>-4,1</u> | <u>-4,3</u> | <u>-4,6</u> |
| 2                                                  | -4,6        | -5        | -4,5        | -3,9      | -4,1        | -4,2        | -4,3        |
| 3                                                  | -4,6        | -4,9      | -4,5        | -3,9      | -4          | -3,9        | -4,1        |
| 4                                                  | -4,4        | -4,9      | -4,4        | -3,9      | -3,8        | -3,8        | -4          |
| 5                                                  | -4,4        | -4,9      | -4,4        | -3,9      | -3,6        | -3,8        | -4          |
| 6                                                  | -4,4        | -4,9      | -4,3        | -3,8      | -3,5        | -3,7        | -3,9        |
| 7                                                  | -4,4        | -4,8      | -4,3        | -3,8      | -3,5        | -3,7        | -3,8        |
| 8                                                  | -4,4        | -4,7      | -4,3        | -3,7      | -3,5        | -3,7        | -3,8        |
| 9                                                  | -4,3        | -4,7      | -4,1        | -3,6      | -3,5        | -3,5        | -3,8        |
| Blind (U)                                          |             |           |             |           |             |             |             |
| <u>1</u>                                           | <u>-4,3</u> |           |             |           |             |             |             |

| Table 5. Binding affinity results of PU-TDI26-PucB |             |             |             |             |             |             |             |
|----------------------------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Affinity (kcal/mol)                                |             |             |             |             |             |             |             |
| Modes/AminoAcid                                    | SER212      | SER253      | SER401      | SER416      | SER554      | SER83       | SER58       |
| <u>1</u>                                           | <u>-3.7</u> | <u>-4.7</u> | <u>-5.6</u> | <u>-4.5</u> | <u>-4.2</u> | <u>-3.7</u> | <u>-4.7</u> |
| 2                                                  | -3.5        | -4.5        | -5.5        | -4.4        | -4.1        | -3.7        | -4.5        |
| <u>3</u>                                           | <u>-3.5</u> | <u>-4.3</u> | <u>-5.1</u> | <u>-4.4</u> | <u>-4</u>   | <u>-3.7</u> | <u>-4.5</u> |
| 4                                                  | -3.5        | -4.3        | -4.8        | -4.3        | -3.9        | -3.5        | -4.4        |
| <u>5</u>                                           | <u>-3.4</u> | <u>-4.3</u> | <u>-4.7</u> | <u>-4.2</u> | <u>-3.9</u> | <u>-3.5</u> | <u>-4.3</u> |
| 6                                                  | -3.3        | -4.3        | -4.7        | -3.9        | -3.9        | -3.5        | -4.3        |
| <u>7</u>                                           | <u>-3.3</u> | <u>-4.2</u> | <u>-4.7</u> | <u>-3.9</u> | <u>-3.8</u> | <u>-3.4</u> | <u>-4.2</u> |
| 8                                                  | -3.2        | -4.2        | -4.6        | -3.8        | -3.8        | -3.3        | -4.2        |
| <u>9</u>                                           | <u>-3.2</u> | <u>-4.2</u> | <u>-4.6</u> | <u>-3.8</u> | <u>-3.8</u> | <u>-3.2</u> | <u>-4.2</u> |
| <i>Blind (U)</i>                                   |             |             |             |             |             |             |             |
| <u>1</u>                                           | <u>-4.7</u> |             |             |             |             |             |             |