

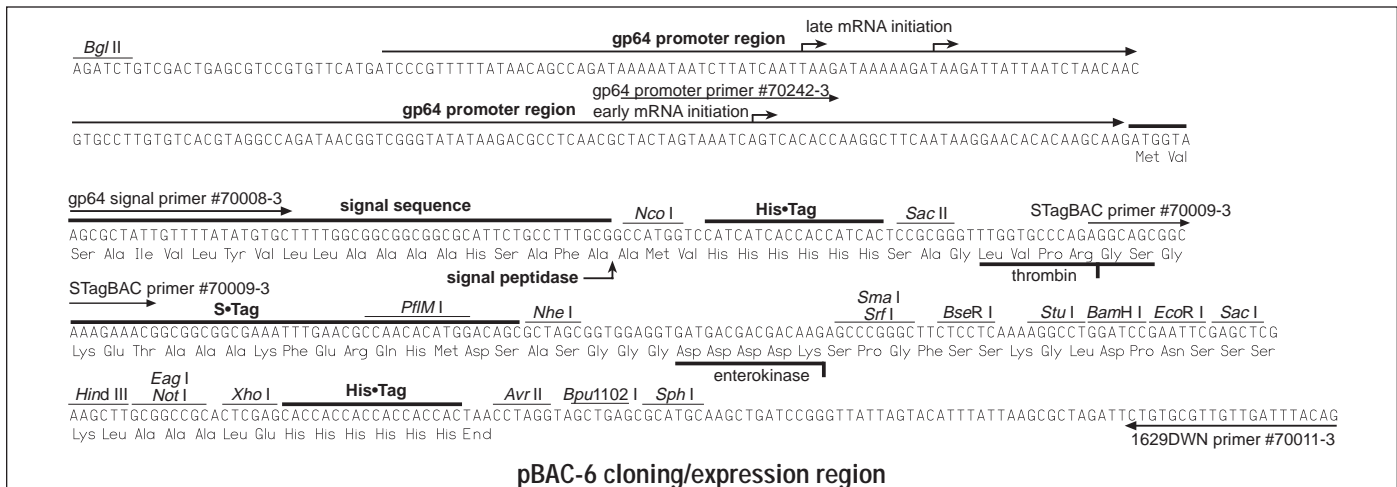
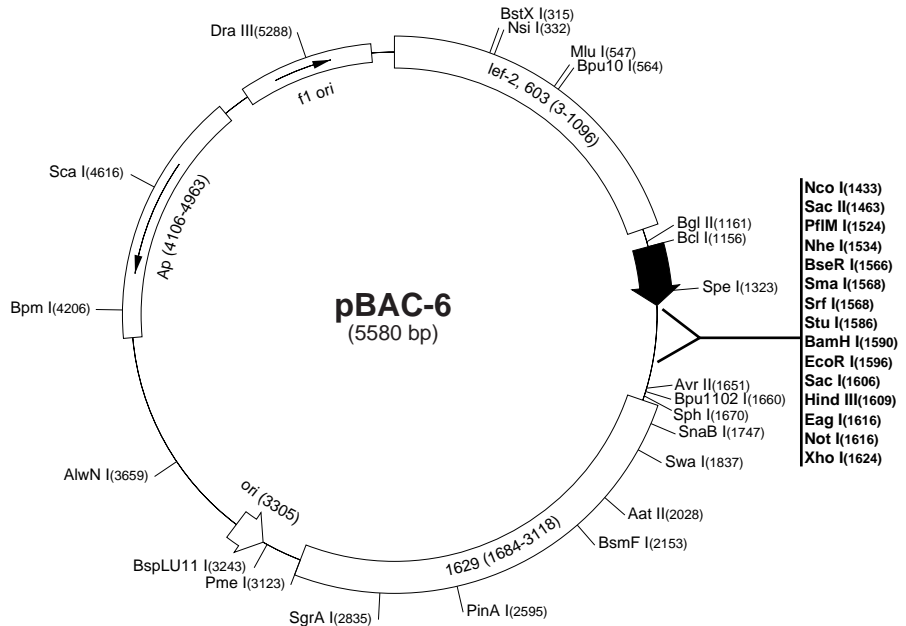
# pBAC-6 Transfer Plasmid

|          |                          |                     |
|----------|--------------------------|---------------------|
| <b>b</b> | Locus                    | polh                |
|          | Promoter                 | gp64                |
|          | secretion signal         | gp64 signal peptide |
|          | N-terminal fusion        | His•Tag, S•Tag      |
|          | C-terminal fusion option | His•Tag             |
|          | Cloning options          | polylinker          |

pBAC™-6 is a baculovirus transfer plasmid (Cat. No. 70224-3) compatible with BacVector™ -1000, -2000, or -3000 Triple Cut Virus DNA for low background transfection. A modified gp64 tandem promoter contains both an immediate early promoter for expression beginning immediately after infection and a late promoter for continued expression in the late phase of infection. The pBAC-6 Transfer Plasmid enables isolation of stably-transfected insect cell lines expressing target proteins. A 20 amino acid gp64 signal peptide sequence is capable of directing high levels of protein into the secretory pathway of infected insect cells. Cloning sites are provided for the creation of N-terminal fusions of an insert with His•Tag® and/or S•Tag™ sequences. Unique restriction sites are indicated on the circle map. The cloning/expression region of the coding strand transcribed from the gp64 promoter is shown below. The f1 origin is oriented so that infection with helper phage will produce virions containing single stranded DNA that corresponds to the coding strand. Single stranded sequencing of phage-derived DNA can be performed using the 1629DWN primer (Cat. No. 70011-3).

### pBAC-6 sequence landmarks

|  |            |
|--|------------|
| gp64 promoter region                                     | 1167-1371  |
| gp64 early transcription start                           | 1334       |
| gp64 late transcription starts                           | 1233, 1246 |
| wt gp64 5'UTR position                                   | 1371       |
| signal peptide coding region                             | 1372-1431  |
| His•Tag coding sequence                                  | 1441-1458  |
| S•Tag coding sequence                                    | 1489-1533  |
| Multiple cloning sites<br>( <i>Sma</i> I - <i>Xho</i> I) | 1566-1629  |
| His•Tag coding sequence<br>(C-terminal option)           | 1630-1647  |



# pBAC-6 Restriction Sites

| Enzyme   | # Sites | Locations                | Enzyme   | # Sites | Locations                | Enzyme  | # Sites | Locations                |
|----------|---------|--------------------------|----------|---------|--------------------------|---------|---------|--------------------------|
| AatII    | 1       | 2028                     | EaeI     | 3       | 1430 1616 4524           | SrfI    | 1       | 1568                     |
| AccI     | 3       | 107 392 1168             | EagI     | 1       | 1616                     | SspI    | 7       | 155 573 1841 2038 2911   |
| AcII     | 66      |                          | EarI     | 4       | 199 695 4931 5569        |         |         | 4940 5493                |
| AflIII   | 5       | 547 2098 2248 2353 3243  | Eco47III | 4       | 1380 1533 1705 2888      | StuI    | 1       | 1586                     |
| AhdI     | 3       | 647 1175 4136            | Eco57I   | 2       | 3791 4803                | StyI    | 3       | 1341 1433 1651           |
| AluI     | 23      |                          | EcoRI    | 1       | 1596                     | Swal    | 1       | 1837                     |
| AlwI     | 15      |                          | EcoRII   | 5       | 1586 3269 3390 3403 5577 | TalI    | 17      |                          |
| Alw26I   | 8       | 433 1097 2534 2597 3033  | FauI     | 10      | 1455 2292 2306 2454 2663 | TaqI    | 17      |                          |
|          |         | 3092 4197 4973           |          |         | 2693 3055 5077 5146 5550 | TfiI    | 3       | 594 1710 3218            |
| AlwNI    | 1       | 3659                     | Fnu4HI   | 43      |                          | ThaI    | 15      |                          |
| ApaLI    | 2       | 3557 4803                | FokI     | 3       | 4102 4283 4570           | TseI    | 19      |                          |
| ApoI     | 12      |                          | FspI     | 3       | 807 4358 5531            | Tsp45I  | 5       | 1275 1334 4392 4603 5107 |
| AvaI     | 2       | 1566 1624                | HaeII    | 10      | 1382 1535 1707 2581 2746 | Tsp509I | 41      |                          |
| Avall    | 3       | 1437 4274 4496           |          |         | 2848 2890 3491 5130 5138 | TspRI   | 10      | 838 2071 3145 3651 3664  |
| AvrII    | 1       | 1651                     | HaeIII   | 15      |                          |         |         | 3935 4084 4189 4536 4563 |
| BamHI    | 1       | 1590                     | Hgal     | 11      |                          | VspI    | 5       | 20 128 1254 2232 4308    |
| BanI     | 8       | 1470 2550 2577 2781 2832 | HhaI     | 32      |                          | XhoI    | 1       | 1624                     |
|          |         | 2844 4084 5244           | HincII   | 3       | 108 393 1169             | XmnI    | 2       | 2288 4735                |
| BanII    | 3       | 1567 1606 5214           | HindIII  | 1       | 1609                     |         |         |                          |
| BbsI     | 2       | 646 2206                 | HinfI    | 10      | 304 594 1710 2086 3143   |         |         |                          |
| BbvI     | 19      |                          |          |         | 3218 3614 4131 5333 5355 |         |         |                          |
| BcgI     | 2       | 2562 4641                | HphI     | 11      |                          |         |         |                          |
| BcgI'    | 2       | 2528 4675                | MaeIII   | 14      |                          |         |         |                          |
| BclI     | 1       | 1156                     | MbolI    | 15      |                          |         |         |                          |
| BfaI     | 11      |                          | MluI     | 1       | 547                      |         |         |                          |
| BglI     | 3       | 1429 4256 5524           | MnlI     | 29      |                          |         |         |                          |
| BglII    | 1       | 1161                     | MseI     | 44      |                          |         |         |                          |
| BpmI     | 1       | 4206                     | MslI     | 7       | 70 1098 2826 3090 4388   |         |         |                          |
| Bpu10I   | 1       | 564                      |          |         | 4547 4906                |         |         |                          |
| Bpu1102I | 1       | 1660                     | MspI     | 17      |                          |         |         |                          |
| BsaI     | 2       | 433 4197                 | MspA1I   | 8       | 803 1462 1484 2670 2850  |         |         |                          |
| BsaAI    | 3       | 1280 1747 5285           |          |         | 3585 3830 4771           |         |         |                          |
| BsaHI    | 7       | 643 1310 2025 2225 2578  | MunI     | 4       | 1736 2081 2642 3007      |         |         |                          |
|          |         | 2845 4673                | MwoI     | 21      |                          |         |         |                          |
| BsaJI    | 6       | 1341 1433 1460 1566 1651 | NarI     | 2       | 2578 2845                |         |         |                          |
|          |         | 3403                     | NciI     | 6       | 1567 1568 1681 3623 4319 |         |         |                          |
| BsaWI    | 5       | 981 2595 3449 3596 4427  |          |         | 4670                     |         |         |                          |
| BseRI    | 1       | 1566                     | NcoI     | 1       | 1433                     |         |         |                          |
| BsgI     | 3       | 2392 2686 2840           | NgoAIV   | 2       | 929 5180                 |         |         |                          |
| BsiEI    | 7       | 1297 1619 3159 3583 4506 | NheI     | 1       | 1534                     |         |         |                          |
|          |         | 4655 5552                | NlaIII   | 13      |                          |         |         |                          |
| BsiHKAI  | 5       | 1606 1631 3561 4722 4807 | NlaIV    | 19      |                          |         |         |                          |
| BsII     | 11      |                          | NottI    | 1       | 1616                     |         |         |                          |
| BsmI     | 2       | 85 1416                  | NsiI     | 1       | 332                      |         |         |                          |
| BsmBI    | 2       | 1097 2597                | NspI     | 3       | 114 1670 3247            |         |         |                          |
| BsmFI    | 1       | 2153                     | PfiMI    | 1       | 1524                     |         |         |                          |
| Bsp1286I | 10      | 1475 1567 1606 1631 2335 | PinAI    | 1       | 2595                     |         |         |                          |
|          |         | 2835 3561 4722 4807 5214 | PleI     | 7       | 298 2080 3137 3622 4125  |         |         |                          |
| BspLU11I | 1       | 3243                     |          |         | 5341 5349                |         |         |                          |
| BsrI     | 11      |                          | PmeI     | 1       | 3123                     |         |         |                          |
| BsrBI    | 5       | 2700 2995 3176 4977 5141 | Psp1406I | 3       | 4362 4735 5498           |         |         |                          |
| BsrDI    | 3       | 227 4197 4371            | PvuI     | 2       | 4506 5552                |         |         |                          |
| BsrFI    | 6       | 929 2595 2787 2835 4216  | RcaI     | 3       | 1186 3963 4971           |         |         |                          |
|          |         | 5180                     | RsaI     | 11      |                          |         |         |                          |
| BsrGI    | 3       | 197 916 2077             | SacI     | 1       | 1606                     |         |         |                          |
| BssSI    | 2       | 3416 4800                | SacII    | 1       | 1463                     |         |         |                          |
| BstXI    | 1       | 315                      | SalI     | 3       | 106 391 1167             |         |         |                          |
| BstYI    | 8       | 1161 1590 3884 3895 3981 | Sau3AI   | 23      |                          |         |         |                          |
|          |         | 3993 4761 4778           | Sau96I   | 7       | 1437 4178 4257 4274 4496 |         |         |                          |
| Cac8I    | 24      |                          |          |         | 5291 5559                |         |         |                          |
| Clal     | 2       | 2719 3042                | Scal     | 1       | 4616                     |         |         |                          |
| CviJI    | 67      |                          | ScrFI    | 11      |                          |         |         |                          |
| Ddel     | 8       | 564 1172 1660 2601 3518  | SfaNI    | 6       | 339 2492 3340 4392 4583  |         |         |                          |
|          |         | 3927 4093 4633           |          |         | 4832                     |         |         |                          |
| DpnI     | 23      |                          | Sfcl     | 5       | 3080 3508 3699 4377 5062 |         |         |                          |
| DraI     | 7       | 45 577 1837 3123 4002    | SgrAI    | 1       | 2835                     |         |         |                          |
|          |         | 4021 4713                | SmaI     | 1       | 1568                     |         |         |                          |
| DrallI   | 1       | 5288                     | SnaBI    | 1       | 1747                     |         |         |                          |
| DrdI     | 2       | 3351 5332                | SpeI     | 1       | 1323                     |         |         |                          |
| Dsal     | 2       | 1433 1460                | SphI     | 1       | 1670                     |         |         |                          |

|                                 |          |          |        |         |
|---------------------------------|----------|----------|--------|---------|
| Enzymes that do not cut pBAC-6: |          |          |        |         |
| AflIII                          | Apal     | AscI     | BsaBI  | BspEI   |
| BspMI                           | BssHII   | Bst1107I | BstEII | Bsu36I  |
| EcoNI                           | EcoO109I | EcoRV    | FseI   | HpaI    |
| KpnI                            | MscI     | NdeI     | NruI   | NspV    |
| PacI                            | PmlI     | PshAI    | Psp5II | PstI    |
| PvuII                           | RsrII    | SanDI    | SapI   | SexAI   |
| SfiI                            | SgfI     | Sse8387I | SunI   | Tth111I |
| UbaEI                           | XbaI     | XcmI     |        |         |