MultiBac Expression System

User Manual

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Illustrations:

A. Synopsis:

We describe here in detail new baculovirus transfer vectors constructed specifically for multigene applications. We present a modified recipient baculovirus DNA for these transfer vectors engineered for improved protein production, and a simple and rapid method to integrate genes via two access sites (attTn7 and LoxP) into this baculoviral DNA in *E. coli* cells tailored for this purpose.

An intense focus of biological research efforts in the post-genomic era is the elucidation of protein interaction networks (interactome). Since many of the identified multiprotein complexes are not present in sufficient quantities in their native cells for detailed molecular biological analysis, their study is dependent on recombinant technologies for large-scale heterologous protein production. Currently, recombinant expression methods require a disproportionate investment in both labor and materials prior to multiprotein expression, and subsequent to expression do not provide flexibility for rapidly altering the multiprotein components for revised expression studies.

Our invention, using the baculovirus expression system, introduces three major advances which are instrumental to fully exploit the potential of this heterologous protein production system:

1. New transfer vectors (pFBDM and pUCDM) that contain a multiplication module. These vectors greatly facilitate modular combination of heterologous genes with a minimum requirement for unique restriction sites. Viral promoters (currently p10 and polh very late promoters) can be exchanged in our vectors to other promoter sequences (early, late, mammalian) if required. Likewise, terminator sequences (currently SV40, HSVtk) can be substituted.

2. Engineered baculovirus genome (MultiBac) with improved protein production properties. Two baculoviral genes were disrupted which leads to improved maintenance of cellular compartments during infection and protein production. The quality of proteins produced by our system is significantly improved through a reduction of viral dependent proteolytic activity and reduced cell lysis.

3. New protocol for rapid combinatorial generation of recombinant baculovirus DNA by accessing the viral genome via two specific sites. In addition to the commercially available Tn7 transposon site, we introduced a LoxP sequence at a separate site on the baculovirus genome which can accept multigene expression cassettes from our pUCDM plasmid by site-

specific recombination. We developed a protocol for carrying out Tn7 transposition (from pFBDM derivatives carrying multigene cassettes) and LoxP site-specific recombination (from pUCDM derivatives carrying multigene cassettes) efficiently in a single step in *E.coli*. These protocols can be used not only to integrate multigene cassettes with coding sequences for multiprotein complex subunits into MultiBac, but also to integrate specific enzymes (kinases, acetylases etc.) for modifying the proteins under investigation.

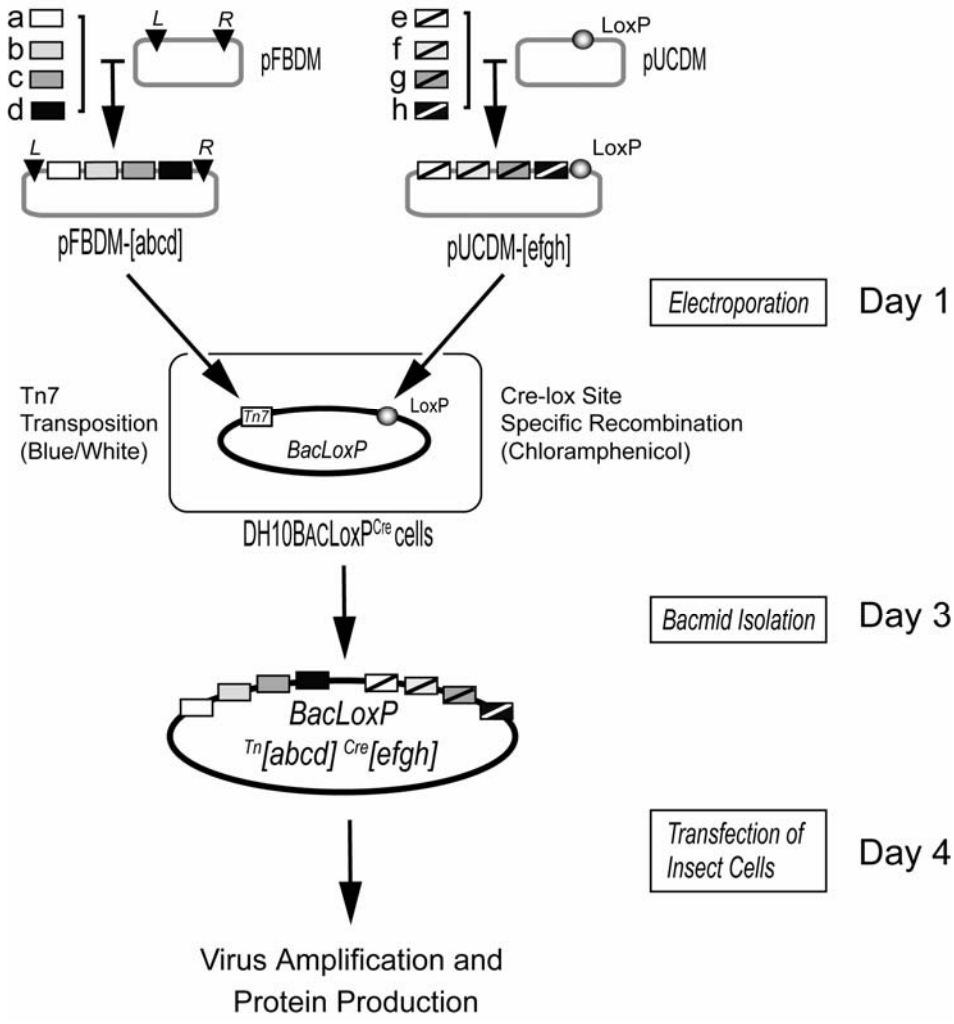


Figure 1: The MultiBac system in a schematic view. Genes of interest are assembled into multigene expression cassettes using the multiplication module present on transfer vectors pFBDM and pUCDM. The resulting vectors are introduced into MultiBac baculoviral DNA in DH10MultiBac^{Cre} *E. coli* cells which contain the factors for Tn7 transposition (for pFBDM derivatives) and *cre-lox* site-specific recombination (for pUCDM derivatives). Colonies containing bacmid carrying integrated multigene cassettes are identified by blue/white screening (Tn7 transposition disrupts a lacZα gene) and chloramphenicol resistance (conferred by Cre catalyzed integration of pUCDM derivative). Transposition and site-specific recombination are carried out either sequentially or, alternatively, concomitantly in a one-step reaction. Bacmid DNA is prepared from selected clones and used to transfect insect cells for protein production.

B. New Baculovirus Tools for Multigene Applications

B.1. Transfer vectors pFBDM and pUCDM.

The transfer vector pFBDM contains two expression cassettes in a head-to-head arrangement with multiple cloning sites MCS1 and MCS2 flanked by polh or p10 promoters and SV40 or HSVtk polyA signal sequences, respectively. Multiplication module M is located in between the promoter sequences. The sequences used for Tn7 transposition (Tn7L and Tn7R) encompass the expression cassettes and a gentamycin resistance marker.

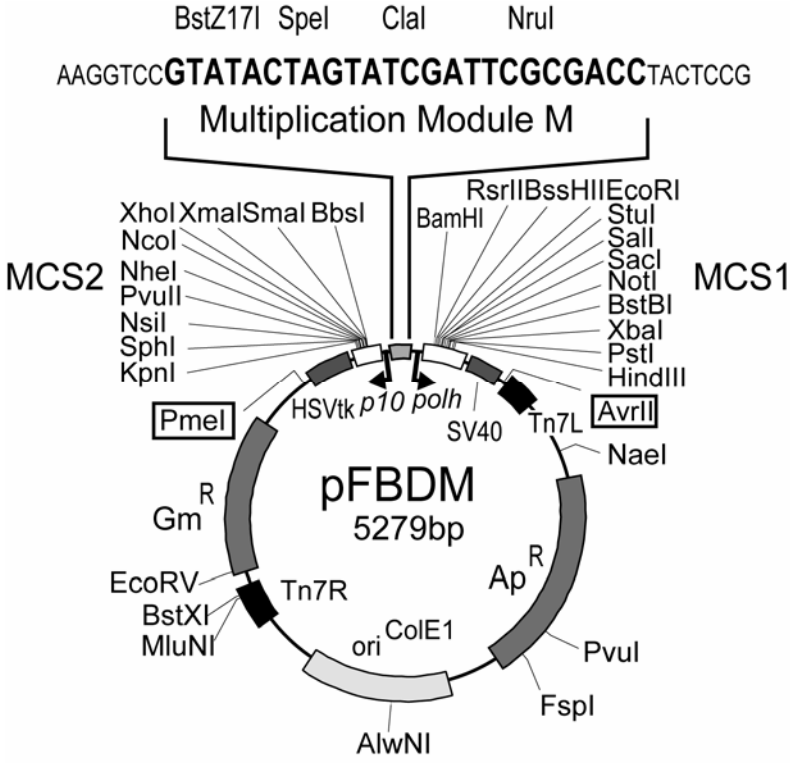


Figure 2: Transfer vector pFBDM. The circle map of pFBDM shows promoters (polh, p10), terminators (SV40, HSVtk), multiple coling sites (MCS1, MCS2), transposon elements (Tn7L, Tn7R) and resistance markers (ampicillin and gentamycin). Genes of interest are cloned into MCS1 or MCS2 using unique restriction sites. The multiplication module (M) is located in between the p10 and polh promoters.

The transfer vector pUCDM has an identical expression cassette including a multiplication module as pFBDM. This expression cassette is flanked by a LoxP inverted repeat. Vector pUCDM contains a chloramphenicol resistance marker and a conditional R6Kγ

origin of replication which makes its propagation dependent on the expression of the *pir* gene in the prokaryotic host.

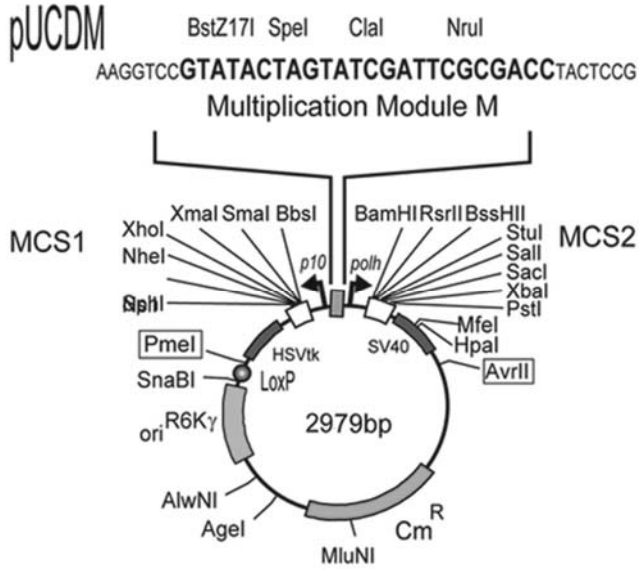


Figure 3: Transfer vector pUCDM. The circle map of pUCDM shows promoters (polh, p10), terminators (SV40, HSVtk), multiple cloning sites (MCS1, MCS2), the inverted repeat for *cre-lox* site-specific recombination (LoxP) and a resistance marker (chloramphenicol). Genes of interest are cloned into MCS1 or MCS2 using unique restriction sites. The multiplication module (M) is located in between the p10 and polh promoters.

B.2. Generating multigene expression cassettes.

Our vectors pFBDM and pUCDM are particularly suited for generating multigene expression cassettes due to the multiplication module inserted in between the two promoters. The logic of multiplication is illustrated below. The only prerequisite for assembling multigene expression cassettes is that the restriction enzymes used for multiplication (e.g. PmeI, AvrII, SpeI, and either BstZ17I or NruI) are unique, which can be easily accomplished for instance by site directed mutagenesis prior to multigene cassette assembly. Genes are cloned into MCS1 and MCS2 of pFBDM. The entire expression cassette is then excised by PmeI and AvrII digestion. The resulting fragment is placed into the multiplication module of a pFBDM derivative containing further sets of genes via either SpeI/BstZ17I or SpeI/NruI sites. SpeI produces a cohesive end compatible with AvrII, while BstZ17I, NruI and PmeI are blunt-

cutters). The restriction sites involved are eliminated in the process and multiplication can be repeated iteratively using the module present in the inserted cassette. The same logic applies also for generation of pUCDM derivatives with multigene expression cassettes. Also, promoter and terminator sequences can be easily modified if desired using appropriate restriction sites in our vectors.

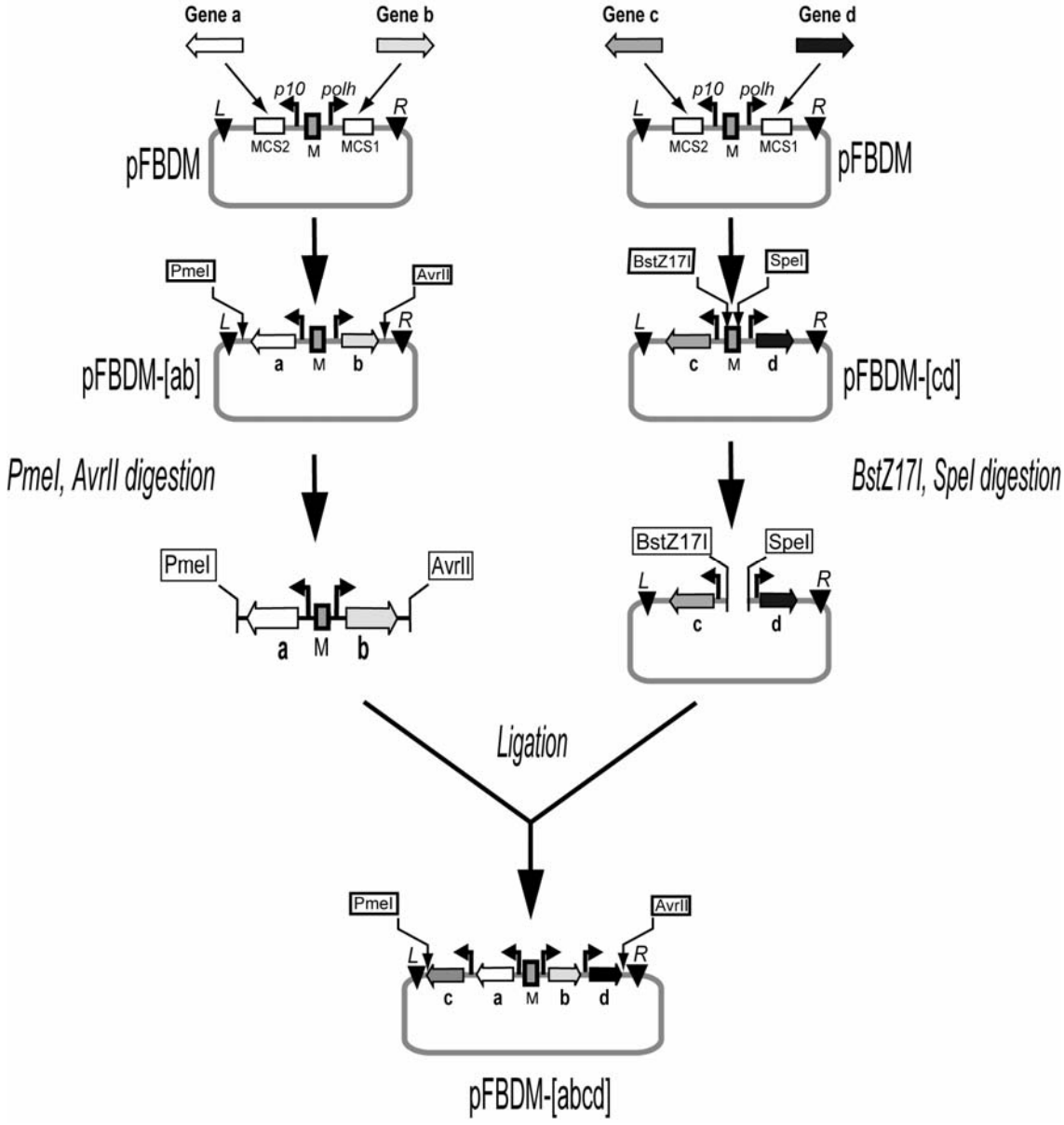


Figure 4: Assembling multigene expression cassettes. The logic of multiplication is shown for pFBDM. The expression cassette containing two genes of choice (denoted a,b) is excised by digestion with AvrII and PmeI (boxed) and placed into a multiplication module of a construct containing further genes (c,d) via BstZ17I/SpeI (or, alternatively NruI/SpeI) sites present in the multiplication module (M). SpeI produces a cohesive end compatible with AvrII, while BstZ17I, NruI and PmeI are blunt-cutters. Multigene derivatives of pUCDM can be generated following the same logic.

B.3. Baculovirus engineered for improved protein production.

We modified the baculovirus genome to obtain improved protein production properties. Two baculoviral genes, *v-cath* and *chiA*, were disrupted which leads to improved maintenance of cellular compartments during infection and protein production. The *v-cath* gene encodes for a viral protease, V-CATH, which is activated upon cell death by a process dependent on a juxtaposed gene on the viral DNA, *chiA*, which encodes for a chitinase. We disrupted both genes to eliminate V-CATH activity and to gain the option of utilizing chitin-affinity chromatography for purification without interference from the *chiA* gene product. The quality of proteins produced by our MultiBac baculovirus is significantly improved through a reduction of viral-dependent proteolytic activity and reduced cell lysis. In place of the disrupted viral DNA sequence, we placed a LoxP sequence for *cre-lox* site-specific recombination.

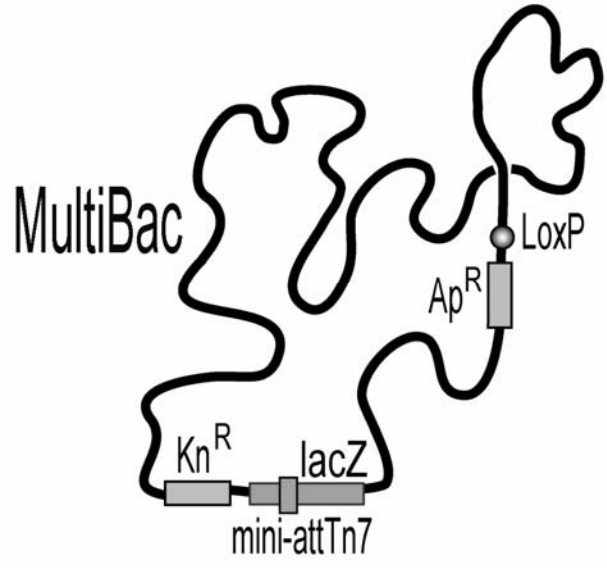


Figure 5: MultiBac baculoviral DNA. The modified viral genome is shown in a schematic representation. The Tn7 attachment site is located within a LacZα gene; insertion of Tn7 elements from pFBDM derivatives therefore produces a white phenotype when plated on agar containing BluoGal and IPTG. In place of the disrupted *v-cath* and *chiA* viral genes, a LoxP sequence was inserted to accept pUCDM derivatives by Cre catalysis, producing a chloramphenicol resistant phenotype.

C. Protocols

C.1. Cloning into pFBDM or pUCDM transfer vectors.

Reagents:

Restriction endonucleases

DNA ligase

E. coli competent cells

Ampicillin

Chloramphenicol

The genes of choice are cloned using standard cloning procedures into the multiple cloning sites MCS1 or MCS2 (see *Supplementary Information*) of pFBDM and pUCDM. Ligation reactions for pFBDM derivatives are transformed into standard *E. coli* cells for cloning (such as TOP10, DH5α, HB101) and plated on agar containing ampicillin (100 μg/ml). Ligation reactions for pUCDM derivatives are transformed into *E. coli* cells expressing the *pir* gene (such as BW23473, BW23474) and plated on agar containing chloramphenicol (25 μg/ml). Correct clones are selected based on specific restriction digest and DNA sequencing of the inserts.

C.2. *Cre-lox* site-specific recombination protocol.

Reagents:

Electro-competent DH10MultiBac^{Cre} cells

Ampicillin

Kanamycin

Chloramphenicol

Approximately 5-10 ng of the sequenced pUCDM derivative is incubated on ice (15 min) with 50-100 μl electro-competent DH10MultiBac^{Cre} cells. Following electroporation (200 ohms, 25 μF, 1.8 kV pulse), cells are incubated at 37 °C for 8 hours and plated on agar containing kanamycin (50 μg/ml), chloramphenicol (25 μg/ml) and ampicillin (100 μg/ml). Colonies appear after incubation at 37 °C (12-15 hours). Proceed to bacmid preparation for insect cell infection (see C.6.) or prepare clones for integration of a pFBDM derivative by Tn7 transposition (see C.5.).

C.3. Transposition protocol for pFBDM derivatives.

Reagents:

Electro-competent DH10MultiBac^{Cre} cells

Ampicillin

Kanamycin

Tetracyclin

Gentamycin

BluoGal

IPTG

Approximately 5-10 ng of the sequenced pFBDM derivative is incubated on ice (15 min) with 50-100 µl electro-competent DH10MultiBac^{Cre} cells. Following electroporation (200 ohms, 25 µF, 1.8 kV pulse), cells are incubated at 37 °C for 8 hours and plated on agar containing kanamycin (50 µg/ml), gentamycin (7 µg/ml), ampicillin (100 µg/ml), tetracyclin (10 µg/ml), BluoGal (100 µg/ml) and IPTG (40 µg/ml). White colonies are selected after incubation at 37 °C (18 - 24 hours). Proceed to bacmid preparation for insect cell infection (C.6.).

C.4. One-step transposition / *cre-lox* site-specific recombination.

Reagents:

Electro-competent DH10MultiBac^{Cre} cells

Kanamycin

Tetracyclin

Gentamycin

Chloramphenicol

BluoGal

IPTG

Cre-lox site-specific recombination and Tn7 transposition can be carried out simultaneously in DH10MultiBac^{Cre} cells if desired. Since the efficacy of a double transformation is reduced as compared to transformation with one plasmid only, significantly larger amounts of DNA have to be utilized in this reaction. Approximately 2-4 µg of the pFBDM derivative **and** 2-4 µg of the pUCDM derivative of choice are incubated on ice (15 min) with 50-100 µl electro-competent DH10MultiBac^{Cre} cells. Following electroporation

(200 ohms, 25 μF, 1.8 kV pulse), cells are incubated at 37 °C for 8 hours and plated on agar containing chloramphenicol (25 μg/ml), kanamycin (50 μg/ml), gentamycin (7 μg/ml), tetracyclin (10 μg/ml), BluoGal (100 μg/ml) and IPTG (40 μg/ml). White colonies are selected after incubation at 37 °C (18 - 24 hours). Proceed to bacmid preparation for insect cell infection (C.6.).

C.5. Preparation of *cre-lox* integrands for Tn7 transposition.

Reagents:

DH10MultiBac^{Cre} cells with integrated pUCDM derivative
Ampicillin
Kanamycin
Tetracyclin
Chloramphenicol
BluoGal
IPTG
2xTY medium
glycerol

For certain applications, it can be advantageous to add protein genes for expression to a MultiBac baculoviral DNA which already carries a set of foreign genes integrated by Cre catalysis. Then, the DH10MultiBac^{Cre} cells harboring recombinant MultiBac with an integrated pUCDM derivative (see C.2.) are restreaked on agar containing chloramphenicol (25 μg/ml), kanamycin (50 μg/ml), ampicillin (100 μg/ml), tetracyclin (10 μg/ml), BluoGal (100 μg/ml) and IPTG (40 μg/ml). A blue colony with a transposition-competent MultiBac derivative is then incubated in 500 ml 2xTY medium containing chloramphenicol (25 μg/ml), kanamycin (50 μg/ml), ampicillin (50 μg/ml), and tetracyclin (10 μg/ml) at 37 °C until OD_{600} reaches 0.5. The culture is then cooled on ice (15 min), and centrifuged (4000 rpm, 8 min). The cell pellet is resuspended in 250 ml ice-cold 10% glycerol solution (sterile) and centrifuged (4000 rpm, 8 min). The cell pellet is then resuspended in 200 ml ice-cold 10% glycerol solution (sterile) and centrifuged again (4000 rpm, 8 min). Then, the cell pellet is resuspended in 50 ml ice-cold 10% glycerol solution (sterile), and finally after centrifugation in 1 ml 10% glycerol solution (sterile). Cells are frozen in 50-100 μl aliquots in liquid nitrogen and stored at –80 °C. For transposition of pFBDM derivatives, proceed as described in C.3.

C.6. Bacmid preparation and infection of insect cells.

Preparation of bacmid DNA, infection of insect cells and protein expression is carried out according to established protocols (e.g. O'Reilly, D.R., Miller, L.K. & Luckow, V.A. "Baculovirus expression vectors. A laboratory manual." Oxford University Press, New York - Oxford, 1994; "Bac-to-Bac™ Baculoviorus Expression Systems Manual." Invitrogen, Life Technologies Incorporated, 2000).

D. The MultiBac System Kit

Reagents to be supplied in a MultiBac system kit:

DH10MultiBac^{Cre} cells
BW23474, BW23474 cells†
pFBDM vector
pUCDM vector
control plasmid for Tn7 transposition*
control plasmid for *cre-lox* site-specific recombination*
a generic transfectant reagent#

† *E. coli* strains expression the *pir* gene for propagation of pUCDM derivatives (any other strain with *pir*+ background can be used) .

* In our experiments, we used vectors carrying genes for fluorescent proteins ECFP and EYFP as controls. These genes are marketed under license by Molecular Probes. They are particularly useful as controls since the observation of fluorescence either by fluorescent microscopy or by using a fluorescence spectrophotometer is entirely straight forward. However, any type of control plasmid carrying a gene encoding for a protein that can be identified with ease (glucurodinase, catechol dioxygenase XylE, luciferase etc.) can be utilized.

For example CellFECTIN (Invitrogen), LipoTAXI® Transfection Reagent (Stratagene) etc.

E. Supplementary Information

E.1. Restriction endonuclease sites in pFBDM.

MCS1 of pFBDM

```
                              BamHI  RsrII   BssHII EcoRI
ATTCATACCGTCCCACCATCGGGCGCGGATCCCGGTCCGAAGCGCGCGGAATTCA
   StuI  SalI      SacI      NotI     BstBI XbaI
AAGGCCTACGTCGACGAGCTCACTAGTCGCGGCCGCTTTCGAATCTAGAGCCTGC
PstI     HindIII
AGTCTCGACAAGCTTGTCGAGAAGTACTAGAGGATCATAATCAGCCATACCACAT
```

MCS2 of pFBDM

```
                                          SmaI
                                    BbsI  XmaI    XhoI  NcoI
TTACATTTTATTTACAATCACTCGACGAAGACTTGATCACCCGGGATCTCGAGCCA
    NheI   PvuII   NsiI   SphI  KpnI
TGGTGCTAGCAGCTGATGCATAGCATGCGGTACCGGGAGATGGGGGAGGCTAACTG
```

Enzymes that cut pFBDM once

229	NaeI	4304	SphI	4487	ClaI
4695	SacI	1107	PvuI	4311	NsiI
4487	Bsp106I	4705	NotI	1253	FspI
4315	PvuII	4494	NruI	4714	BstBI
1955	AlwNI	4318	NheI	4647	BamHI
4719	XbaI	2809	MluNI	4325	NcoI
4654	RsrII	4731	PstI	2817	BstXI
4331	XhoI	4662	BssHII	4740	HindIII
2925	EcoRV	4339	XmaI	4669	EcoRI
4855	MfeI	3992	PmeI	4341	SmaI
4679	StuI	4868	HpaI	4298	KpnI
4345	BbsI	4685	SalI	5019	AvrII
4304	PaeI	4478	BstZ17I		

Enzymes that cut pFBDM twice

876	XmnI	2810	StyI/1	3901	XmnI
4755	ScaI	995	ScaI	3117	BglII
4480	SpeI	4856	BsmI	2647	BglII
3700	StyI/1	4697	SpeI	4949	BsmI
2810	StyI/2	3700	StyI/2		

Enzymes that do not cut pFBDM

AflII	AgeI	ApaI	BclI
BstEII	MluI	NarI	NdeI
SfiI	SnaBI		

E.2. Restriction endonuclease sites in pUCDM.

MCS1 of pUCDM

```
                                    BamHI  RsrII   BssHII
CCGGATTATTCATACCGTCCCACCATCGGGCGCGGATCCCGGTCCGAAGCGCGCGG
         StuI  SalI      SacI                    XbaI
AATTCAAAGGCCTACGTCGACGAGCTCACTAGTCGCGGCCGCTTTCGAATCTAGAG
     PstI
CCTGCAGTCTCGACAAGCTTGTCGAGAAGTACTAGAGGATCATAATCAGCCATACC
```

MCS2 of pUCDM

```
                                                        SmaI
                                                  BbsI  XmaI
ACTATACTGTAAATTACATTTTATTTACAATCACTCGACGAAGACTTGATCACCCG
                                        PaeI
    XhoI         NheI           NsiI   SphI
GGATCTCGAGCCATGGTGCTAGCAGCTGATGCATAGCATGCGGTACCGGGAGATGG
```

Enzymes that cut pUCDM once

358	SnaBI	792	SmaI	1105	RsrII
1306	MfeI	755	PaeI	796	BbsI
1113	BssHII	1319	HpaI	755	SphI
929	BstZ17I	1130	StuI	1551	StyI/2
762	NsiI	938	Bsp106I	1136	SalI
1551	StyI/1	769	NheI	938	ClaI
1146	SacI	2408	MluNI	782	XhoI
945	NruI	1170	XbaI	2745	AgeI
790	XmaI	1098	BamHI	1182	PstI
2870	AlwNI	453	PmeI	1581	AvrII

Enzymes that cut pUCDM twice

114	HindIII	776	NcoI	1156	EagI
2141	EcoRI	402	EagI	931	SpeI
1165	BstBI	2442	NcoI	402	NotI
1120	EcoRI	1191	HindIII	2558	ScaI
413	KpnI	1148	SpeI	1206	ScaI
2671	BstBI	749	KpnI	1156	NotI

Enzymes that do not cut pUCDM

AflII	ApaI	BclI	BglII
BstEII	BstXI	EcoRV	FspI
MluI	NaeI	NarI	NdeI
PvuI	SfiI	XmnI	

E.3. Generation of DH10MultiBac^{Cre} electro-competent cells.

Expression of Cre Recombinase protein and generation of electro-competent DH10MultiBac^{Cre} cells:

1. Electroporate pBADZ-His6Cre plasmid into DH10MultiBac cells (25 microFD, 2.0 kV, 200 Ohm).
2. grow in 2xTY medium for 4h at 37^{o}C.
3. Plate on LS plates (Low Salt Medium Agar) with antibiotics (Kan, Tet, Zeocin) and BluoGal/IPTG.
4. Grow a 500 ml LS (Low Salt) culture from one blue colony. Antibiotics: Kan/Tet/Zeo. Temp. 37^{o}C or RT.
5. Grow to OD_{600}=0.25 at 37^{o}C or RT.
6. Take 500 µl sample („Minus"-probe)
7. Add L-Arabinose to 0.1% (0.5 g in 500 ml).
8. Grow to OD_{600}=0.5.
9. Take 250 µl sample („ Plus"-probe)
10. Cool culture on ice for 15 min.
11. Resuspend in 500 ml ICE COLD STERILE 10% glycerol sol.
12. Centrifuge at 4000rpm, 4^{o}C, 15 min.
13. Resuspend in 250 ml ICE COLD STERILE 10% glycerol sol.
14. Centrifuge at 4000rpm, 4^{o}C, 15 min.
15. Resuspend in 10 ml ICE COLD STERILE 10% glycerol sol.
16. Centrifuge at 4000rpm, 4^{o}C, 15 min.
17. Resuspend in 1 ml ICE COLD STERILE 10% glycerol sol.
18. Prepare 80 µl aliquots (sterile Eppendorfs).
19. Shock-freeze in liq. nitrogen, store at –70^{o}C.

20. Centrifuge „Minus"-probe und „Plus" probe (14 krpm, 5 min, RT).
21. Resuspend in 150 ul PGLB. Analyze by 15% SDS-PAGE (load 5-10 ul)

A strong induced band at around 35 kDa shows presence of Cre protein. Strong Cre expression is mandatory for successful integration of pUCDM derivatives !

Low Salt Medium/Agar for Zeocin cultures:

LS culture medium (Low Salt):

10 g Tryptone

5g NaCl

5g Yeast Extract

Water (dd) to 950 ml

pH to 7.5 with 1N NaOH

Add water (dd) to 1L

For plates add 15g/L agar

Autoclave (liquid cycle)

Add Zeocin to 25 µg/ml below 55^{o}C (same for other antibiotics)

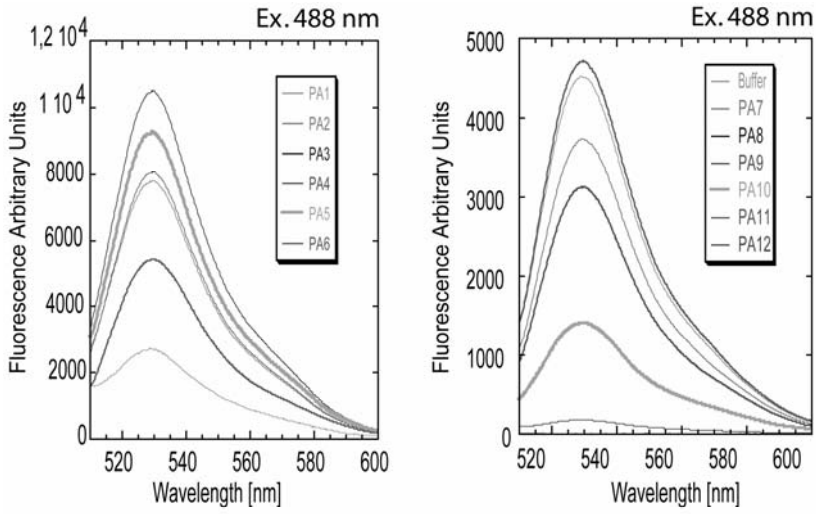
BlouGal: 100mg/ml stock (1000x)

IPTG: 0.5 M stock (1000x)

Store plates at 4^{o}C in the dark (BluoGal is light sensitive).

E.4. Efficiency of *cre-lox* site-specific recombination into MultiBac.

The propagation of MultiBac in DH10MultiBac^{Cre} cells is dependent on the presence of the F-replicon on the bacmid. A function of the F-replicon is the tight control of the copy-number (one or two), reducing the potential for undesired recombination. Introduction of pFBDM derivatives into MultiBac disrupts the *lacZ*α gene thus allowing for unambiguous identification of cells containing only composite bacmid. In case of Cre-catalyzed integration of pUCDM derivatives, however, a co-existence of one composite bacmid and one parent MultiBac molecule can not be ruled out based on chloramphenicol resistance. Virus from initial transfections with MultiBac containing a gene for yellow fluorescent protein EYFP inserted by Cre catalysis was therefore clonally separated by plaque purification. 29 of 32 (91 %) plaque purified specimens expressed EYFP, arguing for a *cre-lox* site-specific recombination reaction with close to saturating efficacy.



PA1 +	PA6 +	PA11 +	PA17 +	PA22 +	PA27 -	PA32 +
PA2 +	PA7 +	PA12 +	PA18 -	PA23 +	PA28 +	PA33 +
PA3 +	PA8 -	PA14 +	PA19 +	PA24 +	PA29 +	
PA4 +	PA9 +	PA15 +	PA20 +	PA25 +	PA30 +	
PA5 +	PA10 +	PA16 +	PA21 +	PA26 +	PA31 +	

Figure 6:. **Efficiency of *cre-lox* site-specific recombination in MultiBac.** The gene encoding for yellow fluorescent protein EYFP was cloned into pUCDM and integrated into MultiBac by Cre catalysis. Virus was clonally separated by plaque assay. 32 plaques were tested for EYFP expression by fluorescence spectroscopy. Excitation was at 488 nm. Spectra recorded from cell lysate from viruses 1 to 12 are shown (above). In this experiment, 91% (29 of 32) of the specimens showed strong fluorescence emission indicating expression of the EYFP protein (Table below).

E.5. Effect of *chiA* and *v-cath* deletion in MultiBac.

During heterologous protein production using a commercially available baculovirus expression system, we observed viral-dependent proteolytic breakdown consistent with the action of a cysteine protease. The baculoviral *v-cath* gene encodes for a cysteine protease which is directly involved in liquefaction of the insect cell host. V-CATH is activated upon cell death by a process dependent on a juxtaposed gene on the viral DNA, *chiA*, which encodes for a chitinase. We disrupted both genes to eliminate V-CATH activity and to gain the option of utilizing chitin-affinity chromatography for purification without interference from the *chiA* gene product. We assayed the effect of the disruption by comparing samples from cells infected with MultiBac virus to cells infected with a commercially available baculovirus carrying *v-cath* and *chiA*. We observed strongly reduced proteolytic activity in lysate from cells infected with MultiBac.

E.6. Production of a 275 kD protein complex using MultiBac.

In a test experiment, we expressed the yeast Isw2 chromatin remodeling complex consisting of the 130 kDa Isw2p and the 145 kDa Itc1p proteins. We created one version of MultiBac with both proteins integrated at the attTn7 site, and a second version with Isw2p integrated at the attTn7 site and Itc1p inserted at the LoxP site. Spatial decoupling of the two genes on the virus did not adversely affect either relative production levels of the subunits or functional assembly of the Isw2 complex in insect cells, exemplifying the utility of both Cre-lox and Tn7 sites for simultaneous integration and expression of proteins.

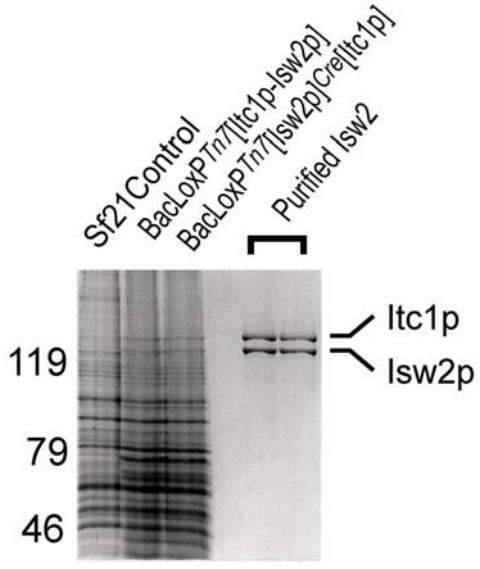


Figure 8: Expression of the 275 kDa chromatin remodeling complex Isw2 using MultiBac. Isw2 complex was expressed from the attTn7 site or, alternatively, from the attTn7 and LoxP sites of MultiBac (‘*BacLoxP’*). Cell lysate as well as protein complex purified from both composite bacmids exhibits virtually identical protein production levels. Sample from uninfected Sf21 cells is included as a control.