epermit_attachment

Attachment for e-permit application to import *Drosophila* carrying transgenic constructs lacking plant pest sequences

I. Purpose

The purpose of this attachment is to describe the sequence components present in the *Drosophila* strains covered by this e-permit application for a courtesy import permit.

Since most *Drosophila* transgenic constructs are based on a limited number of transformation vectors and relatively few species donate sequences to these constructs, it will be possible to describe all but a few constructs with this document. Users have been instructed to provide details in the e-permit application for any nonpest sequence components that are not covered by this attachment.

This attachment is not intended for use with standard permit applications for importing transgenic strains containing plant pest sequences or select agent sequences. Guidance for determining whether a courtesy permit or a standard permit is needed is provided at How do I know if my construct carries sequences from a plant pest?

II. Description of transgenic construct components covered by this permit application

A. Plant pest sequences

Transgenic *Drosophila* strains in this application contain no sequences from plant pests listed in the Code of Federal Regulations Title 7, chapter III, part 340 "Introduction of organisms and products altered or produced through genetic engineering which are plant pests or which there is reason to believe are plant pests" or classified more recently by USDA Plant Protection and Quarantine.

B. Transformation vectors

Transgenic constructs covered by this application were based on one of the commonly used vectors listed below. The specific sequence components present in each vector detailed in a downloadable spreadsheet and the components are described in the following sections. All hyperlinks lead to FlyBase entries where references and more complete descriptions may be obtained.

H{FLN}	P{3xP3-EGFP}	P{Car20}	P{CaSpeR-act(B)}
H{hawN}	P{3xP3-EYFPaf}	P{Car20-Act5C-FRT}	P{CaSpeR-act(R)}
Herm{3xP3-ECFP}	P{6.1}	P{Car20X}	P{CaSpeR-alphaTub84B}
Herm{3xP3-ECFPaf}	P{6.1C}	P{Car3}	P{CaSpeR-AUG-betagal}
Herm{3xP3-EGFPaf}	P{BLUEi}	P{Car30}	P{CaSpeR-hs/act}
Herm{3xP3-EYFPaf}	P{BSR}	P{Car30A}	P{CaSpeR-hs}
M{3xP3-ECFPaf}	P{BSRN}	P{Car30B}	P{CaSpeR-Hsp70/SV40}
M{3xP3-ECFPafm}	P{C20K}	P{Car3w}	P{CaSpeR-lacZ.III}
M{3xP3-EGFPaf}	P{C20NX}	P{Car4}	P{CaSpeRlacZ.M}
M{3xP3-EGFPafm}	P{C4-AUG-betagal}	P{Car4-ro}	P{CaSpeR-lacZ}
M{3xP3-EYFPaf}	P{C4betagal}	P{CaSpeR(NotI)}	P{CaSpeR-βgal}
M{3xP3-EYFPafm}	P{C4cat}	P{CaSpeR}	P{CaSpW18}
M{M789[mini-white]}	P{C4PLZ}	P{CaSpeR-2}	P{CGC.A}
M{M789}	P{Car1.1}	P{CaSpeR-3}	P{COG}
M{Mos1}	P{Car1}	P{CaSpeR-4}	P{cosPer}
Mi{Not}	P{Car2}	P{CaSpeR4-lo-}	P{CV}
P{3xP3-ECFPaf}	P{Car20.1}	P{CaSpeR4-lo+}	P{DM23}

P{DM24}
P{DM25}
P{DM26}
P{DM30}
P{DM30-hs}
P{DM79}
P{Express}
P{Express-elav}
P{Express-ey1x}
P{Express-ey3.5}
P{Express-ey4x}
P{Express-FRT.2.1}
P{Express-FRT.2.2}
P{Express-FRT.2.3}
P{Express-glass}
P{Express-sev1x}
P{Express-sev3x}
P{Express-UAS}
P{Express-vgM}
P{Express-vgMQ}
P{FlipG}
P{FRT(X97)}
P{FRT.G}
P{GalW}
P{GawB}
P{Germ70}
P{Germ80}
P{Germ90}
P{GMR}
P{GreenH-Pelican}
P{GREENi}
P{GreenPelican}
P{GS.ry[+]}
P{GS.ry[+]EGFP}
P{GS.ry[+]hs}
P{GS.ry[+]hsEBFP}
P{GS.ry[+]hsEGFP}
P{GS.ry[+]hslexA}
P{GS.ry[+]lexA}
P{GS.ry[+]UAS-EGFP}
P{GS.v[+]}
P{GS.v[+]EGFP}
P{GS.v[+]hsEGFP}
P{GS.v[+]hslexA}
P{GS.v[+]lexA}
P{GUS}
P{H-Pelican}
P{hs43-lacZ-Cahsneo}
P{HS85}
P{HSBJCaSpeR}
P{hsneo.ry[+]}
P{hsneo}
P{hsneo-act(B)}
P{hsneo-act(R)}
P{hsp(-44)lacZ}
P{Hsp70-CaSpeR}
P{HspCaSpeR1}
P{H-Stinger}
P{HT4}
P{HZ50}
P{HZ50PL}
P{HZCaSpeR}
P{HZR}
P{ICon}
P{lacH}
P{lacW}
P{lacW-DeltaR18}
P{lacW-DeltaR27}
P{lacW-DeltaR33}
P{lacW-DeltaR38}
P{MBO140}
P{MBO141}
P{neobeta-b}
P{neobeta-c}
P{neobeta-gal}
P{neoR.A}
P{neoR}
P{opd-A}
P{opd-B}
P{PA-1}
P{PA-2}
P{PCW}
P{Pelican}
P{PFHW}
P{PFMW}
P{PFW}
P{PGW}
P{PHW}
P{PMW}
P{PRW}
P{PTGAL}
P{pUCPlac-1}
P{pUCPlac-11}
P{pUCPlac-12}
P{pUCPlac-13}
P{pUCPlac-1B}
P{pUCPlac-2}
P{pUCPlac-2B}
P{pUCPlac-3}
P{pUCPlac-3B}
P{PVW}
P{PWC}
P{PWF}
P{PWG}
P{PWH}
P{PWM}
P{pwnbetaE}
P{PWR}
P{PWV}
P{RDC4}
P{RedH-Pelican}
P{RedH-Stinger}
P{RHT}
P{RIC4}
P{RMC4}
P{RNC4}
P{RRC4}
P{smart2}
P{SSRN}
P{Stinger}
P{Switch1}
P{Switch2}
P{SXhLac-7}
P{TCW}
P{TFHW}
P{TFW}
P{TGW}
P{THW}
P{TMW}
P{TRW}
P{TVW}
P{TWC}
P{TWF}
P{TWG}
P{TWH}
P{TWM}
P{TWR}
P{TWV]
P{UASp}
P{UAST}
P{Ubi-CaSpeR}
P{UdsGFP}
P{V10}
P{V11}
P{W5}
P{W5-hsp70-lacZ}
P{W5N}
P{W6}
P{W7}
P{W8}
P{W8H}
P{W8HL}
P{W-ATG-lac1}
P{W-ATG-lac2}
P{wbeta-gal}
P{wFl}
P{WIZ}
P{wlo+GS}
P{wlo+hsGS}
P{wlo+hsinGS}
P{wlo+inGS}
P{wlo-GS}
P{wlo-hsGS}
P{wlo-hsinGS}
P{wlo-inGS}
P{WTP}
P{WUM}
P{WUM2}
P{WUM-B}
P{YC1.8}
P{YELLi}
P{YellowH-Pelican}
P{YES}
P{YTP}

1. *Transposable element end sequences*

Transposable element system	Origin
Hermes	*Musca domestica*
hobo	*D. melanogaster*
mariner	*Drosophila mauritiana*

Minos	*Drosophila hydei*
P	*D. melanogaster*

2. Marker genes

Gene	Description	Origin
Adh	Alcohol dehydrogenase	*D. melanogaster*
amp^R	beta-lactamase	*E. coli*
lacZ	beta-galactosidase	*E. coli*
neo^R	Neomycin resistance	*E. coli*
opd	Organophosphate insecticide resistance	*Flavobacterium sp.*
rosy	eye color	*D. melanogaster*
rough	eye facet organization	*D. melanogaster*
vermilion	eye color	*D. melanogaster*
white	eye color	*D. melanogaster*
yellow	body color	*D. melanogaster*

3. Polylinker sequences

These sequence elements are engineered and did not originate in an organism. A large variety of different polylinkers are present in *Drosophila* vectors.

4. Promoter/Enhancer sequences

Gene	Description	Origin
actin	Ubiquitously-expressed gene	*D. melanogaster*
alpha Tubulin84B	Ubiquitously-expressed gene	*D. melanogaster*
elav	Nerve-specific gene	*D. melanogaster*
ey	Eye-specific gene	*D. melanogaster*
gl	Eye-specific gene	*D. melanogaster*
Hsp26	Heat shock protein	*D. melanogaster*
Hsp27	Heat shock protein	*D. melanogaster*
Hsp70	Heat shock protein	*D. melanogaster*
Hsp83	Heat shock protein	*D. melanogaster*
ninaE	Eye-specific gene	*D. melanogaster*
otu	Ovary-specific gene	*D. melanogaster*
Pax6	Eye-specific gene	Human
Scr	Developmentally regulated gene	*D. melanogaster*
sev	Eye-specific gene	*D. melanogaster*
Sgs3	Salivary gland-specific gene	*D. melanogaster*
SP6	Promoter for bacterial polymerase	*E. coli*
SV40	General eukaryotic promoter	*Simian virus 40*
T7	Promoter for bacterial polymerase	*E. coli*
UAS	Binding site for GAL4 activator protein	*Saccharomyces cerevisiae*
ubiquitin	Ubiquitously-expressed gene	*D. melanogaster*
Ubx	Developmentally regulated gene	*D. melanogaster*
vg	Wing-specific gene	*D. melanogaster*

5. Transcription termination and polyadenylation sequences

Gene	Description	Origin
Actin	Ubiquitously-expressed gene	*D. melanogaster*
alpha tubulin	Ubiquitously-expressed gene	*D. melanogaster*
fs(1)K10	Ovary-specific gene	*D. melanogaster*
Hsp70	Heat shock protein	*D. melanogaster*
SV40	General eukaryotic terminator	*Simian virus 40*

6. Protein binding sites

Site	Description	Origin
attB	Binding site for *phiC31* integrase (converted to attL site by action of integrase)	*Streptomyces*
FRT	Binding site for FLP recombinase	*Saccharomyces cerevisiae*
loxP	Binding site for cre recombinase	*E. coli*
su(Hw)	Insulator sequence	*D. melanogaster*
tetO	Binding site for tetracycline regulator (tetR) protein	*E. coli*

7. Epitope tags and fusion proteins

Gene	Description	Origin
CAT	Chloramphenicol O-acetyltransferase	*E. coli*
DsRed	Fluorescent protein	*Discosoma sp.*
FLAG	Epitope	Not from an organism
GFP and spectral variants	Fluorescent protein	*Aequorea victoria*
HA	Hemagglutin	*Influenza virus*
lacZ	beta-galactosidase	*E. coli*
lexA DNA binding domain	Transcriptional activation domain	*E. coli*
Myc	Myc oncogene	Human
Progesterone receptor ligand binding domain	Hormone receptor	Human
RELA transcriptional activation domain	transcriptional activation domain	Human
SV40 nls	nuclear localization sequence	*Simian virus 40*
tra nls	nuclear localization sequence	*D. melanogaster*
VP16	transcriptional activation domain	*Herpes simplex*

8. Protein-coding genes

Gene	Description	Origin
ccdB	Gene necessary for selection in Gateway cloning system. Present in vectors, but eliminated upon cloning of unique sequences.	*E. coli*
Dt-A	Temperature-sensitive variant of diphtheria toxin A chain	*Corynephage beta*
FLP	FLP recombinase	*Saccharomyces cerevisiae*
GAL4	Transcriptional activator	*Saccharomyces cerevisiae*
tetR	Tetracycline operator protein. Transcriptional regulator	*E. coli*

9. Unidentified sequence components

To the best of our knowledge, all the sequence components present in the listed transformation vectors have been identified. Nevertheless, some vectors may contain additional *E. coli*, *Saccharomyces cerevisiae* or *Drosophila melanogaster* DNA fragments leftover from progenitor plasmids during cloning manipulations.

C. Donor sequences

In the transgenic strains covered by this application, all sequences cloned into the standard transformation vectors derive from one of the nonpest species listed below or are one of the sequences listed in the table of assorted sequences below. Sequences from other nonpest organisms will be described in detail in the e-permit application.

1. Donor species

a. Flies of the Genus Drosophila

Sequences donated by any fly of the genus *Drosophila* may be present in transgenic constructs covered by this application. Users must provide details for sequences originating in flies outside this genus, because BRS evaluates these species on a case-by-case basis. One fly in the Family Drosophilidae, *Zaprionus indianus*, is a

recognized pest of U.S. fruit crops and constructs containing sequences from this organism require a standard permit.

b. Vertebrate species.

No vertebrates are classified as plant pests, so *Drosophila* constructs with vertebrate sequences may be imported under the courtesy permit mechanism. Nevertheless, BRS wishes to have vertebrate donors identified in the e-permit application for all species except the following commonly studied organisms:

Danio rerio (zebrafish)
Gallus gallus (chicken)
Homo sapiens (Human)
Mus musculus (mouse)
Rattus norvegicus (rat)
Xenopus laevis (African clawed toad)

c. Arthropod species

Aedes aegypti (mosquito)
Anopheles gambiae (mosquito)
Apis mellifera (honeybee)
Chironomus tentans (midge)
Musca domestica (housefly)

d. Other invertebrate species

Caenorhabditis elegans (roundworm)
Chlamydomonas reinhardtii
Helobdella robusta (leech)
Hirudo medicinalis (leech)
Hydra vulgaris (hydra)

e. Plant species

Arabidopsis thaliana (cress)
Oryza sativa (Rice)
Zea mays (corn)

f. Fungal species

Coprinus cinereus (ink cap mushroom)
Neurospora crassa (bread mold)
Saccharomyces cerevisiae (budding yeast)
Schizosaccharomyces pombe (fission yeast)

g. Bacterial species

Bacillus subtilis
Escherischia coli

h. Viruses

Bacteriophages of *E.coli* (*e.g. lambda, M13, P1, T7*)
Baculovirus
Herpes simplex
Simian Virus 40

2. Assorted sequences from other organisms and engineered epitope tags

Most sequences encoding proteins to be fused to a protein of interest or encoding short peptide epitope tags were donated by one of the donor species listed above or were engineered *in vitro*. The table below lists epitope tags and fusion proteins donated by other species that have been used in *Drosophila* constructs in the past and may be present in transgenic constructs covered by this application. The table also lists engineered tags that may be present. Other epitope tags not originally isolated from an organism may also be present.

Name	Description	Origin
1D4	Epitope tag ASKTETSQVAPA from rhodopson	*Bos taurus*
CAG	Polyglutamine (CAG) repeat	Not from an organism
CYP2C1	Transmembrane domain that anchors proteins to the endoplasmic reticulum	*Oryctolagus cuniculus* (rabbit)
DsRed	Red fluorescent protein.	*Discosoma sp.*
DT-A	Diphtheria toxin A chain	*Corynephage β*
E1a	Early gene 1a. Induces cell death	*Human adenovirus type 5*
FLAG	epitope tag DYKDDDDK	Not from an organism
GFP and spectral variants	Green Fluorescent Protein	*Aequorea victoria*

GST	Glutathione-S-transferase	*Schistosoma japonicum*
HA1	Hemagglutin 1 epitope tag (M)YPYDVPDYA(SS)	*Influenza virus*
His6	Epitope tag. Six Histidine residues.	Not from an organism
HPC4	Epitope tag. EDQVDPRLIDGK.	Not from an organism
HRP	Horseradish peroxidase	*Armoracia rusticana*
LUC	Luciferase	*Photinus pyralis* (firefly)
LUC	Luciferase	*Renilla reniformis* (sea pansy)
LUC	Luciferase	*Photuris pennsylvanica* (firefly)
MAPT	Microtubule-associated protein tau	*Bos taurus*
nes	Nuclear export sequence from cAMP-dependent protein kinase inhibitor	*Oryctolagus cuniculus* (rabbit)
nls1	Nuclear localisation signal KKKRKV.	Not from an organism
nls4	Nuclear localisation signal	*Polyoma virus*
opd	Organophosphate insecticide resistance	*Flavobacterium sp.*
Pym	Polyoma DNA fragment	*Polyoma virus*
SIP1	Epitope tag from Seed inhibition protein 1	*Hordeum vulgare*
V5	Epitope tag GKPIPNPLLGLDST from P/V proteins	*Simian virus 5*
Xpress	Epitope tag DLYDDDDK	Not from an organism

3. Common laboratory pest species

The following species that are commonly used in laboratory research are classified as pest species by the USDA. Transgenic constructs incorporating sequences from these species cannot be imported under the courtesy permit mechanism; consequently, they are not covered by this attachment.

Antheraea pernyi (Silkmoth)
Bombyx mori (Silkmoth)
Ceratitis capitata (Medfly)
Heliothis virescens (Tobacco budworm)
Manduca sexta (Tobacco hornworm)
Oncopeltus fasciatus (Milkweed bug)
Sciara coprophila (Fungus fly)
Tenebrio molitor (Meal worm)
Tribolium castaneum (Flour beetle)
Trichoplusia ni (Cabbage looper moth)

Note that sequences from animal pathogens such as *Clostridium tetani* are also not covered by this attachment.

III. Other information for users

This document was assembled by Kevin Cook, Ph.D. of the Bloomington *Drosophila* Stock Center. He welcomes suggestions for future improvements. This version of this attachment was reviewed by Biotechnology Regulatory Services in August 2007. This document must not be altered by users. Any comments or clarifications should be included elsewhere in the e-permit application.