Information about *Pb*GEM gene targeting vectors

Working with *Pb*GEM vectors:

- If sent as agar stabs, inoculate liquid cultures and make master glycerol stocks.
- Re-inoculate cultures for DNA prep from master glycerol stocks, avoid serial culturing.
- Verify your vectors by PCR and / or restriction digest prior to transfection.
- Prepare your vector for transfection by NotI restriction digest.
- Genotype your transgenic lines thoroughly.

Vector information:

- *Pb*GEM vectors are genetically modified clones from a *P. berghei* ANKA genomic DNA library (PbG) that was constructed in the pJAZZ®-OK NotI vector from Lucigen (Godiska et *al.*, 2010).
- The pJAZZ vector was derived from bacteriophage N15. It is a linear, low copy vector (~5 copies per cell) with hairpin telomers that requires the telN telomerase encoded on the plasmid to replicate in *E. coli*.
- The PbG genomic library clones are converted into a gene targeting vectors using a combination of recombineering and Gateway technology (Pfander et *al.* 2011), resulting in the introduction of the *P. berghei* selective marker *hdhfr/yfcu* (Braks et *al*. 2006).
- *Pb*GEM vector propagation is restricted to TSA™ cells and is selected for by kanamycin in *Ecoli.* For re-transformation of DNA use BigEasy® TSA™ Electrocompetent Cells (Lucigen).
- A NotI mediated restriction digest releases the 10 kb and 2 kb constant arms of the pJAZZ® vector backbone, as well as an additional band of variable size as determined by the clone insert-size.

Growth conditions and vector preparation:

Recommendations

- The vector copy number can be increased by 5-10 X by L-arabinose induction. However, **we recommend <u>not</u> growing the vectors in the presence of L-arabinose** since maintaining a low copy number is important for vector sequence stability.

- All *Plasmo*GEM vectors are clonal and sequence-verified prior to dispatch. However, please be aware that **serial culturing of *E.coli* harboring large fragments of low-complexity, AT-rich DNA is <u>not</u> recommended,** since this may induce sequence instability. For the same reason it is **<u>not</u> recommended to clonally re-select constructs** by streaking out cultures and picking single colonies. This may inadvertently select for a rare clone having acquired mutation post-sequencing.

- In our lab we make one master glycerol stock for each construct and re-inoculate from this stock for all subsequent DNA preparations.

1 x TB Medium (per litre)

11.8 g Bacto-tryptone
23.6 g yeast extract
9.4 g dipotassium hydrogen phosphate (anhydrous)
2.2 g potassium dihydrogen phosphate (anhydrous)

Autoclave

Add 0.4% glycerol after autoclaving.
Supplement with Kanamycin (10 mg/mL stock) to a final concentration of 30 µg/ml.

Alternatively, use Invitrogen Terrific Broth 2211-022. Dissolve 47g powder in 1L dH20 and add 4ml glycerol before autoclaving.

DNA preparation

***Pb*GEM is a low copy vector (~5 copies / cell).** If a large quantity of DNA is required for transfection, the Qiagen MAXI Prep Kit can be used by following the manufacturer's recommendations for low copy plasmids, adjusting inoculum for using the nutrient-rich TB medium:

- *Inoculate a 2-3 mL starter culture (supplemented TB medium as above) from agar stabs.*
- *Grow at 37°C, shaking over-night.*

- *The following afternoon, inoculate 250 mL supplemented TB medium with 100 µL (1/2500*) of your starter culture and *grow at 37°C, shaking over-night (12-16 hours) prior to MAXI prep purification.*

If a smaller amount of DNA is required, MIDI preps from >50mL cultures yield sufficient material.

Quality controls for *Plasmo*GEM vectors

All *Plasmo*GEM vectors are subjected to PCR and sequenced-based quality control prior to dispatch. Nevertheless, following vector prep, prior to transfection **we recommended that a PCR is run to check for the presence of the modified target locus** in your vector by using **QCR2 and GW2 primers** (primer sequences are available for each construct from http://plasmogem.sanger.ac.uk).

Alternatively or complementary:

Perform a small scale diagnostic **NotI digest** under standard restriction digest conditions, which should produce the following restriction digest pattern during gel electrophoresis:

2 kb	Constant, vector short arm
10 kb	Constant, vector long arm
X kb	Variable, dependent on insert size

PCR master mix (per reaction):

H2O	9.5µl
Template	1µl
2xGoTag Green	12.5µl
Primers (2µM) each	1µl
DNA template	1µl

PCR conditions:
95°C 5’ // 95°C 30’’ / 50°C 30’’ / 68°C 1’ (x30) // 68°C 10’ // 4°C hold

PbGEM vector *P. berghei* transfections

Before *P. berghei* transfection, the vector DNA must be prepared by a NotI digest followed by standard ethanol precipitation. This releases the *P. berghei* insert from its vector backbone and makes it ready for parasite transfection. There is no need to purify the insert away from the 2 and 10 kb constant arms of the vector before transfection.

Please note that due to the increased transfection efficiency often observed with the long-homology arm *Pb*GEM targeting vectors, **it is advisable to check the parasitaemia of your host mice already at day 7 post-transfection.**

Sequence files are available for each construct from http://plasmogem.sanger.ac.uk. These can be downloaded in form of .embl or .gbk files containing feature and sequence information.

As a general guide:

A. *Pb*G-XXX indicates library clone number, which carries the modified gene of interest.
B. Flanking genomic sequence is given to allow design of post-transfection genotyping primers (an additional 1 kb, up- and downstream of the *Pb*G genomic clone, not carried by the clone itself).
C. Primer information: Gene specific QCR2 and genotyping (GT) primers, as well as the generic GW2 primer, are indicated on the maps.

P.berghei genotyping

Integration: For genotyping by long-range PCR use the gene specific genotyping (**GT**) primer (annealing up- or downstream of clone homology region) together with the generic primer **GW2 or GW1**, depending on positioning of the GT primer annealing site as shown in the annotated sequence files.

The transfection vector can be detected using **QCR2 and GW2 primers.** The **QCR2-GW2** product in the majority of cases is expected to be 400-800 bp.

WT locus: To detect the *wt* locus, use primers **QCR1** and **QCR2**. Please note that in the majority of cases QCR1 is not annotated in your sequence files since it anneals within the deleted / modified region of your target gene. The QCR1-QCR2 product in the majority of cases is expected to be 400-800 bp.

PCR based conclusions regarding vector integration should always be followed up by complementary genotyping methods such as Southern hybridisation of restricted genomic DNA or of whole chromosomes separated by pulsed field gel electrophoresis.

Generic primer sequences

GW2: 5'-ctttggtgacagatactac-3'
GW1: 5'-catactagccattttatgtg-3'