Generating Stable Transfection in Bodo saltans Version 2

Fatma Gomaa,Zuhong Li,Roberto Docampo,Peter Girguis,Virginia Edgcomb

Abstract

- *B. saltans* cells were electroporated using a square-wave electroporator (Nepa21, Bulldog Bio, Inc.) using one poring pulse of 200 volts with a pulse duration of 25 ms and five transfer pulses of 60 volts with a pulse duration of 99 ms, with plasmid targeting the 18S region (18S-GFP). A schematic representation of the plasmid, the target locus and the expected site of integration into the *B. saltans* genome is shown in Figure 1.

- Electroporated cells were selected with 1 µg/ml of G418, added 24 hours after electroporation. Cells were washed and subcultured into fresh selection medium every 3-4 days. G418 resistant cells started to emerge 7-9 days post-electroporation.

- Cells were processed for genotyping analysis to confirm plasmid integration 3 weeks post-electroporation. DNA was extracted from pools of transfected and wild cells using the Qiagen DNeasy Blood & Tissue kit.

- PCR analyses were used to characterize the 18S-GFP tagging using 6 sets of PCR primers, as shown in Figure 1 C.

- Gel electrophoresis image (Figure 2) showing the amplified PCR products at the expected sizes.

- Amplified PCR#1 with primer sets Ribo_tag_forward & GFP reverse (800 bp)
- Amplified PCR #2 with primer sets Neo_forward & Ribo_tag_reverse (1000 bp)
- Amplified PCR #3 with primer sets TubR & IG forward (3 bands)
- Amplified PCR #4 with primer sets Tub_forward & IG reverse (3 bands)
- Amplified PCR #5 with primer sets Ribo_tag_forward & Ribo_tag_reverse (Wild (C) cells band at 350 bp, transfected cells S1 and S2 two bands, 350 bp and 2800 bp)

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Figure 1: Schematic representation of the (A); the 18S- GFP plasmid; (B) Ribosomal operon in *B. saltans* genome (C); and the expected site of plasmid integration in *B. saltans* genome through homologous regions 1 and 2 (HR1, HR2).

Figure 2: Agarose gel electrophoresis image of the amplified PCR products for *B. saltans* cells transfected with 18S-GFP Cassette (S1 and S2) and the wild type cells (C). The primers sets and the expected product sizes are mentioned above in the text.

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Figure 3: *B. saltans* cells transfected with 18S-GFP tagging cassette. On the left: a light microscopy image showing the *B. saltans* cells (around 4 cells at different level). On the right: GFP fluorescent signal detected in the transfected cell. Scale bar 10 µm.

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Protocol

Step 1.

saltans cells were electroporated using a square-wave electroporator (Nepa21, Bulldog Bio, Inc.) using one poring pulse of 200 volts with a pulse duration of 25 ms and five transfer pulses of 60 volts with a pulse duration of 99 ms, with plasmid targeting the 18S region (18S-GFP). A schematic representation of the plasmid, the target locus and the expected site of integration into the *B. saltans* genome is shown in Figure 1.

Step 2.

Electroporated cells were selected with 1 µg/ml of G418, added 24 hours after electroporation. Cells were washed and subcultured into fresh selection medium every 3-4 days. G418 resistant cells started to emerge 7-9 days post-electroporation.

Step 3.

Cells were processed for genotyping analysis to confirm plasmid integration 3 weeks post-electroporation. DNA was extracted from pools of transfected and wild cells using the Qiagen DNeasy Blood & Tissue kit.

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Step 4.
PCR analyses were used to characterize the 18S-GFP tagging using 6 sets of PCR primers, as shown in Figure 1 C.

Step 5.
Gel electrophoresis image (Figure 2) showing the amplified PCR products at the expected sizes.

Step 6.
Amplified PCR#1 with primer sets Ribo_tag_forward & GFP reverse (800 bp)

Step 7.
Amplified PCR #2 with primer sets Neo_forward & Ribo_tag_reverse (1000 bp)

Step 8.
Amplified PCR #3 with primer sets TubR & IG forward (3 bands)

Step 9.
Amplified PCR #4 with primer sets Tub_forward & IG reverse (3 bands)

Step 10.
Amplified PCR #5 with primer sets Ribo_tag_forward & Ribo_tag_reverse (Wild (C) cells band at 350 bp, transfected cells S1 and S2 two bands, 350 bp and 2800 bp)

Step 11.

Figure 1: Schematic representation of the (A); the 18S- GFP plasmid; (B) Ribosomal operon in *B. saltans* genome (C); and the expected site of plasmid integration in *B. saltans* genome through homologous regions 1 and 2 (HR1, HR2)

Step 12.

Figure 2: Agarose gel electrophoresis image of the amplified PCR products for *B. saltans* cells transfected with 18S-GFP Cassette (S1 and S2) and the wild type cells (C). The primers sets and the expected product sizes are mentioned above in the text.

Step 13.

Figure 3: *B. saltans* cells transfected with 18S-GFP tagging cassette. On the left: a light microscopy image showing the *B. saltans* cells (around 4 cells at different level). On the right: GFP fluorescent signal detected in the transfected cell. Scale bar 10 µm.

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