Resource Summary Report

Generated by dkNET on Sep 8, 2026

Generating Stable Transfection in Bodo saltans

DOI:10.17504/protocols.io.sh3eb8n
Type: Protocol

Proper Citation

Fatma Gomaa, Zuhong Li, Roberto Docampo, Peter Girguis, Virginia Edgcomb 2018. Generating Stable Transfection in Bodo saltans. protocols.io dx.doi.org/10.17504/protocols.io.sh3eb8n

Protocol Information

URL: https://dx.doi.org/10.17504/protocols.io.sh3eb8n

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Group: Protist Research to Optimize Tools in Genetics (PROT-G)

Summary: 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)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

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Version: 1

Publication Date: 2018

Proper Citation: Fatma Gomaa, Zuhong Li, Roberto Docampo, Peter Girguis, Virginia Edgcomb 2018. Generating Stable Transfection in Bodo saltans. protocols.io dx.doi.org/10.17504/protocols.io.sh3eb8n

Record Creation Time: 20210329T220531+0000

Record Last Update: 20210329T220955+0000

Data and Source Information

Source: