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CRISPR knock-in validation V.1

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We use this protocol and it's working

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Abstract

CRISPR knock-in validation

Troubleshooting

1

Cultured hippocampal neurons tagged with oScarlet were prepared with Lucigen QuickExtract DNA extraction solution (Biosearch Technologies, cat# QE09050). Briefly, neurons were lysed by adding 50 ul of QuickExtract solution to each sample.

2 The samples were mixed by pipetting and incubated at 68 °C for 15 min followed by 95 °C for 10 min in a thermocycler before being stored at -20 °C for downstream analysis.

3 Two different PCR reactions were performed to amplify DNA products of ~500 bp corresponding to the 5′ and 3′ integration junctions. PCR#1 used the α-syn forward primer: TGTGCTTTCTCTTCCCTCTCTG and the reverse oScarlet primer CCGTCCTCGAAGTTCATCAC, whereas PCR#2 used the α-syn forward primer:
ATAACACTTCGTGCAGCACC and the reverse oScarlet primer ACAGGATGTCCCAGGAGAAG.

4 PCR products were extracted from the agarose gel using the Monarch DNA gel extraction kit (New England Biolabs, cat# T1020S), and samples were submitted for sequencing for analysis at MCLAB.