NOV 12, 2023

OPEN ACCESS

CRISPR knock-in validation

Leonardo A Parra-Rivas[1]

[1]University of California, San Diego

Subhojit Roy Lab

Leonardo A Parra-Rivas
University of California, San Diego

ABSTRACT

CRISPR knock-in validation

DOI:
dx.doi.org/10.17504/protocols.io.n2bvj3qb5lk5/v1

Protocol Citation: Leonardo A Parra-Rivas 2023. CRISPR knock-in validation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvj3qb5lk5/v1

Protocol status: Working
We use this protocol and it's working

Created: Nov 12, 2023

Last Modified: Nov 12, 2023

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.