Supplementary materials

Challenges in visualizing endogenous *loci* in the human genome using CRISPR imaging systems

Materials and methods

Materials

Cells

For experiments, the HEK293T cell line with 41 CAG repeats on both alleles of the *HTT* gene was used (Dabrowska et al. 2020).
For lentivirus production HEK293TN cell line was used.

Plasmids

encoding dCas9-KRAB	pHR-EF1a-dCas9-HA-BFP-KRAB-NLS (#102244)
encoding MCP-YFP	UbC NLS-HA-MCP-YFP (#31230)
encoding sgRNA with MS2 sites	sg2.02 16x(MS2) MUC4.1 (#101154)
encoding sgRNA for CD47 assay	pCRISPRia-v2 (#84832)
plasmids for lentiviral production	pCMV-dR8.2 dvpr (#8455); pCMV-VSV-G (#8454)

Protospacer sequence targeting CD47 was taken from hCRISPRi-v2 library (Addgene ID #83969; http://n2t.net/addgene:83969; RRID:Addgene_83969)

Methods

Plasmids encoding sgRNAs with 16 MS2 sites

sgRNAs targeting flanking regions of the CAG tract in the *HTT* gene (sgRNA_16xMS) were designed using CRISPOR software (Concordet and Haeussler 2018).
The oligonucleotides used for sgRNA assembly were synthesized by the Laboratory of DNA Sequencing and Synthesis (Institute of Biochemistry and Biophysics, PAS, Warsaw). Assembled oligonucleotides were cloned into the sg2.02 16x(MS2) MUC4.1 plasmid and propagated in transformed DH5α bacteria. To verify correct cloning, plasmids isolated from bacterial clones were sequenced using the Sanger method at the Molecular Biology Techniques Laboratory (Adam Mickiewicz University Poznan).

Lentivirus production and transduction

For lentivirus production, HEK293TN cells were co-transfected with the plasmids: pCMV-dR8.2 dvpr and pCMV-VSV-G. Culture medium was collected at days 2 and 3, and the viral supernatant was filtered through 0.45 μm filters and concentrated using PEGit Virus Precipitation Solution (System Biosciences). The concentrated lentiviral particles were resuspended in Opti-MEM (GIBCO). Transduction of HEK293T cells was performed in the presence of polybrene (4 μg/mL).

Cell transduction with the lentiviral cocktail

The concentration of each lentivirus encoding sgRNA_16xMS was determined in a separate transduction experiment, based on BFP expression measured by flow cytometry.

MOI of each lentivirus encoding MOI was determined by flow cytometry. Lentiviral cocktails with equimolar amounts of each virus were prepared at an MOI of 10 for subsequent transduction.

Plasmid transfection

HEK293T cells (3 x 10^5) were seeded on 20 mm glass coverslips coated with 1% Geltrex in 6-well plates for transfection on the following day. Three consecutive co-transfections, each involving six plasmids encoding sgRNAs_16xMS, were performed using PEI. Each plasmid was used at a concentration of 0.5 μg per transfection.

Cell sorting and flow cytometry analysis

Cells were sorted on a FACSAria Fusion cell sorter (Becton Dickinson) and flow cytometry analyses were performed on a Guava easyCyte 12HT flow cytometer (Cytek Biosciences) at the Laboratory of Single Cell Analyses (Institute of Bioorganic Chemistry, PAS, Poznan).

CD47 assay

HEK293T clones propagated after transduction with lentivirus encoding dCas9-KRAB and subsequently sorted based on BFP expression were seeded (6 x 10^4 per well) for transduction on the following day with lentivirus encoding an sgRNA targeting the *CD47* gene, in the presence of polybrene (4 μg/mL). Cells were subjected to puromycin selection 48 h post-transduction and maintained under selection for four days. Seven days after transduction, cells were harvested and resuspended in anti-CD47 antibody (B6H12; Invitrogen) diluted in PBS (1:200), followed by incubation at 4 °C for 20 min in the dark. Samples were then centrifuged, washed three times with FACS buffer, and acquired on a flow cytometer. Clones in which CD47 expression decreased to below 10% relative to the unsilenced control sample were selected for further experiments.

Cell fixation

Cells were fixed in 4% paraformaldehyde and permeabilized with 0.5% Triton-X-100 in PBS for 10 min, followed by a 5 min wash with PBS. The coverslips were then mounted on glass slides using SlowFade Diamond Antifade Mountant with DAPI (Life Technologies).

Microscopic analysis

Micrographs were taken using a Leica TCS SP5 II confocal microscope (63x 1.4 objective, oil immersion). Laser line 514 with ~15% of the total laser power. The study utilized a standard PMT detector, which is included in the microscope equipment. All images were captured under the same parameters during a single session. Based on the observed background fluorescence, the detection threshold for YFP was set to 50 arbitrary units to visualize the number of extraneous fluorescence peaks in the micrographs. From each sample, we obtained three micrographs on which we marked three linear profiles. Line profiling and measurement of fluorescence intensity were performed in Leica Application Suite X version 3.4.1.18368 and plotted in Graphpad Prism version 9.0

References

Concordet J-P, Haeussler M. 2018. CRISPOR: intuitive guide selection for CRISPR/Cas9 genome editing experiments and screens. Nucleic Acids Research. 46(W1):W242–W245. https://doi.org/10.1093/nar/gky354

Dabrowska M et al. 2020. Generation of New Isogenic Models of Huntington's Disease Using CRISPR-Cas9 Technology. International Journal of Molecular Sciences. 21(5):1854. https://doi.org/10.3390/ijms21051854