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# DNA imaging with CRISPRdelight labeling system

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**We use this protocol and it's working**

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## Disclaimer



## Abstract

Tracking the dynamics of genomic loci is very important to know genome organization and gene function regulation. However, tools for imaging genomes, especially non-repetitive locus have still been limited. This protocol uses endonuclease-dead, programmable RNA-guided DNA-targeting Cas12 DNases (d)Cas13 proteins fused with fluorescent proteins to visualize and track DNA dynamics in live cells. This protocol details several aspects of the procedure, including gRNA design, CRISPR array construction and imaging.

## Materials

### Reagents

Cell culture
1. Modified Eagle's Medium (DMEM, Thermo Fisher Scientific, cat. no. 11965118)
2. FBS (Thermo Fisher Scientific, cat. no. 10099141)
3. Trypsin 0.25% EDTA (Thermo Fisher Scientific, cat. no. 25200072)
4. DPBS (Thermo Fisher Scientific, cat. no. 14190136)
5. FluoroBrite DMEM (Thermo Fisher Scientific, cat. no. A1896701)

Cell line
1. HeLa (ATCC, cat. no. CCL-2)

Molecular cloning

1. T4 DNA ligase (NEB, cat. no. M0202L)
2. Hieff Clone™ One Step Cloning Kit (Yeasen, cat. no. 10905ES25)
3. Ampicillin (Sangon Biotech, cat. no. A100339-0025)
4. Agar (Sangon Biotech, cat. no. A505255)
5. NucleoBond Xtra Midi kit (MACHEREY-NAGEL, cat. no. 740410.5)
6. Trans2K Plus II DNA marker (TransGen Biotech, cat. no. BM121-02)
7. Trans1-T1 chemically competent cell (Transgene, cat. no. CD501-02)

Plasmid

1. pHAEG-IRES-puro-NLS-HyperdLbCas12a-StayGold-NLS ()
2. pHAGE-CAG-CRISPR array ()

Transfection

Lipofectamine 3000 transfection reagent (Thermo Fisher Scientific, cat. no. L3000015)

General molecular biology reagents including KCl, Tris, NaCl, etc.

### Equipments

Tissue culture dish, 10 cm (Greiner, cat. no. 664160)

Tissue culture plate, 24-wells (Biological Hope, cat. no. H181-24)

Kirgen 1.5-ml tubes (Kirgen, cat. no. KG2211)

Falcon 15-ml conical centrifuge tubes (Falcon, cat. no. 14-959-49B)

Falcon 50-ml conical centrifuge tubes (Falcon, cat. no. 352070)

Filtered sterile pipette tips (Thermo Fisher Scientific QSP)

NanoDrop 2000 (Thermo Fisher Scientific)

PCR instrument (Bio-Rad, cat. no.1861096)

Dry bath (e.g., Eppendorf)

Digital gel imaging system (Tanon, cat. no. 3500B)

35 mm no.1.5 glass-bottomed dishes (Cellvis, cat. no. D35-2001.5-N)

DeltaVision Elite imaging system (GE Healthcare N/A)

## crRNA designing

1 Find ATAC-seq data for targeted cell lines, like the ATAC-seq data for HeLa (**GEO Accession viewer (nih.gov)**). Download the .bw file

2 Open IGV application, choose the associated genome (GRCh38/hg38), load ATAC-seq .bw file to get the accessibility of each gene or locus.

3 Jump to the wanted gene or locus, choose the most accessible region around the gene or locus, get the coordination of region of interest.

4 Input the coordination of region of interest to CHOPCHOP (**CHOPCHOP (uib.no)**), choose the associated specie and genome, using CRISPR/Cpf1 or CasX, using the For repression or nanopore enrichment mode. In the part of options, set **sgRNA length without PAM** as 20, **5'-PAM:** as TTTN, **Efficiency score:** as Kim et al. 2018, and Check for self-complementarity. Click the **Find Target Sites!** to get the target site candidates. Download the excel file of target sites

5 Remove target sites with MM0>5, MM1>5, MM2>25 and MM3>50, remove target sites with GC content more than 80% and less than 30%, remove target sites that contains potential poly(A) signals ("ATTAAA","AATAAA","CATAAA","AATACA","AATATA","GATAAA","AAGAAA","TATAAA","AGTAAA","ACTAAA","AAAAAG","AATAGA") or can form potential poly(A) signals with DR sequence. Save as .csv file

6 Extract the sequence of target region in genome browser (**Get DNA in Window (hg38/Human) (ucsc.edu)**) and construct the snapgene file, import target sites sequences as primers, pick out the sites with higher efficiency when the distance between two target sites is shorter than 50 bp. Export primer data. Sort the left target sites from highest efficiency to lowest efficiency. Assemble the first 48 target sequences with DR sequences (remember to remove the PAM sequence (TTTN) at the 5' of target sequences) to form the CRISPR array

## CRISPR array construction

7 For synthesizing CRISPR array, we recommend Genscript (**Gene synthesis & DNA synthesis Service - Fast Turnaround Time | GenScript**) or Twist Bioscience (**Twist Bioscience (twistdna.com)**). Genscript provide the service of synthesizing intact 48x CRISPR array comparably more expensive but help construct the array into target plasmid for free, while Twist Bioscience can only synthesize CRISPR arrays shorter than 1 kb but in a much cheaper way. If choosing Twist Bioscience, we need split the 48x CRISPR array into two part and kindly select a suitable restriction enzyme cutting site (X=BbsI/Esp3I/BsaI/existing site in the middle of the array) for the assembly of the two parts using molecular cloning.

8 To construct the CRISPR array into expressing plasmid, using NotI and ClaI to linearize plasmid, NotI and X to digest the array-1, and X, ClaI to digest array-2.

10× CutSmart Buffer . . . . . 4 µl
pCAG plasmid DNA/Array (1 µg) . . . . . —.– µl
NotI . . . . . 1 µl
ClaI . . . . . 1µl
H2O up to 40 µl. . . . . —.– µl
incubate at 37 ◦C for >3 hours
Resolve the digest production on 1.0 % agarose gel in 1× TAE buffer. Cut out the ~1 kbp fragment of array-1 and array-2, and 7.567 kb fragment of pHAGE-CAG fragment from the agarose gels. Purify the DNA from the gel slabs with gel clean-up kit

9 Using T4 DNa liagse (NEB) to ligate vector, array-1 and array-2 together:

T4 DNA ligase buffer. . . . . 1 µl
pHAGE-CAG fragment (20 ng). . . . . –.— µl
Array-1 (20 ng) . . . . . –.— µl
Array-2 (20 ng) . . . . . –.— µl
T4 DNA ligase . . . . . 0.5 µl
H2O up to 10 µl . . . . . –.— µl

Incubate at room temperature for 30 min.

Transform the ligation product into E. Coli competent cells, spread them on the Amp+ agar plate

10 Colony PCR:
Fprimer（CAG-F）：ggttcggcttctggcgtgtgacc
Rprimer: design reverse primer targeting the last spacer of the CRISPR array
Pick 8 clones, pipette them separately in 10 µL Amp+ LB medium, use 1 µL for PCR:

2x Taq Master mix: . . . . . .10 µL
Fprimer (10 µM): . . . . . .1 µL
Rprimer (10 µM): . . . . . .1 µL
colony . . . . . .1 µL
H2O . . . . . 8 µL

**PCR program:**

1) 3 min at 95°C
2) 20 sec at 98°C Denaturation
3) 30 sec at 58°C Annealing

4) 2 min at 72°C extension
5) Go to 2) for 25 cycles
6) 5 min at 72°C finishing up
7) Stop at 12°C for 5 min

Checked the product on the agarose gel.

Choose the PCR positive clones for culturing and plasmid extraction, send them for sequencing.

## Transfection

11 Day 1: Passage ~2x10^5 cells to one well of the 24-well plate, starting with about 40% confluency

12 Day 2: Do the transfection when the cell confluency reaches 80-90%. We use Lipofectamine 3000 Transfection Reagent (Invitrogen) for HeLa cells, and Lipofectamine stem reagent (Invitrogen) for mESCs.

For HeLa cells:
1) Opti-MEM. . . . . 25 µL
HyperdLbCas12a-Staygold plasmid: . . . . . 100 ng
pHAGE-CAG-array plasmid: . . . . . 400 ng
P3000: . . . . . 1 µL
2)Opti-MEM. . . . . 25 µL
Lipo 3000. . . . . 1.5 µL
Prepare solution 1) and solution 2) separately, then mix them thoroughly. Incubate for 10 min, add to the cells.

For mESCs:
1) Opti-MEM. . . . . 25 µL
HyperdLbCas12a-Staygold plasmid: . . . . . 300 ng
pHAGE-CAG-array plasmid: . . . . . 1700 ng

2)Opti-MEM. . . . . 25 µL
Lipofectamine stem reagent. . . . . 4 µL
Prepare solution 1) and solution 2) separately, then mix them thoroughly, incubate for 10 min, add to the cells.

Note: The transfection amount may need be optimized according to the transfection efficiency. Since expression of hyperdLbCas12a should be enough to process CRISPR array efficiently, and not too much to avoid high background. Expression of CRISPR array should be as high as possible.

13 Day 3: Passage cells to the glass-bottom dish, incubate overnight.

## Imaging

14 After transfection 48 hours, change the medium with pre-warmed FluoroBrite DMEM (complemented with 10% FBS) and prepare for imaging.
When using DeltaVision Elite imaging system (GE Healthcare), image cells in the FITC channel using 60×/1.42NA Plan Apo oil-immersion objective with the parameters of 50%T, 0.2 second, average of 3 and 0.2 μm z-step, images are then deconvolved by the inner deconvolution mode of the system.
When using Nikon Eclipse-Ti (inverted widefield), image cells in the 488-channel using 60×/1.42 NA CFI Plan Apochromat Lambda D oil-immersion objective with the parameters of 100%T, 300 ms, Dual Gain 1/4, and 0.3 μm z-step, images are then deconvolved using Richardson-Lucy method.

Note: the parameters for imaging should be adjusted according to the quality of signals.