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Generation of Stable STING-GFP cells using retrovirus

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This method describes the generation of HeLa cells stably expressing STING-GFP using retroviral transduction in order to study the localization of STING under different conditions.

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STING, STING-GFP, Retrovirus, Retroviral transduction, ASAPCRN

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DNA Reagents:

STING-

- V1 **addgene Catalog #124262**
- pEGFP-N1 (Clontech)

pMXs-IRES-Bsd Retroviral Expression Vector **Cell Biolabs,**

- **Inc Catalog #RTV-016**

The sequences for the primers used are listed in Table S1 of our manuscript.

Solutions to prepare:

DMEM (-P/S) solution:

A	B
FBS	10%
L-glutamine	2 mM

All appropriate biosafety precautions should be observed when handling retrovirus.

Cloning of pMX-STING-GFP retroviral vector 2d 16h

1

Amplify the coding sequence for human STING using PrimeSTAR GXL DNA polymerase (Takara Bio) according to manufacturer protocol. Primers include a XhoI restriction site at the 5' end and a SacII restriction site at the 3' end.

2

Purify the amplicon from PCR reaction mixture using a NucleoSpin Gel and PCR Cleanup kit (Macherey-Nagel) and run amplicon in an agarose gel to confirm expected size.

3

Digest the hSTING PCR product and pEGFP-N1 plasmid using XhoI and SacII restriction enzymes (New England BioLabs) in CutSmart buffer (New England BioLabs) according to manufacturer protocol.

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4

Run digested products in an agarose gel to confirm expected size and purify the DNA from gel using a NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel).

5 Ligate the digested hSTING amplicon and linearized pEGFP-N1 backbone using T4 ligase (New England BioLabs) according to manufacturer protocol.

6 Transform product of ligation reaction into competent E. coli, and plate on kanamycin resistant agar plates. Incubate at **37 °C** for **16:00:00** . 16h

7 Pick single bacterial colonies and expand. Grow in **5 mL** LB media at **37 °C** for **16:00:00** . 16h

8 Purify plasmid by Mini-Prep (Qiagen) and sequence.

9

Digest the hSTING-EGFP-N1 and pMXs-IRES-Blasticidin Retroviral Vector backbone using XhoI and NotI-HF restriction enzymes (New England BioLabs) in CutSmart buffer (New England BioLabs) according to manufacturer protocol.

10

Run digested products in an agarose gel to confirm expected size and purify the DNA from gel using a NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel)

11 Ligate the digested hSTING-EGFP amplicon and linearized pMXs-IRES-Blasticidin Retroviral Vector backbone using T4 ligase (New England BioLabs) according to manufacturer protocol.

12 16h

Transform product of ligation reaction into competent E. coli, and plate on kanamycin resistant agar plates. Incubate at **37 °C** for **16:00:00** .

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13 Pick single bacterial colonies and expand. Grow in **5 mL** LB media at **37 °C** for **16:00:00** . 16h

14 Purify plasmid by Mini-Prep or Maxi-Prep (Qiagen) and sequence.

Transduction of HeLa cells with pMX-STING-GFP retrovirus 2d

15 Plate 5 x 10^6 Plat-A cells (Cell Biolabs) on a **10 cm** plate in DMEM (-P/S).

16 The following day, transfect cells with **9 µg** of pMX-STING-GFP using Fugene HD (Promega).

17 At **48:00:00** post-transfection, plate target HeLa cells at 2.5 x 10^5 in DMEM (-P/S) 6 well format. 2d

18 At **72:00:00** post-transfection, collect retroviral supernatant into a falcon tube and supplement with **8 µg/ml** Polybrene (Millipore). 3d

19 

Pass supernatant through **0.22 µm** filter to remove cellular debris and add to target HeLa cells.

20 At **24:00:00** post-transduction, remove retroviral supernatant and replace with fresh DMEM complete. 1d

21 At **48:00:00** post-transduction, sort HeLa cells by FACS to enrich for GFP positive cells. 2d

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