Generation of Stable STING-GFP cells using retrovirus

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Abstract

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.

Keywords

STING, STING-GFP, Retrovirus, Retroviral transduction

WARNING: All appropriate biosafety precautions should be observed when handling retrovirus.

DNA Reagents:

STING-V1 (Addgene #124262)
pEGFP-N1 (Clontech)
pMXs-IRES-Blasticidin Retroviral Vector backbone (RTV-016, Cell Biolabs)
The sequences for the primers used are listed in Table S1 of our manuscript.

Solutions to prepare:

DMEM (-P/S) containing 10% FBS and 2 mM L-glutamine (all from Gibco).

Cloning of pMX-STING-GFP retroviral vector

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 Clean-up 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.
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 hours.
7. Pick single bacterial colonies and expand. Grow in 5 mL LB media at 37˚C for 16 hours.
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. Transform product of ligation reaction into competent E. coli, and plate on kanamycin resistant agar plates. Incubate at 37˚C for 16 hours.
13. Pick single bacterial colonies and expand. Grow in 5 mL LB media at 37˚C for 16 hours.
14. Purify plasmid by Mini-Prep or Maxi-Prep (Qiagen) and sequence.

Transduction of HeLa cells with pMX-STING-GFP retrovirus

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1. Plate 5 x 10^6 Plat-A cells (Cell Biolabs) on a 10 cm plate in DMEM (-P/S).
2. The following day, transfect cells with 9 μg of pMX-STING-GFP using Fugene HD (Promega).
3. At 48 hours post-transfection, plate target HeLa cells at 2.5 x 10^5 in DMEM (-P/S) 6 well format.
4. At 72 hours post-transfection, collect retroviral supernatant into a falcon tube and supplement with 8 μg/mL Polybrene (Millipore).
5. Pass supernatant through 0.22 μm filter to remove cellular debris and add to target HeLa cells.
6. At 24 hours post-transduction, remove retroviral supernatant and replace with fresh DMEM complete.
7. At 48 hours post-transduction, sort HeLa cells by FACS to enrich for GFP positive cells.