

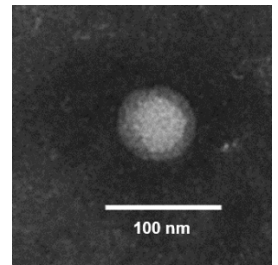
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Version 1

AdenoBuilder: modular assembly of recombinant adenovirus genomes from high-copy plasmids V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.qt5dwq6>



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Seamless assembly of recombinant adenoviral genomes from high-copy plasmids

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Protocol status: Working

We use this protocol and it's working

Created: June 09, 2018

Last Modified: July 12, 2018

Protocol Integer ID: 12893

Keywords: recombinant adenovirus, in vitro assembly, modular assembly of recombinant adenovirus, customized adenoviral, copy plasmid, crude preparations of infectious virus, copy plasmids the diverse application, infectious virus, hek293 cell

Abstract

The diverse applications of mutant and recombinant adenoviruses demand facile methods for their generation. This protocol allows the user to rapidly assemble customized adenoviral genomes from high-copy plasmids in a two-step isothermal reaction. Following *in vitro* assembly, full-length genomes can be directly packaged in HEK293 cells. Crude preparations of infectious virus can be prepared in less than one week.

Attachments



AdenoBuilder Protoco...

20KB



Materials

MATERIALS

- ✕ Phusion Hot Start Flex 2X Master Mix - 100 rxns **New England Biolabs Catalog #M0536S**
- ✕ BstBI - 2,500 units **New England Biolabs Catalog #R0519S**
- ✕ DMEM, High Glucose **Life Technologies Catalog #11965-092**
- ✕ NEBuilder HiFi DNA Assembly Master Mix - 10 rxns **New England Biolabs Catalog #E2621S**
- ✕ Gel Loading Dye, Purple (6X), no SDS - 4.0 ml **New England Biolabs Catalog #B7025S**
- ✕ Lonza Nucleofector 4d **Lonza Catalog #AAF-1002X**
- ✕ TE buffer
- ✕ Buffered Phenol Chloroform Isoamyl alcohol (P:C:I) ((25:24:1, saturated with 10 mM Tris, pH 8.0 and 1 mM EDTA **Merck MilliporeSigma (Sigma-Aldrich) Catalog #Sigma P2069**
- ✕ Trypsin EDTA **Gibco - Thermo Fisher Scientific Catalog #25-051-CI.**
- ✕ BSA **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7906**
- ✕ Paraformaldehyde fixative: 4% paraformaldehyde in phosphate buffered saline (PBS)
- ✕ Costar® 24-Well Flat-Bottom Plate, Tissue Culture-Treated 50 Plates **STEMCELL Technologies Inc. Catalog #38017**
- ✕ T25 or T75 Flask
- ✕ SF Cell Line 4D-Nucleofector X Kit L (12 RCT) **Lonza Catalog #V4XC-2012**
- ✕ FITC-conjugated anti-Hexon antibody **Thermo Fisher Scientific Catalog #MA1-7330**
- ✕ Ammonium acetate solution for molecular biology, 7.5 M **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A2706**
- ✕ HEK293 **ATCC Catalog #CRL-1573**
- ✕ Gibco™ Penicillin-Streptomycin (10,000 U/mL) **Fisher Scientific Catalog #15-140-122**

Safety warnings

- ! Adenovirus cause generally mild respiratory tract infections which are self-limiting and generally asymptomatic despite virologic and serologic proof of infection. BSL-2 facilities, equipment, and operational practices are required for work involving infectious or potentially infectious materials. Contact your institutional biosafety office for additional information.

