

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

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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.


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Materials

 Phusion Hot Start Flex 2X Master Mix - 100 rxns M0536S by New England Biolabs

 BstBI - 2,500 units R0519S by New England Biolabs


 DMEM, High Glucose 11965-092 by Life Technologies

 NEBuilder HiFi DNA Assembly Master Mix - 10 rxns E2621S by New England Biolabs


 Gel Loading Dye, Purple (6X), no SDS - 4.0 ml B7025S by New England Biolabs

 Lonza Nucleofector 4d AAF-1002X by Lonza

✓ TE buffer by Contributed by users

 Buffered Phenol Chloroform Isoamyl alcohol (P:C:I) ((25:24:1, saturated with 10 mM Tris, pH

8.0 and 1 mM EDTA Sigma P2069 by Sigma


 Trypsin EDTA 25-051-CI. by Gibco - Thermo Fischer


 BSA A7906 by Sigma Aldrich


✓ Paraformaldehyde fixative: 4% paraformaldehyde in phosphate buffered saline (PBS) by Contributed by users

 Costar® 24-Well Flat-Bottom Plate, Tissue Culture-Treated 50 Plates 38017 by Stemcell Technologies


✓ T25 or T75 Flask by Contributed by users

 SF Cell Line 4D-Nucleofector X Kit L (12 RCT) v4XC-2012 by Lonza

 FITC-conjugated anti-Hexon antibody MA1-7330 by Thermo Fisher Scientific

 Ammonium acetate solution for molecular biology, 7.5 M A2706 by Millipore Sigma

 HEK293 CRL-1573 by ATCC

 Gibco™ Penicillin-Streptomycin (10,000 U/mL) 15-140-122 by Fisher Scientific

Protocol

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.