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

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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.

**Attachments**

*AdenoBuilder*  
Protocol.io.docx

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

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Last Modified: Jul 11, 2018

PROTOCOL integer ID: 12893

Keywords: recombinant adenovirus, in vitro assembly

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 mI New England Biolabs Catalog #B7025S
- Lonza Nucleofector 4d Lonza Catalog #AAF-1002X
- TE buffer 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 Catalog #Sigma P2069
- Trypsin EDTA Gibco - Thermo Fischer Catalog #25-051-CI.
- BSA Sigma Aldrich Catalog #A7906
- Paraformaldehyde fixative: 4% paraformaldehyde in phosphate buffered saline (PBS) Contributed by users
- Costar® 24-Well Flat-Bottom Plate, Tissue Culture-Treated 50 Plates Stemcell Technologies Catalog #38017
- T25 or T75 Flask Contributed by users
- 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 Millipore Sigma Catalog #A2706
- HEK293 ATCC Catalog #CRL-1573
- Gibco™ Penicillin-Streptomycin (10,000 U/mL) Fisher Scientific Catalog #15-140-122

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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.