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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](#)

MANUSCRIPT CITATION:

[Miciak JJ, Hirshberg J, Bunz F.](#)

Seamless assembly of recombinant adenoviral genomes from high-copy plasmids

[PLoS One](#). 2018 Jun 27;13(6):e0199563

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Protocol status: Working
We use this protocol and it's working

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PROTOCOL integer ID:
12893

Keywords: recombinant adenovirus, in vitro assembly

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 **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**

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.