

BAC (Bacterial Artificial Chromosome) Clone Collections

Catalog nos. 96012, 96012D, 96022, RPCI-11.C,
RPCI-22.C, RPCI-23.C

Version D
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IMPORTANT!

Beginning in 2009,
all Invitrogen clone
manuals will only be
available online at
www.invitrogen.com

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Contents and Storage

Types of Products

This manual is included with the following products. For more details on each BAC clone collection, see page 1.

Product	Catalog no.
CITB Human B & C BAC Clones	96012
CITB Human D BAC Clones	96012D
RPCI-11 Human BAC clones	RPCI-11.C
CITB Mouse BAC Clones	96022
RPCI-22 Mouse BAC Clones	RPCI-22.C
RPCI-23 Mouse BAC Clones	RPCI-23.C

Shipping and Storage

The BAC (Bacterial Artificial Chromosome) Clones are supplied as glycerol stocks and shipped on dry ice. Upon receipt, store at -80°C .

Contents

Each tube of BAC Clone contains genomic DNA cloned into an appropriate vector and transformed into GeneHogs[®] (also known as HS996) or DH10B[™] *E. coli* (see page 1 for vector and host strain information). Each clone is supplied in 500 μl of LB media containing 8% glycerol and 12.5 $\mu\text{g}/\text{ml}$ chloramphenicol.

Information for European Customers

These cells are genetically modified and contain the plasmid, pBeloBAC11 or pBACe3.6. As a condition of sale, this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

Contents and Storage, Continued

Genotype of GeneHogs® E. coli

The genotype of GeneHogs® *E. coli* (also known as HS996) is:
F⁻ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ80*lacZ*ΔM15 Δ*lacX74 recA1 araD139* Δ(*ara-leu*)7697 *galU galK rpsL* (Str^R) *endA1 nupG fhuA::IS2* (confers phage T1 resistance)

Genotype of DH10B™

The genotype of DH10B™ *E. coli* is:
F⁻ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ80*lacZ*ΔM15 Δ*lac74 recA1 araD139* Δ(*ara-leu*)7697 *galU galK rpsL* (Str^R) *endA1 nupG λ*-

Additional Products

Additional products that may be used with BAC Clones are available separately from Invitrogen. Ordering information is provided below.

Item	Quantity	Catalog no.
PureLink™ HiPure Plasmid Maxiprep Kit	10 preps	K2100-06
	25 preps	K2100-07
PureLink™ HiPure BAC Buffer Kit	1 kit	K2100-18

Overview

BAC Clone Collection Overview

General information on each BAC (Bacterial Artificial Chromosome) clone collection is provided in this section. The table below provides an overview of the different BAC Clone Collections, source, coverage, and vector information.

BAC Clone Collection	CITB Human B & C	CITB Human D1	CITB Human D2	RPCI-11
Source	978SK cell line (1–194, 384-well plates) Human sperm (195–768, 384-well plates)	Human sperm (2001–2423, 384-well plates)	Human sperm (2501–3253, 384-well plates)	Human male white blood cell (1–1152, 384-well plates)
Vector	pBeloBAC11	pBeloBAC11	pBeloBAC11	pBACe3.6
Average Insert Size	130 kb	129 kb	129–202 kb	174 kb
Host	GeneHogs® (HS996)	GeneHogs® (HS996)	GeneHogs® (HS996)	DH10B™
Coverage	15×	17×	17×	25×

BAC Clone Collection	CITB Mouse	RPCI-22	RPCI-23
Source	CJ7 ES cell line/129Sv (1–612, 384-well plates)	Female, mouse spleen (129/SvEvTACC/Br) (1–576, 384-well plates)	Female, mouse brain/kidney (C57BL/6J) (1–480, 384-well plates)
CITB Mouse Vector	pBeloBAC11	pBACe3.6	pBACe3.6
Average Insert Size	130 kb	154 kb	197 kb
Host	GeneHogs® (HS996)	DH10B™	DH10B™
Coverage	8×	10.9×	11.2×

Overview, Continued

CITB

The CITB BAC Libraries were constructed through collaboration with Hiroaki Shizuya at the California Institute of Technology.

The DNA source used for constructing the library is listed on the previous page. The CITB Human B & C, CITB Human D1, and CITB Mouse libraries were constructed by cloning the DNA into the *Hind* III sites of pBeloBAC11 vector and transforming into GeneHogs® (HS996) *E.coli*. The CITB Human D2 library was cloned into the *Eco*R 1 site of pBeloBAC11 vector and transformed into GeneHogs® (HS996) *E.coli*. The library is arrayed into 384-well plates.

RPCI

The RPCI-11, 22, and 23 BAC Libraries were constructed in Pieter deJong's lab at Children's Hospital Oakland Research Institute (CHORI) by Kazytoyo Osoegawa and Minako Tateno.

The DNA source used for constructing the library is listed on the previous page. The DNA was cloned into the *Eco*R I sites of pBACe3.6 vector and transformed into DH10B™ *E.coli*. The library is arrayed into 384-well plates. For details on library construction, visit <http://www.chori.org/bacBAC/home.htm>

Vector Information

The BAC Clones are cloned into the following vectors:

- pBeloBAC11
For vector map, see page 5. The complete sequence is available on our website at www.invitrogen.com
 - pBACe3.6
For vector map and sequence, visit <http://www.chori.org/bacBAC/vectorsdet.htm>
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Using BAC Clones

Introduction

General guidelines for using the BAC Clones are described in this section.

Preparing Glycerol Stocks

We recommend you prepare a set of master stocks prior to using the BAC Clones. To prepare 5–10 glycerol master stocks for long-term storage:

1. Streak a small portion of the glycerol stock you received on a LB plate containing 12.5 µg/ml chloramphenicol.
 2. Incubate the plate at 37°C overnight.
 3. Isolate a single colony and inoculate into 5–10 ml of LB containing 12.5 µg/ml chloramphenicol.
 4. Grow the culture to stationary phase ($OD_{600} = 1-2$).
 5. Mix 0.8 ml of culture with 0.2 ml of sterile glycerol and transfer to a cryovial.
 6. Store at –80°C. Use one master stock to create working stocks for regular use.
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BAC Plasmid Preparation

To isolate BAC plasmid DNA, you need to grow a culture of GeneHogs® or DH10B™ *E. coli* containing your clone. Use LB medium containing 12.5 µg/ml chloramphenicol to select single colonies or to grow a culture. Use a culture volume appropriate for the amount of plasmid needed for your plasmid isolation method of choice.

We recommend isolating BAC plasmid DNA using a resin based method, such as the PureLink™ HiPure Plasmid Maxiprep Kit (see page vi for ordering information). Other methods may also be suitable. For detailed instructions, refer to the literature provided with the product. Individual manuals are also available on our website at www.invitrogen.com or by contacting Technical Support (see page 6).

Sequencing or PCR of Clones

You may verify the clone by PCR, sequencing, or a method of choice using primers to T7 and Sp6 sites on the plasmid. The T7 and Sp6 priming sites flank the DNA cloning site for the BAC vectors, pBeloBAC11 and pBACe3.6. The cloning site of pBeloBAC11 is shown on the next page.

Using BAC Clones, Continued

Cloning Site of pBeloBAC11

The cloning site for pBeloBAC11 is shown below. Restriction sites are labeled to indicate the cleavage site. The inserts are cloned into the *Hind* III site, except for the CITB Human D2 library; the insert was cloned into the *Eco*R 1 site.

266 AGGGTTTTCC CAGTCACGAC GTTGTA AAAAC GACGGCCAGT

306 GAATTGTAAT ACGACTCACT ATAGGGCGAA TTCGAGCTCG

T7 Promoter Primer EcoR I

346 GTACCCGGGG ATCCTCTAGA GTCGACCTGC AGGCATGCAA

Kpn I *Sma* I *Bam* H I *Xba* I *Sal* I *Pst* I *Sph* I *Hind* III

386 GCTTGAGTAT TCTATAGTGT CACCTAAATA GCTTGCCGTA

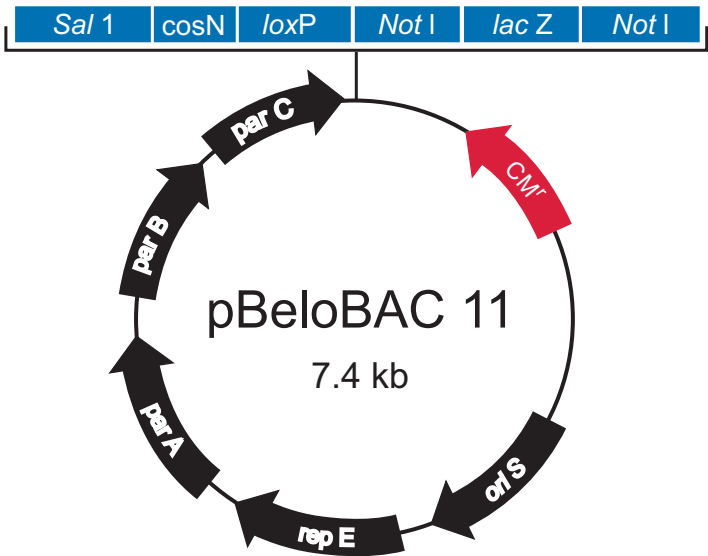
Sp6 Promoter Primer

426 ATCATGGTCA TAGCTGTTTC CTGTGTGAAA TTGTTATCCG

Map of pBeloBAC11

Map of pBeloBAC11

The figure below shows the features of pBeloBAC11 vector. The complete sequence of pBeloBAC11 is available for downloading from our website at www.invitrogen.com or by contacting Technical Support (see next page).



Technical Support

World Wide Web



Visit the Invitrogen website at www.invitrogen.com for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
 - Complete technical support contact information
 - Access to the Invitrogen Online Catalog
 - Additional product information and special offers
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Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our website (www.invitrogen.com).

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MSDS

Material Safety Data Sheets (MSDSs) are available on our website at www.invitrogen.com/msds.

Certificate of Analysis

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at www.invitrogen.com/support, and is searchable by product lot number, which is printed on each box.

Continued on next page

Technical Support, Continued

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