Invitrogen™
life technologies

Instruction Manual

ProQuest™ Pre-made cDNA Libraries

For detecting protein-protein interactions

Version B
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25-0617

Table of Contents

General Information

Contents and Storage

2 x 0.5 ml aliquots

Each cDNA library is supplied in 80% SOB medium, 20% (v/v) glycerol.

Store the library at -80°C. The cDNA library is stable for six months when stored properly.

Titer of the Libraries

Each library has greater than 5 x 10^9 cfu (colony forming units) derived from > 10^7 primary clones to ensure complete representation of rare sequences.

ProQuest™ Pre-made cDNA Libraries

The following ProQuest™ Pre-made cDNA Libraries are available from Invitrogen. For more information on preparing the library, see page 7.

Product	Avg. Insert Size	Vector	Catalog no.
Human			
Brain (27 years, male)	1.9 kb	pEXP-AD502	11376-027
Brain (Fetal)	2.3 kb	pEXP-AD502	11386-026
Brain (Fetal)	2.1 kb	pPC86	11386-018
Heart (26 years, male)	1.4 kb	pPC86	11378-015
Heart (23 years, male)	1.7 kb	pEXP-AD502	11378-023
HeLa cells	1.5 kb	pPC86	11287-018
Liver (28 years, male)	2.0 kb	pPC86	11381-019
Skeletal muscle (24 years, male)	1.6 kb	pPC86	11367-018
Prostate (25 years, male)	1.3 kb	pPC86	11579-018
Prostate, poorly differentiated adeno-carcinoma, (63 years, male)	1.3 kb	pPC86	11582-012
Prostate, moderately, differentiated leimyosarcoma (29 years, male)	1.3 kb	pPC86	11583-010

Continued on next page

ProQuest™ Pre-made cDNA Libraries, continued

Product	Avg. Insert Size	Vector	Catalog no.
Mouse			
Brain	1.5 kb	pPC86	11298-015
Embryo (8.5 day)	1.2 kb	pPC86	11291-010
Embryo (10.5 day)	1.4 kb	pPC86	11292-018
Liver	1.5 kb	pPC86	11297-017
Lymph nodes (*in vivo* activated, see **RNA Source**, next page)	1.7 kb	pPC86	11289-014
Rat			
Brain	1.2 kb	pPC86	11290-012
Liver	1.3 kb	pPC86	11296-019
Caenorhabditis elegans (see **RNA Source**, next page)	1.4 kb	pPC86	11288-016

Continued on next page

General Information, Continued

RNA Source

Mouse Lymph Nodes

The RNA was obtained from mesenteric lymph node tissue at day 8 after oral inoculation of BALB/c mice with third stage larvae of the nematode, *Heligmosomoides polygyrus*. *H. polygyrus* triggers a strong Th2 mucosal response at day 8 after immunization (Greenwald *et al.*, 1997; Svetic *et al.*, 1993). The mesenteric lymph node at day 8 is composed of about 60% T cells, 30% B cells, 10% dendritic cells, macrophages, and other tissue-associated cells.

C. elegans

C. elegans (Bristol N2) RNA (supplied by Monique A Kreutzer and Sander van den Heuvel of the Massachusetts General Hospital Cancer Center, Charlestown, Massachusetts) isolated from populations of non-synchronously staged L1 to adult nematodes (including dauer larvae and embryos) was combined to generate approximately equal representation of RNA expressed at different developmental stages. The nematodes were mated to ensure representation of sex-specific RNAs.

Quality Control

The ProQuest™ Pre-made cDNA Libraries are qualified by PCR amplification of 23 randomly selected clones using primers flanking the multiple cloning site. Twenty out of twenty-three clones must contain inserts.

Overview

Introduction

ProQuest™ Pre-made cDNA Libraries are suitable for use with the ProQuest™ Two-Hybrid System (see page 11). Each library is constructed using the SuperScript™ II Plasmid System for cDNA Synthesis and Plasmid Cloning to generate full-length and high-yield cDNA.

Libraries are constructed in a standard vector pPC86 or the Gateway® vector, pEXP-AD502 (see next page). For information on library preparation, see page 7.

ProQuest™ Libraries

ProQuest™ Pre-Made cDNA Libraries may be screened for interacting proteins using the ProQuest™ Two-Hybrid System (see page 11), PCR or plate screening procedures, further amplified (Sambrook *et al.*, 1989), or used for the production of single-stranded plasmid DNA *in vivo* (Li *et al.*, 1994). For more information on the ProQuest™ Two-Hybrid System, refer to the manual. This manual is available for downloading from our Web site at www.invitrogen.com or by contacting Technical Service (see page 22).

Libraries prepared in the Gateway® vector pEXP-AD502 allow you to shuttle DNA sequences of interest into a variety of Gateway®-compatible expression and analysis vectors using the Gateway® recombinational cloning Technology (see page 12).

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pPC86 and pEXP-AD502

The vectors pPC86 and pEXP-AD502 are activation domain expression vectors. pEXP-AD502 is similar to pPC86 (Chevray and Nathans, 1992) except pEXP-AD502 contains the Gateway® recombination sites *att*B1 and *att*B2. The major features of the two vectors are listed below.

- The constitutive, moderate-strength yeast alcohol dehydrogenase (*ADH1*) promoter for expression of *GAL4* fusions
- The SV40 large T antigen nuclear localization sequence (NLS)
- The *GAL4* activation domain (AD) allowing expression of the reporter gene which is activated when brought into proximity with the DNA binding domain by interacting bait and prey proteins
- The recombination sites, *att*B1 and *att*B2 (only in pEXP-AD502) for transfer of cDNA into Gateway®-compatible vectors
- The *ADH1* transcription termination (TT) for efficient transcription termination and stabilization of the mRNA
- An f1 origin for ss DNA production
- The *TRP1* promoter for expression of the *TRP1* gene
- The *TRP1* gene for auxotrophic selection of the plasmid in Trp^- yeast hosts
- An *ARSH4*/*CEN6* sequence for replication and low-copy number maintenance of plasmid in yeast
- Ampicillin resistance gene for selection of transformants in *E. coli*
- The pUC origin for high copy replication and maintenance of the plasmid in *E. coli*

For maps of vectors, see pages 13-15.

Continued on next page

Preparing ProQuest™ Libraries

ProQuest™ Pre-made cDNA Libraries are prepared as follows:

- mRNA is isolated using two steps. First, total RNA is isolated from tissues or cells using the TRIzol® Reagent. Second, mRNA is isolated from total RNA using oligo (dT) in a filter syringe.
- First-strand cDNA is synthesized using SuperScript™ Plasmid System for cDNA Synthesis and Plasmid Cloning with *Not* I primer-adapter (D'Alessio, J. M., Gruber, C. E., Cain, C., and Noon, M. C. 1990) *Focus*® **12**: 47)
- Second-strand cDNA is synthesized using *E. coli* RNase H, *E. coli* DNA polymerase I, and *E. coli* DNA ligase
- Blunt-end cDNA is created using T4 DNA polymerase
- cDNA is adapted with *Sal* I adapter and digested with *Not* I
- cDNA is size-selected using column chromatography
- Size-selected cDNA is directionally cloned downstream of the G*AL4* activation domain into the *Sal* I-*Not* I region of the vector (pPC86 or pEXP-AD502) to generate *GAL4* AD-cDNA fusion proteins
- Ligation mixture is transformed into competent ElectroMAX™ DH10B™ *E coli* and the number of primary recombinants is determined
- cDNA library is amplified once using a semi-solid procedure (Kriegler, 1990) to minimize representational biases.

Genotype of DH10B™

F′ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC)ϕ80*lac*ZΔM15 Δ*lac*X74 *deo*R *rec*A1 *end*A1 *ara*D139 Δ(*ara,leu*)7697 *gal*U *gal*K *rps*L *nup*G

Using ProQuest™ Libraries

Introduction

ProQuest™ Pre-made cDNA Libraries may be screened for interacting proteins using the ProQuest™ Two-Hybrid System (see page 11), PCR or plate screening procedures.

General procedures for preparing DNA from the library and colony PCR are provided in this section. For detailed information on library screening refer to published references (Ausubel *et al.*, 1994; Sambrook *et al.*, 1989).

Preparing dsDNA from a Plasmid cDNA Library

You will need the following items.

- Terrific Broth (see page 17 for a recipe)
- Buffer I with RNase (15 mM Tris-HCl, pH 8.0; 10 mM EDTA; 100 µg/ml RNase A; 1200 U/ml RNase T1)
- Buffer II (0.2 M NaOH, 1% SDS)

1. Inoculate 100 ml Terrific Broth containing 100 µg/ml ampicillin with 2.5 x 10^9 cells from the library in a 500-ml flask.
2. Incubate the culture for 16 hours at **30 °C** with shaking at 275 rpm. **Clones are prone to deletions when the culture is grown at > 30°C.**
3. Read the A_{590} of the culture. For accurate A_{590} determination, dilute the cells 1:10-1:20, so that the observed value is between 0.2 and 0.8. In two 50-ml centrifuge tubes, process ~ 500 OD_{590} units.
4. Centrifuge the tubes at 4800 x g for 15 minutes at 4°C. Discard the supernatant.
5. Resuspend the cell pellets in a total volume of 10 ml Buffer I with RNase. The cells must be < 50 OD/ml.
6. Add 10 ml of Buffer II to the resuspended cells. Invert the tubes to mix the cells and incubate for 5 minutes at room temperature.

Continued on next page

Preparing dsDNA from a Plasmid cDNA Library, continued

7. Add 10 ml cold 7.5 M ammonium acetate to the cell mixture. Invert the tubes to mix the cells and incubate for 10 minutes on ice.
8. Centrifuge the tubes at 3,000 x g for 15 minutes at 4°C. Pour the supernatant through cheesecloth or a clean, DNase-free, porous filter into a fresh 50-ml centrifuge tube. Avoid the white flocculant material.
9. Add an equal volume of cold isopropanol to the tube, mix well, and centrifuge the tubes at 3,000 x g for 15 minutes at 4°C. Discard the supernatant.
10. Resuspend the cell pellet in 1 ml of Buffer I with RNase and transfer it to a microcentrifuge tube.
11. Centrifuge the tubes at 14,000 x g for 1 minute at 4°C. Transfer the supernatant to a fresh microcentrifuge tube. Incubate the tube at 37°C for 10 minutes.
12. Incubate the tubes at 65 °C for 5 minutes. Split each sample into two equal parts (~ 500 µl each) in 1.5-ml microcentrifuge tubes.
13. Add an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) to each sample and vortex the tubes. Centrifuge the tubes at 14,000 x g for 5 minutes at room temperature.
14. Avoiding the interface, transfer 450 µl of the upper (aqueous) phase to a fresh microcentrifuge tube.
15. Repeat the phenol : chloroform : isoamyl alcohol extraction at least twice. If an interface remains, repeat steps 13 and 14 until the supernatant is clear.
16. Add an equal volume of isopropanol (4 °C) to each tube. Centrifuge the tubes at 14,000 x g for 15 minutes at 4°C. Discard the supernatant.
17. Add 500 µl 70 % ethanol to each tube. Centrifuge the tubes at 14,000 x g for 5 minutes at 4°C. Discard the supernatant.
18. Dry the pellet for 10 minutes at room temperature.
19. Completely dissolve the two pellets in 200 µl TE buffer. The plasmid DNA library concentration must be approximately 1 µg/µl. Store the DNA at -20°C.

Continued on next page

Colony PCR Screening

A colony PCR procedure to screen for the presence of specific cDNA is described below. You may also use this method to identify desired cDNA clones.

The recommended primers for PCR and their sequences are shown on page 14 for pPC86 and on page 16 for pEXP-AD502.

1. Add 10 µl TE to each labeled, 0.5 ml microcentrifuge tube.
2. Pick individual colonies using a pipette tip and place the colonies directly into separate tubes containing TE. Pipette up and down to mix.
3. Incubate the tubes in a pre-warmed thermal cycler at 99°C for 5 minutes.
4. Incubate the tubes on ice for 2 minutes.
5. Centrifuge briefly to collect the sample at the bottom of the tube. Replace the tubes on ice.
6. Prepare the appropriate amount of the following reaction mix and add 40 µl of reaction mix to each tube.

   1X PCR Buffer (contains no $MgCl_2$)

   0.2 mM dNTP mix (see page 18)

   0.5 µM primers

   2.4 mM $MgCl_2$

   2.5 units Platinum® *Taq* DNA polymerase (see page 18)
7. Bring the volume to 50 µl with sterile water.

Continued on next page

Colony PCR Screening, continued

8. Perform PCR using the following cycling parameters:

Temperature	Time	Cycles
95°C	2 minutes	1
94°C	1 minute	40
55°C	1 minute	
72°C	1 minute	
72°C	5 minutes	1

9. Transfer 10 µl of each reaction to a new tube containing 2 µl 10X gel loading buffer.
10. Electrophorese the samples on a 1.5% agarose gel and analyze your results.

ProQuest™ Two-Hybrid System

The ProQuest™ Two-Hybrid System is an *in vivo* yeast-based system for identifying protein-protein interactions (Chevray and Nathans, 1992). The major features of the system are:

- Low copy (ARS/CEN) vectors for reduced toxicity
- Three reporter genes with independent promoters to reduce false positives due to non-specific interactions
- Large panel of control yeast strains
- Gateway®-compatible vectors for transferring your DNA sequences of interest into a variety of expression and analysis vectors using recombinational cloning

For more details on screening ProQuest™ Pre-made cDNA Libraries using the ProQuest™ Two-Hybrid System, refer to the ProQuest™ Two-Hybrid System manual. This manual is available for downloading from our Web site at www.invitrogen.com or by contacting Technical Service (see page 22).

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Gateway® Cloning

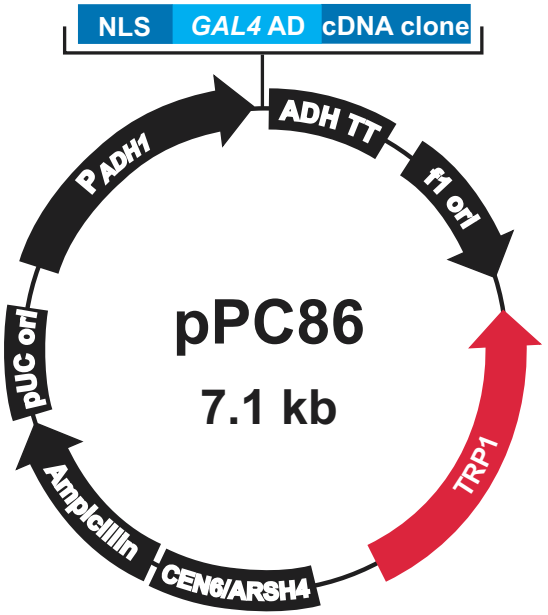
The vector, pEXP-AD502 contains *att*B1 and *att*B2 recombination sites flanking the cDNA cloning site. The cDNA insert can be transferred into other Gateway®-compatible vectors for expression by performing a BP recombination reaction with a pDONR™ vector (see page 18).

For details on Gateway® technology, refer to the Gateway® Technology Manual on our Web site at www.invitrogen.com or contact Technical Service (see page 22).

pPC86

Map of pPC86

The figure below shows the features of pPC86 vector. **The complete sequence of pPC86 is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 22).**



Comments for pPC86 (no insert)
7093 nucleotides

ADH1 promoter: bases 114-1567
Initiation ATG: bases 1573-1575
SV40 nuclear localization signal (NLS): bases 1576-1596
GAL4 activation domain: bases 1606-1953
ADH1 transcription termination region: bases 2042-2508
f1 origin: bases 2700-3155
TRP1 gene (c): bases 3252-3926
TRP1 promoter (c): bases 3947-4029
CEN6/ARSH4 (c): bases 4458-4976
bla promoter: bases 5009-5113
Ampicillin (*bla*) resistance gene: bases 5108-5968
pUC origin: bases 6113-6786
(c) = complementary strand

Continued on next page

pPC86, Continued

Cloning Site of pPC86

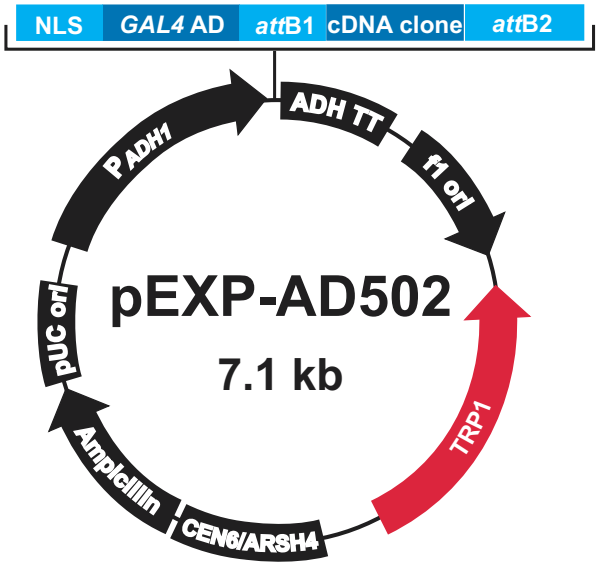
The cloning site for pPC86 is shown below. Restriction sites are labeled to indicate the cleavage site.

*This *Mlu* I site is contained within the *Sal* I adapter introduced into the vector upon ligation of the cDNA insert.

pEXP-AD502

Map of pEXP-AD502

The figure below shows the features of pEXP-AD502 vector. **The complete sequence of pEXP-AD502 is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 22).**



Comments for pEXP-AD502 (no insert)
7146 nucleotides

ADH1 promoter: bases 64-1517
Initiation ATG: bases 1523-1525
SV40 nuclear localization signal (NLS): bases 1526-1548
GAL4 activation domain: bases 1556-1903
*att*B1: bases 1910-1934
*att*B2: bases 1979-2003
ADH1 transcription termination region: bases 2045-2511
f1 origin: bases 2703-3158
TRP1 gene (c): bases 3255-3929
TRP1 promoter (c): bases 3930-4031
CEN6/ARSH4 (c): bases 4461-4979
bla promoter: bases 5012-5116
Ampicillin (*bla*) resistance gene: bases 5111-5971
pUC origin: bases 6116-6789
(c) = complementary strand

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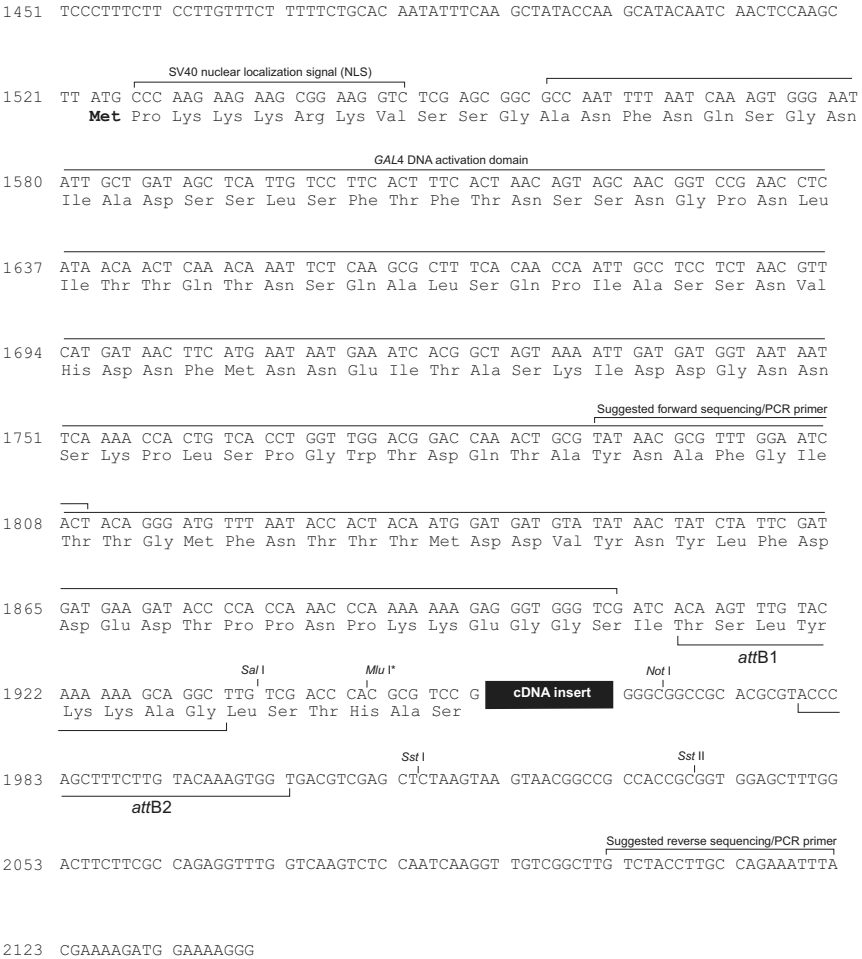
pEXP-AD502, Continued

Cloning Site of pEXP-AD502

The cloning site for pEXP-AD502 is shown below. Restriction sites are labeled to indicate the cleavage site.



*This *Mlu* I site is contained within the *Sal* I adapter introduced into the vector upon ligation of the cDNA insert.

Recipe

Terrific Broth

Terrific Broth is available from Invitrogen (see page 18 for ordering information).

1. Dissolve the following reagents in 800 ml of distilled water:

Tryptone	12 g
Yeast Extract	24 g
Glycerol	4 ml

2. Mix well and adjust the volume to 900 ml with distilled water.
3. Autoclave on liquid cycle for 20 minutes. Allow solution to cool to ~55°C
4. Dissolve the following reagents in 80 ml of distilled water:

KH_2PO_4 (monobasic)	2.3 g
K_2HPO_4 (dibasic)	12.5 g

5. Mix well and adjust the volume to 100 ml with distilled water.
6. Autoclave on liquid cycle for 20 minutes. Allow solution to cool to ~55°C. Mix this solution with the solution prepared in Step 3.
7. After the media is cooled, add antibiotic to the desired concentration.
8. Store at +4°C.

Accessory Products

Additional Products

The table below lists additional products that may be used with the ProQuest™ Pre-made cDNA Libraries.

Product	Quantity	Catalog no.
ProQuest™ Two-Hybrid System with Gateway™ Technology	5 reactions	10835-031
MaV203 Competent Yeast Cells; Library Scale	2 x 0.55 ml	11281-011
Terrific Broth	500 g	22711-022
2.5 mM dNTP Mix	1 ml	R725-01
Platinum® *Taq* DNA Polymerase	100 reactions	10966-018
pDONR™221	6 µg	12536-017
pDONR™207	6 µg	12213-013
pDONR™221	6 µg	12536-017
Phenol:Chloroform:Isoamyl Alcohol, (25:24:1, v/v/v)	100 ml	15593-031

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Purchaser Notification, Continued

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Technical Service

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- View and download vector maps and sequences
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- Obtain citations for Invitrogen products
- Request catalog and product literature

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http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

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For more information or technical assistance, please call, write, fax, or email. Additional international offices are listed at www.invitrogen.com.

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Technical Service, Continued

MSDS Requests

To request an MSDS, visit our Web site (www.invitrogen.com).

1. On the home page, go to the left-hand column under 'Technical Resources' and select 'MSDS Requests'.
2. Follow instructions on the page and fill out all the required fields.
3. To request additional MSDSs, click the 'Add Another' button.
4. All requests will be faxed unless another method is selected.
5. When you are finished entering information, click the 'Submit' button. The MSDS will be sent within 24 hours.

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