



Express Biotech International

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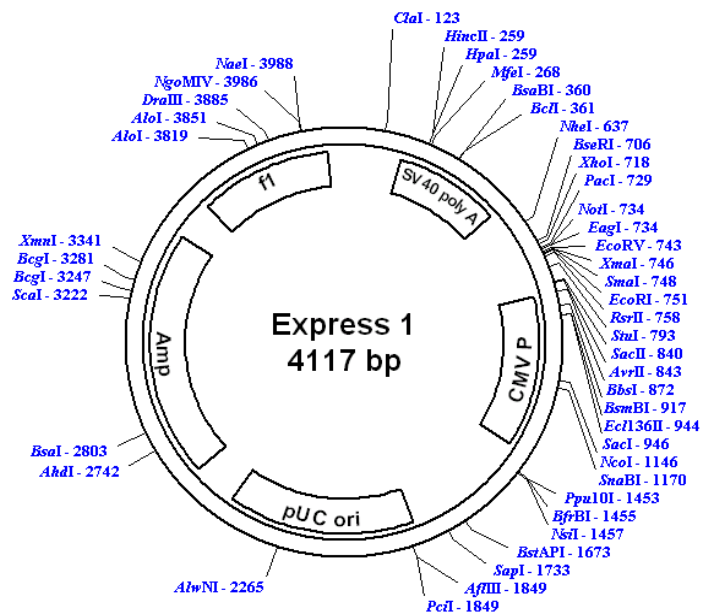
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cDNA Library Services

The scientists at XpressBio have constructed hundreds of cDNA libraries over the last twenty years and are responsible for many of the current critical improvements in cDNA library technology, including 70% full-length clones, greater than 95% productive clones in a library, large insert libraries, high quality libraries made from nanoquantity amounts of mRNA (no PCR), libraries made in more than 50 custom vectors, normalization of the expression pattern of clones in a library to equivalent gene copies, subtraction of clones from one library (normal) from another library (diseased) to reveal the genes that make-up the difference between the diseased and normal state, and the synthesis of large quantities of 454 ready DNA from nanoquantity amounts of mRNA. Normalized libraries constructed for the National Cancer Institute's Mammalian Genome Collection have been sequenced up to 40,000 clones deep into the library. The sequence data demonstrated that the discovery rate of novel clones in these libraries was the same from beginning to end of the sequencing of the 40,000 clones. The multifunctional and high copy number pExpress 1 cloning vector is a super discovery tool and gives sequencing reads per reaction that average 850 bases into each end of the cDNA.

XpressBio will construct cDNA libraries from customer supplied tissues, cells or RNAs in pExpress 1 or custom provided vectors and will deliver high quality libraries within 4 to 6 weeks. In addition, we have a large offering of primary and normalized catalog cDNA libraries constructed from several different human, mouse, rat, monkey, zebra fish, and xenopus tissues. The catalog libraries are available for immediate delivery at very economical prices. We have the highest quality cDNA libraries in the market place and guarantee customer satisfaction.

Express 1 vector



The features of our 4 kb Express 1 vector are as follows:

- Puc based, Amp resistant vector
- CMV promoter for eukaryotic expression
- SP6 and T7 RNA polymerase promoters flanking the MCS for RNA synthesis
- Amersham ET and M13 primer sites for sequencing
- F1 ori for single-stranded DNA production
- cDNA directionally cloned for expression, antibody screening, subtraction and for normalization



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High Quality 454-Ready cDNA

High Quality 454-Ready cDNA is made from our custom or catalog cDNA libraries. Briefly, library DNA is digested with *Not I* and *in vitro* RNA transcripts are produced using the SP6 RNA polymerase promoter. Then, first strand cDNA is made from these transcripts using a modified primer adapter that reduces the size of the poly A sequence (to about 20 As). After the second strand is synthesized, the double stranded (ds) cDNA is blunt ended and size fractionated. This ds cDNA is now ready to be used with the 454 sequencing method or with other next generation sequencing technology (i.e., illumina). We guarantee at least 6 µg of ds cDNA, 150 ng/µl concentration in TE and 1 kb average cDNA size, however, we generally produce 8 µg of cDNA, 200 ng/µl concentration and 1.5 kb average cDNA size.

Features list:

- High quality size fractionated cDNA.
- Reduced poly A size to improve sequencing results.
- Large amount of cDNA can be produced from nanoquantity levels of tissue or RNA.
- Ideal substrate for next generation sequencing methods.

Quality testing: Gel analysis of cDNA population.



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High Quality Normalized cDNA Libraries

High Quality Normalized cDNA Libraries are produced using our primary cDNA libraries. Biotinylated driver cRNA produced from the T7 RNA polymerase promoter and single-stranded (ss) target cDNA produced from the F1 ori are hybridized to each other at a low Cot (concentration of driver times the time of hybridization) value. The cRNA:cDNA hybrids are removed by phenol extraction and the remaining ss target cDNA is converted to double-stranded DNA (dsDNA) with a repair oligo and Taq DNA polymerase. After electroporation of the dsDNA into T1 phage resistant *E. coli*, normalized clones are produced.

The normalization process reduces the level of abundant and moderately abundant genes and enriches for rare genes. To determine the reduction of an abundant gene, we compare the relative representation of the actin gene in the primary library with the normalized library by hybridization of different dilutions of the primary and normalized dsDNA with an actin probe. Using this method, we determine that the actin gene is reduced at least 20 fold in the normalized library.

We guarantee at least 1×10^6 normalized clones with > 87% recombinant clones containing an average insert size of at least 1 kb for standard normalized libraries and 3 kb for large insert normalized libraries. However, we generally produce libraries of 3×10^6 normalized clones with >95% recombinant clones containing an average insert size of 1.5 kb average insert size for standard normalized libraries and 4 kb for large insert normalized libraries.

Features list:

- High quality size fractionated cDNA
- Directionally cloned cDNA for expression, antibody screening, subtraction and normalization.
- Large numbers of normalized clones
- Low vector background
- Abundant and moderately abundant genes reduced and rare genes enriched in the normalized library

Quality testing: Percentage of recombinant clones and average insert size determined by gel analysis of 24 clones picked at random. Actin gene reduction determined by hybridization of primary and normalized library.



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High Quality Standard cDNA Libraries

High Quality Standard cDNA Libraries are produced using our proprietary technology. First strand cDNA is made from mRNA that is primed using oligo dT. After the second strand is synthesized, the double stranded cDNA is size fractionated, cloned directionally into our Express I vector and transformed into T1 phage resistant *E. coli*. We guarantee at least 3×10^6 primary clones with $\geq 87\%$ recombinant clones containing an average insert size of at least 1kb, however, we generally produce libraries of 10×10^6 primary clones with $>95\%$ recombinant clones containing ≥ 1.5 kb average insert size.

We will make standard libraries from customer supplied tissue (≥ 1 g), cells (at least 1×10^8), total RNA (≥ 1 mg) or mRNA (≥ 5 μ g). We provide quality controlled primary or amplified libraries.

Features list:

- High quality size fractionated cDNA.
- Directionally cloned cDNA for expression, antibody screening, subtraction and normalization.
- Large numbers of primary clones.
- Low vector background.

Quality testing: Percentage of recombinant clones and average insert size determined by gel analysis of 24 clones picked at random.

Characteristics of a cDNA Library Constructed From a Standard Amount of HeLa mRNA

mRNA (ng)	Total # of primary clones	Average insert size (kb)*	Percent recombinants	Insert size range (kb)*
5,000	12.0×10^7	2.24	96	0.5 - 4.4

*Average insert size and insert size range determined by restriction enzyme digestion of 24 clones picked at random from each library.



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High Quality Large Insert cDNA Libraries

High Quality Large Insert cDNA Libraries are constructed using our state-of-the-art methods for cDNA synthesis and size fractionation to produce libraries containing the largest cDNA inserts available. Although these libraries are made using a different process, they share the same advantageous features as our standard libraries (directional cloning into Express I and T1 phage resistant *E. coli*). We guarantee at least 1×10^6 primary clones with $\geq 87\%$ recombinant clones containing an average insert size of at least 3kb, however, we generally produce libraries of 3×10^6 primary clones with $>95\%$ recombinant clones containing ≥ 4 kb average insert size.

We will make large insert libraries from customer supplied tissue (≥ 2 g), cells (at least 2×10^8), total RNA (≥ 2 mg) or mRNA (≥ 10 μ g). We provide quality controlled primary or amplified libraries.

Features list:

- High quality size fractionated cDNA.
- Directionally cloned cDNA for expression, antibody screening, subtraction and normalization.
- Large numbers of primary clones.
- Low vector background.

Quality testing: Percentage of recombinant clones and average insert size determined by gel analysis of 24 clones picked at random.

Characteristics of a cDNA Library Constructed From a Large Insert Amount of HeLa mRNA

mRNA (ng)	Total # of primary clones	Average insert size (kb)*	Percent recombinants	Insert size range (kb)*
5,000	2.1×10^7	4.26	96	1.0 - 6.2
2,000	2.5×10^7	4.46	100	2.4 - 7.2

*Average insert size and insert size range determined by restriction enzyme digestion of 24 clones picked at random from each library.



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High Quality Microquantity cDNA Libraries

High Quality Microquantity cDNA Libraries are constructed using our state-of-the-art methods for cDNA synthesis and cloning to produce libraries from small amounts of starting material. Like our standard libraries, these libraries are directionally cloned into Express I and then transformed into T1 phage resistant *E. coli*. We guarantee at least 1×10^6 primary clones with $\geq 87\%$ recombinant clones containing an average insert size of at least 1kb, however, we generally produce libraries of 3×10^6 primary clones with $>95\%$ recombinant clones containing ≥ 1.5 kb average insert size.

We will make microquantity libraries from customer supplied tissue (<1 g), cells (at least 1×10^7), total RNA (≥ 50 μ g) or mRNA (≥ 500 ng). We provide quality controlled primary or amplified libraries.

Features list:

- High quality cDNA clones.
- Directionally cloned cDNA for expression, antibody screening, subtraction and normalization.
- Large numbers of primary clones.
- Low vector background.

Quality testing: Percentage of recombinant clones and average insert size determined by gel analysis of 24 clones picked at random.

Characteristics of a cDNA Library Constructed From a Microquantity Amount of HeLa mRNA

mRNA (ng)	Total # of primary clones	Average insert size (kb)*	Percent recombinants	Insert size range (kb)*
2,000	7.3×10^7	2.43	100	1.7 - 5.5
1,000	8.0×10^7	2.43	100	0.9 - 5.2
500	6.7×10^7	1.75	100	0.8 - 2.9

*Average insert size and insert size range determined by restriction enzyme digestion of 24 clones picked at random from each library.



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High Quality Nanoquantity cDNA Libraries

Recently, we developed methods that enable us to produce High Quality Nanoquantity cDNA Libraries from such small amounts of tissue and cells that material prepared by laser capture microdissection (LCM) or FACS can be used. Like our standard libraries, these libraries are directionally cloned into Express I and then transformed into T1 phage resistant *E. coli*. We guarantee at least 2×10^5 primary clones with $\geq 87\%$ recombinant clones containing an average insert size of at least 800 bp, however, we generally produce libraries of 5×10^5 primary clones with $>95\%$ recombinant clones containing ≥ 1.0 kb average insert size.

We will make nanoquantity libraries from customer supplied tissue (at least 250 μ g), cells (at least 25,000), total RNA (≥ 250 ng) or mRNA (≥ 5 ng). We provide quality controlled primary or amplified libraries.

Features list:

- High quality cDNA clones.
- Directionally cloned cDNA for expression, antibody screening, subtraction and normalization.
- Large numbers of primary clones.
- Low vector background.

Quality testing: Percentage of recombinant clones and average insert size determined by gel analysis of 24 clones picked at random.

Characteristics of a cDNA Library Constructed From a Nanoquantity Amount of HeLa mRNA

mRNA (ng)	Total # of primary clones	Average insert size (kb)*	Percent recombinants	Insert size range (kb)*
100	7.2×10^7	1.20	100	0.5 - 3.2
50	1.5×10^7	1.35	100	0.6 - 3.5
10	1.7×10^6	1.00	100	0.4 - 2.9
5	9.0×10^5	1.01	96	0.4 - 3.0
2	2.0×10^5	0.74	94	0.2 - 2.3
1	4.5×10^4	0.92	94	0.2 - 3.6

*Average insert size and insert size range determined by restriction enzyme digestion of 24 clones picked at random from each library.



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High Quality Subtracted cDNA Libraries

High Quality Subtracted cDNA Libraries are produced using our primary target and driver cDNA libraries. Biotinylated driver cRNA produced from the T7 RNA polymerase promoter and single-stranded (ss) target cDNA produced from the F1 ori are hybridized to each other at a high Cot (concentration of driver times the time of hybridization) value. The cRNA:cDNA hybrids are removed by phenol extraction and the remaining ss target cDNA is converted to double-stranded DNA (dsDNA) with a repair oligo and Taq DNA polymerase. After electroporation of the dsDNA into T1 phage resistant *E. coli*, subtracted clones are produced.

The subtraction process removes genes that are in common (i.e., actin) between two different libraries (i.e., normal versus disease) and enriches for unique and highly induced genes. To determine the effectiveness of the subtraction process, we compare the relative representation of the actin gene in the primary library (target) with the subtracted library by hybridization of different dilutions of the primary and subtracted dsDNA with an actin probe. Using this method, we determine that the actin gene must be reduced at least 20 fold in the subtracted library. Generally, we see at least 100 fold reduction of the actin gene in the subtracted library.

We guarantee subtracted libraries of at least 1×10^5 subtracted clones with > 87% recombinant clones containing an average insert size of at least 1 kb. However, we generally produce libraries of $>5 \times 10^5$ subtracted clones with >95% recombinant clones containing an average insert size of 1.5 kb.

Features list:

- High quality size fractionated cDNA.
- Directionally cloned cDNA for expression, antibody screening, subtraction and normalization.
- Large numbers of subtracted clones.
- Low vector background.
- Unique and highly induced genes enriched in the subtracted library.

Quality testing: Percentage of recombinant clones and average insert size determined by gel analysis of 24 clones picked at random. Actin gene reduction determined by hybridization of primary and subtracted libraries.



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Sample of a Certificate of Analysis

Murine Pancreatic Islets Amplified Primary cDNA Library

Customer:

Date: June 15, 2004

Catalog No.: 1000-1-1, Amplified Microquantity cDNA library

Customer ID No.: PO# 747469

Tissue description: mRNA isolated from mouse pancreatic islet cells. Primary clones generated for amplification were 800,000.

Vector: pExpress 1 (Not I –Eco RV)

Cells used for Electroporation: DH10B *E. coli* bacterial cells, T1 phage resistant

Diluent: 80% SOC:20%Glycerol

Number of Tubes: 18

Total Volume: 25 ml

Volume per tube: 1.4 ml

Titer: 8.0×10^{10} cfu/ml

Average insert size: 1.0 kb

Clone size range of the QC: 0.4 to 3.2 kb

Recombinants: 100%

Storage conditions: -80° C

Primer(s) for sequencing:	M13FWD	GTA AAA CGA CGG CCA GT
	M13REV	CAG GAA ACA GCT ATG AC

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