# MegaMan Human Transcriptome Library

## INSTRUCTION MANUAL

Catalog #790000 MegaMan Human Transcriptome Library (20 reactions)
#790001 MegaMan Human Transcriptome Library (100 reactions)
Revision B.0

**For Research Use Only. Not for use in diagnostic procedures.**
790000-12

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# ORDERING INFORMATION AND TECHNICAL SERVICES

## United States and Canada

**Agilent Technologies**
**Stratagene Products Division**
11011 North Torrey Pines Road
La Jolla, CA 92037
**Telephone** (858) 373-6300
**Order Toll Free** (800) 424-5444
**Technical Services** (800) 894-1304
**Internet** techservices@agilent.com
**World Wide Web** www.stratagene.com

## Europe

| Location | Telephone | Fax | Technical Services |
|---|---|---|---|
| **Austria** | 0800 292 499 | 0800 292 496 | 0800 292 498 |
| **Belgium** | 00800 7000 7000 | 00800 7001 7001 | 00800 7400 7400 |
| | 0800 15775 | 0800 15740 | 0800 15720 |
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| | 0800 182 8232 | 0800 182 8231 | 0800 182 8234 |
| **Netherlands** | 00800 7000 7000 | 00800 7001 7001 | 00800 7400 7400 |
| | 0800 023 0446 | +31 (0)20 312 5700 | 0800 023 0448 |
| **Switzerland** | 00800 7000 7000 | 00800 7001 7001 | 00800 7400 7400 |
| | 0800 563 080 | 0800 563 082 | 0800 563 081 |
| **United Kingdom** | 00800 7000 7000 | 00800 7001 7001 | 00800 7400 7400 |
| | 0800 917 3282 | 0800 917 3283 | 0800 917 3281 |

## All Other Countries

Please contact your local distributor. A complete list of distributors is available at www.stratagene.com.

# MegaMan Human Transcriptome Library

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# MegaMan Human Transcriptome Library

## MATERIALS PROVIDED

**MegaMan Human Transcriptome Library (Catalog # 790000)**

| Materials provided | Concentration | Quantity |
|---|---|---|
| MegaMan Library | 160 ng DNA/µl | 20 µl |
| Control Primer Set<br>forward primer 5′ AATGTGCATGTTCCAGCTGC 3′<br>reverse primer 5′ AAAGAGCAGGCTCTGTACTC 3′ | 10 µM of each | 10 µl |

**MegaMan Human Transcriptome Library (Catalog # 790001)**

| Materials provided | Concentration | Quantity |
|---|---|---|
| MegaMan Library | 160 ng DNA/µl | 100 µl |
| Control Primer Set<br>forward primer 5′ AATGTGCATGTTCCAGCTGC 3′<br>reverse primer 5′ AAAGAGCAGGCTCTGTACTC 3′ | 10 µM of each | 10 µl |

## STORAGE CONDITIONS

**All Reagents:** –20°C

## ADDITIONAL MATERIALS REQUIRED

Acetamide
DMSO
DNA Polymerase
Recommended: *PfuUltra* DNA Polymerase [Stratagene Catalog #600380 (100 U), #600382 (500 U), and #600384 (1000 U)]
Temperature cycler
PCR tubes[||]
PCR primers
Deoxynucleoside triphosphates (dNTPs)

## NOTICES TO PURCHASER

For use in PCR amplification only.

Manufactured for Agilent by Genofi.

[||] Thin-walled PCR tubes are highly recommended for use with Stratagene thermal cyclers. These PCR tubes are optimized to ensure ideal contact with the multiblock design to permit more efficient heat transfer and to maximize thermal-cycling performance.

Revision B.0

# INTRODUCTION

The Stratagene MegaMan human transcriptome library is a collection of cDNA created using mRNA from 66 diverse sources, including 32 from human tissues and 34 from human cancer cell lines (Table 1). This human transcriptome library is ideal for cloning both well-characterized genes and transcripts previously identified by expressed sequence tag (EST) studies. Additionally, this library can be used for the validation of predicted genes and alternative splice isoforms generated from The Human Genome sequencing data. The library contains full-length cDNA sequences, providing for downstream applications, and provides a quick screen for gene validation and discovery.

The MegaMan human transcriptome library is the largest comprehensive template of the human transcriptome in a single tube, and can be used in an extensive variety of cloning projects. This library is a complex mixture of high-quality plasmid libraries representing tens of millions of primary clones (Figure 1A). In order to maximize the probability of good representation of even the rarest, longest and most fragile transcripts, the construction of these libraries includes an enrichment protocol which singles out and pools the longest cDNAs. Further, because adapters are not ligated onto the ends of the cDNA, genomic DNA false inserts as well as fusion cDNAs from two different genes are nearly nonexistent.

In order to construct the MegaMan human transcriptome library, each individual library is size fractionated, and each fraction (low-sizing: >800 bp and high-sizing: >2000 bp) is cloned separately (Figure 1B). By treating the class sizes separately, competition at the cloning steps (i.e. ligation, transformation, and growth), which would normally select for smaller species, is eliminated. The removal of that competition increases representation of the longest transcripts. Additionally, the cloning technique used to create these libraries is extremely efficient for directional cloning of full-length cDNAs without blunting and ligating linkers onto the ends of the cDNA molecules. As a result, the cloning of genomic DNA or chimeric cDNAs from different transcripts is extremely rare.

This template is the perfect source to quickly clone any human gene, and is ideal for obtaining full-length cDNAs to be used for downstream expression analysis and functional studies (Figure 2).

**TABLE 1**

## Messenger RNA sources represented in the MegaMan Human Transcriptome Library

| Human Tissues (32) | Human Cancer Cell lines (34) |
|---|---|
| Bone Marrow | 29SR (Lymphoblast, Leukemia) |
| Brain, portion: | 786-O (Renal, Adenocarcinoma) |
| Amygdala | A549 (Lung, Carcinoma) |
| Caudate Nucleus | ACHN (Renal, Adenocarcinoma) |
| Cerebellum | BT-549 (Mammary Gland, Carcinoma) |
| Corpus Callosum | CCF-STTG1 (Brain, Astrocytoma) |
| Hippocampus | CCRF-CEM (T Lymphoblast, Leukemia) |
| Insula | COLO 205 (Colon, Adenocarcinoma) |
| Medulla Oblongata | D341 Med (Brain, Medulloblastoma) |
| Nucleus Accumbens | DBTRG-05MG (Brain, Glioblastoma) |
| Paracentral Gyrus | DLD-1 (Colon, Adenocarcinoma) |
| Pons | DMS 114 (Lung, Carcinoma) |
| Postcentral Gyrus | DU 145 (Prostate, Adenocarcinoma) |
| Putamen | HCN-1A (Brain, Cortical neuron) |
| Subthalamic Nucleus | HCT-15 (Colon, Adenocarcinoma) |
| Thalamus | HCT-116 (Colon, Carcinoma) |
| Brain, whole | HL-60 (Promyeloblast, Leukemia) |
| Heart | Hs 578T (Mammary Gland, Carcinoma) |
| Kidney | K-562 (Bone Marrow, Leukemia) |
| Liver | M059K (Brain, Glioblastoma) |
| Lung | MCF7 (Mammary Gland, Adenocarcinoma) |
| Lymph Node | MDA-MB-231 (Mammary Gland, Adenocarcinoma) |
| Placenta | MDA-MB-435S (Mammary Gland, Carcinoma) |
| Prostate | MDA-MB-468 (Mammary Gland, Adenocarcinoma) |
| Skeletal Muscle | MOLT-4 (T Lymphoblast, Leukemia) |
| Small Intestine | NCI-H460 (Lung, Carcinoma) |
| Spinal Cord | OVCAR-3 (Ovarian, Adenocarcinoma) |
| Spleen | PC-3 (Prostate, Adenocarcinoma) |
| Stomach | PFSK-1 (Brain, Neuroectodermal tumor) |
| Testis | RPMI 7951 (Skin, Melanoma) |
| Thyroid | RPMI 8226 (B Lymphocyte, Leukemia) |
| Trachea | SHP-77 (Lung, Carcinoma) |
| Uterus | SW620 (Colon, Adenocarcinoma) |
| | T-47D (Mammary Gland, Carcinoma) |

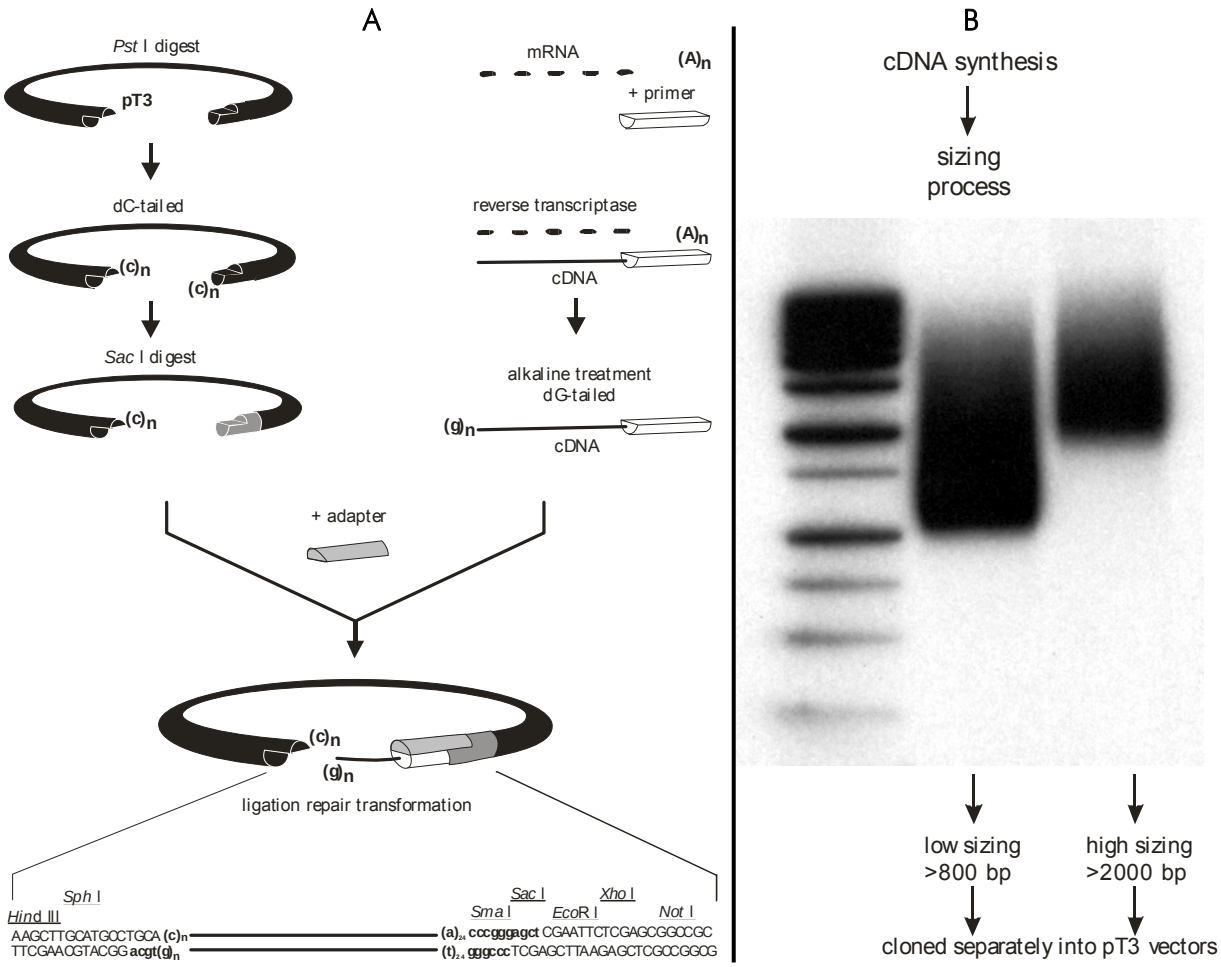


**FIGURE 1** Cloning technique used to make the MegaMan Human Transcriptome Library (A) and size fractionation of cDNA used for library construction (B).

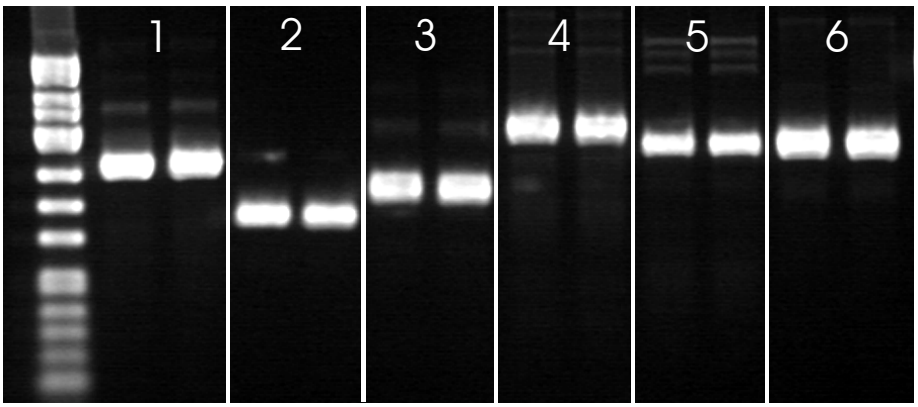


**FIGURE 2** Successful amplification in duplicate of gene fragments using *PfuUltra* DNA Polymerase. Gene fragments from low (30-60 copies per cell), medium (300-600 copies per cell) and high abundance genes (>1500 copies per cell) were used to test representation in the MegaMan library. Medium abundance genes human ADP ribosylation factor 1 and human ADP ribosylation factor 3 are represented in lanes 1 and 2, respectively; high abundance genes human cytoskeletal β actin and human cytoskeletal γ actin in lanes 3 and 4, respectively; low abundance genes human protein phosphatase 1 and human ornithine decarboxylase in lanes 5 and 6, respectively. These data show that even the rarest mRNA transcripts, such as the ornithine decarboxylase gene transcript, have been preserved.

# PROTOCOL

## PCR Protocol

1. Prepare the amplification reaction by adding the following components to a PCR tube:

***Notes*** *Acetamide and DMSO are optional components that can be added separately to reactions if less-than-satisfactory results are obtained using the standard protocol. Acetamide should be added to a final concentration of 5% (w/v) and DMSO should be added to a final concentration of 3% (v/v).*

*We offer Stratagene* PfuUltra *HF DNA Polymerase for the highest fidelity amplification [Stratagene Catalog #600380, 600382, 600384].*

### Control Reaction

1.0 µl MegaMan human transcriptome library
0.4 µl dNTP mix (25 mM of each dNTP)
2.0 µl Control Primer Set
X µl DNA polymerase
5.0 µl PCR reaction buffer (10×)
X µl nuclease-free $dH_2O$
50 µl Total reaction volume

### Experimental Reaction

1.0 µl MegaMan human transcriptome library
0.4 µl dNTP mix (25 mM of each dNTP)
2.0 µl gene-specific primers (10 µM of each primer)
X µl DNA polymerase
5.0 µl PCR reaction buffer (10×)
X µl nuclease-free $dH_2O$
50 µl Total reaction volume

### Reaction Amplification

1. Amplify the DNA using the following PCR cycling conditions:

| Number of Cycles | Temperature | Duration |
|---|---|---|
| 1 cycle | 94°C | 3 minutes |
| 35 cycles | 94°C | 45 seconds |
| | 58°C | 30 seconds |
| | 72°C | 1 minute/kb[a] |
| 1 cycle | 72°C | 7 minutes |

[a] A one minute minimum is required for the 72°C elongation step.

### Expected Control Amplification Results

Amplification of the control primer amplicon should result in a PCR product of 753 bp in length.

## TROUBLESHOOTING

| Observations | Suggestions |
|---|---|
| Poor amplification of low-abundance mRNAs | Increase the amount of MegaMan human transcriptome library to 2 µl per amplification reaction. |
| Low or no yield | Increase the number of PCR amplification cycles to 40. |
| | Amplify using either acetamide (5% final concentration) or DMSO (3% final concentration). |
| | Amplify using 2.5 U/reaction of *PfuUltra* HF DNA Polymerase |
| | Increase the amount of DNA Polymerase used per reaction (e.g. increase *PfuUltra* HF DNA Polymerase to 5.0 U/reaction). |

## MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.

STRATAGENE
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# MegaMan Human Transcriptome Library

Catalog #790000 and 790001

## QUICK REFERENCE PROTOCOL

### PCR Protocol

- Prepare the amplification reactions by adding the following components to a PCR tube:

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*Agilent offers Stratagene* PfuUltra *HF DNA Polymerase for the highest fidelity amplification [Stratagene Catalog #600380, 600382, 600384].*

### Control Reaction

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2.0 µl Control Primer Set
X µl DNA polymerase
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X µl nuclease-free $dH_2O$
50 µl Total reaction volume

### Experimental Reaction

1.0 µl MegaMan human transcriptome library
0.4 µl dNTP mix (25 mM of each dNTP)
2.0 µl gene-specific primers (10 µM of each)
X µl DNA polymerase
5.0 µl PCR reaction buffer (10×)
X µl nuclease-free $dH_2O$
50 µl Total reaction volume

### Reaction Amplification

- Amplify the DNA from the PCR protocol using the following cycling conditions:

| Number of Cycles | Temperature | Length of Time |
|---|---|---|
| 1 cycle | 94°C | 3 minutes |
| 35 cycles | 94°C | 45 seconds |
| | 58°C | 30 seconds |
| | 72°C | 1 minute/kb[a] |
| 1 cycle | 72°C | 7 minutes |

[a] *A one minute minimum is required for the 72°C elongation step.*

### Expected Control Amplification Results

- Amplification of the control primer amplicon should result in a PCR product 753 bp in length.