Transcriptome Discovery

ExactSTART™ Full-Length cDNA Library Cloning Kit*

Create a directionally cloned full-length cDNA library from as little as 1 µg of total RNA in only 2 days. The kit facilitates selective cloning of cDNA made from full-length polyadenylated RNA, permitting identification of the exact 5′ ends of RNA transcripts. Tags are added to 5′ and 3′ ends of the first-strand cDNA, providing priming sites for PCR amplification. The amplified dsDNA products can be used to generate templates for sequencing or to generate labeled target cDNA for microarray expression analysis. Alternatively, they can be cloned into the vector included in the kit for generating a full-length-selected cDNA library.

Cat. #	Quantity
ES0907	10 Reactions

Covered by issued and/or pending patents; see www.EpiBio.com/legal.

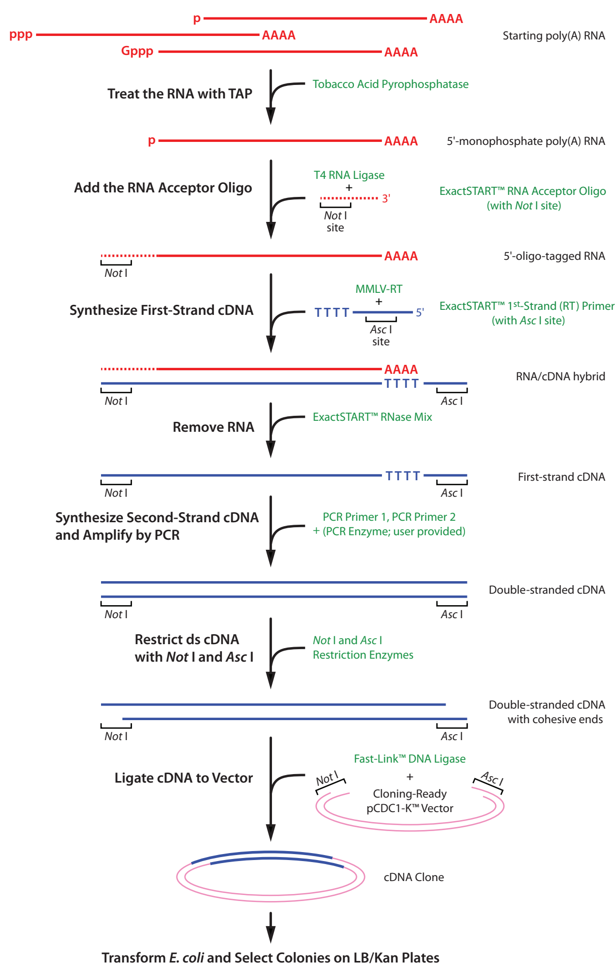

Figure 1. Schematic overview of the process for the ExactSTART™ Full-Length cDNA Library Cloning Kit. The simplified protocol can produce a library in as little as 2 days.

ExactSTART™ Mammalian Small RNA Cloning Kit*

Generate a cloned library derived from small mammalian RNA transcripts in 2 days, starting with a total RNA sample. The kit selectively tags and converts small RNAs that contain 5′-monophosphate/3′-hydroxyl ends or 5′-triphosphate/3′-hydroxyl ends to amplified dsDNA. Species of RNA tagged by the kit include mammalian miRNA, other small noncoding RNAs, and small primary transcripts. A simple RNA size-selection procedure removes large RNAs to ensure that only small RNAs are converted to dsDNA and subsequently cloned. An optimized RNA ligation reaction is used to tag the exact 5′ end of the transcript, and a high-efficiency polyadenylation/cDNA synthesis reaction is used to tag the 3′ end. The amplified dsDNA is ligated into the pCDC1-K™ Cloning-Ready Vector provided and then transformed into a competent *E. coli* host.

Cat. #	Quantity
ES81005	5 Reactions

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ExactSTART™ Eukaryotic mRNA 5′- & 3′-RACE Kit*

Precisely identify the 5′ and 3′ends of eukaryotic mRNAs in less than 1 day with this kit. Treatment with APex™ Heat-Labile Alkaline Phosphatase and Tobacco Acid Pyrophosphatase is used to select RNA with a 5′ cap, triphosphate, or monophosphate. A T4 RNA Ligase-mediated tagging process selectively adds an RNA oligonucleotide, containing a PCR priming site, to the 5′ end of this RNA. The 3′ end of the RNA is tagged by reverse transcription using an oligo(dT) primer with a PCR priming site at its 5′ end. The tagged cDNA is converted to dsDNA and amplified by PCR. Preservation of the extreme 5′ end of the mRNA molecule allows for accurate mapping of transcriptional start sites.

Cat. #	Quantity
ES80910	10 Reactions

Covered by issued and/or pending patents; see www.EpiBio.com/legal.

RNA Amplification

TargetAmp™ 1-Round Biotin-aRNA Amplication Kit 105

Produce microgram amounts of biotin-aRNA for microarray analysis from as little as 25 ng of total cellular RNA in this single-tube, 6-hour protocol. The kit uses a linear amplification method to achieve at least 5,000-fold amplification of poly(A) RNA present in a total cellular RNA sample. The biotin-aRNA produced by the kit detects more transcripts than competitive kits, and shows high correlation with MAQC TaqMan® data. Multiple RNA samples can be amplified and labeled

simultaneously, and the linear amplification process preserves the transcript profile of the sample.

Cat. #	Quantity
TAB1R80510	10 Reactions
TAB1R80524	24 Reactions

RiboMultiplier™ Sense-RNA Amplication Kit*

Generate amplified sense-strand RNA (sRNA) from the poly(A) RNA contained in a total RNA sample in 1 day with this kit. Microgram amounts of sRNA can be generated from as little as 10 ng of input total RNA with essentially no background amplification. Using a unique terminal-tagging process, the kit produces amplified sRNA with a greatly improved 3′/5′ ratio and greatly reduced 3′-bias compared to other oligo(dT)-primed RNA amplification processes. Both sRNA-derived and unamplified RNA-derived targets produced microarray results that correlated very well with MAQC TaqMan® results, indicating a faithful representation of the original gene expression profile. Use the aRNA for archiving RNA samples for future use, real-time PCR studies, or microarray analysis requiring sRNA target.

Cat. #	Quantity
RM80510	10 Reactions

Covered by issued and/or pending patents; see www.EpiBio.com/legal.

dsDNA Conversion Kit for RiboMultiplier™ RNA

Convert the sRNA generated with the RiboMultiplier™ Sense-RNA Amplification Kit to dsDNA with this kit and use as a target for DNA expression arrays, templates for next-gen sequencing, or cDNA library construction. The kit is designed to convert up to 500 µg of RiboMultiplier sRNA to dsDNA. The kit should only be used to prepare dsDNA from sRNA produced by the RiboMultiplier Kit.

Cat. #	Quantity
RMD80625	25 Reactions

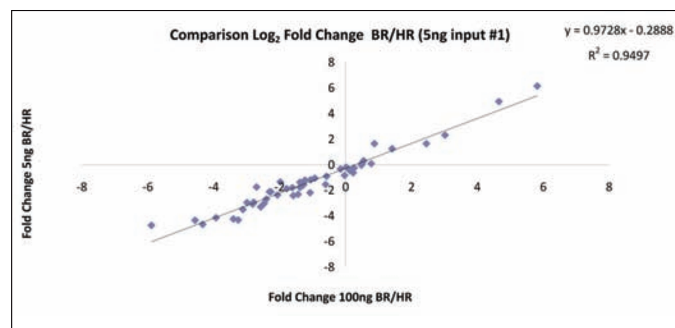

Figure 2. Fidelity of RiboMultiplier™ amplification. Using the Nanostring nCounter® Analysis System, hybridizations were performed using 100 ng of Universal Human Reference (HR) and Brain Reference (BR) RNA from either amplified (test) or unamplified (control) samples. Raw data were normalized to internal positive spike-in controls present in every reaction. The correlation of Log_2 fold-change values (Brain Reference/Human Reference RNA) obtained with test and with control samples was determined. The amount of input total RNA in the RiboMultiplier amplification process was 5 ng.

DNA & RNA Purification

QuickExtract™ Seed DNA Extraction Solution

Rapidly extract PCR-ready genomic DNA from most ground or fragmented seed samples using a simple, one-tube protocol that takes only 8 minutes. Process one to hundreds of samples simultaneously, without centrifugation, spin columns, or use of any toxic organic solvents. Simply add the QuickExtract Seed Solution to the ground sample and perform two sequential heating steps. A small aliquot of the sample mix is then used as a template for PCR or qPCR. Most ground seed material under 10 mg is acceptable for DNA extraction, and the procedure is suitable for high-throughput or automated workflows.

Cat. #	Quantity
QES08095T	5 ml (50 Extractions)
QES080950	50 ml (500 Extractions)

End-Point PCR

PlantAmp™ PCR System

PCR amplification of genomic DNA from plants such as sunflower and cotton is challenging due to the presence of inhibitory compounds. The presence of polyphenols also makes it difficult to detect DNA of spoilage bacteria in wine samples. The PlantAmp™ PCR System is formulated to optimize PCR amplification from samples containing polyphenols, as well as GC-rich templates. The PlantAmp PCR Enzyme Mix is an enzyme blend that includes 3′→5′ proofreading activity for high PCR fidelity. The PlantAmp 2X PCR PreMix includes all four dNTPs and $MgCl_2$, and is formulated for optimal PCR results from samples containing polyphenols and other plant-derived inhibitors.

Cat. #	Quantity
PA0809100	100 Units
PA08091K	1,000 Units

TAQXpedite™ PCR System (FAST End-Point)

Using a standard thermocycler, the TAQXpedite PCR System (FAST End-Point) achieves fast PCR results with total reaction time as little as 16 minutes for a 500-bp amplicon. The kit contains a unique blend of thermostable DNA polymerases, an optimized $MgCl_2$ concentration, and two MasterMixes (for universal amplications and difficult/long amplifications). The MasterMix contains EPICENTRE's patented PCR Enhancer which improves the yield, efficiency, and specificity of amplication of many target sequences, especially those with high GC content (up to 80%) or secondary structure. The kit is useful for high-throughput formats and multiplex PCR.

Cat. #	Quantity
TXP78200	200 x 25-µl Reactions
TXP78001	1,000 x 25-µl Reactions

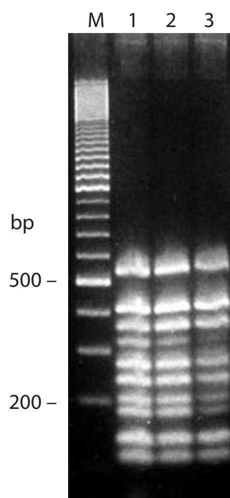

Figure 3. Efficient multiplex PCR of purified blood DNA. A 100-ng sample of human genomic DNA purified with the MasterPure™ DNA Purification Kit for Blood Version II was amplified using the TAQXpedite™ PCR System with nine primer sets representing different exons of the dystrophin gene. All nine amplicons were produced in three amplification reactions (lanes 1-3). Lane M, 100-bp DNA ladder.

Vector and Insert Preparation

End-It™ DNA End Repair Kit

The End-It™ DNA End-Repair Kit converts DNA containing damaged or incompatible 5′- and/or 3′-protruding ends to 5′-phosphorylated, blunt-ended DNA with high efficiency. The kit can be used to repair sheared, nebulized, or restriction enzyme-digested genomic DNA for preparation of templates for next-gen sequencing, blunt-end cloning into plasmid, cosmid, or fosmid vectors, or shotgun library preparation. It can also be used to prepare BAC or fosmid clones for subclone library construction. The end-repaired, 5′-phosphorylated DNA can be efficiently ligated into a blunt-ended, dephosphorylated cloning vector or to next-gen sequencing adapters using EPICENTRE's Fast-Link™ DNA Ligation Kit.

The new 50-reaction End-It DNA End-Repair Kit contains reagents for repair of up to 250 µg of genomic DNA.

Cat. #	Quantity
ER0720	20 Reactions (100 µg genomic DNA)
ER81050 *New!*	50 Reactions (250 µg genomic DNA)

Enzymes for Molecular Biology

DisplaceAce™ DNA Polymerase

A recombinant DNA polymerase derived from the thermophilic bacterium *Geobacillus kaustophilus*, this enzyme has been altered by truncation to remove the 5′→3′ exonuclease activity of the full-length enzyme. It has strong strand-displacing DNA polymerase activity similar to that of *Bacillus* DNA polymerases. In addition to its DNA-dependent DNA polymerase activity (optimal at 65°C), it also has RNA-dependent DNA polymerase activity. Use this enzyme for first-strand cDNA synthesis from primed RNA templates, or in most applications that call for strand-displacing DNA polymerases.

Cat. #	Concentration*	Quantity
D0806250	5 U/µl	100 Units
D08061K	5 U/µl	1,000 Units

**Higher concentrations available soon; see www.EpiBio.com.*

rGka DNA Polymerase

A recombinant DNA polymerase derived from thermophilic bacterium *Geobacillus kaustophilus*, this enzyme has both DNA and RNA-dependent DNA polymerization activity and 5′→3′exonuclease activity. Use this enzyme for high-temperature DNA synthesis, and first-strand cDNA synthesis from primed RNA templates.

Cat. #	Concentration*	Quantity
G0808250	5 U/µl	100 Units
G08081K	5 U/µl	1,000 Units

**Higher concentrations available soon; see www.EpiBio.com.*

RNA 5′ Polyphosphatase

A Mg^{2+}-independent phosphohydrolase discovered and characterized by EPICENTRE scientists, RNA 5′ Polyphosphatase** is the only commercially available phosphohydrolase that sequentially removes the γ- and β- phosphates from 5′-triphosphorylated RNA transcripts (uncapped primary RNA transcripts) such as bacterial mRNA and some noncoding eukaryotic RNAs. RNAs with 5′-diphosphorylated ends are also converted to 5′-monophosphorylated RNA by the enzyme for use in 5′-RNA ligation-tagging methods. The enzyme has no activity on RNA with a 5′-capped or a 5′-monophosphorylated end. However, both NTPs and dNTPs are substrates for RNA 5′ Polyphosphatase, yielding the corresponding NMPs and dNMPs, and inorganic phosphate.

The unique activity of the enzyme and its strict substrate specificity make it an important component of some of EPICENTRE's new ExactSTART™ Platform for transcriptome discovery and analysis (see p. 4). The 5′-monophosphorylated RNA produced can be used for RNA Ligase-Mediated (RLM) RACE and other oligoribonucleotide acceptor strategies, for preparing RNA transcripts for high-throughput sequencing (RNA-Seq), or to analyze the 5′-end structure of RNA.

Cat. #	Concentration	Quantity
RP8092H	20 U/µl	200 Units

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Tobacco Acid Pyrophosphatase

EPICENTRE's Tobacco Acid Pyrophosphatase (TAP) is the most frequently cited TAP enzyme in the scientific literature. TAP is most commonly used to remove the 5′- cap structure from eukaryotic mRNAs. However, TAP will also remove the γ,β-phosphates from 5′-triphosphorylated RNAs (e.g., uncapped primary transcripts such as bacterial mRNAs and some noncoding eukaryotic RNAs). The 5′-monophosphorylated RNA produced by a TAP reaction readily serves as "donor" for RLM 5′ tagging of RNAs for transcriptome analysis methods. Its unique activity and specificity makes the enzyme an important component of EPICENTRE's new ExactSTART™ Kits for transcriptome discovery and analysis (see p. 4).

TAP can be used to characterize the 5′-end moiety of RNA transcripts or prepare templates for RLM 5′-tagging of eukaryotic mRNA and other primary RNA transcripts, or for 5′-RACE. It can also be used to prepare RNA transcripts for high-throughput sequencing (RNA-Seq), such as next-gen sequencing.

Cat. #	Concentration	Quantity
T81050 *New!*	5 U/µl	50 Units
T19050	10 U/µl	50 Units
T19100	10 U/µl	100 Units
T19250	10 U/µl	250 Units
T19500	10 U/µl	500 Units

FailSafe™ PROBES Real-Time PCR Optimization Kit

Designing probes for real-time PCR can be time-consuming and expensive. Given this effort, it is also important that PCR conditions are optimized to ensure the best results for a specific set of probes. The FailSafe™ PROBES Real-Time PCR System enables rapid, precise optimization of PCR experiments in which the product is detected using fluorescent, target-specific labeled probes.

The FailSafe PROBES Real-Time PCR System uses the FailSafe™ PCR Enzyme Blend, which has been proven to amplify the most difficult templates with extremely high specificity, sensitivity, and fidelity, and a set of eight specially chosen PCR 2X PreMixes representing a complete range of optimal real-time PCR conditions. Each PCR PreMix contains everything needed for optimal real-time PCR for a certain group of template and primer sequences, including EPICENTRE's FailSafe PCR Enhancer with betaine,* which improves amplification specificity, efficiency, and sensitivity. In addition, the specially designed PreMixes, in combination with the robust FailSafe PCR Enzyme Blend, enable setup of reactions at room temperature, so hot-start PCR is not necessary.

Cat. #	Quantity
FSP51048	48 x 25-µl reactions

**Covered by issued and/or pending patents; see www.EpiBio.com/legal.*

Real-Time PCR

TAQXpedite™ GREEN Real-Time PCR MasterMix Kit

Fast PCR can be achieved using specialized thermocyclers, or by using enzyme/reagent combinations that improve PCR efficiency. Using any standard real-time thermocycler, the TAQXpedite™ GREEN Real-Time MasterMix Kit is able to achieve fast PCR results in as little as 30 minutes.

The TAQXpedite GREEN Real-Time PCR MasterMix Kit includes a unique blend of thermostable DNA polymerases with an optimized 2X MasterMix solution containing all four dNTPs, $MgCl_2$ and Green Dye 12. The solution also contains EPICENTRE's patented PCR Enhancer (with betaine*), which substantially improves the yield and specificity of amplification of many target sequences, especially those containing a high GC content or secondary structure. In addition, betaine also may enhance PCR by protecting DNA polymerases from thermal denaturation. The TAQXpedite™ GREEN Kit can also be used in high-throughput real-time PCR applications.

Cat. #	Quantity
TXG70796	96 x 25-µl reactions
TXG707400	400 x 25-µl reactions

**Covered by issued and/or pending patents; see www.EpiBio.com/legal.*

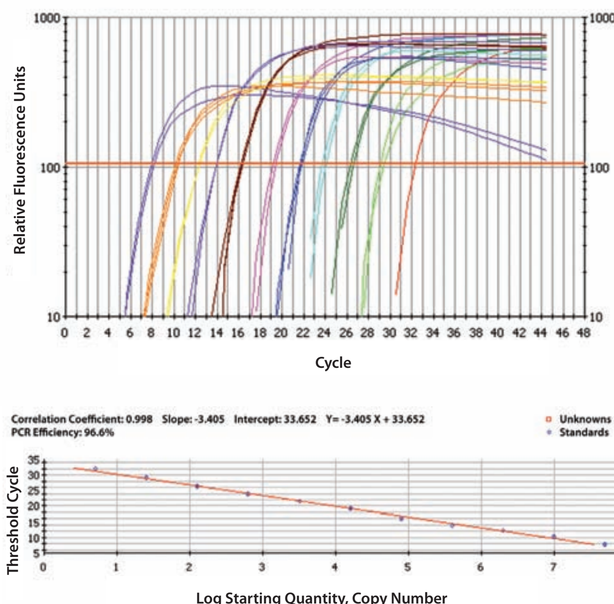

Figure 4. Sensitivity and dynamic range of the TAQXpedite™ GREEN Kit. Serial dilutions of lambda DNA from 4.88 x 10^7 to 5 copies were amplified. Each real-time PCR included 1X MasterMix, 12.5 pmol each of forward and reverse primers, and template. Cycling conditions were 30 seconds at 98°C, followed by 45 cycles of 1 second at 92°C and 6 seconds at 70°C. The entire reaction was completed in 30 minutes. This serial dilution demonstrated a consistent qPCR and a reaction efficiency of 96.6%.