

## Using ImProm-II™ Reverse Transcriptase to Construct cDNA Libraries

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### Abstract

Promega's new ImProm-II™ Reverse Transcription System, in conjunction with components from the Universal RiboClone® cDNA Synthesis System, can be used to generate high-titer, full-length cDNA libraries that are comparable to those generated using other cDNA synthesis systems.

**Table 1. Comparison of First- and Second-Strand cDNA Synthesis for ImProm-II™ and SuperScript™ II Reverse Transcriptases.**

	ImProm-II™ Reaction	SuperScript™ II Reaction
Yield of First-Strand cDNA	17.3%	21.0%
Yield of Second-Strand cDNA	90.6%	82.0%

The ImProm-II™ Reverse Transcriptase allows for robust and efficient synthesis of first-strand cDNA for use in cDNA library construction.

### Introduction

Promega's ImProm-II™ Reverse Transcription System (Cat.# A3800) combines a newly formulated reverse transcriptase, an optimized reaction buffer and associated reagents qualified for efficient synthesis of first-strand cDNA. Previous work has demonstrated the use of the ImProm-II™ Reverse Transcription System for generation of first-strand cDNA for either uncoupled (1) or coupled (2) RT-PCR<sup>(a)</sup>. An additional use for this robust reverse transcriptase is the construction of high-titer, full-length cDNA libraries. Generating and screening cDNA libraries is a common step in cloning novel genes, species-specific gene homologs, and new gene family members.

In this article, we use the ImProm-II™ Reverse Transcriptase System in combination with components of the Universal RiboClone® cDNA Synthesis System<sup>(b,c)</sup> (Cat.# C4360) to generate high-quality, double-stranded cDNA suitable for cloning into an appropriate lambda or plasmid cDNA library vector. ImProm-II™ Reverse Transcriptase generates cDNA comparable to that generated using the SuperScript™ II Choice System for cDNA Synthesis (Invitrogen).

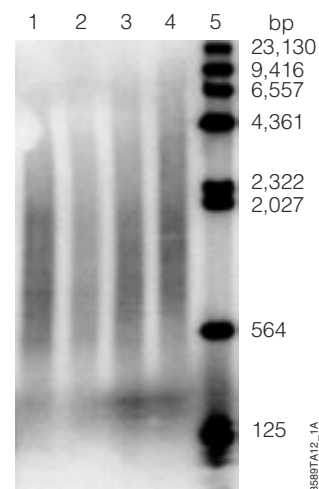
### Phage cDNA Library Yields

We used TCA precipitation and radiolabeled first- and second-strand cDNA reactions to determine incorporation and calculate the yield of first-strand and second-strand cDNA (3). The ImProm-II™ and SuperScript™ II Systems produced comparable yields for both first- and second-strand cDNA (Table 1). Alkaline agarose gel analysis (3) of both the radiolabeled first- and second-strand cDNA products also showed comparable results for both ImProm-II™ and SuperScript™ II Systems (Figure 1). We observed the expected smear, migrating from approximately 200 to 9,500 bases, with the predominant products between 500 and 4,300 bases.

We purified each double-stranded cDNA sample using the Wizard® DNA Clean-Up System<sup>(d)</sup> (Cat.# A7280), following the protocol in the accompanying Technical Bulletin, #TB141 (4). The purified double-stranded cDNA generated using either ImProm-II™ or SuperScript™ II was then ligated to *Eco*R I Adaptors (Cat.# C1291), phosphorylated, and size-selected using Sephacryl® S-400 resin as described in reference 3. We inserted the purified, adaptor-ligated cDNA into the Lambda ZAP II vector, packaged the library, and determined the titer using blue/white screening according to the manufacturer's directions (Stratagene). The cDNA libraries generated using either ImProm-II™ or SuperScript™ II gave similar titers and percentage of plaques with inserts (Table 2).

### Generation of a Plasmid cDNA Library

To further demonstrate the utility of ImProm-II™ Reverse Transcriptase for generating cDNA libraries, we purified mRNA from hemocyte cells of the caterpillar



**Figure 1. Alkaline agarose gel analysis of first- and second-strand cDNA products synthesized by either ImProm-II™ Reverse Transcriptase (lanes 1 and 3) or SuperScript™ II Reverse Transcriptase (lanes 2 and 4). Lane designations: lanes 1 and 2, 10,000cpm first-strand cDNA; lanes 3 and 4, 10,000cpm second-strand cDNA; lane 5, Lambda Hind III Marker (Cat.# G1711). The dried gel was exposed to film for 3 hours.**

**Table 2. Titers and Characterization of cDNA Libraries Created Using ImProm-II™ Reverse Transcriptase or SuperScript™ II Reverse Transcriptase for First-Strand cDNA Synthesis.**

	ImProm-II™ Reverse Transcriptase	SuperScript™ II Reverse Transcriptase
cDNA Library Titer	6.9 × 10 <sup>6</sup> pfu/ml	5.3 × 10 <sup>6</sup> pfu/ml
Percent of Plaques with Insert (blue in total number of blue + white plaques)	85.0%	80.0%

species *Pseudoplusia includens*. We reverse transcribed the purified RNA using either ImProm-II™ Reverse Transcriptase or SuperScript™ II Reverse Transcriptase in the presence of methyl dCTP to protect against subsequent restriction enzyme digestion of the synthesized cDNA. Second-strand cDNA was synthesized, ligated to *EcoR* I linkers, digested with *EcoR* I, and finally ligated into the plasmid vector pCMV-Script™ (Stratagene). We transformed electrocompetent DH5α-E™ cells with the ligated cDNA and vector and plated the entire ligation and transformation onto large LB/kanamycin (30μl/ml) plates. We overlaid the cDNA colony containing plates with LB medium, pooled the eluted colonies, and purified the plasmid DNA. This pool of plasmid constitutes the unamplified plasmid cDNA library. Agarose gel analysis of an aliquot of each purified plasmid library revealed comparable smears between 4–9kbp for both ImProm-II™- and SuperScript™ II-generated libraries, with the majority of the plasmid clones between 4–7kb, representing cDNA inserts of up to 3kb. However, both libraries did have some clones up to 12kb in length, which would represent inserts of approximately 8kb. We gel-purified the plasmid smear greater than 4kb and retransformed electrocompetent DH5α-E™ cells with the purified DNA. Both the ImProm-II™ and SuperScript™ II libraries yielded approximately 1 × 10<sup>7</sup>cfu/μg.

## Methods for Constructing Phage Library

**mRNA Purification:** We used the PolyATtract® System 1000 (Cat.# Z5420) to purify mRNA from 2 × 10<sup>8</sup> Jurkat cells (5). An aliquot of this mRNA (1μg) provided the template for generating first-strand cDNA using either ImProm-II™ Reverse Transcriptase or SuperScript™ II Reverse Transcriptase (see Table 3).

**Table 3. First-Strand cDNA Synthesis Annealing Reaction.**

Component	ImProm-II™ System	SuperScript™ II System
Oligo(dT) (0.5μg/μl)	2.0μl	2.0μl
Jurkat mRNA (200ng/μl)	5.0μl	5.0μl
Nuclease-Free Water	7.25μl	8.75μl

**First-Strand Synthesis:** The reactions were assembled and incubated first at 70°C for 5 minutes then on ice for 5 minutes. Table 4 lists the components that were added to each reaction. We removed an aliquot of each first-strand cDNA reaction (5μl) and labeled it with 5μl [ $\alpha$ -<sup>32</sup>P]-dCTP (10mCi/ml; 400Ci/mmol) (3) to allow for visualization of the first-strand reaction products. The assembled reactions were incubated for 5 minutes at 25°C, followed by 60 minutes at 42°C. The radiolabeled reaction was terminated by adding 95μl of 50mM EDTA.

**Table 4. First-Strand cDNA Synthesis Reaction.**

Component	ImProm-II™ System	SuperScript™ II System
5X Reaction Buffer (provided with reverse transcriptase)	5.0μl	5.0μl (3mM final MgCl <sub>2</sub> )
0.1M DTT	–	2.0μl
25mM MgCl <sub>2</sub>	3.0μl (3.0mM final MgCl <sub>2</sub> )	–
10mM dNTP Mix	1.25μl	1.25μl
RNasin® Ribonuclease Inhibitor <sup>(c)</sup>	0.5μl	–
Reverse Transcriptase	1.0μl	1.0μl
<b>Final Reaction Volume (including annealing reaction)</b>	<b>25.0μl</b>	<b>25.0μl</b>

**Second-Strand Synthesis:** Table 5 outlines the second-strand synthesis reaction. The Universal RiboClone® cDNA Synthesis System contains the Second Strand 2.5X Reaction Buffer, BSA, DNA Polymerase I, and RNase H. We removed an aliquot of each assembled second-strand cDNA synthesis reaction (10μl) and labeled it with [ $\alpha$ -<sup>32</sup>P]-dCTP (10mCi/ml; 400Ci/mmol) (3) to allow for visualization of the generated double-stranded cDNA. The reactions were incubated for 4 hours at 14°C, followed by 10 minutes at 70°C. We added two units of T4 DNA Polymerase to each reaction and incubated the reactions for 10 minutes at 37°C. The reactions were terminated with 10μl of 200mM EDTA or 90μl of 50mM EDTA for the radioactive tracer reaction.

**Table 5. Second-Strand cDNA Synthesis.**

Component	Volume
First-Strand cDNA Reaction	20.0μl
Second-Strand 2.5X Reaction Buffer	40.0μl
BSA (1mg/ml)	5.0μl
DNA Polymerase I (23 units)	2.6μl
RNase H (0.8 units)	0.8μl
<i>E. coli</i> DNA Ligase (8 units, New England Biolabs)	0.8μl
Nuclease-Free Water	30.8μl
<b>Final Reaction Volume</b>	<b>100.0μl</b>

## Summary

ImProm-II™ Reverse Transcriptase compares to SuperScript™ II Reverse Transcriptase in the generation of first-strand cDNA and results in similar cDNA library construction with respect to yield of first- and second-strand cDNA, library titer, percent of plaques with inserts and average insert size. This is true for both lambda and plasmid vectors. Thus the ImProm-II™ Reverse Transcriptase allows for robust and efficient synthesis of first-strand cDNA for use in cDNA library construction, and second-strand synthesis can be accomplished using components of the Universal RiboClone® cDNA Synthesis System.

## References

1. *ImProm-II™ Reverse Transcription System Technical Manual #TM236*, Promega Corporation.
2. Miller, K. (2001) *Promega Notes* **79**, 15–17.
3. *Universal RiboClone® cDNA Synthesis System Technical Manual #TM038*, Promega Corporation.
4. *PolyAtract® System 1000 Technical Manual #TM228*, Promega Corporation.
5. *Wizard® DNA Clean-Up Technical Bulletin #TB141*, Promega Corporation.

## Protocols

- ◆ *ImProm-II™ Reverse Transcription System Technical Manual #TM236*, Promega Corporation.  
([www.promega.com/tbs/tm236/tm236.html](http://www.promega.com/tbs/tm236/tm236.html))
- ◆ *PolyAtract® System 1000 Technical Manual #TM228*, Promega Corporation.  
([www.promega.com/tbs/tm228/tm228.html](http://www.promega.com/tbs/tm228/tm228.html))
- ◆ *Universal RiboClone® cDNA Synthesis System Technical Manual #TM038*, Promega Corporation.  
([www.promega.com/tbs/tm038/tm038.html](http://www.promega.com/tbs/tm038/tm038.html))
- ◆ *Wizard® DNA Clean-Up Technical Bulletin #TB141*, Promega Corporation.  
([www.promega.com/tbs/tb141/tb141.html](http://www.promega.com/tbs/tb141/tb141.html))



## Ordering Information

Product	Size	Cat.#
ImProm-II™ Reverse Transcription System*	1,000 reactions	A3800
ImProm-II™ Reverse Transcriptase*	10 reactions	A3801
	100 reactions	A3802
	500 reactions	A3803
Universal RiboClone® cDNA Synthesis System	1 system	C4360
PolyAtract® System 1000*	Scalable	Z5420
Wizard® DNA Clean-Up System*	100 preps	A7280

\*For Laboratory Use.

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*pCMV-Script is a trademark of Stratagene. DH5α-E and SuperScript are trademarks of Invitrogen Corporation. Sephacryl is a registered trademark of Amersham Pharmacia Ltd.*

<sup>(a)</sup> The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.

<sup>(b)</sup> U.S. Pat. No. 5,552,302, Australian Pat. No. 646803 and other patents.

<sup>(c)</sup> U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

<sup>(d)</sup> U.S. Pat. Nos. 5,658,548, 5,808,041, Australian Pat. No. 689815 and other patents pending.