Recovery of productive antibody variable regions from hybridoma RNA using SuperScript V Reverse Transcriptase

Introduction

Monoclonal antibodies generated from hybridoma cell lines are important tools for research, diagnostic, and therapeutic applications, and determining their immunoglobulin heavy- and light-chain variable-region sequences provides a molecular record that supports recombinant expression, antibody engineering, clone verification, and long-term preservation. However, recovering these sequences from hybridoma RNA can be challenging due to the high diversity of antigen-binding regions, while conventional amplification methods often require multiple variable-region or degenerate primers. Template-switching reverse transcription offers a streamlined alternative by using an immunoglobulin constant-region RT primer and a template-switching oligonucleotide (TSO) to introduce a defined sequence at the 3′ end of the cDNA [1]. This TSO-derived sequence serves as a universal priming site for downstream PCR, reducing the need for broad primer panels while enabling amplification across diverse antibody transcripts (Figure 1).

This application note demonstrates the use of Invitrogen™ SuperScript™ V Reverse Transcriptase in a customizable template-switching workflow for sequencing mouse hybridoma antibody variable regions. Using chain-specific constant-region primers and amplicon sequencing, the workflow supports recovery of heavy-chain and light-chain variable regions from hybridoma RNA without prior knowledge of the antibody variable-region sequence (Figure 2).

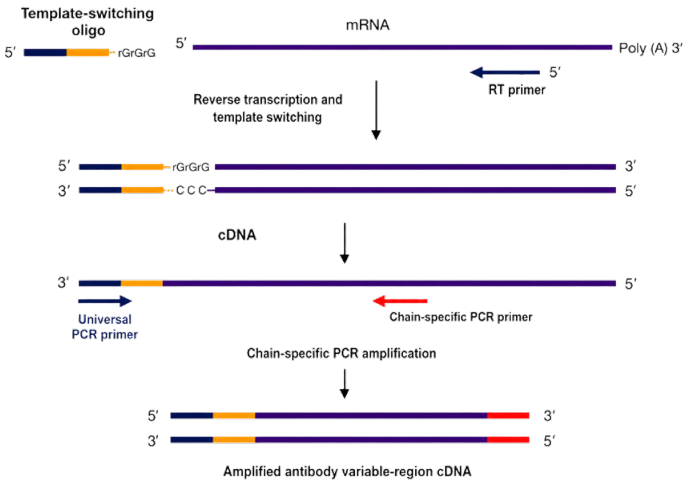

Figure 1. Principle of template-switch reverse transcription and antibody variable-region amplification. A chain-specific reverse transcription primer initiates first-strand cDNA synthesis from the immunoglobulin transcript. Upon reaching the 5′ end of the RNA template, SuperScript V Reverse Transcriptase adds non-templated cytosine residues to the 3′ end of the cDNA. These residues anneal to the TSO, enabling incorporation of a universal primer-binding sequence at the cDNA terminus. The resulting cDNA is amplified using a universal primer complementary to the incorporated template-switch sequence and a chain-specific PCR primer.

Hybridoma antibody sequencing workflow

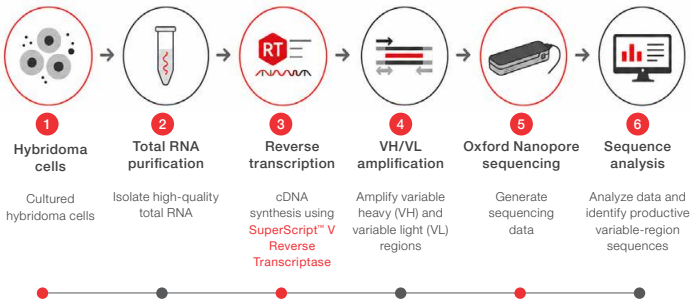

Figure 2. Workflow for recovery and sequencing of antibody variable regions from hybridoma RNA. Total RNA isolated from cultured hybridoma cells is used for template-switch reverse transcription with SuperScript V Reverse Transcriptase. Heavy-chain and kappa-chain variable-region cDNA are amplified by chain-specific PCR, purified, and sequenced using the Oxford Nanopore Technologies™ platform. Consensus sequences were analyzed using the National Center for Biotechnology Information (NCBI) IgBLAST tool to identify productive antibody variable-region rearrangements.

Methods

Total RNA purification from hybridoma cells

Three hybridoma cell cultures designated H1, H2, and H3 were used for total RNA isolation. Approximately 1 × 10^7 cells were processed per sample. Total RNA was purified using the Invitrogen™ RiboPure™ RNA Purification Kit according to the manufacturer's instructions, with chloroform used for phase separation. Purified RNA was eluted in 200 µL of elution buffer.

RNA concentration and purity were assessed by UV spectrophotometry, and integrity was evaluated using the Agilent™ RNA 6000 Nano assay. RNA concentrations ranged from 528 to 607 ng/µL. All samples showed high purity and high integrity, with RNA integrity numbers of 9.9–10.0.

Template-switch reverse transcription

First-strand cDNA synthesis was performed in a 20 µL template-switch reverse transcription reaction using SuperScript V Reverse Transcriptase. Reactions were prepared according to the recommendations in the user guide, using 5.6 µM of TSO and 4.35 µM of a specific reverse transcription primer.

Separate reactions were prepared for immunoglobulin kappa-chain and heavy-chain transcripts using the corresponding reverse transcription primers listed in Table 1. RNA inputs ranging from 5 to 250 ng were evaluated. Reverse transcription was performed at 50°C for 5–30 min, followed by enzyme inactivation at 95°C for 1 min.

Table 1. Primers used for template switching RT and PCR reactions.

Oligonucleotide	Sequence (5′→3′)	Function	Cat. No.
TSO	AAGCAGTGGTATCAACGCAGAGTACATrGrGrG	Incorporates TSO sequence to 3′ end of cDNA	A65427
mIGKRT*	TTGTCGTTCACTGCCATCAATC	Kappa-chain reverse transcription primer	Custom
mIGHGRT*	AGCTGGGAAGGTGTGCACAC	Heavy-chain reverse transcription primer	Custom
Preamplification primer	AAGCAGTGGTATCAACGCAGAGT	Used for amplification from incorporated TSO sequence	A65428
mIGKPCR*	ACATTGATGTCTTTGGGGTAGAAG	Kappa-chain PCR primer	Custom
mIGHGPCR*	GGGATCCAGAGTTCCAGGTC	Heavy-chain PCR primer	Custom

* Primer sequences adapted from Meyer L, López T, et al. (2019) [2].

Amplification of antibody variable-region cDNA

Template-switched cDNA was amplified with Invitrogen™ Platinum™ SuperFi™ II Green PCR Master Mix according to the manufacturer's recommendations. Each 20 µL reaction contained 10 µL 2X Platinum SuperFi II Green PCR Master Mix, 0.25 µL commercial preamplification forward primer (Cat. No. A65428), 0.5 µM of the appropriate immunoglobulin chain-specific reverse primer (Table 1), and 2 µL of cDNA. The reverse primer was selected according to the primer used for reverse transcription: mIGKPCR1 was used for kappa-chain reactions, and mIGHGPCR1 was used for heavy-chain reactions. PCR was performed with an initial denaturation at 98°C for 30 sec, followed by 25 cycles of 98°C for 10 sec, 60°C for 10 sec, and 72°C for 15 sec, with a final extension at 72°C for 5 min.

Agarose-gel analysis and amplicon purification

PCR products were initially evaluated by electrophoresis on 1% agarose gels prepared in TAE buffer. Additional PCR reactions were then performed under the same cycling conditions to generate sufficient amplicon for preparative purification. Bands corresponding to the expected amplicon sizes were excised from the gel and purified using the Thermo Scientific™ GeneJET™ Gel Extraction and DNA Cleanup Micro Kit according to the manufacturer's instructions. Purified DNA concentration was measured by UV spectrophotometry, and fragment size and quality were assessed using the Agilent™ High Sensitivity DNA assay.

Oxford Nanopore sequencing

DNA fragments excised and purified from the gel were sequenced using the Oxford Nanopore Technologies platform. Consensus sequences were obtained for each amplicon and analyzed using NCBI IgBLAST against mouse immunoglobulin reference databases.

Results

Template-switch RT-PCR of antibody variable regions

SuperScript V Reverse Transcriptase was evaluated in chain-specific template-switch reactions targeting immunoglobulin kappa-chain and heavy-chain transcripts. Reactions were tested using 5–250 ng of total RNA and reverse transcription times of 5–30 min at 50°C. The resulting cDNA was amplified using a universal primer complementary to the incorporated template-switch sequence, together with a kappa-chain- or heavy-chain-specific PCR primer.

PCR amplification for 25 cycles generated products in the expected size range for both chain types (Figure 3). Amplification was observed across all tested reverse transcription times and RNA inputs, including reactions containing 5 ng of total RNA. Based on the overall amplification profiles, a 10 min reverse transcription time and an RNA input of 50 ng were selected for subsequent experiments and applied to RNA from hybridomas H1, H2, and H3. To generate sufficient material for preparative electrophoresis and purification, PCR was performed in replicate reactions, and the replicate products were pooled before gel separation and purification.

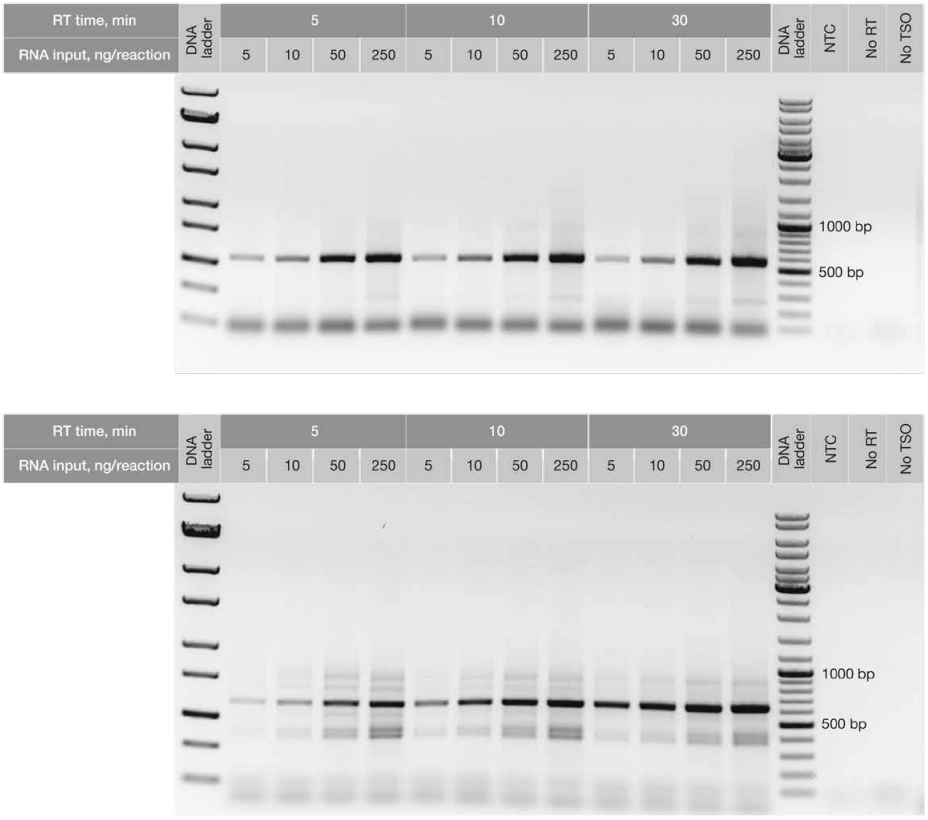

Figure 3. Amplification of antibody variable kappa-chain and heavy-chain regions. RT-PCR products corresponding to kappa-chain **(A)** and heavy-chain **(B)** variable regions were analyzed by electrophoresis on 1% agarose gels prepared in TAE buffer. Negative controls included a no-template control (NTC), a no-RT control containing all reaction components except SuperScript V Reverse Transcriptase, and a no-TSO control containing all reaction components except the TSO. Thermo Scientific™ ZipRuler™ Express DNA Ladder 1 and GeneRuler™ DNA Ladder Mix were used as size standards.

The pooled amplicons were resolved on 1% agarose gels, excised at the expected sizes, and purified for downstream sequencing. Purified amplicons were analyzed using the Agilent High Sensitivity DNA assay (Figure 4) and sequenced using Oxford Nanopore technology.

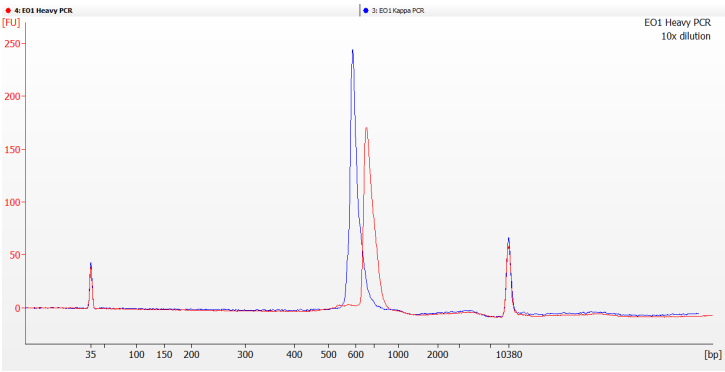

Figure 4. Capillary electrophoresis analysis of purified antibody variable-region amplicons. Representative electropherograms of purified kappa-chain (blue) and heavy-chain (red) variable-region amplicons analyzed using the Agilent High Sensitivity DNA assay. The main peaks correspond to products of approximately 580 bp and 660 bp, respectively.

Nanopore sequencing and IgBLAST analysis of antibody variable regions

Using the selected conditions of a 50 ng RNA input and a 10 min reverse-transcription reaction, kappa- and heavy-chain variable-region amplicons were recovered from all three hybridomas and sequenced on an Oxford Nanopore Technologies platform. Peak-derived consensus sequences were analyzed using NCBI IgBLAST against mouse immunoglobulin reference databases [3].

IgBLAST identified productive kappa-chain and heavy-chain variable-region sequences for all three hybridomas. All analyzed consensus sequences were in frame and contained no stop codons or V-region frameshifts. The nucleotide identity of the variable regions to the closest mouse germline matches ranged from 95.6% to 98.5% for kappa chains and from 93.9% to 96.9% for heavy chains. An identifiable CDR3 was present in each recovered variable-region sequence.

Exact V(D)J assignments, CDR3 sequences, and complete variable-region sequences are proprietary and are therefore not disclosed. Instead, productivity, reading-frame status, germline identity, and CDR3 recovery are reported as non-proprietary indicators of sequence quality (Table 2).

Table 2. Summary of non-proprietary IgBLAST results for Nanopore consensus sequences.

BLAST analysis outcome	Kappa chain	Heavy chain
Hybridomas with a productive sequence identified	3/3	3/3
In-frame rearrangement	3/3	3/3
Sequences containing a stop codon	0/3	0/3
Sequences containing a V-region frameshift	0/3	0/3
Hybridomas with an identifiable CDR3	3/3	3/3
V-region identity to the closest mouse germline V-gene match	95.6–98.5%	93.9–96.9%
Distinct variable-region assignment for each hybridoma	Yes	Yes

Independent verification of antibody variable regions

Antibody samples were submitted for independent liquid chromatography-mass spectrometry (LC-MS/MS) analysis to support sequence confirmation. For multiple samples, experimentally determined peptides from tryptic or Glu-C digests mapped to candidate heavy-chain and kappa-chain sequences, supporting the sequences derived from nucleic acid–based analysis. For the sequences evaluated, peptide-level evidence confirmed greater than 96% sequence coverage.

Conclusions

This proof-of-concept study demonstrates the use of SuperScript V Reverse Transcriptase for template-switch-assisted recovery of antibody variable regions from hybridoma RNA. Rapid template-switch reverse transcription generated kappa- and heavy-chain amplicons suitable for sequencing, enabling identification of productive, sequence-resolved variable regions. The resulting sequence information can support downstream antibody cloning, recombinant expression, and sequence archiving.

The template-switching capability of SuperScript V Reverse Transcriptase may support workflows that benefit from incorporating a universal 3′ sequence during cDNA synthesis.

References

1. Zhu YY, Machleder EM, Chenchik A, Li R, Siebert PD. 2001. Reverse transcriptase template switching: A SMART™ approach for full-length cDNA library construction. *BioTechniques.* 30(4):892–897. doi:10.2144/01304pf02.
2. Meyer L, López T, Espinosa R, Arias CF, Vollmers C, DuBois RM. 2019. A simplified workflow for monoclonal antibody sequencing. *PLOS One.* 14(6):e0218717. doi:10.1371/journal.pone.0218717.
3. Ye J, Ma N, Madden TL, Ostell JM. 2013. IgBLAST: an immunoglobulin variable domain sequence analysis tool. *Nucleic Acids Research.* 41:W34–40. doi:10.1093/nar/gkt382.

Ordering information

Product	Quantity	Cat. No.
RiboPure RNA Purification Kit	50 preps	AM1924
SuperScript V Reverse Transcriptase	50 reactions	19090050
Invitrogen™ RNaseOUT™ Recombinant Ribonuclease Inhibitor	5,000 units	10777019
Invitrogen™ dNTP Mix, 10 mM each	100 µL	18427013
Invitrogen™ Template Switching Reverse Transcription Oligonucleotides	48 reactions	A65427
Preamplification primer	48 reactions	A65428
Platinum SuperFi II Green PCR Master Mix	100 reactions	12369010
GeneJET Gel Extraction and DNA Cleanup Micro Kit	50 preps	K0831

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