# Improved Universal Vectorette System and Pre-Made Vectorette Genomic DNA Libraries

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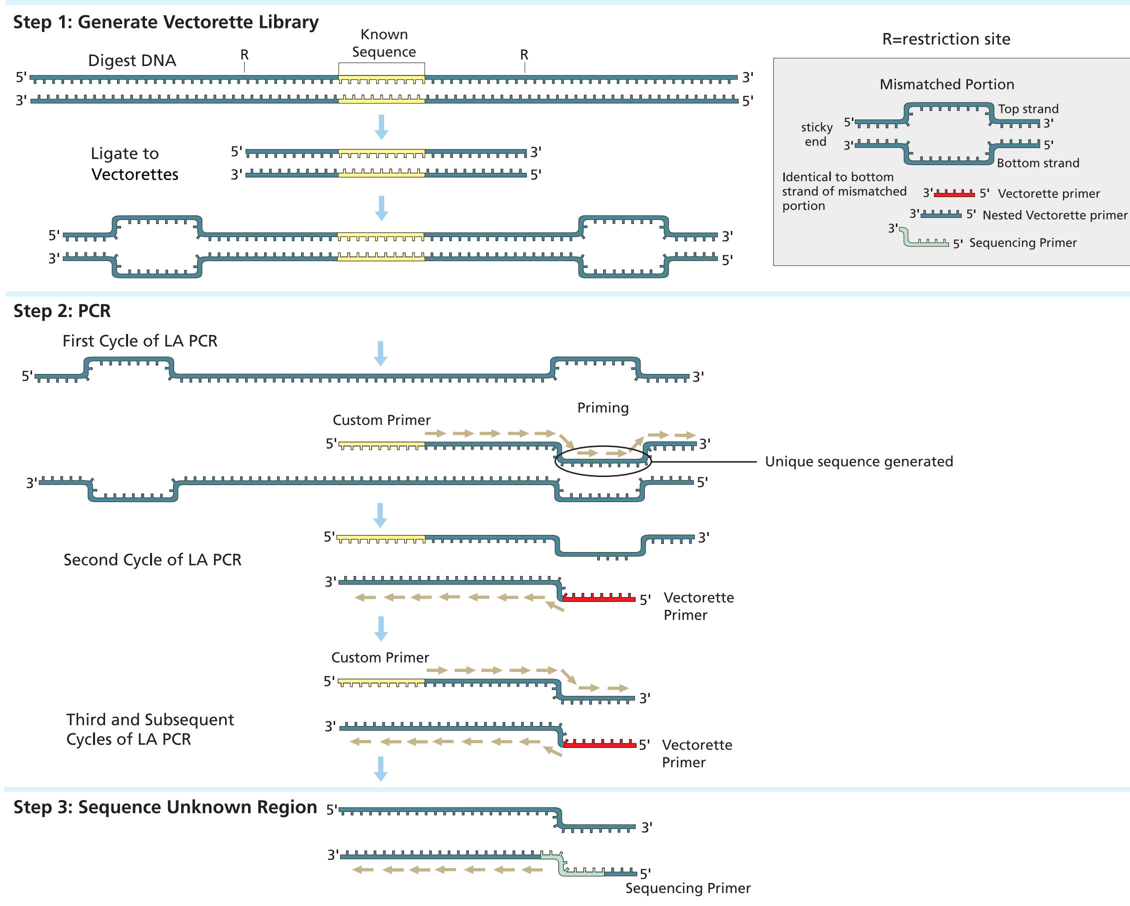
## Introduction

Sigma's Vectorette system facilitates the PCR amplification of DNA where the primer sequence site of only one end is known. Researchers use the Vectorette system because it allows specific amplification of any uncharacterized sequence adjacent to a known region.[1] Specific applications of the Vectorette system include genome walking,[2,3] determination of genomic sequence at YAC/vector junctions,[4-7] isolation and characterization of end sequences in BAC DNA inserts to facilitate contig formation,[8] identifying and sequencing intron/exon boundaries in genes,[9,10] revealing 5' and 3' untranscribed sequence of promoter/enhancer regions that control transcription[11] and most recently, identifying genomic sequences flanking a transgene in genetically modified organisms.

Sigma's Universal Vectorette System has three steps, as shown in Figure 1. The first step is to generate Vectorette DNA libraries by digesting the target DNA with a restriction enzyme that produces a compatible overhang with one of the Vectorette units supplied in the Universal Vectorette System. Preparing several libraries with different restriction enzymes practically ensures the user their uncut region of interest is contained in one or more of the libraries. The first step is complete with the addition of Vectorette unit adapter ends which are ligated onto the previously generated fragments. These adapters have a unique composition, which increases the specificity of the second step - PCR amplification. In the final step (Step 3), the PCR fragment can be directly sequenced yielding the desired sequence information.

The key to the Vectorette system lies in the design of the Vectorette unit adapter. This DNA duplex consists of a non-complementary region flanked by complementary DNA on either side. The Vectorette primer is identical to a sequence in the unpaired region (the bottom strand in Figure 1, Step 3), is not complementary to any portion of DNA in the mixture, and therefore cannot prime PCR. The user-defined, specific primer thus provides the only primed region, and allows first strand synthesis to occur. Once a complementary strand is produced, the Vectorette primer has a perfect spot to anneal and the newly generated, doubly primed DNA region goes through PCR. The Vectorette system essentially places all specificity in one user-defined primer.

Figure 1. Vectorette System Process



The kit is built with flexibility in mind, to accommodate the needs of the researcher. Several different types of Vectorette libraries will soon be available from Sigma-Aldrich, allowing the researcher to bypass this first step of creating Vectorette libraries. By starting out with the pre-made Vectorette libraries, the researcher is able to immediately go into Vectorette primary PCR. In some cases, Vectorette primary PCR may be followed by secondary PCR, further amplifying the product of interest with a nested specific primer and the nested Vectorette primer supplied in the kit. In the final step, the amplified DNA sequence of interest may be directly sequenced or cloned into a suitable vector by using Vectorette unit adapter sequences specifically engineered for this purpose.

The Vectorette system has several advantages over existing technologies. Vectorette is a system that allows for cell-free manipulation. Vectorette PCR replaces conventional library construction and screening which is tedious and time consuming. A small amount of sequence information is all that is needed to design target-specific primers, and only a limited amount of starting material is needed because of the sensitivity and specificity of Vectorette PCR.

This report describes recent improvements to the Universal Vectorette System and shows data using the Vectorette libraries available from Sigma-Aldrich. A list of the improvements to the Universal Vectorette System include using a recommended touchdown cycling protocol, using a high specific primer to Vectorette primer ratio in Vectorette primary PCR, designing primers with a 3′ GC clamp, using a long and accurate DNA polymerase blend with a hot start mechanism and adding Lambda DNA positive control reagents to the system. The positive control reagents can be used to verify whether all of the steps in the Universal Vectorette System are working correctly. We offer pre-made Vectorette libraries for human, mouse, and rat genomic DNA giving researchers the ability to go right into Vectorette primary PCR. With these libraries the researcher only needs to obtain a specific primer in order to perform Vectorette primary PCR.

## Materials and Methods

All materials were supplied by Sigma-Aldrich Corporation (St. Louis, MO) and Sigma-Genosys (Woodlands, TX) unless otherwise stated. Human genomic DNA was obtained from a commercial source.

### *Vectorette Conditions*

Vectorette experiments were conducted using different procedures for either Lambda DNA or human genomic DNA. The methods are described in the Universal Vectorette System technical bulletin (Product Code UVS-1.) and the Vectorette Library technical bulletin (Product Codes HUMAN-VS, MOUSE-VS and RAT-VS). Sanger DNA sequencing methods were used to sequence Vectorette PCR amplicons directly using either the specific primer or the Vectorette sequencing primer.

The Lambda DNA digestion was performed in a 50-µl reaction for one hour using 1X restriction enzyme buffer, 10 units of restriction enzyme and approximately 1 µg of Lambda DNA (Product Code D 3779) using the buffer and temperature suggested by the supplier. Digestion was immediately followed by a Lambda DNA-Vectorette unit ligation in a final volume of 30 µl containing 20 µl of the restriction enzyme digest, 0.1 µM of the appropriate Vectorette units, 1 unit of T4 DNA ligase and 3.3 mM of ATP. The reaction was incubated at 16 °C for one hour and at the recommended restriction enzyme temperature for 30 minutes for two cycles, followed by a final 16 °C for one hour incubation period. The ligation reaction was then diluted up to 100 µl with sterile water. Two microliters of the diluted ligation reaction was added to a 50-µl PCR containing 1X AccuTaq™ buffer (50 mM Tris-HCl, pH 9.3, 15 mM ammonium sulfate, 2.5 mM $MgCl_2$), 1 µM Lambda specific primer, 0.1 µM Vectorette primary primer, 2.5 units of JumpStart™ REDAccuTaq™ LA DNA Polymerase Mix or AccuTaq LA and 200 µM each dNTP. Vectorette Lambda DNA touchdown PCR consisted of 7 cycles of a 15-second denaturation at 94 °C and a 1 minute per kb of expected amplicon annealing/extension at 72 °C followed by 32 cycles of a 15-second denaturation at 94 °C and a 1 minute per kb of expected amplicon annealing/extension at 68 °C. A final extension period of 1 minute per kb of amplification product at 68 °C was applied in the last cycling step. Vectorette Lambda DNA standard PCR consisted of 35 cycles of a 30-second denaturation at 94 °C, a 30-second annealing at 65 °C and a one minute per kb of amplification product at 68 °C. A final extension period of 1 minute per kb of amplification product was applied in the last cycling step.

Genomic DNA was digested using an identical procedure to the Lambda DNA Vectorette digestion reaction except the incubation time was extended to two hours. Reactions designed to find the optimal conditions for genomic DNA ligation to the Vectorette unit adapters was accomplished in a series of reactions consisting of 30 µl containing 16 µl of the above digestion, 4.2 - 0.006 picomoles of the appropriate Vectorette units, 2 - 0.001 units of T4 DNA ligase, 23.3 - 3.3 mM ATP, and 3.3 mM DTT. The ligation cycling protocol used for Lambda DNA was also used for genomic DNA. When analyzing these reactions for ligation yield, individual reactions were diluted 3.5-fold with sterile water, and 2 µl of this diluted genomic DNA ligation reaction was added to a 50 µl PCR using the same components at the same concentrations that were used in the Vectorette PCR using Lambda DNA. Genomic DNA Vectorette PCR used the same touchdown cycling protocol as previously described, but the length of the denaturation step was shortened from 15 to two seconds. Nested PCR was performed with 1 µl of a 1:1000 dilution of the primary PCR as the template. The cycling parameters and reaction conditions in the nested Vectorette genomic PCR are identical to those for Lambda DNA, with the exception that both the nested specific and nested Vectorette primers are at 0.4 µM.

Vectorette PCR amplicons for direct sequencing were generated by Vectorette primary and secondary (nested) PCR, which had been purified after agarose gel fractionation. The PCR amplicons were purified using the GenElute™ Gel Purification kit (Product Code: GEN-PCR). The amount of PCR amplicon added to each of the 20 µl sequencing reactions contained 7 fg of DNA. Each 20 µl sequencing reaction contained either a specific primer or a Vectorette sequencing primer at 0.5 µM final concentration, 4 µl of ABI PRISM® BigDye™ (Applied Biosystems, Foster City, CA) sequencing mix and 4 µl of SeqSaver (Product Code: S 3938). An aliquot of each sequencing reaction was run on an ABI PRISM 377 DNA Sequencer following standard protocols and the sequence data generated was compared against other sequence data by performing a BLAST search at the National Center for Biotechnology web site (http://www.ncbi.nlm.nih.gov/BLAST/).

## Results

### *Specificity is improved by using a touchdown PCR cycling protocol*

Vectorette primary PCR was performed using *Eco*R I and *Hin*d III Lambda DNA Vectorette libraries using either a standard PCR cycling protocol or a touchdown PCR cycling protocol[12] as described in the Materials and Methods. A single specific primer was used for both the *Eco*R I and *Hin*d III Lambda DNA Vectorette libraries. The expected primary PCR amplicon size for the *Eco*R I Lambda DNA Vectorette library was approximately 12 kb and the expected primary PCR amplicon size for the *Hin*d III Lambda DNA Vectorette library was 14 kb. Under the standard PCR cycling conditions only a large smear is observed at the expected fragment sizes on an ethidium-stained agarose gel. When the touchdown PCR cycling protocol was used a distinct band was seen for the *Eco*R I Lambda DNA Vectorette library 12 kb fragment size and several distinct bands approximately 14 kb in size were seen when the *Hin*d III Lambda Vectorette library was amplified in Vectorette primary PCR (Figure 2 ).

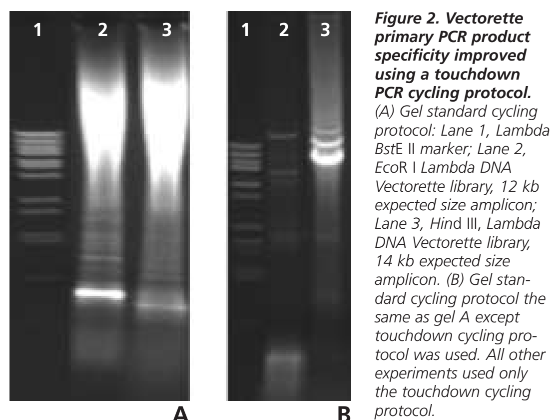



***Figure 2. Vectorette primary PCR product specificity improved using a touchdown PCR cycling protocol.*** *(A) Gel standard cycling protocol: Lane 1, Lambda Bst*E II *marker; Lane 2, Eco*R I *Lambda DNA Vectorette library, 12 kb expected size amplicon; Lane 3, Hin*d III, *Lambda DNA Vectorette library, 14 kb expected size amplicon. (B) Gel standard cycling protocol the same as gel A except touchdown cycling protocol was used. All other experiments used only the touchdown cycling protocol.*

### *A high specific primer to Vectorette primer ratio gives increased yield in Vectorette primary PCR*

Vectorette primary PCR using the *Cla* I Lambda DNA Vectorette library was run with three different ratios of specific primer to Vectorette primer. In this experiment, PCR with an equal amount of specific primer to Vectorette primary only gave negative results. As the amount of specific primer was increased and the amount of Vectorette primer was decreased, a significant yield of the expected 6-kb amplicon was observed (Figure 3).

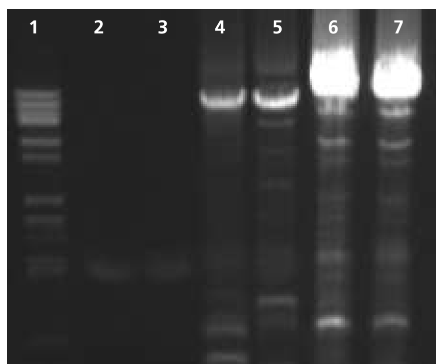



***Figure 3. Vectorette primary PCR product sensitivity and yield is improved by utilizing a high-specific primer to Vectorette primer ratio.*** *Vectorette primary PCR expected amplicon is an 8-kb fragment from a Cla* I *Lambda DNA Vectorette library. Lane 1, Lambda Bst*E II *marker; Lanes 2 and 3, Specific primer 0.4 μM, Vectorette primer 0.4 μM; Lanes 4 and 5, Specific primer 0.8 μM, Vectorette primer 0.1 μM; Lanes 6 and 7, Specific primer 1 μM, Vectorette primer 0.1 μM.*

### *Specific primers with an unconventional primer design give greater Vectorette PCR yield*

A series of specific primers were designed against the human β-globin gene. Vectorette primary PCR using differently designed primers was compared against specific primers designed using conventional primer design criteria. The *Cla* I human genomic DNA Vectorette library was used for these experiments. Primers used in these experiments were designed at approximately the same position in the human β-globin gene, differing only in the position of the GC clamp. In all cases, primer length was adjusted so the $T_m$ and GC content of the primers were equivalent. In Figure 4, the results from lanes 2 and 3, with a 5′ GC clamp primer, are compared against the results from lanes 8 and 9, with a 3′ GC clamp primer. Lanes 8 and 9, with the 3′ GC clamp primer, show more yield. The results from lanes 4 and 5, with a 3′ GC clamp primer, are compared against the results from lanes 10 and 11, with a 3′ and a 5′ GC clamp primer. Lanes 10 and 11, with the 3′ and 5′ GC clamp primer, show more yield. The results from lanes 6 and 7, with a 3′ GC clamp primer, are compared against the results from lanes 12 and 13, with a 5′ GC clamp primer. Lanes 6 and 7, with the 3′ GC clamp primer, show more yield. These results show two major points: (1) Primers with a 3′ GC clamp give greater yields than primers with the conventional 5′ GC clamp, and (2) Primers with two GC clamps (3′ clamp and 5′ GC clamp) show greater yields than a primer with only one GC clamp (in this case at the 3′ end).

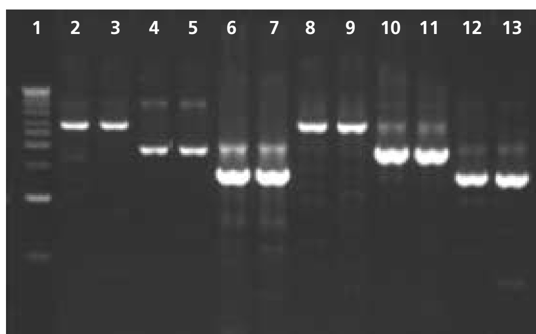



***Figure 4. Vectorette primary PCR yield improved by unconventional primer design.*** *Lane 1, DNA Ladder, 1 kb; Lanes 2 and 3, 5′ GC clamp, primer 1; Lanes 4 and 5, 3′ GC clamp, primer 2; Lanes 6 and 7, 3′ GC clamp, primer 3; Lanes 8 and 9, 3′ GC clamp, primer 1B; Lanes 10 and 11, 5′ & 3′ GC clamp, primer 2B; Lanes 12 and 13, 5′ GC clamp, primer 3B. Expected PCR product sizes from Human Cla* I *Vectorette library using the following β-globin primers: Primers 1and 1B, 3 kb; Primers 2 and 2B, 1.9 kb; Primers 3 and 3B, 1.4 kb.*

*Vectorette PCR sensitivity and yield is improved by using PCR Hot Start mechanism*

Five Lambda DNA Vectorette libraries were made using the appropriately paired restriction enzyme and Vectorette unit adapter combinations. Each was subjected to Vectorette primary PCR using one specific primer. Each primer was expected to generate a unique product of differing size, depending upon the restriction enzyme used to generate the library. The experiment showed that reactions using AccuTaq LA with JumpStart antibody afforded greater yields and improved sensitivity over reactions performed with just AccuTaq LA (Figure 5).

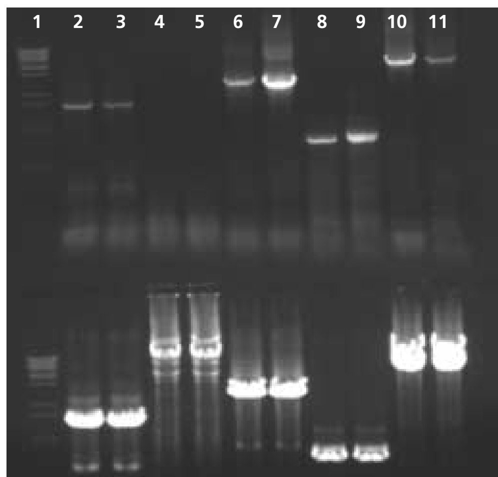



***Figure 5. Universal Vectorette system Lambda DNA positive control primary PCR yield and sensitivity is improved by adding a PCR Hot-Start mechanism.*** *Lambda DNA Vectorette Library PCR products using control primer provided in kit. Top Level AccuTaq LA: Lane 1, Lambda* BstE II *marker; Lanes 2 and 3,* BamHI, *1.9 kb; Lanes 4 and 5,* Cla I, *8.1 kb; Lanes 6 and 7,* EcoR I, *3 kb; Lanes 8 and 9,* Hind III, *1.1 kb; Lanes 10 and 11,* Sma I, *4.8 kb. Bottom level: Same as top level but with JumpStartRED AccuTaq LA.*

*Vectorette PCR product can be sequenced from both ends directly by using the Vectorette sequencing primer or the specific primer*

*Cla* I, *Nar* I, *Acc* I, and *Hpa* II are all alternative restriction enzymes generating an overhang compatible with the *Cla* I Vectorette unit. These enzymes were tested for their ability to generate PCR amplicons which, after Vectorette primary and secondary PCR, could be directly sequenced. Specific primers directed against exons in the human tissue plasminogen activator gene and the human β-globin gene were used for primary Vectorette PCR and nested Vectorette PCR as described in Materials and Methods. After secondary PCR the products were fractionated on an agarose gel and bands of the expected size were excised. These bands were purified using GenElute Gel Purification kit, then subjected to sequencing reactions as described in the Materials and Methods section using both the specific- and the Vectorette-sequencing primer. Sequencing in this manner allowed data to be generated on both ends of the PCR amplicon. Seven PCR amplicons were sequenced resulting in fourteen sequencing reactions. Each set of sequences gave the expected matches for either the human TPA gene or the human β-globin gene after a BLAST search. All seven PCR amplicons were of the expected size (data not shown).

*Optimization of the Vectorette ligation reaction using quantitative PCR for Vectorette library production*

Following the digestion of genomic DNA with a restriction enzyme, Vectorette unit adapters specific to the restriction enzyme were ligated to both ends of the digested fragments producing a Vectorette library. A series of experiments was executed to determine the optimal amount of T4 DNA ligase, Vectorette units, and ATP needed in the Vectorette ligation reaction to achieve maximum efficiency. The degree of ligation was measured using quantitative PCR with primers spanning an amplicon across the ligation site. In these experiments an early detection of the threshold cycle ($C_T$, i.e. the cycle at which PCR product is detected over noise[13]) corresponds to higher levels of starting (ligated) template, indicating a more efficient ligation condition. Ligation reactions were run with different amounts of T4 DNA ligase ranging between 2 units and 0.001 units. One unit of T4 DNA ligase gave the lowest threshold cycle in Vectorette primary quantitative PCR, indicating this amount as the most effective ligation reaction condition. Vectorette units were added in a range between 4.2 picomoles and 0.06 picomoles to 320 ng of DNA digest. ATP was also added in a range between 23 mM and 3.3 mM to 320 ng of DNA digest. Vectorette units at the 3-picomole level and ATP at the 3.3-mM level gave the lowest threshold cycles indicating these levels provided the best conditions to generate Vectorette libraries (data not shown).

## Discussion

In this study we have demonstrated improvements to the Universal Vectorette System, which have enhanced the system's specificity, sensitivity, yield, and convenience to the user. The use of stringent primer design, touchdown PCR, and an optimized primer ratio greatly improve Vectorette specificity, sensitivity, and yield. Including a Hot Start mechanism, by adding JumpStart AccuTaq LA, likewise improves reaction sensitivity and yield. A set of ligation conditions provides optimized yields. The kit includes a set of controls so that each step can be analyzed, an addition to assist both the first-time user and the researcher troubleshooting their process. Finally, we have been able to show that the Vectorette procedures can be successfully applied to get sequencing data.

Primer design is the most user-controlled variable in Vectorette, because one primer effectively controls the specificity of the reaction. Conventional PCR primers are designed with a GC clamp at the 5' end of the primer, which ensures that the primer will bind tightly to the complementary sequence while mispriming is minimized. Designing a primer with a 3' GC clamp would normally cause excessive mispriming because any binding of the 3' end of primer to template will allow extension.[14] The primer design software used to design these primers with GC clamps at different locations calculated the internal stability of five base pair segments in each primer. Lower $\Delta G$, or free energy values, indicate a more stable region around those five nucleotides when bound in the DNA duplex. In these experiments, stretches of five nucleotides in primers that had continuous free energy values below $-8\ \Delta G$ were identified as GC clamps.[15] In Vectorette primary PCR, primers designed with a 3' GC clamp have been found to increase yield without increasing the amount of nonspecific product formation. We speculate that this is due to the asymmetrical nature of Vectorette PCR. Specific primer design of primers that bind stronger than normal is needed because the annealing temperature in touchdown PCR is elevated and the specific primer is the only primer binding during the first cycle of PCR. This specific primer is the main factor responsible for the specificity and sensitivity of primary Vectorette primary PCR. The goal in specific primer design

for Vectorette primary PCR is to balance the binding efficiency and specificity at the 3' end of the primer with the touchdown cycling protocol. Other specific primer design criteria that improve the results of Vectorette primary PCR include: (1) designing primers that avoid hairpin formations, (2) designing primers which do not form primer-dimers, (3) designing primers with a $T_m$ between 72 °C and 74 °C, and (4) designing primers in conserved exons and designing primers where the 3' ends of the primers land on the first or second position of a codon to avoid mismatches caused by synonymous mutations.

The touchdown cycling protocol recommended for Vectorette primary PCR has several initial cycles at an annealing/extension temperature approximately equal to the $T_m$ of the specific and Vectorette primers. These seven cycles enhance the specificity of PCR by allowing annealing only under highly stringent conditions. The remaining cycles, which are carried out at an annealing/extension temperature at 5 °C below the $T_m$ of the specific and Vectorette primers, is useful to increase the yield of the PCR amplicon in Vectorette primary PCR. In general, touchdown PCR biases the system to specificity, which is more important in the Vectorette system than overall yield.

The high specific primer to Vectorette primer ratio in Vectorette primary PCR further contributes to increase sensitivity and yield. This strategy is effective because of the design of the Vectorette adaptor. As described in the Introduction, Vectorette units are double-stranded DNA with a stretch of mismatched nucleotides in the middle. The system is set so that only the user specified primer can anneal to the DNA, and so the first cycle of PCR is asymmetrical. The first cycle generates a compliment to the Vectorette primer, so that in the second and subsequent cycles, PCR continues normally to amplify the targeted sequence. A greater amount of specific primer allows more synthesis of the complementary strand to the bottom strand of the Vectorette unit in the first cycle of PCR. This means more template is available for both of the primers in the second cycle of Vectorette primary PCR. Less Vectorette primer ensures less mispriming in the first cycle of Vectorette primary PCR.

Quantitative PCR was used to determine the optimal Vectorette ligation conditions. By designing a set of primers that spanned the ligation site, we were able to determine the amount of ligated template generated under a variety of ligase, adapter and ATP concentrations. The best conditions found are those detailed in the bulletin - one unit of ligase, 3.3 mM ATP and 3 picomoles of Vectorette units in a 30-µl reaction.

Proper controls are of the utmost importance in the Vectorette system protocol. A positive control reaction using Lambda DNA was added to the Universal Vectorette System so every step of the Vectorette process can be evaluated. Lambda DNA was chosen because the genome is small enough to give discrete bands after a restriction enzyme digest, and the observance of a single large molecular weight band suggests incomplete digestion. The results observed from the positive control ligation reaction with Vectorette units, after immediately fractionating on an ethidium stained agarose gel, should be similar to the restriction enzyme digest. Positive results from the control ligation must be compared to a no adapter mock ligation to ensure the reaction is working correctly. If a separate ligation reaction with Vectorette units has a positive result and a high molecular weight band is seen in a ligation reaction without Vectorette units, then the reagents in the supplied kit are able to perform Vectorette ligation. The addition of a positive control in the Vectorette kit does not preclude the user from performing controls with the DNA of interest. The technical bulletin (Product Code UVS-1) suggests a series of such controls, including the design of two or more nested, target specific primers to provide several primer sets designed to amplify the region of interest. We also recommend the routine set up of three control reactions containing (1) both the specific primer and the Vectorette primer, (2) the Vectorette primer alone and (3) the specific primer alone, as any bands unique to the one reaction with both primers are likely to be desired products because either primer alone may prime or misprime at two sites generating a product.

In brief, we have demonstrated an optimized system for determining sequence data neighboring a region of known sequence. The Vectorette system, designed for this purpose, is robust, flexible, and convenient. Yield, sensitivity and specificity in Vectorette primary PCR is improved by using touchdown cycling conditions, stringent primer conditions, optimized primer ratio and a Hot Start PCR mechanism. Vectorette ligation conditions provide optimized yields. The Universal Vectorette System includes a set of controls so that each step can be analyzed. Finally, the convenience of this system is increased with pre-made Vectorette libraries for human, mouse, and rat genomic DNA (Figure 6).

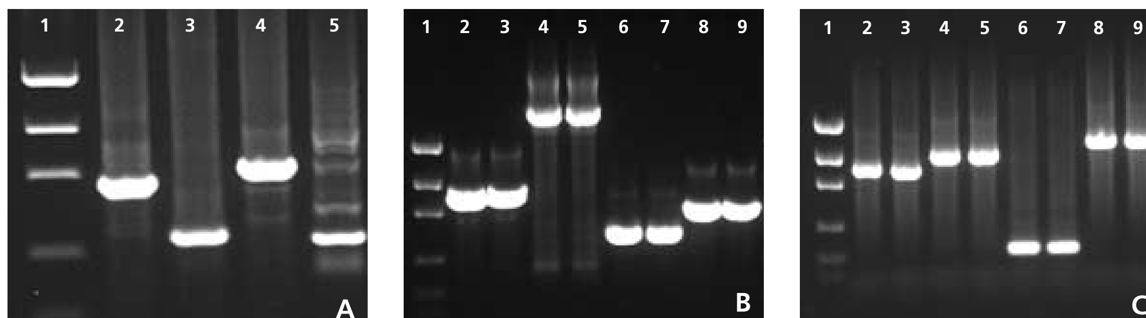



***Figure 6. Human, Mouse, and Rat Genomic Vectorette Libraries after secondary PCR using the provided control primers. (A)*** *Human genomic Vectorette library PCR products after nested PCR. Nested primers used were designed using the G3PDH gene. Lane 1, DNA marker 2 kb, 1.2 kb, 800 bp, 400 bp, 200 bp; Lane 2, Bgl* II, *747 bp; Lane 3, Eco*R I, *427 bp; Lane 4, Hin*d III, *837 bp; Lane 5, Pvu* II, *407 bp.* ***(B)*** *Mouse genomic Vectorette library PCR products after nested PCR. Nested primers designed using the murine Interleukin 1-beta gene. Lane 1, DNA marker 2 kb, 1.2 kb, 800 bp, 400 bp, 200 bp; Lanes 2 and 3, Bgl* II, *1 kb; Lanes 4 and 5, Eco*R I, *3 kb; Lanes 6 and 7, Hin*d III, *540 bp; Lanes 8 and 9, Pvu* II, *800 bp.* ***(C)*** *Rat genomic Vectorette library PCR products after nested PCR with nested primers designed using the Rat Interleukin 6 gene. Lane 1, DNA marker 2 kb, 1.2 kb, 800 bp, 400 bp, 200 bp; Lanes 2 and 3, Bgl* II, *1 kb, Lanes 4 and 5, Eco*R I, *1.2 kb; Lanes 6 and 7, Hin*d III, *2000 bp; Lanes 8 and 9, Pvu* II, *1.5 kb.*

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## References


1. Lilleberg, S., and Patel, S., Isolation of DNA flanking retroviral integration sites using Vectorette II. Genosys Origins, **1,** 5-6 (1998).

2. Arnold, C., and Hodgson, I. J., Vectorette PCR: a novel approach to genomic walking. PCR Methods Appl., **1,** 39-42 (1991).

3. Nielsen, K., et al., Sequencing of the *rpoB* Gene in *Legionella pneumophila* and characterization of mutations associated with rifampin resistance in the *legionellaceae.* Antimicrob. Agents Chemother., **44,** 2679-2683 (2000).

4. Riley, J., et al., A novel, rapid method for the isolation of terminal sequences from yeast artificial chromosome (YAC) clones. Nucleic Acids Res., **18,** 2887-2890 (1990).

5. Shearman, J.D., et al., Rapid mapping of markers applying vectorette technology to YAC fragmentation allows easy assembly of a high-density STS bacterial clone contig spanning the markers D6S1260-D6S1918. Mamm. Genome, **9,** 220-225 (1998).

6. Cowley, C.M., et al., A YAC contig joining the desmocollin and desmoglein loci on human chromosome 18 and ordering of the desmocollin genes. Genomics, **42,** 208-216 (1997).

7. Monani, U., and Burghes, A. H., Structure of the human alpha 2 subunit gene of the glycine receptor-use of vectorette and Alu-exon PCR. Genome Res., **6,** 1200-1206 (1996).

8. Asakawa, S., et al., Human BAC library: construction and rapid screening. Gene, **191,** 69-79 (1997).

9. Dolphin, C. T., et al., Structural organization of the human flavin-containing monooxygenase 3 gene (FMO3), the favored candidate for fish-order syndrome, determined directly from genomic DNA. Genomics, **46,** 260-267 (1997).

10. Villard, L., et al., Determination of the genomic structure of the XNP/ATRX gene encoding a potential zinc finger helicase. Genomics, **43,** 149-155 (1997).

11. Wiemann, S., et at., Promoter of the gene encoding the bovine catalytic subunit of cAMP-dependent protein kinase isoform C beta 2. Biochim Biophys Acta., **1309,** 211-220 (1996).

12. Don, R. H., et al., 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucl. Acid Res., **19,** 4008 (1991).

13. Heid, C. A., et al., Real Time Quantitative PCR. Genome Res., **6,** 986-994 (1996).

14. Rychlik, W., Priming efficiency in PCR. Biotechniques, **18,** 84-86, 88-90. (1995).

15. Rychlik, W., Oligo Primer analysis software version 5.0 for windows, (National Biosciences, Inc., 1994).



*Purchase of these products is accompanied by a limited license to use them in the Polymerase Chain Reaction (PCR) process in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Perkin-Elmer or as purchased, i.e., an authorized thermal cycler. These products are sold under licensing arrangements with F. Hoffmann-LaRoche Ltd., Roche Molecular System, Inc. and the Perkin-Elmer Corporation.*



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## ORDERING INFORMATION

| Product Code | Product Name | Unit |
|---|---|---|
| UVS1 | Universal Vectorette System | 1 kit |
| HUMAN-VS | Human Genomic Vectorette | 1 kit |
| MOUSE-VS | Mouse Genomic Vectorette | 1 kit |
| RAT-VS | Rat Genomic Vectorette | 1 kit |