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[11] Screening Transposon Mutant Libraries Using Full-Genome Oligonucleotide Microarrays

By Kelly M. Winterberg and William S. Reznikoff

Abstract

The experimental details for a high-throughput microarray-based screening technique for both detecting and mapping Tn*5* insertion mutants in parallel within a library are presented. Following Tn*5* mutagenesis, viable mutants are pooled and grown competitively under selective conditions. Chromosomal DNA is then isolated from each mutant pool. Biotin-labeled run-off *in vitro* RNA transcripts, representing the neighboring chromosomal DNA for each insertion remaining in the population, are generated using T7 promoters located at the ends of the transposon. Custom-designed, whole-genome oligonucleotide microarrays are used to analyze the labeled RNA transcripts and to detect each mutant in the library. Microarray data comparisons for each growth condition allow the identification of mutants that failed to survive the imposed growth selection. In addition, due to the density of the microarrays the genomic locations of the individual transposon insertions within each library can be identified to within 50 base pairs. Details for the *in vivo* Tn*5* mutagenesis procedure, mutant library construction and competitive outgrowth, T7 *in vitro* transcription/labeling, and microarray data analysis will be provided.

Introduction

In 1995 sequencing of the first bacterial genome of *Haemophilus influenzae* was completed (Fleischmann *et al.*, 1995). Since then 344 microbial genomes have been fully sequenced and over 550 more are in various stages of completion (http://www.ncbi.nlm.nih.gov). On average 30 to 40% of the

METHODS IN ENZYMOLOGY, VOL. 421
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0076-6879/07 $35.00
DOI: 10.1016/S0076-6879(06)21011-5

genes in any sequenced genome are characterized as having unknown or hypothetical functions (Judson and Mekalanos, 2000; Lehoux *et al.*, 2001). A portion of these genes have homologues to other genes with unknown functions, but the remainder of these genes has no similarity to any genes in the available databases. Although genomic sequence data provide the basic genetic information for a given organism, they do not tell us the functions of each gene, when or how the genes are regulated, or under what growth conditions each gene is required by the organism. To use genomic sequence information to answer these questions, the development of high-throughput molecular and genetic approaches is needed.

Classically, gene functions have been characterized one gene at a time using conventional genetic techniques such as transposon mutagenesis and phenotype and/or loss-of-function screening. Although somewhat labor intensive, these methods have been quite effective for identifying gene functions. Recently, new high-throughput screening techniques have also been developed. Some of these techniques that have been used for studying phenotypes of transposon mutants in parallel have included *in vivo* expression technology (IVET) (Slauch *et al.*, 1994), signature-tagged mutagenesis (STM) (Hensel *et al.*, 1995), size-marker integration technique (SMIT) (Benton *et al.*, 2004), genetic footprinting (Smith *et al.*, 1995), transposon site hybridization (TraSH) (Sassetti *et al.*, 2001), and similar methods (Badarinarayana *et al.*, 2001; Winterberg *et al.*, 2005). Collectively, these high-throughput approaches have the potential to address the fitness of individual unknown bacterial mutants in a pool under certain conditions. This provides an initial starting point for choosing interesting genes to study individually in greater detail.

Advances in DNA microarray technology have also made it possible to not only measure changes in gene expression patterns for a given strain but to also perform comparative genomic hybridizations, genome resequencing, and DNA binding site analysis on a genome-wide scale. Two of the high-throughput methods mentioned above (Badarinarayana *et al.*, 2001; Sassetti *et al.*, 2001) use spotted DNA microarrays to analyze transposon mutant pools grown under different defined growth conditions. Following a PCR amplification of chromosomal DNA flanking each transposon insertion contained in the library, samples are hybridized to spotted DNA microarrays. The features/probes that were spotted onto the microarrays in these studies were either complete (Sassetti *et al.*, 2001) or partial (Badarinarayana *et al.*, 2001) open reading frames (ORFs). In both cases the microarray probes represented only a portion of the genome, and therefore were limited in detecting transposon insertions throughout the genome.

Here we present the experimental details for a technique that was developed to monitor transposon insertion mutants in parallel using

custom-designed, high-density, whole-genome oligonucleotide microarrays (Winterberg *et al.*, 2005). In this chapter, we will describe this technique as it applies to the generation and screening of Tn*5* insertion libraries in *Escherichia coli* K-12. The principle and experimental details for this technique will be discussed, including Tn*5* *in vivo* transposon mutagenesis, transposon mutant library construction, competitive outgrowth of transposon libraries, whole-genome oligonucleotide microarray design, mapping genomic locations of transposon insertions, and microarray data analysis methods. We will also indicate what types of modifications can be made and which parts of the protocols should be further optimized for application to other organisms.

Principle

This technique was developed to allow high-throughput parallel screening of transposon mutants under various *in vivo* and *in vitro* growth conditions using whole-genome oligonucleotide microarrays. Following transposon mutagenesis, libraries of viable mutants are constructed and competitively grown in both a control and a test condition (Fig. 1). The composition of each mutant library is analyzed by first isolating chromosomal DNA and digesting it to 1- to 1.5-kb fragments using an appropriate restriction enzyme. T7 promoters located at the ends of the transposon are used to generate in parallel biotin-labeled runoff *in vitro* transcripts of the chromosomal DNA flanking each transposon insertion contained in the library (Fig. 2). The labeled *in vitro* transcripts are hybridized to whole-genome oligonucleotide microarrays to identify which transposon insertions (and thus which mutants) were present in the original library (control condition), and which mutants have been lost from the library following competitive outgrowth in the test condition. In each case, the loss or outcompetition of a mutant is inferred from the loss of *in vitro* transcript signal from a given transposon insertion. By using high-density, whole-genome oligonucleotide microarrays, the genomic locations of each transposon insertion for each mutant screened can be identified to within 50 bp of the insertion site. Using this technique, genes essential for growth in a variety of conditions can be easily identified.

The two key features that allow this technique to be employed are the T7 promoters contained within the transposon and the whole-genome oligonucleotide microarrays. Outward-facing T7 promoters are cloned into the 5′ and 3′ ends of the transposon prior to mutagenesis. These promoters are designed in a way that does not interfere with the transposition process, that is, the transposase binding sites should be left intact, but should allow run-off *in vitro* transcripts of the neighboring chromosomal DNA to be

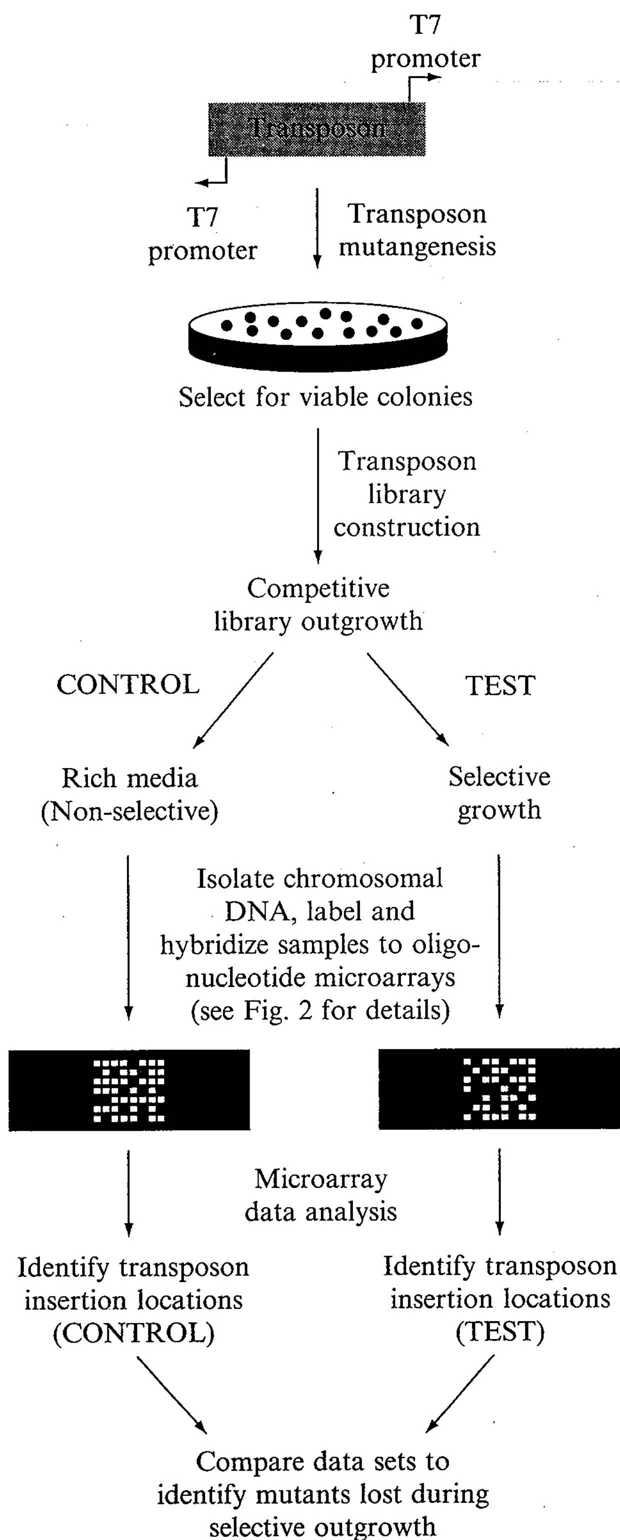

FIG. 1. Technique overview.

generated. Because the T7 transcripts are generated in a 5′ to 3′ direction, the transcripts generated from the left and right sides of the transposon will be homologous to the top (5′ to 3′) and bottom (3′ to 5′) strands of the

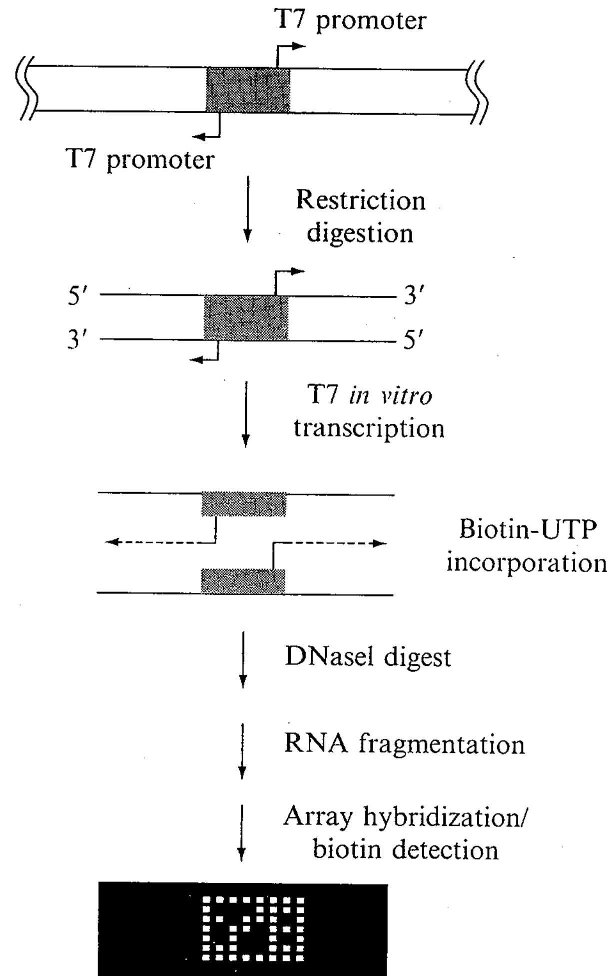

FIG. 2. *In vitro* transcription/biotin labeling schematic. Adapted and used with permission from the American Society for Microbiology.

neighboring DNA, respectively. When these transcripts are hybridized to whole-genome microarrays, the junction between the left- and right-side transcripts can easily be identified.

Second, whole-genome oligonucleotide microarrays are used to determine if a mutant is present in the library, and more specifically, where the transposon insertion within that mutant is located. The oligonucleotide probes representing the microarray are designed to represent both the top and bottom strands (5′ to 3′ and 3′ to 5′) of a given organism's genome, and are not specific to open reading frames or intergenic regions within the genome. This design strategy allows transposon insertions to be detected regardless of their location within the genome (intragenic or extragenic). Furthermore, the *in vitro* transcripts generated from each side of a given transposon insertion can be detected and depending on how closely spaced the oligonucleotide probes are located within the genome, transposon insertions can be mapped to within several base pairs of their genomic

location. Many currently available commercial microarrays are designed specifically for gene expression studies and are thus limited in their representation of an organism's total genomic DNA. In the original development of this technique (Winterberg *et al.*, 2005), commercial microarrays using both oligonucleotide probes and spotted open reading frames were tested. In both cases, the design of the microarrays, although suitable for gene expression, failed to allow the detection of more than 50% of the insertions within a given transposon mutant library. When a custom-designed, whole-genome oligonucleotide microarray was implemented, each insertion in the library could easily be detected, suggesting that using whole-genome microarrays for tracking transposon insertion mutants was more appropriate.

Transposon Mutagenesis Protocol

In general, transposon mutagenesis is performed using a transposon containing outward-facing T7 promoters cloned into the 5′ and 3′ ends of the transposon and a selectable marker. In the original development of this technique (Winterberg *et al.*, 2005), *in vivo* Tn*5* mutagenesis (Goryshin *et al.*, 2000) was used to deliver a modified transposon to competent *E. coli* cells by electroporation. The pMOD2 transposon-cloning vector (Epicentre Inc., Madison, WI) was used to clone two T7 promoters and a kanamycin-resistance gene into the Tn*5* transposon. Transposome complexes were formed between the transposon DNA and purified hyperactive EK/LP Tn*5* transposase (Bhasin *et al.*, 1999). Following electroporation and a short recovery period, kanamycin-resistant colonies were selected on agar plates. Approximately 10^5 kanamycin-resistant colonies per milliliter of transformed culture can typically be generated using this method.

Other transposon mutagenesis strategies can also be used with this method as long as the transposon contains a selectable marker and outward-facing T7 promoters. Care should be taken to choose a transposon that transposes fairly randomly to allow more complete coverage of the genome and to choose mutagenesis conditions that will minimize the occurrence of multiple transposon insertions within a single mutant. Southern blot analysis of several of the resulting transposon mutants can be used to verify that the mutagenesis resulted in single random insertions.

Tn5 Transposome Complexes

1. Perform a plasmid purification of the pMOD2 plasmid vector containing the transposon of interest. Alternatively, polymerase chain reaction (PCR) can be performed to amplify the transposon from the plasmid vector.
2. Digest approximately 10 μg of plasmid DNA or PCR product with *Psh*AI to release the transposon from the surrounding DNA.

3. Separate the transposon from the plasmid backbone using agarose gel electrophoresis. Purify the DNA from the transposon DNA band using either a gel extraction kit from Qiagen or other appropriate kit. (If PCR was used to generate the transposon DNA, a Qiagen PCR cleanup is sufficient to remove the digested ends away from the transposon DNA). Elute the DNA in deionized water.

4. Check the DNA concentration and purity by measuring the A_{260} and A_{260}/A_{280} ratio.

5. On ice, set up transposome complex reactions containing: 0.5 μg pre-cut transposon DNA, 4 μl of 10× binding buffer (10× concentration: 250 m*M* of Tris-acetate, pH 7.5, 1 *M* of potassium glutamate), and deionized water to 36 μl. Add different amounts of EK/LP hyperactive transposase ranging from 0.25 μg to 1 μg to each reaction. (The volume of transposase added to the reaction should never exceed 10% of the final reaction volume.) Deionized water should be added to each reaction to make the final volume 40 μl. A no-transposase control reaction should be included each time new transposome complexes are formed. (Optimization may be needed to determine the proper amounts of transposase to be used. Although hyperactive Tn*5* transposase is commercially available [Epicentre Inc. Madison, WI], Tn*5* transposase purified in the lab was used for this method.)

6. Incubate reactions at 37° for 2 hr.

7. Prior to electroporation, check the transposome complexes by running 4 μl of each reaction along with transposon DNA alone on a 1% agarose gel. The transposon DNA-only lane will provide a guide for where the transposon band within the transposome complex reactions should migrate. Transposome complexes will appear as a ladder of bands migrating slower than the transposon alone. The transposon band will appear diminished, concurrent with an increase in transposome complex bands.

8. Buffer exchange is used to decrease the amount of salt in each reaction prior to electroporation. This is done by spotting samples onto 0.05-μm filter discs floating in either 10% glycerol/5 m*M* of Tris-acetate (pH 7.5) or deionized water. This will help minimize arcing when the complexes are electroporated into electrocompetent cells. Transposome complexes exchanged into 10% glycerol/5 m*M* of Tris-acetate (pH 7.5) can be stored at –20° for up to 1 year.

In Vivo *Tn5 Mutagenesis*

Growth conditions are given for *E. coli* and should be changed as needed for other organisms.

1. Make transposome complexes with the transposon of interest and hyperactive EK/LP Tn*5* transposase (as described above).

2. Using standard electroporation conditions, electroporate 1 to 2 μl of complexes in binding buffer into 50 μl of electrocompetent cells. If complex reactions were buffer exchanged into 10% glycerol/5 m*M* of Tris-acetate (pH 7.5), up to 10 μl can be electroporated. If water was used for buffer exchange, presumably the entire binding reaction can be electroporated into the cells. This may be needed if the competency of the recipient strain is low.

3. Recover cells in 1 ml of Luria-Bertani (LB) medium at 37° for 1 hr with shaking (250 rpm). Incubations of more than 1 hr can lead to the accumulation of sibling insertions that are most likely due to the outgrowth/doubling of the recovering cells.

4. Isolate transformants by plating 50 to 100 μl of cells per LB plate containing the appropriate antibiotic. Incubate at 37° overnight. Typically 10^5 individual transposon mutants can be obtained from 1 ml of electroporated culture, but this will depend on the competency of the recipient cells and the amount of transposome complexes that were electroporated.

5. Perform Southern blot analysis of several selected mutants to verify that random single insertions were isolated in the mutagenesis.

Library Construction Protocol

Following transposon mutagenesis, mutant libraries can either be made directly from the selection plates or by first stocking individual mutants in 96-well format and then combining individual mutants. For the former method colonies should be dislodged and scraped together with ~2 to 3 ml of LB broth (per plate) using a glass spreading rod. The size of the library can be increased by combining the colonies from several plates. Aliquots of this mixture can be stored in 15% glycerol at –80°.

Mutant libraries can also be constructed by first picking individual mutants into separate wells of a polystyrene 96-well plate containing LB broth (~200 μl per well). Incubate 96-well plates at 37° in a stationary incubator overnight. Stock each plate of mutants in 15% glycerol (final concentration) by transferring the overnight culture by multichannel pipetor into a sterile polypropylene 96-well plate containing the appropriate amount of glycerol. Store plates at –80°. Libraries of various sizes can be constructed from these 96-well stock plates. To do this, mutants should be inoculated from the frozen stock plates into fresh LB broth in 96-well format using a 48- or 96-well replica pinner transfer device. Following overnight growth at 37°, combine equal volumes of cell culture in a sterile reservoir or tube to create libraries of various sizes. A few individual OD_{600} culture readings should be taken to verify that the cultures are at similar densities prior to pooling. Aliquots of this library can be stored in 15% glycerol at –80°. Although this method is a bit more labor intensive, the

number of mutants being combined into a library can be controlled easier and it provides a stock of individual transposon mutants that can be used for additional experiments.

In some cases, the size limit of the library may need to be determined empirically. In the development of this technique, mutant libraries containing 94 mutants allowed scoring of 100% of the members. Larger libraries containing 188, 376, and 564 mutants permitted the detection of only 79.8%, 72.9%, and 59% of the library members, respectively (Winterberg *et al.*, 2005). This was most likely due to the dilution of T7 promoter sequences as more mutant chromosomal DNA is added to the population. Modifications to enhance the T7 promoter containing DNA fragments in the template population prior to the *in vitro* transcription labeling procedure could be added. This might include modifying the stated procedure to include a separation step where all DNA fragments containing a T7 promoter and transposon end sequence could be separated away from the "nonspecific" chromosomal DNA. This could be done by running the digested DNA over a column that would specifically bind the T7 and/or transposon end sequences. Additionally, the competitive outgrowth test condition may also restrict the size of the mutant library. For example, some *in vivo* animal screening models have a limit to the complexity of the mutant library that can successfully establish an infection and be screened in a single animal (Bahrani-Mougeot *et al.*, 2002). Therefore, the experimental design may affect the complexity of the transposon library that can be screened effectively using this method.

Competitive Outgrowth of Mutant Libraries Protocol

Competitive outgrowth of mutant libraries is used to screen many transposon mutants in parallel to identify mutants that cannot survive an imposed (test) growth condition as compared to a control condition. Mutants from the test condition that fail to grow or are out-competed by the other mutants in the library are identified by microarray analysis (see below). The control condition should be a nonselective rich media that preferably is similar to the conditions originally used for the selection of transposon insertion mutants. This control condition should permit all of the transposon insertion mutants to grow. A test condition such as growth in minimal media, high/low pH, heat shock, and *in vivo* growth in animals, plants, and/or insects should be chosen to identify specific classes of mutants. Additionally, as a reverse approach, mutant pools that are specific for certain pathways or cellular functions presumably could be used with this technique to determine the composition and/or nutrient make-up of an unknown environmental growth condition.

The following procedure can be used for performing competitive outgrowth in rich and minimal media to identify M9 minimal-glucose auxotrophs.

1. Thaw a transposon mutant library aliquot on ice and dilute it 1/10 in LB broth to ~4.5 ml. Incubate for 1 to 3 hr at 37° with shaking (250 rpm). (This recovery of the frozen library introduces a short period of competitive growth and possible cell doubling, and should therefore be minimized to prevent the loss of any mutants within the population.) Alternatively, if mutants were stocked individually, frozen 96-well plates should be used to inoculate 96-well plates containing fresh media. Grow mutants overnight at 37°, pool them in a sterile reagent reservoir, and mix well.
2. Pellet the cells by centrifugation and discard the supernatant.
3. Wash the pellet three times with ~4.5 ml of phosphate-buffered saline, pelleting the cells after each resuspension.
4. Resuspend the final cell pellet in 4.5 ml of fresh phosphate-buffered saline.
5. Dilute the resuspended library cells 1:50 into ~100 ml each of both rich (control condition) and M9 minimal media supplemented with 0.2% glucose (test condition).
6. Incubate the cultures at 37° with shaking (250 rpm).
7. Take OD_{600} readings to determine doubling times/generations of each culture.
8. When the cells have reached late log phase/early stationary phase, harvest the cells in each condition by pelleting the cells in a tabletop centrifuge.
9. Harvest chromosomal DNA separately from each cell pellet in preparation for *in vitro* transcription and biotin labeling.

Mutant Labeling Protocol

Chromosomal DNA isolated from competitively grown mutant libraries is prepared for *in vitro* transcription/biotin labeling by first digesting it with a restriction enzyme that leaves blunt ends and will result in average DNA fragment sizes of ~1 to 1.5 kb. Run-off *in vitro* transcription is used to generate biotin-labeled RNA transcripts of the DNA directly flanking each transposon insertion still remaining in the mutant library following competitive outgrowth.

DNA Template Preparation

1. Quantify and check the quality of the isolated DNA by measuring the A_{260}/A_{280} ratio. The A_{260}/A_{280} reading should be ~1.8 to 1.9.

2. Digest 40 to 50 μg of DNA to completion using an enzyme that leaves blunt ends. (A blunt-end cutter is used because overhangs of DNA can be used as nonspecific templates by T7 polymerase.) Typically, *Hinc*II or *Fsp*I is used for digestion of *E. coli* strains resulting in average fragment sizes ranging from ~1 to 1.5 kb in length.

3. Add an equal volume of phenol chloroform isoamyl alcohol to the digested DNA and vortex mix for ~30 sec. Transfer mix to phase-lock gel tubes (Eppendorf) for easier phase separation. Spin tubes at 13,000 rpm (~17,900 × g) in a conventional table-top microcentrifuge for ~1 min to separate the phases. Transfer the aqueous phase to an RNase-free 1.5-ml Eppendorf tube.

4. Ethanol precipitate the digested DNA by adding 1/10 volume of RNase-free, 3 *M* sodium acetate (NaOAc), pH 5.2, and 2–1/2 volumes of cold 95% ethanol (RNase-free). Invert the tube 20 to 30 times until well mixed. Precipitate at –20° for at least 2 to 3 hr (preferably overnight). Spin samples at 13,000 rpm (~17,900 × g) in a conventional table-top microcentrifuge for 10 min at 4°. Wash pellets once with 1 ml of RNase-free 70% ethanol. Allow pellets to dry for 10 to 15 min at room temperature.

5. Resuspend the DNA pellet to a concentration of ~2 μg/μl in diethylpyrocarbonate (DEPC)-treated/nuclease-free water. (Typically, this takes ~10 to 15 μl of DEPC-treated water for each 40 to 50 μg of digest). Measure the A_{260} and A_{260}/A_{280} ratio following resuspension of the DNA pellet.

In Vitro *Transcription Reaction*

This step uses the Epicentre Ampliscribe T7 High Yield *in vitro* transcription kit to generate biotin-labeled RNA transcripts from the ends of each transposon insertion within the population. Other *in vitro* transcription kits should also work at this step.

1. Mix the following at *room temperature*:
   - 3 μl of 10X T7 reaction buffer
   - 10 μl of 10 m*M* Biotin-16-UTP (Enzo)
   - 1.5 μl of 100 m*M* ATP
   - 1.5 μl of 100 m*M* GTP
   - 1.5 μl of 100 m*M* CTP
   - 0.5 μl of 100-m*M* UTP
   - 2.5 μl of 100-m*M* DTT
   - 10 μg of digested, precipitated DNA (in a volume of less than 6.5 μl)
   - Add DEPC-treated water as needed to 27 μl
2. Add 3 μl of T7 RNA polymerase (from –20°) and mix well. (Total volume should now be 30 μl.)

3. Incubate at 37° overnight in a heat block. (Shorter times may yield less RNA, but may still be sufficient.)
4. Add 2 μl of DNaseI and incubate for 15 min at 37°. This removes all chromosomal DNA that may compete with the labeled RNA during microarray hybridization.
5. To clean up the labeled samples prior to microarray hybridization, add 470 μl of DEPC-treated water to each 32 μl sample, transfer to a YM10 microcon column, and spin as recommended until ~20 μl remain. Recover the retentate in a fresh RNase-free collection tube.
6. Quantify the RNA by measuring the A_{260}, and check the purity by measuring the A_{260}/A_{280} ratio. Typically, this is done on a NanoDrop spectrophotometer to minimize sample loss.

Microarray Design and Hybridization Protocol

A significant aspect of this technique is to use a microarray that represents most of the genomic DNA of an organism. During the optimization of this technique, it was determined that microarrays designed specifically for gene expression analysis (both spotted arrays and oligonucleotide arrays, and specifically, Affymetrix GeneChip arrays) failed to detect over 50% of the insertions from a transposon insertion library containing precharacterized transposon insertion mutants (Winterberg *et al.*, 2005). For this reason, care should be taken to design and/or use microarrays that provide near-complete genomic coverage of the strain or chromosomal regions being investigated.

1. Prior to hybridization, RNA samples should be fragmented. To do this, mix DEPC-treated water, 5 to 10 μg of RNA, and 5X fragmentation buffer (200 m*M* of Tris-acetate, pH 8.1, 500 m*M* of potassium acetate, and 150 m*M* of magnesium acetate) to a final volume of 30 μl. Fragmentation buffer should be used at a final concentration of 1X. Incubate for 10 min at 95°. Hold on ice until prehybridization and hybridization mixtures are prepared.
2. Prehybridization and hybridization to whole-genome oligonucleotide microarrays or other suitable microarray should be performed as recommended by the manufacturer.
3. Following microarray hybridization, biotin-labeled transcripts hybridized to oligonucleotide microarrays are detected by first staining the microarrays with a Cy3-streptavidin conjugated fluorophore dye, and then scanning with a confocal scanning laser.
4. Signal intensities of each oligonucleotide probe on the microarray are extracted from the scanned microarray image based on the X and Y positions on the microarray.

Data Analysis

Although many microarray data analysis programs exist, it is important to use a program that allows analysis of the individual probe signal intensities, rather than a program that calculates the average probe signal intensity for each individual gene, as is often the case for gene expression analysis software programs. Most microarray data sets will contain over 65,000 records, and therefore cannot be analyzed using a spreadsheet program such as Microsoft Excel. Database programs like Microsoft Access or other custom programs written in PERL and/or MySQL can be used to organize and sort the microarray data.

Probe signal intensities are extracted from each microarray based on X and Y coordinates from the microarray. The signal intensity data and genome position for each probe can be linked together using the original microarray library file that describes each probe's X and Y position on the microarray and the DNA sequence and genome position for each probe. When the genome position and signal intensity data for each oligonucleotide probe have been linked, the microarray data can be sorted in genome order. Both strands of DNA can be analyzed together, but a common factor of (–1) should be multiplied to each probe signal intensity for one of the strands (typically the 3′ to 5′ or bottom strand). This will allow differentiation between probes that are located at the same genome position but are homologous to opposite strands.

Software programs that allow several histograms of data to be plotted relative to each other are very effective for visualizing this type of microarray data. GenVision (DNASTAR) is an example of such a program, and was used during the development of this technique. Files representing (1) the gene names, (2) gene lengths and coding strand (sense or antisense), (3) microarray probe-signal intensities, and (4) restriction enzyme digestion sites all relative to the position in the genome can be constructed using gene annotation tables and the extracted microarray data. As shown in Figs. 3 and 4, GenVision allows the visualization and comparison of each of these histograms of information.

In the development of this technique (Winterberg *et al.*, 2005), it was determined that mutants diluted 10^{-1} in a library of 50 total mutants will show a noticeable signal intensity decrease, and mutants diluted 10^{-3} will no longer be detected on the microarray. Thus, the limit of detection for this technique is between 10^{-1} and 10^{-3}. A decrease in signal may also represent a region of the chromosome that T7 RNA polymerase does not transcribe well, but this decrease should not change between the two growth conditions being tested.

Array normalization and/or specific algorithms to pick out stretches of higher signal intensities may or may not be necessary depending on the