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An Affymetrix Microarray Design for Microbial Genotyping

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Abstract

There is an ongoing requirement for development of high-density or multiplex assays for detection or identification of microbes. There is also a need to develop assays or toolsets that can detect or identify microbial threats without prior knowledge of the target microbe(s) in a given sample. Indeed, some samples that contain no culturable material (e.g. viable but non-culturable cells) will nonetheless contain detectable DNA fragments which might be of value with respect to forensics or attribution of source. For many pathogenic microbes, various specific tests already exist, but there are few general methods wherein a single adaptable tool can be applied to multiple species or to previously uncharacterized organisms. The high-density DNA microarray has the potential to address many of these requirements and thus complements existing identification tools. The microarray platform has for example, the ability to detect microbial DNA that is not a perfect match to known genomic DNA sequences, thus making it possible to detect microbial variants that might otherwise be missed. In this report, the design and preliminary testing of a high-density DNA microarray for the purpose of microbial identification and detection is described.

Résumé

Les méthodes à haute densité et les techniques multiplex pour la détection et l'identification des microorganismes sont des outils toujours en demande. Il faut aussi des méthodes pour détecter et identifier les dangers microbiens savoir quels microorganismes peuvent être présents dans les échantillons. En fait, certains échantillons ne contenant que des espèces non cultivables (c.-à-d. qui sont viables mais qui ne peuvent être mises en culture) peuvent contenir des fragments d'ADN détectables qui pourraient être utiles à des fins criminalistiques ou pour la détermination de l'origine du matériel. Diverses méthodes spécifiques existent déjà pour de nombreux microorganismes pathogènes, mais il y a peu de méthodes générales avec lesquelles une même technique adaptable peut être appliquée à de multiples espèces ou à des microorganismes qui n'ont pas été préalablement caractérisés. La puce à ADN à haute densité pourrait satisfaire à un bon nombre de ces critères et être un complément aux outils d'identification dont nous disposons actuellement. Elle peut servir, par exemple, à détecter l'ADN microbien qui ne correspond pas entièrement aux séquences d'ADN génomique connues, ce qui permet de détecter des variants microbiens qui, autrement, auraient pu passer inaperçus. Dans le rapport présenté ici, nous décrivons la conception et les essais préliminaires d'une puce à ADN à haute densité mise au point pour la détection et l'identification des microorganismes.

Executive summary

An Affymetrix Microarray Design for Microbial Genotyping

Barry Ford; Doug Bader; Yimin Shei; Cindy Ruttan; David Mah; DRDC Suffield TM 2009-183; Defence R&D Canada – Suffield; October 2009.

Background: DNA and RNA (nucleic acids) are the genetic material of bacterial and viral species. The composition (e.g. DNA sequence) of the genetic material can be used to determine unambiguously, the identity of the species down to the level of individual unique strains. There are in existence assays useful for the detection and identification of single target nucleic acid sequences in microbial samples. In surveillance or diagnosis, it may be necessary to screen for many biological agents without knowing which in particular is of interest. While it is possible to run multiple single assays in parallel, the ability to execute multiple assays simultaneously within a single assay run is limited. Thus testing for multiple species of micro-organism currently requires multiple assay runs.

There is also a need to develop toolsets that can assay microbial targets without extensive microbiological culture or analysis of the specific sample. For example, some samples that can't be cultured at all will nonetheless contain detectable DNA fragments which might be of value with respect to forensics or attribution of source. There are currently few general methods wherein a single adaptable tool can be applied to multiple species or to previously uncharacterized organisms.

The high-density DNA microarray has the potential to complement existing identification tools, especially for multiple species or strains, or samples which can't be cultured using conventional microbiology. Microarrays are a technology which permits the detection of many nucleic acid sequences in a single run, with identification of each detected sequence. The basic microarray is comprised of many individual DNA sequence targets on a tiny microscope slide. The Affymetrix platform represents the current state of the art in microarray density (more than 221,000 individual targets) and throughput. An advantage of the microarray platform is the ability to detect microbial DNA sequences that are not a perfect match to the DNA sequences on the microarray chip.

Results: In this report, the design and preliminary testing of a high-density Affymetrix DNA microarray for the purpose of microbial identification and detection is described. The microarray can discriminate between multiple species of interest using qualitative analysis.

Significance: The DNA microarray is a single adaptable high-density platform useful for detection, identification and discrimination of multiple threat agents simultaneously. It is a complementary diagnostic technology to existing low-density microbiological and assay systems.

Future plans: Detailed validation of the current microarray and comparison to other microarray systems is planned. Additional testing is required to fully assess the real-world value of the DNA microarray as a tool for diagnosis, detection, and identification of microbial samples. Mixed microbial DNA samples and samples with human DNA (a frequent element in diagnostic samples) will be evaluated.

Sommaire

An Affymetrix Microarray Design for Microbial Genotyping

Barry Ford; Doug Bader; Yimin Shei; Cindy Ruttan; David Mah; DRDC Suffield TM 2009-183; R & D pour la défense Canada – Suffield; Octobre 2009.

Contexte : L'ADN et l'ARN (acides nucléiques) constituent le matériel génétique des espèces bactériennes et virales. La composition (c.-à-d. la séquence des acides nucléiques) du matériel génétique peut servir à déterminer sans aucune ambiguïté l'identité d'une espèce jusqu'au niveau des souches individuelles uniques. Diverses méthodes servent à la détection et à l'identification de séquences d'acides nucléiques cibles dans les échantillons microbiens. Pour des besoins de surveillance ou de diagnostic, il peut être nécessaire de rechercher de nombreux agents biologiques sans savoir lesquels cibler plus particulièrement. Il est possible de faire plusieurs analyses parallèlement, mais peu de méthodes permettent de rechercher simultanément plusieurs microorganismes par une seule analyse. Ainsi, actuellement, lorsqu'il faut rechercher plusieurs microorganismes dans un échantillon, il faut le soumettre à plusieurs analyses.

Il faudrait aussi mettre au point des méthodes qui permettent de cibler des microorganismes sans qu'il soit nécessaire de faire des cultures ou des analyses avancées. Par exemple, certains échantillons ne se prêtant pas à la culture peuvent contenir des fragments d'ADN détectables qui pourraient être utiles à des fins criminalistiques ou pour la détermination de leur origine. Actuellement, il existe peu de méthodes générales avec lesquelles une technique adaptable peut être appliquée à de multiples espèces ou à des microorganismes qui n'ont pas été préalablement caractérisés.

La puce à ADN à haute densité pourrait être un complément utile des outils d'identification actuels, surtout pour les espèces ou souches multiples ou encore pour les échantillons qui ne se prêtent pas aux méthodes culturales de la microbiologie classique. La puce à ADN est une technologie qui permet la détection et l'identification de nombreuses séquences d'acide nucléique en une seule analyse. Essentiellement, une puce à ADN est une petite lamelle de microscope sur laquelle ont été déposées un grand nombre de séquences individuelles d'ADN cible. La plate-forme Affymetrix est actuellement le dernier cri de la technologie des puces à ADN à haute densité (plus de 221 000 cibles individuelles) et à débit élevé. L'un des avantages de cette plate-forme vient de ce qu'elle permet de détecter des séquences d'ADN microbien qui ne correspondent pas entièrement aux séquences utilisées sur la puce.

Résultats : Dans le rapport présenté ici, nous décrivons la conception et les essais préliminaires d'une puce à ADN à haute densité Affymetrix mise au point pour la détection et l'identification des microorganismes. Cette puce permet de différencier un grand nombre d'espèces d'intérêt par une analyse qualitative.

Importance : La puce à ADN est une plate-forme adaptable à haute densité qui peut servir pour la détection, l'identification et la différenciation simultanées de multiples agents dangereux. Cette technologie de diagnostic est un complément des systèmes de détection et d'analyse microbiologiques à faible densité.

À venir : Validation détaillée de la puce à ADN actuelle et comparaison avec d'autres systèmes à puce. D'autres essais seront nécessaires pour l'appréciation exacte de la valeur opérationnelle de la puce à ADN comme outil de diagnostic, de détection et d'identification des échantillons microbiens. Des échantillons d'ADN microbien mixtes et des échantillons contenant de l'ADN humain (dont la présence est fréquente dans les échantillons de diagnostic) seront évalués.

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1 Introduction

In the area of microbial genotyping there are multiple platforms that can identify one or a few microbial targets in a single assay iteration. For most pathogenic microbes, various specific methods exist, but there are few general methods wherein a single adaptable tool can be applied to multiple species or to previously uncharacterized organisms. There is a continuing need for the capability to detect or identify many possible microbial agents without having prior knowledge of the offending agent in a given sample [1,2]. Indeed, some samples that contain no live or culturable cells could contain detectable DNA fragments which may prove to be useful for clinical, forensic or attribution purposes [3]. One platform with potential to aid identification of multiple species or strains without culturing or specific prior sequence knowledge is the high-density microarray.

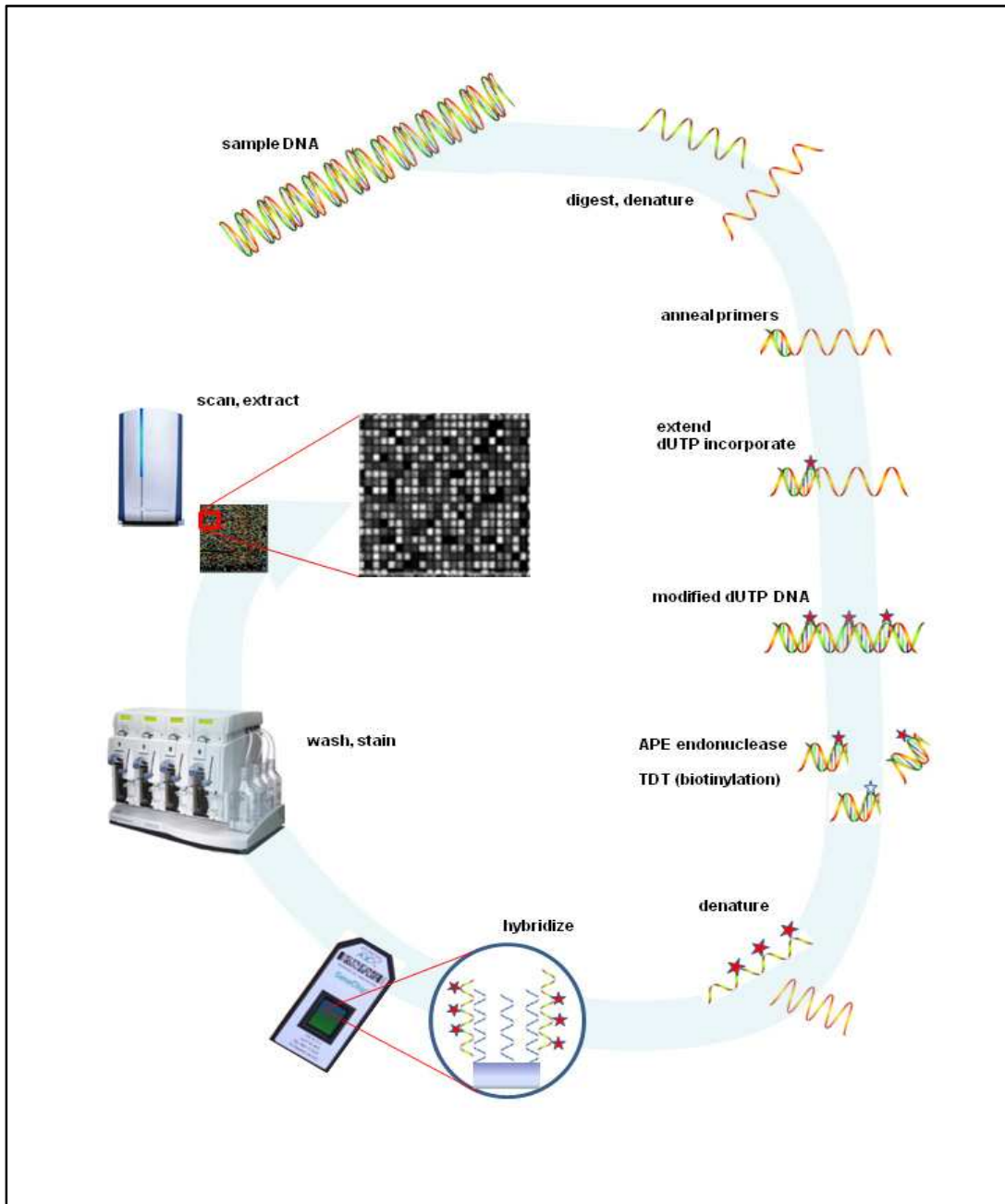
Each microarray can carry from a few hundred to a few hundred thousand individual target DNA sequences. Choice of specific array platform is driven by a combination of cost, density, and usability. For maximal utility, the ideal microarray should have as many features as possible, each feature representing one unique DNA sequence fragment. In the current work, the Affymetrix platform was exploited towards the development of a broad spectrum multi-species, multi-strain microarray, on a single microarray chip containing over 200,000 individual features. The Affymetrix system is closed source, meaning that the applied technologies for array fabrication, labeling, and data extraction are integrated into a pre-packaged system purchased from Affymetrix. Basic methods are established by the vendor, such that standardization of techniques takes much less time than with open source platforms. There are additional hardware costs for choosing Affymetrix relative to open source platforms, which are largely balanced by the reduced hands-on time for the pre-hybridization and post-hybridization manipulations, as well as for the extraction of data from the scanned microarray image. Figure 1 is a summary of the Affymetrix microarray processing system used at DRDC Suffield in support of this work. The protocol is itemized in detail in the Annexes.

In order to assess the utility of DNA microarrays for identification of bacterial pathogens to the species and strain level, a multipathogen chip was designed for the Affymetrix platform. Organisms included on the chip were derived from the National Institute of Allergy and Infectious Diseases Category A and B list of priority pathogens [4]. Also selected were *Haemophilus influenzae*, *Acinetobacter baumannii*, *Chaetomium* species, *Rickettsia* species, plasmids pBC16 and pLS1. Sequences representing bacterial toxins and antimicrobial resistance (e.g. antibiotic markers) were also sampled. Targets for viral pathogens were not included in this chip. The sequences thus chosen constituted approximately 16,000 individual sequence targets, which, allowing for sequence variants and internal controls, included over 81,000 unique probes. The remaining capacity of the chip surface was used to deploy some 140,000 probes from the Affymetrix "antigenomic library" to serve as non-targeted probes, essentially a random target library.

The number of microbial genomic targets thus did not equal the number of individual probes on the array. This is due to the redundancy built into the Affymetrix microarray technology, wherein variants of specific probe sequence differing by one or a few bases from the specific probe, are used to assess non-specific or variant binding to probe sites. In general, each specific target is represented by 3–20 individual probe sequences, varying by length, sequence, or single base pair

differences. In typical applications, only one signal is reported from a probe set, the remaining features serving as quality assurance and quality control indicators. For genomic fingerprinting, the variants related to the primary probe may also contain useful signals, and are also reported.

Figure 1: Overview of the Affymetrix microarray system.



APE : apurinic endonuclease; TDT : terminal deoxynucleotidyl transferase

2 Methods

2.1 Microarray platform selection

The two types of custom Affymetrix DNA microarray formats which could be used for genotyping are the Tiling Arrays and Resequencing Arrays. Choice of format in general governs the default reagent and protocols which can be accessed directly from Affymetrix. Probes on tiling arrays are spaced at a specified regular distances across a given genomic sequence (*e.g.* one probe every 500 bp). For resequencing arrays, the sequence for a region of interest is provided and four 25 base probes are designed for every base pair for the entire length of the sequence. Each member of a four probe set differs at the central (13th) bp at which an A, C, G or T will be incorporated. Probes are designed for both strands so that each base pair within the sequence is interrogated eight times.

Since the purpose of the multipathogen chip is to use probes derived from multiple source organisms, neither of the two standard custom designs fit our direct requirements. Through discussions with Affymetrix, a modified Tiling Array format was selected such that up to five probes would be provided for each sequence submitted for evaluation. In addition to control probes, whatever space remained on the array design would be filled with nonspecific probes selected from the existing Affymetrix probe library. The design contract with Affymetrix (executed as a subcontract with Canada West Biosciences) allows DRDC to retain ownership of our own probe designs, while using part of the Affymetrix probe library under licence.

2.2 Selection of targets

In principal, given the 25 base pair size of the oligonucleotide probes on the Affymetrix microarray, an ideal array could sample any possible sequence (known or unknown) if all possible 25-base oligonucleotides were spotted on the array. This would be A,C,G or T at all possible positions, which is 4^{25} oligonucleotide sequences, or $\sim 1.126 \times 10^{16}$ individual sequences. Current maximal capacity of the Affymetrix array system is approximately 1 million probes per array. It would require 1×10^{10} microarrays to cover most of the possible sequences. Thus, designing all possible 25 base pair sequences was not a practical nor an affordable approach.

Targeted probes are categorized as SNP (single nucleotide polymorphism) or non-SNP. SNP probes are included to better differentiate between strains of the same species. The target sequence submitted is 49 bp or less and contains one or more SNPs. For a sequence with one SNP, a set of 5 probes covering different segments of the target sequence is created for each of A, C, G and T at the variant base, resulting in a total of 20 probes. Thus, an organism with 'A' at the target site would register high intensity signal for the 5 'A' probes and low intensity for the remaining 15 probes. For sequences containing more than one variant within a 49 base pair region, the number of probes increases accordingly. The mismatch SNP probe variants of a specific probe sequence (differing by one or a few bases from the specific probe), are used to assess non-specific or variant binding to probe sites and are useful for genomic fingerprinting.

Non-SNP probes have little sequence commonality and are used to differentiate at the species level. The target sequences are much longer than those used for SNP design and the probes, ranging from 1 to 15 unique sequences, can be spread over a large section of genomic DNA. Multiple target sequences (SNP and non-SNP) were submitted for each organism of interest to

ensure detection even if some probes failed to perform as expected. Ideally, all probes designed using a specific organism's DNA sequence should produce high intensity signal while the remaining probes (off-target) should have little to no signal.

In order to rationally develop a library of probes suitable for identifying the maximal number of agents, we focused on regional microvariation within sequenced genomes of interest. Sequences for probe design included those that encompassed regions that differed between strains of the same species, especially those from Category A bacteria. Also included were regions that were constant within a species but differed between species, virulence genes, and antibiotic resistance genes.

2.3 Target sequence extraction

The first step in identifying regions of interest was to review the existing literature on bacterial microarray genotyping and strain differentiation. This provided a partial list of genes to include in our search. Next, various online databases were investigated for genes of interest. Initially, the NCBI Protein Clusters database [5] was used. Protein Clusters provides curated and non-curated clusters of related protein reference sequences. The database was searched by species and the protein clusters of that species were targeted by the level of conservation. For example, strain variants in *Bacillus anthracis* were identified by selecting clusters conserved to the *Bacillus cereus* or *Bacillus anthracis* group level. Selecting a cluster of interest revealed the list of all strains included in the cluster. Variants within the sequence were then identified by viewing the detailed alignment. When variants were found, the DNA sequence was retrieved by clicking on the locus tag, then on the sequence viewer.

Antibiotic resistance genes were obtained from the Antibiotic Resistance Genes Database [6]. The majority of the sequences used for probe selection were obtained from VFDB, the Virulence Factors of Pathogenic Bacteria database [7]. This database provided FASTA formatted (plaintext for database searches) sequences of virulence genes and sequences that can be used for comparative genomics. Additional strains of interest for inclusion in the microarray design were provided by Dr Kingsley Amoako (Canadian Food Inspection Agency, Lethbridge, Alberta). In addition, the coding sequences for hypoxanthine guanine phosphoribosyltransferase (HPT) and adenine phosphoribosyltransferase (APRT) from multiple species of origin were included for future applications. Species and strains represented on the microarray are itemized in Annex A.

A master Excel file was created in order to manage the selected sequence segments. This file contained a number of data points: probe name, organism used to obtain the sequence, gene ID/Accession Number/Locus ID used to locate the gene, start and end base positions of the sequence used, length of the sequence segment used, first and last 8 bp of the selected sub-sequence, gene name and description, the strain the sequence was derived from, and the complete sequence segment. To determine which strains matched which sequence (beyond and including the sequence source strain), sequences were initially tested using nucleotide BLAST tool [8] against nucleotide reference sequences, then whole-genome shotgun sequences. For strains that differed by single base pair variations, single-nucleotide polymorphism (SNP) target sequences were prepared. These probe sequences were 49 bases in length with the variant, designated by an "n", in the 25th (central) position.

From this master file, two files were prepared for Affymetrix. The first was the instruction file listing the probe name, start and end positions of the probe sequence, first and last 8 bp of the

probe sequence and a description of the probe. The second file included all the probe sequences in FASTA format, each identified by the probe name provided in the instruction file. Once Affymetrix received the instruction and FASTA sequence files, five 25-mer probes were designed for each probe sequence submitted, using Affymetrix proprietary software. Degenerate or redundant probes were removed and a list of the proposed microarray design was returned for evaluation. The final microarray design was assembled using 81,678 probes from 11,516 unique microbial sequences, 24,660 probes from 264 SNP sequences, and approximately 140,000 non-specific probes along with controls to fill in the 220,678-probe chip. Annex A contains the listing of species and strain-specific represented, and how many probes that were used in the final design.

2.4 Microarray in silico verification

The straight text listing of oligonucleotide sequences printed onto the array was analyzed by testing all the sequences in the design against the entire NCBI non-redundant nucleotide database using iterative BLAST searches. PERL scripts were developed to run serial segments of the dataset (being too large to submit as a single set), and to log the returned sequence data, predicted species (and strain), and general annotations including accessions, of all hits against the submitted oligonucleotide sequences.

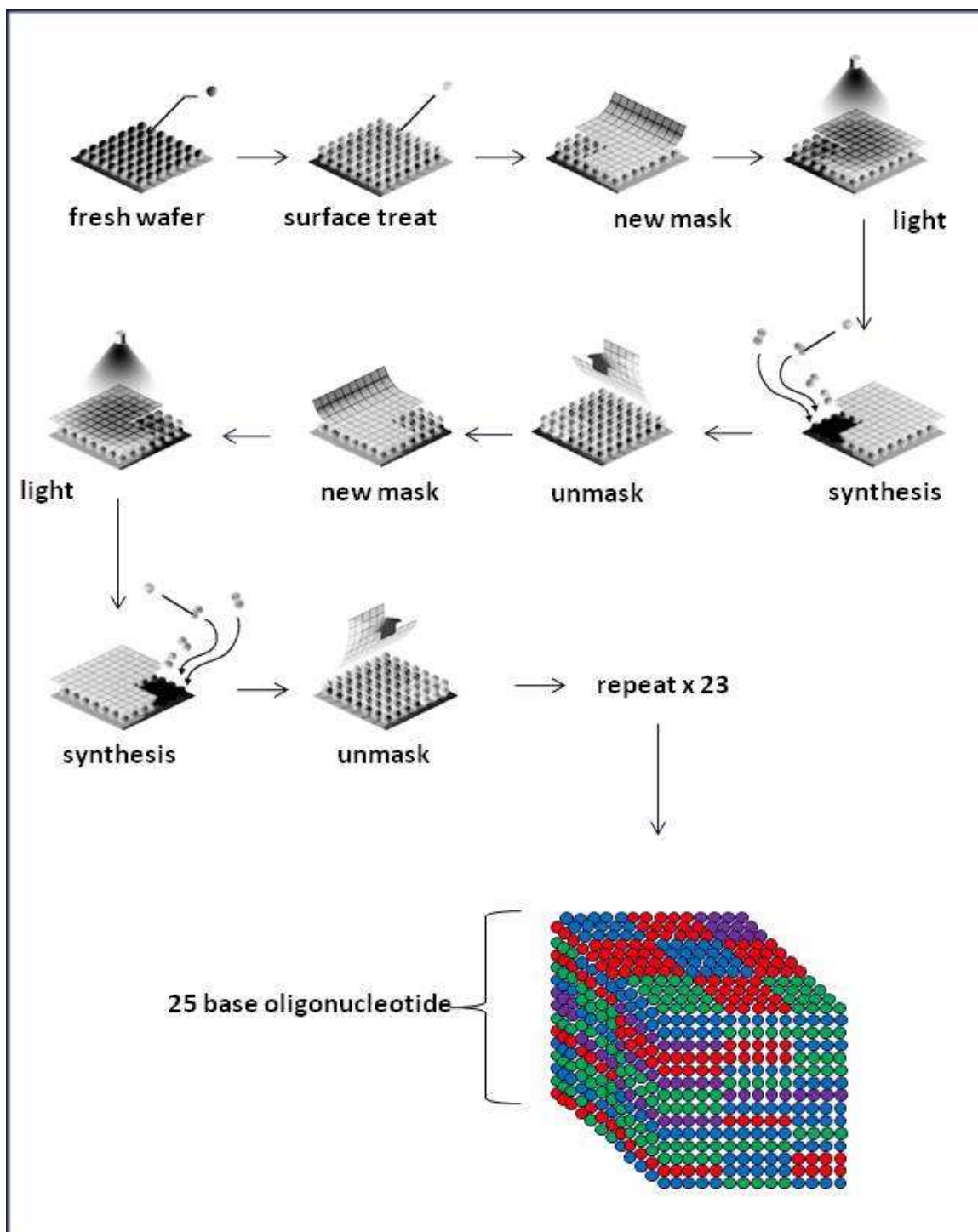
Since the entire feature sequence set was designed using publicly available databases, of which NCBI comprises a large, if not exhaustive aggregation, it was anticipated that *in silico* testing would recapitulate the designed species and strain identifications. It was also expected that due to sequence data errors and accession-specific variations within the database, that some sequences designed as unique probes (single species, single target) would actually align to accessions other than the record of origin.

2.5 Microarray fabrication

Affymetrix photolithography

The fabrication of the microarray per se is one key to the Affymetrix product package. Driven by the availability of photomicroolithography in microfabrication of microcircuitry, Affymetrix developed the method to template microarray chips using multiple lithographic overlays combined with photo-activatable oligonucleotide synthesis chemistry (Figure 2, adapted from Affymetrix Inc.). The technique allows for submicron precision in placement of oligonucleotide synthesis reactions on the surface of the silicon microarray wafer. Using multiple overlays, each site can be photoactivated differentially, and the different oligonucleotides synthesized stepwise. The more features on the array, the more overlays are required. Although photomicroolithography has been reported to produce some truncated probe sequences within each feature, the chip design includes truncation variants which can be used to verify signals from the features, or the absence of signal from the truncated probes as a set. This is generally done within the signal extraction software.

Figure 2: Photomicrolithography of microarrays. (adapted from Affymetrix Inc., Santa Clara, CA)



2.6 Microarray testing

Microbial DNA samples

Table 1 lists the microbial DNA samples that were used for preliminary testing of the custom microarray design. DNA samples from *E. coli* were prepared by the contractor, while DNA from level 2 and level 3 microbes were prepared by DRDC Suffield staff in the DRDC Suffield BSL2 or BSL3 labs respectively. BSL2 and BSL3 DNA extracts were tested for product sterility using standard procedures in the containment facility prior to release for microarray testing.

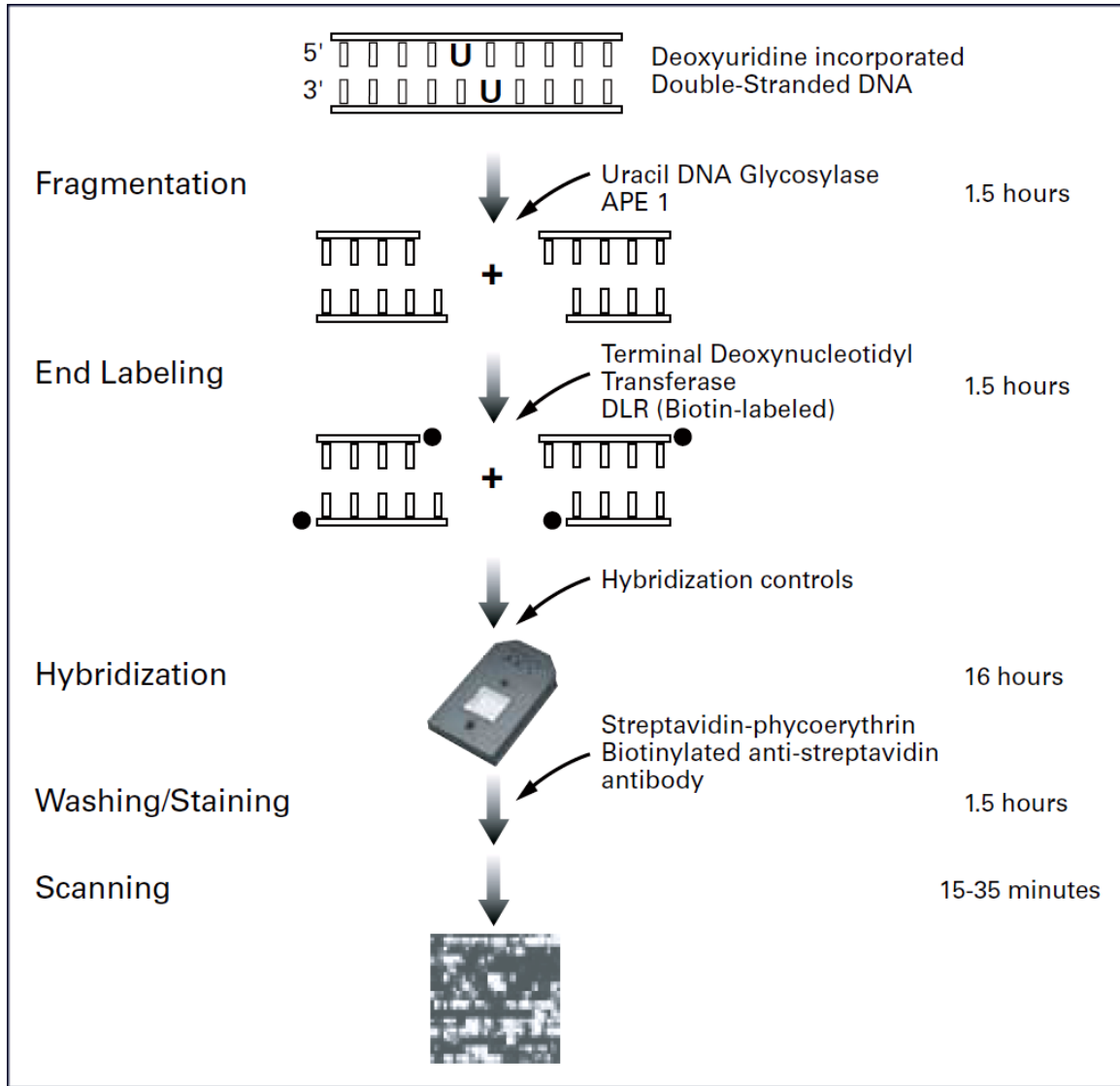
Table 1: DNA extracts used in initial testing of the microarray

Genus	Species	Strain / Isolate
<i>Escherichia</i>	<i>coli</i>	JM109
<i>Bacillus</i>	<i>anthracis</i>	RP42
<i>Bacillus</i>	<i>cereus</i>	ATCC 11778
<i>Yersinia</i>	<i>pestis</i>	19428
<i>Yersinia</i>	<i>enterocolitica</i>	

Affymetrix DNA labeling

The sample labeling method used in this study involved preparing a random-primed synthesis reaction incorporating uracil instead of thymine into the newly synthesized DNA using genomic DNA as the template, followed by direct end-labeling of the product DNA with biotinylated nucleotide, as shown in Figure 3. The detailed protocol used to label target DNA (per sample) is reproduced in Annex B:

Figure 3: End labelling sample DNA with terminal deoxynucleotide transferase (TdT).



2.7 Data reduction and analysis

Much of the data reduction work involved with microarrays involves feature extraction from the image to a spreadsheet, aligning the signals to the annotated target list, and data curation. These elements are contained within and managed by the Command Console software. Subsequent analysis involves normalization of data sets within the experimental series (between arrays), followed by comparison of test data to control data. In the case of genomic identification or fingerprinting, as on our array design, comparison to control arrays is not required for initial

assignment of genera and species. For the purposes of preliminary array design testing, no exhaustive comparison was undertaken.

Using tools developed for the open source microarray system (Chromablast) [9], data were reviewed without requiring prior normalization of signals. We compared data sets from *E. coli*, *B. anthracis*, *Y. pestis* and *Y. enterocolitica*, to determine qualitatively whether the target-specific array elements could discriminate between the samples. Data from the non-specific probe sets were not considered in this initial assessment. Student's t tests were performed pairwise on data sets to determine whether this analysis was informative relative to the heat map display.

3 Results and Discussion

Data were obtained on the Affymetrix custom-designed microarray for DNA from *Escherichia coli*, *Yersinia pestis*, *Yersinia enterocolitica* and *Bacillus anthracis*. Although a number of analytical tools are available for comparing and estimating distance in genomic fingerprint data [10,11], for this verification of concept and function, qualitative comparison was sufficient.

Using Chromablast [9], a heat map representing relative signal values was developed for a series of technical replicates of *E. coli* used for the microarray testing. An excerpt showing a region of the resultant heat map is shown in Figure 4. Uniform heat map colour across the replicates would indicate perfect concordance between replicates. The excerpted region shows some examples of this, as well as some replicates with varying colour, indicating some variation across the replicates. In Figure 4, green represents low value intensities (*i.e.* background to about 12% of maximum intensity, 0 to 6 in log base 2), and bright red indicates maximal intensity, as indicated in the scale below the heat map. The absolute scale of variation between non-normalized array data sets is thus seen to be about $\pm 30\%$ within individual probe sets. This is verified by numerical analysis of the raw intensity data. Most of this variation is concentrated within the lower intensity values, where the standard deviation as a fraction of the mean is maximal. Above the mean signal intensity (~ 7.0 in log 2), the maximum signal variation per probe set is about $\pm 5\%$ (Figure 5).

In practice, this suggests that a pruning of low-intensity signals may be useful to refine discrimination between samples versus knowns. Alternatively, a weighting factor could be applied to bias discriminatory decisions towards higher intensity signals. One method to compensate for signal variation between replicate arrays is to use the Student's t test to compare knowns to unknowns, or to detect outliers within the replication set for a known sample. In the case of the *E. coli* replicated data set, for the complete data set, including the lowest value probe intensities (15,533 probe sets), less than 2% of all signals in a pairwise comparison have a t-test value of less than 0.05. If only the signals greater than background are considered (9335 probe sets), the number of t-test values less than 0.05 falls to $\sim 1\%$ (72 probe sets). "Significant" t-test results obtained for low-intensity signals (low confidence) are removed by this strategy. Small occurrences of outlier or systematically unreliable signal sets, as indicated by this analysis, are unlikely to interfere with discrimination between different genera or species, but may complicate detailed discrimination between closely related strains.

An initial survey of *E. coli*, *B. anthracis*, *Y. pestis* and *Y. enterocolitica* on the microarray revealed that even at a high level view, the array could easily discriminate *E. coli* from *B. anthracis* (Figure 6-A,C). Note that these plots are of data not normalized post extraction, since the Affymetrix signal processing software applies in-process normalization using the internal controls. Comparison of the *Y. pestis* versus *E. coli* data suggested that the *Y. pestis* DNA sample contained DNA from *E. coli* or a related species.

Figure 4: Comparison of DNA from various *E. coli* replicates.
See text for detailed description.

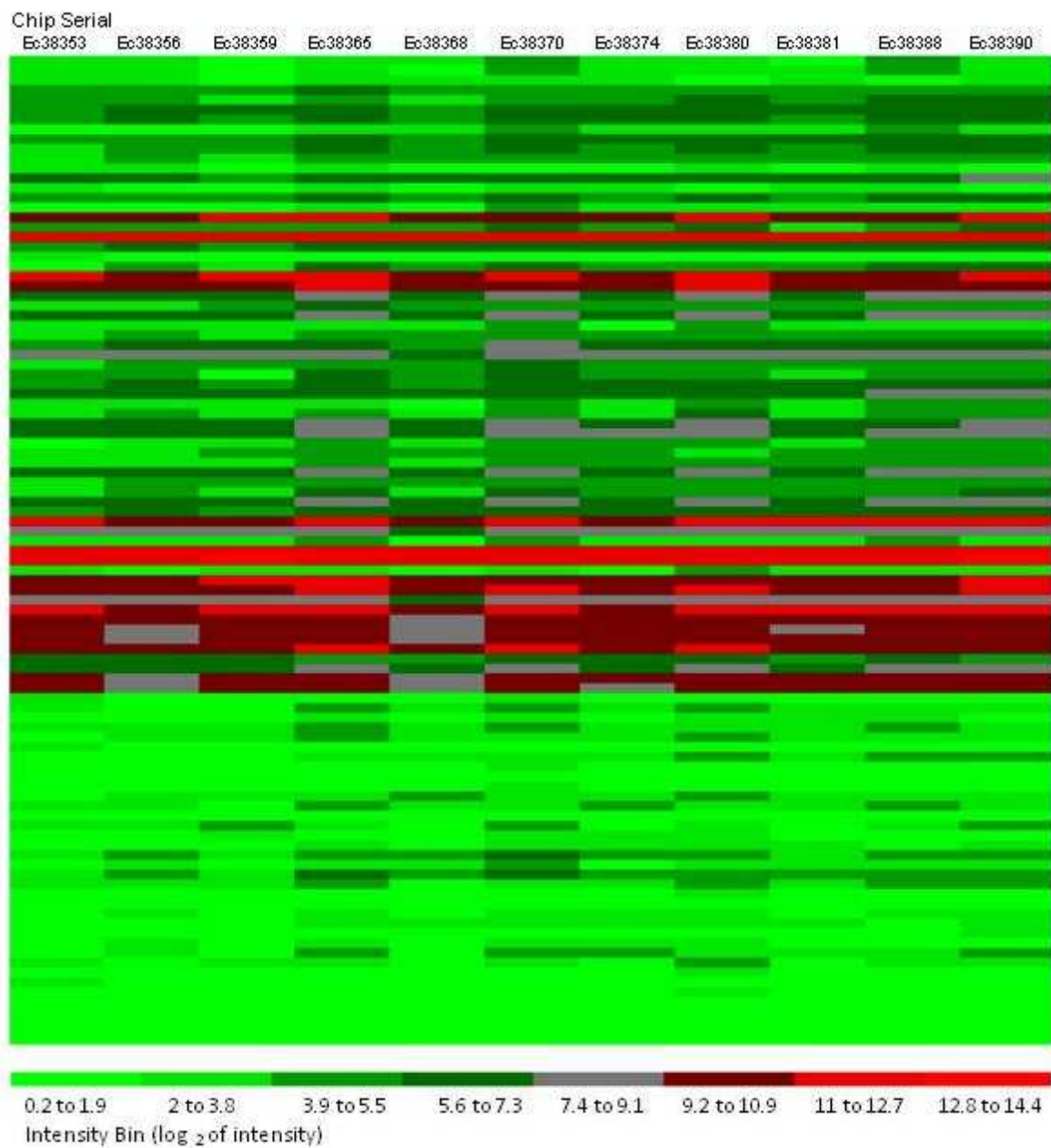
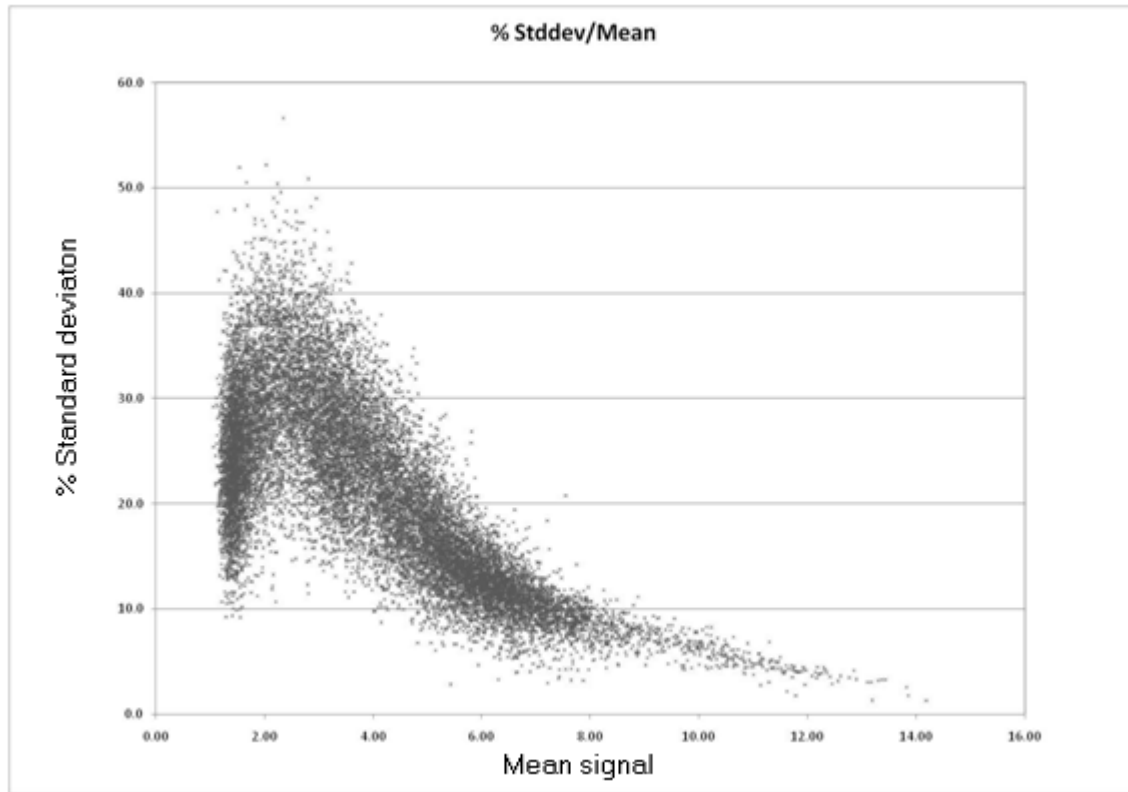
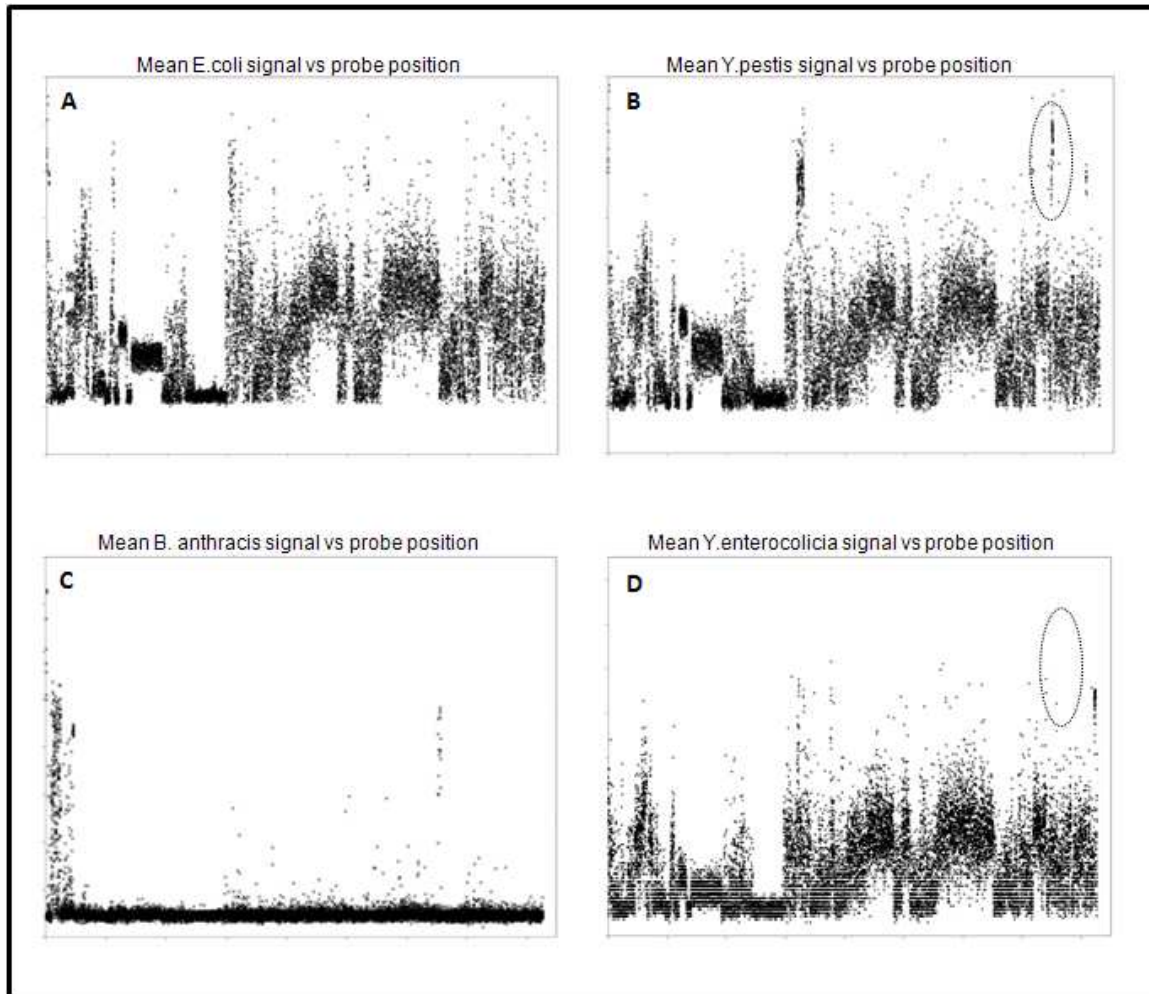


Figure 5: Standard deviation versus mean signal value.



Closer inspection of the plot in Figure 6-B showed the presence of signals not seen in the *E. coli* or the *Y. enterocolitica* samples (circled region in 6-B,D), indicating that this sample contained some unique DNA, and thus was either contaminated during preparation, or in the original culture stock. Upon detailed review of the signal data, it was apparent that even though the putative *Y. pestis* sample did indeed contain many signals similar to the *E. coli* pattern, a unique series of features designed to yield *Y. pestis*-specific signals, verified by in silico analysis, did indeed result in uniquely high signals for the *Y. pestis* data set, but not for any other data set. Our conclusion is that this sample did indeed contain *Y. pestis* DNA, but had suffered some contamination event either in original culture or in subsequent DNA preparation. Signals from both the intended DNA (*Y. pestis*) and from the contaminant were identified in this sample. In these plots, the *Y. enterocolitica* sample also appears to very like the *E. coli* sample, but differs noticeably from the *Y. pestis*.

Figure 6: Plots of signal intensity versus position.
See text for discussion.



4 Conclusions

The application of microarrays to microbial genotyping or fingerprinting is a technical compromise of time and difficulty versus data density. Single target or multiplex real-time PCR assays are faster and can be quantitative. Real-time PCR assays can in principle detect 2–4 targets per assay reaction, based on positive detection of specific sequences in known genomic targets. Routine PCR assays however, are not the best method of choice for detecting recombinants, variants, or the presence of non-target organisms. If an assay system could run hundreds of PCR reactions for each test sample, the analytical density of the microarray could be equaled. Typical microarray open source platforms can detect 20–50,000 targets per array, using a single labeling or amplification reaction. Open source microarray systems typically take 18–26 hours for a single execution, but each run encompasses the equivalent of 1–2 thousand multiplex PCR reactions.

Compared to PCR or gel electrophoresis assays, microarrays appear to be very expensive [13–15]. Microarray platforms are, for now at least, clear winners when the multiplex capabilities of the array systems are compared to comparable efforts on other platforms. Operating costs between open source microarray platforms and the Affymetrix system are similar, despite the higher hardware and consumables costs for the Affymetrix system. For example, the Affymetrix software package Command Console contains an integrated suite of tools for feature extraction, system quality assurance, and data curation. Required hands-on time for the feature extraction (from image to signal data on a spreadsheet) is measured in minutes, compared to the open source microarray system, where each array requires 1-3 hours of manual image data extraction. With the minimal hands-on time required for the post-hybridization, the automation features of the Affymetrix system represent its greatest operational advantage over the open source microarray platforms. Although initial costs are greater with the Affymetrix system, it seems likely that the cost differential will be very small once the accumulated savings in time and labour are considered.

The number of assays executed per microarray has the drawback that for some material sources, DNA from multiple species is likely to be present and may contribute to the measured signals [15]. If the microarray contains sufficient numbers of features and has a high degree of automation, endemic species are always going to give a signal, thus the mere presence of a signal of such a species in a given environmental or clinical context is not in itself meaningful [14-16]. Assays must be combined with other indices of suspicion (clinical signs, known exposure, suspect samples) in order to determine whether a given positive represents a real diagnosis or threat [1, 2, 17]. This is also true for most other molecular or microbiological assays currently in use. Simple detection of agent is insufficient to establish a diagnosis in a clinical setting.

In addition, as the sensitivity of assay systems improves (due to non-specific genomic amplification for example), out-of-context true positive signals (not within the normal range of endemics) may be detected [3,15,17]. Such signals may be due to sample contamination by workers, gratuitous sampling of infrequent (but locally intense) organism populations, or previously undetected genetic similarity between lab strains and endemic strains. Use of confirmatory assays of high-specificity (e.g. real-time PCR) will complement such data.

Given the requirement for technical expertise in operating a microarray system, and given the sensitivity to multiple targets in some samples, microarray systems will continue to require laboratory support. Microarray systems are in use in clinical centers, but point-of-care microarray systems are not imminent. On the other hand, time-to-result times are comparable to or better than conventional microbiology. Detailed testing of the current microarray and comparison to other microarray systems is underway. Additional testing with an expanded library of DNA samples and a wider sampling of species is required to fully assess the value of the microarray as a tool for diagnosis, detection, and identification of microbial samples.

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Annex A Species and strain-specific probes in the final array design.

Organism	Strain / details	Probes on Array
<i>Acinetobacter baumannii</i>	ACICU	38
<i>Acinetobacter baumannii</i>	ATCC 17978	54
<i>Acinetobacter baumannii</i>	AYE	143
<i>Acinetobacter baumannii</i> SNP	baumannii	20
<i>Acinetobacter baumannii</i>	baumannii	15
<i>Acinetobacter baumannii</i>	plasmid pSUN-5	5
<i>Acinetobacter baumannii</i>	SDF	44
<i>Acinetobacter baumannii</i> HPT	ATCC 17978	5
<i>Acinetobacter baumannii</i> HPT	AYE	5
<i>Acinetobacter baumannii</i> HPT	baumannii	5
<i>Bacillus anthracis</i>	Ames ancestor	140
<i>Bacillus anthracis</i>	Ames ancestor plasmid pX01	5
<i>Bacillus anthracis</i>	Ames ancestor plasmid pX02	25
<i>Bacillus anthracis</i>	anthracis	45
<i>Bacillus anthracis</i>	Australia 94	6
<i>Bacillus anthracis</i>	Kruger	5
<i>Bacillus anthracis</i>	Sterne	55
<i>Bacillus anthracis</i> APRT	A2012 plasmid pX01	5
<i>Bacillus anthracis</i> APRT	Ames	5
<i>Bacillus anthracis</i> HPT	A0442	5
<i>Bacillus anthracis</i> HPT	anthracis	5
<i>Bacillus anthracis</i> HPT	anthracis	10
<i>Bacillus anthracis</i> plasmid	Sterne plasmid pX01+pX02-	10
<i>Bacillus anthracis</i> SNP	A2012	20
<i>Bacillus anthracis</i> SNP	anthracis	780
<i>Bacillus anthracis</i> SNP	other anthracis	200
<i>Bacillus anthracis</i> SNP	W. North America	20
<i>Bacillus cereus</i>	ATCC 10987	179
<i>Bacillus cereus</i>	ATCC 14579	200
<i>Bacillus cereus</i>	B. cereus plasmid pBCX01	5
<i>Bacillus cereus</i>	E33L	45
<i>Bacillus cereus</i>	G9241	5
<i>Bacillus cereus</i> group SNP	Bacillus	1800
<i>Bacillus cereus</i> HPT	E33L	5
<i>Bacillus cereus</i> SNP	ATCC 10987	80
<i>Bacillus cereus</i> SNP	ATCC 14579	320

<i>Bacillus cereus</i> SNP	B. cereus plasmid pBCXO1	320
<i>Bacillus cereus/anthracis</i> SNP	B. cereus plasmid pBCXO1	20
<i>Bacillus amyloliquefaciens</i> APRT	FZB42	5
<i>Bacillus clausii</i>	KSM-K16	5
<i>Bacillus clausii</i> APRT	KSM-K16	5
<i>Bacillus halodurans</i>	C-125	5
<i>Bacillus halodurans</i> APRT	C-125	5
<i>Bacillus licheniformis</i>	ATCC 14580	35
<i>Bacillus licheniformis</i> APRT	ATCC 14580	5
<i>Bacillus pumilus</i> APRT	SAFR-032	5
<i>Bacillus subtilis</i>	168	25
<i>Bacillus subtilis</i> APRT	168	5
<i>Bacillus thuringiensis</i>	97-27	115
<i>Bacillus thuringiensis</i>	AI Hakam	130
<i>Bartonella bacilliformis</i>	ATCC 35685	175
<i>Bartonella henselae</i>	Houston-1	270
<i>Bartonella quintana</i>	Toulouse	245
<i>Bartonella tribocorum</i>	CIP 105476	330
<i>Bordetella</i> SNP	Bordetella	20
<i>Bordetella avium</i>	197N	440
<i>Bordetella avium</i> APRT	197N	5
<i>Bordetella bronchiseptica</i> APRT	RB50	5
<i>Bordetella bronchiseptica</i>	RB50	75
<i>Bordetella parapertussis</i>	12822	268
<i>Bordetella pertussis</i> APRT	Tohama I	5
<i>Bordetella pertussis</i>	Bordetella	5
<i>Bordetella pertussis</i>	Tohama I	615
<i>Bordetella petrii</i> APRT	DSM 12804	5
<i>Borrelia afzelii</i> APRT	PKo	5
<i>Brucella</i> SNP	9-941	20
<i>Brucella</i>	all brucella	250
<i>Brucella</i> HPT	all brucella	5
<i>Brucella abortus</i>	9-941	30
<i>Brucella abortus</i>	S19	45
<i>Brucella abortus</i> SNP	melitensis/abortus	40
<i>Brucella abortus</i> APRT	9-941	5
<i>Brucella abortus/melitensis</i> SNP	abortus/melitensis	20
<i>Brucella abortus/suis</i> SNP	abortus/suis	20
<i>Brucella canis</i>	ATCC 23365	15
<i>Brucella canis</i>	S19	5
<i>Brucella canis</i> HPT	ATCC 23365	10
<i>Brucella melitensis</i>	16M	427
<i>Brucella melitensis</i>	2308 bv Abortus	210
<i>Brucella melitensis</i>	bv Melitensis	10
<i>Brucella melitensis</i>	bv Suis 686	5
<i>Brucella ovis</i>	ATCC 25840	82

<i>Brucella ovis</i>	bv Abortus 2308	35
<i>Brucella suis</i>	1330	25
<i>Brucella suis</i>	ATCC 23445	5
<i>Brucella suis</i>	ATCC 23447	5
<i>Brucella suis</i>	ATCC 25840	10
<i>Brucella suis</i>	bv. 4 str. 40	15
<i>Brucella suis/abortus</i> SNP	suis/abortus	80
<i>Burkholderia</i> SNP	Burkholderia	1160
<i>Burkholderia</i> HPT	all burkholderia	5
<i>Burkholderia mallei</i> APRT	ATCC 23344	5
<i>Burkholderia mallei</i>	ATCC 23344	40
<i>Burkholderia mallei</i>	PRL-20	5
<i>Burkholderia multivorans</i> APRT	ATCC 17616	5
<i>Burkholderia pseudo/mallei</i> SNP	pseudomallei/mallei	20
<i>Burkholderia pseudo/mallei</i>	Burkholderia	15
<i>Burkholderia pseudomallei</i>	668	10
<i>Burkholderia pseudomallei</i>	1710b	5
<i>Burkholderia pseudomallei</i>	392f	5
<i>Burkholderia pseudomallei</i> SNP	B7210	40
<i>Burkholderia pseudomallei</i>	K96243	75
<i>Burkholderia pseudomallei</i> SNP	pseudomallei	340
<i>Burkholderia pseudomallei</i>	pseudomallei	5
<i>Burkholderia pseudomallei</i>	T18-1984	5
<i>Burkholderia pseudomallei</i> HPT	91	5
<i>Burkholderia pseudomallei</i> HPT	668	5
<i>Burkholderia pseudomallei</i> HPT	NCTC 13177	5
<i>Burkholderia pseudomallei</i> APRT	668	5
<i>Burkholderia thailandensis</i> APRT	E264	5
<i>Campylobacter concisus</i> APRT	13826	5
<i>Campylobacter fetus</i>	82-40	440
<i>Campylobacter hominis</i> APRT	ATCC BAA-381	5
<i>Campylobacter jejuni</i> APRT	doylei 269.97	5
<i>Campylobacter jejuni</i>	269.97 ss doylei	476
<i>Campylobacter jejuni</i>	81116 (NCTC 11828)	351
<i>Campylobacter jejuni</i>	81-176	349
<i>Campylobacter jejuni</i>	jejuni	60
<i>Campylobacter jejuni</i>	NCTC 11168	560
<i>Campylobacter jejuni</i>	plasmid pCjA13 t	5
<i>Campylobacter jejuni</i>	RM 1221	304
<i>Campylobacter jejuni</i> APRT	81-176	5
<i>Campylobacter jejuni</i> plasmid	81-176 plasmid pVir	5
<i>Chaetomium atrobrunneum</i>	atrobrunneum	5
<i>Chaetomium funicola</i>	funicola	29
<i>Chaetomium funicola</i>	OC13	5
<i>Chaetomium funicola</i>	olrim130	5
<i>Chaetomium thermophilum</i>	CT2	20

<i>Chaetomium thermophilum</i>	MTCC 6350	5
<i>Chaetomium thermophilum</i>	thermophilum	85
<i>Chlamydia abortus</i>	S26/3	115
<i>Chlamydia caviae</i>	GPIC	115
<i>Chlamydia felis</i>	Fe/C-56	120
<i>Chlamydia muridarum</i>	Nigg (MoPn)	115
<i>Chlamydia pneumoniae</i>	AR39	115
<i>Chlamydia pneumoniae</i>	CWL 029	5
<i>Chlamydia trachomatis</i>	D/UW-3/CX	175
<i>Chlamydia trachomatis</i>	HAR-13	15
<i>Chlamydia trachomatis</i>	trachomatis	5
<i>Clostridium botulinum</i> APRT	Alaska E43	10
<i>Clostridium botulinum</i> APRT	ATCC 3502	5
<i>Clostridium botulinum</i> APRT	Eklund 17B	5
<i>Clostridium botulinum</i> APRT	Okra	5
<i>Clostridium botulinum</i>	A str. ATCC 19397	5
<i>Clostridium botulinum</i>	ATCC 3502	40
<i>Clostridium botulinum</i>	B str. Eklund 17B	5
<i>Clostridium botulinum</i> SNP	B1 str. Okra plasmid pCLD	20
<i>Clostridium botulinum</i>	B1 str. Okra plasmid pCLD	5
<i>Clostridium botulinum</i>	Bf	5
<i>Clostridium botulinum</i> SNP	botulinum	1860
<i>Clostridium botulinum</i>	C str. Eklund	5
<i>Clostridium botulinum</i> SNP	C. botulinum A strains	100
<i>Clostridium botulinum</i>	C. botulinum A strains	5
<i>Clostridium botulinum</i>	<i>Clostridium botulinum</i>	15
<i>Clostridium botulinum</i>	Hall 183	5
<i>Clostridium botulinum</i> HPT	Alaska E43	15
<i>Clostridium botulinum</i> HPT	Eklund 17B	10
<i>Clostridium botulinum</i> HPT	Loch Maree	20
<i>Clostridium botulinum</i> HPT	Okra	5
<i>Clostridium botulinum</i>	A3 str. Loch Maree	5
<i>Clostridium acetobutylicum</i>	ATCC 824	25
<i>Clostridium beijerinckii</i>	NCIMB 8052	20
<i>Clostridium difficile</i>	630	45
<i>Clostridium difficile</i>		15
<i>Clostridium difficile</i> HPT	difficile	5
<i>Clostridium kluyveri</i> APRT	DSM 555	5
<i>Clostridium novyi</i>	ATCC19402	45
<i>Clostridium novyi</i>	NT	40
<i>Clostridium perfringens</i> APRT	SM101	5
<i>Clostridium perfringens</i>	13	111
<i>Clostridium perfringens</i>	ATCC 13124	66
<i>Clostridium perfringens</i> S		20
<i>Clostridium perfringens</i>	SM101	65
<i>Clostridium perfringens</i> HPT	13	5

<i>Clostridium perfringens</i> HPT	ATCC 13124	10
<i>Clostridium perfringens</i> HPT	SM101	10
<i>Clostridium perfringens</i> plasmid	plasmid pCP13	5
<i>Clostridium tetani</i>	E88	55
<i>Clostridium tetani</i> HPT	tetani	5
<i>Clostridium thermocellum</i>	ATCC 27405	15
<i>Corynebacterium diphtheriae</i>	diphtheriae	5
<i>Corynebacterium diphtheriae</i>	NCTC 13129	165
<i>Corynebacterium efficiens</i>	YS-314	65
<i>Corynebacterium glutamicum</i>	ATCC 13032	20
<i>Corynebacterium glutamicum</i>	R	69
<i>Corynebacterium glutamicum</i> APRT	ATCC 13032	5
<i>Corynebacterium jeikeium</i>	K411	110
<i>Coxiella burnetii</i>	CbuG Q212	15
<i>Coxiella burnetii</i>	Dugway 5J108-111	25
<i>Coxiella burnetii</i>	MSU Goat Q117	29
<i>Coxiella burnetii</i>	RSA 331	15
<i>Coxiella burnetii</i>	RSA 334	5
<i>Coxiella burnetii</i>	RSA 493	178
<i>Coxiella burnetii</i> HPT	Dugway	5
<i>Coxiella burnetii</i> HPT	burnetii	10
<i>Enterococcus faecalis</i>	faecalis	5
<i>Enterococcus faecalis</i>	MMH594	5
<i>Enterococcus faecalis</i>	V583	145
<i>Enterococcus faecalis</i> APRT	V583	5
<i>Enterococcus faecalis</i> HPT	faecalis	5
<i>Escherichia coli</i>	536	1035
<i>Escherichia coli</i>	1226	5
<i>Escherichia coli</i>	1334	5
<i>Escherichia coli</i>	55989	20
<i>Escherichia coli</i>	042	70
<i>Escherichia coli</i>	17-2	25
<i>Escherichia coli</i>	536 (UPEC)	30
<i>Escherichia coli</i>	B171	85
<i>Escherichia coli</i>	C1845	5
<i>Escherichia coli</i>	CFT 073 (UPEC)	516
<i>Escherichia coli</i>	coli	182
<i>Escherichia coli</i>	coli/shigella	5
<i>Escherichia coli</i>	E. coli plasmid pC15-1a_016	5
<i>Escherichia coli</i>	E/99 3-2 SHV	10
<i>Escherichia coli</i>	E2348/69	285
<i>Escherichia coli</i>	E45035	5
<i>Escherichia coli</i>	EC7372	5
<i>Escherichia coli</i>	EU2657	5
<i>Escherichia coli</i>	EU4855 plasmid	5
<i>Escherichia coli</i>	H11128	25

<i>Escherichia coli</i>	H11129	5
<i>Escherichia coli</i>	K12	38
<i>Escherichia coli</i>	K12 substr. MG1655	25
<i>Escherichia coli</i>	K983802	5
<i>Escherichia coli</i>	KS52	5
<i>Escherichia coli</i>	O157:H7 EDL933	345
<i>Escherichia coli</i>	plasmid	15
<i>Escherichia coli</i>	plasmid p541	5
<i>Escherichia coli</i>	plasmid pEC365	5
<i>Escherichia coli</i>	plasmid pGR2439	5
<i>Escherichia coli</i>	plasmid pMEL2	3
<i>Escherichia coli</i>	plasmid RZA92	5
<i>Escherichia coli</i>	Sakai(EHEC O157:H7)	11
<i>Escherichia coli</i>	SMS-3-5	20
<i>Escherichia coli</i>	Str. 01 (APEC)	50
<i>Escherichia coli</i>	Toho-1	5
<i>Escherichia coli</i>	UTI89 (UPEC)	65
<i>Escherichia coli</i>	YMC02/08/U310	5
<i>Escherichia coli</i>	SMS-3-5	5
<i>Escherichia coli</i> APRT	O157:H7 EDL933	5
<i>Escherichia coli</i> HPT	ATCC 8739	5
<i>Escherichia coli</i> HPT	E24377A	5
<i>Escherichia coli</i> HPT	F11	4
<i>Escherichia coli</i> HPT	HS	5
<i>Escherichia coli</i> plasmid	plasmid pAPEC-O1-ColBM	40
<i>Escherichia coli</i> strain EO 516	EO 516	5
<i>Francisella holarctica</i> APRT	OSU18	5
<i>Francisella holarctica</i>	FTNF002-00	15
<i>Francisella holarctica</i>	holarctica	31
<i>Francisella holarctica</i>	LVS	35
<i>Francisella holarctica</i>	OSU18	25
<i>Francisella holarctica</i> HPT	holarctica	10
<i>Francisella holarctica</i> SNP	FSC022	40
<i>Francisella holarctica</i> SNP	FTNF002-00	80
<i>Francisella holarctica</i> SNP	HOL 257	20
<i>Francisella holarctica</i> SNP	holarctica	240
<i>Francisella holarctica</i> SNP	LVS	20
<i>Francisella holarctica</i> SNP	OSU18	100
<i>Francisella novicida</i>	U112	105
<i>Francisella novicida</i> HPT	U112	5
<i>Francisella novicida</i> SNP	GA99-3548	700
<i>Francisella novicida</i> SNP	novicida	7480
<i>Francisella novicida</i> SNP	U112	620
<i>Francisella tularensis</i>	ATCC 6223	46
<i>Francisella tularensis</i>	francisella	5
<i>Francisella tularensis</i>	fsc033	5

<i>Francisella tularensis</i>	FSC198	15
<i>Francisella tularensis</i>	plasmid pOM1	5
<i>Francisella tularensis</i>	SCHU S4	411
<i>Francisella tularensis</i>	tularensis	52
<i>Francisella tularensis</i>	WY96-3418	55
<i>Francisella tularensis</i> SNP	SCHU	180
<i>Francisella tularensis</i> SNP	tularensis	580
<i>Francisella tularensis</i> SNP	WY96	100
<i>Francisella tularensis</i> SNP	WY96-3418	20
<i>Francisella</i>	Francisella	15
<i>Francisella holarctica/novicida</i>	holarctica/novicida	5
<i>Francisella holarctica/tularensis</i>	holarctica/tularensis	25
<i>Francisella novicida/tularensis</i>	novicida/tularensis	30
<i>Francisella tularensis/holarctica</i> SNP	tularensis/holarctica	20
<i>Haemophilus ducreyi</i>	35000 HP	405
<i>Haemophilus influenzae</i> APRT	86-028NP	5
<i>Haemophilus influenzae</i> APRT	Rd KW20	5
<i>Haemophilus influenzae</i>	12	30
<i>Haemophilus influenzae</i>	1007	89
<i>Haemophilus influenzae</i>	3179B	5
<i>Haemophilus influenzae</i>	86-028NP	336
<i>Haemophilus influenzae</i>	AM30	25
<i>Haemophilus influenzae</i>	C54	5
<i>Haemophilus influenzae</i>	influenzae	5
<i>Haemophilus influenzae</i>	N187	5
<i>Haemophilus influenzae</i>	Pitt EE	275
<i>Haemophilus influenzae</i>	Pitt GG	299
<i>Haemophilus influenzae</i>	Rd	95
<i>Haemophilus influenzae</i>	Rd KW20	375
<i>Haemophilus somnus</i>	2336	205
<i>Haemophilus somnus</i>	129 PT	380
<i>Helicobacter acinonychis</i>	Sheeba	279
<i>Helicobacter hepaticus</i>	ATCC 51449	250
<i>Helicobacter pylori</i> APRT	J99	5
<i>Helicobacter pylori</i>	26695	438
<i>Helicobacter pylori</i>	HPAG1	377
<i>Helicobacter pylori</i>	J99	484
Human	Human	100
<i>Klebsiella pneumonia</i> APRT	MGH 78578	5
<i>Lactobacillus delbrueckii</i> APRT	subsp. bulgaricus ATCC 11842	5
<i>Legionella pneumophila</i>	Philadelphia 1	793
<i>Legionella pneumophila</i> HPT	Corby	3
<i>Legionella pneumophila</i> HPT	Lens	5
<i>Legionella pneumophila</i> HPT	Paris	10
<i>Legionella pneumophila</i> HPT	Philadelphia 1	5
<i>Legionella pneumophila</i>	Corby	296

<i>Legionella pneumophila</i>	Lens	378
<i>Legionella pneumophila</i>	Paris	399
<i>Legionella pneumophila</i>	pneumophila	5
<i>Listeria innocua</i>	Clip 11262	105
<i>Listeria ivanovii</i>	ATCC 19119	5
<i>Listeria monocytogenes</i>	monocytogenes	10
<i>Listeria monocytogenes</i> APRT	EGD-e	5
<i>Listeria monocytogenes</i> HPT	4b 2365	10
<i>Listeria monocytogenes</i> HPT	EGD-e	5
<i>Listeria monocytogenes</i>	4b 2365	260
<i>Listeria monocytogenes</i>	EGD-e sv 1/2A	453
<i>Listeria monocytogenes</i>	F2365	95
<i>Listeria monocytogenes</i> APRT	F2365	5
<i>Listeria monocytogenes</i> SNP	J1-194	1280
<i>Listeria monocytogenes</i> SNP	J2-064	80
<i>Listeria monocytogenes</i>	J2-064	5
<i>Listeria monocytogenes</i> SNP	monocytogenes	5180
<i>Listeria welshimeri</i> APRT	SLCC 5334	5
<i>Listeria welshimeri</i>	SLCC 5334	100
<i>Mycobacterium avium</i> APRT	K-10 ss paratuberculosis	5
<i>Mycobacterium avium</i>	104	263
<i>Mycobacterium avium</i>	K-10 ss paratuberculosis	743
<i>Mycobacterium bovis</i> APRT	BCG str. Pasteur 1173P2	5
<i>Mycobacterium bovis</i>	AF2122/97	15
<i>Mycobacterium bovis</i>	BCG Pasteur 1173P2	15
<i>Mycobacterium gilvums</i>	PYR-GCK	619
<i>Mycobacterium leprae</i> APRT	TN	5
<i>Mycobacterium leprae</i>	TN	379
<i>Mycobacterium marinum</i> APRT	M	5
<i>Mycobacterium smegmatis</i>	MC2155	543
<i>Mycobacterium tuberculosis</i> APRT	CDC 1551	5
<i>Mycobacterium tuberculosis</i>	CDC 1551	120
<i>Mycobacterium tuberculosis</i>	F11	15
<i>Mycobacterium tuberculosis</i>	H37 Ra	5
<i>Mycobacterium tuberculosis</i>	H37 Rv	682
<i>Mycobacterium tuberculosis</i>	tuberculosis/bovis	5
<i>Mycobacterium ulcerans</i>	Agy 99	504
<i>Mycobacterium ulcerans</i> APRT	Agy99	5
<i>Mycobacterium ulcerans</i> plasmid	Agy99 plasmid pMUM001	20
<i>Mycobacterium van baalenii</i>	PYR-1	702
<i>Mycobacterium</i> sp.	JLS	650
<i>Mycobacterium</i> sp.	KMS	120
<i>Mycobacterium</i> sp.	MCS	45
<i>Mycoplasma agalactiae</i>	PG2	45
<i>Mycoplasma capricolum</i>	ATCC 27343	10
<i>Mycoplasma gallisepticum</i>	R	230

<i>Mycoplasma genitalium</i>	G37	50
<i>Mycoplasma hyopneumoniae</i> APRT	7448	4
<i>Mycoplasma hyopneumoniae</i> APRT	J	7
<i>Mycoplasma hyopneumoniae</i>	232	70
<i>Mycoplasma hyopneumoniae</i>	7448	35
<i>Mycoplasma hyopneumoniae</i>	J	30
<i>Mycoplasma mobile</i>	163K	105
<i>Mycoplasma mycoides</i> APRT	PG1	5
<i>Mycoplasma mycoides</i>	PG1	90
<i>Mycoplasma penetrans</i>	HF-2	250
<i>Mycoplasma pneumoniae</i> APRT	M129	5
<i>Mycoplasma pneumoniae</i>	M129	50
<i>Mycoplasma pneumoniae</i>	pneumoniae	5
<i>Mycoplasma pulmonis</i> APRT	UAB CTIP	5
<i>Mycoplasma pulmonis</i>	UABCTIP	74
<i>Mycoplasma synoviae</i>	53	10
<i>Neisseria gonorrhoeae</i>	FA 1090	205
<i>Neisseria meningitidis</i>	FAM18	188
<i>Neisseria meningitidis</i>	MC58	274
<i>Neisseria meningitidis</i>	neisseria	5
<i>Neisseria meningitidis</i>	str. 053442	164
<i>Neisseria meningitidis</i>	Z2491	281
Plasmid pBC16	Plasmid pBC16	5
Plasmid pLS1	Plasmid pLS1	5
<i>Pseudomonas aeruginosa</i> HPT	2192 Paer2_01_70	5
<i>Pseudomonas aeruginosa</i> HPT	PA01	10
<i>Pseudomonas aeruginosa</i> HPT	PA7	5
<i>Pseudomonas aeruginosa</i>	aeruginosa	5
<i>Pseudomonas aeruginosa</i>	PA01	1274
<i>Pseudomonas aeruginosa</i>	PA7	1015
<i>Pseudomonas aeruginosa</i>	UCBPP-PA14	317
<i>Pseudomonas entomophila</i> HPT	L48	5
<i>Pseudomonas entomophila</i>	L48	558
<i>Pseudomonas fluorescens</i> HPT	Pf-5	5
<i>Pseudomonas fluorescens</i> HPT	PfO-1	5
<i>Pseudomonas fluorescens</i>	Pf-5	710
<i>Pseudomonas fluorescens</i>	PfO-1	590
<i>Pseudomonas mendocina</i> HPT	ymp	5
<i>Pseudomonas mendocina</i>	ymp	645
<i>Pseudomonas putida</i> APRT	KT 2440	5
<i>Pseudomonas putida</i> HPT	GB-1	5
<i>Pseudomonas putida</i> HPT	KT 2440	5
<i>Pseudomonas putida</i>	F1	430
<i>Pseudomonas putida</i>	GB-1	607
<i>Pseudomonas putida</i>	KT 2440	706
<i>Pseudomonas putida</i>	W619	560

<i>Pseudomonas stutzeri</i>	A1501	480
<i>Pseudomonas stutzeri</i> HPT	A1501	5
<i>Pseudomonas syringae</i> APRT	pv. phaseolicola 1448A	5
<i>Pseudomonas syringae</i>	1448A	1042
<i>Pseudomonas syringae</i>	B728a	1021
<i>Pseudomonas syringae</i>	DC3000	1214
<i>Pseudomonas syringae</i> HPT	pv. phaseolicola 1448A	7
<i>Pseudomonas syringae</i> HPT	pv. syringae B728a	5
<i>Pseudomonas syringae</i> HPT	pv. tomato str. DC3000	5
<i>Pseudomonas syringae</i> plasmid	1448A large plasmid	50
<i>Pseudomonas syringae</i> plasmid	plasmid pDC3000A	20
<i>Ricinus communis</i>	communis	20
<i>Rickettsia prowazekii</i>	Madrid E	55
<i>Rickettsia prowazekii</i>	prowazekii	5
<i>Rickettsia rickettsii</i>	Iowa	70
<i>Rickettsia rickettsii</i> SNP	rickettsiae	60
<i>Rickettsia rickettsii</i>	rickettsii/afraicae/sibirica	5
<i>Rickettsia typhi</i>	Wilmington	55
<i>Salmonella enterica</i> APRT	Typhi str. CT18	5
<i>Salmonella enterica</i>	ATCC 9150 sv paratyphi A	168
<i>Salmonella enterica</i>	CT18	332
<i>Salmonella enterica</i>	enterica	5
<i>Salmonella enterica</i>	LT2	520
<i>Salmonella enterica</i>	RSK 2980 ss arizona sv 62	544
<i>Salmonella enterica</i>	SC-B67 sv Choleraesuis	201
<i>Salmonella enterica</i>	SPB7 sv Paratyphi B	207
<i>Salmonella enterica</i>	sv typhimurium	239
<i>Salmonella enterica</i>	Ty2	10
<i>Salmonella enterica</i> plasmid	pSN254	125
<i>Salmonella enterica</i> plasmid	SC-B67 plasmid pSCV50	10
<i>Salmonella typhimurium</i>	LT2	253
<i>Salmonella typhimurium</i> plasmid	LT2 plasmid pSLT	5
<i>Shigella dysenteriae</i>	plasmid pmK105	5
<i>Shigella boydii</i>	227	43
<i>Shigella boydii</i>	0-1392	20
<i>Shigella boydii</i>	CDC 3083-94	93
<i>Shigella boydii</i>	Sb227	300
<i>Shigella boydii</i> HPT	boydii	5
<i>Shigella boydii</i> plasmid	plasmid pSB4_227	15
<i>Shigella dysenteriae</i> APRT	Sd197	5
<i>Shigella dysenteriae</i>	197	107
<i>Shigella dysenteriae</i>	Sd197	130
<i>Shigella dysenteriae</i> plasmid	plasmid pSD1_197	171
<i>Shigella flexneri</i>	301	866
<i>Shigella flexneri</i>	8401	60
<i>Shigella flexneri</i>	2457T	80

<i>Shigella flexneri</i>	flexneri	45
<i>Shigella flexneri</i>	M90T	248
<i>Shigella flexneri</i>	multiple species	5
<i>shigella flexneri</i> HPT	flexneri	5
<i>Shigella flexneri</i> plasmid	M90T plasmid pWR501	15
<i>Shigella flexneri</i> plasmid	plasmid pPCP301	35
<i>Shigella sonnei</i>	Ss046	66
<i>Shigella sonnei</i> plasmid	str. 046 plasmid pSS_046	15
<i>Staphylococcus aureus</i> APRT	N315	5
<i>Staphylococcus aureus</i> HPT	aureus	5
<i>Staphylococcus aureus</i>	aureus	45
<i>Staphylococcus aureus</i>	COL	140
<i>Staphylococcus aureus</i>	JH1	15
<i>Staphylococcus aureus</i>	JH9	15
<i>Staphylococcus aureus</i>	MRSA 252	255
<i>Staphylococcus aureus</i>	MSSA 476	2
<i>Staphylococcus aureus</i>	Mu3	10
<i>Staphylococcus aureus</i>	Mu50	140
<i>Staphylococcus aureus</i>	MW2	350
<i>Staphylococcus aureus</i>	N315	20
<i>Staphylococcus aureus</i>	NCTC 8325	29
<i>Staphylococcus aureus</i>	Newman	15
<i>Staphylococcus aureus</i>	RF122	203
<i>Staphylococcus aureus</i>	USA 300_TCH 1516	10
<i>Staphylococcus aureus</i>	USA 3000	27
<i>Staphylococcus epidermidis</i> APRT	RP62A	5
<i>Staphylococcus epidermidis</i>	ATCC 12228	62
<i>Staphylococcus epidermidis</i>	RP62A	60
<i>Staphylococcus epidermidis</i> HPT	epideridis	5
<i>Staphylococcus haemolyticus</i>	JCSC 1435	80
<i>Staphylococcus haemolyticus</i> HPT	haemolyticus	5
<i>Staphylococcus saprophyticus</i> HPT	saprophyticus	5
<i>Staphylococcus saprophyticus</i>	ATCC 15305	95
<i>Streptococcus agalactiae</i> APRT	A909	5
<i>Streptococcus agalactiae</i>	2603 V/R	145
<i>Streptococcus agalactiae</i>	A909	200
<i>Streptococcus agalactiae</i>	agalactiae	5
<i>Streptococcus agalactiae</i>	FM027022	5
<i>Streptococcus agalactiae</i>	NEM316	75
<i>Streptococcus agalactiae</i> HPT	agalactiae	5
<i>Streptococcus agalactiae</i> HPT	CJB111	10
<i>Streptococcus gordonii</i>	Challis	150
<i>Streptococcus mutans</i>	UA 159	135
<i>Streptococcus pneumoniae</i> APRT	Hungary 19A-6	5
<i>Streptococcus pneumoniae</i> APRT	R6	5
<i>Streptococcus pneumoniae</i> HPT	Hungary 19A-6	6

<i>Streptococcus pneumoniae</i> HPT	pneumoniae	5
<i>Streptococcus pneumoniae</i> HPT	TIGR4	2
<i>Streptococcus pneumoniae</i>	CGSP14	87
<i>Streptococcus pneumoniae</i>	D39	154
<i>Streptococcus pneumoniae</i>	Hungary 19A-6	130
<i>Streptococcus pneumoniae</i>	pneumoniae	5
<i>Streptococcus pneumoniae</i>	R6	5
<i>Streptococcus pneumoniae</i>	TIGR4	185
<i>Streptococcus pyogenes</i> APRT	M1 GAS	5
<i>Streptococcus pyogenes</i>	Manfredo st M5	50
<i>Streptococcus pyogenes</i>	MGAS 10270 st M2	95
<i>Streptococcus pyogenes</i>	MGAS 10394 st M6	95
<i>Streptococcus pyogenes</i>	MGAS 10750 st M4	83
<i>Streptococcus pyogenes</i>	MGAS 2096 st M12	57
<i>Streptococcus pyogenes</i>	MGAS 315 st M3	85
<i>Streptococcus pyogenes</i>	MGAS 5005 st M1	35
<i>Streptococcus pyogenes</i>	MGAS 6180 st M28	80
<i>Streptococcus pyogenes</i>	MGAS 8232 st M18	65
<i>Streptococcus pyogenes</i>	MGAS 9429 st M12	10
<i>Streptococcus pyogenes</i>	pyogenes	5
<i>Streptococcus pyogenes</i>	SF370	150
<i>Streptococcus pyogenes</i>	SSI-1 st M3	36
<i>Streptococcus pyogenes</i> HPT	MGAS 10394	5
<i>Streptococcus pyogenes</i> HPT	MGAS 10750	5
<i>Streptococcus pyogenes</i> HPT	MGAS 8232	5
<i>Streptococcus pyogenes</i> HPT	pyogenes	5
<i>Streptococcus sanguinis</i>	SK36	232
<i>Streptococcus sanguinis</i> HPT	sanguinis	5
<i>Streptococcus suis</i>	05ZYH33	138
<i>Streptococcus suis</i>	98 HAH33	65
<i>Streptococcus thermophilus</i> HPT	LMG 18311	4
<i>Streptococcus thermophilus</i>	CNRZ1066	117
<i>Streptococcus thermophilus</i>	LMD-9	150
<i>Streptococcus thermophilus</i>	LMG 18311	135
<i>Streptococcus thermophilus</i> HPT	thermophilus	5
<i>Treponema pallidum</i>	Nichols	5
<i>Treponema pallidum</i>	pallidum	5
<i>Treponema pallidum</i>	SS14	30
<i>Ureaplasma parvum</i> APRT	ATCC 27815	5
<i>Vibrio cholerae</i> APRT	O395	5
<i>Vibrio cholerae</i> HPT	623-39	5
<i>Vibrio cholerae</i> HPT	RC385	5
<i>Vibrio cholerae</i>	1587	5
<i>Vibrio cholerae</i>	623-39	10
<i>Vibrio cholerae</i>	all other <i>Vibrio cholerae</i>	60
<i>Vibrio cholerae</i>	cholerae	5

<i>Vibrio cholerae</i>	MAK 757	5
<i>Vibrio cholerae</i>	MZO-2	5
<i>Vibrio cholerae</i>	MZO-3	5
<i>Vibrio cholerae</i>	N16961	1144
<i>Vibrio cholerae</i>	NCTC 8457	5
<i>vibrio cholerae</i>	O395	145
<i>Vibrio cholerae</i>	plasmid pTLC -1	5
<i>Vibrio cholerae</i>	plasmid pTLC -2	5
<i>Vibrio cholerae</i>	plasmid pTLC -3	5
<i>Vibrio cholerae</i>	plasmid pTLC -4	5
<i>Vibrio cholerae</i>	plasmid pTLC -5	5
<i>Vibrio cholerae</i>	RC385	5
<i>Vibrio cholerae</i>	V51	10
<i>Vibrio cholerae</i> HPT	cholerae	5
<i>Vibrio cholerae</i> HPT	V51	5
<i>Vibrio fischeri</i>	ES114	554
<i>Vibrio parahaemolyticus</i>	AQ3810	5
<i>Vibrio parahaemolyticus</i>	parahaemolyticus	5
<i>Vibrio parahaemolyticus</i>	RIMD 2210633	830
<i>Vibrio vulnificus</i>	CMCP6	764
<i>Vibrio vulnificus</i>	Vibrio vulnificus	5
<i>Vibrio vulnificus</i>	YJ016	443
<i>Xanthomonas axonopodis</i> APRT	pv. citri str. 306	5
<i>Yersinia enterocolitica</i>	8081	560
<i>Yersinia enterocolitica</i>	84-50	5
<i>Yersinia enterocolitica</i>	A127	177
<i>Yersinia enterocolitica</i>	W1024	10
<i>Yersinia enterocolitica</i> APRT	8081	5
<i>Yersinia enterocolitica</i> HPT	8081	10
<i>Yersinia enterocolitica</i> plasmid	8081 plasmid pYVe8081	94
<i>Yersinia pestis</i>	91001 bv Microtus	20
<i>Yersinia pestis</i>	Angola	38
<i>Yersinia pestis</i>	Antiqua	50
<i>Yersinia pestis</i>	bv Microtus str. 91001	15
<i>Yersinia pestis</i>	CO92	614
<i>Yersinia pestis</i>	KIM	65
<i>Yersinia pestis</i>	Nepal 516	20
<i>Yersinia pestis</i>	Pestoides F	15
<i>Yersinia pestis</i>	Y. pestis	5
<i>Yersinia pestis</i> APRT	Angola	5
<i>Yersinia pestis</i> APRT	CO92	5
<i>Yersinia pestis</i> APRT	KIM	5
<i>Yersinia pestis</i> HPT	CO92	10
<i>Yersinia pestis</i> plasmid	pIP1202	90
<i>Yersinia pestis</i> plasmid	91001 bv Microtus plasmid pCD1	10
<i>Yersinia pestis</i> plasmid	Angola plasmid pCD	5

<i>Yersinia pestis</i> plasmid	Pestoides F plasmid pCD	13
<i>Yersinia pseudotuberculosis</i>	IP 31758	115
<i>Yersinia pseudotuberculosis</i>	IP 32953	68
<i>Yersinia pseudotuberculosis</i>	pseudotuberculosis	5
<i>Yersinia pseudotuberculosis</i>	YP111	56
<i>Yersinia pseudotuberculosis</i> HPT	PB1/+	10
<i>Yersinia pseudotuberculosis</i> plasmid	IP32953 plasmid YV	12
<i>Yersinia pseudotuberculosis</i> plasmid	plasmid pYps IP31758.1	195
<i>Yersinia pseudotuberculosis</i> plasmid	plasmid pYps IP31758.2	45
<i>Yersinia pestis/pseudotuberculosis</i>	pestis/pseudotuberculosis	10
<i>Yersinia pestis/pseudotuberculosis</i> SNP	IP 31758	20
<i>Yersinia pestis/pseudotuberculosis</i> SNP	pestis/pseudotuberculosis	520

Annex B Detailed Protocols for microarray labeling

dUTP Incorporation - Using ROCHE Random Priming kit (no amplification)

1. Add 5 µg sample DNA (16 µl volume) to PCR tube. Incubate at 95 °C for 10 min.
2. During the above incubation, prepare the following (Incorporation mix):

- 0.8 µl dH₂O
- 0.8 µl of 1 mM dUTP
- 1.6 µl of 0.5 mM dTTP
- 2.0 µl of 0.5 mM dATP
- 2.0 µl of 0.5 mM dCTP
- 2.0 µl of 0.5 mM dGTP

3. Vortex the mixture briefly.
4. When the incubation in step 1 is almost finished, add the following to the incorporation mix:
 - 2.0 µl of the hexamer primer reaction mix
 - 1.0 µl of Klenow enzyme (keep on ice until use)
5. Vortex the mixture briefly and keep on ice until use.
6. Add 12.2 µl (total volume of the incorporation mixture) to the cooled sample DNA from step 1. Vortex briefly.
7. Incubate the sample at 37 °C for 2 hrs (program AFFY1).
8. After 2 hrs, take sample from incubator and add sufficient RNase-free H₂O to make a final volume of 28.2 µl (if required).
9. Incubate sample tube at 95 °C for 10 min

Fragmentation - Using AFFY GeneChip WT Terminal Labelling Kit

10. When step 9 is almost finished, prepare the following (Fragmentation mix):

- 10.0 µl RNase-free H₂O
- 4.8 µl of Fragmentation buffer
- 1.0 µl of 10 U/µl UDG enzyme
- 1.0 µl of 1000 U/µl APE1 enzyme
- Total volume 16.8 µl

11. Vortex briefly and keep on ice until needed.
12. Add 16.8 µl of Fragmentation mix to the cooled sample DNA. Total volume should be 45 µl.
13. Incubate mixture at 37 °C for 1 hour (program AFFY2)

Labeling - Using the AFFY GeneChip WT Terminal Labelling Kit

14. When step 13 is almost finished, prepare the following (Labeling Mix):

10.0 μ l 5x TdT buffer
2.0 μ l TdT
1.0 μ l DNA labelling reagent
Total volume 15.0 μ l

15. Vortex briefly and keep on ice until needed.

16. Add the total volume of labelling mix to the cooled sample mixture from step 13.

17. Incubate at 37 °C for 1 hour (program AFFY3)

Microarray hybridization, post-treatment, scanning, feature extraction

After preparing the biotinylated sample probe, a hybridization mixture is prepared as follows:

60.0 μ l sample mixture
11.0 μ l warmed (65 °C) 20x Eukaryote Hybridization Control
3.7 μ l B2 Oligo control
15.4 μ l DMSO
110.0 μ l 2X Hybridization Buffer Mix
20.0 μ l dH₂O

The entire labeled sample reaction is denatured at 99 °C, then cooled to 45 °C for 5 minutes. 200 μ l is applied to the microarray, then the array is incubated in a hybridization oven in a rotating holder for 16–18 hours at 45 °C. After hybridization, the array is transferred to the fluidics station which performs a post-hybridization wash followed by an automated labeling with streptavidin-phycoerythrin, a fluorescent chromophore complex that binds to the biotin in the sample probe.

Post-hybridization arrays were scanned in the 3000-7G Affymetrix array imager. This unit scans the barcode of the array, then applies the appropriate colour and resolution settings automatically, scans the array, and downloads the recorded data to the workstation for analysis. Feature extraction takes place automatically following the array scan download.

List of symbols/abbreviations/acronyms/initialisms

APE	Apurinic endonuclease; cleaves DNA adjacent to apurinic sites
APRT	adenine phosphoribosyltransferase
ATCC	American Type Culture Collection; an organization supplying standard microbial strains and samples
BLAST	Basic Local Alignment Search Tool
bp	base pair
DNA	deoxyribonucleic acid
DRDC	Defence Research & Development Canada
HPT	hypoxanthine guanine phosphoribosyltransferase
mM	millimolar
NCBI	National Center for Biotechnology Information (also referred to as Genbank)
PERL	a high level programming language for scanning text files, extracting data, and generating reports from the data
R&D	Research & Development
SNP	single nucleotide polymorphism; a sequence variant at one base position which may be different between populations or individuals
TDT	terminal deoxynucleotidyl transferase

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There is an ongoing requirement for development of high-density or multiplex assays for detection or identification of microbes. There is also a need to develop assays or toolsets that can detect or identify microbial threats without prior knowledge of the target microbe(s) in a given sample. Indeed, some samples that contain no culturable material (e.g. viable but non-culturable cells) will nonetheless contain detectable DNA fragments which might be of value with respect to forensics or attribution of source. For many pathogenic microbes, various specific tests already exist, but there are few general methods wherein a single adaptable tool can be applied to multiple species or to previously uncharacterized organisms. The high-density DNA microarray has the potential to address many of these requirements and thus complements existing identification tools. The microarray platform has for example, the ability to detect microbial DNA that is not a perfect match to known genomic DNA sequences, thus making it possible to detect microbial variants that might otherwise be missed. In this report, the design and preliminary testing of a high-density DNA microarray for the purpose of microbial identification and detection is described.

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