charles river

MICROBIAL SOLUTIONS

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AccuGENX-ST®

Advantages

- High discriminatory power
- High reproducibility
- Most informative data in the industry
- Greater reliability than the Dupont RiboPrinter®
- Easily databased information for use in tracking and trending

Production facilities often find themselves needing to determine the source of a contamination, if a certain production strain is the correct one, or even what the normal flora are for the facility. Now it is possible to reliably track and trend some of the most difficult microorganisms encountered in aseptic and non-sterile production facilities by combining the accuracy of Charles River AccuGENX-ID® rDNA genotypic identification with AccuGENX-ST®, a cGMP service for single- or multi-locus sequence typing.

Strain-Level Characterization

AccuGENX-ST® is a microbial characterization process used to distinguish closely related microorganisms to the strain level by utilizing well-established, highly accurate sequencing methods, based on single- and multi-locus sequence typing (S/MLST). Once a genetic description is generated using AccuGENX-ST®, the S/MLST raw sequence data are electronically retained in compliance with cGMP guidelines and are accessible for comparison among facilities for accurate tracking and trending.

Applications

- Sterility failures
- Tracking contamination events
- Root cause analysis
- QA/QC-OOS investigations
- Microbial mapping of production area
- Ensuring stability of QC organisms
- Certifying strain identity for cell banks and dietary supplements
- Research and development projects

Methodology

Increased discrimination at the strain level is achieved by sequencing, analyzing, and comparing single or multiple gene loci (regions) that show higher variability within a species. These regions include essential outer membrane protein coding genes or housekeeping genes that encode for proteins necessary for normal cellular functions of the organism, all of which contain more variability in their sequence. The principle of S/MLST is simple: the technique involves PCR amplification followed by DNA sequencing. Nucleotide differences between strains are evaluated at a variable number of gene targets, depending on the species being investigated.

Process Steps

To determine the appropriate gene targets and primer sets, the process begins with an AccuGENX-ID® identification analysis to the genus/species level. Target genes are then amplified using specific primers and subjected to comparative DNA sequence analysis. In the final report, sequence similarities between organisms are displayed using a phylogenetic tree (referred to as the neighbor-joining tree) and an observation is made on their relatedness.

Figure 1: Multi- and single-locus sequence typing

Methods for sequence-based strain typing

Microbial identification using the rRNA regions is a robust technology. There are times, however, when additional information is needed and it is essential to be able to differentiate organisms below the species level. Increased discrimination at the strain level can be achieved by sequencing, analyzing, and comparing highly variable loci (regions) in the organism's genome. MLST and SLST analyze essential protein coding genes, or housekeeping genes, that encode for proteins necessary for the normal cellular functions of the bacterium, all of which contain more variability in their sequences.

After extracting DNA from the sample, which can be viable or non-viable, the first step in this process is an accurate ID to the species level. This must be performed before starting MLST or SLST to determine appropriate targets and primer sets as the target genes will not be the same for each species being characterized. The target genes are PCR amplified using the gene-specific primers and subjected to comparative DNA sequence analysis. All the sequences from each gene target are aligned and compared to the other organisms' sequence data and the level of divergence or conservation between the organisms is calculated and displayed with a phylogenetic tree. We will interpret the data for the client and state whether the isolates are indistinguishable by the sequencing of the particular gene targets or whether the isolates are different sequence types.

AccuGENX-ST® Versus RiboPrinter®

Often, organisms do not have sufficient diversity in the ribosomal region to result in strain-level resolution after testing with the RiboPrinter®. Coupled with subjective reading and interpretation of the fragment banding patterns, reproducibility of results is often compromised. Sequencing protein coding genes with AccuGENX-ST® provides more differentiation for strain typing. Sequence data is more objective, easily analyzed, and, because it is based on sequencing, yields consistent, reproducible results.

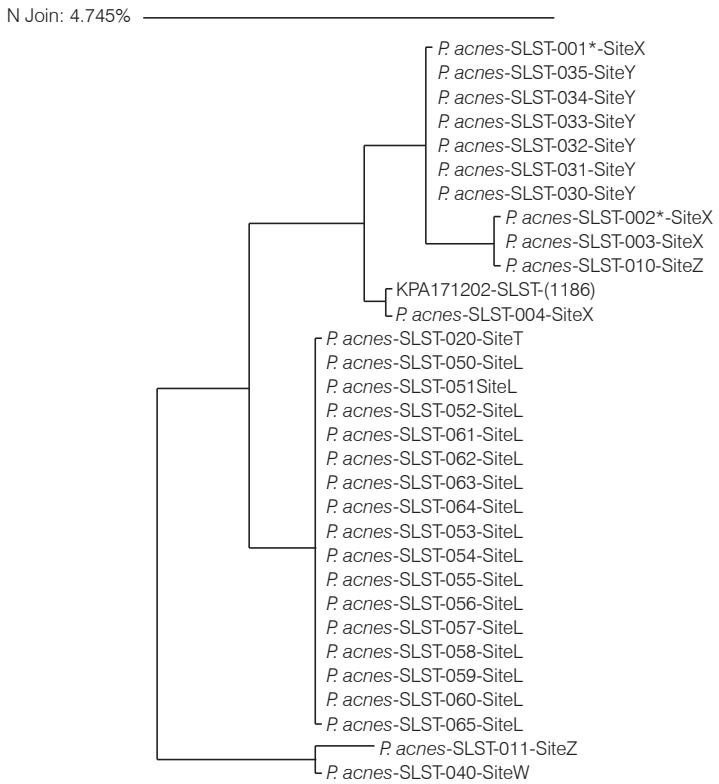
AccuGENX-ST® makes it possible to strain-type some of the more challenging organisms encountered in environmental monitoring programs and cell banks that are difficult to reliably type using the RiboPrinter. Commonly found organisms like *B. cereus, P. acnes, M. luteus,* and *S. aureus* consistently yield high-confidence strain typing data. For a complete listing of organisms, go to our website, www.criver.com/accugenix.

As an example, during our internal feasibility and validation studies, we tested 31 isolates of *Propionibacterium acnes,* a highly clonal species that exhibits very little genetic variability between strains, using both the RiboPrinter® and AccuGENX-ST®. Although this assay was run at its highest level of discrimination by using two restriction enzymes, *Eco*RI and *PvuII*, ribotyping was ineffective in discriminating between *P. acnes* strains. AccuGENX-ST®, however, was able to differentiate between the strains and revealed that the 31 *P. acnes* isolates fell into eight separate sequence types. For additional information on AccuGENX-ST® versus RiboPrinter®, please see our technical note, "Highly Accurate Strain Analysis Through Sequence Typing."

Table 1: Ribotype pattern of *P. acnes* isolates showing two RiboGroups

Label	Protocol Cutter	RiboGroup	RiboPrint™ Pattern
001*-SiteX	EcoRI-KHA	250-S2	
001*-SiteX	EcoRI-KHA	250-S2	
001*-SiteX	EcoRI-KHA	250-S2	
002*-SiteX	EcoRI-KHA	250-S2	
002*-SiteX	EcoRI-KHA	250-S2	
002*-SiteX	EcoRI-KHA	250-S2	
003*-SiteX	EcoRI-KHA	130-S6	
001*-SiteX	PvuII-KHB	141-S2	
001*-SiteX	PvuII-KHB	141-S2	
001*-SiteX	PvuII-KHB	141-S2	
002*-SiteX	PvuII-KHB	141-S2	
002*-SiteX	PvuII-KHB	141-S2	
002*-SiteX	PvuII-KHB	141-S2	
003*-SiteX	PvuII-KHB	141-S2	

Figure 2: Dendrogram showing genetic relatedness of 31 *P. acnes* isolates based on the *nif3* gene sequences



Ordering AccuGENX-ST®

AccuGENX-ST® can be conveniently ordered through our customer web portal at myaccount.accugenix.com. We have established cGMP processes for the most frequently occurring species. Go to our website, www.criver.com/accugenix, to see our complete listing. If you do not see your species of interest, contact Technical Support at +1.302.292.8888 to find out what organisms are under R&D investigation and when they will be available as a cGMP process.

Service	Turnaround Time	Code
AccuGENX-ST® sequence-based strain typing	5 day	AccuGENX-ST-5
AccuGENX-XGST® multi-locus sequence-based strain typing	5 day	AccuGENX-XGST-5