

ABSTRACT



The availability of quality genomic reference materials is essential to the defense community for biological detection efforts, development of biotechnology, and basic research. In an effort to fulfill this need and support the warfighter, the Edgewood Chemical Biological Center (ECBC) Biosensors Branch provides the Critical

Reagents Program (CRP) with genomic reference materials isolated from biological threat agents and simulants. All produced materials must pass strict conformance testing in compliance with ISO 17025 accredited procedures to ensure that the highest standards of quality are met. Currently, the Biosensors Genomic Repository has over 140 certified genomic reference materials available. As production continues into FY16, the goal is to further improve consistency, quality and throughput of the production and conformance testing process to reduce the cost of CRP genomic products.

BACKGROUND

As the principal national resource for the biological defense community, the CRP supports the Warfighter, federal agencies, and the Joint Program Executive Office for Chemical Biological Defense. The CRP provides high quality, validated, and standardized biological detection assays and reagents.

Several government facilities serve as repositories for CRP products. These products include antibodies, inactivated antigens, genomic materials, electrochemiluminescence assays, polymerase chain reaction assays, lateral flow immunoassays, and biological sampling kits. Since 2009, the ECBC has served as the repository for genomic CRP products. Members of ECBC BioSensors Branch and BioDefense Branch are responsible for the production, isolation, purification, and conformance testing of CRP genomic materials. ECBC BioTechnology Branch members are responsible for the proper storage and shipment of these genomic materials to CRP customers worldwide.

CRP Genomic Materials

<i>Acinetobacter baumannii</i>	<i>Neisseria lactamica</i>
<i>Bacillus spp.</i> (50+ organisms)	<i>Ochrobactrum anthropi</i>
<i>Brucella spp.</i> (20+ organisms)	<i>Pasteurella multocida</i>
<i>Burkholderia spp.</i> (30+ organisms)	<i>Proteus spp.</i>
<i>Clostridium spp.</i>	<i>Providencia stuartii</i>
<i>Comamonas spp.</i>	<i>Pseudomonas aeruginosa</i>
<i>Corynebacterium spp.</i>	<i>Ralstonia picketti</i>
<i>Enterobacter spp.</i>	<i>Rickettsia prowazekii</i>
<i>Erysipelothrix rhusiopathiae</i>	<i>Salmonella enteritidis</i>
<i>Escherichia coli</i>	<i>Serratia marcescens</i>
<i>Francisella spp.</i> (20+ organisms)	<i>Shigella spp.</i>
<i>Haemophilus influenzae</i>	<i>Staphylococcus spp.</i>
<i>Klebsiella pneumoniae</i>	<i>Stenotrophomonas maltophilia</i>
<i>Listeria monocytogenes</i>	<i>Streptococcus spp.</i>
<i>Moraxella catarrhalis</i>	<i>Yersinia spp.</i> (40+ organisms)



QUALITY



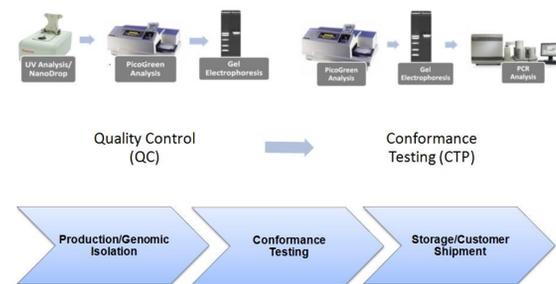
The BioSensors Branch continues to conduct all conformance testing in support of the production of genomic reference materials as an ISO/IEC 17025 accredited testing laboratory. Over the past three years, the BioSensors Branch has continued to maintain an ISO Guide 34 accreditation for the production, storage, and distribution of certified genomic reference materials. The Branch is also compliant with the applicable requirements of ISO Guide 35 and the definitions outlined in ISO Guide 30.

In addition to routine conformance testing, the BioSensors Branch conducts the following evaluations of the certified genomic reference materials:

- Short-term Stability
- Long-term Stability
- Homogeneity
- Method Validation
- Performance Verification

Continued compliance to ISO/IEC 17025 and ISO Guide 34 accredited procedures is a labor and cost intensive commitment. Therefore, the BioSensors Branch strives to streamline these processes through the integration of automation in FY16.

Conformance Testing Process



OSCAR

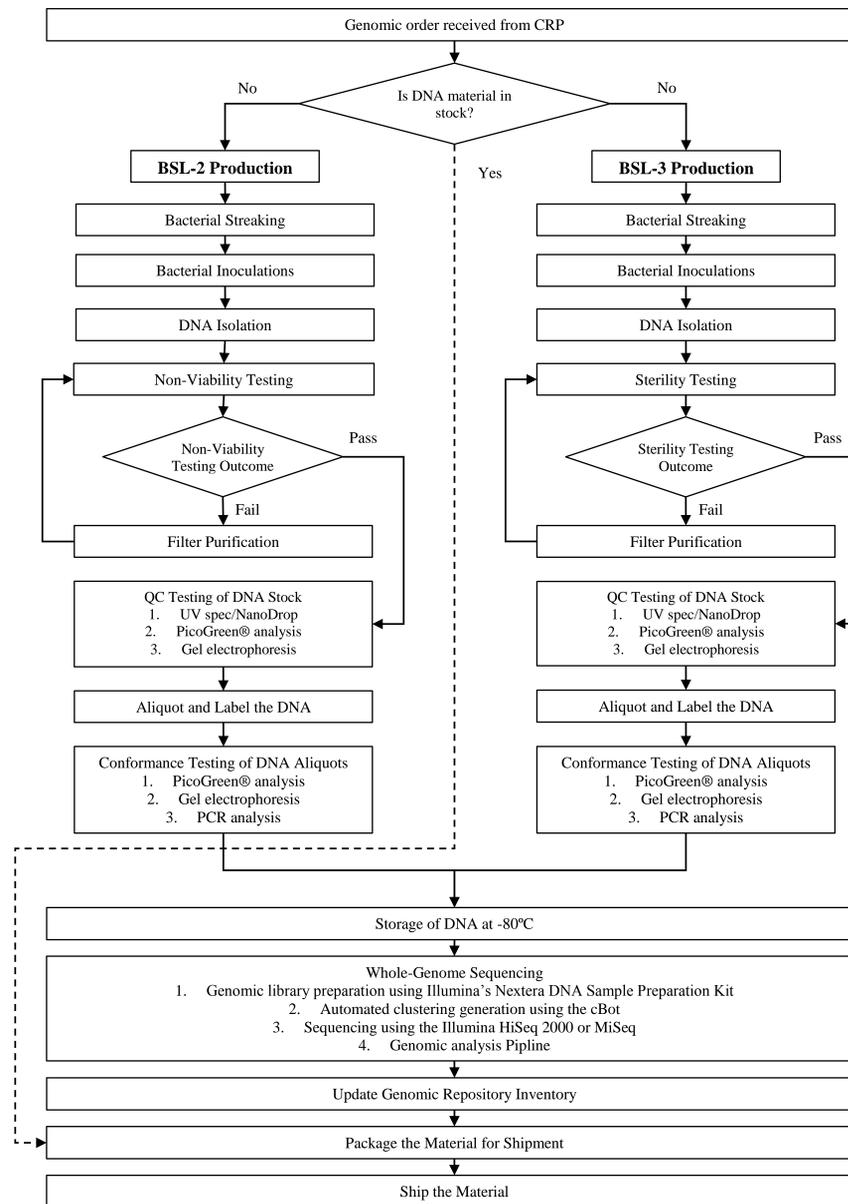
In FY14, the CRP Genomic Repository Database transitioned to the Order System For CRP Assays and Reagents (OSCAR)

OSCAR is web-based, user-friendly, and allows convenient ordering directly from the CRP Online Catalog along with order tracking

This database was established by the Joint Program Executive Office for Chemical and Biological Defense (JPEO-CBD) to support the Homeland Security and Warfighting communities as an effective and powerful resource in quickly accessing CBRN defense product and support information.



GENOMIC REPOSITORY BACTERIAL DNA PRODUCTION PROCESS FLOW



SYSTEM IMPROVEMENTS

In FY16, the CRP Genomic Repository plans to automate and streamline the conformance testing procedures with the following procured automated platforms:

- Qubit® 3.0 Fluorometer
- Agilent 2200 TapeStation
- QuantStudio™ 6 Flex Real-Time PCR System



WHOLE-GENOME SEQUENCING (WGS)

Currently, quality control procedures for CRP genomic reference materials are limited to verification of inclusivity by real-time PCR using agent-specific primers. To accurately verify the identity of these reference materials and detect any possible contaminants without the need of gene-specific primers, ECBC incorporated WGS to the CRP as a suitable replacement for exclusivity PCR and 16S procedures. The Genomics group of ECBC performs WGS of the reference materials using the Illumina HiSeq 2000 or MiSeq. The HiSeq is the high-throughput standard model and the MiSeq is the smaller, quicker, less expensive desktop version. The WGS process consists of genomic library preparation, automated cluster generation using the cBot, sequencing using the HiSeq 2000 or MiSeq, and genome analysis using an automated pipeline. Whole-genome sequencing is an excellent way to verify the genomic material and detect the presence of any genomic contaminants.

ACKNOWLEDGEMENTS

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- Dr. Shanmuga Sozhamannan (CRP Technical Coordinator)
- Ms. Demitra Drake (CRP Quality Manager)

