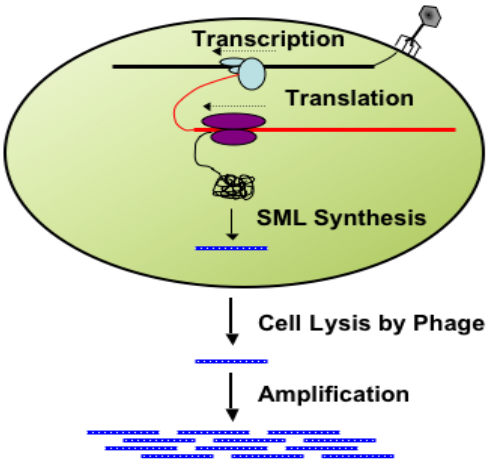
B-SMART™: Biological System for Molecular Antibiotic Resistance Testing

Development Status: Proof-of-Principle Completed.

B-SMART™ is a fully automatable nucleic acid-based system that measures bacterial viability and drug resistance. By combining the accuracy of functional drug susceptibility assays with the speed and sensitivity of molecular diagnostics, it can accurately and simultaneously report microbial identification and drug susceptibility in a matter of hours or days, depending upon the bacterial species. B-SMART™ is the first rapid nucleic acid-based test that can detect drug resistance without any prior knowledge of the genes or mutations that confer drug resistance.

How B-SMART™ works. Viruses rely on the protein and nucleic acid synthesis machinery of their host cell to manufacture their progeny because they don't carry these processes in their genes. The extent to which a virus can produce new viral components depends on the metabolic activity of the cell it infects.

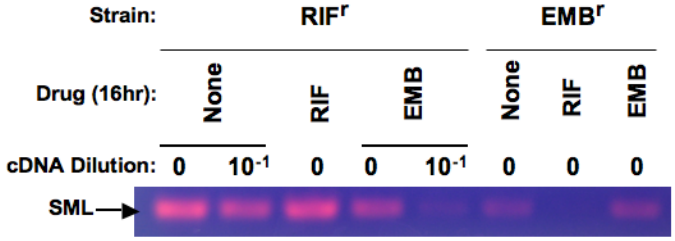
B-SMART™ uses bacteriophage (viruses that infect bacteria) to assess the metabolic capacity of bacteria in a sample exposed to one or more antimicrobial antibiotics. These phages are designed to create a new and novel nucleic acid (NA), the Surrogate Marker Locus (SML). Because phage infection requires a metabolically active cell, synthesis of the SML is compromised in drug-susceptible bacteria exposed to an effectuve antibiotic. If the organism is drug-resistant, the antibiotic has no effect on bacterial metabolism and the SML is synthesized efficiently. SML is the bridge between two general strategies for determining cellular viability or detecting drug resistance: functional assays (to assess the ability of bacteria to grow in the absence or presence of antibiotics) and molecular assays (to amplify and detect pathogen-specific nucleic acid sequences or resistance-conferring mutations). B-SMART™ can be adapted to determine antibiotic susceptibility of any microorganism infected by a bacteriophage. It is especially useful for pathogens that are difficult to culture, for which the gene(s) that encode drug resistance are unknown, or for which drug resistance is controlled by multiple genes. The SML-generation module used in B-SMART™ functions in diverse bacterial species, including *Mycobacteria* and *E. coli*, as well as in eukaryotic cells.



B-SMART™ Assay. *Following infection of a metabolically active cell, a new NA is created (the SML) that can be detected by NA amplification and detection technologies.*

Proof-of-principle is completed. We showed that B-SMART™ has the following promising characteristics:[1]

- It can detect Mtb with at least the sensitivity of the sputum smear (<1000 cells), and with optimization we believe that it will have significantly higher sensitivity (<50 cells);
- It can determine the susceptibility and/or resistance of Mtb to the four front-line anti-TB drugs tested: rifampicin (RIF), isoniazid, streptomycin, and ethambutol (EMB). The ability of the phage to detect drug resistant Mtb is demonstrated in the figure to the right. Two Mtb strains, one RIF-resistant (RIF^r) and the other EMB-resistant (EMB^r), were either untreated or treated with RIF and EMB and infected with the phage,



Proof-of-principle. *B-SMART™ can detect Mtb resistance to RIF and EMB.*

and then nucleic acid was purified and amplified. For the RIF^r strain, treatment with EMB results in a 10-fold reduction in SML generation compared to the untreated control, whereas there is no observable reduction after treatment with RIF. For the EMB^r strain, treatment with RIF results in no detectable SML, while SML generation is not decreased after EMB treatment.

- We believe that this success would extend to all anti-TB drugs, including Sequella's drug in development, SQ109. This means that B-SMART™ could determine the susceptibility or resistance of Mtb to any anti-TB drugs or drug candidate, whether or not the mechanism of resistance is known. **No other rapid molecular assay has been shown to be capable of this achievement.**
- It can produce a susceptibility result for RIF, a marker for MDR-TB, after only 3 hr of drug treatment, suggesting that this technology has the potential to be developed into a very rapid test for MDR-TB.

Product Format. Under the support of an R21 grant from the National Institutes of Health, we are creating a panel of phage that each express a distinct SML, which will facilitate multiplexed amplification and detection of many different SML in a single reaction. One clinical sample can be split into several vessels and exposed to both a drug and to a specific drug-associated SML-phage. All SML synthesized after exposure to different drugs can be amplified and detected in one reaction on a real-time PCR machine using a variety of detection techniques.

Market Opportunity. Nucleic acid-based diagnostics are well established in laboratory medicine, and many are used to screen blood supplies for a variety of infectious diseases. In the U.S., the market for hospital and laboratory-based infectious disease nucleic acid tests is a $1.1 billion industry. We believe that B-SMART™ has significant advantages over existing competing technologies, as outlined in the table below.

System	Type	Detection	Automation	Drug(s)	Time to Detection
FastPlaque	Phenotype	Plaque formation	No	RIF	2-3 days
Genotype MTBDRplus	Nucleic Acid	Line Probe Assay	Automatable	RIF+Isoniazid	1 day
GeneXpert-TB	Nucleic Acid	Fluorescent Probes	Yes	RIF	1 day
BACTEC	Phenotype	Fluorescent metabolites	Yes	Any	2-4 weeks
B-SMART	**Phenotype + Nucleic Acid**	**Adaptable to a variety of detection systems**	**Automatable**	**Any**	**Hours to <2 days**

Intellectual Property. Sequella has an issued patent (7,919,234) and a pending patent application (20110212457) with the USPTO. Additionally, we have pending patents in Australia, Canada, Japan, and the EPO and made a PCT filing to expand protection to other countries earlier this year. We are unaware of any complementary or blocking patents or technology that would need to be licensed to successfully commercialize this product.

References. Copies of the referenced paper are available upon request: please contact katherinesacksteder@sequella.com.

1. Mulvey MC, Sacksteder KA, Einck L, Nacy CA. Generation of a Novel Nucleic Acid-Based Reporter System To Detect Phenotypic Susceptibility to Antibiotics in Mycobacterium tuberculosis. MBio 2012;3.