

EpiSwitch™ Technology Overview

Background, concepts and application areas

Grounded in Epigenetics

Oxford BioDynamics' *EpiSwitch* technology is based on epigenetics: mechanisms that alter gene expression without altering the underlying DNA sequence and whose deregulation plays a role in the development of cancer, autoimmune diseases, neurologic diseases and other conditions. Although DNA is often illustrated as a simple linear strand, the reality is that it is packaged into a complex three-dimensional structure that brings DNA sequences that are distant from each other in linear genomic space into close physical proximity. These long-range interactions, occurring both within and between gene loci, reflect genetic epistasis and the regulatory network imposed on the genome and as result directly modulate gene expression in the context of the established phenotype. An example of these conformations is shown below.

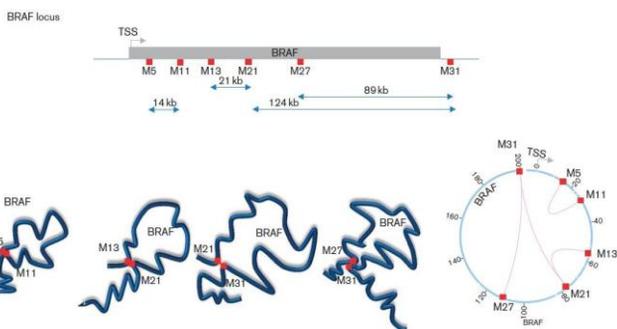


Figure 1: The linear DNA strand for the BRAF gene (grey bar) containing potential chromosomal interacting sites (red boxes) can take on different states of genomic organization (blue loops) depending on which interacting sites are active. This phenomenon occurs throughout the entire human genome. Image source: Jakub et al., *Melanoma Research*, 2015.

Collectively, functionally relevant combinations of discrete chromosomal conformations across the genome (chromatin “bar-codes”)² are called a

chromosome conformation signature (CCS). CCSs can be monitored by means of molecular biology and represent an ideal biological marker for assessing disease pathophysiology and response to therapeutic intervention.

Fast, simple and reliable detection

EpiSwitch is a proprietary industrial platform for identifying CCSs. It uses well-accepted principles of detection, proprietary molecular biology techniques, and operates under ISO compliant standards. The platform generates readouts within hours and can handle diverse types of source material (peripheral blood, tissue biopsies and cellular isolates) in high throughput at high resolution and sensitivity, using a robotic/automated platform. Detection is based on chromosome conformation capture, an established molecular biology technique (Figure 2). In short, without disrupting the cells, the genome is chemically stabilized and extracted as a chromatin template. Linearly distant genomic sites found in close physical proximity are converted into sequence tags. Quantification of the segments can be done by comparative genomic hybridization (CGH) on an Agilent array platform, by next generation sequencing, by nested PCR or by MIQE-compliant qPCR and provides the readout of the CCS present in a sample.

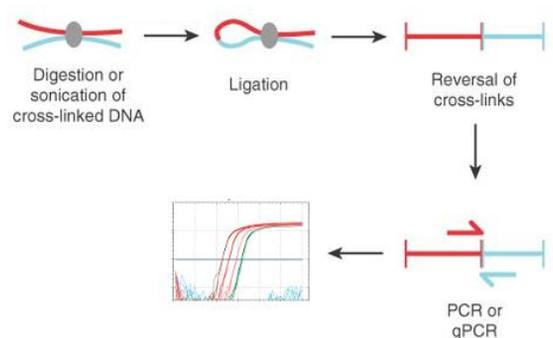


Figure 2: Detection of chromosomal conformation signatures.

Application Areas

Biomarker Identification: A biomarker is a characteristic that can be objectively measured and acts as an indicator of normal biological processes, pathogenic processes, or biological responses to therapeutic intervention. The stability and binary nature of chromosome conformations (it's either there or it isn't) makes CCSs ideal biomarkers. Epigenetic-based biomarkers have the potential to improve early diagnosis and prognosis of disease and help to monitor epigenetic profiles in order to predict the likelihood of response to treatment, both before and during treatment with either approved or development stage products. There are three types of *EpiSwitch* applications for which validated stratification signatures have already been successfully developed (Figure 3).

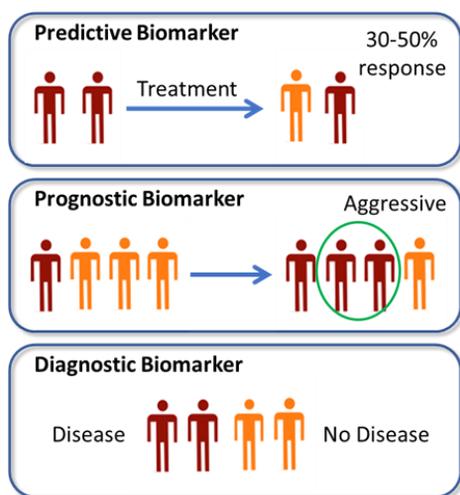


Figure 3: Biomarker discovery using *EpiSwitch*.

Predictive Biomarkers: Developed on the retrospective analysis of patient cohorts and then used to monitor stable epigenetic profiles in patients at baseline (prior to therapeutic intervention), one can use *EpiSwitch* to categorize patients by their likelihood of response to a particular treatment. *EpiSwitch* monitoring can be used for patient enrichment strategies, as well as patient exclusion for propensity to develop adverse side effects, and to ensure efficacious response to treatment².

Prognostic Biomarkers: The measurement of CCSs can categorize patients by the degree of risk for disease occurrence or progression of a specific aspect of disease. This could be critical for patient recruitment into clinical trials and can offer advantages in improving trial design to maximize treatment response and therapeutic efficacy.

Diagnostic Biomarkers: Measurement of CCS can reveal disease characteristics that categorize people by the presence or absence of a specific physiological or pathophysiological state, even at the early symptomatic or pre-symptomatic state³.

Target Discovery: As drug pipelines face attrition from the high failure rates of lead molecules in clinical stages, the identification of novel, disease-relevant therapeutic targets is critical to sustain R&D efforts in the pharmaceutical industry. *EpiSwitch* can be applied to samples from healthy and diseased patients to identify disease-specific chromosomal conformations. The genomic areas found to differ in disease states generate a rich pool of potential therapeutic targets. Importantly, since *EpiSwitch* is not reliant on the abundance of a particular molecule, many targets identified using this approach would not be detected by the conventional target discovery techniques based on continuous readouts of RNA or protein abundance as used in the industry today.

Drug Rescue: Clinical trials are expensive, time-consuming and prone to failure. Only about 1% of drugs make it from Phase 1 studies to FDA approval, and the process can take between 12-15 years and cost billions of dollars. Many drugs fail due to a lack of efficacy, which isn't typically known until the end of Phase II trials. A recent trend in the pharmaceutical industry is to include additional molecular markers in the trial design to enable retrospective analysis and identification of subsets of patients that are most likely to respond to therapy.

In fact, the FDA has issued guidance on enrichment strategies to help pharmaceutical companies maximize their chance for successful trials⁴. Utilizing *EpiSwitch* measurements as part of clinical trial design provides OBD's partners with a rich pool of data on individual patients to better understand therapeutic response.

References

1. Bastonini E, Jeznach M, Field M, Juszczak K, Corfield E, Dezfouli M, Ahmat N, Smith A, Womersley H, Jordan P, Ramadass A, Akoulitchev A, Goding CR. Chromatin barcodes as biomarkers for melanoma. *Pigment cell & melanoma research.*, 2014, Sep;27(5):788-800
2. Carini C, Hunter E; Scottish Early Rheumatoid Arthritis Inception cohort Investigators, Ramadass AS, Green J, Akoulitchev A, McInnes IB, Goodyear CS. Chromosome conformation signatures define predictive markers of inadequate response to methotrexate in early rheumatoid arthritis. *Journal of Translational Medicine.* 2018 Jan 29;16(1):18.
3. Jakub JW, Grotz TE, Jordan P, Hunter E, Pittelkow M, Ramadass A, Akoulitchev A, Markovic S. A pilot study of chromosomal aberrations and epigenetic changes in peripheral blood samples to identify patients with melanoma. *Melanoma Research*, 2015, Oct;25(5):406-11.

Authors

Willem Westra, PhD

Ewan Hunter, PhD

Alexandre Akoulitchev, PhD

Oxford BioDynamics Plc

Oxford, United Kingdom

info@oxfordbiodynamics.com

Oxford BioDynamics Plc
26 Beaumont Street
Oxford, OX1 2NP
United Kingdom
Ph: +44 (0)1865 518 910
www.oxfordbiodynamics.com



INNOVATION
SAVING LIVES



OXFORD BIODYNAMICS

For a complete listing of our global offices, visit <https://www.oxfordbiodynamics.com/contact/>

Copyright © 2018, Oxford BioDynamics Plc. All rights reserved. EpiSwitch™ is a registered trademark of Oxford BioDynamics Plc.