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, and neurologic diseases. 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.

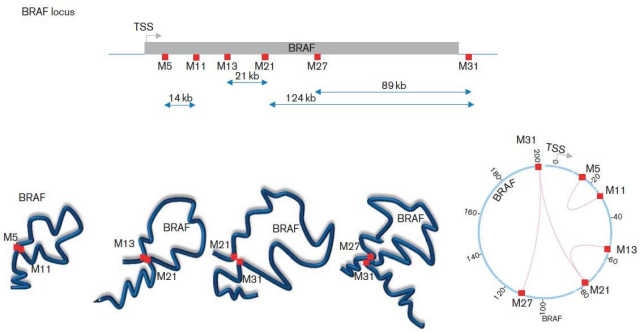

Figure 1: The linear DNA strand for the BRAF gene (grey bar) containing potential chromosomal interacting sites (red boxes) can take on different states of genomic organization (blue loops) depending on which interacting sites are active. This phenomenon occurs throughout the entire human genome. Image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

Collectively, functionally relevant combinations of discrete chromosomal conformations (CC) across the genome (chromatin "bar-codes")[2] are called a chromosome conformation signature (CCS). CCSs can be monitored by means of molecular biology and therefore represent an ideal biological marker for assessing disease pathophysiology and response to therapeutic intervention.

Fast, simple and reliable detection

EpiSwitch™ proprietary industrial platform for detecting CCSs uses well-accepted principles of CC 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 established molecular 3C biology techniques (Figure 2). In short, without disrupting the cells, the CCs are chemically stabilized, extracted as a chromatin template and the distant genomic sites found in proximity by stable juxtaposition are converted into adjacent sequence tags on an artificially generated chromatin template. Quantification of the segments can be done by comparative genomic hybridization (CGH) on an Agilent array platform, by NGS, by nested PCR or by MIQE-compliant qPCR and provides the readout of the CCS present in a sample.

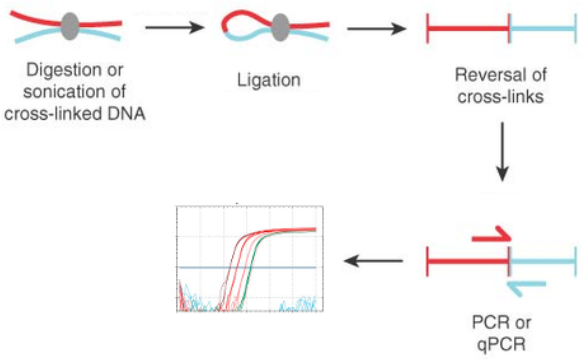

Figure 2: Detection of chromosomal conformation signatures.

Application Areas

Biomarker Identification: A biomarker is a characteristic that can be objectively measured and acts as an indicator of a normal biological processes, pathogenic processes, or biological responses to therapeutic intervention. The stability and binary nature of CC structure (it's either there or it isn't) makes them 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).

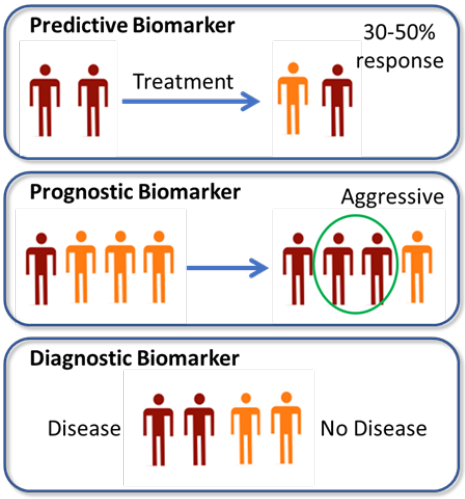

Figure 3: Biomarker discovery using EpiSwitch™.

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 by *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 treatment[2].

Prognostic Biomarkers: The measurement of CCS 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 state[3].

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 are 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 1 in 5,000 of pre-clinical drug leads will make it to the market, 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 isn't known until the end of the trial. 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 trials[4]. Utilizing *EpiSwitch™* measurements as part of clinical trial design provides OBD partners with a rich pool of data on individual patients to better understand therapeutic response.

References

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