EpiSwitch™ Biomarker Discovery

Leveraging the power of the epigenome to enable precision medicine

Epigenetics for Precision Medicine

The last decade has seen an explosion in the application of precision medicine strategies in the life science industry, from drug development to clinical trial design. The ability to use molecular tools to identify patients who are more or less likely to respond to therapeutic intervention has tremendous commercial, social, and economic benefits. While several approaches using different molecular measurement techniques have historically been used with different levels of success, one that has gained particular traction in recent years is the assessment of epigenomic changes.

The packaging of chromosomal DNA plays a critical role in the epigenetic regulation of the whole genome. It ensures effective storage, access to genetic information and its regulation by the complex protein machinery utilized in gene expression. Known also as 'gene loops', 'long-range chromosomal interactions' and 'chromatin domains', chromosome conformations have been recognized as an essential high-level framework of epigenetic regulation imposed across the whole genome.

EpiSwitch is a proprietary industrial platform for the discovery, evaluation, validation and monitoring of a novel class of epigenetic biomarkers known as 'chromosome conformation signatures' ("CCSs"). CCSs provide a compelling, stable framework from which changes in the regulation of a genome can be analyzed, long before the results of these epigenetic changes manifest themselves as obvious abnormalities.

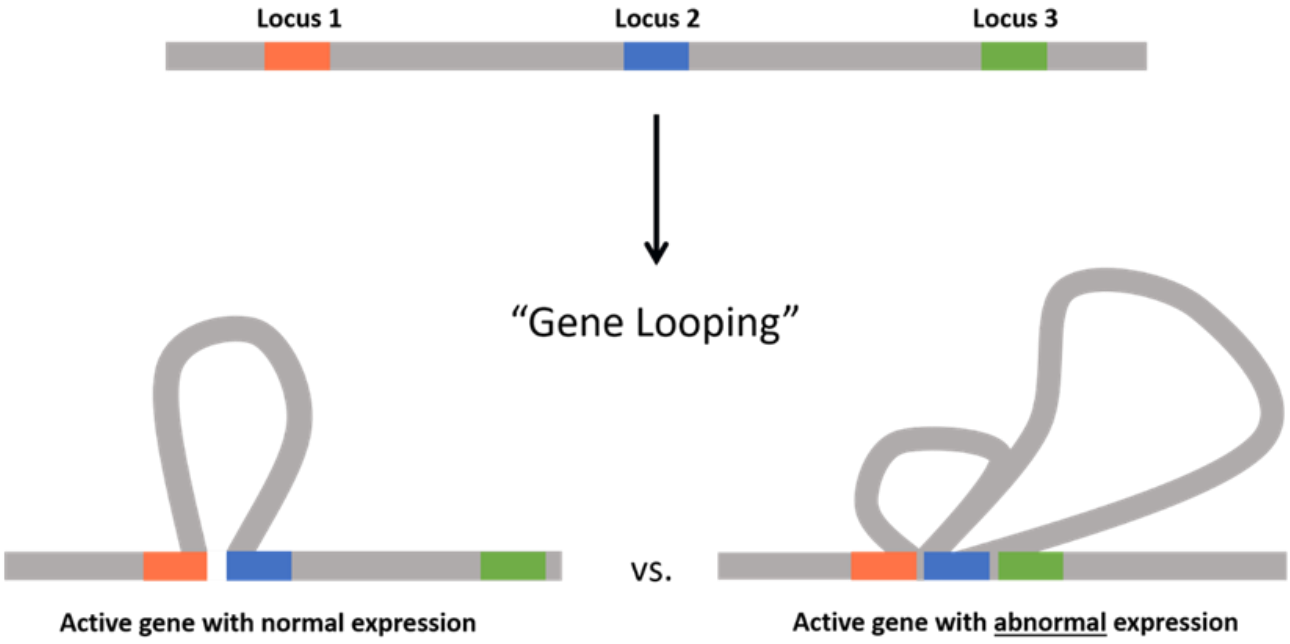

Figure 1: The higher order, three-dimensional structure of DNA includes chromosome conformations, or "gene loops" that bring genomic loci that are distant from each other in linear space into close spatial proximity.

When considering both cis (between genomic regions on the same chromosome) and trans interactions (between genomic regions on different chromosomes), there are on the order of billions of potential interactions that can be experimentally measured. While this makes for a rich pool of candidate biomarkers, the sheer size of the pool makes measuring every single potential interaction experimentally overbearing. There are, however, ways to screen a large pool of potential interactions in a high-throughput fashion to identify a subset of markers of interest for any given stratification question, whether it's identifying responders and non-responders to a therapeutic intervention, identifying markers of disease onset or progression, or identifying novel disease targets.

OBD uses a three-stage approach to screen, identify, evaluate and validate CCSs, ensuring that the biomarker panels that emerge from this pipeline are biologically vetted and statistically robust to be used in pre-clinical and clinical practice. The following provides a step-by-step explanation of OBD's *EpiSwitch* biomarker discovery platform, from initial design to validation.

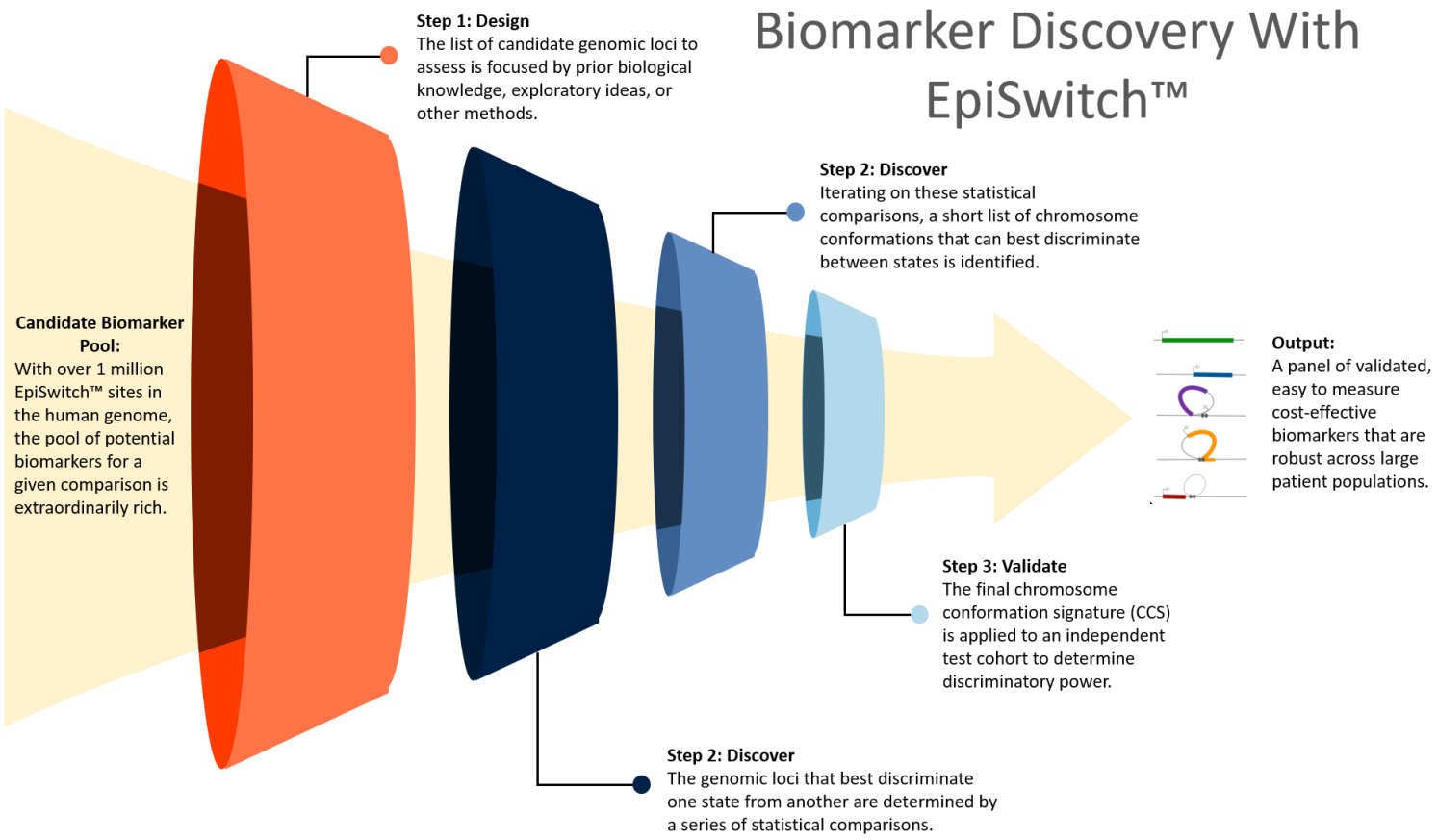

Figure 2: Oxford BioDynamics' three-step biomarker discovery workflow using the *EpiSwitch* platform technology.

STAGE 1 – DESIGN

EpiSwitch Design Annotation

In this stage, the proprietary annotations of over 1 million *EpiSwitch* sites hidden within the genome, are mapped for the loci of interest, matching them as anchoring points of framework regulation across the genes, single nucleotide polymorphisms, enhancers, sites of transcriptional relevance, sites of regulatory histone modifications and other loci of potential functional importance. This generates a reliable and manageable list of chromosomal interactions for further analysis. In essence, this step serves as a feature reduction exercise to home in on the most biologically meaningful interactions. The approach to deriving the short list is defined collaboratively between OBD and its partners and depends on the nature and scope of the research question at hand. For example, if the goal is to identify a set of markers that shed light on the mechanism of response to an immuno-oncology drug with a

defined target (e.g. PD-1 or PD-L1), genomic loci encoding proteins implicated in the known pathways and signaling cascades of PD-1/PD-L1 may be selected. In contrast, if the goal is to identify a biomarker of disease onset in a condition with a known inflammatory component, such as ALS, genomic loci encoding proteins involved in regulating the immune system (cytokines, chemokines, MHC proteins) may be selected. At this stage, between 200-400 genomic regions are identified and processed by OBD's proprietary pattern recognition algorithm to identify the *EpiSwitch* sites contained in these regions, typically on the order of around 14,000 sites. Importantly, the genomic regions selected in this stage need not be restricted only to those that are protein-encoding; non-coding regions, regulatory regions, regions encoding non-coding RNAs and genetic variants can be used as well.

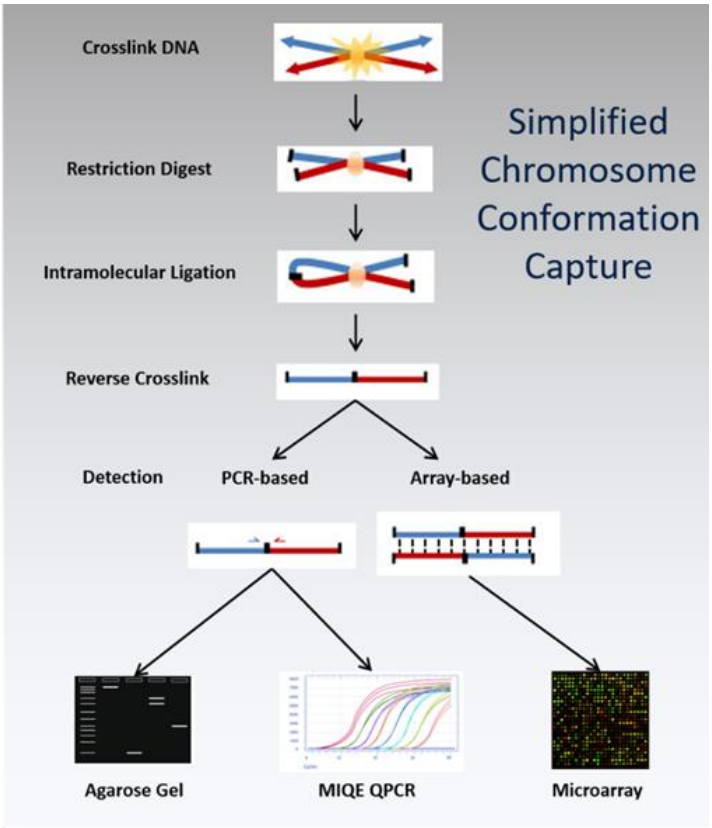

Figure 3: A simplified workflow for detecting chromosome conformations by microarray or PCR.

STAGE 2 – DISCOVER

EpiSwitch Arrays and qPCR

Once the *EpiSwitch* sites for the study are identified, the next step is to measure which of these sites form long range chromosome interactions and which are conditional. Remember, the *EpiSwitch* sites identified in Stage 1 are predicted interaction sites and need two additional steps. First is the experimental confirmation of detectable chromosome loops (typically over 90% of annotated sites form detectable interactions when tested) and second, is to determine if the chromosome loops are conditional (can they differentiate between compared phenotypes (e.g. disease vs. non-disease, responder vs. non-responder, fast progressors vs slow progressors)).

Molecular biology offers several methods to detect long range chromosomal conformations. The underlying principle allows for the capture of distant genomic juxtapositions by first stabilizing distant sites within intact cell nuclei, then isolating them and converting them into an artificial template with distant fragments of the genome ligated to each other in one DNA product and detecting the new products as evidence of long range interactions. Early research protocols called chromosome conformation capture (3C) were developed in 2002, which were followed over the years by several other variants aimed at assessing different scopes and levels of chromosome conformations[2-3]. These protocols are used widely in academic settings today. Although all are very useful in providing insights and evidence for specific interactions, they remain time and labor consuming, often with low resolution and frequent variability in readouts. The proprietary protocols used in processing and detection of long range interactions used by *EpiSwitch* in the analysis of clinical samples and other cellular inputs have taken screening and monitoring of chromosome conformations to a different level in terms of industry standards: speed, cost, sensitivity, resolution, accuracy and robustness. Conversion of epigenetic analytes into sequence-based readouts is conducted within hours, on robotic platforms and in high throughput. Final readouts for individual markers and validated stratifying signatures are performed either in the simple format of binary nested PCR or in the format of the industry standard MIQE-compliant qPCR format using proprietary hydrolysable probe design.

Stage 2 is dedicated to screening for biomarker leads in a high-throughput approach using a customized microarray containing readouts for the ~14,000 potential interactions annotated in the design stage (Stage 1).

Next, biospecimen samples (whole blood, peripheral blood mononuclear cells, cell lines) from two phenotypic comparisons (responders and non-responders at baseline, or fast and slow progressing disease patients, for example) are hybridized to a custom-designed comparative genomic hybridization (CGH) array. Resulting readouts from biological and technical repeats are put through a series of statistical filters to determine the subset of interactions that can discriminate between the different conditions. The top statistically significant disseminating marker leads of conditional chromosome conformations (typically on the order of a few hundred) are then translated into *EpiSwitch* PCR detection, enabling faster and lower cost marker reduction on the expanded patient cohorts. By iteratively comparing the interactions that can differentiate between two conditions in this sequence-based approach, a final set of interactions (typically between 5 and 10) is identified with machine learning-based methodology. This resulting 'chromosome conformation signature' is a molecular barcode that discriminates the epigenetic landscape and network regulation between the two compared patient groups.

STAGE 3 – VALIDATE

Episwitch PCR Platform

In the final stage, the signature identified in Stage 2 is validated on an independent sample cohort. This meets the criteria of independent validation or "proof" required by industry and ensures that the resulting biomarker panel is robust across the broad, real-world patient populations that will be assessed in future studies.

Case Study: Therapeutic Response Biomarker

Now that we've learned about how OBD's *EpiSwitch* discovery platform works from a conceptual standpoint, let's see how the process comes together in a real-world example. Recently, OBD partnered with a Top 10 pharmaceutical company and a leading academic consortium to solve one of the major unmet clinical needs in rheumatoid disease. Rheumatoid arthritis (RA) is an autoimmune inflammatory disorder that manifests clinically as painful, swollen, tender and stiff joints. For patients diagnosed with RA, methotrexate (MTX) is the first choice of disease modifying anti-rheumatic drug (DMARD) as recommended by European League against Rheumatism (EULAR) and American College of Rheumatology (ACR). While MTX has therapeutic benefit in some patients, approximately 35–59% of patients do not achieve clinically meaningful responses after starting therapy. Over the last several years, a range of newer targeted biologic drugs have been developed and approved for RA, many of which show substantial clinical benefit. Thus, having a mechanism to identify patients who are likely non-responders to MTX would facilitate earlier access to these more effective therapies, thus avoiding disease progression, unnecessary exposure to potentially toxic drugs and diminished quality of life.

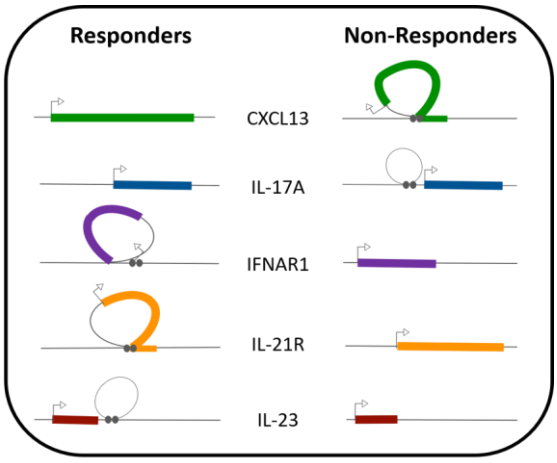

Figure 3: An example of a chromosome conformation signature (CCS) used to identify non-responders to methotrexate in Rheumatoid Arthritis.

Using blood samples from the Scottish Early Rheumatoid Arthritis Inception cohort, in the **Design** step, we evaluated chromosomal interactions at 123 genomic loci selected from a systematic literature review for genes that have been associated with RA. These loci were processed through the OBD pattern recognition software, generating 13,322 chromosomal interactions that were screened for association with response to MTX in RA. In the **Discovery** step, we used blood samples from RA patients and control samples in a series of comparisons that used statistical filters to refine the 13,332 chromosomal interactions to five interactions (in IFNAR1, IL-21R, IL-23, IL-17A, and CXCL13 loci)

that could accurately predict response to MTX. In the **Validation** step, we applied the 5-marker panel to an independent, blinded cohort of RA patients and asked how well the biomarker panel could identify non-responders to MTX prior to initiating therapy. In this independent cohort, we were able to attain a positive predictive value of over 90%, showing that the use of a CCS has practical utility as a predictive clinical tool.

This study was published[4] in **The Journal of Translational Medicine** and can be found here.

Oxford Biodynamics has built extensive expertise and knowledge on using CCSs as biomarkers from a broad spectrum of successful research collaborations in a variety of therapeutic areas including oncology (Melanoma, Lung cancer, Breast cancer, DLBCL and AML), immunology (Rheumatoid Arthritis, Multiple Sclerosis, Systemic Lupus Erythematosus, Ulcerative Colitis and Fibrosis) and neurology (Amyotrophic Lateral Sclerosis, Alzheimer's Disease, Huntington's Disease and Depression).

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