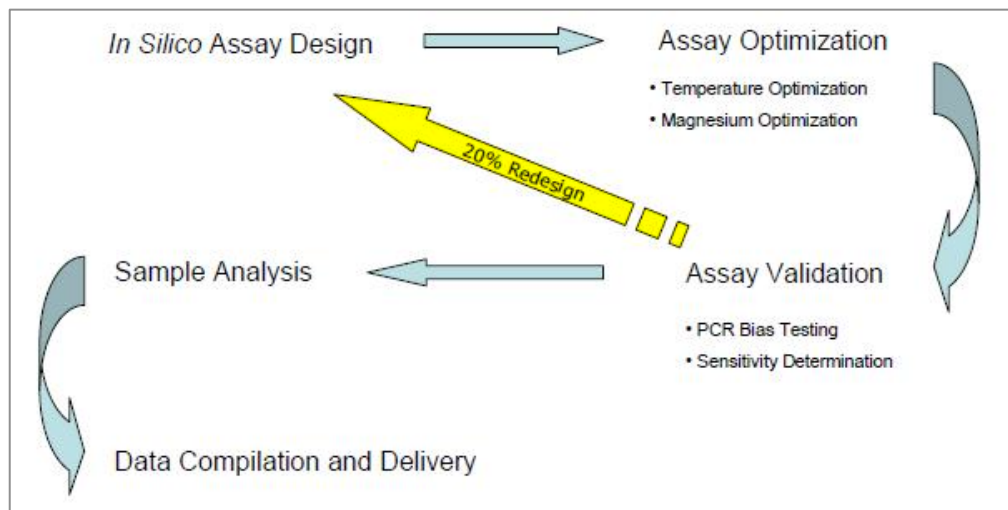


Workflow for DNA Methylation Services

Workflow Summary

- **Incoming Sample Preparation**
 - DNA Extraction & Purification
 - Bisulfite Modification & Purification
- **In Silico Assay Design**
 - In silico target sequence analysis
 - PCR primer design & biotinylation
 - Sequencing primer design
- **Assay Optimization**
 - PCR Temperature Optimization
 - PCR Magnesium Optimization
- **Assay Validation**
 - PCR Bias Testing
 - Sensitivity Determination
- **Sample Analysis**
 - Streptavidin Bead Immobilization & Washing
 - Pyrosequencing Analysis
- **Data Compilation and Delivery**
 - Data Analysis
 - Report Creation



Detailed Description

Incoming Sample Preparation

See our Sample Submission Form for recommended incoming sample QC values, along with shipping instructions. Samples can be shipped as 96-well plates, individual tubes, cell pellets, tissue segments, or FFPE samples. EpigenDx can perform the associated DNA extraction, purification, and QC procedures. EpigenDx also performs bisulfite modification and purification for individual tubes as well as 96-well plates. These processes can all be ordered as independent services.

In Silico Assay Design

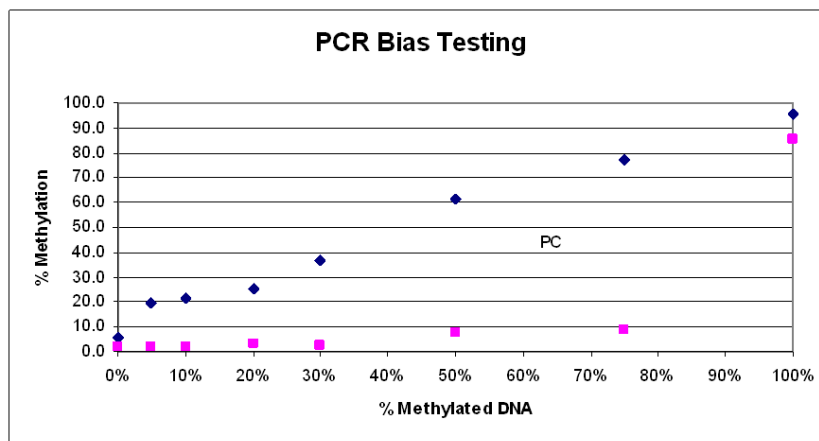
Customer provides the target region/sequence to be analyzed. EpigenDx either utilizes an existing primer set from a pre-developed assay (ADS), or designs a custom new PCR and sequencing primer set to suit the sequence. Each assay is specially designed to take into consideration the difficulties encountered with the loss of genetic variability due to the bisulfite

conversion process, wherein most cytosine residues are converted into uracil/thymidine. EpigenDx also offers validated bisulfite sequencing primer kits that can be independently purchased.

Assay Optimization and Validation

EpigenDx takes significant measures to ensure quality and accuracy. PCR bias is common in many methylation studies, so EpigenDx monitors many QC checkpoints to guarantee PCR conditions do not select unfairly for either methylated or un-methylated CpG sites.

Figure 2 demonstrates how a difference in annealing temperature (pink series) can create significant bias in methylation studies. EpigenDx includes bias testing and validation results with all reports.



Data Analysis and Report Delivery

As part of EpigenDx's standard Methylation Analysis service, EpigenDx's data analysis team summarizes the data and provides a report outlining the results so you can continue easily to the next stage of your scientific investigations and experiments. EpigenDx delivers analyzed quantitative Pyrosequencing results in Microsoft Excel, and raw Pyrogram data in Microsoft Word (other delivery options also available). An assay report, along with validation results are included in the package.

Turn-around Time

General EpigenDx DNA methylation services have a turn-around time of 5–15 days, depending the number of assays and samples sent. Custom assay design projects have a turn-around time of 3–6 weeks. EpigenDx pursues aggressive timelines with weekly project updates and high quality technical support and consultation from design phase through project completion and data delivery.