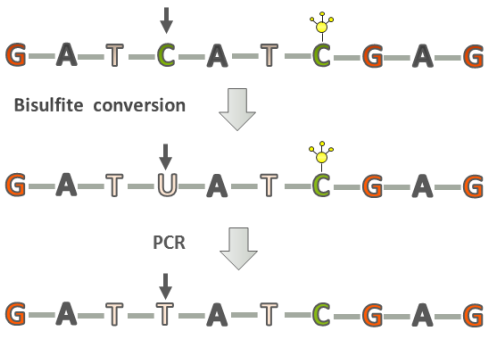
Bisulfite Sequencing

Background:
DNA methylation is an epigenetic modification, which involves a methyl group being added to the 5-carbon position in the base, cytosine. Highly methylated genes tend to have low gene expression. Methylation is also important during development and differentiation. Bisulfite sequencing enables the identification of regions of DNA methylation

Method:

- The DNA is treated with sodium bisulfite.
- Bisulfite converts cytosine to uracil.
- Any cytosine that is methylated (5-methylcytosine) will not be affected by the bisulfite and will remain cytosine.
- The treated DNA is sequenced and aligned to a reference genome for comparison.



http://www.gatc-biotech.com/fileadmin/Kundendaten/Upload_8.0/Bilder/Bisulfite_conversion.png

Examples of bisulfite sequencing in literature:

- Saint-Carlier and Riviere (2015) used bisulfite sequencing to determine the patterns of DNA methylation within promoter regions and the regulation of a specific target gene in genome of the Pacific oyster (*C. gigas*) throughout various developmental stages.
- Smith et al. (2015) discovered which genes were highly methylated within the stickleback (*G. aculeatus*) genome using bisulfite sequencing.
- Shang et al. (2015) used bisulfite sequencing to examine the differences in methylation patterns of an immune-related gene between susceptible and resistant grass carp (*C. idella*) in response to a pathogen.

Limitations of bisulfite sequencing:
The treatment of DNA with bisulfite may not be completely efficient. There may be unmethylated cytosines that have not been converted to uracil. Otherwise, this is one of the best ways to visualize DNA methylation.

References:

Song, F.; Smith, J.; et al. (2005). Association of Tissue-Specific Differentially Methylated Regions (TDMs) with Differential Gene Expression. *PNAS* 102(9): 3336-3341.

Shang, X.; Su, J.; et al. (2015). CpA/CpG methylation of CiMDA5 possesses tight association with the resistance against GCRV and negatively regulates mRNA expression in grass carp, *Ctenopharyngodon idella*. *Developmental and Comparative Immunology* 8(1): 86-94.

Smith, G.; Smith, C.; et al. (2015). Genome-Wide DNA Methylation Patterns in Wild Samples of Two Morphotypes of Threespine Stickleback (*Gasterosteus aculeatus*). *Molecular Biology and Evolution* 32(4): 888-895.

Saint-Carlier, E.; Riviere, G. (2015). Regulation of *Hox* orthologues in the oyster *Crassostrea gigas* evidences a functional role for promoter DNA methylation in an invertebrate. *FEBS Letters* 589(13): 1459-1466.