X Chromosome Inactivation in Pluripotent Stem Cells

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Purpose of the Experiment X chromosome inactivation (XCI) is an essential epigenetic silencing process established during early embryonic development of female mammals, guaranteeing a proper dosage compensation of X linked genes between males and females. Activation of the lncRNA XIST, which triggers XCI, occurs early during differentiation, therefore pluripotent stem cells (PSCs) can be used as a model for studying this process. Different studies demonstrated variable states of XCI in human PSCs (hPSCs). They however focused on a limited number of specific cell-lines. Therefore, an unbiased global view on XCI in hPSCs is required. Procedures Used To analyze XCI in hundreds of human PSCs samples, we utilized published RNA-sequencing data. First, a quantitative method to assess pluripotency and sex of sample was established. Then, a bioinformatics pipeline was developed to determine the XCI of female samples based on three parameters: XIST expression level, Allelic ratio of X chromosomes and X linked genes expression ratio compared to male samples. Results Striking differences in XIST expression were found when comparing embryonic stem cells (ESCs) with reprogrammed PSCs. All the analyzed parameters indicate most reprogrammed PSCs maintain an inactive X (XaXi) while ESCs are partially reactivated (eroded, XaXe), where only the center of the chromosome remains inactive. Cancer female cell lines were found to be different in their XCI state; four were XaXi or XaXa and one was XaXe. Conclusions XCI is globally extremely distinct between ESCs and reprogrammed PSCs. Overall, the protocols developed during this research can determine the X inactivation state of cells and the geography of inactivation based on RNA-Seq data.