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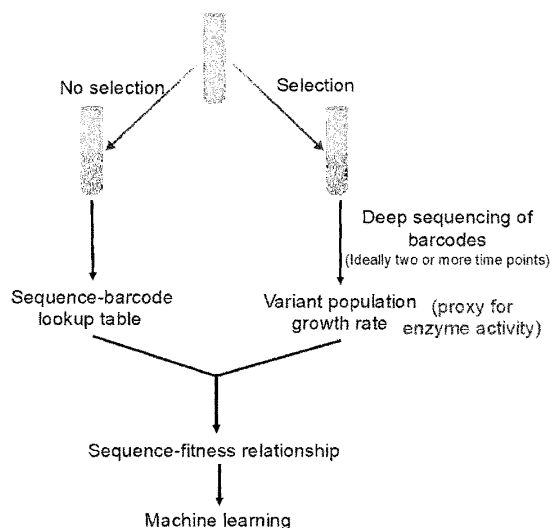


Fig. 2

(57) **Abstract:** The present invention relates to a method for estimating or determining fitness of enzyme variants and/or optimizing a nucleotide sequence encoding an enzyme variant comprising generating a DNA variant library and linking a unique DNA barcode to each DNA variant, wherein the DNA variants are subjected to long-read and short-read sequencing. In particular, the present invention relates to a method for predicting or determining fitness of enzyme variants and/or optimizing a nucleotide sequence encoding an enzyme variant by combining DNA barcodes, selection pressure, long-read sequencing, and short-read sequencing. Furthermore, the present invention relates to application of machine learning for predicting enzyme fitness and/or optimizing a nucleotide sequence encoding an enzyme variant based on data obtained from said method.

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