**A Shiny App for Determining Labeling Duration for Two-Sample Experiments**

In this study, we report a web application developed using the R programming language and the Shiny framework. This application assists researchers in planning heavy water labeling experiments using a two-sample approach. It effectively determines the optimal labeling duration for a given peptide based on essential experimental parameters. The tool features two input modes, as illustrated in Figure A and Figure B. A summary of the inputs and outputs is presented in Table 1.

In Figure A, the application utilizes a single expected turnover rate along with the peptide sequence, body water enrichment (BWE), and ΔI₀ (the smallest detectable change in mono-isotope relative abundance). Users can also specify the maximum labeling duration using a slider. With these inputs, the application simulates the monoisotopic RA depletion curve over time and identifies the time window during which the relative monoisotope abundance, I₀(t), falls between the theoretical upper and lower bounds (represented by red and black dashed lines, respectively). This interval is visually indicated by the green shaded region on the plot, which corresponds to the recommended minimum and maximum labeling durations for the specified peptide and conditions.

In Figure B, the application incorporates a range of turnover rates (the slowest and fastest expected rates), which is particularly useful when turnover is uncertain or varies across conditions. The labeling curves are simulated for both the slowest and fastest turnover rates (shown as black and blue points, respectively). The intersection of these simulations with the detection thresholds defines the valid labeling window, indicated by the shaded region in the plot.

For both input modes, the application provides a graphical plot and a text summary that indicate the recommended minimum and maximum labeling durations for reliable estimation of turnover rates using a two-sample approach. This tool guides the design of a single-deuterium labeled experiment by identifying the earliest and latest plausible durations that can be used to accurately determine turnover rates.

A screenshot of a computer

AI-generated content may be incorrect.

**Figure A)**

A screenshot of a computer

AI-generated content may be incorrect.

**Figure B)**

**Figure XXX**. Screenshots of the web application developed in this study, which estimates optimal labeling durations for heavy water labeling experiments using a two-sample approach. The application supports two input modes: (A) a single estimated turnover rate for a given peptide, and (B) a range of expected turnover rates. Both modes generate visual and numerical outputs to guide experimental design.

**Table XXX.** Summary of the web application's components: (A) Inputs, including peptide information and experimental parameters; and (B) application-generated outputs, including graphical and numerical estimates of optimal labeling durations.

**Table XXX A)**

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| --- | --- |
| Input | Description |
| Peptide sequence | The peptide sequence is used to determine the number of exchangeable hydrogens (NEH). |
| Expected Turnover Rate (k) | The estimated turnover rate of the peptide (used in single-rate mode, Figure A). |
| Expected Slowest Turnover Rate | Lower bound of expected turnover rate range (used in range mode, Figure B). |
| Expected Fastest Turnover Rate | Upper bound of expected turnover rate range (used in range mode, Figure B). |
| Body Water Enrichment (BWE) | Fraction of enriched water in the system |
| ΔI₀ | Minimum detectable change in the relative abundance of the mono isotope. |
| Max. Labeling Duration | User-defined upper limit on the number of days for labeling simulation. |

**Table XXX B)**

|  |  |
| --- | --- |
| Output | Description |
| I₀ Depletion Curves | Simulated I₀(t) values for one or more turnover rates, shown as black/blue dots. |
| Detection Bounds | Theoretical upper (red) and lower (black) detection thresholds. |
| Valid Labeling Window | Time range (green shaded area) where I₀(t) falls between detection bounds. |
| Minimum Labeling Duration | The plausible earliest labeling duration for the two-sample approach |
| Maximum Labeling Duration | The plausible latest labeling duration for the two-sample approach |