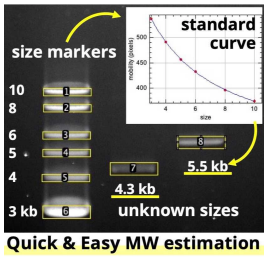
Sep 19, 2024 Version 2

Quick estimation of molecular weight from gel images with an ImageJ macro, MolecularWeightEstimator V.2



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We use this protocol and it's working

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Abstract

This protocol shares an ImageJ macro that enables easy and quick estimation of molecular weights from gel electrophoresis images, including the agarose gel electrophoresis for nucleic acids and the SDS-PAGE for proteins. By selecting the bands (size markers and samples of unknown size) and running this macro, you can estimate the size of your bands based on the curve fitted to your size marker data.

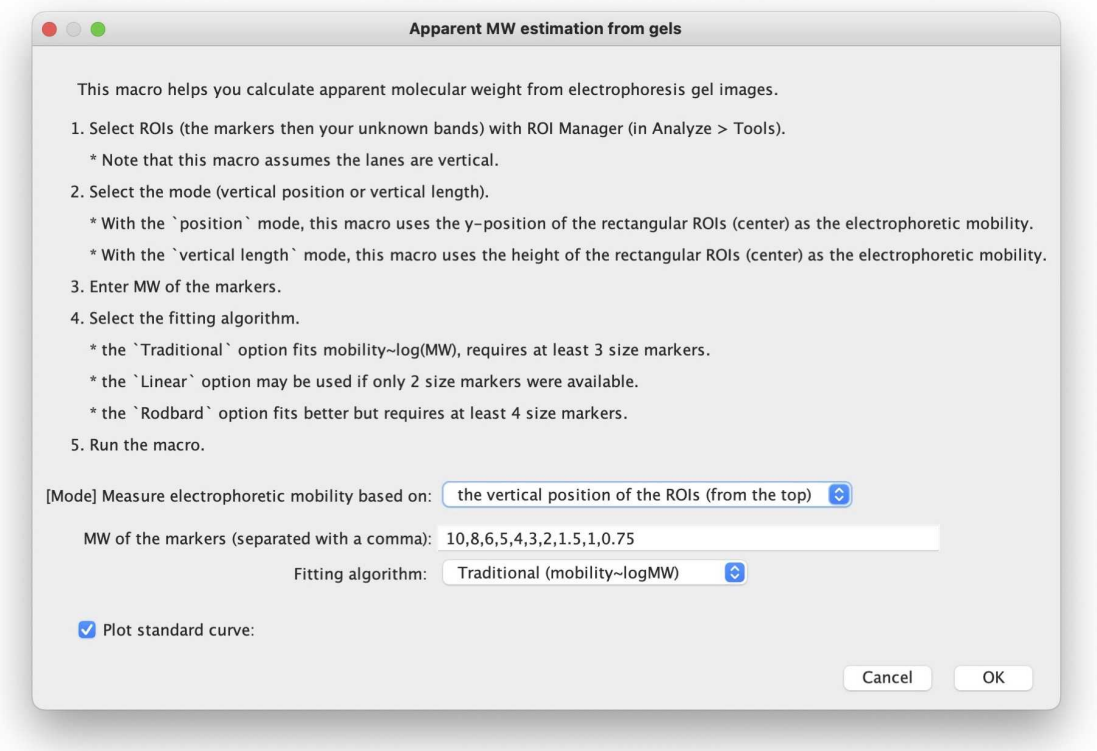

Figure 1. The main window of the Molecular Weight Estimator macro.

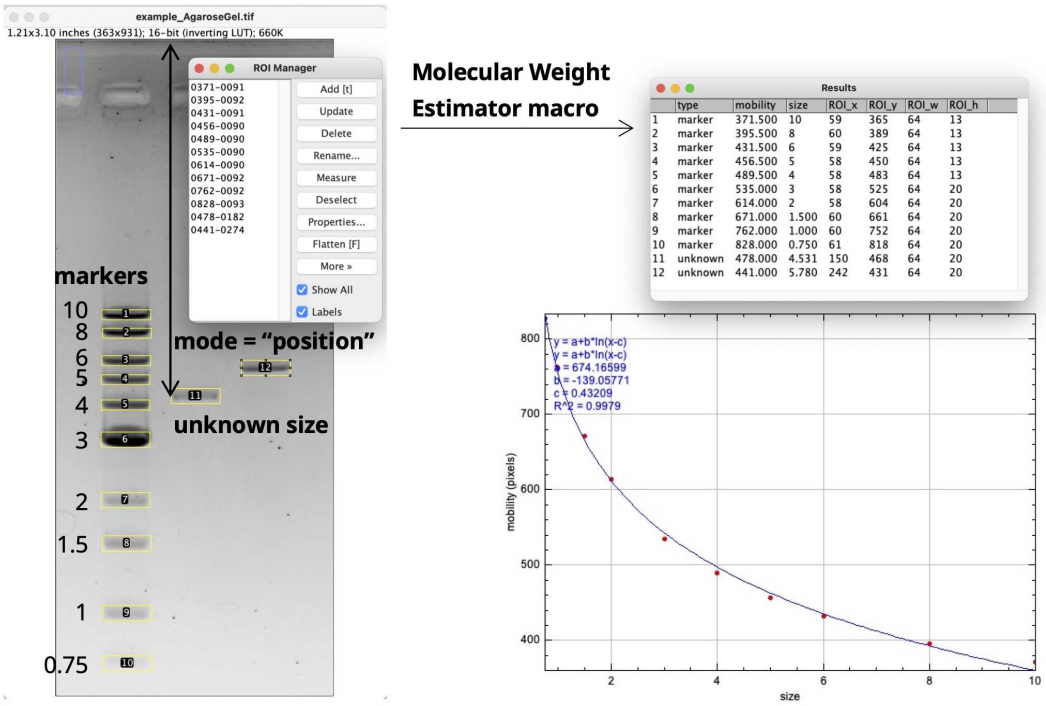

Figure 2. MW of two bands on the agarose gel image can be easily estimated by selecting the bands and running this macro. The fitting was performed with the traditional "mobility~log(MW)" model.

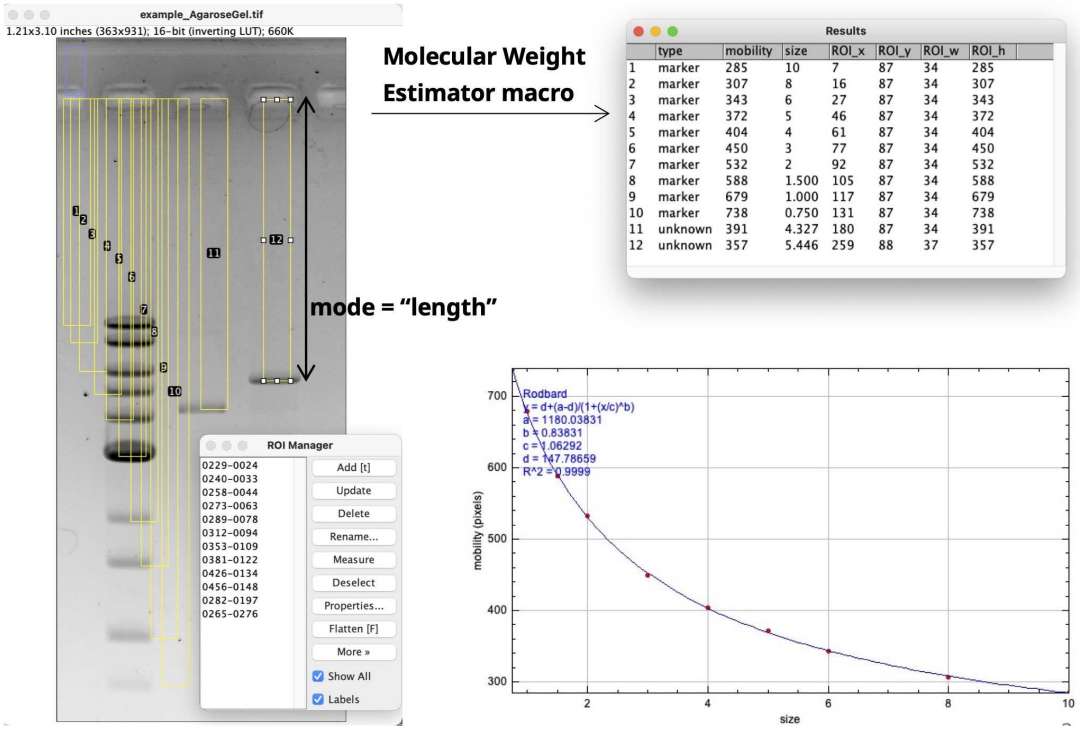

Figure 3. The start of the electrophoresis can also be clearly marked by using the "vertical length" mode.

Installation of MolecularWeightEstimator macro into ImageJ

1 Download the macro file ("_MolecularWeightEstimator.ijm") anywhere in your local disk.

_MolecularWeightEstimator.ijm

2 Start ImageJ.

3 From the menu bar, select `Plugins > Install...`. Open the downloaded macro file, and save the macro in the `plugins` folder in the ImageJ directory.

4 Restart ImageJ.

Estimating MW from gel images.

5 Open an image file.

Note

Check if the lanes in your gel image are vertical. If not, rotate the image by `Image > Transform > Rotate ...`. Also, if you use the "vertical position" mode (see below), the electrophoretic mobility is calculated from the vertical position (the center) of ROIs from the top of the image. So, crop the image beforehand to remove unnecessary regions outside of the gel (or at the well where the samples start migrating).

6 Start `ROI Manager', which can be found in the ImageJ menu, Analyze > Tools > ROI Manager.

Note

In the ROI Manager, check the "show all" and "show labels" options to mark the selected ROIs.

7 Select the rectangular tool, and select the bands of the size markers. To register each ROI into the ROI Manager, select a band and then click "Add" in the ROI Manager (or use a shortcut key like "Command+t" or "Ctrl + t").

Note

Place the ROIs on the bands if you use "the vertical position" mode. To use "the vertical length" mode, ROIs must be defined so that the top of the ROI marks the loading well of the gel and the bottom of the ROI marks the band position.

8 Also, register the bands with unknown molecular weight.

9 Start the "Molecular Weight Estimator" plugin from the ImageJ menu (Plugins > MolecularWeightEstimator).

10 Select the mode from either "vertical position" mode which uses the y-coordinate of the ROIs from the top of the image, or "vertical length" mode which uses the vertical length of the ROIs as the electrophoretic mobility. Normally, the vertical position mode would be more easy and quick.

11 Enter the molecular weights of your size markers. Use a comma to separate the values.

12 Select the algorithm (function to fit).

Note

In most cases, the default "Traditional (mobility~logMW)" is the most common method and would be sufficient, requiring at least 2 size markers. The `Rodbard` algorithm performs better over a wider range but requires more than 4 data points. The `linear` option is not commonly used to estimate molecular weight.

13 Click `OK` to run the macro to see the fitting result and a table showing the estimated molecular weights of your samples.