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Integrated protocol for reliable and fast quantification and documentation of electrophoresis gels

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ABSTRACT

Quantitative analysis of electrophoresis gels is an important part in molecular cloning, as well as in protein expression and purification. Parallel quantifications in yield and purity can be most conveniently obtained from densitometric analysis. This communication reports a comprehensive, reliable and simple protocol for gel quantification and documentation, applicable for single samples and with special features for protein expression screens. As major component of the protocol, the fully annotated code of a proprietary open source computer program for semi-automatic densitometric quantification of digitized electrophoresis gels is disclosed. The program ("GelQuant") is implemented for the C-based macro-language of the widespread integrated development environment of IGOR Pro.

Introduction

Quantitative analysis of electrophoresis gels is a critical element in molecular cloning (restriction analysis, optimization of polymerase chain reactions, etc.) as well as in protein expression and purification. Densitometric analysis of band intensities from stained agarose-gels for correctly evaluating polynucleotide molecular weights and quantities represents a most fundamental cornerstone in molecular biology and in general nucleic acid research (e.g., structural biology of ribonucleic acids).

After successful cloning, maximizing the yield of heterologously expressed proteins is an important task, especially in structural biology, which demands for large amounts of purified protein. Optimization of the expression typically involves the empirical determination of the optimal growth conditions and medium composition (screening approach). Here, densitometric analysis of band intensities from SDS polyacrylamide gel electrophoresis (SDS–PAGE[1]) can serve as a convenient method for analyzing data from expression screens and also to monitor progress during protein purification efforts. Densitometry is especially useful when spectroscopic determination is too tedious (e.g., high throughput approaches or safety considerations with toxic proteins) or when absorbance coefficients for the protein of interest are not readily available. In any case, proper documentation is also of great importance, since huge amounts of data are often produced.

Considering these demands, an integrated protocol for gel evaluation by densitometry was developed: Stained and scanned gels usually feature a baseline or background staining of highly uneven distribution which is imperative to be removed before any further quantitative analysis is possible. The key aspect of the described methodology is therefore the implementation of an algorithm for background subtraction and subsequent semi-automatic quantitative analysis of the corrected gel. Additionally, accommodating functions for automatic gel documentation of the gel save time and labor. The complete devised protocol is described in the following – a typical protein expression screen performed in our laboratory was chosen to serve as an example to fully demonstrate all features of the program.

Materials and methods

Chemicals

Unless stated otherwise, all chemicals used were from Sigma–Aldrich and of analytical grade.

Molecular cloning

For expression, a construct consisting of residues 121–232 (human numbering) of the C-terminal domain of the murine major

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[1] *Abbreviations used:* SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; RNA, ribonucleic acid.

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prion protein (C-mPrP$^{121-232}$) under control of the T7-promoter with N-terminal hexa-histidine-tag and thrombin-recognition-site for tag-cleavage [1] was used, cloned into a pRSET-vector (Invitrogen, V351-20). For expression, the plasmid was transformed into chemically competent *Escherichia coli* cells (strain BL21(DE3), Invitrogen).

Expression cultures and screening

A single colony was picked from a minimal plate with ampicillin selection and used to inoculate 5 ml of minimal medium, this preculture was grown at 37 °C in an orbital shaker (200 rpm) for 24 h. The preculture was spun down briefly (5 min, 6000 rpm) and cells were washed twice in neutral 0.1 M potassium phosphate buffer. 5 µl each of resuspended cells where used to inoculate the expression cultures of the screening, which was performed in small disposable bioreactors (50 ml, vented cap, Corning, NY, 14831). Auto-inducing minimal medium was used [2], with constant concentration of the inducer lactose. The culture conditions were identical for all 10 cultures, only the glucose concentration was varied (lane 6: 0 mM, lane 7: 5 mM, lane 8: 10 mM, lane 9: 15 mM, lane 10: 20 mM, lane 11: 25 mM, lane 12: 30 mM, lane 13: 40 mM, lane 14: 50 mM, lane 15: 60 mM), see Fig. 1.

Preparation of quantification standards

Four standard concentrations are chosen: 50 mg/l, 100 mg/l, 150 mg/l and 200 mg/l. This calibration range should be suitable for all practical purposes. If protein yields of over 200 mg/l are expected or encountered, the samples can be diluted before application to the gel, ensuring they are covered by the calibration working range. It should be noted here that since color density on gels depends on dye-binding by the analyte and also the maximal packing density of the analyte allowed by the gel matrix, there will be a maximal color density per band that can be obtained ("saturation"), therefore limiting the possible calibration range. At saturation, increasing the amount of analyte will not lead to higher signals, but to overall bigger and unshapely bands with no more than saturated color density ("gel overloading"). Saturation therefore obviously crucially depends on the combination of analyte, dye and gel matrix material, so each staining method should be carefully validated for its calibration range.

Table 1
Preparation of protein standard solutions.

c (protein)/mg/l	50	100	150	200
V (1 mg/ml stock)/µl	20	40	60	80
V (dd. H_2O)/µl	380	360	340	320

The standard solutions are prepared as follows: A 50 mg/ml stock solution of standard protein is prepared on ml-scale – in order to minimize weighing errors. It is desirable that the chosen standard protein is in the same molecular weight range and contains equal amounts of basic amino acids (mainly arginine and lysine) as the protein to be quantified, in order to minimize deviations caused by differences in stainability, which is a known issue with Coomassie-staining [3] and silver-staining. Considering this, the chosen standard protein (lysozyme from chicken egg white (Sigma–Aldrich L6876), MW = 14.3 kDa, basic amino acids = 13%) seems well suited for quantifying the protein of interest in this example (C-terminal domain of murine prion protein, MW = 15.2 kDa, basic amino acids = 11%). Using lysozyme as standard protein, an aliquot of 1 g lysozyme (chicken egg white, Sigma–Aldrich) is dissolved in distilled water to a final volume of 20 ml, yielding a 50 mg/ml stock solution, which is stored at −20 °C in aliquots of 500 µl. A 1 mg/ml stock solution is prepared by adding 20 µl of 50 mg/ml lysozyme to 980 µl distilled water, stored at −20 °C. The four standard solutions are then prepared according to Table 1.

Preparation of samples for electrophoresis

This protocol uses precast gels (NuPAGE® Novex® 4–12% Bis-Tris Gels, Life Technologies). Gels cast in the laboratory are suitable as well, but were not explicitly tested with the described protocol. Unless stated otherwise, this protocol uses the electrophoresis protocols proposed by Invitrogen concerning precast gels and matching buffer system. For a 15-well-gel, 15 microcentrifuge tubes are prepared and filled each with 10 µl of twice concentrated sample buffer (3.03 g Tris-Base, 5.5 g SDS, 25 g glycerol, 100 mg bromophenol blue sodium salt, 0.3 g dithiothreitol, made up to

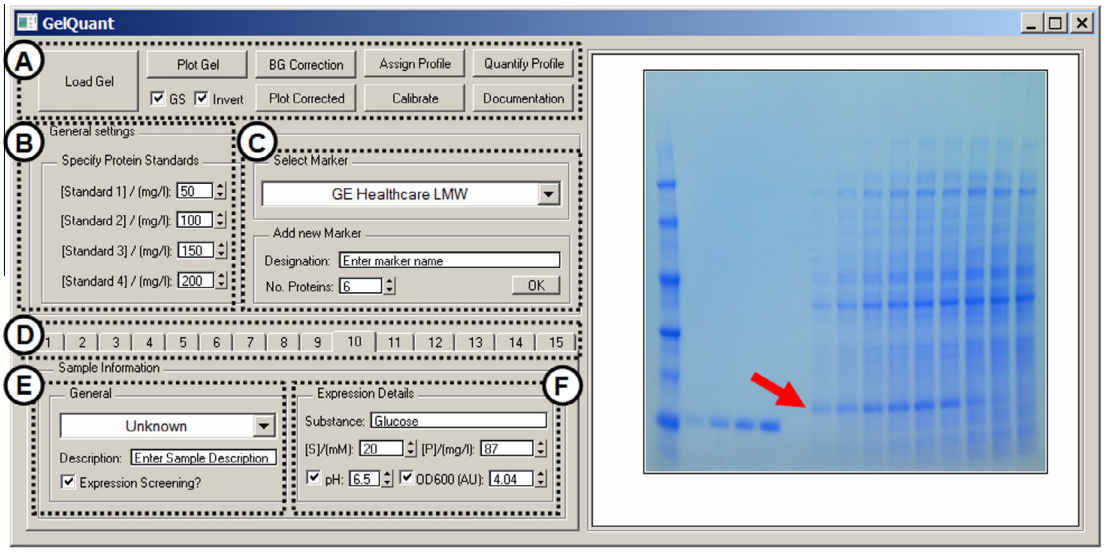

Fig. 1. Main window of the "GelQuant" program with loaded gel. (A) Main functions in sequential order from left to right, (B) concentrations of protein standard solutions, (C) marker section, (D) lane selection, max. 15 (E) sample type selection, (F) expression screening details (optional). The protein of interest of this example expression screening (C-terminal domain of murine major prion protein) is marked (arrow).