SHORT PROTOCOL No. 25 I April 2017

Easy Monitoring of GFP Production in a Fermentation Process by the Eppendorf BioSpectrometer® fluorescence

Bin Li[1], Natascha Weiß[2], Ma Sha[1]
1[1]Eppendorf, Inc., USA
[2]Eppendorf AG, Germany

Introduction

For the development of large scale commercial production of fermentation based biologics, extensive efforts in method optimization at an early stage is required. The aim is to have a time- and cost-effective fermentation process together with high reliability. In a small scale environment, many process parameters must be varied and have to be evaluated in order to achieve optimum quality and yield on the desired product. This includes the selection of most productive strains and media but also topics like vessel and impeller geometry. Afterwards the process can be transferred to pilot scale following established scale-up strategies.

This Short Protocol is based on experiments conducted using a fermentation scale-up approach [1]. Here a green fluorescent protein (GFP) expressing *E. coli* strain was used. GFP is a well-established fluorescent protein and it is easy to monitor. Protein products engineered with GFP tag are frequently used to monitor protein production. Fluorescence measurements for tracking the yield of GFP production were carried out using samples from a shake flask culture and a 10 L bench scale fermentor culture. In addition, the increasing biomass is observed by determination of OD_{600} values.

It is shown that the Eppendorf BioSpectrometer fluorescence is ideal for these applications as it incorporates UV/Vis mode and fluorescence mode in a single instrument.

Material and Methods

Materials

> *Escherichia coli* GFP (ATCC® 25922GFP™)
> New Brunswick™ Innova® 44 shaker with 500 mL baffled shake flask
> BioFlo® 320 bioprocess control station with 10 L stainless steel dished-bottom glass vessel
> Eppendorf BioSpectrometer fluorescence
> Eppendorf UVette®
> Disposable Vis cuvettes, polystyrene

Shake flask culture

A baffled shake flask containing 100 mL TSB (Tryptic Soy Broth) medium was inoculated with an *E. coli* strain which express GFP. The culture was grown in an Innova 44 shaker at 37 °C and 200 rpm for eight hours. To measure the cell growth and protein yield (GFP), samples were taken at four time-intervals (0h, 2h, 4h, 8h).

BioFlo 320 fermentation process

Details on the fermentation and feeding protocol in the BioFlo 320 can be found in Application Note 306 [1]. In brief, 90 % of the vessel maximum working volume was filled by a defined fermentation growth medium. The growth medium was inoculated with an inoculum volume of 10 % of the initial fermentation volume. Continuous fermentation method was used in order to maintain a constant working volume throughout the whole process. To measure the cell growth and protein yield (GFP), samples were taken every hour.

Measurement of OD_{600}

Samples were diluted with deionized water to achieve a reading range from 1 to 2 A. The absorbance at 600 nm was measured in polystyrene semi-micro cuvettes with an Eppendorf BioSpectrometer using the pre-programmed protocol "OD_{600}" (Figure 1).

Protein extraction with concurrent removal of nucleic acids

For the GFP analysis, the samples were treated using a Bacterial Cell Lysis Kit (GB-176, Gold Biotechnology®, USA) following the protocol provided by the kit manufacturer [2] to release the GFP into the supernatant using the following steps:

1. Pellet bacterial cells by centrifugation at 5000 x g for 10 minutes. Suspend the cell pellet in 5-10 x volumes of the Bacterial Lysis Buffer.
2. Gently pipet up and down until the cell suspension is homogeneous. Incubate the suspension for 5 minutes in ice. Gently pipet again to suspend the cells.
3. Vortex the tube containing Lysozyme to mix the frozen suspension. Add 5 µL Lysozyme for each 100 µL cell suspension in Bacterial Cell Lysis Buffer. Gently mix the content.
4. Incubate the suspension at 37 °C for 30 – 60 minutes.
5. At the end of incubation period, vortex the content of the tube several times (30 seconds each) to complete the lysis. Lysis may be further assisted by pipetting the suspension up and down a few times with a narrow bore pipet tip or a 20-gauge syringe needle.
6. Centrifuge the lysate at 20,000 x g and 4 °C for 30 minutes and collect the clear lysate.

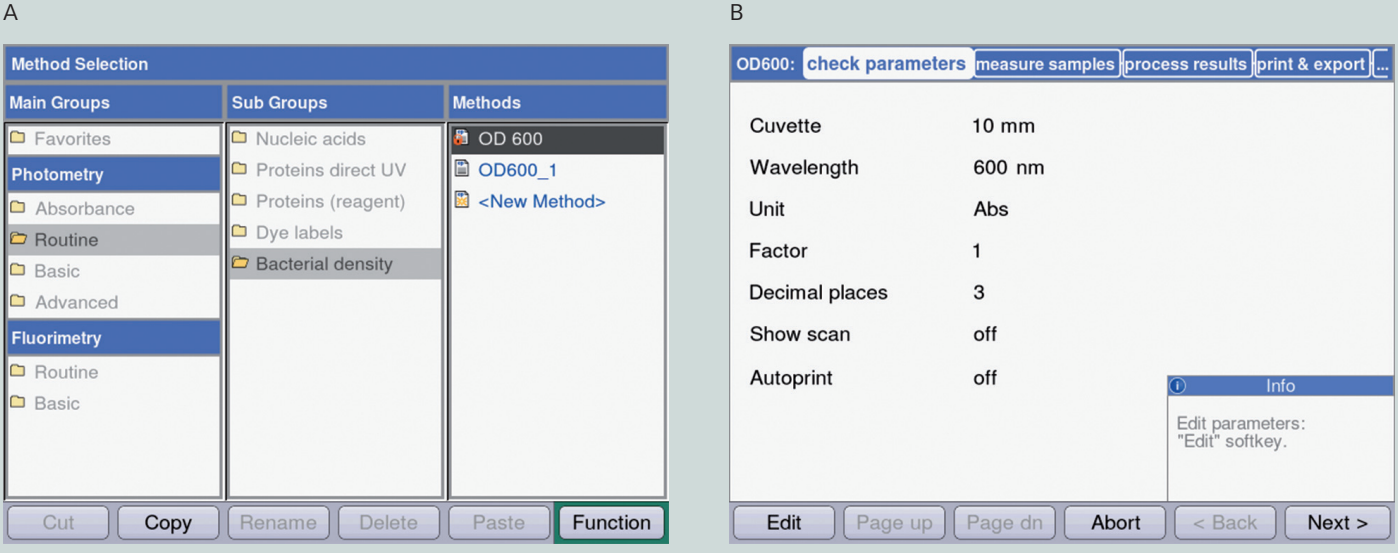

Figure 1: Display Eppendorf BioSpectrometer fluorescence: "Method selection" (A) and "Check parameters" (B) of "OD_{600}" method.

Fluorescence measurement

Fluorescence intensity of the supernatant was measured in a UVette with the Eppendorf BioSpectrometer fluorescence using the pre-programmed protocol "Raw fluorescence" with an excitation wavelength of 470 nm and an emission of 520 nm (Figure 2). These wavelengths overlap sufficiently with the respective excitation and emission spectra of GFP (Figure 3).

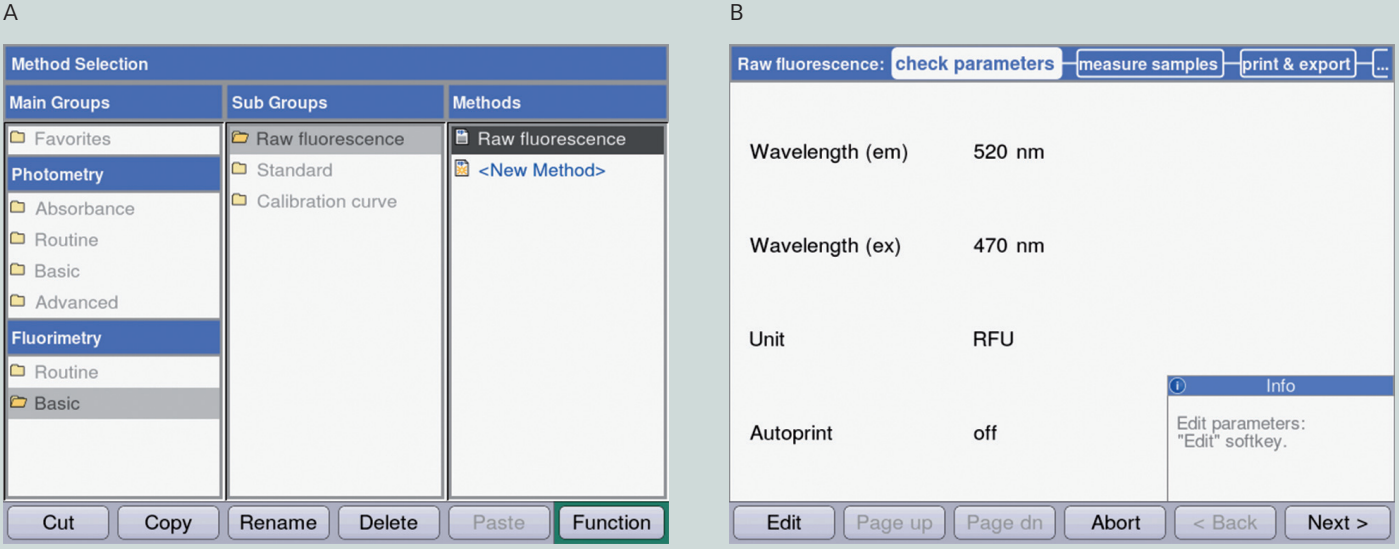

Figure 2: Display Eppendorf BioSpectrometer fluorescence: "Method selection" (A) and "Check parameters" (B) of "Raw fluorescence" method.

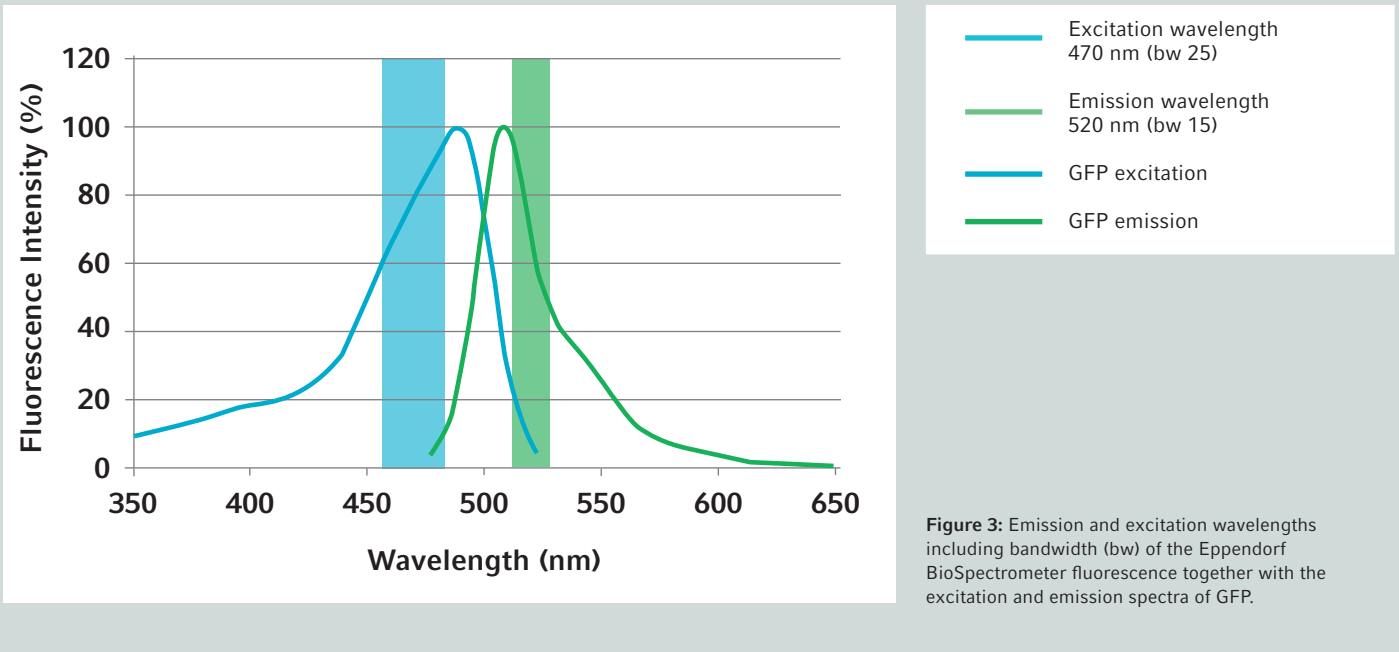

Figure 3: Emission and excitation wavelengths including bandwidth (bw) of the Eppendorf BioSpectrometer fluorescence together with the excitation and emission spectra of GFP.

Results

The OD_{600} measurements show typical growth curves of bacteria from shake flask culture (Figure 4 A) or a continuous fermentation approach (Figure 4 B). In Figure 5 the increasing GFP production which has been tracked by fluorescence measurements is presented.

In this Short Protocol we show the successful monitoring of protein production during a fermentation process. The comparison of GFP production under specific conditions will allow choosing suitable parameters during the optimization of a fermentation process and the technique may be potentially used to monitor fluorescent protein production in general as well as other proteins produced with a fluorescence tag.

The measurement of the GFP expression using the Eppendorf BioSpectrometer fluorescence offers an easy and fast way to determine important bioprocess performance parameters by:

> allowing the determination of the OD_{600} and fluorescence measurement in one device.
> fast handling due to pre-programmed protocols including flexible adaption of methods according to customer needs.
> time-saving conversion of measured data into real values by setting a dilution factor.

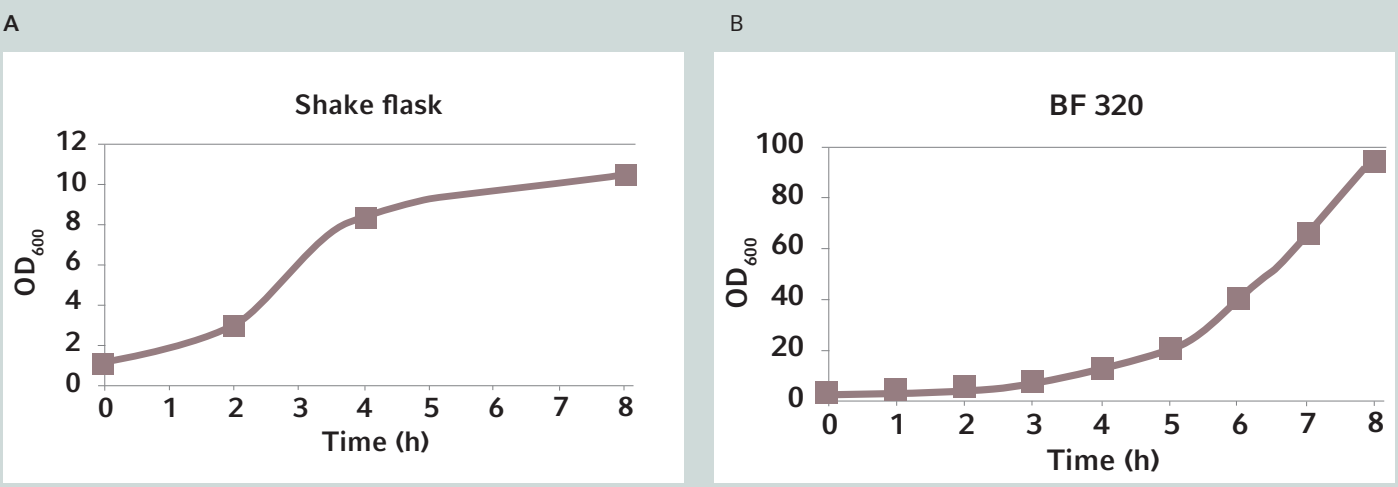

Figure 4: Bacteria growth curves for shake flask culture (A) and BioFlo 320 (B).

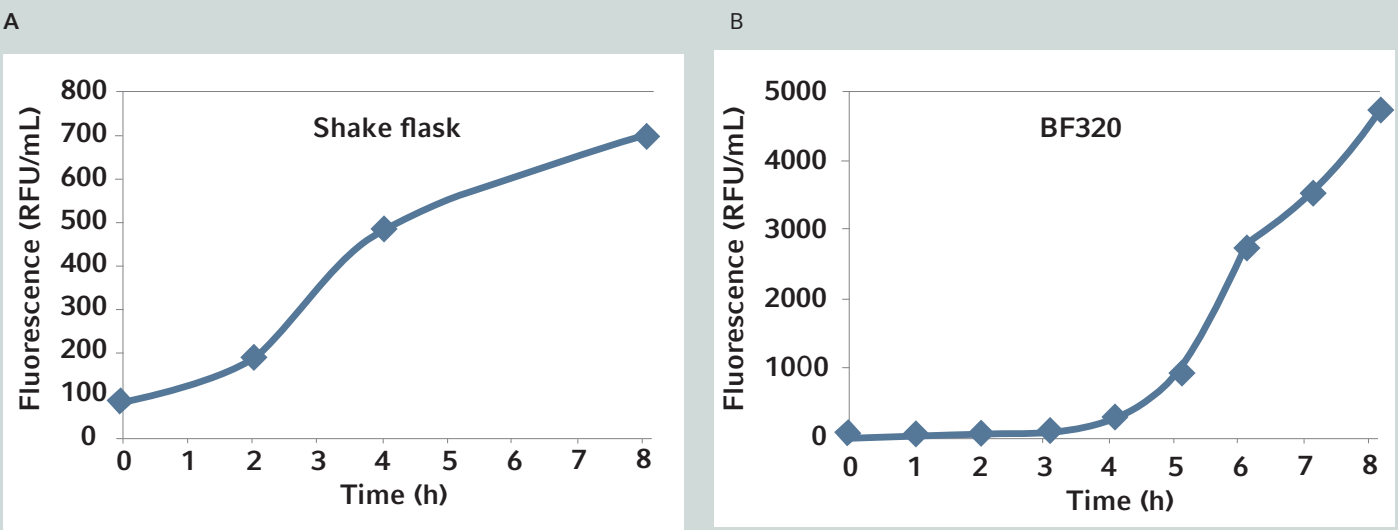

Figure 5: Plot of fluorescence values over time for shake flask culture (A) and BioFlo 320 (B)

Literature

[1] Li Bin, Sha Ma. Scale-Up of Escherichia coli Fermentation from Small Scale to Pilot Scale Using Eppendorf Fermentation Systems. Eppendorf Application Note 306; www.eppendorf.com
[2] Operating Manual Bacterial Cell Lysis Kit (GB-176, GoldBiotechnology®, USA). www.goldbio.com

Ordering information

Description	Order no. international	Order no. North America
Eppendorf BioSpectrometer® fluorescence, 230 V/50-60 Hz, plug Europe	6137 000.006	
Eppendorf BioSpectrometer® fluorescence, 120 V/50-60 Hz, plug North America		6137000014
UVette® 220 nm – 1,600 nm	0030 106.300	952010051
UVette® routine Pack 220 nm – 1,600 nm	0030 106.318	952010069
Eppendorf macro Vis Cuvette 300 – 900 nm	0030 079.345	0030079345
Eppendorf semi-micro Vis Cuvette 300 – 900 nm	0030 079.353	0030079353

Your local distributor: www.eppendorf.com/contact
Eppendorf AG · 22331 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com