CCD-camera gel documentation

INTRODUCTION

CCD, Charge-coupled Device, is as an image sensor system that converts light to a digital signal. The technology is widely used in digital photography and astronomy. In the biotechnology field, due to its great sensitivity, an image system equipped with a CCD-camera is a very useful tool. CCD-elements can capture light emitted from any dyed biological sample. Common dyes include those that are detectable within the visible light range, e.g. silver stain or Coomassie brilliant blue and fluorescent stains such as ethidium bromide and the SYBR stains from Invitrogen. Sensitive CCD-cameras can also be used for the detection of chemiluminescent light emission. The digital signal generated by the capture of light by the CCD-elements is a base for subsequent data analysis, hence good image capture is critical to guarantee optimal performance of automated image analysis packages and generate reliable quantitative data. Factors determining the quality of image capture include image resolution, bit depth and dynamic range. Image resolution relates to the number of pixels displayed per unit length of a digital image, and is often measured in dpi (dots per inch) or in micrometers (the size of the area each pixel represents). A higher number of pixels per unit length will mean better image resolution but also a bigger file-size. The term 'bit depth' describes the number of bits used to define each pixel of a digitized image, and determines how many levels of gray can be generated i.e., an 8-bit grayscale image file has 2^8 possible shades of gray for each pixel. Dynamic range defines the actual range of grayscales that are utilized within a digital image. A large bit-depth enables for a signal to be distinguished from the background more easily than a smaller bit-depth. Thus bit depth is related to sensitivity. Since the limit of gray scale representation possible by conventional computer screens is 256 scales, an image captured by an 8-bit camera will appear identical to one captured by a 12-bit camera. However, analysis software can distinguish between levels of gray that we could not capture by eye. The bit-depth required for a given image system varies depending on application. For applications requiring great sensitivity, e.g. chemiluminescence, higher bit-depth is required, whereas other applications such as day-to-day fluorescent gel-detection may require less bit-depth. In this application note we compare results from fluorescent gel images acquired by an 8-bit CCD-camera with results from a 12-bit camera.

MATERIAL

- Lambda DNA (Invitrogen, Carlsbad, CA, USA) completely digested by Hind III, 0.17 μg/μl (Wealtec, Taipei, Taiwan)
- Lambda DNA (Invitrogen) completely digested by EcoRI, 0.17 μg/μl (Wealtec, Taipei, Taiwan)
- Lambda DNA (Invitrogen) completely digested by Hind III and EcoRI, 0.1 μg/μl (Wealtec)
- GES-equipment (Wealtec)
- 10 mg/ml EtBr (Bio-Rad, Hercules, CA, USA)
- 500 bp DNA ladder (GeNeI, Bangalore, India)
- 1 Kb DNA ladder (New England Biolabs, Ipswich, MA, USA)
- 8-bit CCD camera Dolphin-Doc image system (Wealtec)
- 12-bit CCD camera Dolphin-Doc plus image system (Wealtec)
- Dolphin-1D image system analysis software (Wealtec)

RESULTS

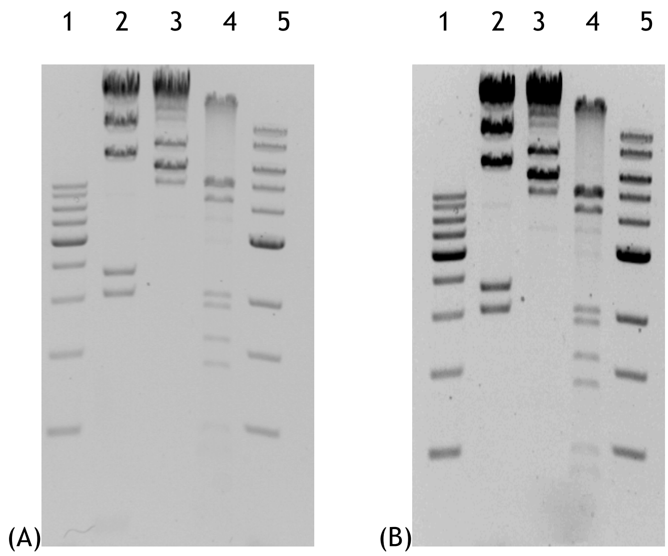

Figure 1. *Agarose gel stained with EtBr. (A) 12-bit CCD camera with normal Amber filter. (B) 8-bit CCD camera with normal Amber filter. Lane 1: 500 bp DNA ladder (0.4 µg), Lane 2: lambda DNA cut by Hind III (1.33 µg), Lane 3: lambda DNA cut by EcoRI (1.33 µg), Lane 4: lambda DNA cut by HindIII and EcoRI (0.83 µg), Lane 5: 1Kb DNA ladder (0.4 µg).*

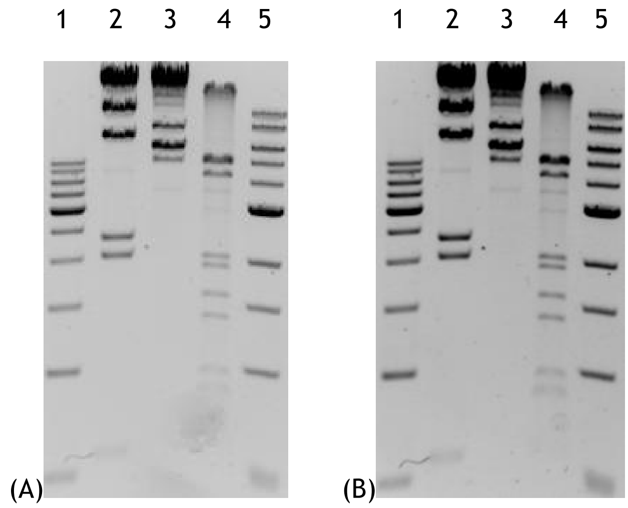

Figure 2. *Agarose gel stained with EtBr. (A) 12-bit CCD camera with high transparent Amber filter. (B) 8-bit CCD camera with high transparent Amber filter. Lane 1: 500 bp DNA ladder (0.4 µg), Lane 2: lambda DNA cut by Hind III (1.33 µg), Lane 3: lambda DNA cut by EcoRI (1.33 µg), Lane 4: lambda DNA cut by HindIII and EcoRI (0.83 µg), Lane 5: 1Kb DNA ladder (0.4 µg).*

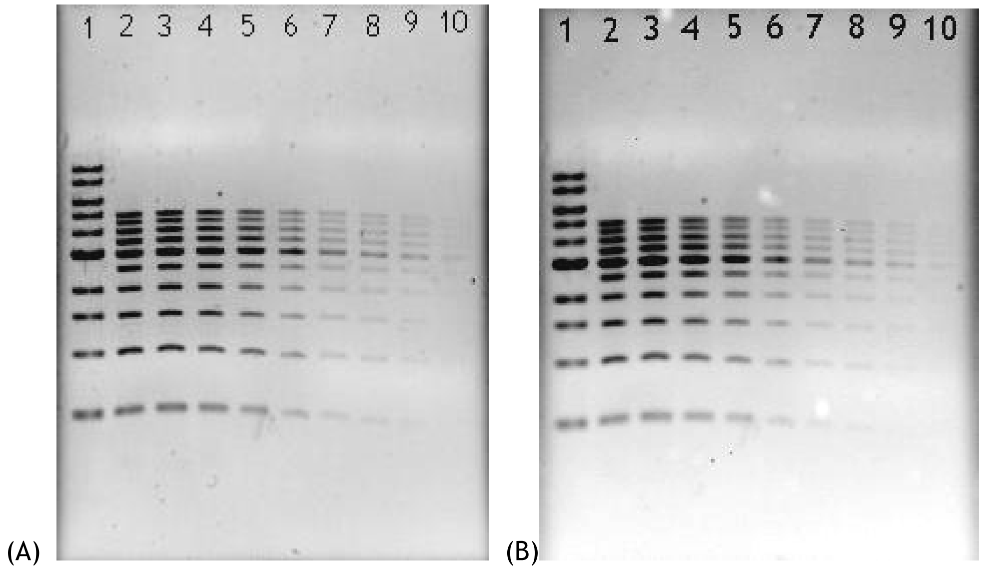

Figure 3. *Agarose gel stained with EtBr. (A) 12-bit CCD camera with high transparent Amber filter 1 second exposure. (B) 8-bit CCD camera with high transparent Amber filter 6 seconds exposure. Lane 1: 1Kb DNA ladder (0.4 µg), Lane 2~Lane 10: 500 bp DNA ladder (0.5 µg, 0.4 µg, 0.3 µg, 0.2 µg, 0.1 µg, 0.075 µg, 0.05 µg, 0.025 µg, 0.01 µg).*

DISCUSSION

Figures 1 to 3 show the images captured by the 8-bit versus 12-bit CCD-cameras. The comparisons displayed are entirely related to the result of differences in bit-depth. Figure 1 and Figure 2 show results from image capturing of gel-images with relatively high concentrations of DNA stained with EtBr and with different exposure times. In image 1 (a and b), a normal amber filter is used, and in image 2 (a and b) a high transparent amber filter is used. The images displayed show that the results are virtually the same in detecting samples whether using an 8 or a 12-bit CCD-camera without regarding to the exposure time. The increase in bit-depth does not markedly improve image quality when using the CCD-camera for this application. As shown in figure 3, where serial dilutions of EtBr-stained DNA are shown, an 8-bit CCD is equally good at detecting small samples as a 12-bit by using longer exposure time. The difference between the cameras becomes slightly more visible as the samples getting smaller; however, it becomes evidence that a smaller bit-depth is also a good option for this application.

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