Cy2, Cy3, Cy5 1D gel imaging using Cy dye lighting unit and epi RGB LED module

Aim

To compare the epi RGB LED module with the Dyversity Cy dye lighting unit in terms of specificity, sensitivity and dynamic range.

Introduction

The Cyanine (Cy) fluorescent dye family are produced by GE Healthcare. This family of fluorophores are a popular choice for labelling proteins in both 1D and 2D gel applications.

The Cy dye lighting unit comprises of a edge illuminator with Red, Green and Blue light capabilities. The epi RGB LED module provides lighting from above with Red, Green and Blue lighting capabilities.

Materials and Methods

Dyversity 4 image capture system has a 4.2m pixel camera with a f1.4 lens, a Cy dye lighting module and Cy bandpass emission filters. The G:Box iChemi XR image capture system has a 1.4m pixel camera with a f1.2 motorised feedback lens, epi RGB LED module and broad band pass emission filters UV, LP and FRLP. The imaging software used was GeneSnap.

1-D Gel

12% acrylamide gel with 5% acrylamide stacking gel, 18cmX16cm. Both carbonic anhydrase (29KDa) (Sigma-aldrich, UK) and bovine serum albumin (BSA) (66KDa) (Sigma-aldrich, UK) were loaded on to the gel. The loading buffer contains 300mM of DTT and 3x50 microgram stock solution of each protein labelled with Cy2™, Cy3™ or Cy5™. From the Cy dyes stock solutions a titration series was used (10,100, 500 and 1000ng). The same dilution series was also used for teach protein loaded on to the gel. For multiplexing between the Cy dyes a protein concentration of 100ng was loaded on to the gel.

Imaging Exposure Times

For the Dyversity image capture system exposure times of 7 seconds were used for Cy2 and Cy5 and 17 seconds for Cy3 dye.

For the G:Box iChemi XR system exposure times of 4 minutes were used for Cy2 and Cy3 and 8 minutes for the Cy5 dye.

Results

Dyversity 4 system

A 12% acrylamide gel was loaded with the following titration series (10,100, 500 and 1000ng) which was used for both the protein sample and the Cy dye.

Distinct dark bands were observed for 1000, 500 and 100ng and a lighter band was observed for 10ng for all Cy dyes tested (Figure 1). For the multiplexed samples bands were detected for the following combinations of Cy dyes; Cy2/Cy3 and Cy2/Cy5 (Figure 1a and b) and Cy3/Cy5 and Cy3/Cy5 (Figure 1c).

G:Box iChemi XR system

A 12% acrylamide gel was loaded with the following titration series (10,100, 500 and 1000ng) which was used for both the protein sample and the Cy dye.

Distinct dark bands were observed for 1000, 500 and 100ng and a lighter band was observed for 10ng for all Cy dyes tested (Figure 2). For the multiplexed samples bands were detected for the following combinations of Cy dyes; Cy2/Cy3 and Cy2/Cy5 (Figure 2a), Cy2/Cy3 and Cy3/Cy5 (Figure 2b) and Cy3/Cy5 and Cy2/Cy5 (Figure 2c).

A small amount of Cy3 labelled BSA was detected under Cy2 imaging conditions and also a small amount of Cy3 labelled BSA was detected under Cy3 imaging conditions (Figure 2a and b).

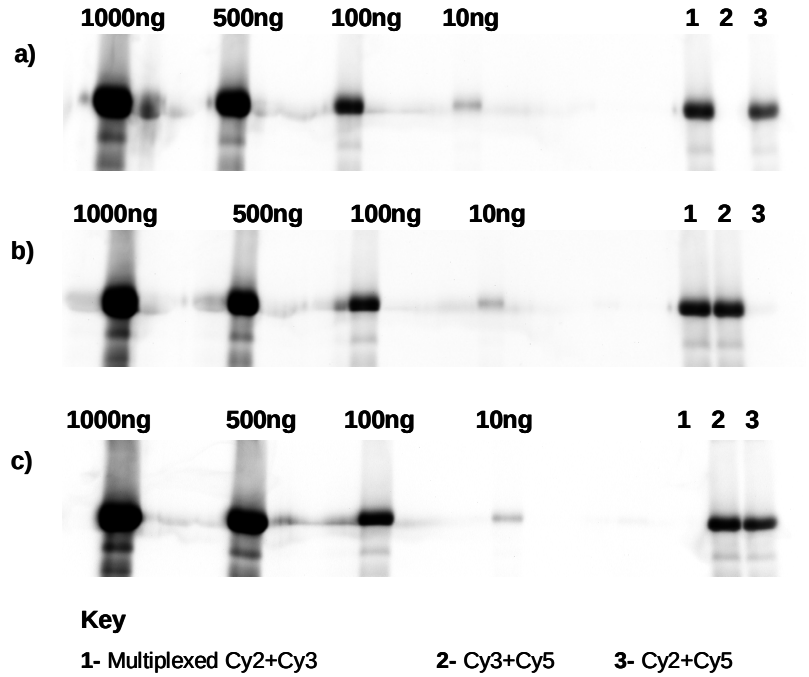

Figure 1- Imaging of Cy dyes using the Dyversity 4 image capture system

a) Cy 2 lighting with a Cy2 emission filter **b)** Cy3 lighting with a Cy3 emission filter and **c)** Cy5 lighting and a Cy5 emission filter.

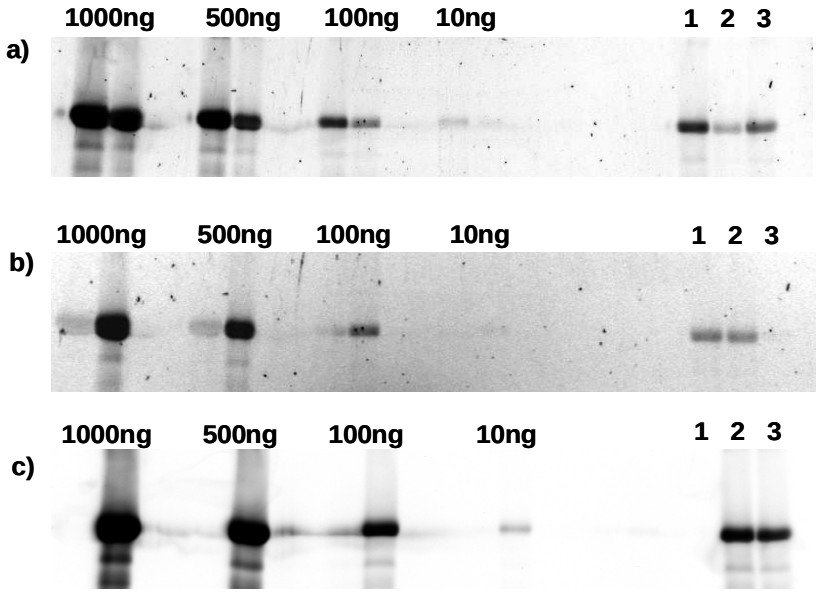

Figure 2- Imaging of Cy dyes using the G:Box iChemi XR image capture system

a) Epi blue with a UV emission filter **b)** Epi green with a LP emission filter and **c)** Epi red and a FRLP emission filter.

Conclusions

Camera signal/noise measurements indicate that Dyversity 4 is similar to the G:BOX iChemi XR when images are acquired at the camera's native resolution (4.2m in the case of Dyversity and 1.4m for G:BOX iChemi XR). Longer exposures for the G:BOX iChemi XR will be due to differences in the excitation light and emission filters.

With the Cy dye lighting unit and Cy emission filters there is 100% specificity of signal for each of the Cy dyes as shown by the multiplexed Cy dyes. The gel image taken using Cy2 conditions shows a band in the Cy3 lane where 1000ng Cy3 labelled BSA has been loaded this could be due to sample carryover or a pipetting error as no signal was detected for any of the other concentrations of Cy3 labelled BSA (Figure 1a).

With the epi RGB module and broad band emission filters (UV, LP and FRLP) there is less than 100% specificity for Cy2 and Cy3 dyes shown by the multiplexed Cy dyes, when Cy2 imaging conditions are used a signal is detected for the Cy3/Cy5 pair (Figure 2a). Additionally, Cy3 labelled BSA at 1000, 500, 100 and 10ng is detectable when Cy2 imaging conditions are used. Furthermore, when using Cy3 imaging conditions some signal is detected for the Cy2 labelled BSA for the titration series.

These results combined show that Cy2 and Cy3 labelled proteins can not be multiplexed. 100% specificity of signal is seen for Cy5 labelled BSA when Cy5 imaging conditions are used as neither Cy2 or Cy3 labelled BSA are detected.

In summary with the epi RGB module and broad band pass filters you are able to multiplex between Cy2 and Cy5 only. With the Cy dye lighting module you are able to multiplex between Cy2, Cy3 and Cy5.

For a multiplex experiment, to image Cy2 use Epi blue and UV filter and to image Cy5 use Epi red and FRLP filter.

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