Accelerate the Semi-dry Transfer with Various Buffer Contents

INTRODUCTION

Semidry transfer technique has been applied for over than 30 years. Buffer recipes had been well established for different purposes, such as Towbin, Bjerrum Schafer-Nielsen buffer, CAPS buffer, discontinuous Tri-CAPS buffer, and Dunn Carbonate buffer. Though buffer system had been used for long time, there still can have some improvements by adjusting buffer contents to accelerate the transfer process. To avoid the problem from bubble formation during the transfer, constant current setting might be varied as long as the buffer recipe changed. Take Towbin buffer as an example to illustrate the different results with different buffer contents and transfer conditions.

MATERIALS

- ELITE 200, Yrdimes, and KETA M imaging systems (Wealtec)
- Thermo Scientific Pageruler Prestainted protein ladder (Thermo)
- 0.45 μm pore size PVDF membrane (Perkin Elmer)
- Towbin Buffer: 25 mM Tris, 192 mM Glycine, pH 8.3 with 20% MetOH (Standard)
  25 mM Tris, 192 mM Glycine, pH 8.3 with 20~40% EtOH (Modified)

PROCEDURES

1. Run the 12% SDS-PAGE with 10, 9, 8, 7, 6, 5, 4, 3, 2, and 1 μl Thermo Scientific Pageruler Prestainted Protein Ladder with 100 V constant voltages for 2h.
2. Transfer the protein ladder onto PVDF membrane with different constant current setting and alcohol contents.
3. Capture the transfer result with KETA M systems.
4. Amplified the image through Magic 1D software.

Result

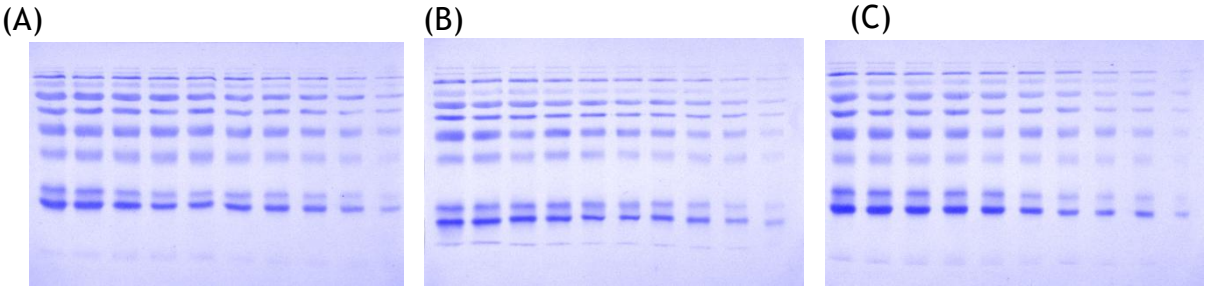

Figure 1. *Transferred with (A) 20% MetOH, 2 mA/cm^2, (B) 20% EtOH, 2 mA/cm^2, and (C) 20% EtOH, 1.7 mA/cm^2 for 45 minutes.*

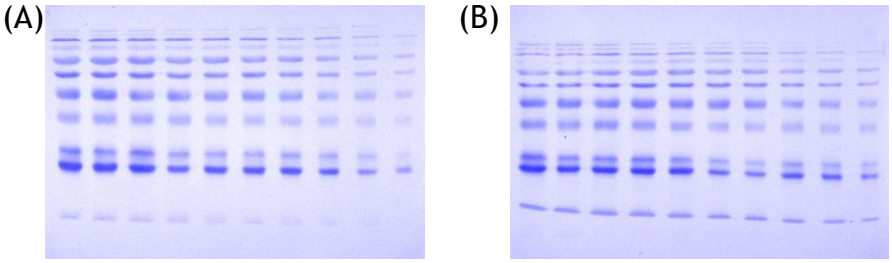

Figure 2. *Transferred with 1.5 mA/cm^2 condition for 45 minutes with (A) 20% EtOH and (B) 30% EtOH.*

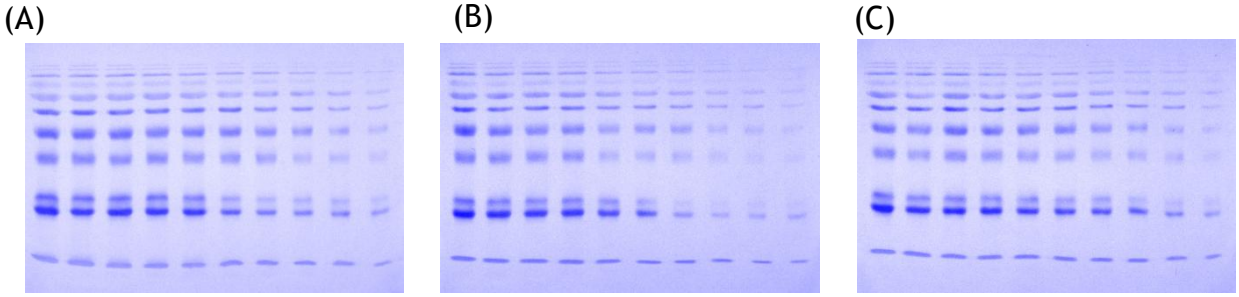

Figure 3. *Transferred with 40% EtOH for 30 minutes with (A) 1.5 mA/cm^2, (B) 1.7 mA/cm^2, and (C) 1.9 mA/cm^2.*

DISCUSSION

It was found that using of MetOH buffer can be replaced by using EtOH under the same transfer condition (Fig. 1). Considering the residue on the SDS-PAGE, using of EtOH can get better transfer efficiency within 60 minutes. However, using of EtOH may increase the possibility of air bubble formation (data not shown). Current setting should be reduced as in Figure 1(c). For transfer buffer contains with 20% EtOH, set with 1.7 mA/cm^2 to transfer for 45 minutes would be the most optimal condition without any bubble formation. Since transfer efficiency was increased in terms of the EtOH concentration increased. In order to decrease the chance of bubble formation, optimal transfer conditions would be set with constant current 1.5 mA/cm^2 for 45 minutes with 30% EtOH transfer buffer and 30 minutes with 40% EtOH transfer buffer.

Protein samples used in this article were all pre-stain markers and might have much lower transfer efficiency than non-stained protein samples. Transfer condition and result would be a reference for user to evaluate how they could modify their original transfer conditions to accelerate the transfer process.

Yi-Ta, Chen Product Manager
Joy, Yi Application Specialist

Wealtec Bioscience Co. Ltd.
Phone: +886-2-8809-8587 FAX: +886-2-8809-8589;
http://www.wealtec.com Email: info@wealtec.com