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# Resolution of Linear DNA Fragments from 23 Kilobases to 6 Megabases Using Biphasic Linear Switch Time Ramping

*Contributed by Donald R. VanDevanter, Tumor Institute, Swedish Hospital Medical Center, 409 Elkind Hall, 747 Summit Avenue, Seattle, WA 98104*

## Introduction

Analyses of uncharacterized DNA fragments using CHEF electrophoresis often require preliminary studies to determine what size ranges DNA fragments of interest fall within. It is not uncommon to run more than one gel in order to fully characterize large DNA fragments from a single cell source[1]. This is because DNA fragments under 200 kilobase pairs (kbp), between 200 kbp and 2 megabase pairs (mbp), and between 2 mbp and 6 mbp are optimally separated by CHEF electrophoretic techniques using different switching frequencies, electrophoretic field strengths, angles of orientation (if using a Bio-Rad CHEF Mapper™ system), and/or agarose type and concentration[2]. Data is presented illustrating that an initial estimate of sizes can be obtained in a single gel by using a biphasic linear switch time ramping procedure.

## Results

A biphasic linear switch time ramping strategy[3] with a fixed electric field strength can be used with the CHEF-DR® II system to resolve linear DNA fragments ranging from 23 kbp to 5.7 mbp in size on a single gel (Figure 1). The gel shown in Figure 1A was 0.5% Chromosomal Grade Agarose made in 0.5x TBE (45 mM Tris borate, 1.0 mM EDTA, pH 8.0), recirculated at 10 °C. The applied electric field was held constant at 1.95 V/cm (65 V), and switch times were ramped linearly from 30 seconds to 2 minutes over 33 hours, then from 2 minutes to 50 minutes over 55 hours. Under these conditions, the chromosomes of *Schizosaccharomyces pombe* (closed circles, lanes 4 and 6), *Saccharomyces cerevisiae* (open squares, lanes 3 and 5), lambda phage concatomers (open circles, lane 2), and the larger fragments of *Hin*d III-digested lambda DNA (closed squares, lane 1) migrate as an approximate linear function of the log of their respective molecular weights (in mbp, Figure 1B).

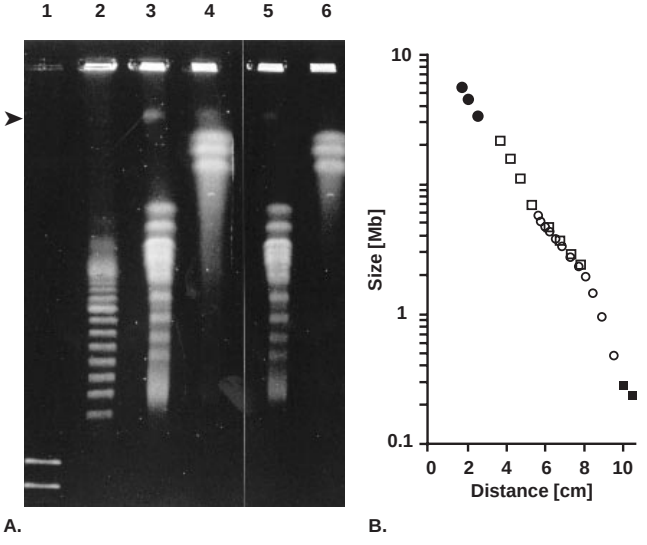


**Fig. 1. Separation of DNA fragments ranging from 23 kbp to 5.7 mbp in size by biphasic linear switch time ramping.** (**A**) Ethidium bromide stain of 27.5 kbp and 23.1 kbp lambda-*Hin*d III fragments (closed squares, lane 1), lambda concatomers (open circles, lane 2), *S. cerevisiae* chromosomes (open squares, lanes 3 and 5), and *S. pombe* chromosomes (closed circles, lanes 4 and 6). The arrow indicates the electrophoretic zone of compression. Specific electrophoretic conditions are described in the text. (**B**) Semi-log plot of the sizes of individual fragments (in mbp) versus distance migrated from the origin (in cm). Only selected chromosomes of *S. cerevisiae* are shown.

Lowering the concentration of agarose in gels can have the effect of decreasing run times without an apparent effect on the linearity of separation (Figure 2). The migration profiles shown in Figure 2 were derived by running the *S. pombe* and *S. cerevisiae* chromosomes on 0.5%, 0.4%, 0.3% and 0.2% Chromosomal Grade Agarose gels.

## Discussion

The CHEF electrophoresis strategy outlined above is not ideal for sharp resolution of bands within a particular narrow range of DNA fragment sizes, but can be extremely useful for determining what range of fragment sizes deserve closer examination. For this reason, it is a preferable approach to running multiple pulsed-field gels under different conditions when size ranges of greatest interest remain unknown.

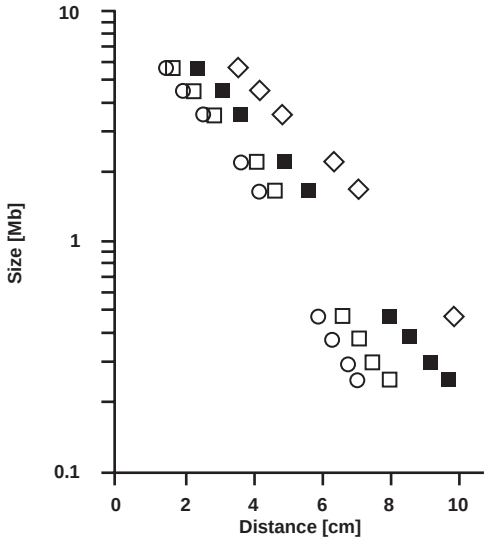


**Fig. 2. Effect of agarose concentration on biphasic linear switch time ramping strategy.** Semi-log plot of the migration of *S. pombe* and select *S. cerevisiae* chromosomes electrophoresed on a single 0.5% agarose gel (open circles) containing internal lanes of 0.4% (open squares), 0.3% (closed squares), and 0.2% (open diamonds) agarose. Gel parameters were identical to those in Figure 1.

## References


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***Life Science Group***

*2000 Alfred Nobel Drive*
*Hercules, California 94547*
*Telephone (510) 741-1000*
*Fax: (510) 741-1060*

***Eastern Regional Office,*** *85A Marcus Dr., Melville, New York 11747 • Phone (516) 756-2575 • Fax (516) 756-2594*
***European Headquarters,*** *Bio-Rad Laboratories, Dreve du Senechal, 19, B-1180 Brussels • Phone 02 375 59 70 • Fax 02 374 61 62*
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***Pacific,*** *Bio-Rad Laboratories, Unit 1111, 11/F., New Kowloon Plaza, 38, Tai Kok Tsui Road, Tai Kok Tsui, Kowloon, Hong Kong • Phone 7893300 • Fax 7891257*
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