MoFlo™ Chromosome Sorting

Introduction

Flow cytometric chromosome classification[1, 2] (flow karyotyping) has been routinely used in the analysis of mammalian karyotypes as well as to assess chromosomal abnormalities.[3, 4] Various chromosome isolation methods[5-7] have been developed in the preparation of isolated chromosomes from mitotic cells for flow karyotyping or flow sorting. To generate a good chromosome preparation with minimal debris formation and large numbers of individual chromosomes for a well-resolved flow karyotype, protocols have made use of stabilizing agents such as divalent cations[7] (magnesium ions) and cationic polyamines[5] (spermine, spermidine) in the isolation buffer. Univariate[8-10] or bivariate[11, 12] flow karyotype analysis on the isolated chromosomes can be performed by labeling with one (propidium iodide, ethidium bromide) or two DNA specific fluorescent dyes (Hoechst 33258, Chromomycin A3). Highly purified fractions of chromosomes can then be isolated rapidly from the labeled chromosomes using the MoFlo high performance cell sorter.

Materials and Methods

Chromosome preparation and staining. Block cells at metaphase using 0.1 µg/ml demecolcine for an optimized amount of time to maximize the percentage of mitotic cells (e.g. ~6 hours for a lymphoblastoid cell line). Pellet (289 g, 5 min) and resuspend cells in hypotonic KCl buffer for 10 mins at room temperature. Pellet (289 g, 5 min) and resuspend cells in ice cold polyamine isolation buffer[5] and vortex for 20 seconds. Spin the chromosome suspension at 200 g for 2 mins, filter the supernatant through 20 µm mesh filter and stain overnight with 5 µg/mL Hoechst 33258, 40 µg/mL Chromomycin A3 and 10 mM $MgSO_4$. Add 10 mM of sodium citrate and 25 mM of sodium sulphite to the stained preparation one hour before flow analysis.

Instrument set up. Analyze the stained chromosome suspension on a MoFlo equipped with two water-cooled argon ion lasers spatially separated at the flow chamber. Tune the first laser to emit multi-line UV (330-360 nm) to excite Hoechst 33258 and the second laser to emit light at 457 nm to excite Chromomycin A3. Maintain the power of both lasers at 300 mW. Trigger the signal based on Hoechst 33258 fluorescence and collect Forward Scatter (FSC) using a 351/10 nm band pass filter, Hoechst 33258 fluorescence using a 400 nm long pass filter combined with a 480 nm short pass filter and Chromomycin A3 flourescence using a 490 nm long pass filter. Prealign the MoFlo for minimum peak coefficient of variance for both fluorescence parameters and forward scatter using 3 µm fluorescent beads. Configure the MoFlo for high speed sorting with optimal settings of the sheath pressure to ~60 psi and the drop drive frequency to ~95 KHz using a 70 µm Cytonozzle tip. Select the high purity sort option of single mode per single drop envelope on a 4WAY™ Sort.

Flow analysis and sorting. For data analysis, acquire a total of 100,000 events at a data rate of 1,000 events per second and analyze on Summit Software. Display data as a bivariate flow karyogram of Hoechst 33258 versus Chromomycin A3 fluorescence (Figure 1) after gating on low forward scatter and high Hoechst 33258 fluorescence to exclude some debris and clumps.

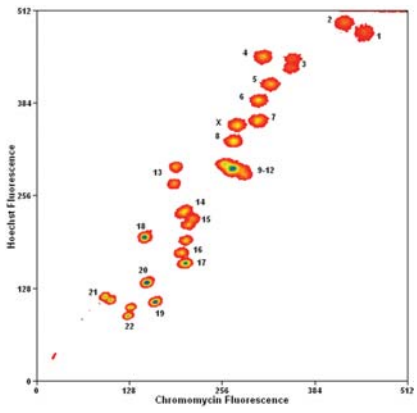

Figure 1

Bivariate flow karyotype of a normal human cell line. The chromosomes are stained with Hoechst 33258 and Chromomycin A3. The position of each chromosome type is indicated. In Summit Software, for analysis, enable smoothing. Smoothing method: Gaussian, # of Passes = 2.

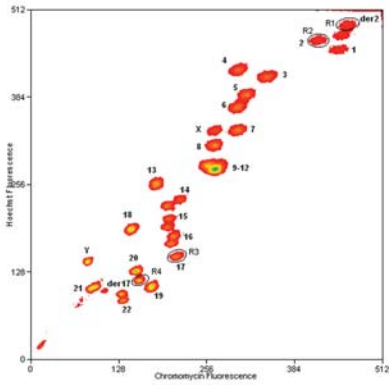

Figure 2

Bivariate plot of Hoechst 33258 vs. Chromomycin A3 fluorescence of a human cell line with translocation between chromosome 2 and chromosome 17. Sort gates are drawn on the translocated chromosomes, derivative 2 (R1) and 17 (R4) and its normal counterpart, chromosome 2 (R2) and 17 (R3). In Summit Software, for analysis, enable smoothing. Smoothing method: Gaussian, # of Passes = 2.

For chromosome sorting, set up the sort decision using the Sort Logic Editor by creating sort gates on the selected chromosome clusters on a bivariate flow karyogram (Figure 2) and isolate the required number of chromosomes into the collection vials.

Discussion

Flow karyotyping and purification of chromosomes isolated from mammalian cell lines can be achieved using the MoFlo high performance cell sorter. The sorting set up of the MoFlo can be customized for rapid isolation of chromosomes with high purity. With the MoFlo, chromosomes can be flow sorted at a data rate of approximately 8,000-12,000 chromosomes per second while still maintaining a good resolution of chromosome peaks. Following purification, DNA as well as proteins can be extracted from the flow sorted chromosomes for genomics[13-15] and proteomics[16] studies.

References

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