Flow Cytometry and Flow Sorting of Metaphase Chromosomes from the Dasyurid Marsupial Dasyurus viverrinus

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Abstract

Metaphase chromosomes (2n = 14) from D. viverrinus were analysed by flow cytometry and flow sorted into six homogeneous groups. Relative chromosomal DNA contents and distribution frequencies of the groups corresponded closely with values for the karyotype obtained by conventional methods.

Introduction

The quantitative classification and purification of isolated metaphase chromosomes is facilitated by the application of flow cytometry and sorting (Gray et al. 1975a, 1975b; Stubblefield et al. 1975; Carrano et al. 1976). Using this approach, individual chromosomes are released from metaphase cells into an isolation buffer to which is added a DNA-specific fluorochrome. The stained chromosomes are then forced to flow through the illumination beam of a flow cytometer (Van Dilla et al. 1974) and a fluorescence frequency distribution is obtained for the total chromosome population, superimposed on an exponential background continuum produced by chromosomal debris, clumps and other fluorescent cellular debris. Typically, such a distribution has several peaks, each representing a distinct group of chromosomes. The mean fluorescence of each peak gives a measure of the relative DNA content of each chromosome type, and the area under each peak is proportional to the frequency of occurrence of chromosomes within each group. These distributions, which may be used in much the same way as conventional karyotypes (Gray et al. 1975b; Carrano et al. 1978, 1979), are particularly sensitive to aberrations which occur homogeneously in all cells. Random aberrations, which occur in only one or a few cells, contribute to the background continuum in the fluorescence distribution.

Flow cytometry, when used in combination with flow sorting (Gray et al. 1975a, 1975b; Carrano et al. 1976), enables the identification and purification of specific metaphase chromosomes to a degree of homogeneity not possible with other chromosome fractionation methods, and such purified chromosomes have been used for a variety of biochemical and genetic investigations (Sawin et al. 1979; Disteche et al. 1981a). For example, genomic DNA libraries have been constructed from purified chromosomes (Davies et al. 1981a; Disteche et al. 1981b), enabling gene sequences from specific chromosomes to be isolated. Purified metaphase chromosomes have been used in several laboratories for gene mapping (Hughes et al. 1979; Lebo et al. 1979, 1982; Collard et al. 1983). The methods involve the extraction of DNA from specific chromosomes.
and subsequent hybridization using nucleic acid probes. Using marsupial globin gene probes, DNA extracted from sorted chromosomes has been analysed to determine the chromosomal localization of the globin sequences (Wainwright and Hope 1985). These approaches to gene mapping are particularly useful where somatic cell hybrids are not available for the species and where low gene sequence copy number makes mapping in situ difficult. The main disadvantages to these approaches are the limited chromosomal resolution for some species.

![Karyotype of the native cat prepared from the dvσ cell line.](image)

Fig. 1. Karyotype of the native cat prepared from the dvσ cell line. Autosomes are numbered from 1 to 6 in order of decreasing size. The sex-determining chromosomes are also indicated.

The native cat, *Dasyurus viverrinus*, has a small number \(2n = 14\) of morphologically distinct chromosomes (Fig. 1) (Martin and Hayman 1967). Because of this low diploid number and their ease of identification, these chromosomes are ideal for use in flow-cytometric investigations on the cytological effects of various clastogenic agents (Carrano et al. 1978), as well as for studies of gene arrangement. This paper presents the results of analysis by flow cytometry of the chromosomes of *D. viverrinus*.

**Materials and Methods**

*Establishment of a Native Cat Cell Line*

A diploid fibroblast cell line (dvσ) was established from a pinna explant from a 1-year-old male native cat. Cells were grown in RPMI 1640 culture medium supplemented with 10% (w/v) fetal calf serum. A karyotype of the dvσ cell line is shown in Fig. 1.

*Chromosome Suspensions for Flow Cytometry*

Chromosomes were prepared from the dvσ cell line by a modification of the method of Sillar and Young (1981). Cell cultures were grown to approximately 50% confluence, and growth then synchronized by the addition of thymidine to a final concentration of 5 mM for 16 h. The cell cultures were then washed and grown in the absence of added thymidine. After 8 h colcemid was added to a final concentration of 0·2 µg/ml for 12 h. Mitotic populations were harvested by vigorous shaking of the culture flasks. Preparations routinely had a mitotic index of greater than 90%. Approximately \(4 \times 10^7\) metaphase cells were resuspended in 0·075 m KCl for 15 min at 37°C, pelleted by centrifugation, and resuspended in chromosome isolation buffer consisting of 15 mM Tris-HCl, 0·2 mM spermine, 0·5 mM spermidine, 2 mM EDTA, 0·5 mM ethyleneglycol-bis-(β-aminoethyl)ether)tetraacetic acid, 80 mM KCl, 20 mM NaCl and 14 mM β-mercaptoethanol, pH 7·2. Cells were washed twice in isolation buffer and the final cell pellet resuspended in approximately 20 times its volume of ice-cold isolation buffer plus 0·1% (w/v) digitonin. Chromosomes were released by vortexing followed by several passages through a 21-gauge needle.
Flow Cytometry and Flow Sorting

Chromosome samples were stained at a final concentration of 100 μg/ml of ethidium bromide and analysed with a Becton–Dickinson FACS IV cell sorter. A Spectra–Physics 164-05 laser, with an output of 0·8 W at 488 nm, was used to excite the stained chromosomes. Scattered laser light was blocked with a Schott SPS 50 filter. Sheath fluid (10 mM Tris-HCl, pH 7·6, 1 mM EDTA, 50 mM NaCl), flow rate and chromosome suspension concentration were adjusted to give a fluorescent particle flow rate of approximately 500-1200 particles per second.

The raw data, in the form of channel values, were transferred to a CDC Cyber computer and the data analysed essentially as described by Moore (1975). Peak composition analysis was performed by sorting 10 000 chromosomes on to dry microscope slides. The chromosomes were then fixed by the dropwise addition of 3 : 1 (v/v) methanol-acetic acid, and stained with a 10% (v/v) solution of Giemsa in phosphate buffer, pH 6·8, for 2 min.

![Graph](image)

Fig. 2. Fluorescence distribution of native cat metaphase chromosomes measured by flow cytometry. Bars indicate the regions of the distribution containing chromosomes that were cytologically examined (see Fig. 3). Chromosomes corresponding to each peak and their relative purity are as follows:

<table>
<thead>
<tr>
<th>Peak</th>
<th>Chromosome No. (see Fig. 1)</th>
<th>Relative purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>72</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>37</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>89</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>86</td>
</tr>
<tr>
<td>E</td>
<td>6</td>
<td>84</td>
</tr>
<tr>
<td>F</td>
<td>X</td>
<td>91</td>
</tr>
</tbody>
</table>

Results

Using a flow rate of 500 chromosomes per second, the fluorescence distribution shown in Fig. 2 was obtained for the dvCf cell line. By application of the least-squares Gaussian distribution program (Moore 1975), an estimate of the peak means and area beneath each fluorescence peak was obtained, taking into account random noise and the underlying exponential continuum. The figure legend shows the chromosome responsible for each fluorescence peak and its corresponding purity. The flow
karyotype derived by flow cytometry corresponds well to the data generated by conventional karyotyping procedures and relative DNA contents based upon length measurements (Table 1). An example of the chromosomes sorted from the areas indicated in Table 1 is shown in Fig. 3.

Fig. 3. Examples of sorted chromosomes. Chromosomes were sorted from areas of the fluorescence distribution indicated in Fig. 2. A, chromosome 1; B, chromosomes 2 and 3; C, chromosome 4; D, chromosome 5; E, chromosome 6; F, X chromosome.
Flow Cytometry of *D. viverrinus* Chromosomes

### Table 1. Chromosome measurement by flow cytometry

<table>
<thead>
<tr>
<th>Peak (see Fig. 2)</th>
<th>Chromosome No. (see Fig. 1)</th>
<th>Relative DNA content as estimated by:</th>
<th>Relative chromosome frequency:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Flow cytometry</td>
<td>Chromosome length</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>As estimated by flow cytometry</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Expected</td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td>1·00</td>
<td>1·00</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>0·92</td>
<td>0·95</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0·87</td>
<td>0·87</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>0·67</td>
<td>0·66</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>0·44</td>
<td>0·48</td>
</tr>
<tr>
<td>E</td>
<td>6</td>
<td>0·34</td>
<td>0·35</td>
</tr>
<tr>
<td>F</td>
<td>X</td>
<td>0·13</td>
<td>0·15</td>
</tr>
</tbody>
</table>

### Discussion

Flow cytometry resolved the native cat chromosomes into six distinct fluorescence distributions. Using ethidium bromide staining, chromosomes 2 and 3 could not be resolved. Whilst the use of a general DNA fluorochrome such as ethidium bromide enables an accurate estimate of the relative DNA content of each chromosome it is possible that some cytochemical differentiation of chromosomes 2 and 3 could be achieved using base-specific fluorochromes such as Hoechst 33258 (Ho) or Chromomycin A3 (CA3). In addition, it has been found that Ho- or CA3-stained chromosomes give lower coefficients of variation than those stained with ethidium bromide and thus lead to better resolution (Carrano *et al*. 1979; Langlois *et al*. 1980). The fluorescence of the Y chromosome was not significantly different from that of background debris and does not appear as a separate fluorescence distribution.

The correspondence of the native cat flow cytometric karyotype to that of the conventional karyotype in both relative chromosome frequency and relative DNA content is consistent with the results obtained for other species (Gray *et al*. 1975a, 1975b; Carrano *et al*. 1978). The relative DNA content of the native cat X chromosome, 0·13, represents an actual value of approximately 3% of the haploid genome. This result is consistent with the findings for another dasyurid marsupial, *Sminthopsis crassicaudata* (Hayman *et al*. 1982; B. Wainwright, unpublished data). These are amongst the smallest mammalian X chromosomes described.

### Acknowledgments

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### References


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