# STANDARDIZATION OF THE AMERICAN MINK *(MUSTELLA VISON)* KARYOTYPE AND SOME COSMID *IN SITU* HYBRIDIZATION RESULTS

# ESTANDARIZACIÓN DEL CARIOTIPO DEL VISÓN AMERICANO (*MUSTELLA VISON*) Y ALGUNOS RESULTADOS DE HIBRIDACIÓN *IN SITU* DE CÓSMIDOS

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### Palabras clave adicionales

Cariotipo del visón americano. FISH.

## SUMMARY

The mink karyotype consists of 14 autosomes and a par of sex chromosomes. A system is proposed where row 1 consists of five large metacentric chromosomes ordered by size, row 2 consist of five submetacentric chromosomes ordered by size, and row 3 consists of three acrocentric ordered by size, 1 small telocentric and the sex chromosomes. The identification of the individual chromosomes is based on R-, Q- and N-banding. A numbering system is proposed for the individual bands covering approximately a total of 100 bands.

Results are given for *in situ* hybridization for more than 50 cosmids selected for containing dinucleotide repeats. By these hybridizations a marker are located on all chromosomes. Five cosmids show strong centromere hybridizations results, including the nuclear organizer region and the Y chromosome. Some more detailed results can be seen on the www server http=//www.husdyr.kvl.dk/mink.htm.

## RESUMEN

El cariotipo del visón consta de 14 autosomas y un par de cromosomas sexuales. Se propone un sistema en el cual la fila 1 consta de cinco grandes cromosomas metacéntricos ordenados por tamaño, la fila 2 consta de cinco cromosomas submetacéntricos ordenados por tamaño y la fila 3 consta de tres acrocéntricos ordenados por tamaño. La identificación de los cromosomas individuales está basada en bandeos R-, Q- y N. Se propone un sistema de numeración para las bandas individuales que cubre un total aproximado de 100 bandas.

Se presentan resultados de hibridación *in situ* para más de 50 cósmidos seleccionados por contener repetición de dinucleótidos. Mediante esas hibridaciones se localiza un marcador sobre todos los cromosomas. Cinco cósmidos muestran resultados de fuerte hibridación en centrómeros, incluyendo la región del organizador nuclear y el cromosoma Y. Resultados mas detallados pueden apreciarse en el servidor www http=//www.husdir.kvl.dk/mink.htm.

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## MATERIALS AND METHODS

### CONSTRUCTION OF MINK COSMID LIBRARY

High molecular weight DNA was extracted from mink spleen cells according to Sambrook *et al.* (1989) DNA was partially digested by the restriction enzyme Sau 3A and size fractioned by Sucrose gradient centrifugation (10-40 p. cent Sucrose, 20 mM Tris, pH 8.0, 5 mM EDTA, pH 8.0, 1 M NaCl, 26,000 rpm, 20°C, 24 hours). Fractions were analyzed by gel electrophoresis (0.7 p. cent NuSieve agarose (FMC), 1 x TAE buffer). Fractions containing DNA of approximately 50,000 bp. were pooled and subcloned in the cosmid vector Supercos (Stratagene) according to the manufactures procedure. Ligated DNA was packaged in phage particles Gigapack II Gold, Stratagene). The library was transfected into NM554 cells and plated on NZY-plates containing 50 mg/ml Ampicillin, 0.2 p. cent Maltose, 10 mM $MgSO_4$). Individual clones were picked at random and processed for mini-prep DNA preparation. The DNA was dot blotted on Hybond-N+ nylon membranes (Amersham) and screened using a gamma-32P-dATP (Amersham) labeled (GT)g oligonucleotide (Pharmacia) for hybridization (44°C, overnight; 5 x SSC, 2 x Denhardt, 50 mM Tris, pH 7.4, 100 mg/ml tRNA). All washes were; 15 min., room temperature, 20 min., 51°C, 15 min., room temperature: 6 x SSC, 0.5 p. cent SDS, 0.05 p. cent PoP(sodium pyrophosphate). Filters were exposed for 3-5 days. Positive clones were digested by either RsaI and PvuII in parallel. Digestions were confirmed by gel electrophoresis (0.8 p. cent agarose, 1 x TAE buffer), followed by Southern blotting (Depurinization in 0.2 M HCI; 10 min., denaturation in 1.5 M NaCl, 0.5 M NaOH; 20 min., followed by transfer using a vacuum blotter from Hybaid and denaturating buffer as transferbuffer). Hybridizations followed the same procedure as described above. The digested cosmid DNA was subcloned into the plasmid vector pCRScribt (Stratagene), transformed into DH5 cells and plated on SOB-plates containing 75 mg/ml Ampicillin, 30 mg/ml x-gal, 150 mM IPTG. Clones were analyzed by colonilifting to Nitrocellulose membranes (Sartorius). Filters were hybridized ad described. Positive clones were processed for mini-prep DNA preparation and sequenced using Sangers chain termination reaction procedure. The sequencing reaction were loaded on a ALF DNA sequencer and data were collected by the associated software.

### CHROMOSOME PREPARATION AND *IN SITU* HYBRIDIZATION

As it is not possible to grow lymphocyte cultures form mink blood in any amounts, some fibroblast cell cultures were established from skin biopsies of young male mink. The cells cultures were grown in media containing BrdU for 7.5 hour before the harvest. After fixation the cells were kept at -20°C. The slides were made by the ordinary air drying technique. The entire procedure for handling the chromosomes were essentially done according to Christensen and Pedersen (1990). And the N-bands were made as described by Blom and Goodpasture (1976). *In situ* hybridization was carried out by incubating the slides with RNase-treated

and denatured metaphase spreads at 42°C for 16 hours. There was used one my-g biotinylated probe in 30 my-l of hybridization solution containing 45 p. cent formamide, 2 x SSC, 10 p. cent dextran sulfate and 10 my-g sheared and denatured salmons sperm DNA. The hybridization mix was supplemented with 8 my-g sheared genomic mink DNA and incubated after denaturation for 30 min. at 37°C. After hybridization, the slides were washed two times in 45 p. cent formamide, and three times in 2 x SSC at 42°C. Visualization of the biotinylated probe was achieved with fluorescein isothiocyanate (FITC) conjugated to avidin, and the signal was amplified with one layer of biotinylated anti-avidin by antibody (Vector Laboratories). The RBP-band pattern generated by propidium iodide in alkaline DABCO antifade solution (Lemieux *et al.,* 1992).

## RESULTS AND DISCUSSION

Q-banded chromosomes. Q-banded chromosomes can be seen on the server (Christensen *et al.,* 1996). The Q-bands observed are in all essential identical to the bands published by Mandahl *et al.* (1975).

### R-BANDED CHROMOSOMES AND RELATIVE CHROMOSOME LENGTH AND CENTROMERE POSITION

The relative mink chromosome lengths out of 1000 units for the female complement are given in **table I**. The

***Table I.*** *Relative chromosome length and centromere position. Chromosome numbers refers to old system (Mandahl* et al., *1975).* (Longitud relativa y posición del centrómero. Los números de los cromosomas se refieren al sistema antiguo (Mandahl *et al.,* 1975)).

| Chromsome number | Relative length | | | | Centromere index p. cent | | | |
|---|---|---|---|---|---|---|---|---|
| | NES | MAND | CHRIS | SEROV | NES | MAND | CHRIS | SEROV |
| 1 | 117 | 117 | 119 | **111** | 52 | 51 | 52 | 52 |
| 2 | 94 | 95 | 94 | **105** | 54 | 54 | 54 | 54 |
| 3 | 87 | 87 | **84** | 87 | 52 | 50 | **54** | 50 |
| 4 | 83 | 84 | 86 | 87 | 52 | 51 | 52 | 50 |
| 5 | 82 | 81 | 83 | **79** | 64 | 66 | 67 | **56** |
| 6 | 80 | 79 | 81 | 76 | 72 | 75 | 76 | **65** |
| 7 | 76 | 76 | 78 | **82** | 60 | 57 | 63 | 57 |
| 8 | 72 | 71 | 70 | 70 | 52 | 50 | **47** | **46** |
| 9 | 60 | 57 | 59 | **52** | 68 | 76 | 73 | **57** |
| 10 | 55 | 54 | 55 | **49** | 69 | 75 | 77 | **60** |
| 11 | 54 | 54 | 53 | **49** | 60 | 60 | **66** | **55** |
| 12 | 38 | 38 | 39 | 38 | 62 | 64 | 62 | **50** |
| 13 | 32 | 32 | 30 | 35 | 58 | 58 | **66** | 58 |
| 14 | **24** | 19 | 17 | 20 | 1.0 | 1.0 | 1.0 | 1.0 |
| X | 48 | 49 | 44 | 52 | 56 | 55 | 61 | **66** |

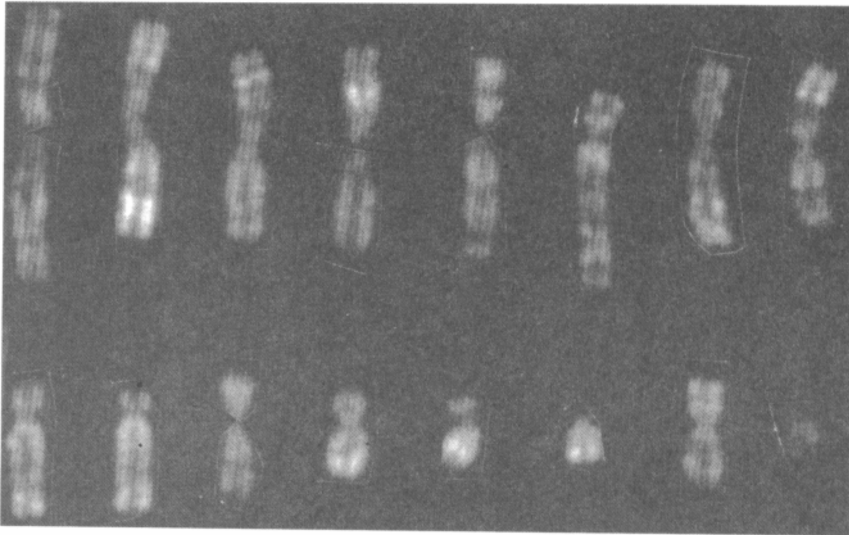

***Figure 1**. R-banded mink chromosomes (old system) ordered by numbers form 1 to 14 and last X and Y. Only one of each pair are shown.* (Cromosomas de visón R-bandeados (sistema antiguo) ordenados de 1 a 14 y finalmente cromosomas X e Y. Sólo se muestra uno de cada par).

chromosomes are numbered according to Mandahl *et al.* (1975). Data are taken from the following studies, Nes (1962) give the actual data (NES), whereas actual measurements were made on ideograms from Mandahl *et al.* (1975) (MAND), and from Serov *et al.* (1987) (SEROV), and present study on R-banded chromosomes (CHRIS). Large deviations are indicated by bold numbers and very large with underscored numbers.

## MINK CHROMOSOME IDENTIFICATION AND NUMBERING SYSTEM

In **figure 1** mink R-banded chromosomes are set up according to the old numbering system proposed by Mandahl *et al.* (1975). The idea behind is ordering in decreasing size in two rows.

In **figure 2** R-banded mink chromosomes are set up with the new system ordered according to size and the centromeric position. The five chromosomes in the first row are large metacentric. The five chromosomes in the second row vary much in size but the p-arm is about one third of the q-arm in length. The last four autosomes in the third row are either sub-telocentric or telocentric, this row also contains the X-Y chromosome pair.

The new system correspond to old numbering as follow:

First row 1, 2, 4, 3 and 8 (8 turned up side down)

Second row 5, 7, 11, 12 and 13

Third row 6, 9, 10, 14 and X,Y

## MOTIVATION FOR THE NEW NUMBERING SYSTEM

When working with many metha-

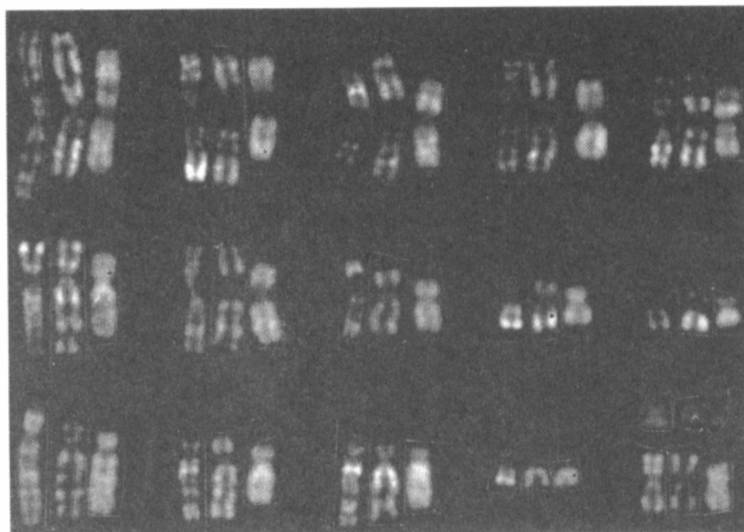

***Figure 2.*** *R-banded mink chromosomes from three cells with different contraction (new system).* (Cromosomas de visón R-bandeados procedentes de tres células con diferente contracción (sistema nuevo)).

phase cells you try to group the one beeing dificult to separate from one another. That is, the five large metacentric chromosomes form a group being difficult to identify. A change between number three and four is necessary as the old number three is smaller than number four, see also Mandahl *et al.* (1975). The chromosome five has a secondary constriction that vary in size. In most cases the constriction is on the longest arm, therfore, the proposed turn over.

### THE MINK STANDARD R-BANDED CHROMOSOME BAND INTERPRETATION

In **figure 3** the interpretation of the R-bands can be seen. The bands correspond to the chromosomes as shown in **figure 2** giving approximately a total of 100 bands.

### COSMID *IN SITU* HYBRIDIZATION RESULTS

The *in situ* hybridization has resulted in the localization of more than 50 single copy sequences with at least one on each chromosome. There has also been found some cosmids with strong centromere repeat signal, and some with weak signal on more chromosomes, these cosmids are listed in **table II**. For a detailed list of all results and some of the pictures see, Christensen *et al.* (1996).

### ALREADY MAPPED GENES IN THE MINK

Serov and Pack (1993) have listed around 50 genes which have been mapped in the mink to specific chromosomes. The mapping have been made by use of a cell hybrid panel. If the percent proposal is accepted for mapping genes in the mink these already mapped

have to be changed accordingly. So if the chromosome numbers has to be changed it is a good time to do so as it

***Figure 3.*** *R-banded karyotype band interpretation made by autocad meassurements. For details of the numbering systems for the bands see, Christensen* et al. *(1996).* (Cariotipo R-bandeado. Interpretación de bandas realizada mediante medidas con autocad. Para detalles sobre el sistema de numeración de bandas ver, Christensen *et al.*, 1996).

***Table II.*** *A list of mink cosmids selected for repeats or localization on more chromosome. HG number and SH numbers refers to the two laboratories involved.* (Lista de cósmidos de visón seleccionados por repeticiones o localización en mas cromosomas. Los números HG y SH se refieren a los dos laboratorios implicados).

| HG | SH | Chromosome number |
|---|---|---|
| AG70 | SH488 | 1,2,3,7,14 weak cen |
| AG34 | SH644 | 2 + 5 Nuclear organizer |
| AG1 | SH8 | 2,3,5,7 weak cen |
| AG71 | SH530 | 2,3 weak cen |
| AG76 | SH571 | 8 strong cen repeat |
| AG23 | SH249 | 9 strong cen repeat |
| AG32 | SH556 | 11 strong cen repeat |
| AG25 | SH376 | Yq strong repeat + Xp |

will not interfere with many earlier publications.

### MINK CHROMOSOME N-BANDS.

R-banded Mink chromosomes, restained with N-bands and set up to identify the two pairs of chromosome with N-bands nº 2 and 5, where nº 5 have the strongest band, see (Christensen *et al.* 1996). The N-band findings are in accordance with Mandahl *et al.* (1975). They found secondary constriction exactly at the same spots where we found the N-bands. The cosmid SH644 give very strong signal in both the N-band regions on chromosome 2 and 5. This cosmid also hybridize to the N-band regions in both cat and pigs.

## REFERENCES


***Christensen, K., S. Malchenko, K. Brusgaard and O. Lohi. 1996.*** The www server http=// www.husdyr.kvl.dk/mink.htm.

***Christensen, K. and J.O. Pedersen. 1990.*** Chromosome C-band polymorphism in relation to fertility in boars of the Duroc breed. *Hereditas* 113: 183-187.

***Blom, S.F. and C. Goodpasture. 1976.*** An improved technique for selective silver staining of nuclear organizer region in human chromosomes. *Hum. Genet.* 34: 199-206.

***Lemieux, N., B. Detrillaux and E. Viegas-Pequignot. 1992.*** A simple method for simultaneous R- or G-banding and florescence in situ hybridization of small single-copy genes. *Cytogenet. Cell Genet.* 59: 311-312.

***Mandahl, N. and K. Fredga. 1975.*** Q-, G- and C-band patterns of the mink chromosomes. *Hereditas* 81: 211-220.

***Nes, N. 1962.*** Chromosome Studies in Heggedal and Standard Dark Mink. *Acta. Vet. Scand.* 3: 275-294.

***Sambrook, J., E.F. Fritsch and T. Maniatis. 1989.*** Molecular cloning. Cold Spring Harbor Laboratory Press.

***Serov, O.L., A.A. Gradov, N.B. Rubtsov, N.S. Zhdanova, S.D. Pack, M.A. Sukoyan, M.R. Mullakandov and S.M. Zakijan. 1987.*** Genetic Map of the American Mink: Gene Conservation and Organization of Chromosomes. Isozymes: *Current Topics in Biological and Medical Research*, eds. C.Markert and J.G. Scandalios, 15: 179-215.

***Serov, O.L. and S.D. Pack. 1993.*** Genetic map of the American mink (*Mustella vison*). In Genetic Maps, ed. S.J.O'Brian. pp 4.279-4.281.