Supplemental data

Material and methods

1. Preparation of chromosomes

Used reagents:

1. ***Distilled water:*** Seralpur DELTA (Seral) produced ultrapure water (deionised, organically pure, no particles), autoclaved in addition
2. ***RPMI 1640 (growth medium):*** RPMI 1640 plus glutamax: L-alanine and L-glutamine (Gibco), fetal calve serum 12%, penicillin (100,000 U/ml) / streptomycin (10,000 U/ml) 1%
3. ***Phytohemagglutinine (PHA):*** Dissolve lyophilized PHA in 5 mL aqua ad injectabilia, aliquot it and store at -20°C
4. ***Hypotone potassium chloride solution:*** KCl 0.56% in distilled water
5. ***Fixation solution:*** Methanol : Glacial acetic acid = 3 + 1
6. ***Colcemid solution:*** Stock solution (10 µg/mL)

1.1 Chromosome preparation from blood

10 mL of medium RPMI 1640 and 100 µL of phytohemagglutinine (PHA) was given into a sterile culture bottle in order to culture lymphocytes from blood. PHA which comes from *Phaseolus vulgaris* stimulates T-lymphocytes specifically whereas there is no significant effect on B-cells. To each bottle 1 mL of heparinized blood was added followed by culturing at 37°C (incubator) over 72 h. 1 h and 30 min before the end of culturing 100 µL colcemid solution was added to each bottle. Colcemid comes from *Colchium autumnale* and prevents the formation of the spindle apparatus in the cell. This way all nuclei that were within the mitosis at that time get into a metaphase arrest.

For harvesting culture bottles were carefully shaken until all cells were in suspension. The suspension was transferred into 14 mL falcon tubes and then centrifuged at 1,500 rpm. The supernatant was carefully removed (water-jet vacuum pump). 10 mL of hypotone potassium chloride solution was heated up to 37°C and added to each falcon tube. The pellet was resuspended by heavy shaking and then incubated at 37°C for 20 min. To hemolyze all erythrocytes 1 mL of cold (4°C) fixation solution was added and then shaken again. After centrifuging again at 1,500 rpm for 5 min the supernatant, which contained the hemolyzed erythrocytes, was removed. The pellet was resuspended again in 10 mL of cold fixation solution and centrifuged at 1,500 rpm for 5 min. This procedure was repeated twice. The suspension was then either stored in the fixation solution at -20°C for 30 min or in the fridge overnight for complete denaturing of proteins. After another centrifugation the supernatant was removed. The sediment was resuspended in about 1 mL of fixation solution (depending on its density). The suspension can be further diluted with fixation solution later on if the density of the suspension is too high. The final suspension was stored in 1.5 mL Eppendorf tubes at -20°C.

This storage handling reduces the DNA damaging action of the fixation solution (depurinization by glacial acetic acid leads to DNA breaking).

1.2 Preparation of chromosomes from cell lines derived from primates

For culturing cell lines of primates (EBV immortalized lymphoblastoid cell cultures) 10 mL aliquots of RPMI 1640 medium were given into sterile culture bottles. Already grown cells were added. Optimally, cells should be incubated at 37°C with 5% CO_2 fumigation over 5 days. Subsequent preparation was performed as described in chapter 1.1.

2. Preparation of object slides for conventional cytogenetics and FISH

Object slides were cleaned using tissue paper. Alternatively, object slides which were stored in a methanol-salt suspension were used. The clean slides were cooled down in distilled water at 4°C in the fridge. One wet chamber was preheated at about 60°C in order to generate a micro climate with higher air moisture. Slides were put at an angle into the wet chamber. After adding the suspension to the slides and slight drying fixation solution was added for rinsing plasma rests and further fixation of the cells. By evaporation of the methanol, glacial acetic acid can develop its hygroscopic effects, which draws water from the atmosphere resulting in a chromosomal swelling and visualization.

For conventional cytogenetic methods object slides were either stored in a drying closet overnight or alternatively stored at 90°C for 1 h.

Object slides for molecular cytogenetic methods were dehydrated in an ascending ethanol series (70%, 95%, 100% ethanol) at first. Slides were kept for a week at room temperature (RT) and then stored further at -20°C.

3. Microdissection of chromosomes

In this work the microdissection procedure after Rubtsov and coworkers (1996; performed by Dr. Trifonov) was used for extraction of interstitial, terminal, and paracentric heterochromatin derived from the species *Pan*. For a better differentiation of heterochromatic regions microdissection was performed using GTG banded chromosomes. In order to avoid contamination all steps were performed under sterile conditions.

Used reagents and processing:

1. ***Trypsin solution:*** Stock solution (5%): Dissolve one ampulla bacto-trypsin (lyophilised) in a*qua ad iniectabilia*; sterile filtration, aliquoting, storing at -20°C. Trypsin solution for general use: 100 µL stock solution + 35 mL phosphate buffer (pH 6.88) in a sterile 50 mL plastic tube
2. ***Giemsa solution:*** Mix 35 mL phosphate buffer (pH 6.88) in a sterile 50 mL plastic tube with 3 mL of sterile Giemsa solution
3. ***Collection solution:*** 10 mM Tris/HCl (pH 7.5), 10 mM NaCl, and *aqua ad iniectabilia*. Irradiate solution with UV and autoclave. Then add 0.1% sodium dodecylsulfate (SDS), aliquote, irradiate with UV again and store at room temperature. Before use: Add 0.5 mg/mL (final conzentration) proteinase K solution, Add 50 µL glycerol
4. ***Production of glass needles:*** Glass needles that were used for collecting chromosome fragments were produced with an average diameter of 2 mm from 10 cm long, massive duran glass rods by a pipette pulling device (Schott Rohrglas GmbH). At level 1 the glass rod was pulled apart under maximal heating temperature. At level 2 the thin part

of the glass rod was ripped off at low heating temperature and the use of additional weights. This way both halves can be used as needles for collecting. Before use the needles were irradiated with UV light.

5. ***Preparation of cover slides:*** Cover slides (60x24 mm) were degreased in 10% SDS solution for a couple of days.
6. ***Preparation of metaphase plates:*** Rinse prepared cover slides with sterile distilled water, put 1-2 drops of cell suspension (depending on cell density) onto wet cover slides, and carefully add fresh fixation solution, air drying
7. ***GTG banding:*** Cover slide in phosphate buffer (pH 6.88) for 1 min, trypsinization for 40-60 s (allow longer time for plasma-rich cultures), staining in Giemsa solution for 2-3 min, rinse with distilled water, air drying

Microdissection was performed using an inverting microscope (Axiovert-135 oder Axiovert-10, Zeiss). Lenses with the following maximization were used: 10x (Plan-Neofluar) and 100x (Plan-Neofluar, oil immersion). Electronically controlled micromanipulators were attached to the microscope stand. Those devices enabled very slow movements into all three directions.

A retractable pencil functioned as a needle holder for a sterile glass needle. The pencil was placed at the right side. The glass needle was centered with the 10x lens at the microscope. At this maximization the adjustment of the object level was performed. A suitable metaphase was identified on the cover slide and adjusted within the middle of the visible area using the 100x lens.

A rotating object table enabled taking the chromosome of interest to the desired position with regards to the needle. After that the needle was manually moved down onto the metaphase using a joystick. The needle was moved down until that point, were the needle touched the cover slide right next to the desired cutting site. For cutting the appropriate fragment out of the chromosome the needle was moved further down so that a forwarding movement was obtained.

Once the fragment was attached to the needle, the needle was moved upward using the joystick first and then using the microscope mechanics. The “loaded” needle was carefully put into a 0.5 mL Eppendorf tube containing the collection solution. Then the tip of the needle was broken off. This procedure was repeated over and over again until an appropriate amount of fragments were collected (about 5 fragments).

After the collection of fragments a protein digestion was performed using proteinase K in the collection solution (incubation at 60°C for 2 h in a PCR machine). After that the DNA library was transferred into the prepared first PCR solution (see chapter 2.4.3.1).

4. Molecular genetic techniques

4.1 DOP-PCR

DOP-PCR (=polymerase chain reaction with degenerated oligonucleotide primers) enables the amplification of DNA of unknown sequence or of the entire genome. This specific PCR works with unspecific primer sequences (22 bp oligonucleotide primers with 6 undefined, central bases flanked by specific bases, which enable that primers bind to many sites of the used DNA together with the low annealing temperature (renaturing temperature).

All used solutions were produced using sterile pyrogen free *aqua ad iniectabilia*. Solutions must not contain nucleases or other DNA material because the use of DOP primers could result in an unspecific amplification which could screw up the results. For that reason it is recommended to aliquot the solutions and to perform PCRs under sterile conditions.

Used reagents:

1. ***DOP primer:*** Sequence: 5’ CCG ACT CGA GNN NNN NAT GTG G 3’
2. ***dNTPs:*** A general solution was produced using single nucleotide solutions. Single nucleotides (10 mM) were mixed in a relation of 1:1:1:1. The final concentration of each nucleotide was 2.5 mM.
3. ***T7 sequenase:*** T7 sequenase 2.0 DNA polymerase (13 U/µL; Amersham Inc.) was diluted in sequenase enzyme dilution buffer (Amersham Inc.; 1:8).
4. ***AmpliTaq DNA polymerase Stoffel fragment (10 U/µL; Perkin Elmer Inc.), 10x Stoffel fragment buffer, 25 mM magnesium chloride ($MgCl_2$) buffer***
5. ***Label mix:*** Using the nucleotide solutions a label mix for the labeling PCRs was produced (dATP, dCTP, dGTP at 2 mM each, plus 1 mM dTTP). For the missing one dTTP a modified nucleotide with a hapten or fluorochrome was built into the DNA during the PCR.
6. ***AmpliTaq DNA polymerase (5 U/µL, Perkin Elmer Inc.):*** For the labeling PCRs
7. *GeneAmp 10x PCR buffer II (Perkin Elmer; 100 mM Tris-HCl; pH 8.3; 500 mM KCl):* Buffer for AmpliTaq DNA polymerase

4.1.1 Amplification of microdissected chromosome fragments

Due to the low amount of initial DNA microdissected chromosome fragments 8 sequenase cycles were performed as low-temperature cycles. The low annealing temperature at the beginning enables a more common and more unspecific binding of the DOP primer. Then the DNA was amplified at high temperature to achieve a more specific amplification. The protocol used here was modified after Rubtsov and coworkers (1996). In order to avoid a DNA contamination PCR was performed in a PCR machine under a hood.

Solution A (low-temperature cycles)

	Stock solution	Final conc.	Per probe
Aqua ad			3.37 µL
Sequenase buffer	5x	0.6x	0.60 µL
DOP primer	40 µM	5.0 µM	0.63 µL
dNTPs	2,5 mM	200 µM	0.40 µL

Per microdissected fragment 5 µL were added to a 0.5 mL Eppendorf tube. After adding the collection drop of microdissected DNA PCR was started.

Solution B

	Stock solution	Final conc.	Per probe
Sequenase	13 U/µL	1.6 U/µL	0.25 µL
Sequenase dilution buffer			1.75 µL

Since sequenase is a thermo labile enzyme and gets inactivated during denaturing 0.25 µL of this solution was added after denaturing in each of the 8 cycles.

Solution C

	Stock solution	Final conc.	Per probe
Aqua ad			34.22 µL
Stoffel fragment buffer	10x	1x	5.00 µL
dNTPs	each 2.5 mM	220.0 µM	4.40 µL
DOP primer	40.0 µM	1.1 µM	1.38 µL
$MgCl_2$	25.0 mM	2.5 mM	5.00 µL

Per sample 45 µL of this solution was added to enlarge the volume of the sample. Subsequent high-temperature cycles were performed in a final volume of 57 µL per sample.

Solution D (high-temperature cycles)

	Stock solution	Final conc.	Per probe
Aqua ad			3.5 µL
Stoffel fragment buffer	1x	0.10 µL	0.5 µL
$MgCl_2$	25 mM	2.50 mM	0.5 µL
Stoffel taq polymerase	10 U/µL	1.0 U/µL	0.5 µL

Adding of 5 µL of solution D was done at last because primers were required to be denatured to prevent binding of degenerated sequences.

DOP-PCR process (modified after Rubtsov *et al*., 1996)

DOP-PCR was performed on a sterile work bench which was irradiated with UV light to get it DNA free. In total, 8 low-temperature PCR cycles (annealing temperature of 25°C) using thermo labile sequenase and 32 high-temperature PCR cycles (annealing temperature of 56°C) using Ampli Taq DNA polymerase Stoffel fragment was performed:

1. 92°C 5 min Initial denaturing
2. 25°C 2 min 20 s Adding of 0.25 µL of **solution B**; primer annealing
3. 34°C 2 min DNA elongation
4. 90°C 1 min

   Steps 2-4 were repeated 7x.
5. 30°C 2 min 20 s Adding of 45 µL of **solution C**
6. 92°C 1 min Denaturing
7. 56°C 2 min 20 s Adding of 5 µL of **solution D**, primer annealing
8. 70°C 2 min DNA elongation
9. 92°C 1 min Denaturing
10. 56°C 1 min Primer annealing
11. 72°C 2 min DNA elongation

    Steps 9-11 were repeated 31x.
12. 72°C 10 min Final DNA elongation
13. 4°C forever

To protect DNA from nucleases 1 µL EDTA was added per sample after DOP-PCR. Samples were stored at -20°C.

4.2 PCR reamplification

After a DOP-PCR another PCR reamplification was performed. This was necessary to get a maximum of DNA for subsequent experiments to avoid unnecessary DOP-PCRs. PCR reamplification was done using the DOP primer (see chapter 2.4.2.1.1) and was used for amplification of the microdissected DNA and the BAC DNA.

PCR

	Stock solution	Final conc.	Per probe
Aqua ad.			34.25 µL
Stoffel fragment buffer	10x	1x	5.00 µL
dNTPs	2.5 mM	0.2 mM	4.00 µL
DOP primer	40.0 µM	1.0 µM	1.25 µL
$MgCl_2$	25.0 mM	2.5 mM	5.00 µL
Stoffel taq polymerase	1 U/µL	0.1 U/µL	0.50 µL

0.5 µL of DOP-PCR products were used for each sample.

PCR processing

1. 92°C 3 min Initial DNA denaturing
2. 91°C 1 min DNA denaturing
3. 56°C 1 min Primer annealing
4. 70°C 2 min DNA elongation

   Steps 2-4 were repeated 29x.
5. 72°C 5 min
6. 4°C forever

To protect PCR products from nucleases 1 µL EDTA (5 mM final concentration) was added per sample after DOP-PCR. Samples were stored at -20°C.