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LMD APPLICATION NOTE

Comparative Genome Hybridisation on Single Intestinal Crypts

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Cryoconserved tissue from the small intestine of mice was embedded in OCT Tissue Tek and frozen sections of 12 µm using a cryostat were prepared. These were mounted on PEN slides suitable for laser microdissection. The tissue was fixed in 75% Ethanol for 2 minutes at -20°C and nuclei were stained using 1% (w/v) cresyl violet diluted in 100% Ethanol. Staining was done for exactly one minute followed by washing and dehydration of the section in 75%, 95% and 100% Ethanol, four seconds each, and a final fixation in 100% Ethanol pre-treated with molecular sieve for one min. Consecutively stained sections were dried on silica gel for at least 30 minutes. Single intestinal crypts were identified, laser micro-dissected using a Leica LMD6500 and collected in the cap of a 0.2 ml tube. It was verified that single micro-dissected crypt could be detected in the cap. The addition of 9 µl of nuclease free water to the cap ensured that the crypt would remain in the cap, and the tube was gentle centrifuged for one minute to spin the crypt containing drop to the bottom of the tube.

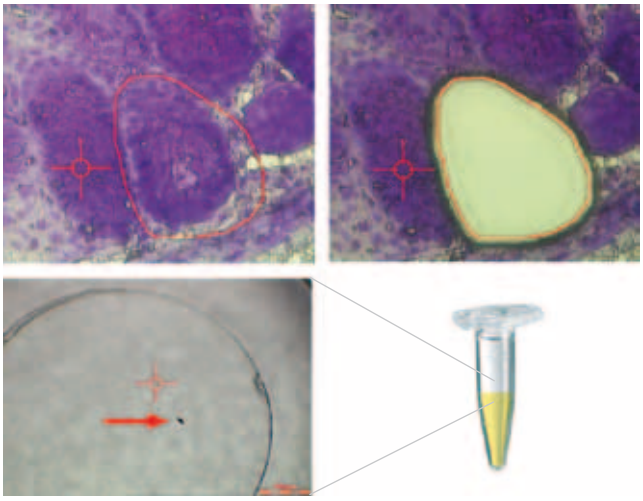

Fig.1: The upper part display the intestinal epithelium stained with cresyl violet. Crypts were selected for laser microdissection (red shape) and cut. The lower part on the left side shows a representative picture taken in the cap control modus. The red arrow points to the dissected crypt in the cap of the collection tube.

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p53 deletion impairs clearance of chromosomal-instable stem cells in aging telomere-dysfunctional mice
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The amplification of the genome was carried out using the GenomePlex Single Cell Whole Amplification Kit (Sigma-Aldrich, #WGA4) following the instructions of the manual provided with the kit. To purifiy the DNA the GenElute PCR clean up Kit (Sigma-Aldrich, #NA1020) was subsequently used. To check for successful amplification the concentration of DNA was measured while the quality of the amplified DNA was determined by a multiplex PCR for GAPDH. ArrayCGH of single crypts requires not only the amplification of DNA but the obtained DNA fragments have to be more than 300bp to allow an efficient labelling and hybridisation to the probes spotted on the array. The primer pairs of the multiplex PCR are designed in a way such that a 100, 200, 300 and 400bp product can be obtained. Amplified samples were used for the multiplex PCR and only samples yielding all the four PCR products were further used for the arrayCGH.

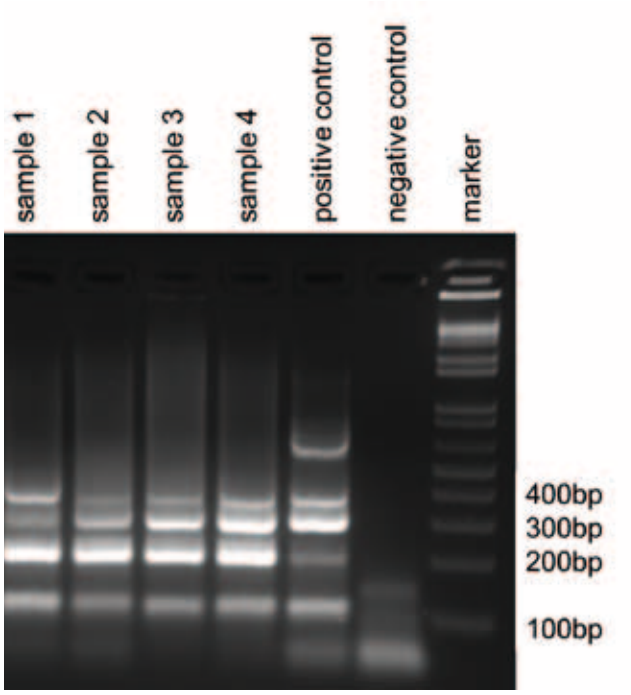

Fig.2: GAPDH PCR for quality check. All samples show bands of 100, 200, 300 and 400bp

For the arrayCGH the microarray platform from Agilent and the mouse 4x44K Microarrays were used. The array consists of 43000 60mer oligos spotted on a glass slide. This results in a maximum resolution of 22kb. As reference DNA we used male DNA obtained from a DNA extraction of a kidney biopsy. 500ng of DNA were labelled using the Bioprime CGH Genomic Labeling" System (Invitrogen, #18095-12) and hybridized following the instruction manual. Hybridized arrays were scanned and analyzed using the CGH Analytics 3.4.40 program. Genomic changes were called by using the Adm-2 statistical algorithm with a stringency setup of 10 points and threshold of 4.

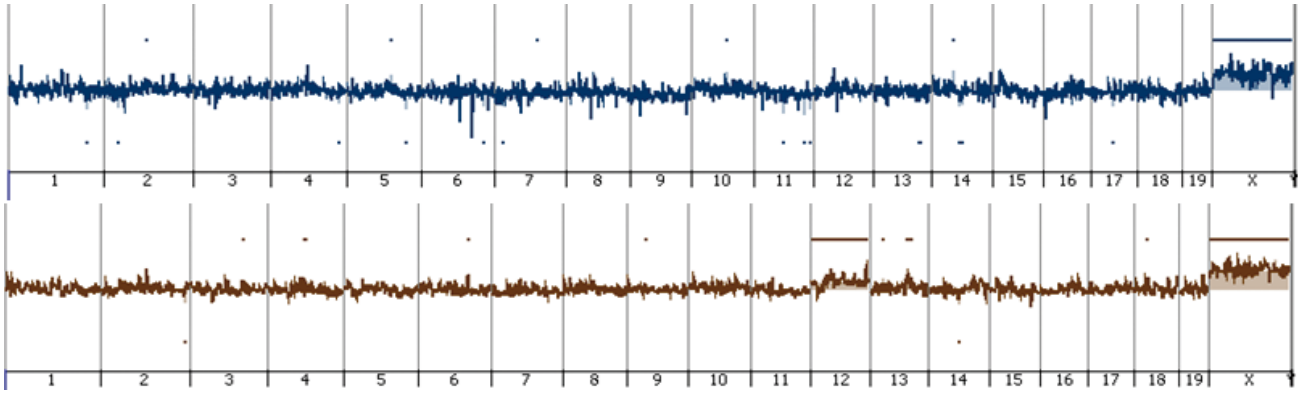

Fig.3: Representative whole genome arrayCGH profiles obtained from a single crypt. While the first profile is genomic stable in the second profile an amplification of chromosome 12 can be detected.

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