

## TUMOR INOCULATION BETWEEN TWO UNRELATED HUMAN INDIVIDUALS: STR ANALYSIS OF PARAFFIN-EMBEDDED TISSUE SECTION

C. Luckenbach\*, V.H. Gärtner\*\*, C. Seidl\*\*\*, H. Ritter\*

\* Inst. f. Anthropologie und Humangenetik, Wilhelmstr. 27, 72074 Tübingen, FRG

\*\* Inst. f. Pathologie, Liebermeisterstr.8, Wilhelmstr. 27, 72074 Tübingen, FRG

\*\*\*Blutspendedienst DRK, Sandhofstr.1, 60528 Frankfurt, FRG

### 1. Introduction

In this study we used the STR (short tandem repeat) technology to check the possibility of tumor inoculation between two unrelated individuals, first described by Gärtner et al. (1995).

Case report: A surgeon injured his hand with a sharp instrument during a resection of an intestinal soft tissue tumor (tumor I). After one year a tumor (tumor II) was detected in his hurt hand. Histological and morphological analysis revealed these two tumors (I and II) as identical.

Only paraffin-embedded tissue of these tumors was available to perform DNA-profiling. We succeeded in DNA extracting from these tissues, but the material was unsuitable for molecular techniques which require high-molecular-weight genomic DNA. Although significant DNA degradation was observed, three STR loci could be detected: CYP 19 (15q21.1), TH01 (11p15.5), SE 33 (ACTBP2).

We present the DNA extracting protocol for paraffin embedded tissue, the STR analysis method and the exceptional findings.

### 2. Materials and Methods

#### 2.1. Samples

##### Tumor tissue

Paraffin embedded tumor tissue from autopsies or biopsies were obtained from the files of the Department of Pathology of the University of Tübingen, FRG.

Samples measuring approximately 5 x 5 x 3 mm were fixed by immersion in 10% buffered formalin, were processed into paraffin blocks and stored at ambient temperature.

##### EDTA-Blood

DNA was prepared from EDTA-blood of the surgeon according to the protocol of Miller et al. (1988)

#### 2.2. Histological Examination

Gärtner et al. (1995)

#### 2.3. DNA Extraction

- according to Goelz et al. (1985) with modifications
- paraffin was cut away before finely mincing the tumor tissue
- suspension in TE-buffer (500mM Tris, 20mM EDTA, 10mM NaCl, pH 9.2)
- homogenization in a 50 ml falcon tube with 10 ml of TE-buffer containing 10% SDS and 0.5% Proteinase K, incubation for 30 hrs at 50 °C
- addition of 1.5 ml 0.5% Proteinase K and 1.5 ml 10% SDS
- vortex with high speed 4 min., incubation for 50 hrs at 50 °C
- DNA extraction once with 1 volume of phenol; three times with 1 volume of phenol-chloroform ( 3 parts phenol, 2 parts TE-buffer, 4 parts chloroform); once with 1 volume of chloroform
- addition of ammonium acetate (0.3 volumes of a 10M solution)
- DNA precipitation by 3 volumes of cold ethanol, incubation for 12-24 hrs at -70 °C, centrifugation 9500 xg for 30 min.
- resuspension of the pellet in TE-buffer (10 mM Tris, 1mM EDTA, pH 8)

## 2.4. PCR and STR-Characterisation

All oligonucleotides were commercially synthesized and 5'primers were labeled with fluorescence dye markers (Applied Biosystems (ABI), Weiterstadt, FRG) according to the allelic size distribution of the different STR-systems. PCR amplification was performed in a 9600 DNA thermal cycler system (Perkin Elmer, Weiterstadt, FRG) using 100 ng genomic DNA, 25 pmol of each primer 200 nmol dNTPs, 1x PCR buffer and 0.5 units Taq polymerase in a final volume of 50 ul.

### HumCYP19:

- name: Human aromatase gene at the **CYP19** locus, Polymeropoulos et al. (1992)
- location: 15q21.1
- repeat: TTTA
- primer (5'-3'): P5 GCAGGTACTTAGTTAGCTAC, P3 TTACAGTGAGCCAAGGTCGT
- cycle conditions: 95 °C 5 min., 95 °C 60 sec (35 cycles), 54 °C 30 sec., 72 °C 60 sec, final extension 72 °C, 5 min.

### HumACTBP2:

- name: Human cytoplasmic beta actin related pseudogene (**SE 33**) Polymeropoulos et al. (1992)
- location: chromosome 6
- repeat: AAAG
- primer(5'-3'): P5 AATCTGGGCGACAAGAGTGA, P3 ACATCTCCCCTACCGTATA
- cycle conditions: 95 °C 5 min., 95 °C 45 sec (35 cycles), 54 °C 30 sec., 72 °C 60 sec, final extension 72 °C, 5 min.

### HumTH01:

- name: Human tyrosine hydroxylase gene, intron 1 (**TC11**), Polymeropoulos et al. (1991)
- location: 11p15.5
- repeat: TCAT
- primer (5'-3'): P5 GTGGGCTGAAAAGCTCCCGATTAT,  
P3 GTGATTCCCATTGGCCTGTCCTC
- labelling: HEX (6-carboxy-2',4',7',4',7'-Hexachlorofluorescin):
- cycle conditions: 95 °C 5 min., 95 °C 60 sec (35 cycles), 54 °C 30 sec., 72 °C 60 sec, final extension 72 °C, 5 min.

## 2.5. Allele Size Analysis:

Electrophoresis: automated DNA sequencer model 373A (ABI), 6% polyacrylamide sequencing gel, 30 W for 8 hrs

Internal standard: generated by amplification using various primer combinations from the vector pGL2-basic (Promega, Heidelberg, FRG), 6 ul

Samples: 2.5 ul of each amplification product, heat denatured

The relative position of fragments are determined by measuring the laserlight induced fluorescence emission. Fragment sizes are calculated automatically with the Genescan 672 software (ABI) using the local reciprocal method described by Southern (1979).

## 3. Results and Discussion

We have tried to determine whether DNA can be prepared from tissue which has been fixed and embedded for routine histopathological examination. Our findings confirm that it is possible to extract DNA from such specimens, but the revealed DNA is not completely intact. The isolated DNA was unsuitable for molecular techniques which require high molecular weight genomic DNA. Although significant DNA degradation was observed, three STR-loci could be detected: CYP19, SE33 and TC11. Because of their small sizes (< 300 bp) STRs are more likely to be successful on old or degraded material.

Figure 1 shows the electropherogram from a 6% denaturing polyacrylamide gel concerning the STR-locus TC11 of three different samples: tumor tissue of the surgeon (lane 13), blood sample of the surgeon (lane 14), tumor tissue of the patient (lane 15). Peaks represent fluorescent intensities of dye-labeled TC11-alleles.

Table 1 summarizes all results of Fig.1 indicating the peak height (measured against an arbitrary scale displayed on the y-axis) and the calculated sizes of the different alleles (shown along the x-axis). Table 2 shows the detected alleles from the analysed samples at the STR-loci CYP19, SE33 and TC11.

Table 1: Summary of the calculated TC11 allele sizes and peak height

Peak	Lane	Allele size	Peak height
1	13	157.68	479
2	13	165.85	474
3	13	173.03	286
1	14	157.68	470
2	14	172.96	487
1	15	165.84	1507
2	15	173.03	344

Table 2: Alleles of the three samples at CYP19, SE33 and TC11

STR-locus	Surgeons tumor	Surgeons blood	Patients tumor
CYP19	168 bp	168 bp	168 bp
	187 bp		187 bp
SE33	252 bp	300 bp	252 bp
	277 bp		277 bp
	300 bp		
TC11	158 bp	158 bp	166 bp
	166 bp	173 bp	173 bp
	173 bp		

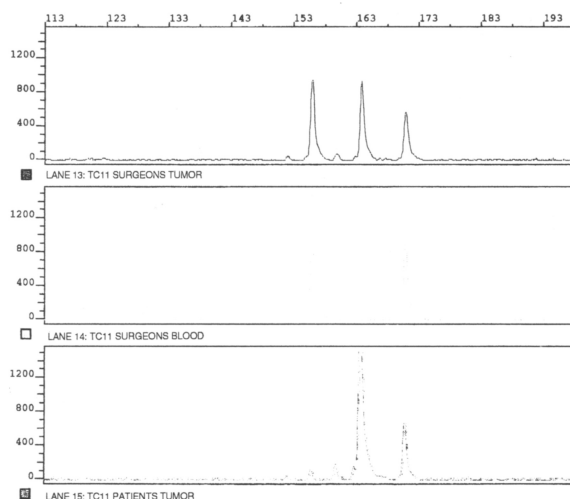


Fig. 1: Electropherogram from a 6% denaturing polyacrylamide gel at the TC11-locus; lane 13: tumor tissue (surgeon); lane 14: blood sample (surgeon); lane 15: tumor tissue (patient). Peaks represent fluorescent intensities of dye-labeled TC11-alleles.

The comparison of the surgeons blood sample with the tissue sections of his tumor (tumor II) and the tumor of the patient (tumor I) gave exceptional findings: tumor II showed at each STR locus all alleles from tumor I. These results point at the chance of tumor inoculation between humans, a new exciting finding in cancer research. Thus we demonstrate the bridge between molecular genetics and classical histology meaning that the world-wide collection of archival paraffin-embedded tissues may be used to study rare cancers and genetic changes which occur in human tumors.

## References

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