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Brazil

Biological Metrology Programm

Development of a Certified Reference Material for DNA

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The aim: To precisely identify individuals through molecular markers.

To study different DNA polymorphism detected through techniques based in eletrophoresis

RFLP – Restriction Amplified Length Polymorphism

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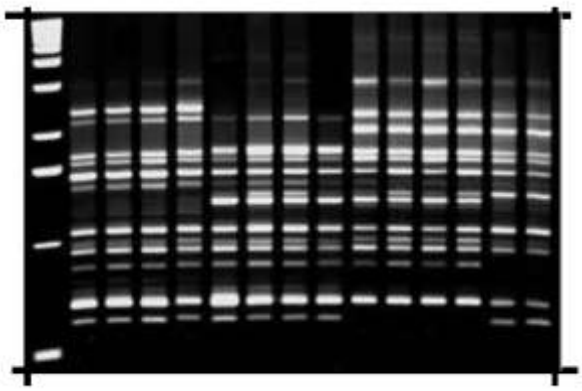
RAPD – Random Amplified Polymorphic DNA

- Caractherized by band ausence or presence



VNTR (variable number of tandem repeats)
- Minisatelites: 500-1000 bp with repetition pattern of 5-35 bp

STR (Short tandem repeats)
- Microsatelites: <200 bp with repetition pattern of 2-7 nucleotides

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Our approach: to use a DNA molecule as a MRC in Biometrology using the DNA length measurement certified by an (calibrated) AFM.

The DNA molecule length can be precisely estimated by knowing the base pair number, once each base pair has about 0.34 nm.

1) To determine the DNA molecule length based on AFM measurements.

2) To determine the AFM measurement uncertainty.

3) To certified the DNA molecule length measurement based on AFM as a MRC in Biometrology following ABNT ISO/IEC 17025:2005 and ISO GUIDE 34.

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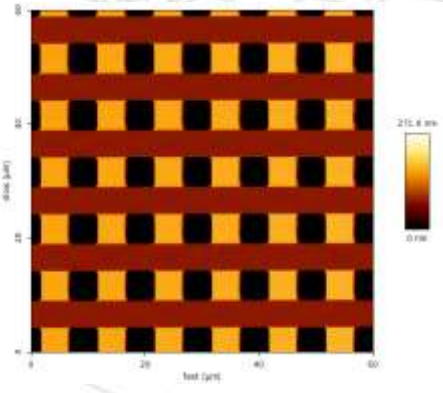
To determine the AFM measurements traceability:
-Traceability – NIST: calibrated AFM;
-AFM uncertainty measurements in x, y and z;



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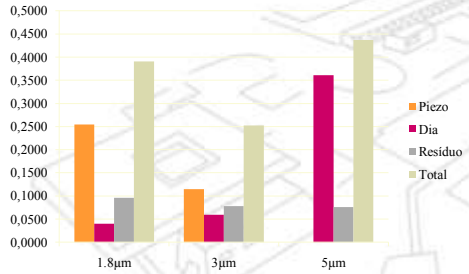
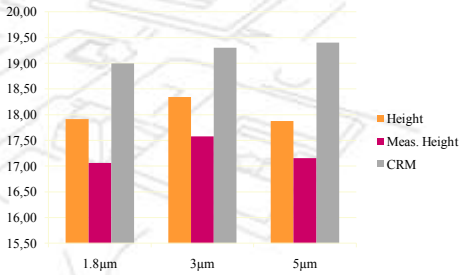
Uncertainty determination x, y and z: STS2

Variance



Mean



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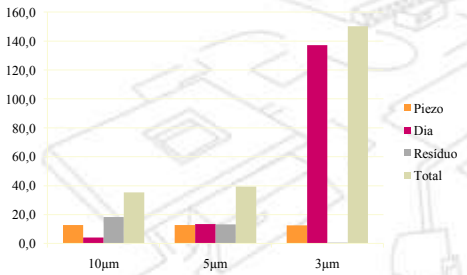
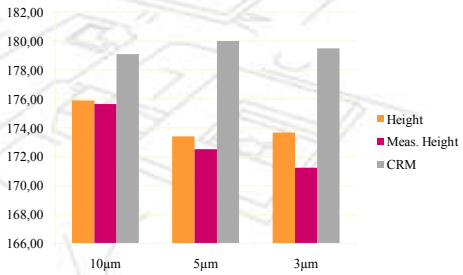
Uncertainty determination x, y and z: STS3

Variance



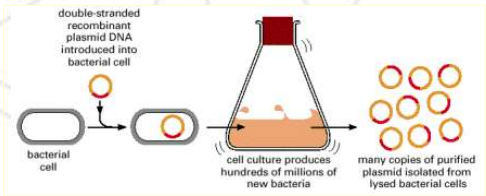
Mean



DNA production:

1ª step: Cloning of target DNA (~ 1kb) into a vector pGEM-T easy.

2ª step: Transformation in *E. coli* (strain DH5α)



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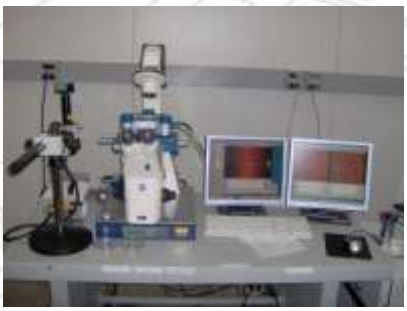
3ª step: Digestion with *Sal*I

4ª step: Purification of DNA from agarose gel.



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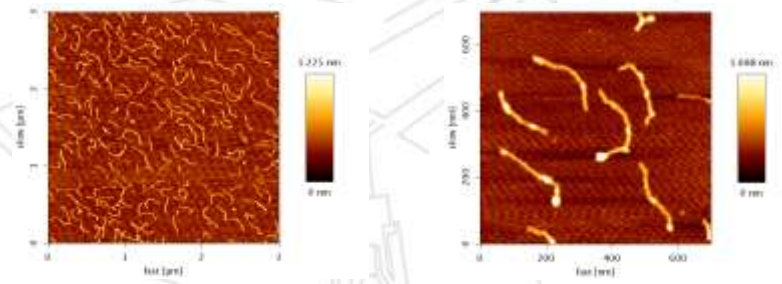
Homogeneous DNA distribution over mica surface



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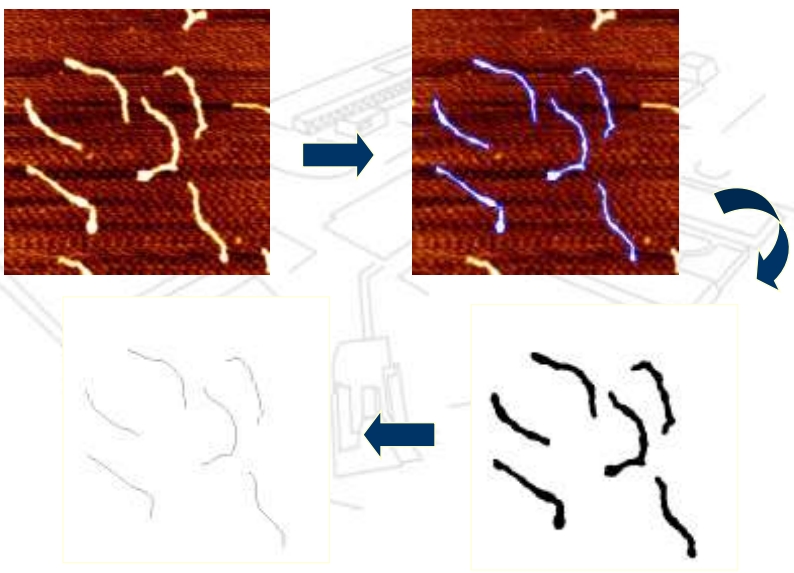

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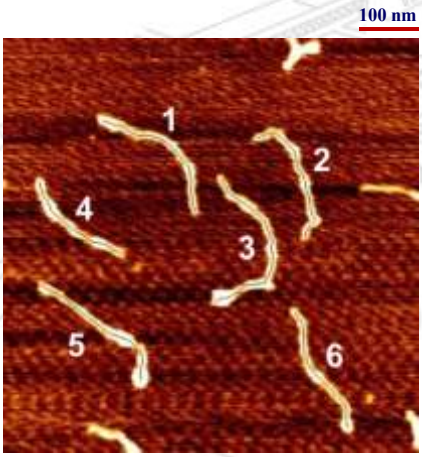
DNA fragments (896 bp)



Fragment	Length (nm)
1	264
2	250
3	316
4	202
5	263
6	230

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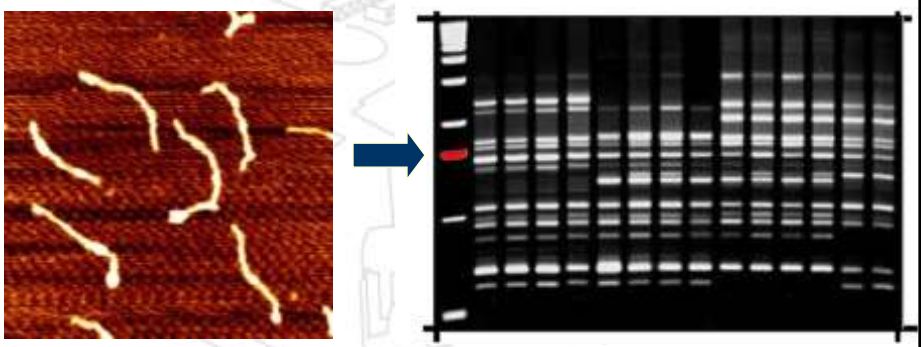
CRM for DNA: SI traceability in electrophoresis



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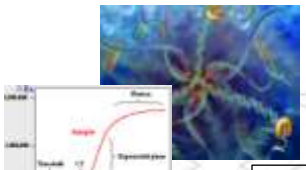
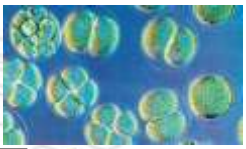
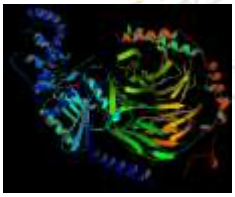
Traceability

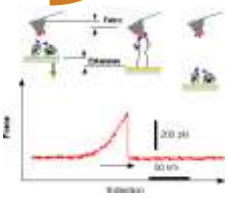




Property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through na unbroken chain of comparisons all having stated uncertainties.

VIM 2009





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