Analysis of high-LET radiation-induced HPRT mutations in mammalian cells

LABORATORY OF RADIATION BIOLOGY

Members

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Pavel Blaha

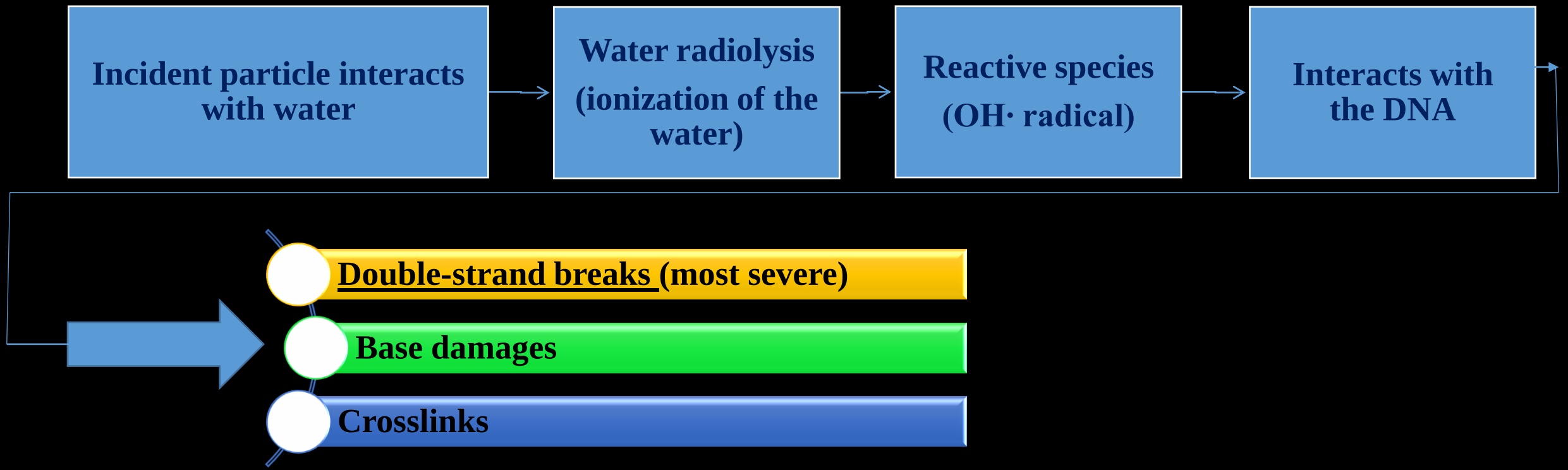
Group of Radiation Cytogenetics

Action of Ionizing Radiation on Living Cells

Ionizing Radiation (mainly) interacts with DNA molecule:

a) Directly

b) Indirectly (H_2O)



HPRT gene

(Chinese hamster cells – V79)

- Location: on the X chromosome
- Number of exons: 9
- Role: synthesis of purines

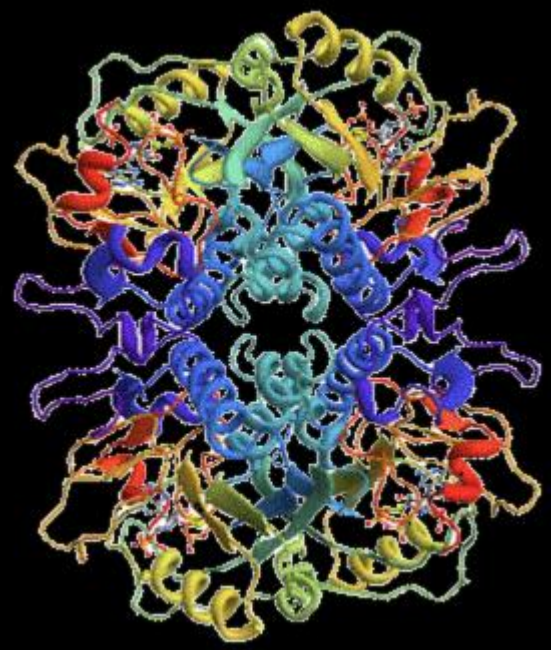
a) "de novo" pathway

b) Salvage pathway

- Consequences of malfunction:

a) Kelley-Seegmiller syndrome

b) Lesch-Nyhan disease



High LET vs. Low LET

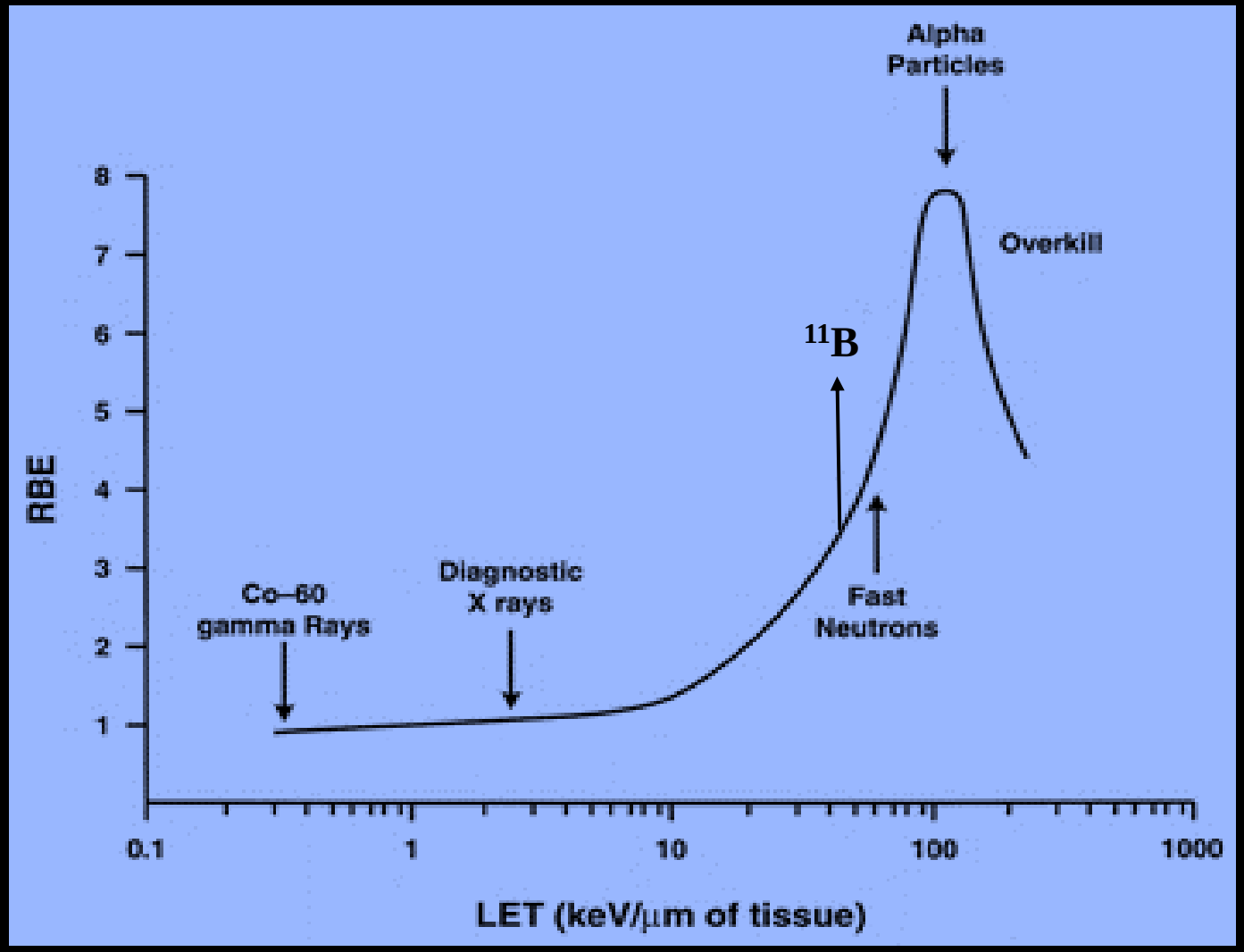
High LET

- For example: heavy charged particles
- More complex damage than low-LET
- Damage more difficult to repair - cluster damage

Low LET

- Gamma ray and X-ray – more penetrating

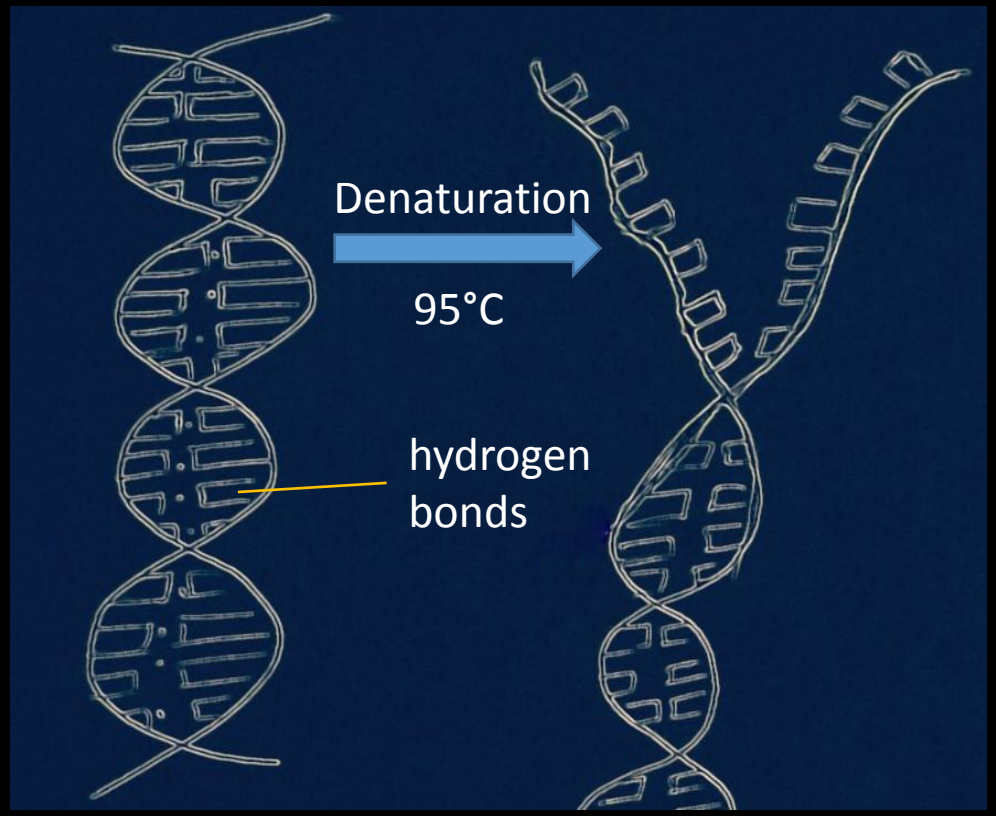
The Dependence of RBE on LET



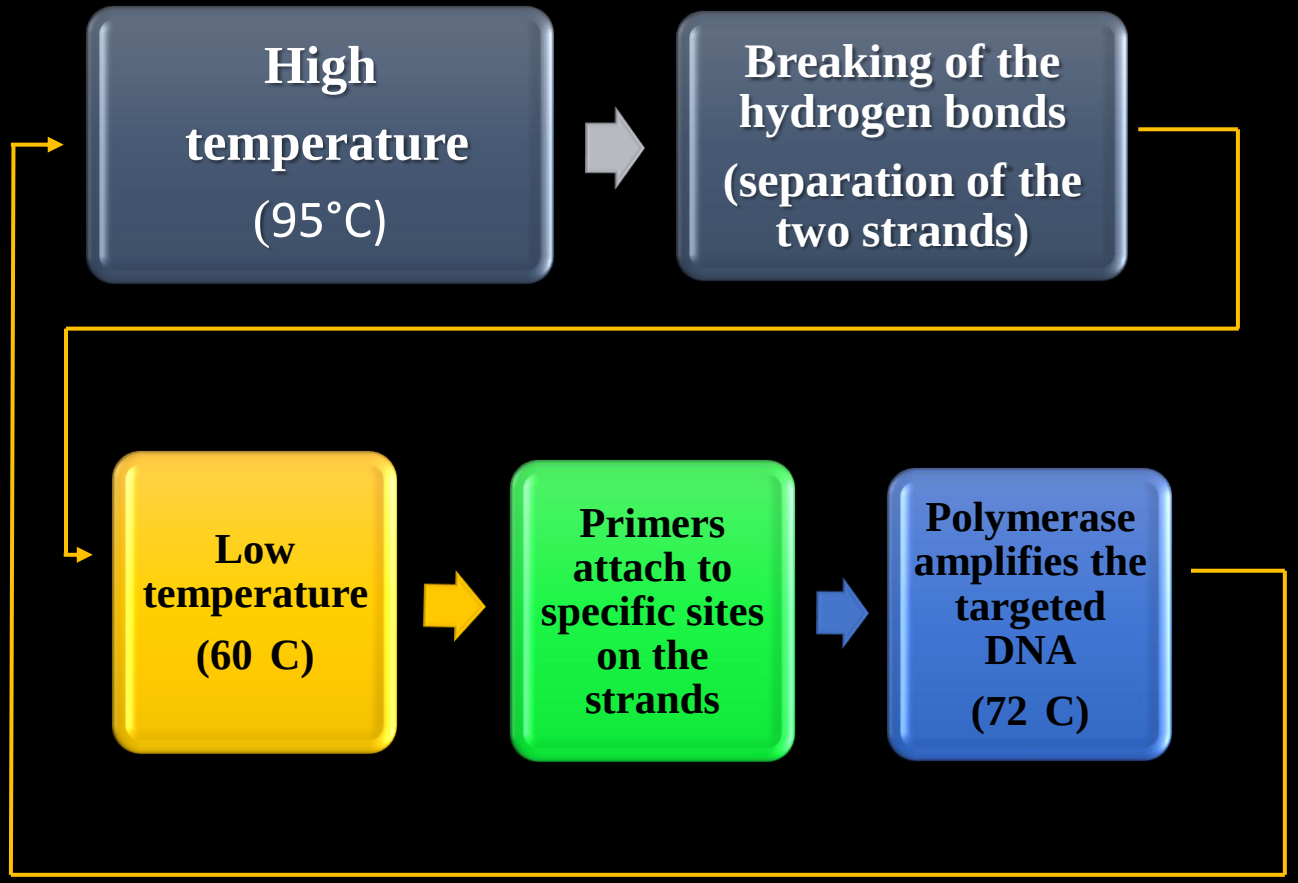
$$\text{RBE} = \frac{Dose\ B}{Dose\ A}$$

PCR (Polymerase Chain Reaction)

- replicates a specific segment of DNA
- based on thermal cycling (repeated **heating** and **cooling**)



Melting = breaking of the hydrogen bonds



STEPS

1. **Preparing the samples**

a) 2 types of primers (+ and – for each of the two strands) ;

b) PCR mixture (PCR mix, DNA sample, deionized water);

2. Putting the samples into the amplifier

3. Setting the right parameters in the software and starting the process

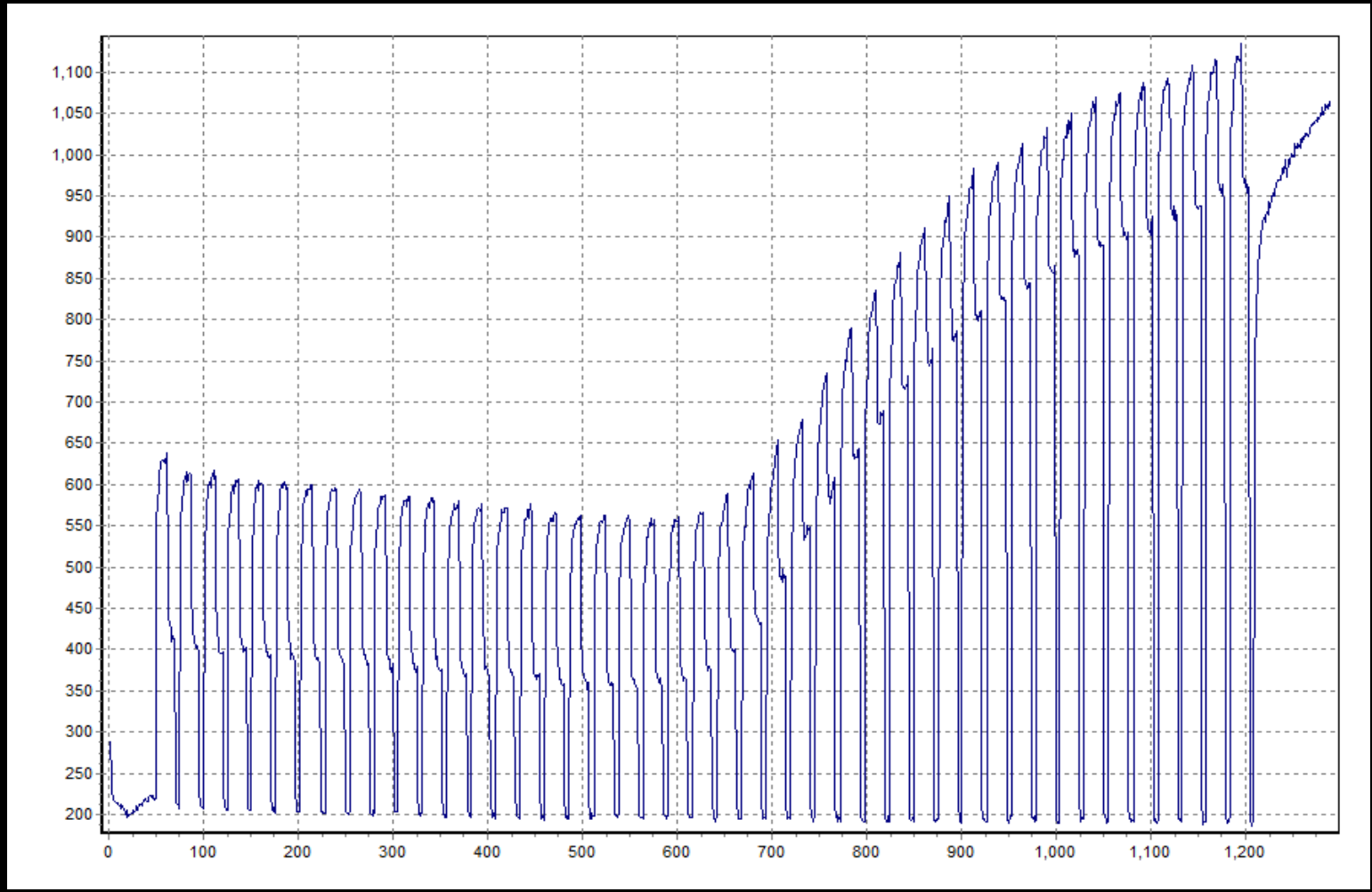
4. Starting the melting procedure

PCR Instruments

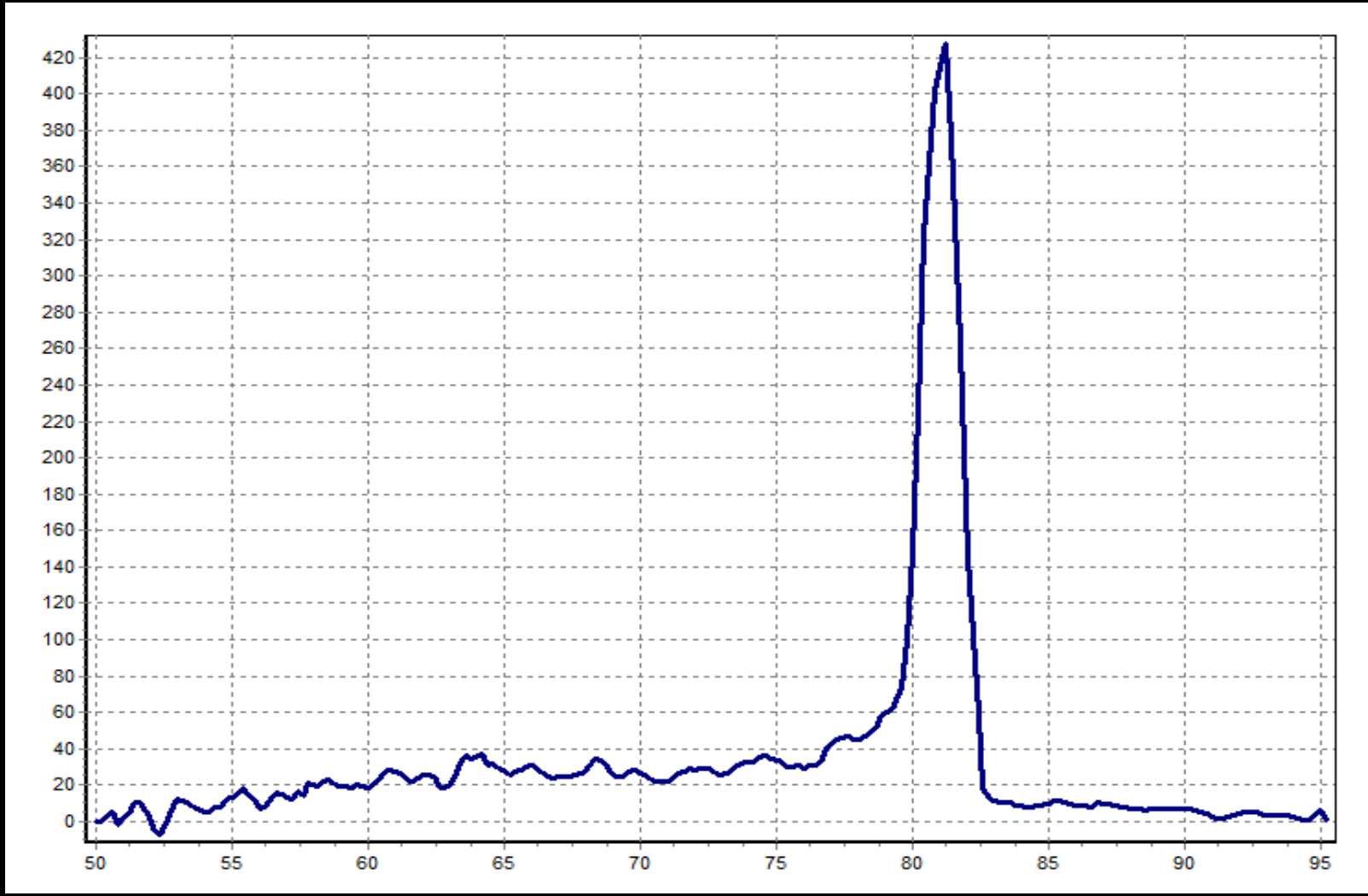
Sample preparation

Amplifier

PCR Results

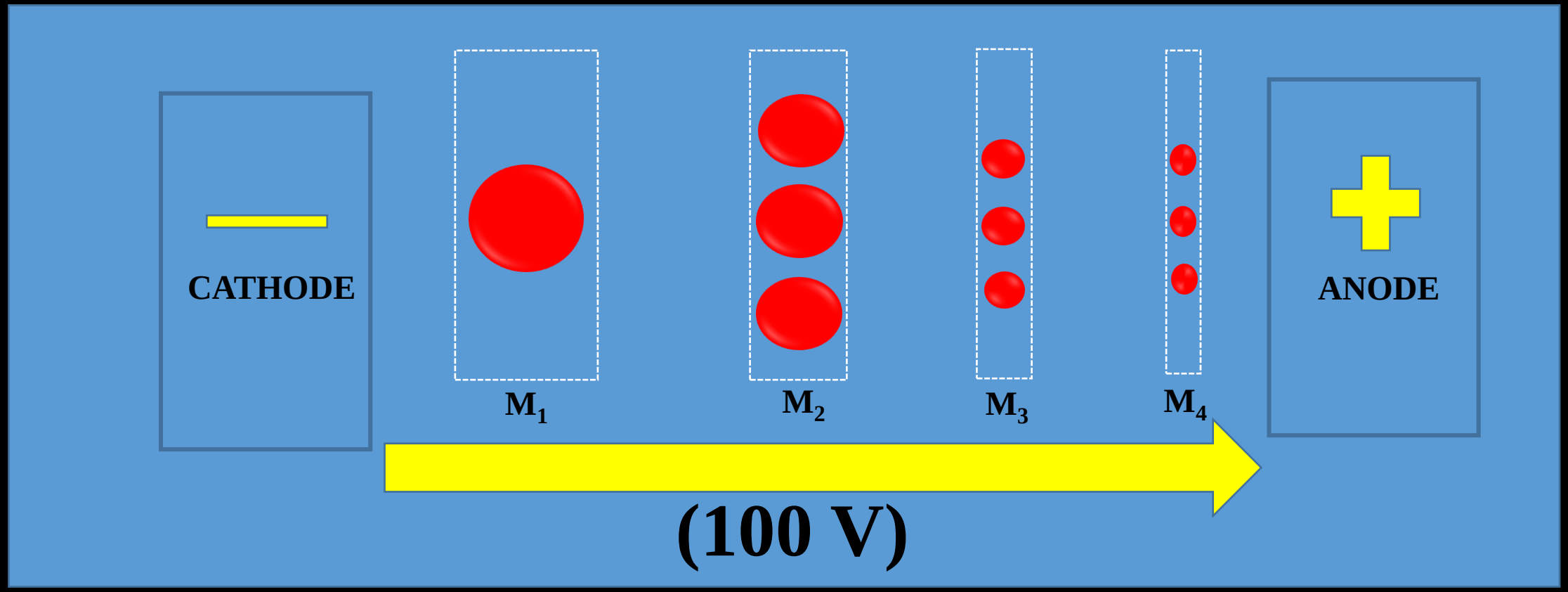

Fluorescent signal
1,100
1,050
1,000
950
900
850
800
750
700
650
600
550
500
450
400
350
300
250
200
0
100
200
300
400
500
600
700
800
900
1,000
1,100
1,200

PCR Results

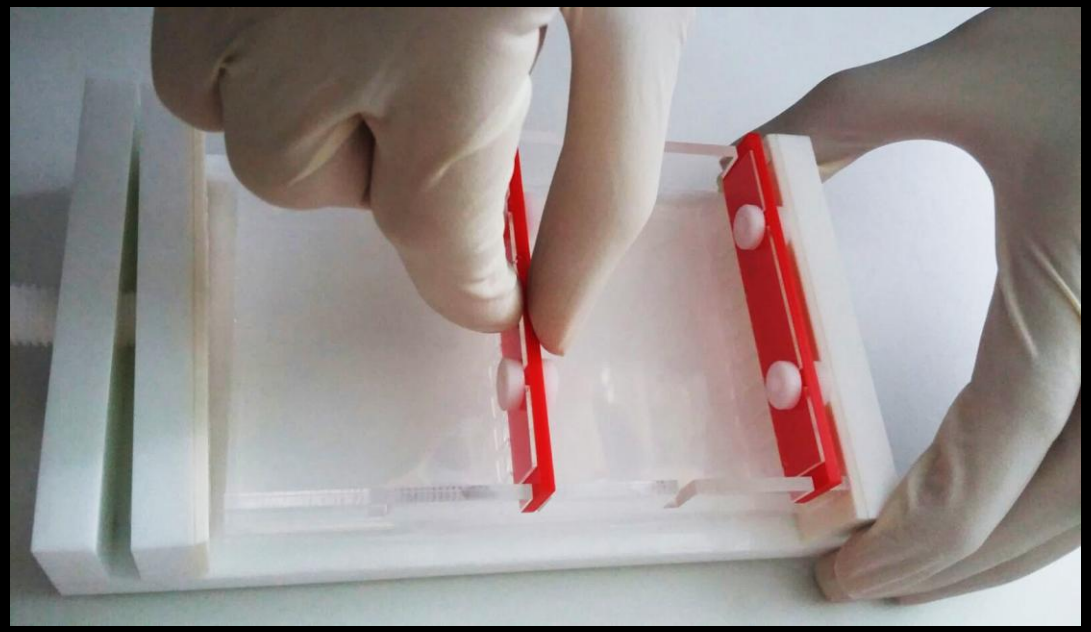

Fluorescent signal
420
400
380
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
50
55
60
65
70
75
80
85
90
95
Temperature (C)

GEL ELECTROPHORESIS

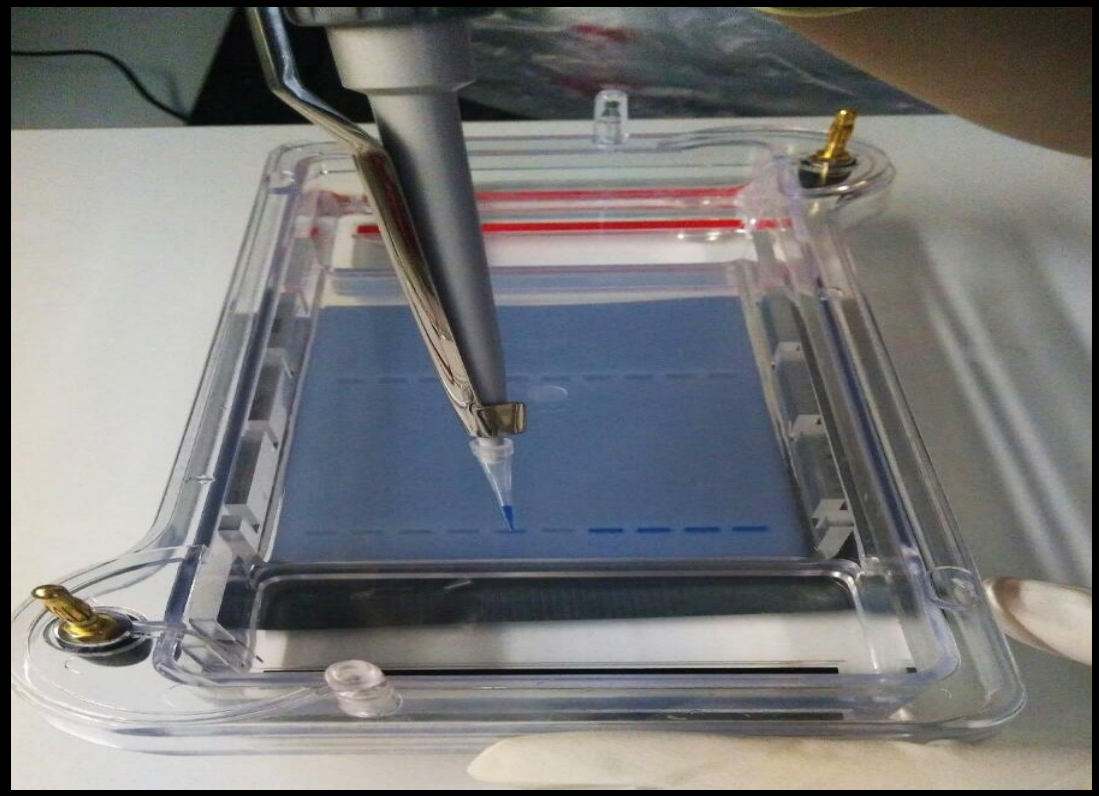
- Separates and analyzes DNA and its fragments, using q/m ratio;
- DNA molecule is negatively charged. Therefore, when applying an electric field:



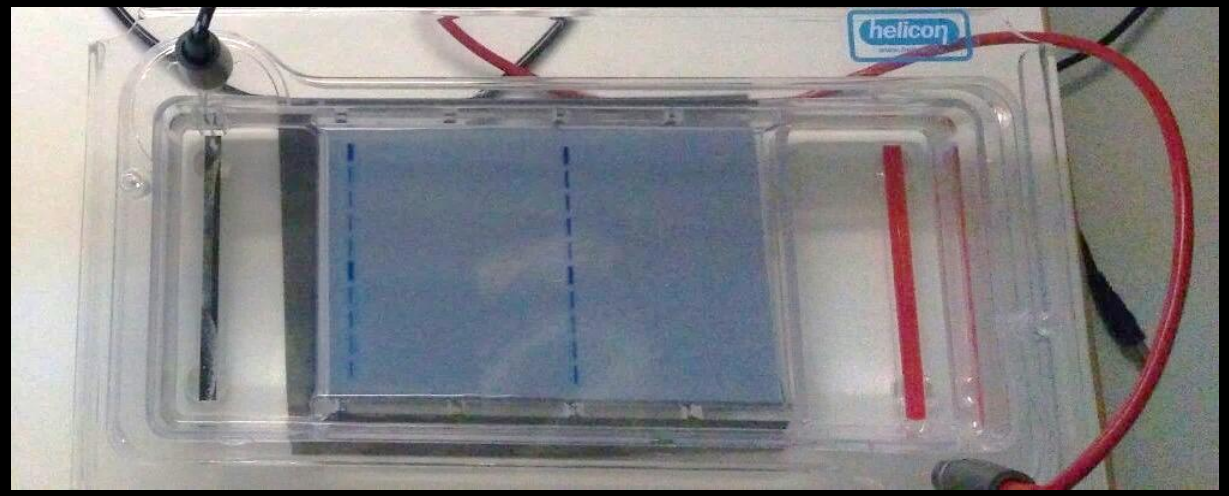
1. Gel preparation (agarose, TAE buffer)

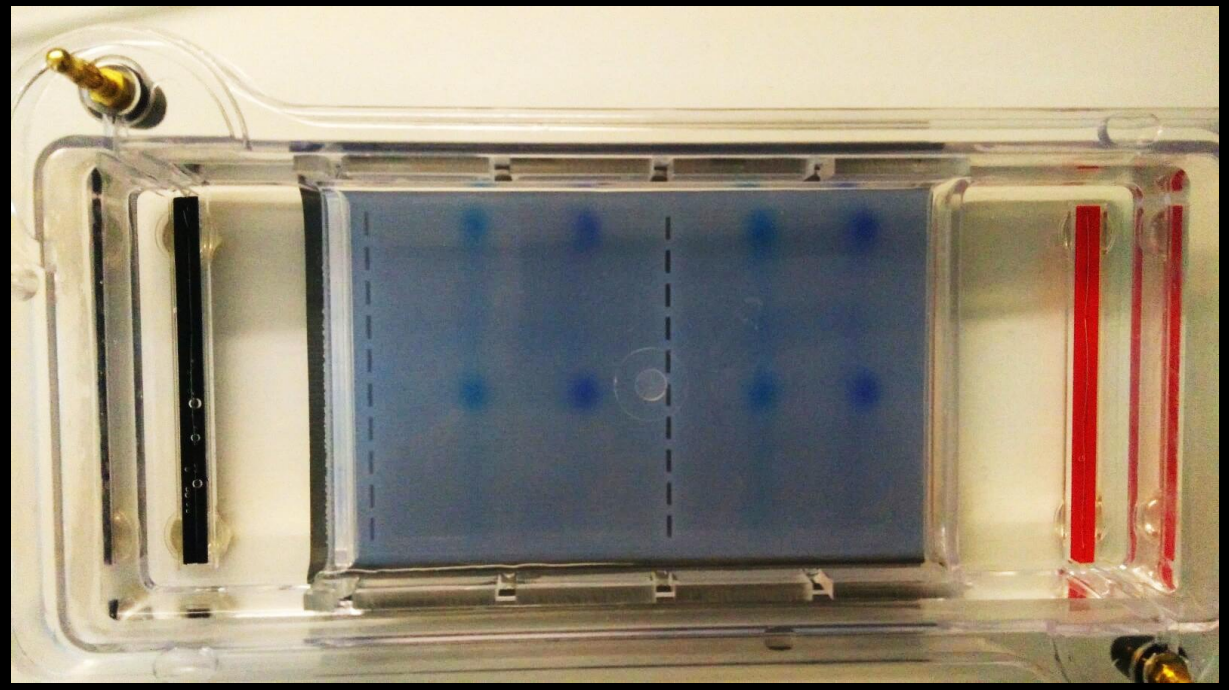


2. Loading the DNA (with dye) into the gel wells



3. Applying an electric field (100 V)





4. Putting the gel on the appropriate tray….

…and then into the Gel Doc EZ System (BioRad)

RESULTS

- We analyzed 25 samples:

1 **control sample**

7 samples of **SM**

12 samples irradiated with **0.5 Gy**

5 samples irradiated with **1 Gy**

Control sample

- We compared all the samples with control sample:

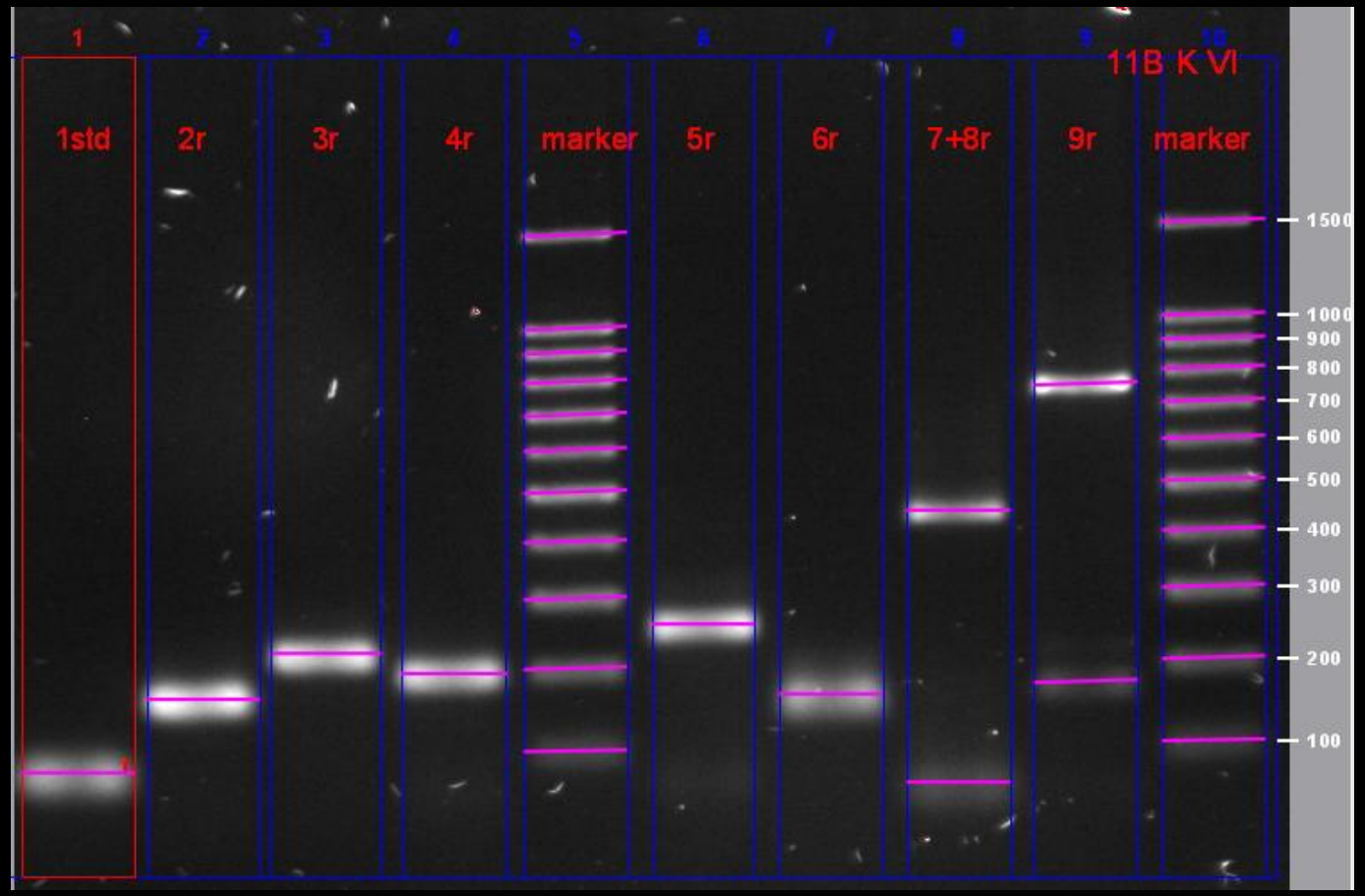
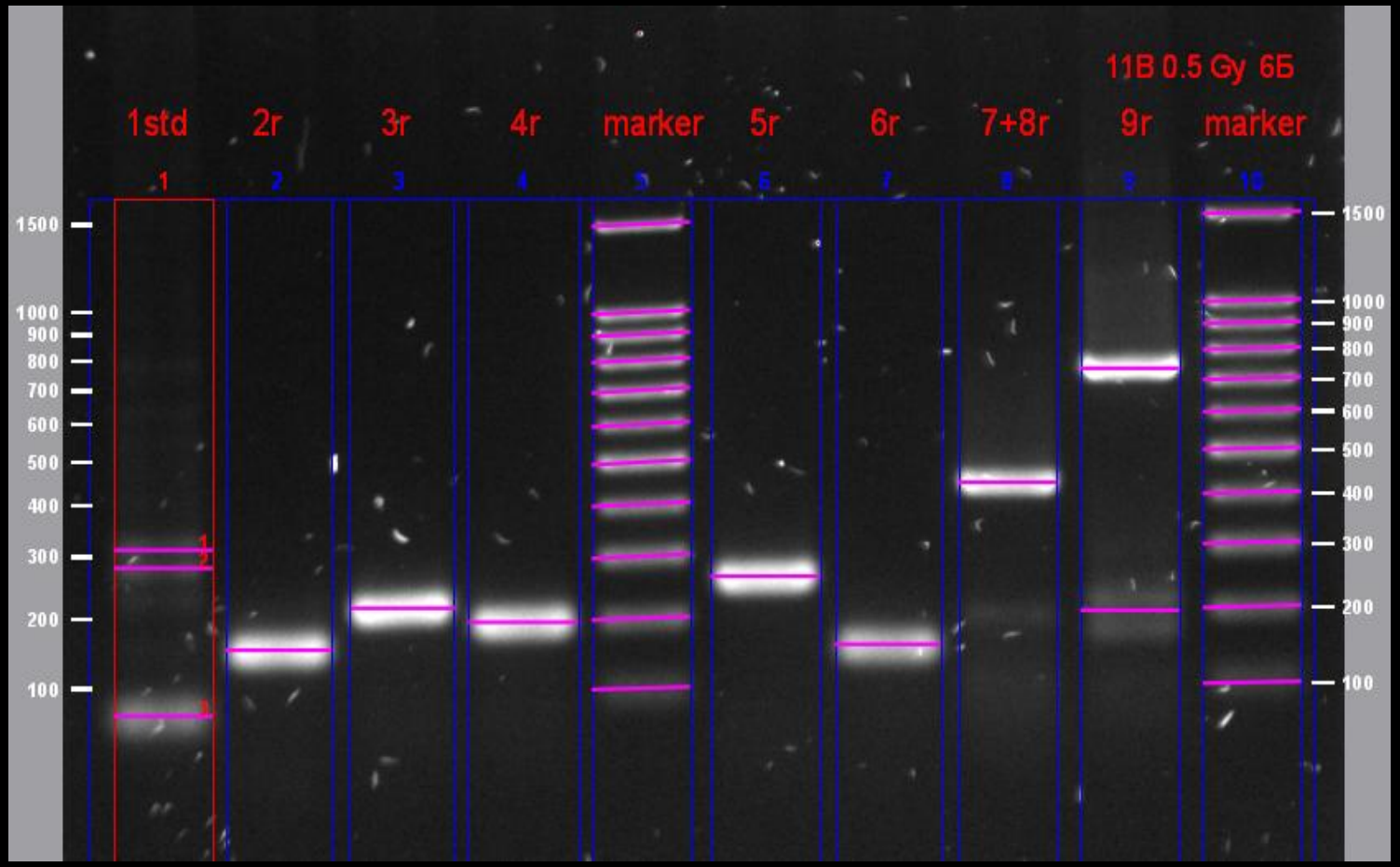


Fig. 1: The control sample ^{11}B

0.5 Gy samples



- 1 exon with some damage (exon 1)
- 8,33 % of samples with some damage

Fig. 2: Sample 6Б with damage at exon 1

1 Gy samples

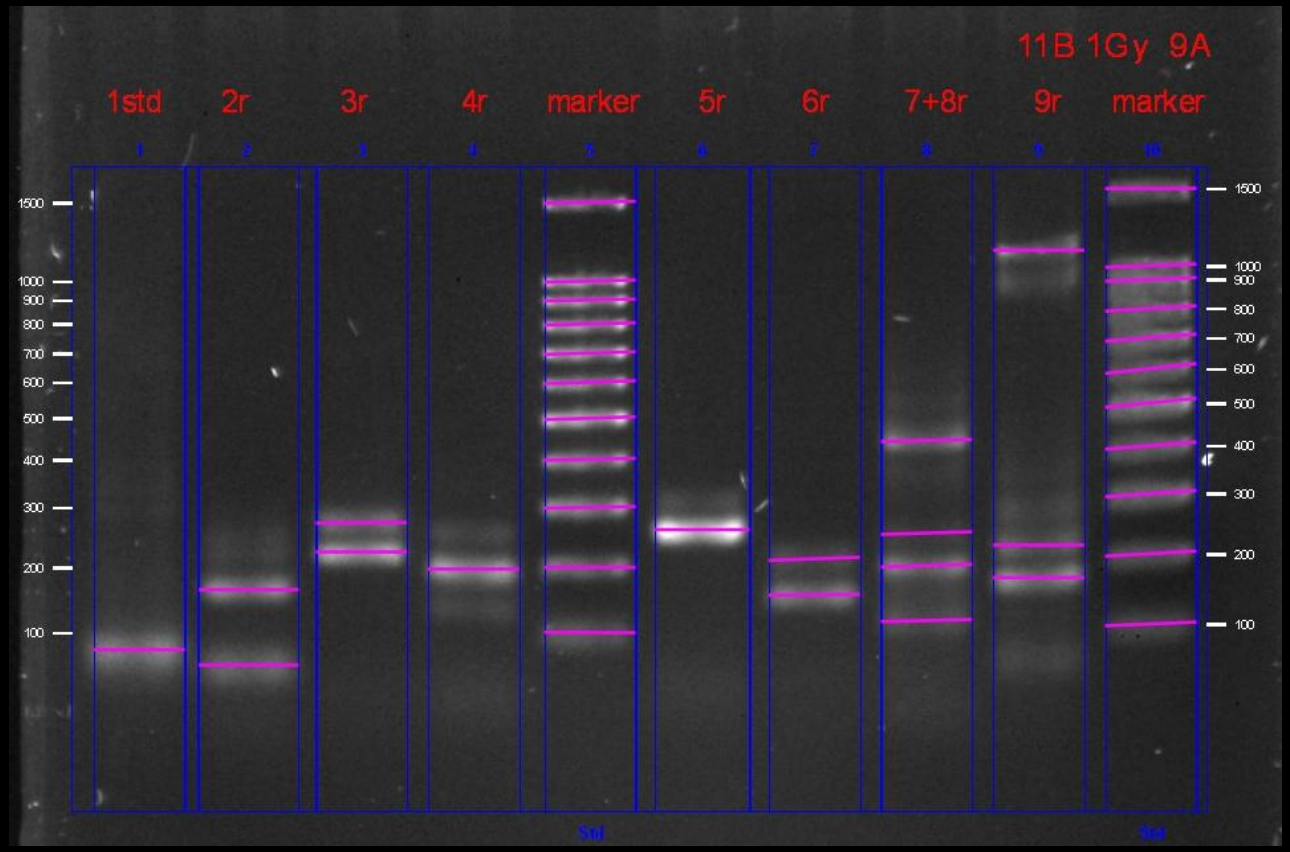


Fig. 3: Sample 9A, 1 Gy

- Damaged exons on each sample
- 80% samples with missing exons

Comparison of 0.5 Gy with 1 Gy

1 Gy ⟶ more damage or missing exons than 0.5 Gy

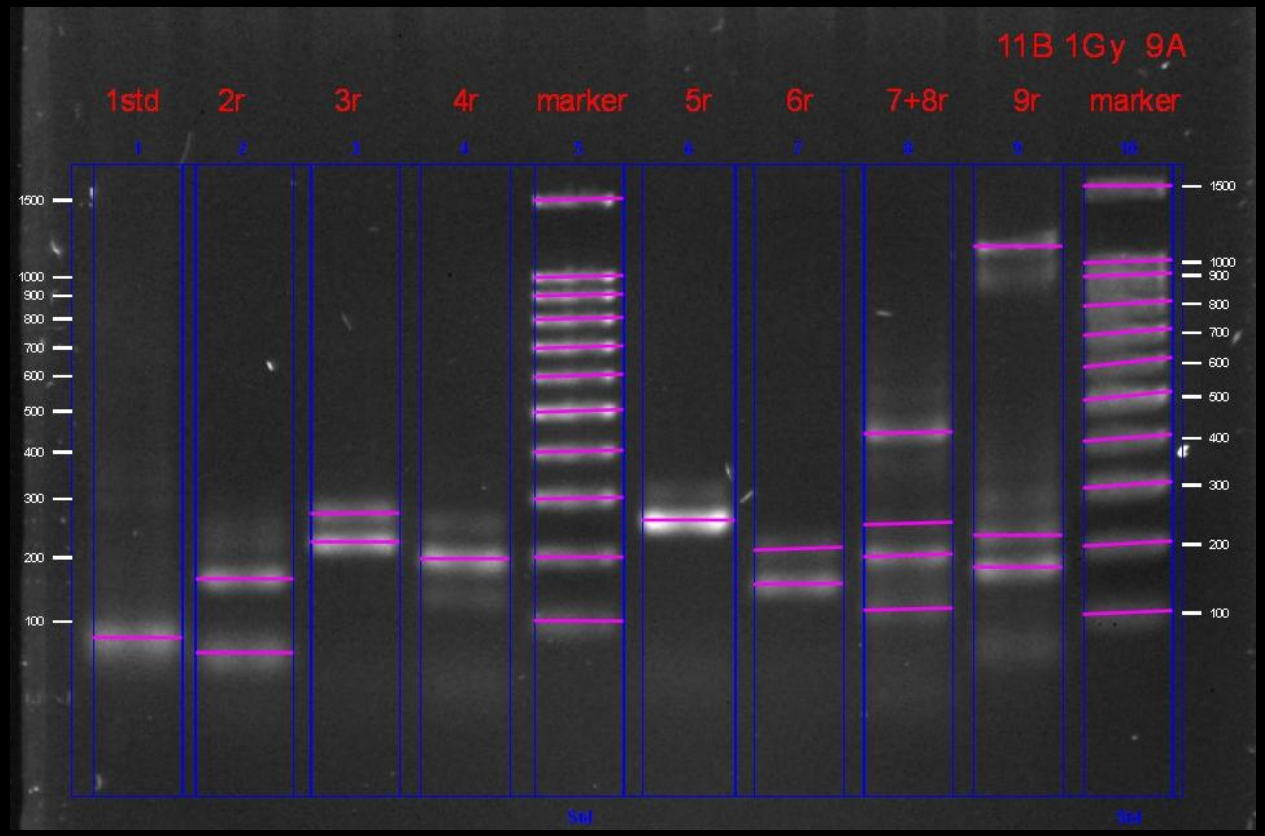


Fig. 4: Sample 9A with damaged exons 2, 3, 7+8, with missing exon 9

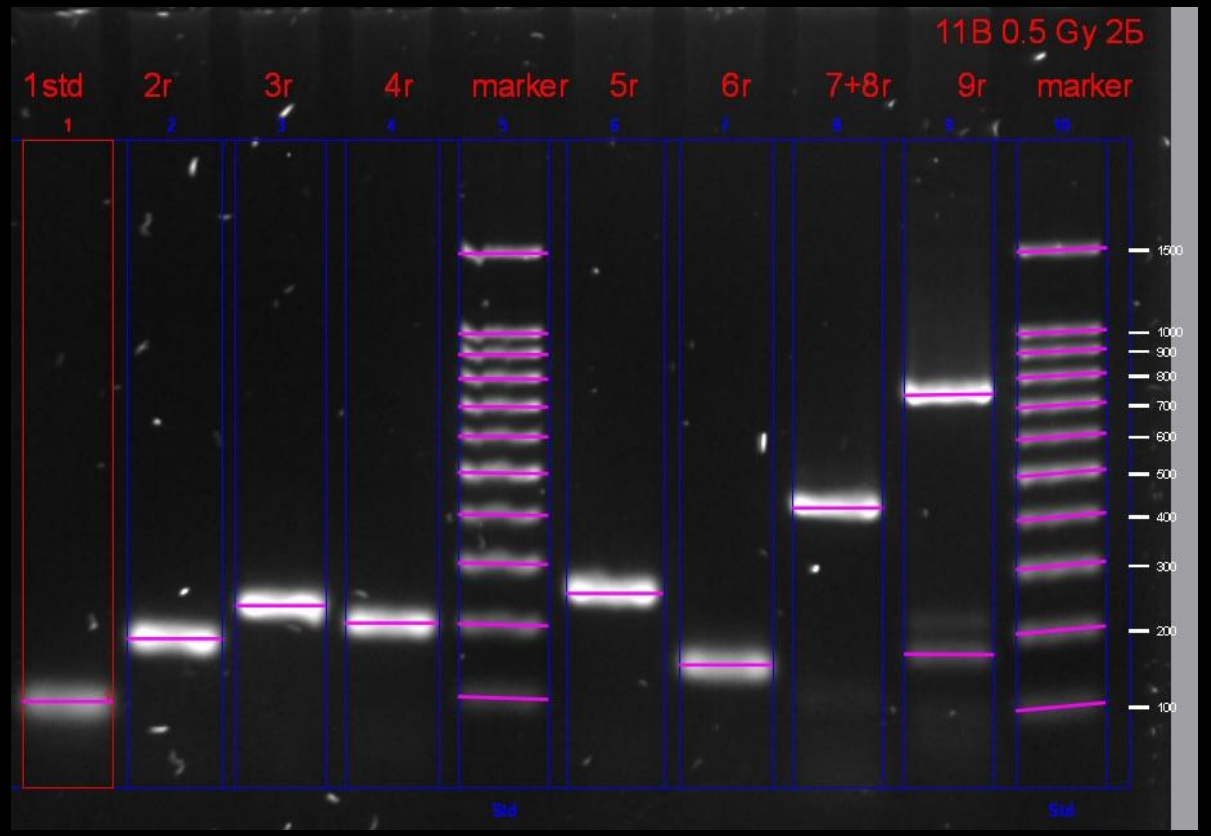


Fig. 5: Sample 2Б

Spontaneous mutants

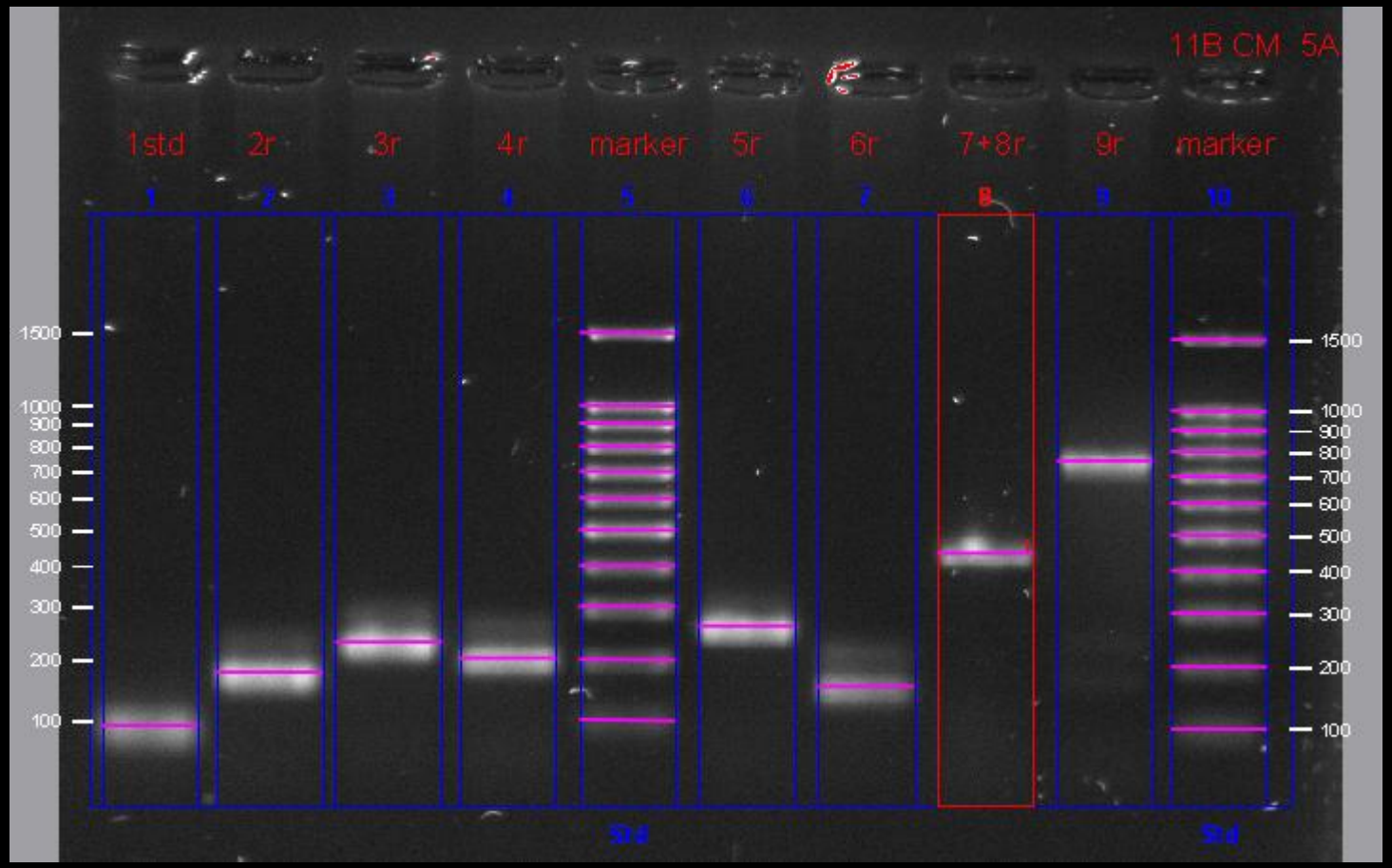


Fig. 6: Sample 5A – spontaneous mutants

- No damage
- No missing exons
- Very good results

Conclusions

1. The SM did not differ from control sample.
2. The sample irradiated with 0.5 Gy had very little deletions/damage.
3. 1 Gy had more deletions than 0.5 Gy.

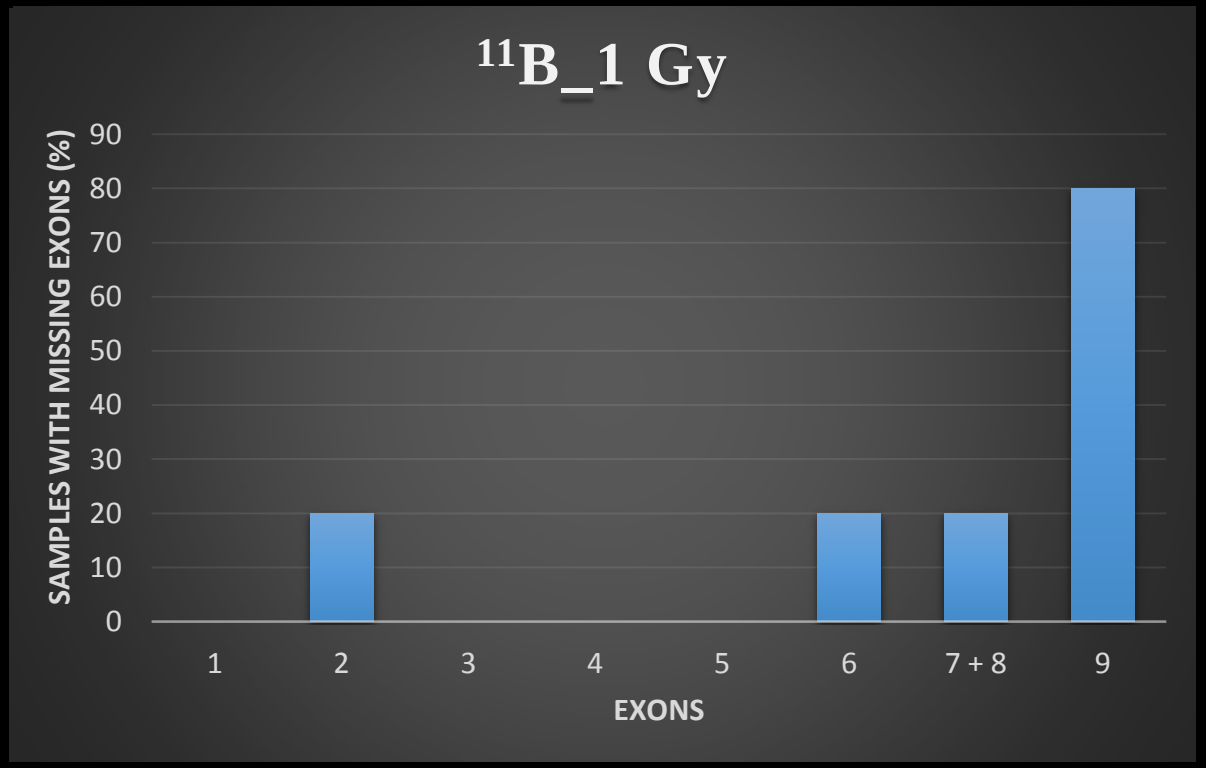


Fig. 7: Samples with missing exons for ^{11}B, 1 Gy

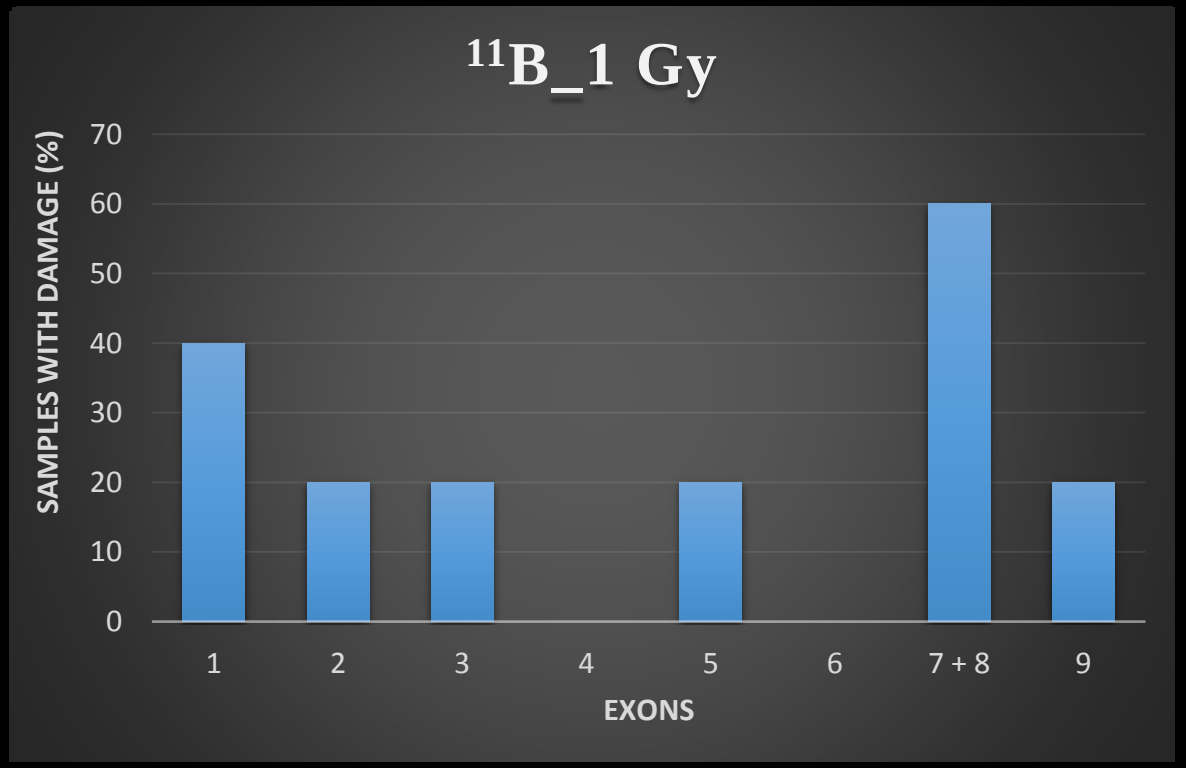


Fig. 8: Samples with some damage for ^{11}B, 1 Gy

Thank you for your attention!