



The comet assay: The procedure



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Comet Assay – a quick overview:

0. Seeding of adherent cells (*A549, Gill fish cells for WP6*)
1. Expose the cells for a certain period of time 3h and 24 h
2. Embedding of the cells into LMP agarose and drop gels on slides
3. Positive controls (e.g. H₂O₂, MMS, KBrO₃, RO^{+/-} cells)
4. Lysis (overnight)
5. Enzyme treatment
6. Alkaline treatment 20 min
7. Electrophoresis 20 min
8. Neutralization PBS and H₂O
9. Fixation is an option
10. Drying
11. Staining
12. Scoring
13. Data analysis



Planning of the week:

Monday 17th:

0. Seeding of adherent cells (A549)

Tuesday 18th:

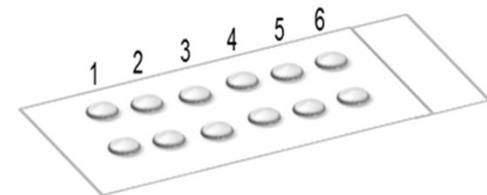
1. Expose the cells (24 h)
 - Positive controls KBrO₃ and MMS
 - NM: JRC TiO₂
 - Solvent control

Wednesday 19th:

2. Embedding of the cells into agarose and drop gels on slides

<http://www.hcomet.eu/video-gallery-tutorials>

3. Positive control H₂O₂
4. Lysis (1h)
5. Enzyme treatment (FPG 30 min)
6. Alkaline treatment 20 min
7. Electrophoresis 20 min
8. Neutralization PBS and H₂O
10. Drying O.N.



<http://www.hcomet.eu/video-gallery-tutorials>

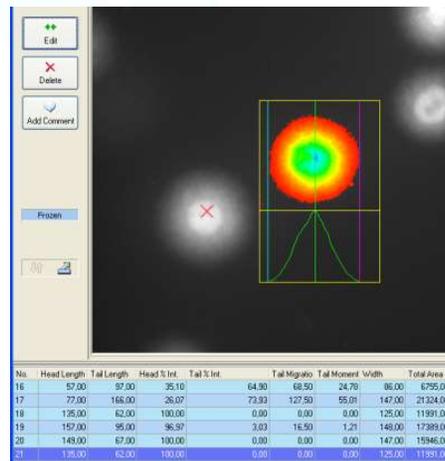
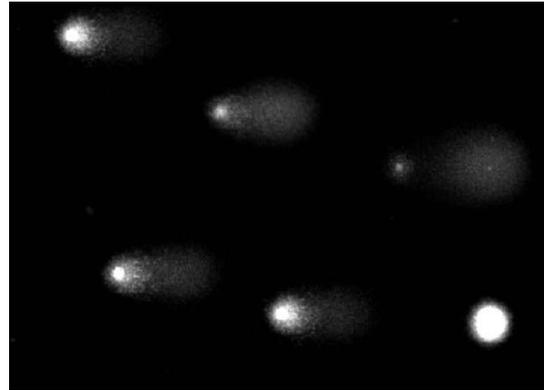


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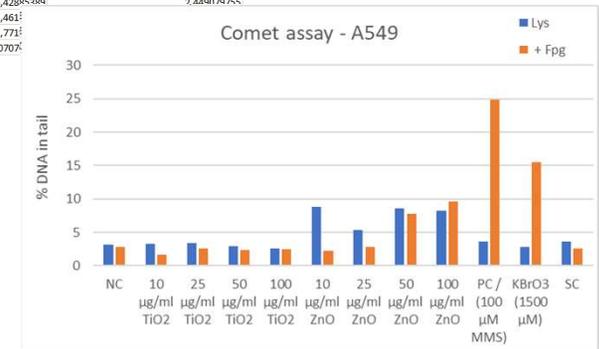
Planning of the week:

Thursday 20th:

- 11. Staining
- 12. Scoring
- 13. Data analysis



| | A | B | C | D | E |
|----|----------------|---------------------------------------|---------------------------------------|---------------------------------------|----------------------|
| 1 | Folder path: | D:\comet\RiskGone | | | |
| 2 | Folder name: | RiskGone | | | |
| 3 | Operator: | ema | | | |
| 4 | Date and time: | 01.27.2020 11:11:07 AM | | | |
| 5 | | | | | |
| 6 | File name: | RG_A549_24h Fpg_R1_Gel10_20200124.xls | RG_A549_24h Fpg_R1_Gel11_20200124.xls | RG_A549_24h Fpg_R1_Gel12_20200124.xls | RG_A549_24h Fpg_R1_G |
| 7 | Count: | 50 | 50 | 50 | |
| 8 | Median: | 19,23823163 | 12,14392654 | 0,740739118 | |
| 9 | Scores: | 40,02262277 | 28,70666586 | 0 | |
| 10 | | 27,44495732 | 16,74762427 | 0 | |
| 11 | | 34,99703977 | 23,64718565 | 0 | |
| 12 | | 55,96617295 | 26,08215221 | 2,454952464 | |
| 13 | | 41,54513857 | 18,71223556 | 0 | |
| 14 | | 53,19599154 | 26,71629859 | 0 | |
| 15 | | 67,33942776 | 15,6961842 | 0,001262871 | |
| 16 | | 29,64952954 | 2,93796207 | 5,25229682 | |
| 17 | | 17,25770885 | 0,567799389 | 1,780708527 | |
| 18 | | 15,11070787 | 2,355524832 | 2,676809431 | |
| 19 | | 28,24602743 | 16,2379036 | 0,001105314 | |
| 20 | | 53,58765546 | 6,349178143 | 3,156264119 | |
| 21 | | 38,30586921 | 26,42885384 | 2,445076275 | |
| 22 | | 37,07504741 | 23,461 | | |
| 23 | | 65,75961019 | 21,771 | | |
| 24 | | 16,21736189 | 8,070 | | |



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THANK YOU!

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