MG labelling Fabiola Garcia

## Media Changes

## 24h and 4h before experiment start exchange the media, leaving 2 mL of previous media on the plate (to keep some growth factors etc there)

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | Tuesday | Wednesday |
|  |  | 24h Change Media | 4h Change Media |
| 1 | MG 12C 15' R1 | 8 | 8 |
| 2 | MG 12C 15' R2 |
| 3 | MG 12C 15' R3 |
| 4 | MG 12C 15' R4 |
| 5 | MG 12C 17' R1 | 08:40 | 08:40 |
| 6 | MG 12C 17' R2 |
| 7 | MG 12C 17' R3 |
| 8 | MG 12C 17' R4 |
| 9 | MG 13C 17' R1 | 09:25 | 09:25 |
| 10 | MG 13C 17' R2 |
| 11 | MG 13C 17' R3 |
| 12 | MG 13C 17' R4 |
| 13 | MG 12C 20' R1 | 10:20 | 10:20 |
| 14 | MG 12C 20' R2 |
| 15 | MG 12C 20' R3 |
| 16 | MG 12C 20' R4 |
| 17 | MG 13C 20' R1 | 11:20 | 11:20 |
| 18 | MG 13C 20' R2 |
| 19 | MG 13C 20' R3 |
| 20 | MG 13C 20' R4 |
| 21 | 12C 2' R1 | 12:20 | 12:20  (Ana) |
| 22 | 12C 2' R2 |
| 23 | 12C 2' R3 |
| 24 | 12C 2' R4 |
| 21 | 13C 2' R1 | 12:50 | 12:50  (Ana) |
| 22 | 13C 2' R2 |
| 23 | 13C 2' R3 |
| 24 | 13C 2' R4 |
| 25 | 12C 5' R1 | 13:30 | 13:30  (Ana) |
| 26 | 12C 5' R2 |
| 27 | 12C 5' R3 |
| 28 | 12C 5' R4 |
| 29 | 13C 5' R1 | 13:55 | 13:55  (Ana) |
| 30 | 13C 5' R2 |
| 31 | 13C 5' R3 |
| 32 | 13C 5' R4 |

2ml of the old media should be left in the plate and only add 3ml?? final volume 5ml

## With MG

* 0' Add MG
  + 12C
    - 15' harvest **(total 30min)**
    - 15' "change" media to 12C containing 7 µm MG
    - 17' harvest **(total 32 min)**
    - 20' harvest **(total 48 min)**
  + 13C
    - 15' change media to 13C containing 7 µm MG
    - 17' harvest **(total 32 min)**
    - 20' harvest **(total 48 min)**

## Without MG

* 12C
  + 0' "change" media to 12C containing no MG
  + 2' harvest **(total 18 min)**
  + 5' harvest **(total 18 min)**
* 13C
  + 0' "change" media to 13C containing no MG
  + 2' harvest **(total 18min)**
  + 5' harvest **(total 18min)**

9 harvests 5 tecreps= 45

Day Schedule

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Samples | | Tuesday | Wednesday | Cell Counting | Experiment starts | Experiment ends |
| 24h change media | 4h change media |
| 1 | MG 12C 15' R1 | 8 | 8 | 12:00 | 12:00 | 12:30 |
| 2 | MG 12C 15' R2 |
| 3 | MG 12C 15' R3 |
| 4 | MG 12C 15' R4 |
| 5 | MG 12C 17' R1 | 08:40 | 08:40 | 12:00 | 12:40 | 13:12 |
| 6 | MG 12C 17' R2 |
| 7 | MG 12C 17' R3 |
| 8 | MG 12C 17' R4 |
| 9 | MG 13C 17' R1 | 09:25 | 09:25 | 13:20 | 13:25 | 13:57 |
| 10 | MG 13C 17' R2 |
| 11 | MG 13C 17' R3 |
| 12 | MG 13C 17' R4 |
| 13 | MG 12C 20' R1 | 10:20 | 10:20 | 14:20 | 14:20 | 15:00 |
| 14 | MG 12C 20' R2 |
| 15 | MG 12C 20' R3 |
| 16 | MG 12C 20' R4 |
| 17 | MG 13C 20' R1 | 11:20 | 11:20 | 15:20 | 15:20 | 16:00 |
| 18 | MG 13C 20' R2 |
| 19 | MG 13C 20' R3 |
| 20 | MG 13C 20' R4 |
| 21 | 12C 2' R1 | 12:20 | 12:20 | 16:20 | 16:20 | 16:38 |
| 22 | 12C 2' R2 |
| 23 | 12C 2' R3 |
| 24 | 12C 2' R4 |
| 21 | 13C 2' R1 | 12:50 | 12:50 | 16:20 | 16:50 | 17:08 |
| 22 | 13C 2' R2 |
| 23 | 13C 2' R3 |
| 24 | 13C 2' R4 |
| 25 | 12C 5' R1 | 13:30 | 13:30 | 17:20 | 17:30 | 17:48 |
| 26 | 12C 5' R2 |
| 27 | 12C 5' R3 |
| 28 | 12C 5' R4 |
| 29 | 13C 5' R1 | 13:55 | 13:55 | 17:20 | 17:55 | 18:13 |
| 30 | 13C 5' R2 |
| 31 | 13C 5' R3 |
| 32 | 13C 5' R4 |

MG 12C 15' (12:00)

|  |  |  |
| --- | --- | --- |
| A | media sample | -0,5 |
| A | label start | 0 |
| B | media sample | 3,5 |
| B | label start | 4 |
| C | media sample | 8,5 |
| C | label start | 9 |
| D | media sample | 11,5 |
| D | label start | 12 |
| A | wash | 14,5 |
| A | label end | 15 |
| A | harvest end | 16 |
| B | wash | 18,5 |
| B | label end | 19 |
| B | harvest end | 20 |
| C | wash | 23,5 |
| C | label end | 24 |
| C | harvest end | 25 |
| D | wash | 26,5 |
| D | label end | 27 |
| D | harvest end | 28 |

MG 12C 17' (12:40) and **MG 13C 17' (13:25) Change the buffer for 13C labelled**

|  |  |  |
| --- | --- | --- |
| A | MG addition | 0 |
| B | MG addition | 5 |
| C | MG addition | 10 |
| D | MG addition | 14 |
| A | media sample | 14,5 |
| A | label start | 15 |
| A | wash | 16,5 |
| A | label end | 17 |
| A | harvest end | 18 |
| B | media sample | 19,5 |
| B | label start | 20 |
| B | wash | 21,5 |
| B | label end | 22 |
| B | harvest end | 23 |
| C | media sample | 24,5 |
| C | label start | 25 |
| C | wash | 26,5 |
| C | label end | 27 |
| C | harvest end | 28 |
| D | media sample | 28,5 |
| D | label start | 29 |
| D | wash | 30,5 |
| D | label end | 31 |
| D | harvest end | 32 |

MG 12C 20' (14:20) and **MG 13C 20' (15:20) Change the buffer for 13C labelled**

|  |  |  |
| --- | --- | --- |
| A | MG addition | 0 |
| B | MG addition | 8 |
| A | media sample | 14,5 |
| A | label start | 15 |
| C | MG addition | 16 |
| D | MG addition | 18 |
| A | wash | 19,5 |
| A | label end | 20 |
| A | harvest end | 21 |
| B | media sample | 22,5 |
| B | label start | 23 |
| B | wash | 27,5 |
| B | label end | 28 |
| B | harvest end | 29 |
| C | media sample | 30,5 |
| C | label start | 31 |
| D | media sample | 32,5 |
| D | label start | 33 |
| C | wash | 35,5 |
| C | label end | 36 |
| C | harvest end | 37 |
| D | wash | 37,5 |
| D | label end | 38 |
| D | harvest end | 39 |

12C 2' (16:20) and **13C 2’ (16:50) Change the buffers for 13C labelled**

|  |  |  |
| --- | --- | --- |
| A | media sample | -0,5 |
| A | label start | 0 |
| A | wash | 1,5 |
| A | label end | 2 |
| A | harvest end | 3 |
| B | media sample | 4,5 |
| B | label start | 5 |
| B | wash | 6,5 |
| B | label end | 7 |
| B | harvest end | 8 |
| C | media sample | 9,5 |
| C | label start | 10 |
| C | wash | 11,5 |
| C | label end | 12 |
| C | harvest end | 13 |
| D | media sample | 14,5 |
| D | label start | 15 |
| D | wash | 16,5 |
| D | label end | 17 |
| D | harvest end | 18 |

12C 5' (17:30) and 13C 5' (17:55)

|  |  |  |
| --- | --- | --- |
| A | media sample | -0,5 |
| A | label start | 0 |
| B | media sample | 2,5 |
| B | label start | 3 |
| A | wash | 4,5 |
| A | label end | 5 |
| A | harvest end | 6 |
| B | wash | 7,5 |
| B | label end | 8 |
| B | harvest end | 9 |
| C | media sample | 9,5 |
| C | label start | 10 |
| D | media sample | 11,5 |
| D | label start | 12 |
| C | wash | 14,5 |
| C | label end | 15 |
| C | harvest end | 16 |
| D | wash | 16,5 |
| D | label end | 17 |
| D | harvest end | 18 |