58758\_R2\_RE2

Thank you for your editing. I edited some to clarify the process. I hope it is more understandable.

|  |  |  |
| --- | --- | --- |
| **Page 5: Commented [A1]** | **Author** | **8/30/18 2:25:00 PM** |
| Centrifugation parameters?  at maximum speed for 2 min using a microcentrifuge |  |  |
| **Page 5: Commented [A2]** | **Author** | **8/30/18 3:33:00 PM** |
| Centrifugal force?  at maximum speed |  |  |
| **Page 5: Commented [A3]** | **Author** | **8/31/18 8:44:00 AM** |

Please note that I highlighted these steps for filming, because we cannot start filming step 2.2.5 without showing the cells first.

I agree with you.

**Page 6 [A4]**

I moved this Note to 2.2.4 where FBS is first mentioned.

I agree with you.

**Page 6 [A5]**

Please note that I have heavily edited 2.3 and sub-steps so they comply with JoVE guidelines. For instance: The Protocol should be made up almost entirely of discrete steps **without large paragraphs** of text between sections and contain only **action items** that direct the reader to do something. Please read carefully to ensure that the intended meaning has remained.

Thank you for your edits. I added some modifications.

**Page 7 [A6]**

As I noticed that these are the only differences between combination (i) and (ii), so I combined the original two

paragraphs here.

Thank you for your edits. I added some modifications.

**Page 7 [A7]**

Please note that calculations are not appropriate for filming. Therefore I unhighlighted calculation steps.

I understand.

**Page 8 [A8]**

It is unclear to me why the volume of transfection reagent in DMEM should be 195 uL. Based on lines 317-318, it should be 210 uL. Please check.

I edited this part.

**Page 8 [A9]**

It is unclear to me why the volume of transfection reagent in DMEM should be 195 uL. Based on lines 317-318, it

should be 210 uL. Please check.

I edited this part.

|  |  |  |
| --- | --- | --- |
| **Page 8: Commented [A10]** | **Author** | **8/31/18 9:00:00 AM** |
| Please specify the antibiotics used in this step.  I added 1% penicillin-streptomycin solution. |  |  |
| **Page 9: Commented [A11]** | **Author** | **8/31/18 9:14:00 AM** |

Do you mean warm to room temperature before use?

Yes. Your edit is right.

**Page 9 [A12]**

Do you mean warm to room temperature before use?

Yes. Your edit is right.