**Protocol Title**

*Indicate the goal of the procedure and include the model system or organism*

Author 11, #, Author 21, 2, #, $, Author 32 and Author 42, \*

*Indicate First name, Middle name initial, and Last name*

1Dept/Center, Institution name, City, Country; 2Dept/Center, Institution name, City, Country; $Current/Present address: Dept/Center, Institution name, City, Country

\*For correspondence: email

#Contributed equally to this work

**[Abstract]** Enter text here.

*Introduce the research field (1-2 sentences), provide context by mentioning the other existing and previous techniques (1-2 sentences), summarize the protocol (3-4 sentences max), and finish by summarizing the advantages of the protocol being presented (1-2 sentences)**.*

**Graphic abstract:** (optional)

Insert the figure here.

*It could be a flowchart of the method/procedures, the highlight of the key steps or the innovative design from this protocol etc.*

1. *Resolution: a minimum of 500 dpi.*
2. *Minimize the usage of text in the picture*

**Keywords:** Keyword 1, Keyword 2, Keyword 3 …

*5-8 keywords to help searches.*

**[****Background]** Enter text here.

*Briefly, introduce the research field that your protocol can be used to advance. Mention previous, related methodologies and summarize the advantages of using your protocol over other published methods. If possible, elaborate on other possible applications of this protocol. (****Reference citation format: “Dow et al., 2018”****).*

**Materials and Reagents**

1. Product 1 name (Manufacturer, Brand, catalog number: XXXX)
2. Product 2 name (Manufacturer, Brand, catalog number: XXXX)
3. …

*Strongly recommended: list all materials/reagents with vendor/manufacturer and catalog number used in the protocol. Provide storage information (i.e., storage temperature, shelf-life) as well.*

**Equipment**

1. Equipment 1 name (Manufacturer, Brand, model/catalog number: XXXX)
2. Equipment 2 name (Manufacturer, Brand, model/catalog number: XXXX)
3. …

*Strongly recommended: list each piece of equipment used in the experiment along with its vendor/manufacturer and catalog number.*

**Software** (if any)

1. Software 1 name (Company or Developer/Provider/Supplier, web address)
2. Software 2 name (Company or Developer/Provider/Supplier, web address)
3. …

*List individual software separately, especially in cases where a long software workflow is used.*

**Procedure**

Enter Step by Step protocol here. For example:

1. Subtitle (e.g., Gene cloning)
2. Isolate DNA from tissues
3. Set up RT-PCR reaction
4. …
5. Cell transformation
6. Put competent cells on ice
7. Add the plasmids into competent cells
8. …
9. ***Write with active voice and verbs in the present tense*** *(e.g., write "Prepare stock solutions and reaction mixtures under anaerobic conditions” instead of “Stock solutions and reaction mixtures were prepared under anaerobic conditions").*
10. ***Provide representative data (intermediary and final) and notes/tips to help others:*** *Your protocol should provide enough information for a first-year graduate student to perform it and be successful.*
11. ***General formatting:*** *(1) Automatic numbered lists: Generally, use five list levels in order of “A. B. C....; 1. 2. 3. …; a. b. c. …; i. ii. iii. (2) A. B. C. …this level only for different big sections –the author normally gives a big title for a set of procedure, then the next big title-procedure.*

1st Level: A. B. C. … [Indentation: Left (0 cm), Hanging (0.63 cm)]

2nd Level: 1. 2. 3. … [Indentation: Left (0.63 cm), Hanging (0.63 cm)]

3rd Level: a. b. c. … [Indentation: Left (1.26 cm), Hanging (0.63 cm)]

4th Level: i. ii. iii. … [Indentation: Left (1.89 cm), Hanging (0.63 cm)]

5th Level: 1). 2). 3). ... [Indentation: Left (2.52 cm), Hanging (0.63 cm)]

1. ***For images:***
2. *Resolution: a minimum of 300 dpi.*
3. *Text in Figure: 8-12 point.*
4. *Panel label: “A, B, C…” (uppercase, bold). Use the same typeface for all figures.*
5. *Each figure should have a title and a comprehensive but concise legend.*
6. *Include scale bars where it applies (e.g. microscope images).*
7. *Figures should be embedded in the text.*
8. ***For tables:***
9. *Each table should have a brief title and a comprehensive but concise legend.* *Put table title above the table. Consistently use “****Table 1. Title.*** *Xxx.”, indicate Table 1. 2... in the text.*
10. *Each table should be editable. Prepare all tables using the table function in a word-processing program.*
11. *Each table should have the following three horizontal lines:*

*One under the title, above the column heading, 1 pound;*

*One between the column headings and the body of the table, 0.75 pounds;*

*One at the bottom of the table, 1 pound.*

1. *Each table should be set off from the text close to where it is first cited.*
2. ***For videos:*** *typical smart phone camera videos are usually adequate*
   * 1. *Each video should have a title.*
     2. *Each video file should be less than 1 GB.*
     3. *Each video must be submitted as a separate file.*

**Data analysis**

Enter text here.

1. *Provide information data processing and analyses: make sure to include statistical tests, criteria for data inclusion/exclusion, and details on the number of replicates in each experiment.*
2. *When detailed information of data analyses already appear in the original research article, summarize the analyses and cite the publication. Please, indicate clearly where the analysis can be found (e.g., Supplemental information or Figure X).*
3. *When the original research paper has restricted access, please request permission to “reprint” the numerical analysis in Bio-protocol or use other unpublished data sets.*

**Notes** (optional)

Enter text here.

*Provide general comments such as notes about reproducibility and variability in your hands and cautionary points.*

**Recipes**

Enter text here.

*Describe ingredients and recipes. Please make sure to carefully review them as small errors can have a profound impact.*

**Acknowledgments**

Enter text here.

*Include the following information: 1) acknowledge funding sources; 2) acknowledge previous work or the original research paper where this protocol was derived from.*

**Competing interests**

Enter text here.

1. *The corresponding author should provide a statement of financial and non-financial competing interests on behalf of all authors.*
2. *Examples include paid employment or consultancy, stock ownership, patent applications, personal relationships with individuals involved in the submission or evaluation of a protocol, and receipt of funding or free products from the vendors of the reagents/equipment or other advertisers.*

**Ethics**

Enter text here.

1. *All protocols that have used human and/or animal subjects must mention the specific ethics committee that approved the described experiment.*
2. *Protocols including human subjects should also indicate that informed consent was obtained from all subjects.*

**References**

Dow, L. E., O'Rourke, K. P., Simon, J., Tschaharganeh, D. F., van Es, J. H., Clevers, H. and Lowe, S. W. (2015). Apc restoration promotes cellular differentiation and reestablishes crypt homeostasis in colorectal cancer. *Cell* 161(7): 1539-1552.

*Strongly recommended: providing the references in our standard format (see above) and listing them in the order of aliphatic.*

**Use the following formats of special symbols where it applies：**

Degree Celsius: °C

Micro: μ

Alpha: α

Beta: β

Gamma: γ

Less than or equal to: ≤

More than or equal to: ≥

Chemical formula: MgSO4·7H2O

Prime: ′