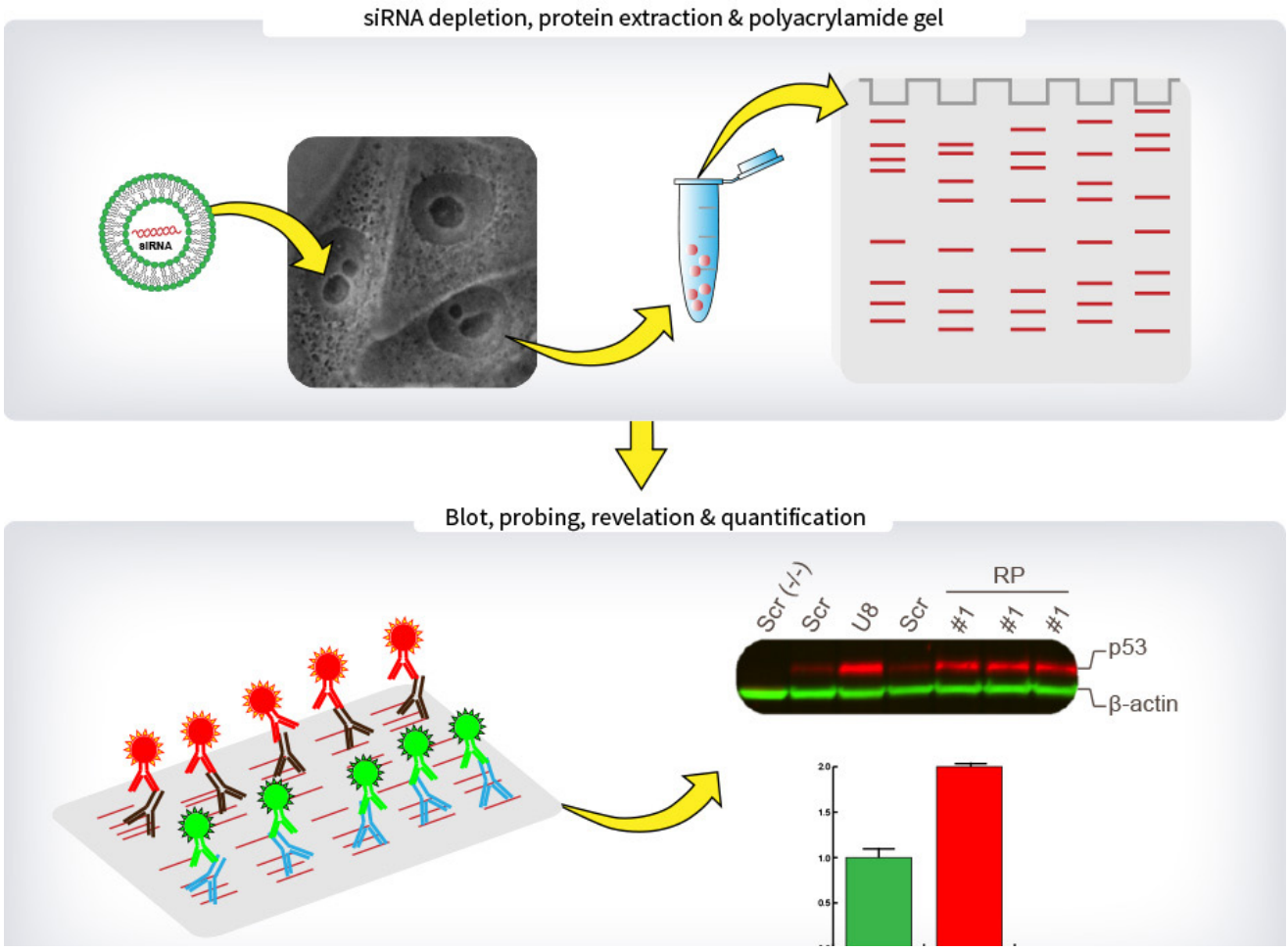
METHODS – p53 Steady-State Level



Legend : METHODS – p53 Steady-State Level

For quantitative Western-blot analysis, HCT116 cells were depleted three times independently with the same siRNA specific to each r-protein. After 2 days of depletion, total proteins were extracted and separated on a polyacrylamide gel. Proteins were then transferred on low-fluorescence PVDF membrane and blocked in Odyssey blocking buffer. Primary antibodies targeting p53 and ß-actin were incubated with the membrane O/N. Specific secondary antibodies carrying fluorescent dyes were then added to detect specifically p53 in red and ß-actin in green. Membranes were finally imaged for the fluorescent signals with the Chemidoc (Biorad). Cellular p53 steady-state level was assessed by calculating a ratio between the red fluorescent signal (corresponding to p53) and the green fluorescent signal (corresponding to ß-actin). For each experiment, 2 independent lanes corresponding to the Scramble condition in HCT116 cell line were loaded on the gel and the results from these two lanes were averaged to determine the level of p53 in this control condition. All data were then harmonized to this averaged value in order to determine the variation in the p53 steady-state level under this reference condition. As another control, we used an antisense oligonucleotide targeting the U8 snoRNA, whose depletion leads to important increase in p53 steady-state cellular level.

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