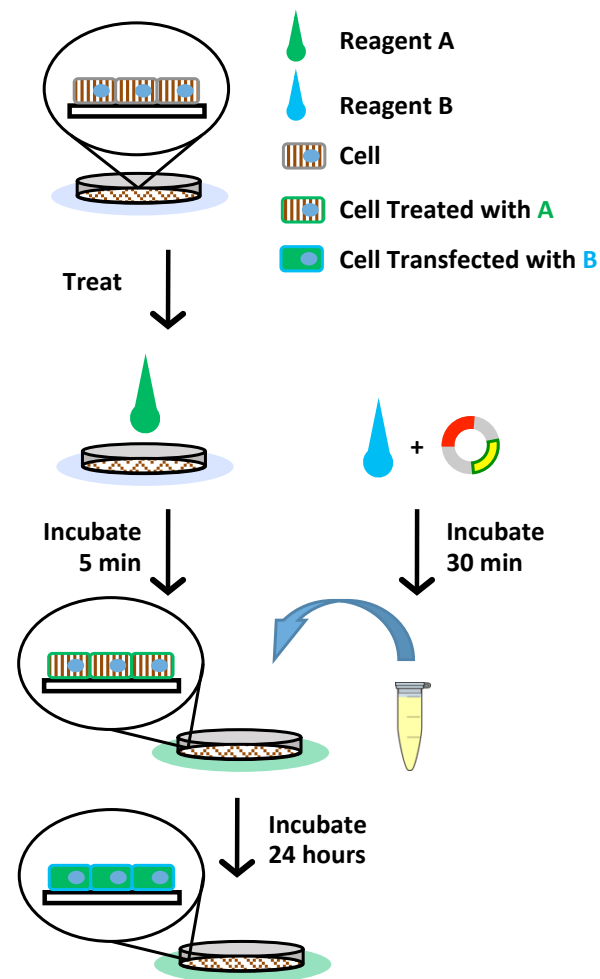









## Snapfect™ CRISPR Transfection Protocol



		Sequential	
Cas9	gRNA		
	+		Co-transfection
Cas9		gRNA	
		Co-transfection	
Cas9 + gRNA			

**Reagent A** – Green cap    **Reagent B** – Blue cap

After seeding cells of interest with a 70-80% density overnight in 6 well culture plates under standard growth conditions, the cells are ready to be transfected. The following protocol is for cell transfection for a single well.

1) Prepare the transfection agent by combining 10  $\mu\text{L}$  **Reagent B** and approximately 3  $\mu\text{g}$  of CRISPR/tRNA plasmid in a DNAase/RNAase free microtube and gently agitate. Then allow the solution to incubate at room temp for 30 minutes.

2) Refresh the target cell media with standard growth media, then add approximately 5% v/v **Reagent A** and gently swirl the plate to mix and incubate the cells for 1-5 min under standard conditions.

3) Aspirate the growth media containing **Reagent A** and wash 1X with PBS or other suitable media once the **Reagent B** transfection agent is prepared.

4) After 30 min has lapsed dilute **Reagent B** by adding 800  $\mu\text{L}$  of SF media.

5) Add 810  $\mu\text{L}$  of **Reagent B** transfection agent to the well and incubate 37°C + 5% CO<sub>2</sub> for 10 minutes

6) Add 2mL of growth media and further incubate for 24hr to 48hr for results.