### Good DNA Transfection Practices
- Use a reporter gene to set up and optimize transfection conditions, as those may vary depending on the cells to transfect. Various reporter systems are commercially available: Renilla/Luciferase and GFP (Green Fluorescent Protein) are the most commonly used.
- Use high quality plasmid purification kits to obtain high grade DNA, without RNA or protein, for higher transfection efficiency and improved reproducibility.
- Passage cells at least twice after thawing to allow recovery before transfection, and use cells at low passage number (< 20 passages). Discard cells if they have become overconfluent. Regularly check for contaminants: yeast, bacteria and mycoplasma.
- Check transfection efficiency before purchasing a new batch of serum or trypsin.
- Store appropriately jetPRIME™ and DNA.

### Prepare the plasmid DNA
- Measure UV absorbance at 280 nm. OD260/280 ratio should reach at least 1.8.
- Resuspend the plasmid in deionized water or TE buffer at a concentration of ca. 1 µg/µl.
- Aliquot the plasmid preparation and store it at -20°C.
- Check for RNA contamination by agarose gel electrophoresis and ethidium bromide staining.

### Transfection tips
- The day before transfection, seed the cells to obtain 60-80% confluency at the time of transfection.
- Prior to transfection, dilute the DNA in the provided jetPRIME™ buffer first, and then add the jetPRIME™ reagent.

### Tips to increase DNA transfection efficiency
- Increase DNA amount up to 2 folds.
- Test higher DNA/jetPRIME™ ratios such as 1:3 or 1:4.
- Just after transfection, centrifuge the plates 5 min at 180 g.

### Tips to increase cell viability
- Replace medium after 4 hours.
- Decrease DNA amount by half or more.
- Analyze transfection at an earlier time point (24 h after transfection instead of 48 h for instance).
- Check that the target gene does not affect cell viability.

### DNA transfection using jetPRIME™ in 6-well plates
- 2 µg of DNA in 200 µl of jetPRIME™ buffer
- Vortex 10 s and spin down
- Add 4 µl of jetPRIME™
- Add 200 µl of transfection mix per well
- Incubate 24 to 48 h and measure gene or protein expression

150,000 to 250,000 cells per well seeded the day before in 2 ml of growth medium
If required, replace medium 4 h after transfection