

# Technical Note

## Guidelines for successful plasmid DNA transfection using jetPRIME™

### + 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™ (4°C) 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

