jetPRIME®

Technical Note

Scope

- Effective and reproducible DNA and siRNA transfection in several adherent cell lines
- Requires only low amounts of nucleic acid
- Suitable for various applications, such as plasmid transfection, gene silencing, co-transfection of different nucleic acids and CRISPR/Cas9-mediated gene knock-outs

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 be approximately 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.

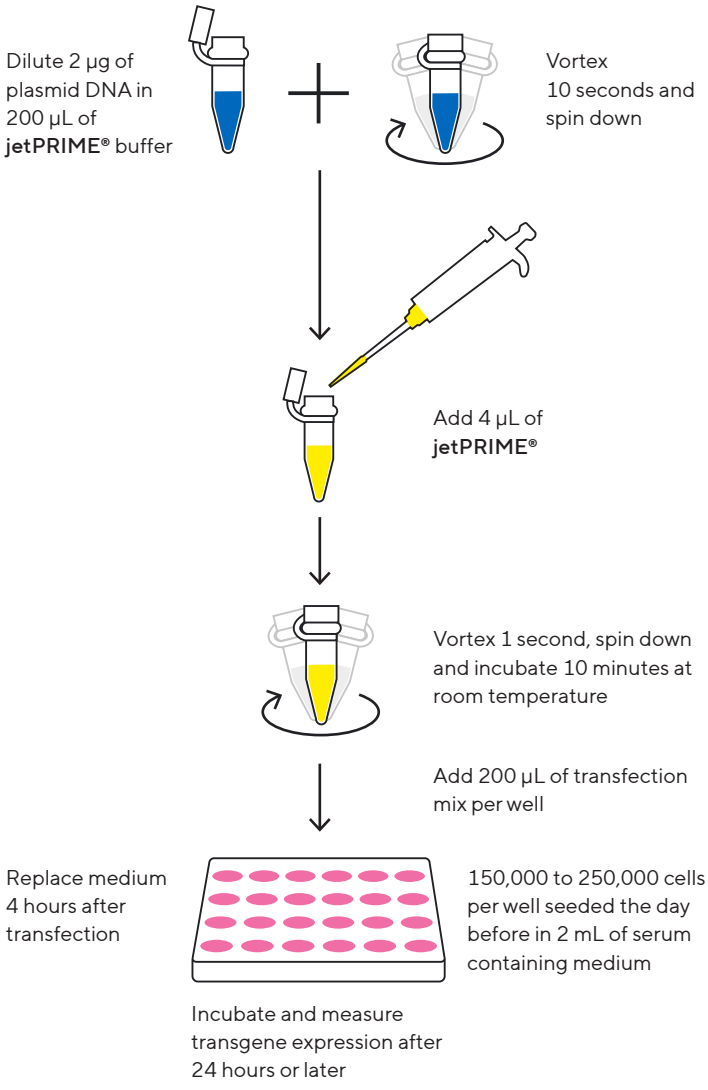
Tips to Increase DNA Transfection Efficiency

- Increase DNA amount up to 2-fold.
- Test higher DNA | jetPRIME® ratios such as 1:3 or 1:4.
- Just after transfection, centrifuge the plates 5 minutes 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 hours after transfection instead of 48 hours, for instance).
- Verify that the expressed protein does not affect cell viability.

DNA transfection protocol using jetPRIME® in 6-well plates



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