

TRANSFECTION TECHNIQUES

Overview and Technical Tips

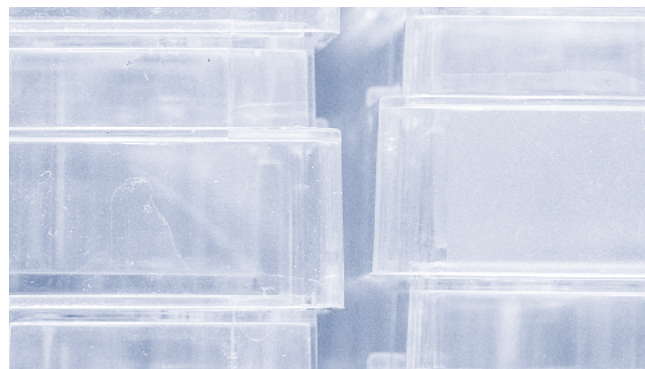
CONTENTS

3-7 Introduction

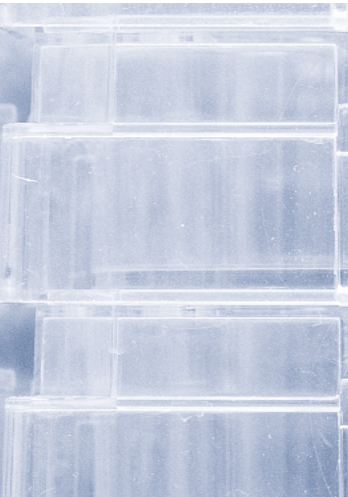
8-14 Transfection Techniques
Cationic-lipid mediated transfection
Calcium phosphate transfection
Viral transfection
Electroporation

15-16 FAQs and Troubleshooting

17 Contact Us



INTRODUCTION



- A transfection is a process that forces nucleic acids into a cell.
- Different methods are used for a transfection (chemical, physical or biological method).
- The transfected nucleic acids may stay transient or stable in the cell, dependent on the method of transfection.

Transfection:

- Nucleic acid transfer into eukaryotic cells.

Transformation:

- Non-viral DNA transfer into non eukaryotic cells, bacteria, plants.

Transduction:

- DNA transfer mediated by a virus.

INTRODUCTION

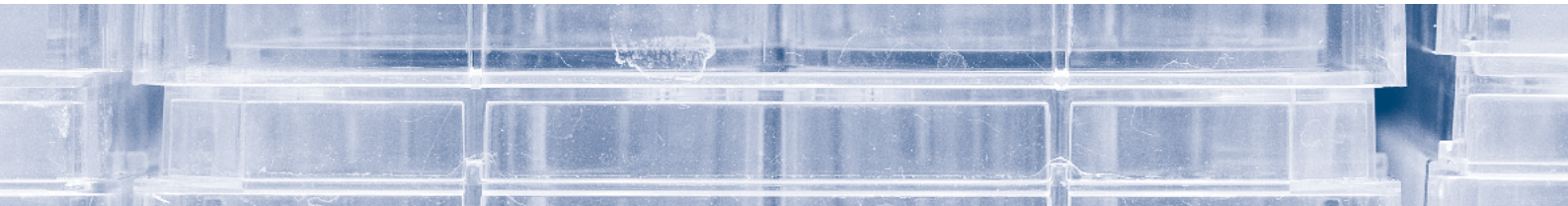
Gene Expression Vs. Gene Inhibition

Gene Expression

- Transfection is used to overexpress a protein-of-interest.

Gene Inhibition

- Transfection is used to down-regulate a protein-of-interest via RNA interference (RNAi).
- miRNA, shRNA or siRNA can induce gene silencing.



INTRODUCTION

Transient Vs. Stable Transfection

Transient Transfection

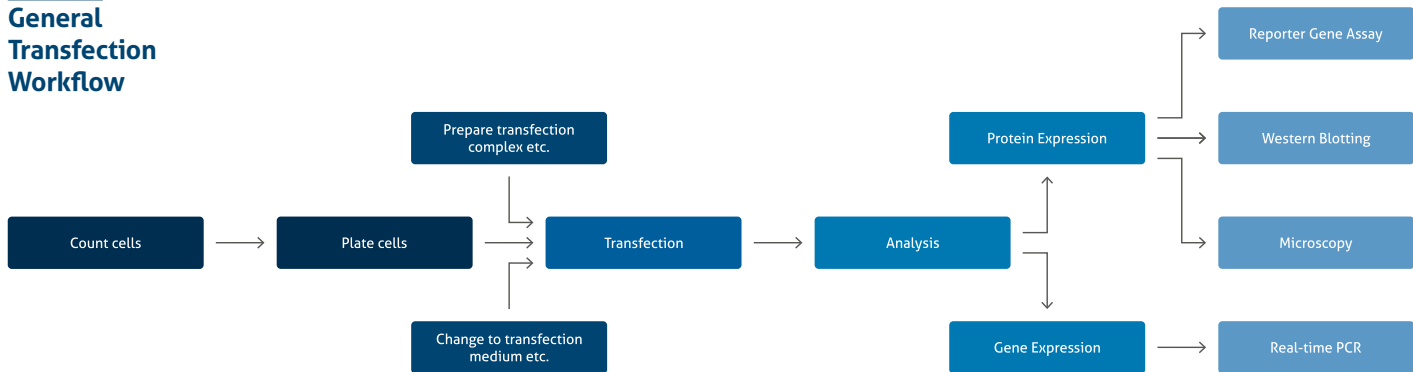
- The transfected nucleic acid stays in the cell for a limited period of time.
- The transfected material gets lost during cell division.
- Transfection effects can be observed normally between 1-7 days after transfection.
- Quick and easy.

Stable Transfection

- The transfected material is integrated into the genome.
- Uptake of foreigner DNA material into the genome requires optimal transfection conditions and selection of positive cells.
- Experiment needs approx. 1 month.

INTRODUCTION

General Transfection Workflow



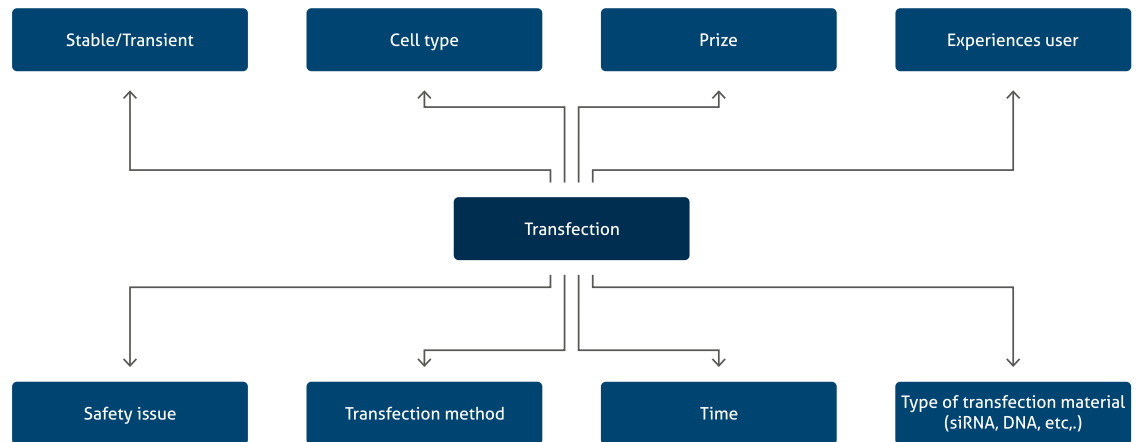
INTRODUCTION

Main Factors Influencing A Transfection

Factor	Suggestion
Cell health	Cell and transfection technique appropriate media should be used.
Cell culture	Correct cell density is needed (40-70%). Low cell passage. Transfection efficiency might change with number of cell passage.
Transfection material	Optimal amount of transfection material has to be validated for each experiment (cell type, cell number, transfection method). Use high quality/pure material.

TRANSFECTION TECHNIQUES

How To Plan A Transfection



In Conclusion

- These factors have to be considered before starting a transfection.

TRANSFECTION TECHNIQUES

Overview of the most common transfection techniques		
Technique	+	-
Cationic-lipid mediated transfection	<p>Fast. Easy. Commercially available. Transient/stable transfection. Also for <i>in vivo</i> use suitable.</p>	<p>Some cell lines can't be transfected. Mainly serum sensitive. Optimization necessary, transfection efficiency highly depends on the cell line.</p>
Calcium phosphate transfection	<p>Fast. Easy. Transient/stable transfection. Also for <i>in vivo</i> use suitable.</p>	<p>Transfection efficiency depends on cell type. Careful preparation of reagents. Cytotoxic. Reproducibility. Not for <i>in vivo</i> use.</p>

TRANSFECTION TECHNIQUES

Overview of the most common transfection techniques

Technique	+	-
Viral transfection	For difficult to transfect cells. High transfection efficiency.	Experienced user. Cytotoxic for cells. Biosafety 2 lab might be required. Laborious experiment. Expensive.
Electroporation	Suitable for primary cells. Quite easy handling. Reproducible results. Fast set-up.	Initial acquisition costs. Optimization for each condition required.

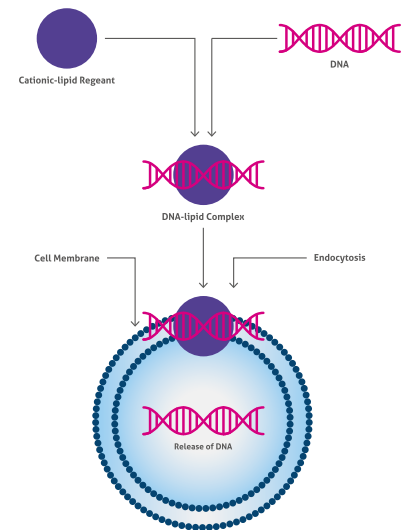
TRANSFECTION TECHNIQUES

Cationic-lipid Mediated Transfection

The method relies on the formation of a complex between the DNA and a cationic lipid-based reagent.

The complex is taken up by the cell via endocytosis.

- Fast and easy.
- Prize range of transfection reagents varies.
- Not working in all cell types.
- Transfection results may vary.
- Transfection success depends on quality of transfection reagent.

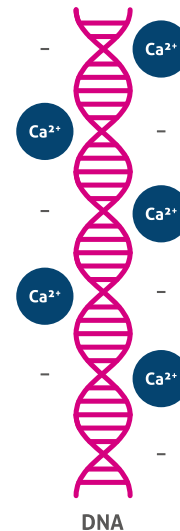


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Calcium Phosphate Transfection

The method is based on the formation of precipitates of calcium phosphate and DNA. The precipitates are taken up by the cell via endocytosis.

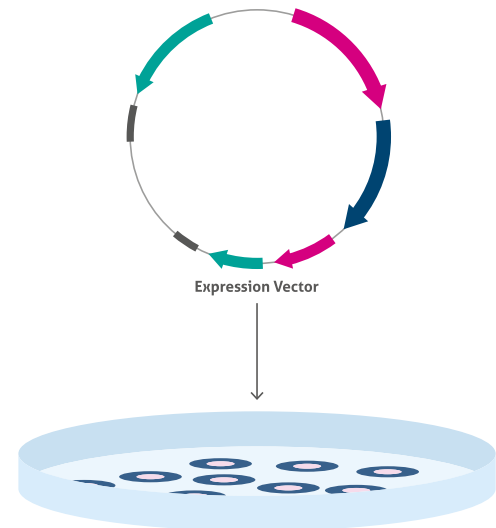
- Easy protocol.
- Not expensive.
- Low transfection efficiency compared to other transfection reagents.
- Works well with most cell lines.
- Toxic for most primary cells.
- For transient and stable transfection.
- Sensitive to small changes in pH/temperature, does not work with high phosphate buffers.



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Viral Mediated Transfection (Transduction)

- Adeno-, Retro- or Lentivirus are used.
- Alternative for difficult to transfect cells.
- Suitable for protein overexpression or protein silencing.
- Additional pre-transduction steps are required:
 - Design of the virus mediating the transgene.
 - Production/purification of virus particles.
 - Virus titer estimation.

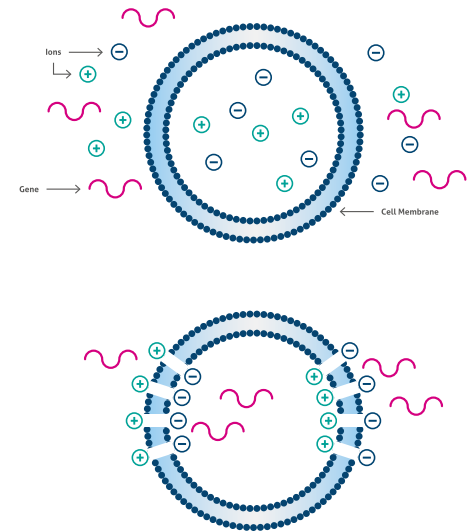


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Electroporation

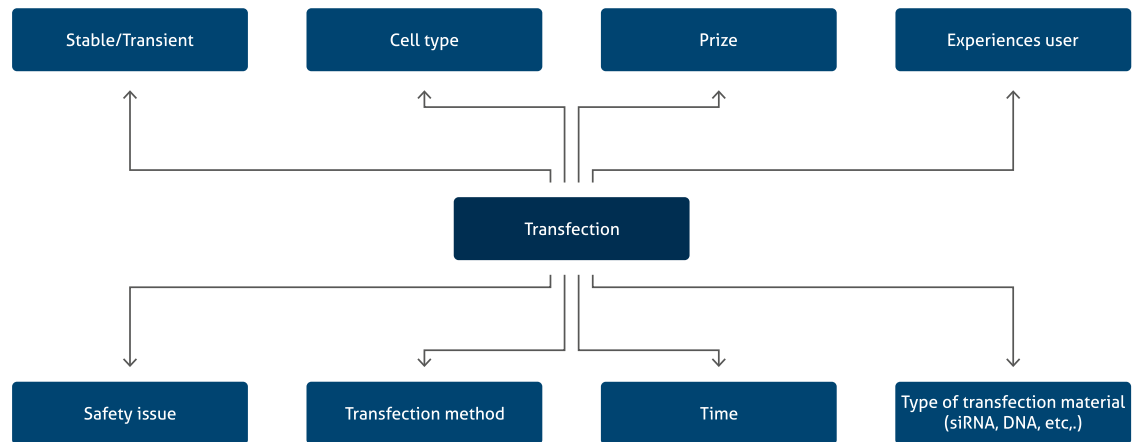
The method uses an electric field that transiently permeabilizes the membrane. During this period the foreigner material can enter the cell.

- Works well with almost all cell types.
- High transfection efficiency.
- Requires optimization as cell permeability can lead to cell damage.
- Experienced user needed.
- Quite expensive method.



FAQS AND TROUBLESHOOTING

How To Plan A Transfection



In Conclusion

- These factors have to be considered before starting a transfection.

FAQS AND TROUBLESHOOTING

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