



Analysis for Plasmid DNA Topologies

The only reliable method to quantify plasmid forms

Quantification of the biologically active ccc (covalently closed circular/supercoiled) plasmid forms for:

- Reproducible transfections
- O Storage stability control of plasmid DNA
- Batch release for DNA vaccination, cell- and gene therapy
- Drug delivery monitoring, e.g. jet injection, gene gun, etc.
- Formulation of DNA
- OAdjustment of plasmid ratio for co-transfections







The CGE analysis is a proprietary analytical tool of PlasmidFactory.

The CGE service is available for any kind of plasmid DNA and for your individual purpose.

It is also offered in combination with PlasmidFactory's manufacturing service. When you order plasmid DNA in *ccc-Grade* qualities, analysis of the topology distribution by CGE is already included in the price.

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CGE Service Analysis for Plasmid DNA Topologies

Background:

Plasmid DNA appears in different topologies. Intact plasmids are covalently closed circular (ccc-form) and negatively supercoiled.

The most important topology with respect to an effective gene transfer *in vitro* as well as *in vivo* is the ccc-form because of its small size which makes it easier to pass the cell membrane and therefore to transfer it into the cell.

Single strand breaks due to enzymatic activity or mechanical stress lead to a relaxation of the DNA molecule. The resulting form is the so called open circular (oc) topology. Restriction endonucleases digest the DNA at certain positions resulting in the linear form. Mistakes in the cellular replication procedure and homologous replication lead to multimeric plasmid forms. These multimers are e. g. concatemers of two or more monomer forms, where also ccc, oc and linear forms can be observed.

The different topologies can be visualized using an agarose gel electrophoresis with subsequent staining of the DNA bands using a fluorescent dye. However, the identification of the respective bands is difficult because the mobility of the different structures changes during the electrophoretical procedure. For most plasmids the ccc monomer is the fastest moving form. The oc monomer and ccc dimer very often appear as one band and cannot be distinguished by an agarose gel electrophoresis. Here, the CGE is the only reliable tool.



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Capillary gel electropherogram and different types of plasmid topology: A-B: ccc-forms (monomer and dimer) C-D: linear-forms (monomer and dimer) E-F: oc-forms (monomer and dimer)

Literature:

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