

## Masterarbeit – Master Thesis

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### Creation of a cell-free chloroplast extract from *Cannabis sativa* and *Nicotiana tabacum* for biotechnological prototyping

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#### Beschreibung des Projekts – Project Description

Chloroplast biotechnology is mainly based on biolistic transformation and afterwards tedious regeneration of homoplasmic plants carrying transgenic chloroplasts. Unfortunately, this time-consuming creation of results easily takes months, creating a detrimental bottleneck during research purposes. Coupled transcription and translation in cell-free chloroplast extract could be a suitable way to shorten this process as a means of prototyping constructs for proper processing before biolistic transformation. Building upon the work of Clark et al. (unpublished) and the project of the Marburg 2021 iGEM project, a working cell-free chloroplast extract from *Cannabis sativa* and *Nicotiana tabacum* shall be created.

#### Aufgabenstellung – Task

During the thesis, you will be isolating chloroplasts using two different protocols. The fitness of the chloroplasts shall be compared between the two isolation protocols. Afterwards, the cell-free extract is created from the isolated chloroplasts and by means of NanoLuc luciferase assay tested for activity. In parallel, heterologous NanoLuc luciferase is produced and isolated from *Nicotiana benthamiana* as a control for the assay. Notable methods include *Agrobacterium* mediated gene transfer, Northern and Western Blotting, chloroplast isolation, *in-vitro* transcription and translation, etc.

#### Vorkenntnisse – Previous knowledge

Not necessary, but a profound interest in plant biotechnology and metabolic engineering is strongly encouraged! Previous knowledge in cell-free transcription and translation is advantageous.

#### Zeitraum – Timeframe

6 months, earliest starting date 3<sup>rd</sup> of November 2022. 9 months when the research internship shall be carried out in advance.

#### Arbeitsgruppe und Ansprechpartner

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