

# WEB318 - COMT von Thomas Vogt

Benjamin Weigel

6th November, 2014

## 1 Introduction

- obtained 4 cultures of COMT expression *E.coli* host from the group of Thomas Vogt
- cultures possess **Ampicillin resistance**
- Culture **COMT 1** was used for plasmid mini prep → sequenced with *pQE\_for* and *pQE\_rev* primers
- COMT1 gene from *Arabidopsis thaliana* (NCBI Accession Number: NM\_124796.3)
- codes for caffeic acid/5-hydroxyferulic acid O-methyltransferase (363 amino acid protein, Accession: NP\_200227)

## 2 Expression

- COMT cultures were incubated over night at 37°C and 200 rpm
- inoculated two 250 mL ZYP-5052 (+ 200  $\frac{\mu g}{mL}$  ampicillin) cultures with 1 mL of COMT 2 each
- incubated at 37°C and 220 rpm from 13.00
- 2030 → OD<sup>600</sup> = 3.8 → cell collection & 1 mL sample
- resuspended cells in 60 mL **lysis buffer** (50 mM Tris/HCl, 0.5 M NaCl, 10% glycerol, 10 mM Imidazole, 1% Tween-20, pH 7.4)
- added a spatula tip of lysozyme and incubated on a shaker platform at room temp for 15 min
- lysed cells by sonication (3x @ 70% amplitude, 30 s, 1 s on-off cycle)
- centrifuged at 10.000 x g, 4°C for 30 min to remove debris → **Prepped IBs from debris**
- filtered the cleared lysate and subjected to metal affinity chromatography (ÄKTA, HiTrap Talon 1 mL FF)  
**Wash Buffer:** 50 mM Tris/HCl, 0.5 M NaCl, 10% glycerol, 10 mM Imidazole, pH 7.4  
**Elution Buffer:** 50 mM Tris/HCl, 0.5 M NaCl, 10% glycerol, 250 mM Imidazole, pH 7.4
- no protein eluted :( → redo with more time and LB, TB, ZY medium