

# WEB321 - COMT expression test

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## 1 Day 1

- 3 mL over night culture (+ 200  $\frac{\mu g}{ml}$  Amp) inoculated with toothpick of COMT (3) from Th. Vogt

## 2 Day 2

- three different media for expression test (**LB**, **TB** and **ZYP-5052**)
- 20 mL cultures (+ 200  $\frac{\mu g}{ml}$  Amp) inoculated to  $OD^{600}=0.075$  from overnight culture ( $OD^{600}=5.59$ )
- started incubation at 10.00

### **LB:**

incubated at 37°C & 220 rpm until  $OD^{600}=0.6 - 1$   
added 1 mM after 2 hours ( $OD^{600}=0.8$ ) & took **pre-induction sample**  
set on ice briefly  
incubate at 30°C & 220 rpm for 5 hours (17.00)  
at harvesting  $OD^{600}=4.9 \rightarrow$  **post-induction sample** & fractionated into soluble and insoluble

### **TB:**

incubated at 37°C & 220 rpm until  $OD^{600}=0.6 - 1$   
added 1 mM after 2 hours ( $OD^{600}=0.9$ ) & took **pre-induction sample**  
set on ice briefly  
incubate at 30°C & 220 rpm for 5 hours (17.00)  
at harvesting  $OD^{600}=8.0 \rightarrow$  **post-induction sample** & fractionated into soluble and insoluble

### **ZYP-5052:**

incubated at 30°C and 220 rpm for 7 hours (17.00)  
at harvesting  $OD^{600}=5.7 \rightarrow$  **post-induction sample** & fractionated into soluble and insoluble

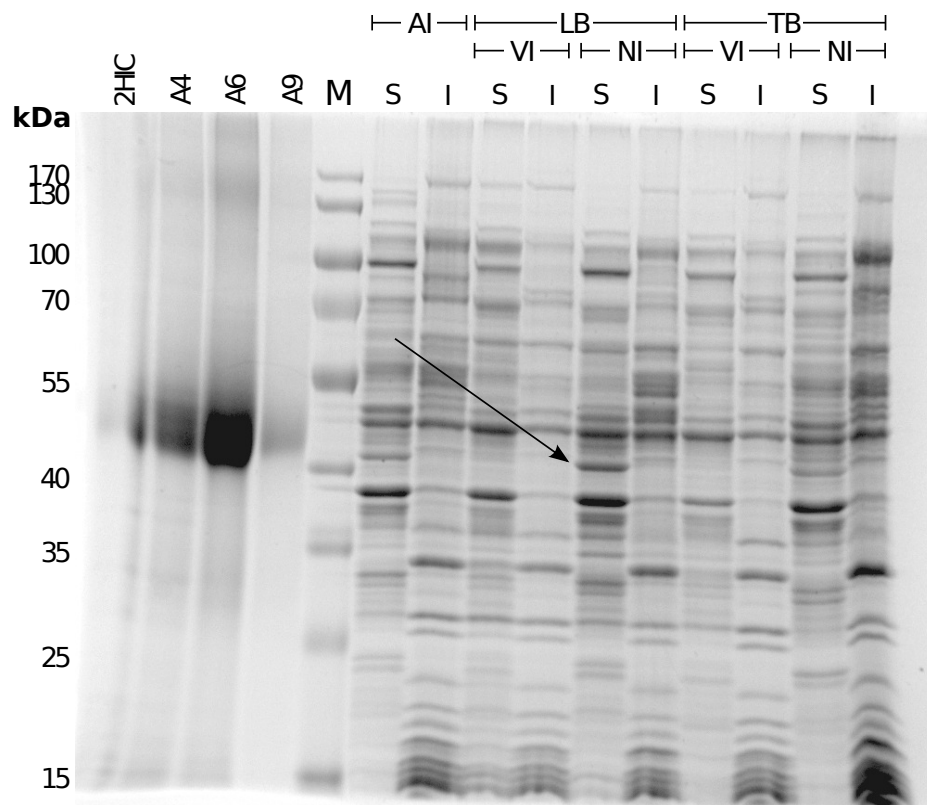


Figure 1: SDS-PAGE of Expressiontest. M - Marker, S - soluble fraction, I - insoluble fraction, AI - autoinduction medium, LB - LB-medium, TB - TB -medium, VI - pre-induction, NI - post-induction. The arrow indicates the suspected COMT protein.