## **PFOMT-Kristallisation 28.10.2013**

Reservoir:

100 mM Hepes/NaOH, 0.2 M CaCl<sub>3</sub>

→ with pH 6.5-7.5 and 15-25% (w/V)PEG-4000

## Other solutions:

250 mM MgCl<sub>2</sub> in ddH<sub>2</sub>O

250 mM Quercetin in DMSO

250 mM Eriodictyol in DMSO

wt - 16.12 mg

Vor. 17:74 14.81 E YS1R NZOZW > E=0.1). 6.858 mg (Var) 71 222 0.71 (wt)

Apl + 1 pm

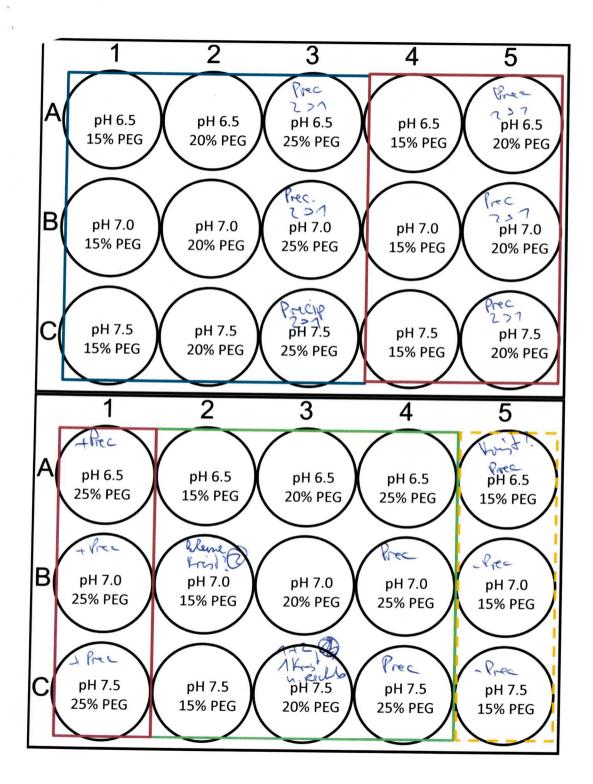
1. Concentrate Protein in centrifugal filter and rebuffer into 10 mM Tris/HCl pH 7.5

2. Readjust concentration to 6.3 mg/mL with 10 mM Tris/HCl pH 7.5

## For crystallization of:

- A) Original (Paper, only wt): add 1:1000 250mM MgCl2 (final 250  $\mu$ M), 1:10.000 250 mM Quercetin (final 25  $\mu$ M), 1:20 5 mM SAM (final 250 µM)
- Without substrate (only wt): add 1:1000 250mM MgCl2
- **SAE + ED (wt and variant):** add 1:1000 250mM MgCl2 (final 250  $\mu$ M), 1:1000 250 mM Eriodictyol (final 250  $\mu$ M), 1:20 5 mM SAE (final 250 μM)
- **SAE + Q (wt and variant):** add 1:1000 250mM MgCl2 (final 250  $\mu$ M), 1:1000 250 mM Quercetin (final 250  $\mu$ M), 1:20 5 mM SAE (final 250 μM)

E) SAM: 250Mh My C12, 250MM SAM



A) wt - 250 μM MgCl2,25 μM Quercetin,250 μM SAM, 2,5% DMSO

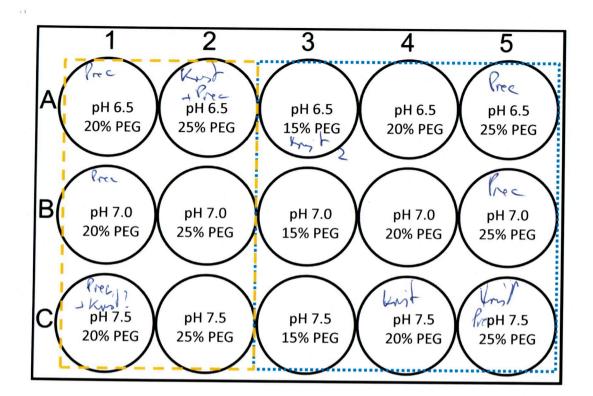
B) wt - 250 μM MgCl2– no other effectors (e.g. substrates)

C1) Wt - 250  $\mu$ M MgCl2, 250  $\mu$ M SAE, 250  $\mu$ M ED

**C2) Variante -** 250 μM MgCl2, 250 μM SAE, 250 μM ED

(D) 1:1:1

2 7 Z



**C2) Var -** 250 μM MgCl2, 250 μM SAE, 250 μM ED

**D) wt** - 250 μM MgCl2, 250 μM SAE,

Wt > 1:2,600 ~ and 12 m/ml VP Vor - 1:200 2.000 > and 12 m/ml VP A) 20,3 we tolal who Portun Puther 40 ml + worl A + 6 Sport Tas 238 18 me (4) 2 pl Owok Zane 200 mb by 12 100 pl 54 M 348 pl Tris 1 ml (3) Zal by C/2 598 pl Tim Butter (O) 1 ml I pl by C/2 In Broodyel JONE SAE 94Pel Tus 1:105-VD) 9 4320 Ens 1.20 19:20 1 +19

· auf 12 mg/nl Wodanne, soolans Kistallisations long: rope Post (12 m/ml) - 10 pl Othertone - 6 m/ml total find core 4x Estelhorlory
A) 1 M Quecete (25 mh) 0,1 mh 1 ml MC/2 (250 m h) sol my SOLL SAM (Jum) 1 mM ad to 250 pl Vos (198) A) Inl Quercete (2, Sum) Inl hyte (25 mm) Spel SAM CSM. 8) sul hale la ad to 250 pel mis (249) C) Int by C/2 (2Pach) I way Int Erischetyof (270 hm) I m/h 144 Soul SAE (Sinh) 1 mm ad to 250 pel Pus (198)

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250 ml 250 ml romb Tois (AC) romh This Hel PHF 6HJ-2

O.Zh Call

wmit Hopes pH 7 20 mm Hepes O.4 M Cally

Paper X-tol Bulle 20% PEG 4000 O.Z.M. Cacl, roomer Hepes (NaOH PH7

- 25 ml 100ml and pt 65-17/75 400mm Hepes +25 ml ddH, 0 0.8M CoCI 2 Ve A Soul ALB Hep. romm Hepes ATTOG Cack . THO O.4mM Callz PH 6.5 /7/7.1 11.76 je 3x Finl + 15/20/25/, PEG + Trme dol #, 0 to some 10 mm Hepes O. 2 mm caclz 15/20/25 /64

pH 65/7/75

5 ml Hepes (pH x) + 5 me PEG (50%) + 4me PEG (TO).)

Sind Hepe) 11%. + 8 ml PEG

Zul ddHro