WEB328 - PFOMT Xtallization

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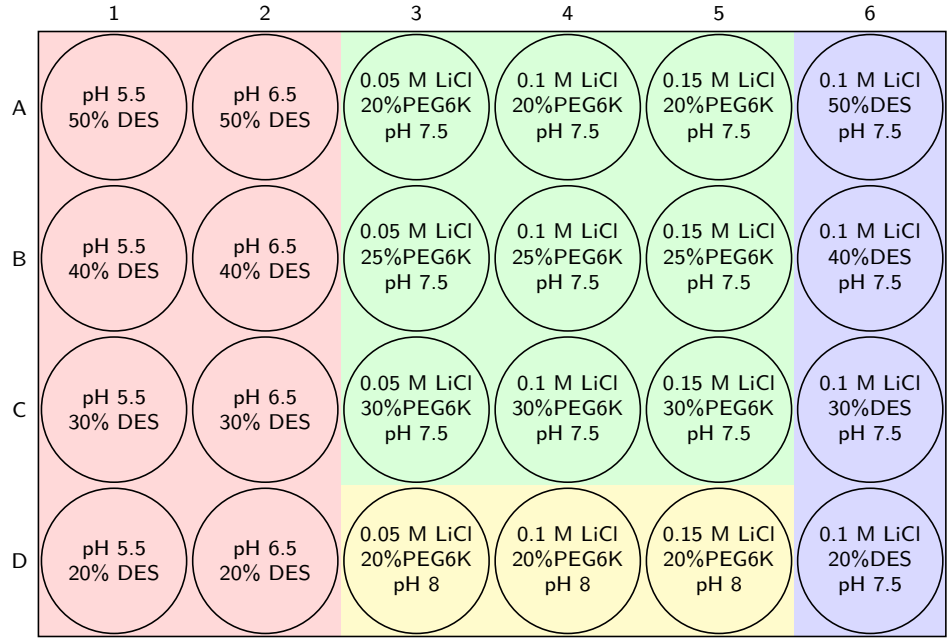

Figure 1: The plate layout for crystallization of proteinase K, lipase B and PFOMT.

component	concentration	volume	stock
SAE	0.25 mM SAE	1 μl	5 mM SAE
$MgCl_2$	0.25 mM	0.2 μl	25 mM $MgCl_2$
eriodictyol	0.25 mM	0.2 μl	25 mM ED
PFOMT	0.262 mM (7.53 mg/mL)	6.4 μl	24 mg/mL PFOMT
	ad to 10 μl water	12.2 μl	

Table 1: freshly prepared PFOMT xtallization solution.

0.1 Buffers used:

A1:D1 → buffer is 0.1 M NaOAc
A2:D2 → buffer is 0.1 M Na-citrate
A3:D5 → buffer is 0.1 M Hepes
A6:D6 → buffer is 0.1 M Hepes

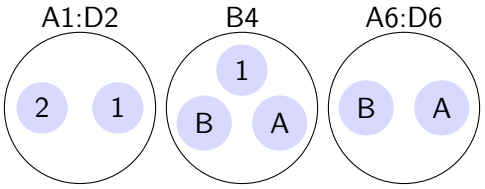

Figure 2: Layout of glass plates in special rows. View from the top. In wells A1 to D1, drop 2 contains proteinase K, drop 1 contains *C. cylindrica* lipase B. In wells A2 to D2, the order of drops is switched. Drop 1 contains Proteinase K and drop 2 contains lipase B. In well B5 drop 1 contains freshly prepared PFOMT (see WEB327) with ED and SAE. Drops B and A contain previously prepared PFOMT xtallization recipes (from WEB293). Wells A6:D6 also contain these preparations. A – 261 uM SAH, $MgCl_2$, ferulic acid and 7.5 mg/mL PFOMT in 10 mM Tris pH 7.5. B – 0.5 mM SAE, 1 mM $MgCl_2$, 10 mg/mL PFOMT.

Well	Drop	Protein
A1:D1	1 (right)	lipase B
	2 (left)	proteinase K
A2:D2	1 (right)	proteinase K
	2 (left)	lipase B

Table 2: Protein in wells.

0.2 Method

500 μl of crystllization buffer was used inside a well. On the glass slide used for crystallization, 1 μl of protein solution was mixed with 1 μl of buffer solution. The small end of a cat whisker was used for seeding. The whisker was dragged along the edges of a previous crystal and then through the crystallization drops. Afterwards the glass slide was put onto the well and placed at 4°C for crystallization.

0.3 Observations

Day 0 - directly after seeding: The drop 1 in wells B1 and C1 already contained crystals shortly after seeding. The drop 2 in wells D2 and C2 also already contained crystals along the seeding line.

Day 4 after seeding: In each well of row 1 and 2 the drops 1 and 2 contain crystals respectively.