WEB324 - Cloning SOMT into GST-tag containing pET41a(+)

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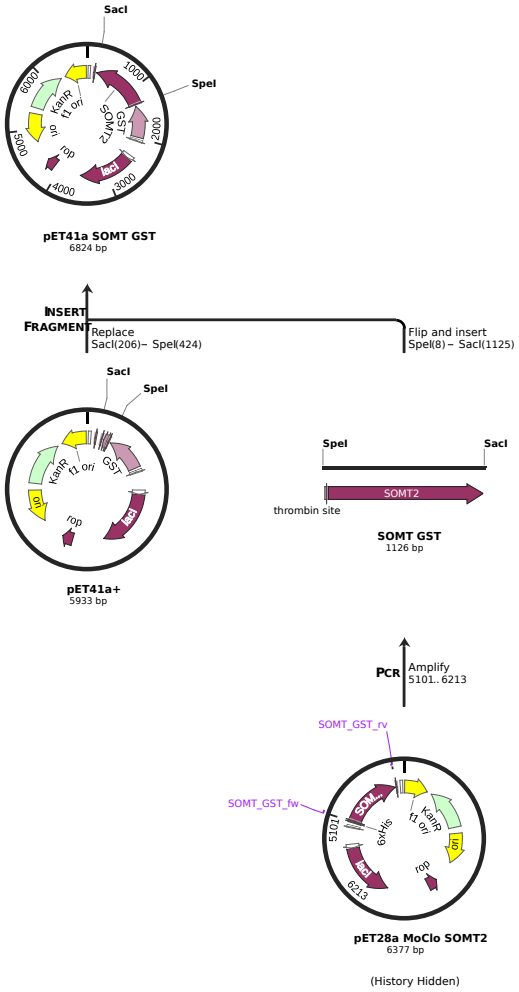

Figure 1: Cloning strategy towards GST-tagged SOMT2. pET41a(+)-SOMT.

1 Cloning to pET41a(+)

ATTENTION: SOMT gene has an endogenous BsaI cleavage site → site needs to be removed

Table 1: The primers used for cloning.

name	sequence [5'→3']
somt_gst_fw	cat cat act agt AGC AGC GGC CTG GTG
somt_gst_rv	tt gaagac aa gag ctc TCA AGG ATA GAT CTC AAT

1.1 PCR SOMT2

- cloning from pET28a MoClo SOMT2 (2) [51 $\frac{ng}{\mu l}$]

Reaction mix:

substance	volume	final concentration
10x KOD Buffer	5 μl	1x
25 mM $MgSO_4$	3 μl	1.5 mM
2 mM dNTPs	5 μl	0.2 mM
51 ng/μl template	0.25 μl	12.5 ng
10 pmol/μl SOMT_GST_fw	1.5 μl	
10 pmol/μl SOMT_GST_rv	1.5 μl	
KOD HS Polymerase	1 μl	1 μl
water	32.75 μl	

PCR Program: theoretical size = 1100 bp

Step	Description	Temperature	Time	
1	Denaturation	95°C	2 min	
2	Denaturation	95°C	20 sec	
3	Annealing	55°C	10 sec	
4	Extension	70°C	21 sec	repeat **2-3** 24x
5	Final Extention	70°C	2 min	
	Pause	4°C	∞	

Figure 2: Agarose gel of PCR to amplify SOMT fragment

2 Digestion of pET41 and fragment by SpeI (BcuI) and SacI

Plasmid digestion:

component	amount	volume
FD Buffer Green		1 μl
pET41a(+)	1 μg	2 μl
SacI FD		0.5 μl
BcuI FD		0.5 μl
ad to 10 μl MQ-H_2O (6 μl)		

fragment digestion:

component	amount	volume
FD Buffer Green		1 μl
SOMT fragment	1 μg	5 μl
SacI FD		0.5 μl
BcuI FD		0.5 μl
ad to 10 μl MQ-H_2O (3 μl)		

- digested plasmid was cleaned by agarose gel > cut band > MN gel cleanup (eluted with H_2O: 40 ng/μl)
- digested fragment was only cleaned by MN PCR cleanup (eluted with H_2O: 46 ng/μl)

2.1 Second Ligation

Ligation:

component	amount	volume
T4 ligase buffer	1x	1 μl
cut pET41a(+)	$\sim$20 ng	0.5 μl
cut fragment	?	0.5 μl
T4 ligase		0.25 μl
ad to 10 μl MQ-H_2O (7.5 μl)		

- ligated at 22°C for 1 h and 4°C over the weekend

Colony PCR: Several small colonies → colony PCR (SOMT1, T7_term as primers) → miniprep of clone 3 and sequencing → Sequencing results were screwed up → Ligation didn't work. → **ligate again**

Colony PCR:

component	amount	volume (* samples)
DreamTaq Buffer	1x	2 μl
SOMT1 primer		0.4 μl
T7_term primer		0.4 μl
dNTP mix		0.4 μl
DreamTaq Polymerase	0.5 U	0.1 μl
ad to 20 μl MQ-H_2O (16.7 μl)		

For more samples the volumes need to be multiplied by the number of samples.

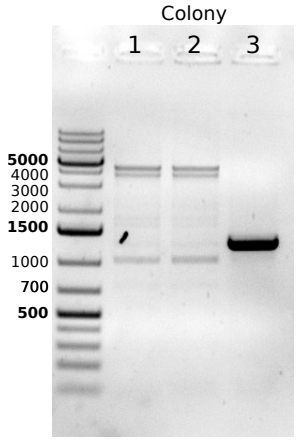

Figure 3: Agarose gel of colony-PCR. The theoretical size of the fragment, if the ligation was successful is 1216 bp. Only clone 3 gave a fragment with s comparable size. This clone was sequenced.

2.2 Second Ligation

Ligation:

component	amount	volume
T4 ligase buffer	1x	1 μl
cut pET41a(+)	~20 ng	0.5 μl
cut fragment	18.98 ng	0.41 μl
T4 ligase		0.25 μl
ad to 10 μl MQ-H_2O (7.84 μl)		

- ligated at 22°C for 1 h and 4°C over night

No colonies grew on the plate.