Strain conservation

Date: 2022-08-17
Tags: *1_Wetlab 3_Microbiology*
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After cloning or receiving of a new genetic construct, every strain is conserved in the {strain collection|aim}. In order to confirm the DNA sequence of the construct, sequencing is performed before conservation.

{27.04.2022|Date of experiment}

The plasmid DNA of a {5 mL LB-Kan|:preculture:} is extracted with the {Macherey-Nagel NucleoSpin|:Plasmid isolation kit:}, following the protocol for {low-copy plasmids|protocol}. Elution was performed in {50|µL|H2O|elution volume} at {70|°C|elution temperature}. The DNA concentration was measured with an {Implen N60 Nanophotometer|device}. Plasmid DNA of three colonies was isolated. The concentrations were {1199.9|ng/µL|(:for:) clone 1|DNA}, {999.3|ng/µL|(:for:) clone 2|DNA}, and {1185.9|ng/µL|(:for:) clone 3|DNA}.

Clone	Concentration [ng/µl]	260/280	260/230
1	1199.8	1.95	2.33
2	999.3	1.94	2.32
3	1185.9	1.94	2.31

Sequencing was performed at {Eurofins Genomics|sequencing service provider} on plasmids of clones 1-3 to confirm the DNA sequence and the success of the mutagenesis. The sequencing primer {TATAAGCAGAGCTGGTTTAGTGAACC|sequencing primer} was used in the premix. According to

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Eurofins instructions, the premix was set up with {5|µL|:plasmid DNA - diluted to 100 ng/µL -:|premix component} and {5|µL| (:of:) 5 µM sequencing primer|premix component}.

Seq. ID	Primer	Sequence	Length [bp]	Tm [°C]	Target	Result
FMA451	FXR_Seq_fw	TATAAGCAGAGCTGGTTTAGTGAACC	26	57	pnoCherry pCMV	G002A
FMA455	FXR_Seq_fw	TATAAGCAGAGCTGGTTTAGTGAACC	26	57	pnoCherry pCMV	G002A
FMA454	FXR_Seq_fw	TATAAGCAGAGCTGGTTTAGTGAACC	26	57	pnoCherry pCMV	G002A
FMA452	FXR_Seq_fw	TATAAGCAGAGCTGGTTTAGTGAACC	26	57	pnoCherry pCMV	WT

Because sequencing was successful for all clones, clone 2 was chosen randomly for cryoconservation.

{05.05.2022|Date of experiment}

A {5 mL LB-Kan|:overnight preculture:} was grown over night for cryoconservation. {Three aliquots per strain|number of cryotubes} of {500|µL|preculture|cryoconservation mix} were mixed with {500|µL|sterile ice cold 50% (w/v) glycerol|cryoconservation mix} each and stored at {-80 °C|storage temperature} in cryotubes. Additionally, {three minipreps of 100 ng/µL|:plasmid DNA:} in {TE buffer|eluent} originating from the same colony were stored at -80 °C in the plasmid collection.