APPENDIX

Buffers and solutions

Table A1. Buffers and solutions used in this study.

Buffers and solutions	Contents	Purpose
PBS (Phosphate Buffered Saline)	0.02 M phosphate, 0.15 M NaCl, pH 7.2	Cell culture, western blotting
0.5 % TBE Buffer	45 mM Tris-borate (Tris base and boric acid), 1 mM EDTA, H_2O	Agarose gel electrophoresis
6 x loading buffer	0.25 % Bromphenol Blue, 40 % Sucrose in H_2O	Agarose gel electrophoresis
SDS 2x sample buffer	1 ml 0.5 M Tris-HCl pH 6.8, 220 μl 87 % Glyserol, 1.6 ml 10 % SDS, 200 μl ß-Mercaptoethanol, 150 μl H_2O	SDS-PAGE
20x MOPS running buffer (available from Invitrogen)	0 mM MOPS, 50 mM Tris base, 0.1 % SDS, 1 mM EDTA, pH 7.7 (for use in electrophoresis, dilute to 1x with water)	SDS-PAGE
Coomassie Blue Solution	40% Ethanol, 10 % Acetic Acid, 0.25 % Coomassie Brilliant Blue R-250, H_2O	Coomassie Blue staining
Destain Solution	30 % Ethanol, 10 % Acetic Acid, H_2O	Coomassie Blue staining
20x Transfer buffer (available from Invitrogen)	25 mM Bicine, 25 mM Bis-Tris (free base), 1 mM EDTA, pH 7.2 (for use in electrophoresis, dilute to 1x with water and ethanol)	western blotting
Blocking buffer	1 % BSA, PBS	western blotting
Wash buffer	0.05 % Tween-20, PBS	western blotting
Buffer for antigen and conjugate solutions	1 % BSA, 0.05 % Tween-20, PBS	western blotting
Substrate-chromogen solution	10 ml TBS buffer, 2 ml alfa-chloro-naftol, 6 μl 30 % H_2O_2	western blotting
TBS (Tris Buffered Saline)	20 mM Tris, 150 mM NaCl, adjust pH to 7.6 with HCl	western blotting
Sucrose solution	20 % (w/v) sucrose, 0.3 M Tris-HCl, pH 8.0, 1 mM EDTA	periplasmic preparation

SDS-PAGE

Figure A1. Precition PLUS Protein™ Standards from BIO-RAD. The All Blue Standard was used as reference for SDS-PAGE, and are marked in red.

AGAROSE GEL ELECROPHORESIS

1 Kb ladder or 100 bp ladder from Fermentas was used as standard for agarose gel electrophoresis. (fig.A2 and A3)

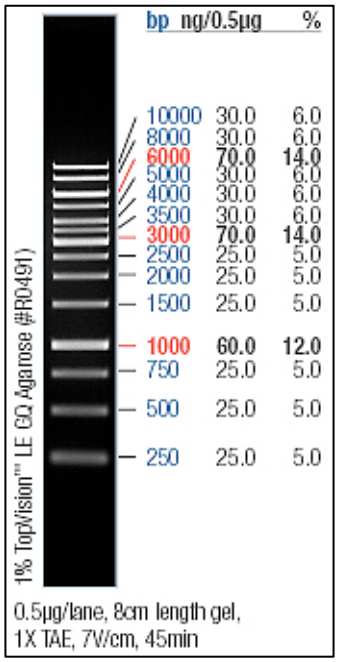

Figure A2. O'gene Ruler 1 Kb DNA ladder.

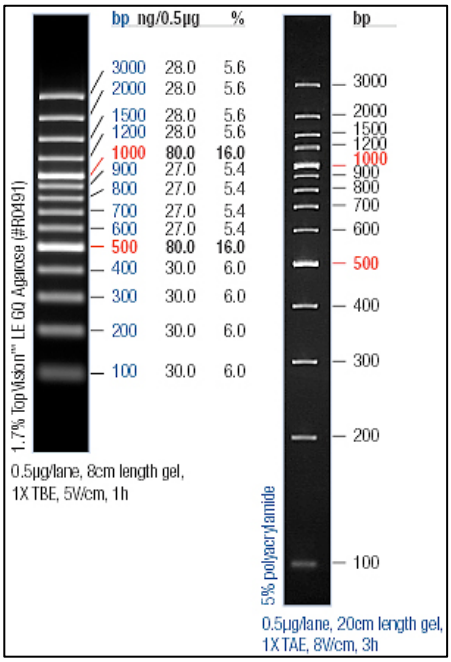

Figure A3. O'gene ruler 100 bp DNA ladder PLUS (right)

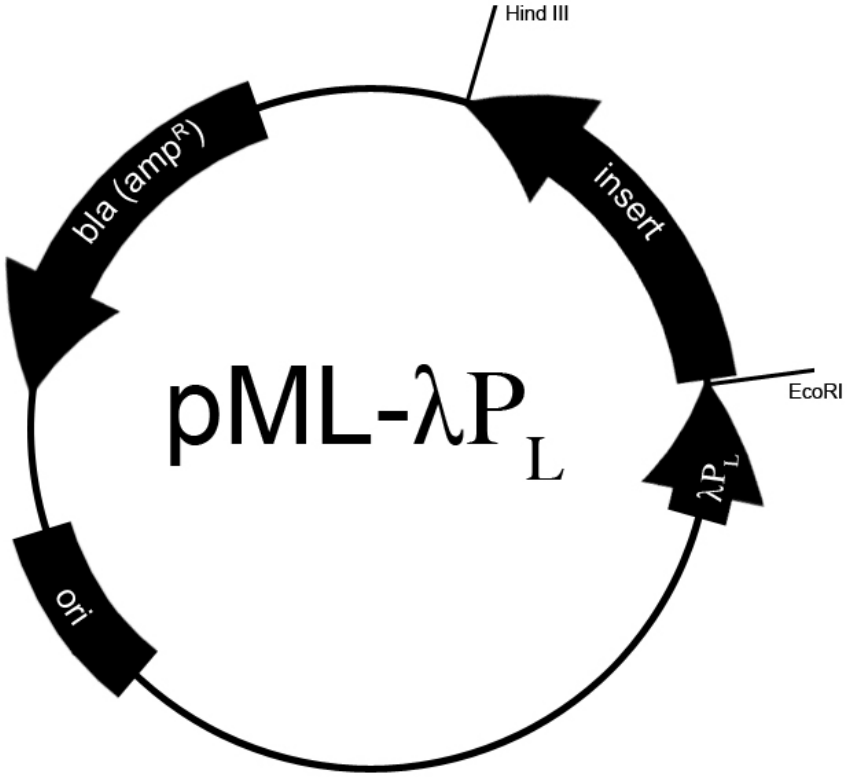

Figure A4: pML- λP_L expression vector:
λP_L promoter
ori
bla (amp^R)

A gene insert cut out with BsaI restriction enzyme from the cloning vector to make EcoRI/HindIII overhangs can be ligated into a pML-λP_L vector pre-cut with EcoRI and HindIII restriction enzymes.