APPENDICES

APPENDIX A
OPTIMIZED DENV-2 prM-E GENE

5’-

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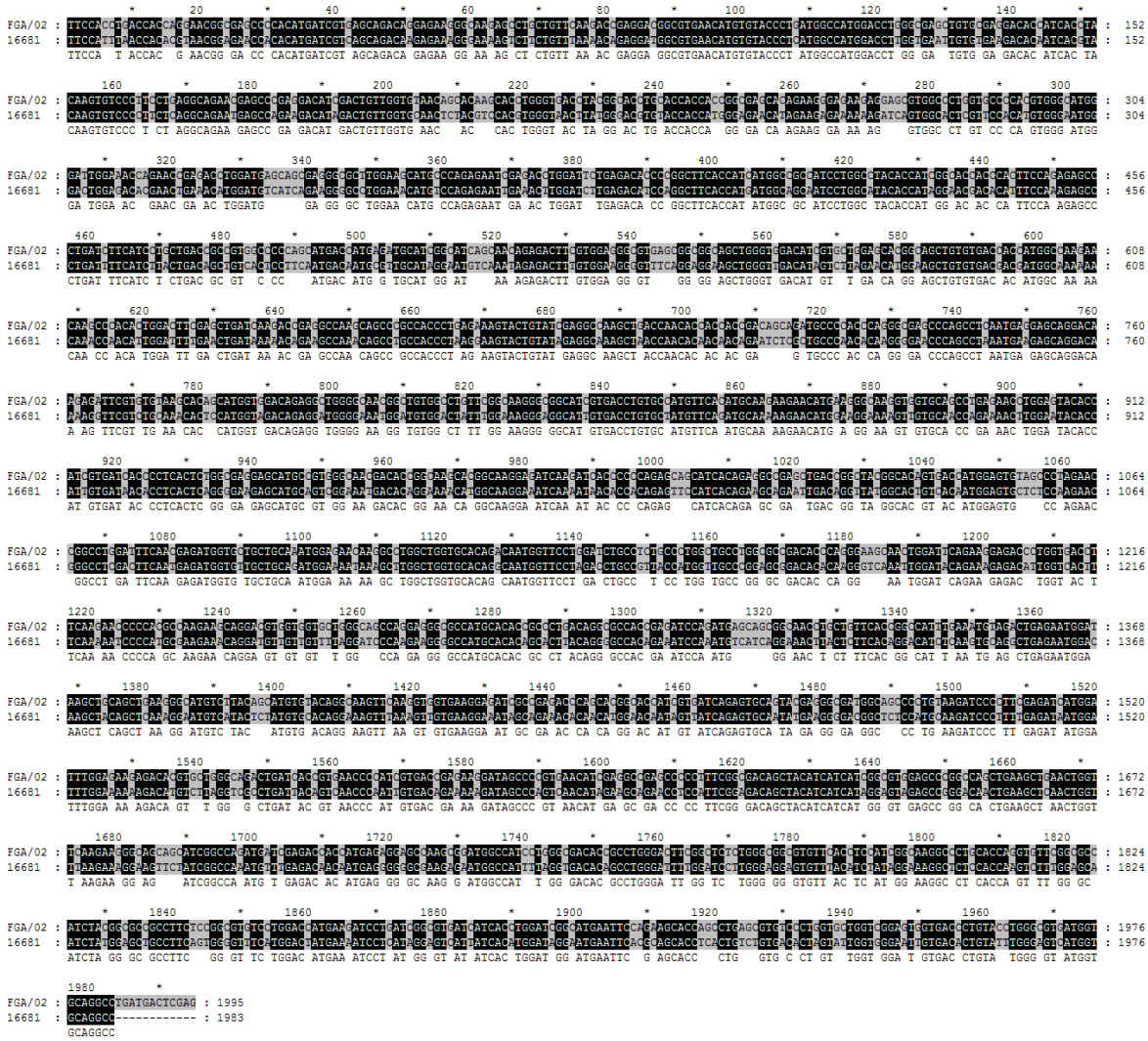
APPENDIX B

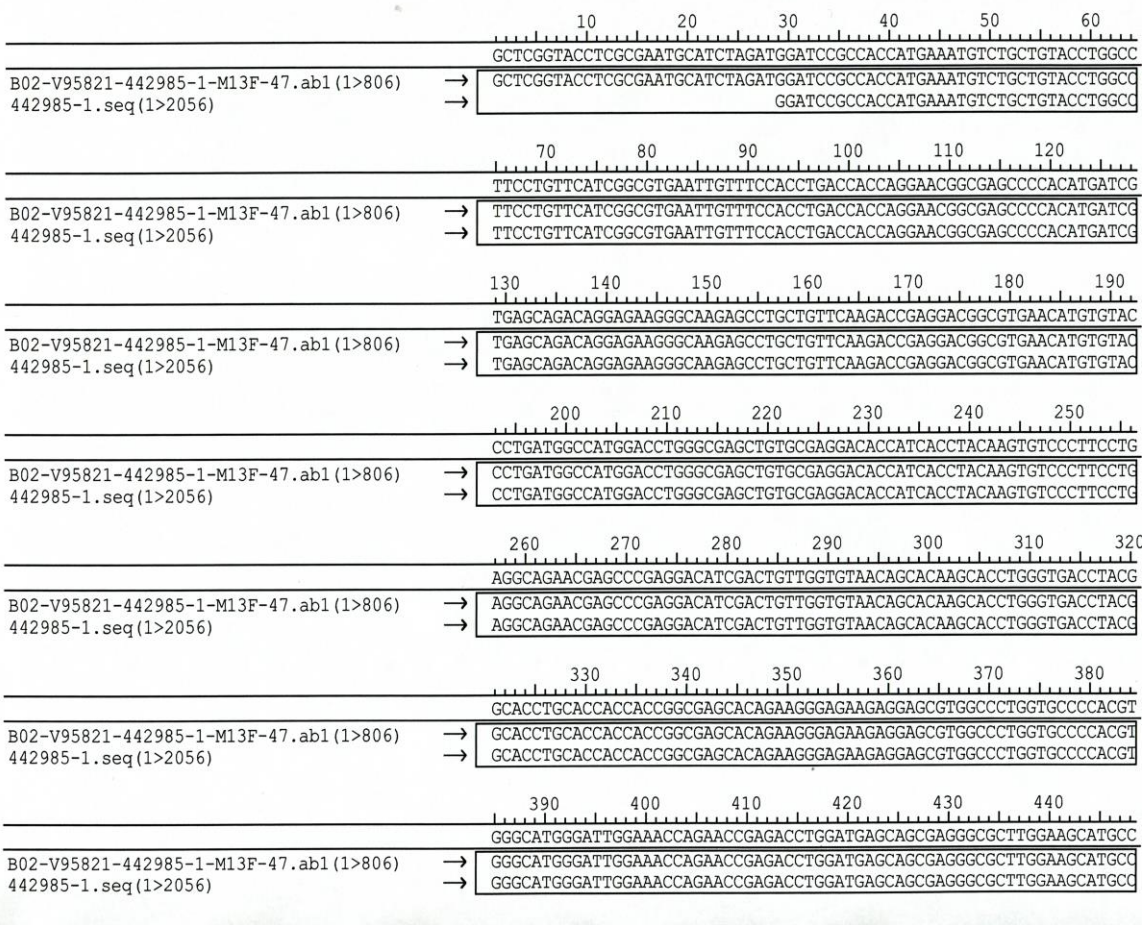
COMPARISON BETWEEN NATIVE DENV-2 prM-E GENE AND OPTIMIZED DENV-2 prM-E GENE

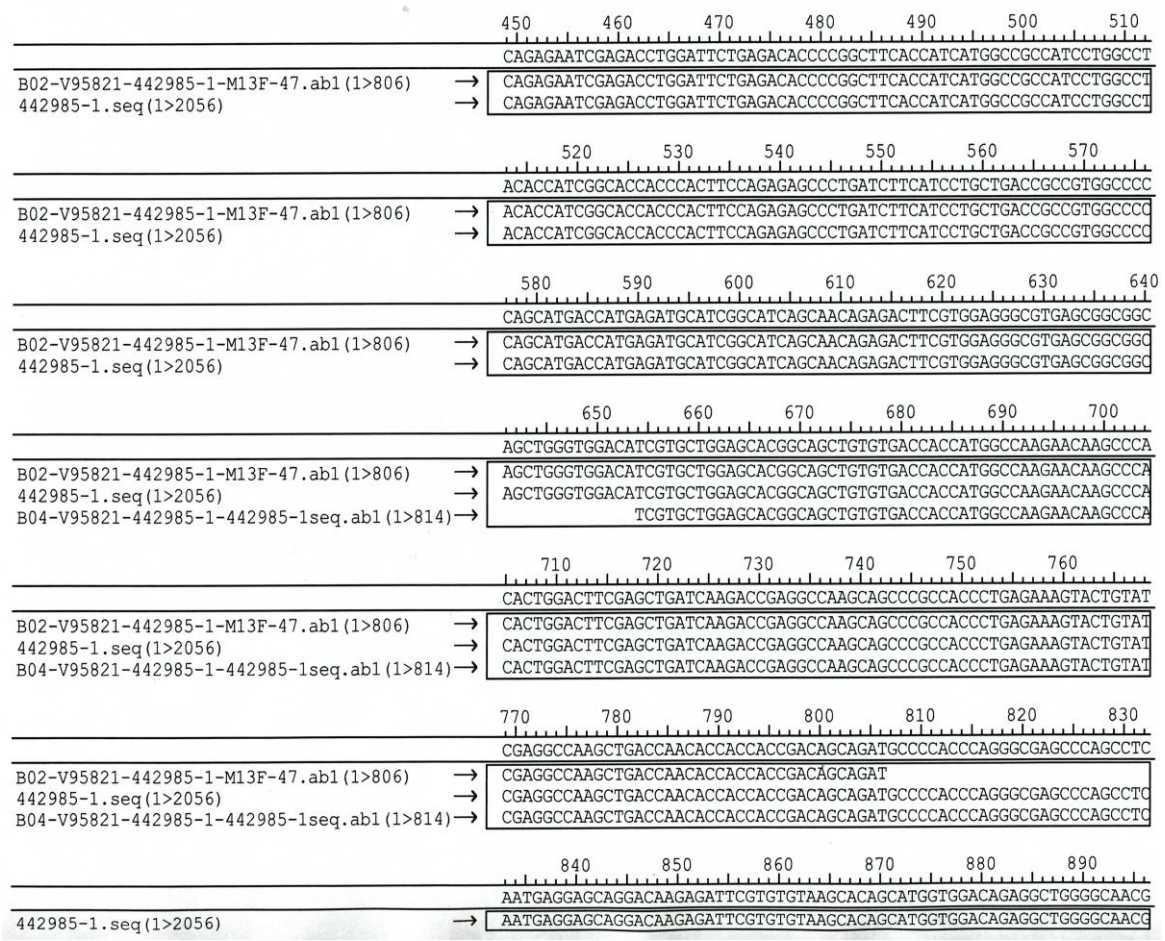


APPENDIX C

NUCLEOTIDE SEQUENCE ALIGNMENT BETWEEN pUC57-D2opt.prM-E CONSTRUCT AND TARGETED INSERTED SEQUENCE

QC Items	Specifications	Results
Sequencing Alignment	Sequencing results are consistent with the targeted insert sequence.	☒ Pass ☐ Fail

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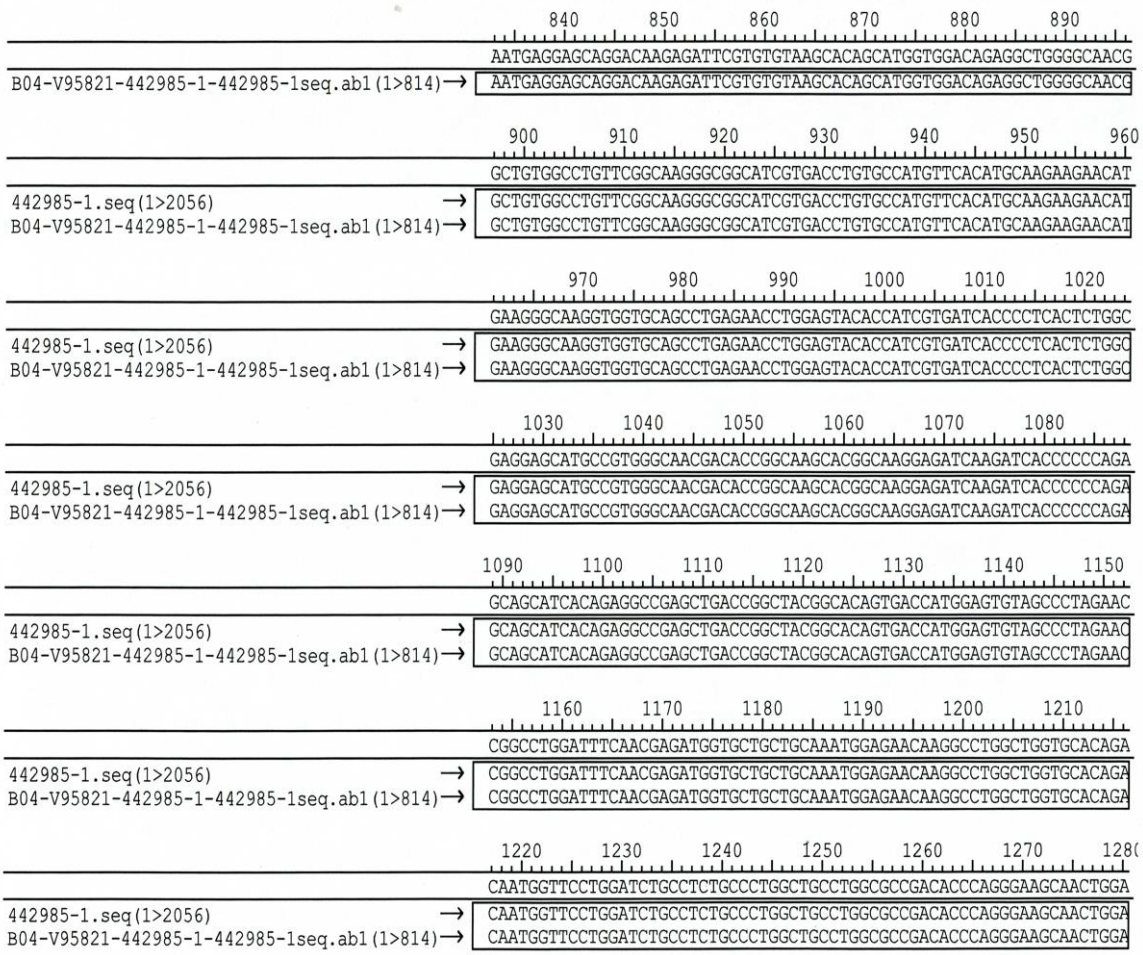
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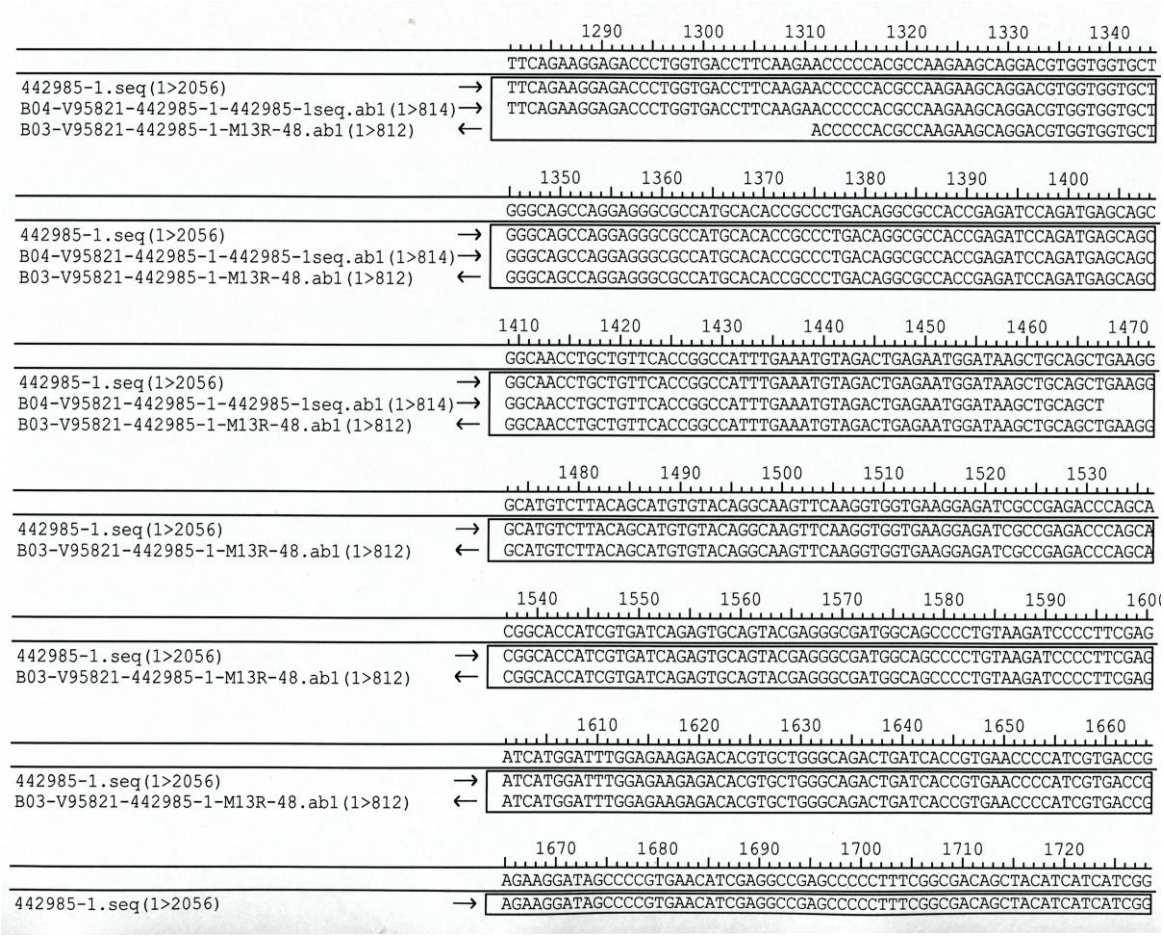
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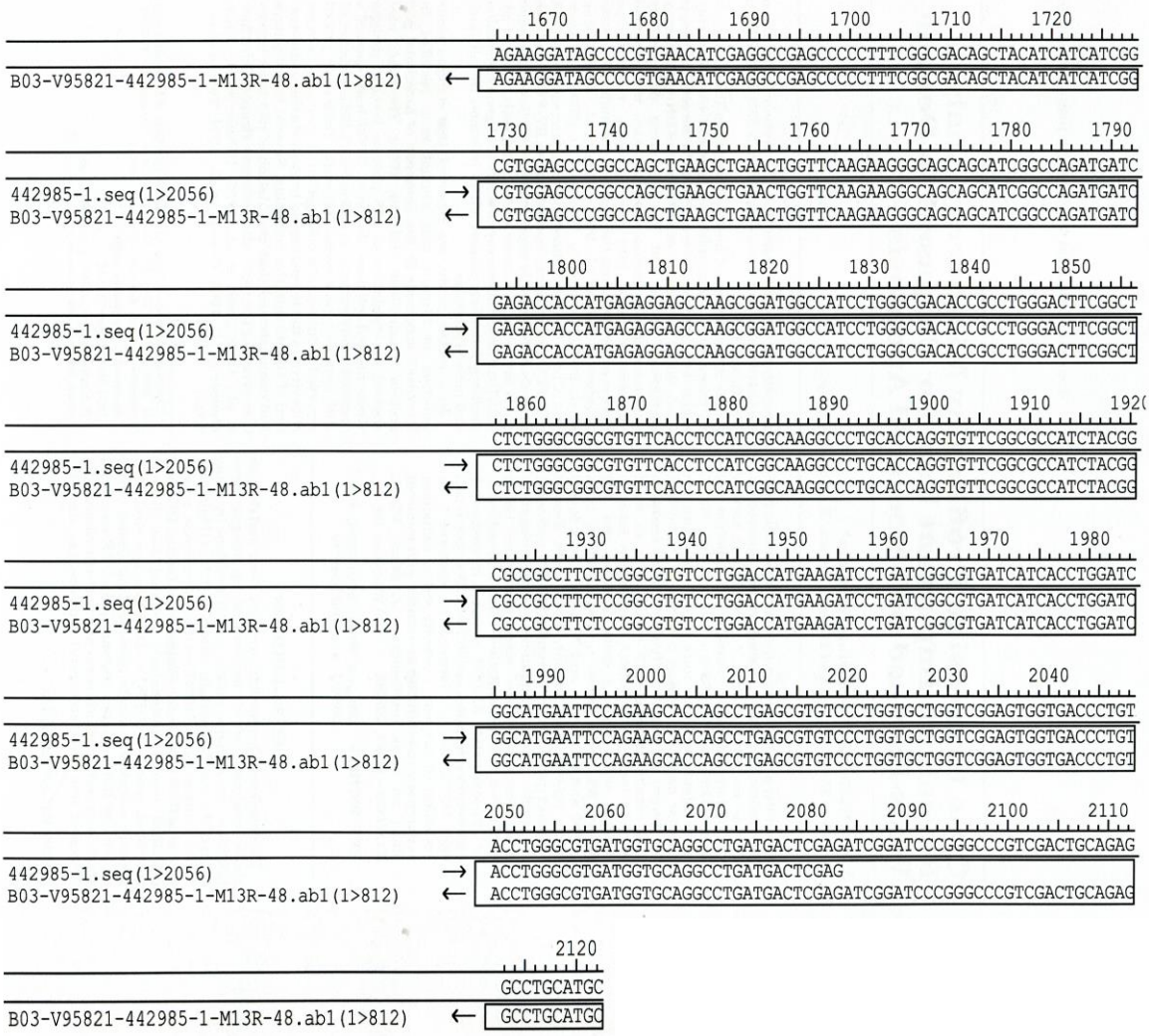
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APPENDIX D
SOLUTION PREPARATION

1. Solutions for agarose gel electrophoresis

1.1	**0.5M EDTA, pH 8.0**	**100**	**ml**
	Na2EDTA (MW 372.2)	18.61	g
	Distilled H_2O	60	ml
	Adjust pH with concentrated NaOH to	8.0	
	Adjust volume with distrilled H_2O up to	100	ml
	store at room temperature		
1.2	**5X TBE**	**1**	**L**
	Tris base	53.89	g
	Boric acid	27.51	g
	0.5 M EDTA, pH 8.0	20	ml
	Adjust volume with distilled H_2O to	1	L
	Store at room temperature		
1.3	**0.5X TBE**	**1**	**L**
	5X TBE	1	L
	Distilled H_2O	100	ml
	Store at room temperature		
1.4	**1% agarose in 0.5X TBE buffer**	**100**	**ml**
	Agarose	1	g
	0.5X TBE buffer	100	ml
	Boil until solution is clear		

2. Solution for bacterial culture and cloning

2.1 LB both	**1**	**L**
NaCl	10	g
Tryptone	10	g
Yeast extract	5	g
Distilled H_2O to	1	L

Sterilize by autoclave at 121 °C, 15 psi for 20 min and store at 4 °C

Add antibiotics as design (Ampicillin 50 or 100 µg/ml, Kanamycin 50 µg/ml final concentration)

2.2 LB agar	**1**	**L**
NaCl	10	g
Tryptone	10	g
Yeast extract	5	g
European bacteriological agar	20	g

Sterilize by autoclave at 121 °C, 15 psi for 20 min and store at 4 °C

To make agar plate, melt LB agar and cool it down to 55-60 °C. Add antibiotics as design (Ampicillin 25, 50 or 100 µg/ml, Kanamycin 50 µg/ml final concentration) and pour into petridishes (approximately 25 ml/plate)

2.3 100 mg/ml Ampicillin (1,000X)	**10**	**ml**
Ampicillin sodium salt	1	g
Sterile MilliQ H_2O	10	ml

Filtrate through 0.22 µm filter and store at -20 °C

2.4 50 mg/ml Kanamycin (1,000X)	**10**	**ml**
Kanamycin	0.5	g
Sterile MilliQ H_2O	10	ml

Filtrate through 0.22 µm filter and store at -20 °C

3. Cell culture media and reagents

3.1	**1X DMEM**	**1**	**L**
	DMEM powder	1	pack
	NaHCO3	3.7	g
	Sterile MilliQ H_2O to	900	ml
	Adjust pH with concentrated HCl to	7.2	
	Adjust volume with sterile MilliQ H_2O to	1	L
	Filtrate through 0.22 µm filter and store at 4 °C		

3.2	**Growth medium for HEK 293 and HEK 393T cell**	**500**	**ml**
	1X DMEM	450	ml
	Heat-inactivated FBS	45	ml
	store at 4 °C		
	Add antibiotics as design (G418 500 – 2000 µg/ml)		

3.3	**1X PBS for cell culture**	**4**	**L**
	NaCl	32.03	g
	KCl	0.81	g
	Na_2HPO_4	2.44	g
	KH_2PO_4	0.76	g
	MilliQ H_2O to	4	L
	Sterilize by autoclave at 121 °C, 15 psi for 20 min and store at room temperature		

3.4	**0.5M EDTA pH 8.0**	**300**	**ml**
	EDTA	55.836	g
	Distilled H_2O to	200	ml
	Adjust pH with concentrated HCl to	8.0	
	Adjust volume with distilled H_2O to	300	ml
	Sterilize by autoclave at 121 °C, 15 psi for 20 min and store at room temperature		

3.5	**0.25 (w/v) Trypsin/EDTA**	**1**	**L**
	Trypsin	2.5	g
	0.5M EDTA pH 8.0	2	ml
	Adjust volume with 1X PBS to	1	L
	Filtrate through 0.22 µm filter and store at -20 °C		

3.6	**0.4% (w/v) Tryphan blue**	**100**	**ml**
	Tryphan blue	0.4	g
	1X PBS	100	ml
	Store at room temperature		

4. Solutions for SDS-PADE and Western blot analysis

4.1	**10% (w/v) SDS**	**100**	**ml**
	Sodium dodecyl sulfate (SDS)	10	g
	Distilled H_2O to	100	ml
	Store at room temperature		

4.2	**1M Tris-HCl pH 6.8**	**100**	**ml**
	Tris base	12.1	g
	distrilled H_2O to	70	ml
	Adjust pH with concentrated HCl to	6.8	
	Adjust volume with distilled H_2O to	100	ml
	Store at room temperature		

4.3	**1.5M Tris-HCl pH 8.8**	**150**	**ml**
	Tris base	27.26	g
	distrilled H_2O to	70	ml
	Adjust pH with concentrated HCl to	8.8	
	Adjust volume with distilled H_2O to	100	ml
	Store at room temperature		

4.4 1M DTT	**32.4**	**ml**
DTT	5	g
distilled H_2O to	32.4	ml
store at -20 °C		

4.5 5X SDS loading buffer	**50**	**ml**
1M Tris-HCl pH 6.8	3.125	ml
10% (w/v) SDS	20	ml
87% glycerol	11.5	ml
Bromophenol blue	7.5	mg
distilled H_2O to	10.375	ml
store at room temperature		
Add 10 µl of 1M DTT before use		

4.6 10X running buffer (stock solution)	**1**	**L**
Tris-base	30.2	g
Glycine	141.4	g
SDS	10	g
Distilled H_2O to	1	L

4.7 1X running buffer (working solution)	**1**	**L**
10X running buffer	100	ml
Distilled H_2O to	900	ml
Freshly preparation before used		

4.8 10% (w/v) APS	**1**	**L**
APS	0.1	g
Distilled H_2O to	1	ml
Store at 4 °C and use within 1 week		

4.9 Polyacrylamide gel

Reagents for 10% separating gel

40% acrylamide/bis-acrylamide	2.5	ml
1.5M Tris-HCl pH 8.8	2.5	ml
Distilled H_2O to	4.8	ml
10% (w/v) SDS	0.1	ml
10% (w/v) APS	0.15	ml
TEMED	0.0125	ml

Reagents for 5% stacking gel

40% acrylamide/bis-acrylamide	0.375	ml
1.5M Tris-HCl pH 6.8	0.38	ml
Distilled H_2O to	2.225	ml
10% (w/v) SDS	0.030	ml
10% (w/v) APS	0.045	ml
TEMED	0.0125	ml

4.10 1X transfer buffer	**1**	**L**
Tris base	3	g
Glycine	14	g
100% methanol	200	ml
Distilled H_2O to	1	L
Freshly preparation and store at -20 °C before used		

4.11 10X TBS (Tris-buffered saline) pH 7.6	**1**	**L**
Tris base	24.2	g
NaCl	80	g
Distrilled H_2O to	800	ml
Adjust pH with concentrated HCl to	7.6	
Adjust volume with distilled H_2O to	1	L
Store at room temperature		

4.12 1X TBS, 0.1% Tween-20	**1**	**L**
10X TBS	100	ml
Tween-20	1	ml
Distrilled H_2O to	1000	ml
Store at room temperature		

4.13 0.2% (w/v) Ponceau S / 1% (v/v) acetic acid	**500**	**ml**
Ponceau S	1	g
Distrilled H_2O to	495	ml
Acetic acid	5	ml
Store at room temperature		

4.14 5% (w/v) Skimmed milk in 1X TBS with 0.1% Tween-20	**100**	**ml**
Skimmed milk	5	g
1X TBS with 0.1% Tween-20	100	ml
store at 4 °C and stir before use		

5. Solution for immunofluorescent assay (IFA)

5.1 1M PBS (10X PBS) for IFA		
NaCl	90	g
Na_2HPO_4	71	g
NaH_2PO_4	60	g
Distrilled H_2O to	800	ml
Adjust pH to	7.4	
Adjust volume with distrilled H_2O to	1	L
store at room temperature		

5.2 0.1M PBS (1X PBS)	**1**	**L**
1M PBS (10X PBS)	100	ml
Distrilled H_2O to	900	ml
store at room temperature		

5.3	**0.3% (v/v) Triton X-100 / 1X PBS**	**100**	**ml**
	Triton X-100	0.3	ml
	1X PBS to	100	ml
	store at room temperature		

5.4	**0.03% (v/v) Triton X-100 / 1X PBS**	**100**	**ml**
	Triton X-100	0.03	ml
	1X PBS to	100	ml
	store at room temperature		