

**Table S1. Recombinant and synthetic peptides and proteins used in this study**

Supplier	Name	Source	Sequence (N – C terminus)	REF
Recombinant production	eGFP	<i>Aequorea victoria</i>	MGSSHHHHHHSSGLVPRGSHMVSKG EELFTGVVPILVELDGDVNGHKFSVSG EGEGDATYGKLTCLKFICTTGKLPVWP TLVTTLYGVQCFSRYPDHMKQHDFE KSAMPEGYVQERTIFFKDDGNYKTRA EVKFEGLTLVNRIELKGIDFKEDGNILG HKLEYNYNSHNVYIMADKQKNGIKVNF KIRHNIEDGSVQLADHYQQNTPIGDGP VLLPDNHYLSTQSALS KDPNEKRDHM VLLLEFVTAAGITLGMDELYK	PMID: 7480149
Recombinant production	17x helix	synthetic	AEEAAAKEAAAKEAAAKA	PMID: 1579220
Recombinant production	TEV site	<i>Tobacco etch virus</i>	ENLYFQG	PMID: 1809930
Recombinant production	LCI	<i>Bacillus subtilis</i>	AIKLVQSPNGNFAASFVLDGTKWIFKS KYYDSSKGYWVGIYEVWDRK	PMID: 1449609
Recombinant production	LFB	<i>Bos taurus</i>	FKCRRWQWRMKKLGAPSITCVRRAF	PMID: 1490908
Recombinant production	TA2	<i>Limulus polyphemus</i>	YSRCQLQGFNCVRSYGLPTIPCCRG LTCRSYFPGSTYGRQRY	PMID: 10473569
Recombinant production	THA	<i>Podius maculiventris</i>	GSKKPVIYCNRRRTGKCQRM	PMID: 8577744
Biomatik (synthetic)	DS01	<i>Phyllomedusa oreades</i>	GLWSTIKQKGKEAAIAAKAAGQAALG AL	PMID: 23029273
Biomatik (synthetic)	THA	<i>Podius maculiventris</i>	GSKKPVIYCNRRRTGKCQRM	PMID: 8577744
Numaferm (recombinant)	DS01-THA	-	MGLWSTIKQKGKEAAIAAKAAGQAAL GALGSKKPVIYCNRRRTGKCQRM	-

**Table S2. PCR programs used in this study**

<b>Step</b>	<b>Temperature (°C)</b>	<b>Time (s)</b>	<b># of cycles</b>
<b><i>Insert PCR – PLICing (primers: F-anchor peptide and R-anchor peptide)</i></b>			
Initial denaturation	98	30	1
Denaturation	98	15	25
Annealing	55	30	25
Elongation	72	30	25
Final elongation	72	180	1
<b><i>Backbone PCR – PLICing (primers: F-pET28a and R-pET28a)</i></b>			
Initial denaturation	98	30	1
Denaturation	98	15	25
Annealing	55	30	25
Elongation	72	210	25
Final elongation	72	300	1
<b><i>Two step PCR – Step 1 (primers: F-eGFP-TAATAA and R-eGFP-TAATAA)</i></b>			
Initial denaturation	98	30	1
Denaturation	98	15	5
Annealing	60	30	5
Elongation	72	210	5
<b><i>Two step PCR – Step 2</i></b>			
Initial denaturation	98	30	1
Denaturation	98	15	20
Annealing	62	30	20
Elongation	72	210	20
Final elongation	72	300	1

**Table S3. Primers used in this study**

<b>Primer name</b>	<b>Sequence 5' – 3'</b>
F-pET28a*	ccg gct gct aac AAA GCC CGA AAG
R-pET28a*	ctt gta cag cTC GTC CAT GCC G
F-anchor peptide*	gct gta caa gGC AGA AGC AGC AG
R-anchor peptide*	gtt agc agc cgg ATC TCA GTG

\*Lower case letters indicate phosphorothioated nucleotides. F, forward; R, reverse.

**Table S4. Extinction coefficient and molecular weight of eGFP-anchor peptides**

<b>Peptide</b>	<b>Extinction coefficient (M/cm)*</b>	<b>Mol weight (kDa)</b>
eGFP	23,380	31.510
eGFP-LCI	45,840	36.956
eGFP-THA	24,870	33.928

\*As calculated with <https://web.expasy.org/cgi-bin/protparam>