

# Subcellular Localization Signals of bHLH-PAS Proteins: their Significance, Current State of Knowledge and Future Perspectives

Beata Greb-Markiewicz <sup>1,\*</sup> and Marta Kolonko <sup>1</sup>

## Supplementary Materials Summary

### Predictors used for NLS prediction:

cNLS Mapper	a
NLStradamus	b
NucPred	c
PSORT II	d
SeqNLS	e
ELM	f

### Predictors used for NES prediction:

ELM	f
NES Finder	g
NetNES	h
LocNES	i

## Prediction summary for Table 1

### hAhR

#### cNLSMapper

11-20 score	11
13-41	5.4
34-62	4.9
78-106	3.0
250-280	3.1
368-398	3.7
640-668	2.7
746-776	2.9

#### NLStradamus

12-42	Posterior 0.3
247-254	

**NucPred**

13-17 score 0.94

**PSORT II**

13-19 score 0.94

35-41

**SeqNLS**

11-24 score 0.887

27-43 0.886

**ELM****NLS**

13-18 1.276e-03

249-254 7.252e-04

**NES**

538-552 7.626e-04

**NES Finder**

47-56

60-69

63-72

259-268

420-429

539-548

735-744

**NetNES**

64-77

577-584?

**LocNES**

59-73 0.746

258-272 0.138

417-431 0.118

732-746 0.174

**hHIF1 $\alpha$** **cNLS Mapper**

8-36 score 2.6

68-97	2.9
245-278	2.8
365-394	2.7
716-725	8.0
717-746	3.2

### **NLStradamus**

3-41    Posterior 0.1  
155-160  
709-723

### **NucPred**

718-721 score 0.94

### **PSORT II**

17-33 score 0.33  
718-721

### **SeqNLS**

10-33	score	0.964
34-39		0.696
58-60		0.568

### **ELM**

#### **NLS**

717-723	7.252e-04
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#### **NES**

82-97	7.626e-04
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### **NES Finder**

37-46  
60-69  
84-93  
559-568

### **NetNES**

L46?  
64-80  
390-399  
559-566?

L583?

L737?

### LocNES

52-66	0.122
443-457	0.284
558-572	0.190
627-641	0.365

### hHIF2 $\alpha$

### cNLS Mapper

5-33	score	2.5
20-42		2.6
65-94		3.8
144-177		2.9
682-711		4.1
705-735		5.4
737-765		3.2
793-822		4.0

### NLStradamus

3-33	Posterior	0.1
156-160		
680-710		
738-741		

### NucPred

14-16	score	0.86
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### PSORT II

14-30	score	0.02
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### SeqNLS

8-30	score	0.916
34-40	score	0.844

### ELM

No NLS/NES

### NES Finder

34-43  
92-101  
498-507  
526-535  
699-708  
773-782

### **NetNES**

56-64  
L92?  
498-501?  
L526?

### **LocNES**

33-47	0.135
49-63	0.190
82-96	0.158
116-130	0.105
497-511	0.234
525-539	0.038
564-578	0.124
698-712	0.354
772-786	0.166

### **hHIF3 $\alpha$**

### **cNLS Mapper**

20-53	score	2.3
153-182		2.6
196-226		3.3
361-392		2.3
438-465		3.6
542-574		3.7
567-598		6.8
590-620		3.0

### **NLStradamus**

7-30    Posterior 0.1  
512-533  
563-597

**NucPred**

569-571 score 0.90

**PSORT II**

No NLS

**SeqNLS**

24-35 score 0.805

**ELM**

No NLS/NES

**NES Finder**

34-43

57-66

487-496

603-612

630-639

**NetNES**

51-61

122-127

L221?

478-494

**LocNES**

56-70	0.265
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114-128	0.256
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477-491	0.387
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603-617	0.500
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629-643	0.547
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**hSIM1****cNLSMapper**

4-33 score	3.0
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74-104	2.3
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163-197	3.4
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235-267	2.8
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347-377	2.7
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600-628	3.6
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703-734            2.6

**NLStradamus**

2-24    Posterior 0.1  
365-386

**PSORT II**

No NLS

**SeqNLS**

No NLS

**ELM**

No NLS/NES

**NES Finder**

20-29  
231-240

**NetNES**

340-347

**LocNES**

19-33	0.254
42-56	0.368
235-249	0.113

**hSIM2**

**cNLS Mapper**

6-35	score	3.7
74-105		3.5
163-195		3.5
235-267		2.8
351-378		6.1
454-484		2.6
556-586		2.4
637-665		2.9

**NLStradamus**

2-25    Posterior 0.1

363-386  
556-572

**NucPred**  
No NLS

**PSORT II**  
No NLS

**SeqNLS**  
5-20 score 0.598

**ELM**  
No NLS/NES

**NES Finder**  
8-17  
65-74  
276-285

**NetNES**  
332-344

<b>LocNES</b>	
19-33	0.228
35-49	0.352
42-56	0.338
102-116	0.130
190-204	0.105
234-248	0.136
275-289	0.083
325-339	0.026

**hClock**

<b>cNLS Mapper</b>		
39-72	score	7.3
256-290		3.4
411-443		3.5
451-481		3.9
526-553		3.1



**NLStradamus**

27-85    Posterior 0.1

**NucPred**

44-47 score 0.94

**PSORT II**

44-47

82-85

Score 0.03

**SeqNLS**

6-16    score    0.522

44-47            0.862

**ELM**

No NLS

NES

546-561            7.626e-04

**NES Finder**

507-516

548-557

588-597

**NetNES**

115-125

**LocNES**

111-127            0.373

414-428            0.119

547-561            0.122

798-812            0.136

**hNPAS1****cNLS Mapper**

33-61    score    3.1

55-87            2.9

89-122	2.2
250-284	3.0
458-487	2.2

### **NLStradamus**

33-76 Posterior 0.1  
96-128  
258-259  
456-475

### **NucPred**

No NLS

### **PSORT II**

No NLS

### **SeqNLS**

41-49	score	0.447
52-60		0.351

### **ELM**

NLS 460-466 7.252e-04  
No NES

### **NES Finder**

65-74  
88-97  
300-309  
304-313  
306-315

### **NetNES**

L97?  
309-318

### **LocNES**

64-78	0.245
87-101	0.295
273-287	0.780
306-320	0.211

## hNPAS2

### cNLS Mapper

14-47	score	7.3
214-242		3.4
231-265		3.7
502-532		2.2
698-730		2.5

### NLStradamus

4-46	Posterior	0.1
803-816		
821-824		

### NucPred

19-22	score	0.89
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### PSORT II

19-22	score	-0.16
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### SeqNLS

19-22	score	0.873
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### ELM

No NLS		
NES 525-539		7.626e-04

### NES Finder

492-501	
527-536	

### NetNES

90-102	
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### LocNES

25-29	0.348
88-102	0.398
526-540	0.150
545-559	0.187

## hNPAS3

### cNLS Mapper

29-58 score	3.4
130-161	3.0
266-297	2.7
360-390	2.5
497-527	2.0
578-608	3.9
606-640	5.1
645-674	3.2
727-755	3.6

### NLStradamus

44-78 Posterior 0.1  
133-136  
273-274  
582-635  
754-773  
596-616 Viterbi Path

### NucPred

599-606 score 0.96

### PSORT II

599-606  
600-618  
Score 3.61

### SeqNLS

1-18 score 0.908

### ELM

NLS  
596-609 score 1.276e-03  
No NES

### NES Finder

71-80  
94-103

285-294  
334-343  
872-881

### **NetNES**

88-98

### **LocNES**

70-84	0.101
88-102	0.507
284-298	0.266
333-347	0.084
871-885	0.079
903-921	0.187

### **hNPAS4**

### **cNLS Mapper**

10-39	score	2.5
158-191		2.1
220-251		3.2
283-314		2.3
593-621		3.0

### **NLStradamus**

8-16    Posterior 0.1  
158-178  
193-211

### **NucPred**

No positive result

### **PSORT II**

No specified NLS

### **SeqNLS**

No positive result

### **ELM**

NLS

No positive result

NES

593-607            7.626e-04

**NES Finder**

20-29

591-600

665-674

**NetNES**

26-45

230-245?

L598?

**LocNES**

19-33            0.104

32-46            0.664

220-234        0.531

230-244        0.343

394-408        0.143

590-604        0.124

664-678        0.602

**Prediction summary for Table 2**

**hARNT**

**cNLSMapper**

39-61 score 8.6

99-130        4.6

151-182       2.3

260-291       2.3

310-339       2.6

390-421       3.1

**NLSradamus**

34-47

83-128

**NucPred**

No NLS

**PSORT II**

42-45  
99-102  
Score 0.09

### **SeqNLS**

39-50 score 0.820

### **ELM**

NLS

39-46 Probability 1.276e-03

No NES

### **NES Finder**

336-345

### **NetNES**

159-178

### **LocNES**

159-173	score	0.222
274-288		0.308
417-431		0.198
671-685		0.104

## **hARNT2**

### **cNLS Mapper**

42-66	score	10.5
73-104		4.0
256-266		6.0
364-394		3.4
591-619		2.1

### **NLStradamus**

34-85  
95-102  
258-269

### **NucPred**

73-76      0.89

### **PSORT II**

73-76 score -0.16

### **SeqNLS**

15-29      0.862

39-51      0.883

### **ELM**

#### **NLS**

42-49      1.276e-03

260-265

#### **NES**

No positive result

### **NES Finder**

310-319

702-711

### **NetNES**

133-150

V319?

### **LocNES**

No NES candidate

## **hBMAL1 (ARNT3)**

### **cNLS Mapper**

34-44 score 5.0

83-113      5.4

174-205      3.1

244-277      4.6

291-322      3.0

471-502      3.8

### **NLStradamus**

37-91 posterior 0.1

258-279

472-499



**NucPred**

score 0.87

37-40

82-85

**PSORT II**

37-40

82-85

Score 0.15

**SeqNLS**

23-45 score 0.879

**ELM****NLS**

37-42      Probability 1.276e-03

**NES**

No positive result

**NES Finder**

105-114

108-117

**NetNES**

V114?

142-159

**LocNES**

107-121      0.636

-115-129      0.150

151-165      0.048

**hBMAL2 (ARNT4)****cNLS Mapper**

47-57 score 7.0

114-123      6.0

209-240      3.7

279-314      3.3

444-474	2.2
554-586	2.6

### **NLStradamus**

35-60  
103-127  
285-322

### **NucPred**

49-52  
117-120  
Score 0.85

### **PSORT II**

49-55  
117-120  
301-307  
NLS score 1.18

### **SeqNLS**

1-10	0.893
19-26	0.791
44-59	0.895

### **ELM**

#### **NLS**

49-55      Probability 1.276e-03

#### **NES**

463-477      Probability 7.626e-04

### **NES Finder**

140-149  
143-152  
239-248

### **NetNES**

140-152?

### **LocNES**

141-156      score 0.614

238-252	0.225
463-477	0.084

### Prediction summary for Table 3

#### MET

##### cNLS Mapper

38-72 score	2.9
98-128	3.8
252-285	3.9
296-330	3.5
489-519	3.0
523-554	2.7
628-662	2.6

##### NLStradamus

38-52  
68-115  
252-260  
636-640

##### NucPred

98-102 score 0.82

##### PSORT II

98-101 score 0.15

##### SeqNLS

34-43 score 0.548

##### ELM

##### NLS

98-104 Probability 1.276e-03

##### NES

No positive result

##### NES Finder

263-272  
431-440

**NetNES**

130-142

L443?

L603?

**LocNES**

130-144 score 0.658

262-276 0.094

425-439 0.064

446-460 0.044

542-556 0.178

**GCE****Full length isoform****cNLS Mapper**

279-323 score 3.3

424-454 3.0

469-499 3.1

521-554 3.2

625-655 2.7

838-860 12.5

**NLStradamus**

278-294

426-458

647-656

**NucPred**

No predicted NLS

**PSORT II**

840-856 score 0.02

**SeqNLS**

12-25 score 0.399

39-56 0.346

## **ELM**

### **NLS**

646-652	Probability 7.252e-04
840-859	Probability 2.588e-04
852-858	Probability 1.276e-03

### **NES**

No positive result

### **NES Finder**

567-576  
650-659

### **NetNES**

44-50  
L329  
L352-359

### **LocNES**

35-49	score	0.073
347-361		0.533
582-596		0.044
778-792		0.016

## **SIMA**

### **cNLS Mapper**

150-180	score	2.9
347-374		3.1
383-414		3.0
561-591		3.3
1102-1131		2.8
1212-1232		6.4
1288-1316		3.3
1409-1417		3.0

### **NLStradamus**

46-91  
545-546

554-581

### **NucPred**

No NLS prediction

### **PSORT II**

63-69

72-88

562-568

1408-1414

NLS score 1.64

### **SeqNLS**

4-32      score    0.902

47-59            0.605

### **ELM**

#### **NLS**

1212-1232      2.588e-04

1408-1413      1.276e-03

#### **NES**

90-105            7.626e-04

173-189

### **NES Finder**

92-101

115-124

121-130

1007-1016

1129-1138

### **NetNES**

L101?

122-124?

L183?

### **LocNES**

91-105    score    0.254

114-128      0.318

120-134      0.139

175-189	0.398
477-491	0.437
636-650	0.226
1006-1020	0.235
1125-1139	0.130

## TANGO

### cNLS Mapper

23-54 score	4.6
167-196	2.2
207-137	2.7
288-320	3.2

### NLStradamus ( cutoff 0.1)

11-52  
170

### NucPred

23-26  
Score 0.76

### PSORT II

23-26  
NLS score -0.16

### SeqNLS

No predicted NLS

### ELM

No NLS/NES positive result

### NES Finder

259-268

### NetNES

83-97  
V268?  
I634?

**LocNES**

83-97	score	0.352
258-272		0.036
624-638		0.539

**CYCLE****cNLS Mapper**

41-71	score	4.8
176-209		3.3
210-240		4.3
232-262		3.4

**NLStradamus** (cutoff 0.1)

25-46  
229-246

**NucPred** score 0.90

40-44

**PSORT II**

40-44  
NLS score -0.16

**SeqNLS**

55-60 score 0.659

**ELM****NLS**

235-240	7.252e-04
236-241	1.276e-03

**NES**

No NES positive result

**NES Finder**

50-59  
63-72  
66-75



## NetNES

63-75?

102-111?

L213?

## LocNES

No NES candidates

### Supplementary Material for Figure 3

Sequences of documented NLS/NES motifs within MET, GCE, AHR and NPAS4 proteins

#### MET:

length	716
HLH	38-87
PAS-1	137-186
PAS-2	403-510

NLS: PAS-1 (98-102), PAS-2 (482-498)

NES PAS-1 (126-139), PAS2 (446-456), C-terminus (598-631)

98-RRRKK-102; NLS-1

126-HLTDTLMQLLDCCFL-139; NES-1

446-VRWVIVALRQM-456; NES-2

482-TKFLEVDRGSNKHVSF-498; NLS-2

598-TPALALVPPAPSSVKSITKSISVVNVTAALKR-631; NES-3

#### GCE:

length	959
HLH	279-325
PAS-1	352-407
PAS-2	539-646

NLS: PAS-2 (618-634), C-terminus (840-859)

NES PAS-1 (343-359), PAS-2 (580-610), C-terminus (761-795)

343-QITDTLMQLLDCCFLTL-359; NES-1

580-DDVRWVIVALRQMYDCNSSYGESTYRLFTRN-610; NES-2

618-SKGYLEIDKETNKHVSF-634; NLS-1

840- KRGSTAHVETEEKLSKRRF-859; NLS-2  
762-TPPLALVPPEASSVKSAITKSISVVNVTAALKR-795; NES-3

### AHR:

length	848
HLH	37-78
PAS-1	113-226
PAS-2	297-382

NLS: bHLH (13-39), C-terminus (648-671)

NES: bHLH (55-75), PAS-1 (214-222), C-terminus (around V647)

13-RKRRKPVQKTVKPIPAEGIKSNPSKRH-39; NLS-1 (human)  
63-KLDKLSVLRSL-73; NES-1 (human) 55-75 mouse  
214-FRCRLRCLL-222; NES-2 (mouse)/ 220-FICRLRCLL-228 human  
V647 indispensable for NES activity; NES-3 (human)  
648-VNGMFENWNSNQFVPFNCPPQDPQQ-671; NLS-2 (human)

### NPAS4:

length	802	mouse
HLH	1-59	
PAS-1	59-144	
PAS-2	194-340	

NLS: bHLH (10-52), PAS-2 x2 (158-191, 285-316?), C-terminus (593-622?)

NES: bHLH (26-45), PAS-2 (227-242), C-terminus x2 (461-580?, 591-600)

10-KARRDQINAEIRNLKELLPLAEADKVRLSYLHIMSLACIYTRK-52; NLS-1  
26-LLPLAEADKVRLSYLHIMSL-45; NES-1  
158-RRQSAGNKLVLIRGRFHAHPPGAYWAGNPVFTAF-191; NLS-2  
227-LALLDISESVLITYLGF-242; NES-2  
285-RLQAKH GGWTWIYCMYSDGPEGPIITANNYPI-316; NLS-3?  
461-580; NES-3?  
591-LAQLRGPLSV- 600; NES-4  
593-QLRGPLSVDVPLVPEGLLTPEASPVKQSFF-622; NLS-4?