

Mutagenesis of RRL-NSD3-Short-3xFLAG Lentiviral Vector

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Overview. The NSD3's PWWP1 domain is present in both long and short isoforms. It has been shown to bind H3K36me2 (Sankaran et al.(2016) - PMID:26912663) and be required for the maintenance of AML (Chen et al.(2015 - PMID: 26912663). However, it is still unclear how this domain contributes to NSD3's function at enhancers. To study this aspect of NSD3's biology, I will mutate W284 to alanine in the RRL-NSD3-3xFLAG-IRES-Puro plasmids I described earlier (exp010) by site-directed mutagenesis. This is the second tryptophan within the PWWP1 motif and is critical for substrate recognition (Qin, S & Min, J(2014) - PMID:25277115). This construct will be useful for understanding how NSD3 recognition of methylated histones influences its putative activity in cancer.

1. Experimental Details

Mutagenic primers were designed using the online tool from NEB, NEBaseChanger (<https://nebasechanger.neb.com/>). Mutagenesis was then performed using NEB's Q5 Site-Directed Mutagenesis Kit (E0554S), following manufacturer's instructions.

Mutagenic Primers

NSD3_W284A_fwd - CTATCCTTGGgcGCCTTGATGGTTCAAG
NSD3_W284A_rev - GTTCCCACCTTGGACCAC

Cycling Conditions

1. Initial Denaturation

95°C - 60s

2. Amplification - 25x

98°C - 10s

68°C - 20s

72°C - 2min

3. Final Extension

72°C - 2min

KLD Digest-Ligation Reaction

1 μL of PCR reaction was added to a 10 μL KLD reaction and incubated 10 minutes at RT, then transformed in E. Coli and plated on LB agar with ampicillin.

Sequencing

Following morning, four clones selected for overnight cultures and mini-prep. Selected clones were sent for sequencing at ACGT Corporation, Toronto to be sequenced with a custom synthesized oligo annealing within NSD3 (below).

Sequencing Primer:

AMF0746 (NSD3fwd) - AAAACCTTCCCTCCACAGC

2. Result

The sequencing results for W284A clones 2 & 3 were correct, clones 1 no change, and 4 was ambiguous with a truncated read. Clone three was selected for future experiments, the results of blastx search against the sequence is shown in Figure 1 below. Sequencing data follows.

histone-lysine N-methyltransferase NSD3 isoform short [Homo sapiens]						
Sequence ID: NP_060248.2 Length: 645 Number of Matches: 2						
► See 7 more title(s)						
Range 1: 147 to 418 GenPept Graphics						
Score	Expect	Method	Identities	Positives	Gaps	Frame
507 bits(1306)	1e-177	Compositional matrix adjust.	271/272(99%)	271/272(99%)	0/272(0%)	+2
Query 2	KTGSPEIKLKITKTIQNGRELFESSLCGDLLNEVQAshtkskhesrkekrkksnkhDSS		181			
Sbjct 147	KTGSPEIKLKITKTIQNGRELFESSLCGDLLNEVQAshtkskhesrkekrkksnkhDSS		206			
Query 182	RSEERKSHKIPKLEPEEQNRPNERVDTVSEKPREEPVLKEEAPVQPILSSVPTTEVSTGV		361			
Sbjct 207	RSEERKSHKIPKLEPEEQNRPNERVDTVSEKPREEPVLKEEAPVQPILSSVPTTEVSTGV		266			
Query 362	KFQVGDLVWSKVGTYPWAPCMVSSDPQLEVHTKINTRGAREYHVQFFSNQPERAWHEKR		541			
Sbjct 267	KFQVGDLVWSKVGTYPWPCMVSSDPQLEVHTKINTRGAREYHVQFFSNQPERAWHEKR		326			
Query 542	VREYKGHKQYEELLAEATKQASNHSEKQKIRKPRPQRERAQWDIGIAHAEKALKMTREER		721			
Sbjct 327	VREYKGHKQYEELLAEATKQASNHSEKQKIRKPRPQRERAQWDIGIAHAEKALKMTREER		386			
Query 722	IEQYTFIYIDKQPEEALSQAKKSVASKTEVKK	817				
Sbjct 387	IEQYTFIYIDKQPEEALSQAKKSVASKTEVKK	418				

TEMPLATE NAME: W284A_1

PRIMER NAME: AMF0747

SAMPLE NAME: SP 2623A

```
## tTccaaagAAgACTGGCTcaCCTGAAATTAAACTAAAATAACCAAAACTATCCAGAATGGCAGGGATTGTTGAGTCT
## TCCCTTGAGACCTTTAAATGAAGTACAGGCAGTGGACACCGAAATCAAAGCATGAAAGCAGAAAAGAAAAGAG
## GAAAAAAAGCAACAAGCATGACTCATCAAGATCTGAAGAGCGCAAGTCACACAAAATCCCCAAATTAGAACCCAGAGGAAC
## AAAATAGACCAATGAGAGGGTTGACACTGTATCAGAAAAACCAAGGGAAAGAACCGTACTAAAGAGGAAGGCCAGTT
## CAGCCAATACTATCTCTGTTCAACAACCGAAGTGTCACTGGTGTAAAGTTTCAGGTTGGCGATCTGTGGTCAA
## GGTGGAACCTATCCTGGCCTTGTATGGTTCAAGTGTACCCAGCTGAGGTTCATACTAAAATTAAACACAAGAG
## GTGCCGAGAATATCATGTCCAGTTTAGCAACCAGCCAGAGAGGGCGTGGGTCATGAAAACGGGTACGAGAGTAT
## AAAGGTCTAAACAGTATGAAGAATTACTGGCTGAGGCAACCAAACGCCAGCAACTCTGAGAAACAAAGATTG
## GAAACCCGACCTCAGAGAGAACGTGCTAGTGGATATTGGCATTGCCATGCAGAGAAAGCATTGAAAATGACTCGAG
## AAGAAAGAATAGAACAGTATACTTTATTTACATTGATAAACAGCCTGAAGAGGGcTTATCCAAGCAAAAAGAGTGTt
## GcCTCCAAAACCGAAGTTAAAAACCCGACGAcCAAgAtCTGTGCTGAATACTCA
```

TEMPLATE NAME: W284A_2

PRIMER NAME: AMF0747

SAMPLE NAME: SP 2624A

```
## gAAgACTGGCTCaCCTGAAATTAAACTAAAATAACCAAAACTATCCAGAATGGCAGGGATTGTTGAGTCTCCCTT
## GTGGAGACCTTTAAATGAAGTACAGGCAGTGGACACCGAAATCAAAGCATGAAAGCAGAAAAGAAAAGAGGAAAAAA
## AGCAACAAGCATGACTCATCAAGATCTGAAGAGCGCAAGTCACACAAAATCCCCAAATTAGAACCCAGAGGAACAAAATAG
## ACCAAATGAGAGGGTTGACACTGTATCAGAAAAACCAAGGGAAAGAACCGTACTAAAGAGGAAGGCCAGTTCAGCAA
## TACTATCTCTGTTCAACAACCGAAGTGTCACTGGTGTAAAGTTTCAGGTTGGCGATCTGTGTGGTCAAAGGTGG
## ACCTATCCTGGCGCCTTGTATGGTTCAAGTGTACCCAGCTGAGGTTCATACTAAAATTAAACACAAGAGGTGCCG
## AGAATATCATGTCCAGTTTTAGCAACCAGCCAGAGAGGGCGTGGGTCATGAAAACGGGTACGAGAGTATAAGGT
## ATAAACAGTATGAAGAATTACTGGCTGAGGCAACCAAACAGCCAGCAATCACTGTGAGAAACAAAAGATTGGAAACCC
## CGACCTCAGAGAGAACGTGCTAGTGGATATTGGCATTGCCATGCAGAGAAAGCATTGAAAATGACTCGAGAAGAAAG
## AATAGAACAGTATACTTTATTTACATTGATAAACAGCCTGAAGAGGGcTTATCCAAGCAAAAAGAGTGTGCCTCCA
## AAACCGAAGTTAAAAACCCGACGAcAGATCTGTGCTGAATACTCAGCcaGAACAGACCC
```

TEMPLATE NAME: W284A_3

PRIMER NAME: AMF0747

SAMPLE NAME: SP 2625A

```
## aAgAAgACTGGCTCaCCTGAAATTAAACTAAAATAACCAAAACTATCCAGAATGGCAGGGATTGTTGAGTCTCCCT
## TTGTGGAGACCTTTAAATGAAGTACAGGCAGTGGACACCGAAATCAAAGCATGAAAGCAGAAAAGAAAAGAGGAAAA
## AAAGCAACAAGCATGACTCATCAAGATCTGAAGAGCGCAAGTCACACAAAATCCCCAAATTAGAACCCAGAGGAACAAAAT
## AGACCAAATGAGAGGGTTGACACTGTATCAGAAAAACCAAGGGAAAGAACCGTACTAAAGAGGAAGGCCAGTTCAGCC
## AATACTATCTCTGTTCAACAACCGAAGTGTCACTGGTGTAAAGTTTCAGGTTGGCGATCTGTGTGGTCAAAGGTGG
## GAACCTATCCTGGCGCCTTGTATGGTTCAAGTGTACCCAGCTGAGGTTCATACTAAAATTAAACACAAGAGGTGCC
## CGAGAATATCATGTCCAGTTTTAGCAACCAGCCAGAGAGGGCGTGGGTCATGAAAACGGGTACGAGAGTATAAGG
## TCATAAACAGTATGAAGAATTACTGGCTGAGGCAACCAAACAGCCAGCAATCACTGTGAGAAACAAAAGATTGGAAAC
## CCCGACCTCAGAGAGAACGTGCTAGTGGATATTGGCATTGCCATGCAGAGAAAGCATTGAAAATGACTCGAGAAGAA
## AGAATAGAACAGTATACTTTATTTACATTGATAAACAGCCTGAAGAGGGcTTATCCAAGCAAAAAGAGTGTGCCTC
## CAAAACcgAAGTTAAAAACCCGACGACCAAGATCTGTGCTGAATACTCAGCcaGAACAGACCC
```

W284A_4

PRIMER NAME: AMF0747

SAMPLE NAME: SP 2626A

Signal drops after 400bp.

```
## aagAAgACTGGCTCACCTGAAATTAAACTAAAATAACCAAAACTATCCAGAATGGCAGGGATTGTTGAGTCTCCCT
## TTGTGGAGACCTTTAAATGAAGTACAGGCAGTGGACACCGAAATCAAAGCATGAAAGCAGAAAAGAAAAGAGGAAAA
## AAAGCAACAAGCATGACTCATCAAGATCTGAAGAGCGCAAGTCACACAAAATCCCCAAATTAGAACCCAGAGGAACAAAAT
## AGACCAAATGAGAGGGTTGACACTGTATCAGAAAAACCAAGGGAAAGAACCGTACTAAAGAGGAAGGCCAGTTCAGCC
```

AATACTATCTTCTGTTCCAACAACCGAACGTGTCCACTGGTGTAAAGTTCAGGTTGGCGATCTTGTGTGG
ExpID-013