

Transcriptomic analysis reveals the role of *Oryza sativa* FOUR LIPS in response to salt stress in rice

Plant Molecular Biology

Chunxia Zhang^{1*}, Jie Zhang^{1,2*}, Huichao Liu^{1,2}, Xiaoxiao Qu^{1,2}, Junxue Wang^{1,2,§}, Qixiumei He^{1,2}, Junjie Zou^{1,3}, Kezhen Yang¹ and Jie Le^{1,2†}

¹Key Laboratory of Plant Molecular Physiology, CAS Center for Excellence in Molecular Plant Sciences, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

²University of Chinese Academy of Sciences, Beijing 100049, China

³Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China

*These authors contributed equally to this work

†Correspondence: Jie Le (lejie@ibcas.ac.cn)

Fig. S1

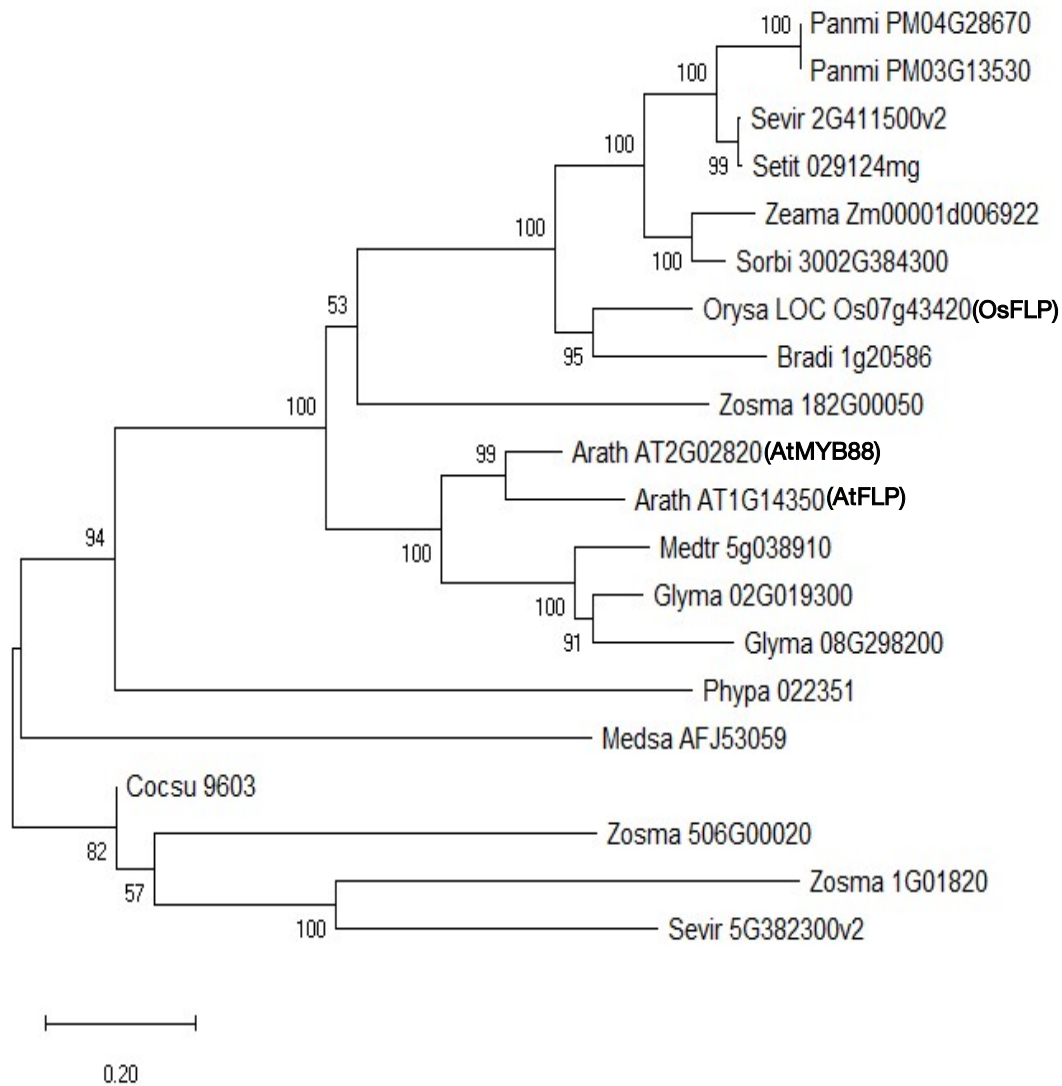


Fig. S1 Phylogenetic tree shows the relationship of FLP proteins from algae, lower plants and higher plants

Amino acid sequences were included in the phylogenetic analysis from the following species: *Coccomyxa subellipsoidea*, *Chlamydomonas reinhardtii*, *Physcomitrella patens*, *Zostera marina*, *Panicum miliaceum*, *Brachypodium distachyon*, *Setaria viridis*, *Setaria italic*, *Zea mays*, *Sorghum bicolor*, *Oryza sativa*, *Arabidopsis thaliana*, *Glycine max*, *Medicago truncatula* and *Medicago sativa*. Phylogenetic analysis was performed with MEGA-X and the phylogenetic tree was constructed by neighbor-joining method with bootstrap analysis (1,000 replicates)

Fig. S2

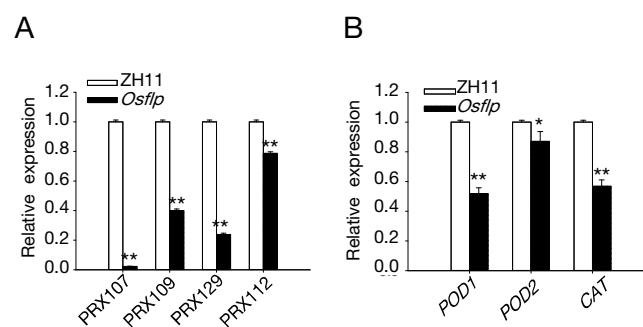


Fig. S2 RT-qPCR was performed to verify the results of the RNA-seq

A. Four randomly selected DEGs were validated their expression levels using RT-qPCR in ZH11 and *Osflp*

B. The expression of three ROS scavenging related genes *POD1*, *POD2* and *CAT* were verified using RT-qPCR in ZH11 and *Osflp*

Fig. S3

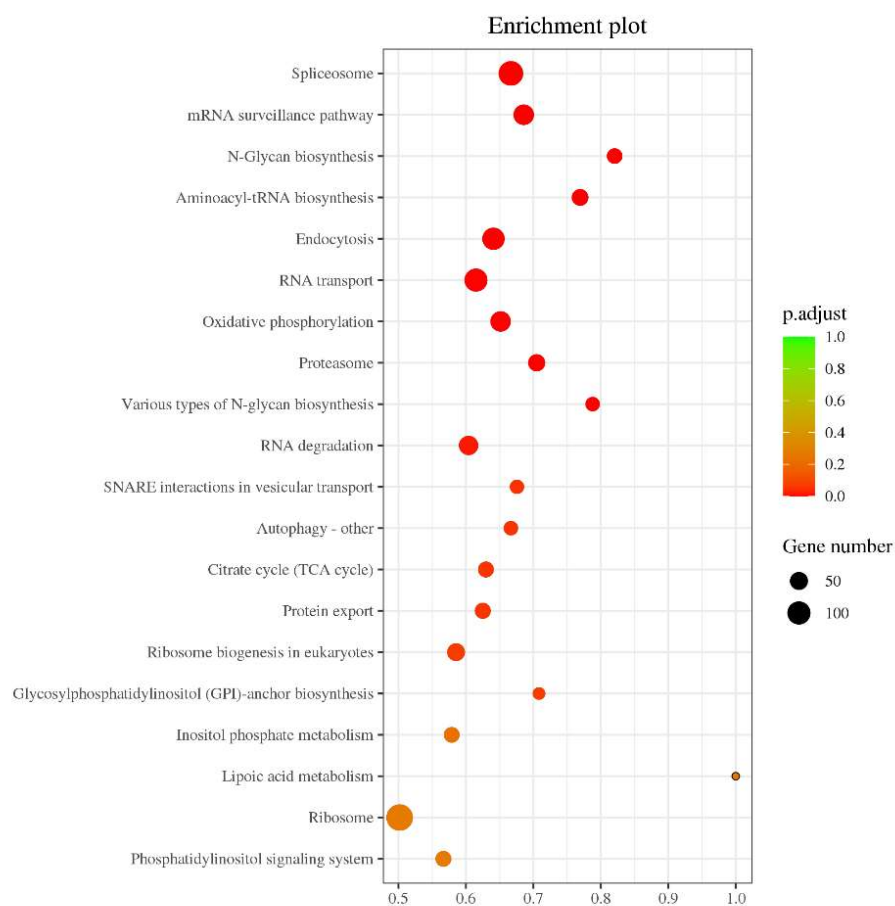


Fig. S3 KEGG enrichment analysis with ChIP-seq

Several potential pathways were enriched with KEGG analysis, including “spliceosome”, “mRNA surveillance pathway”, “N-Glycan biosynthesis”, “endocytosis”, “RNA transport” and “oxidative phosphorylation” etc

Fig. S4

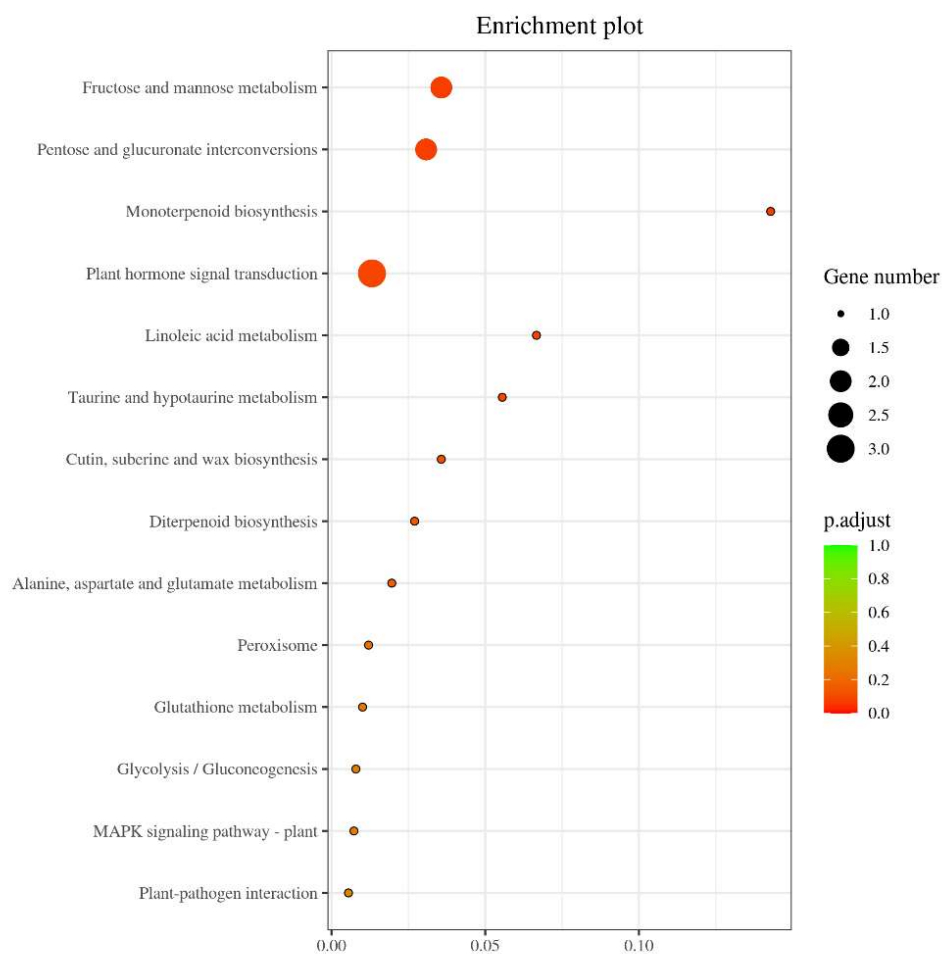


Fig. S4 KEGG enrichment analysis with genes identified by the integrative analysis of RNA-seq data and ChIP-seq data

In all enriched potential pathways, peroxisome related pathway was identified

Fig. S5

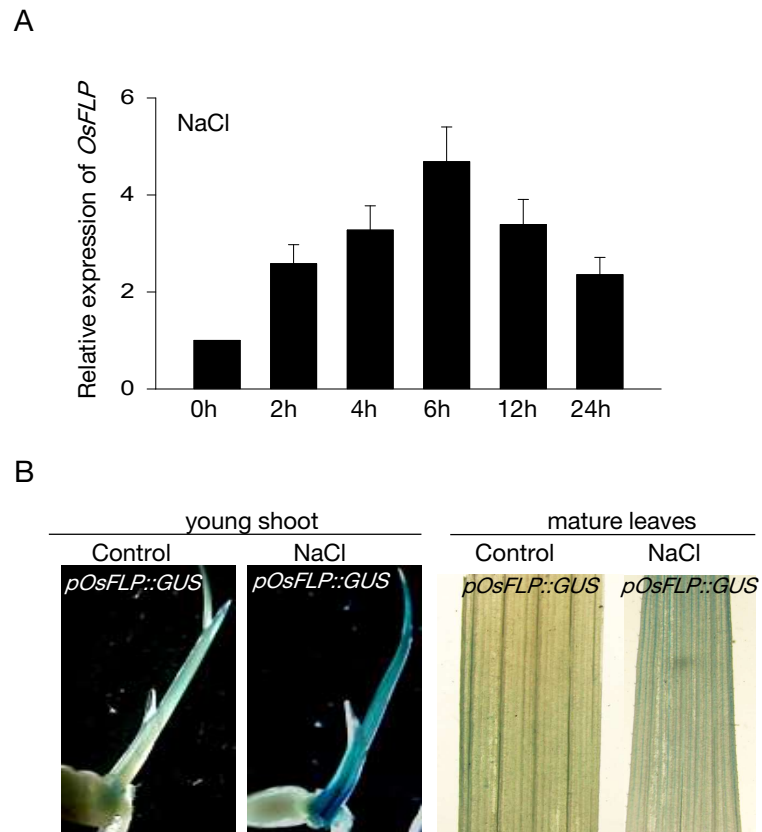


Fig. S5 The expression of *OsFLP* upon salt stress

A. The expression of *OsFLP* was induced by salt treatment

B. The activity of the native promoter of *OsFLP* could be also induced by salt stress in young shoot and mature leaves

Fig. S6

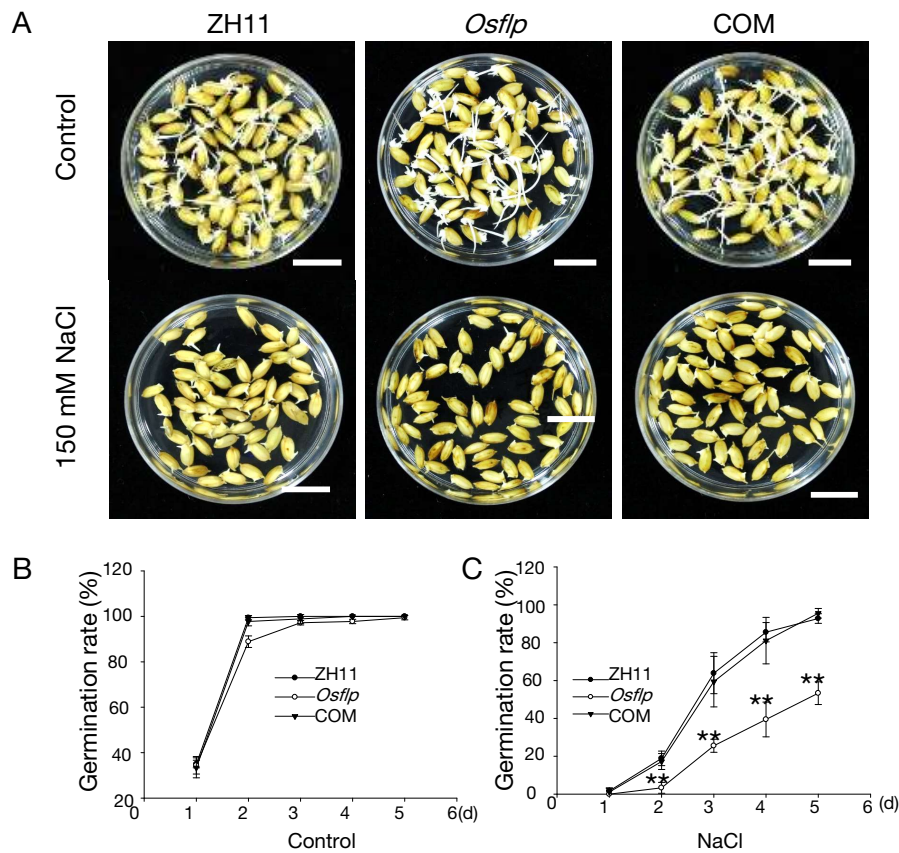


Fig. S6 *Osflp* mutants are more sensitive to salt stress

A-C. Seeds germination rate assay of ZH11 (wild-type), *Osflp* and *OsFLP* complementation line (COM) in the presence of 150 mM NaCl. In red rectangles, the germinated radicle and germ are shown. Scale bars=1 cm

Data are means $\pm$ SD. Student's *t* - test was performed. Statistically significant differences are indicated with asterisks: **, $P < 0.01$

Table S1. Genes are related to ROS metabolism enriched by RNA-seq analysis.

Gene ID	Gene symbol synonym(s)	Gene name synonym(s)	Regulation	Log2FC
Os01g0294500	unknown	unknown	up	3.1
Os12g0541300	rbohH	Respiratory Burst Oxidase	up	2.24
Os07g0499500	prx102	class III peroxidase 102	up	4.5
Os01g0326100	prx13	class III peroxidase 13	up	1.4
Os05g0162000	prx72	class III peroxidase 72	up	1.38
Os05g0135400	prx70	class III peroxidase 70	up	1.17
Os04g0628200	prx56	class III peroxidase 56	down	1.2
Os06g0547100	prx87	class III peroxidase 87	down	1.24
Os03g0121300	prx34	class III peroxidase 34	down	1.26
Os10g0109600	prx126	class III peroxidase 126	down	1.27
Os12g0112000	prx135	class III peroxidase 135	down	1.33
Os07g0677300	prx112	class III peroxidase 112	down	1.88
Os11g0112400	prx130	class III peroxidase 130	down	1.98
Os07g0676900	prx109	class III peroxidase 109	down	2.07
Os07g0639000	prx107	class III peroxidase 107	down	2.3
Os04g0689000	prx64	class III peroxidase 64	down	2.4
Os02g0240100	prx30	class III peroxidase 30	down	2.5
Os07g0677100	prx110	class III peroxidase 110	down	2.7
Os04g0651000	prx57	class III peroxidase 57	down	3.18
Os10g0566800	prx129	class III peroxidase 129	down	3.53

Table S2. Genes are related to salt stress enriched by the integrative analysis of RNA-seq and ChIP-seq.

Gene	Function	Regulation	Fold enrichment
Os04g0353600	response to salt	down	8.5
Os05g0160000	Oxidoreductase activity	down	5.93
Os03g0411300	calcium:sodium antiporter activity	up	5.56
Os01g0294500	peroxidase activity	up	5.06
Os12g0623600	Oxidoreductase activity	up	4.03
Os03g0237100	Oxidoreductase activity	up	3.6
Os01g0303800	response to stress	up	3.10
Os01g0846300	function potassium channel	up	3.06
Os04g0531900	oxidoreductase activity	up	2.88
Os04g0179200	Oxidoreductase activity	down	2.81

Table. S3 Primers were used in this study

Primer name	Forward sequence (5'-3')	Reverse sequence (5'-3')	Purpose
OsActin	GGATCCATCTTGGCATCTCTCA	GGGCCAGACTCGTCGTACTC	RT-QPCR
OsRab16A	GGAACGAGTTGTGTTTGGCC	AGTGGAAGCATGGAAGGGTG	RT-QPCR
OsDREB2A	GACATGGGGTAAGTGGGTGG	CGTGCTGTGGGACCATACAT	RT-QPCR
OsDREB1A	CGGGATCAAGCAGGAGATGA	CCTCGTCTCCCTGAACCTTGG	RT-QPCR
OsDREB1F	GACGCCATCTTCGACATGCC	TCCATAGCTTGTAGTCCATGTG G	RT-QPCR
OsCDKB1;1	TGAAGAAGACGAGGCTGGAG	GAAGACGAGGTAGAGGACGG	RT-QPCR
OsFLP	AACTGGACAATTATAGCGGCAC	GGAATGTTGGGACTGTATGAGC	RT-QPCR
OsPRX107	TTAGGGTTGGGTACTACAAGCG	GCAGTCGTGGAAGAACATGC	RT-QPCR
OsPRX129	CTCCTCCACCTCATCTCCAC	AGCGTGTCTTGATGTCGTC	RT-QPCR
OsPRX109	GCTTCACTTCCATGACTGCT	CGACGACGTTGAATCCTCTG	RT-QPCR
OsPRX112	TGAACTTATCGGCAATTTCTCCA G	GTCTCGTTGTAGAGCCTGTCC	RT-QPCR
OsPOD1	GGTCGGGTTCTACAACAAGAC	CAGCCTCTGACAAAGCAGTC	RT-QPCR
OsPOD2	GAACACCAACCTCCATCCAC	GCTCCAGTGAGTACAACCAG	RT-QPCR
OsCAT	GCCCAAACCTATTTGATGCTTCC	AAGTAGTTAACCTCTTCATCCC TG	RT-QPCR
pOsFLP	CGAGCTCATAAAGGGTCTGACTT GGGT	AACATGCGGGATCCCGGACC	GUS line generation
OsFLP-OE	ATGGCGACCGGACCCGATCTG	TCATAGGCTATTAAGGAGAGC	Over-expression line generation
OsCDKB1;1-OE	ATGGAGAAGTACGAGAAGCTG	CTAGAACTGGGACTTGTCGAG	Over-expression line generation
Probe -137~95	CCGCTGCTTCCCTTCCCCCTTCC ATCCCCCTCCTCCCCACGCTCC	GGAGCGTGGGGAGGAGGGGGG ATGGAAAGGGGAAGGGAAGCA GCGG	EMSA
Mutant Probe 1	CCGCTGC <i>AAAAAAAAA</i> CTTTCCA <i>AAA</i> CCCTCCTCCCCACGCTCC	GGAGCGTGGGGAGGAGGGT <i>TTT</i> <i>TG</i> GAAAGT <i>TTTTTTTT</i> GCAGCG G	EMSA
Mutant Probe 2	CCGCTGC <i>AAAAAAAAA</i> CTTTCCA TCCCCCTCCTCCCCACGCTCC	GGAGCGTGGGGAGGAGGGGGG ATGGAAAGT <i>TTTTTTTT</i> GCAGC GG	EMSA
Mutant Probe 3	CCGCTGC <i>AAAAA</i> TTCCCCCTTCCA <i>AAA</i> CCCTCCTCCCCACGCTCC	GGAGCGTGGGGAGGAGGGT <i>TT</i> TTGGAAAGGGGAATTTTGCAG CGG	EMSA
Mutant Probe 4	CCGCTGCTTCCC <i>AAAA</i> CTTTCCA <i>AAA</i> CCCTCCTCCCCACGCTCC	GGAGCGTGGGGAGGAGGGT <i>TTT</i> <i>TG</i> GAAAGT <i>TTTT</i> GGGAAGCAGC GG	EMSA
Probe -94~45	TCCTCCCCCATTTCAAATCCCCC CCCCCACACACACCCCTTCCA	GCGGTGGAAGGGGGTGTGTGT GGGGGGGGGGGATTTGAAATG	EMSA

	CCGC	GGGGAGGA	
Mutant Probe	TCCTCCCCCATTTCAAAAAACCC CCCCCACACACCCCCCTTCCAC CGC	GCGGTGGAAGGGGGTGTGTGT GGGGGGGGTTTTTTGAAATGG GGGAGGA	EMSA
Probe -44~-1	GCCGCCACCGCCGCCGAATCTAG GGTTCATCGGGGATTCGGGATCG	CGATCCCGAATCCCCGATGAAC CCTAGATTCGGCGGCGGTGGCG GC	EMSA
Mutant Probe 1	GCCGCCACCGCCGCCGAATCTAG GGTTCAAAAAGGAAAAAGATCG	CGATCTTTTTTCCTTTTGAACCC TAGATTCGGCGGCGGTGGCGGC	EMSA
Mutant Probe 2	GCCGGCCACCGCCGCCGAATCTA GGGTTTCATCGGGGAAAAAGATC G	CGATCTTTTTTCCCCGATGAACC CTAGATTCGGCGGCGGTGGCGG C	EMSA
Mutant Probe 3	GCCGCCACCGCCGCCGAATCTAG GGTTCAAAAAGGATTGGGATCG	CGATCCCGAATCCTTTTTGAACC CTAGATTCGGCGGCGGTGGCGG C	EMSA