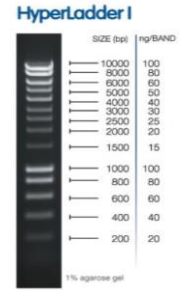
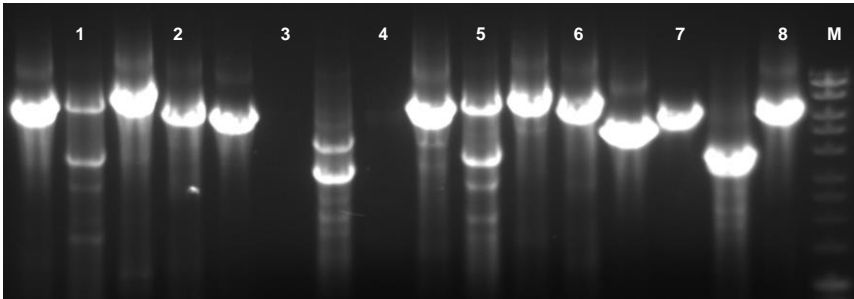


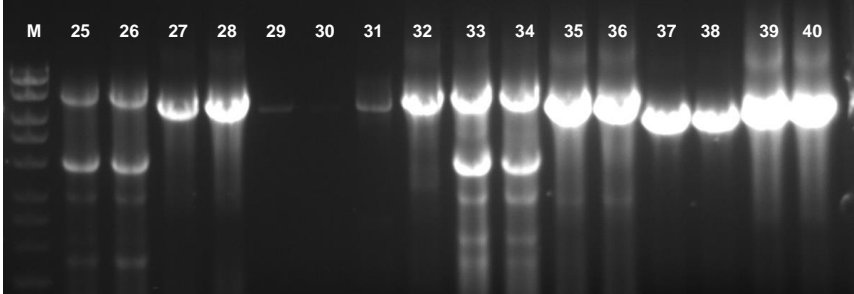
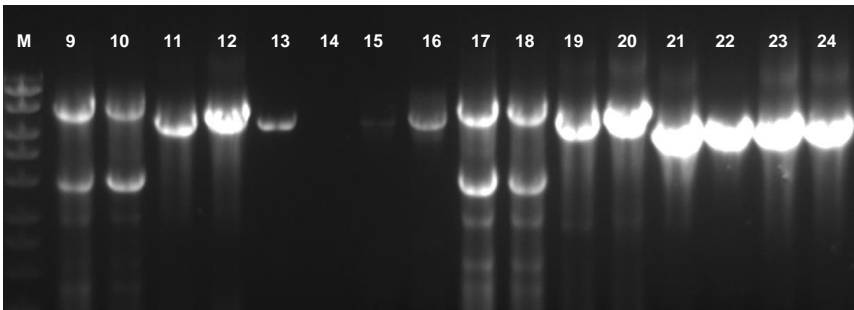
LR-PCR of the **Fra10ac1**

clone EPD0441\_3\_E11, EPD0441\_3\_D12, EPD0441\_3\_D09, EPD0441\_3\_C12, EPD0441\_3\_G10

[Gel picture:](#)



HyperLadder™ I, Biotine



**Corresponding pipetting scheme:**

<b>probe</b>		1	2	3	4	5	6	7	8	
template (5ng/ul)		Fra10ac1 (E11)	Fra10ac1 (E11)	Fra10ac1 (E11)	Fra10ac1 (E11)	Fra10ac1 (E11)	Fra10ac1 (E11)	Fra10ac1 (E11)	Fra10ac1 (E11)	template
primer-for (5uM)		Fra10ac1-GF3	Fra10ac1-GF5	Joel 2	Joel 2	Fra10ac1-GF3	Fra10ac1-GF5	LF	LF	5' primer
constant primer (5uM)		LAR 3	LAR 3			LAR 7	LAR 7			
primer-rev (5uM)				Fra10ac1-GR5	Fra10ac1-GR6			Fra10ac1-GR5	Fra10ac1-GR6	3' primer
<b>Result</b>		-	+	-	-	-	+	+	+	

<b>probe</b>		9	11	13	15	17	19	21	23	
template (5ng/ul)		Fra10ac1 (D12)	Fra10ac1 (D12)	Fra10ac1 (D12)	Fra10ac1 (D12)	Fra10ac1 (D12)	Fra10ac1 (D12)	Fra10ac1 (D12)	Fra10ac1 (D12)	template
primer-for (5uM)		Fra10ac1-GF3	Fra10ac1-GF5	Joel 2	Joel 2	Fra10ac1-GF3	Fra10ac1-GF5	LF	LF	5' primer
constant primer (5uM)		LAR 3	LAR 3			LAR 7	LAR 7			
primer-rev (5uM)				Fra10ac1-GR5	Fra10ac1-GR6			Fra10ac1-GR5	Fra10ac1-GR6	3' primer
<b>Result</b>		-	+	+	-	-	+	+	+	

<b>probe</b>		10	12	14	16	18	20	22	24	
template (5ng/ul)		Fra10ac1 (D09)	Fra10ac1 (D09)	Fra10ac1 (D09)	Fra10ac1 (D09)	Fra10ac1 (D09)	Fra10ac1 (D09)	Fra10ac1 (D09)	Fra10ac1 (D09)	template
primer-for (5uM)		Fra10ac1-GF3	Fra10ac1-GF5	Joel 2	Joel 2	Fra10ac1-GF3	Fra10ac1-GF5	LF	LF	5' primer
constant primer (5uM)		LAR 3	LAR 3			LAR 7	LAR 7			
primer-rev (5uM)				Fra10ac1-GR5	Fra10ac1-GR6			Fra10ac1-GR5	Fra10ac1-GR6	3' primer
<b>Result</b>		-	+	+	-	-	+	+	+	

<b>probe</b>		25	27	29	31	33	35	37	39	
template (5ng/ul)		Fra10ac1 (C12)	Fra10ac1 (C12)	Fra10ac1 (C12)	Fra10ac1 (C12)	Fra10ac1 (C12)	Fra10ac1 (C12)	Fra10ac1 (C12)	Fra10ac1 (C12)	template
primer-for (5uM)		Fra10ac1-GF3	Fra10ac1-GF5	Joel 2	Joel 2	Fra10ac1-GF3	Fra10ac1-GF5	LF	LF	5' primer
constant primer (5uM)		LAR 3	LAR 3			LAR 7	LAR 7			
primer-rev (5uM)				Fra10ac1-GR5	Fra10ac1-GR6			Fra10ac1-GR5	Fra10ac1-GR6	3' primer
<b>Result</b>		-	+	-	-	-	+	+	+	

<b>probe</b>		26	28	30	32	34	36	38	40	
template (5ng/ul)		Fra10ac1 (G10)	Fra10ac1 (G10)	Fra10ac1 (G10)	Fra10ac1 (G10)	Fra10ac1 (G10)	Fra10ac1 (G10)	Fra10ac1 (G10)	Fra10ac1 (G10)	template
primer-for (5uM)		Fra10ac1-GF3	Fra10ac1-GF5	Joel 2	Joel 2	Fra10ac1-GF3	Fra10ac1-GF5	LF	LF	5' primer
constant primer (5uM)		LAR 3	LAR 3			LAR 7	LAR 7			
primer-rev (5uM)				Fra10ac1-GR5	Fra10ac1-GR6			Fra10ac1-GR5	Fra10ac1-GR6	3' primer
<b>Result</b>		-	+	-	-	-	+	+	+	

**Primers used and expected band length:**

<b>LR on 5' end</b>			
Fra10ac1-GF3	GAGGACCGCGCGGACCTGCAGCGGC	30	7928 bp with mutant DNA
LAR3	CACAACGGGTTCTTCTGTTAGTCC	24	
Fra10ac1-GF5	CCTGGACTGGCTCCTTTTCAGCTGCAGG	34	6844 bp with mutant DNA
LAR3	CACAACGGGTTCTTCTGTTAGTCC	24	
Fra10ac1-GF3	GAGGACCGCGCGGACCTGCAGCGGC	30	6891 bp with mutant DNA
LAR7	GGTGTGGGAAAGGGTTCGAAGTTCCTAT	30	
Fra10ac1-GF5	CCTGGACTGGCTCCTTTTCAGCTGCAGG	28	7096 bp with mutant DNA
LAR7	GGTGTGGGAAAGGGTTCGAAGTTCCTAT	28	
<b>LR on 3' end</b>			
Fra10ac1-GR5	GCATCCTAACTCTGGAAGCATGATGTAAGC	30	7853 bp with mutant DNA
Joel2	GCAATAGCATCACAAATTTCAAAATAAGCA	32	
Fra10ac1-GR6	CCTAGTGGCTCAATAGAACTCAAATACAGCC	28	6769 bp with mutant DNA
Joel2	GCAATAGCATCACAAATTTCAAAATAAGCA	32	
Fra10ac1-GR5	GCATCCTAACTCTGGAAGCATGATGTAAGC	30	5969 bp with mutant DNA
LF	GAGATGGGCAACGCAATTAATG	23	
Fra10ac1-GR6	CCTAGTGGCTCAATAGAACTCAAATACAGCC	28	6174 bp with mutant DNA
LF	GAGATGGGCAACGCAATTAATG	23	

**PCR conditions:**

<b>mastermix:</b>	<b>for 1 reaction:</b>	<b>for 15 reactions:</b>			
Gene spec. Primer (8 pmol/ul)	1,8 ul				
DNA (50 ng/ul)	2,0 ul				
Universal Primer (10 pmol/ul)	1,5 ul	LAR3	LAR7	Joel2	LF
5x LongAmp Taq ReactionBuffer	2,0 ul	22,5	22,5	22,5	22,5
100% DMSO	0,2 ul	3,0	3,0	3,0	3,0
dNTP (10mM)	0,3 ul	4,5	4,5	4,5	4,5
Long-Amp polymerase	0,4 ul	6,0	6,0	6,0	6,0
dd H2O	1,8 ul	27,0	27,0	27,0	27,0
		93,0	93,0	93,0	93,0
<b>Mastermix</b>	<b>6,2 ul</b>				
Gene spec. Primer	1,8 ul				
DNA	2,0 ul				
<b>Gesamt:</b>	<b>10,0 ul</b>				

PCR-machine:	3 min	93°C	
	15 sec	92°C	8x
	30 sec	65 °C	
	8 min.	65 °C	
			-1°C/cycle
	15 sec	92°C	30x
	30 sec	55°C	
	8 min.	65°C	
			+ 20sec/cycle
	9 min.	65°C	
	hold	4°C	

**Primer design strategies:**

**5' LR-PCR :**

PCR with gene-specific 5'-forward primer (GF3 or GF5) AND constant cassette specific 3'-reverse primer (LAV1, LAR7, LAR2, LAR3, or LAR5)

**3' LR-PCR :**

PCR with gene-specific 3'-reverse primer (GR5 or GR6) AND constant cassette specific 5'-forward primer (RAF5, Joel2, PNFR, FRTL3 or LF)

