

Functional characterization of a gene cluster responsible for inositol catabolism associated with hospital-adapted isolates of *Enterococcus faecium*

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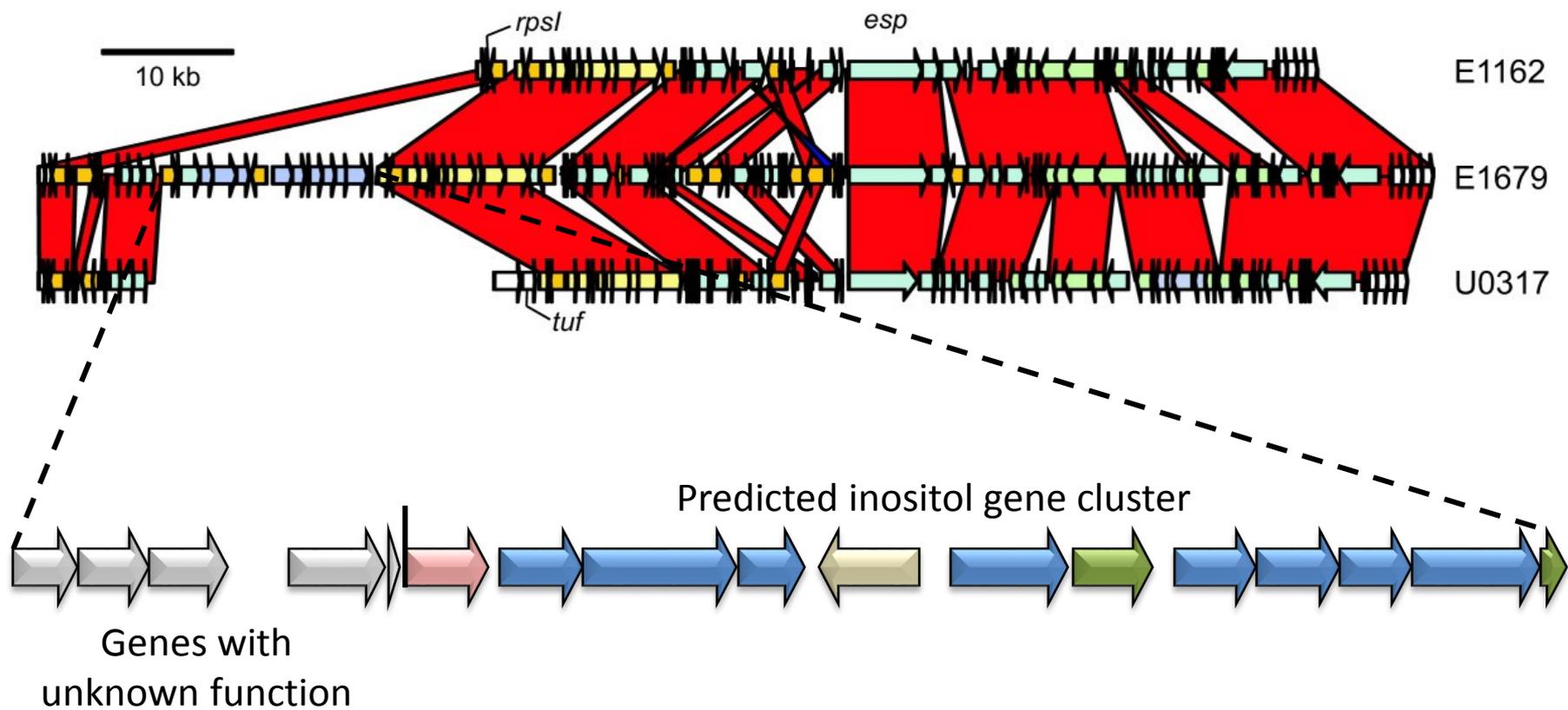
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Suppl. Fig. 1: Organization of the *iol* element encoded on *ICEEfm1* in strain *E. faecium* E1679 (adapted from van Schaik et al. (18)). The *iol* element is encoding for five genes of unknown function (grey arrows) and the *iol* gene cluster, including the *iol* genes indicated in blue, a putative transcriptional repressor (red), a transposase (yellow) and two other genes (green).

A

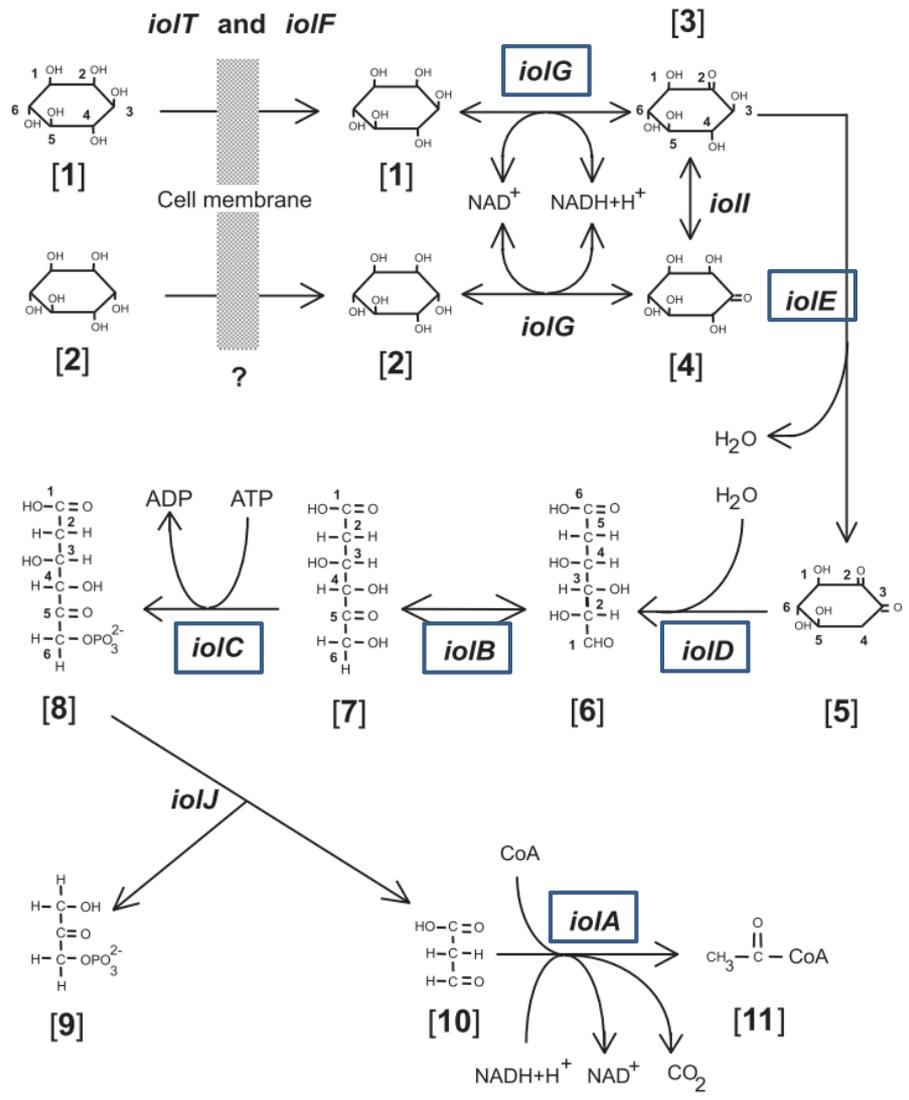
Neighboring genes of first gene <i>iol</i> element in reference E7356	No. of isolates												No. of isolates (with contig breaks)					
	77	38	33	7	3	2	1	2	1	1	2	1	1	1	1	2	2	1
E7356_00308 rpsI	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Yellow	Yellow	Yellow	Yellow	Yellow
E7356_00307 site specific integrase (IntA)	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
E7356_00306 Transposase	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
E7356_00305 Transposase	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
E7356_00304 hypothetical protein	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
E7356_00303 transcriptional regulator	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue
E7356_00302 hypothetical protein	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
E7356_00301 ornithine cyclodeaminase	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
E7356_00300 Putative transposase	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
E7356_00299 hypothetical protein	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
E7356_00298 Putative transposase	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
E7356_00297 hypothetical protein	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
E7356_00296 transcriptional regulator, LacI	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
E7356_00295 <i>iolC</i>	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green

B

Neighboring genes of last gene <i>iol</i> element in reference E7356	No. of isolates					
	161	13	1	3	1	1
E7356_00291 <i>adh</i>	Green	Green	Green	Green	Green	Green
E7356_00290 <i>iolG1</i>	Green	Green	Green	Green	Green	Green
E7356_00289 <i>iolG2</i>	Green	Green	Green	Green	Green	Green
E7356_00288 <i>iolE</i>	Green	Green	Green	Green	Green	Green
E7356_00287 Sodium/myo-inositol cotransporter	Green	Green	Green	Green	Green	Green
E7356_00286 PTS system	Red	Red	Red	Red	Red	Red
E7356_00285 ABC transporter ATP-binding protein	Green	Green	Green	Green	Green	Green
E7356_00284 metal ion ABC transporter permease	Green	Green	Green	Green	Green	Green
E7356_00283 <i>mtsA</i>	Green	Green	Green	Green	Green	Green
E7356_00282 iron dependent repressor	Green	Green	Green	Green	Green	Green
E7356_00281 hypothetical protein	Green	Green	Green	Green	Green	Green

Suppl. Fig. 2: Panaroo gene neighborhood analysis to determine the integration site of the *iol* element using strain *E. faecium* E7356 as reference. A: Neighboring genes for E7356_0303 (light blue), the first gene of the *iol* element as determined for 176/180 isolates. B: Neighboring genes for E7356_0286 (red), the last gene of the *iol* element. Green box: presence of the gene; grey box: absence of gene; blue box: presence of hypothetical gene different from E7356; orange box: presence of a transposase not present in E7356; yellow: gene was not identified as neighboring gene due to contig breaks in these isolates.

A



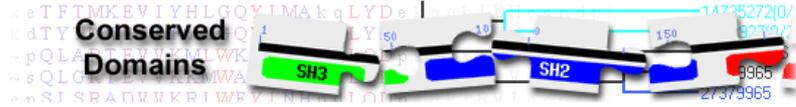
B

Protein/enzyme	Function	inositol encoding genes
IoIT	major inositol transporter	
IoIF	minor inositol transporter	
IoIG	inositol dehydrogenase	
IoII	2-keto-myo-inositol isomerase	
IoIE	2-keto-MI dehydratase	
IoID	THChDO hydrolase	
IoIB	5-deoxy-glucuronate isomerase	
IoIC	5-dehydro-2-deoxygluconokinase	
IoIJ	6-phospho-5-dehydro-2-deoxy-D-gluconate aldolase	
IoIA	malonic semialdehyde dehydrogenase	

C

Step	Compound
1	myo-inositol (MI)
2	D-chiro-inositol
3	2-keto-MI
4	1-keto-MI
5	3D-(3,5/4)-trihydroxycyclohexane-1,2-dione (THChDO)
6	4, 5-deoxy glucuronic acid (5DG)
7	2-deoxy-5-keto-D-gluconic acid (DKG)
8	2-deoxy-5-keto-D-gluconic acid 6-phosphate (DKGP)
9	Dihydroxyacetone phosphate (DHAP)
10	malonic semialdehyde (MSA)
11	acetyl-CoA

Suppl. Fig 3: MI catabolic pathway and functional activities of the *B. subtilis* *iol* genes (adapted from Yoshida et al., 2008 (32)). A: *B. subtilis* *iol* genes proven to encode the enzymes involved in the various reaction steps of the MI catabolic pathway are shown. Blue boxes indicate *iol* genes also identified in the *E. faecium* *iol* gene cluster. C: Overview protein functions *iol* genes. D: Overview of the compounds that are converted by the *iol* enzymes



Conserved domains on [lcl|Query_23218]

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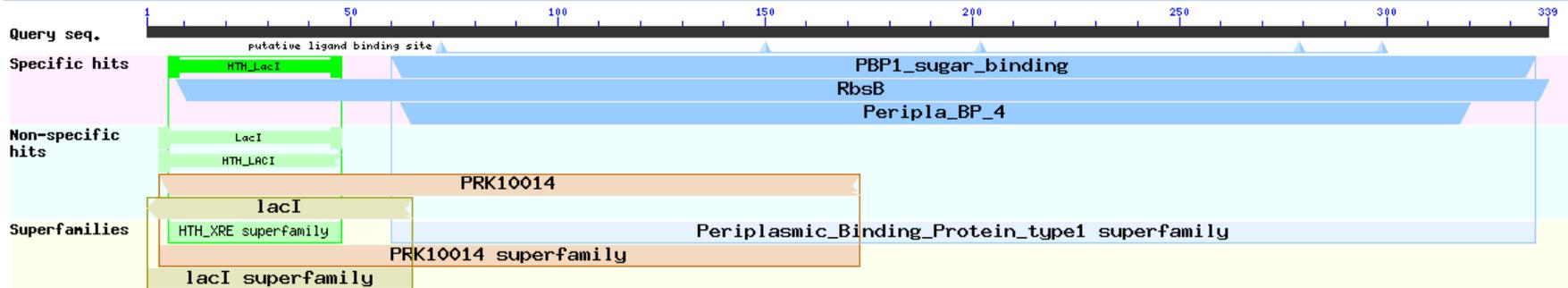
Protein Classification

LacI family DNA-binding transcriptional regulator (domain architecture ID 10105133)

LacI family DNA-binding transcriptional regulator functions as an activator or repressor by binding a specific effector ligand that either decreases (induction) or increases DNA-binding affinity (co-repression)

Graphical summary

Zoom to residue level [show extra options >](#)



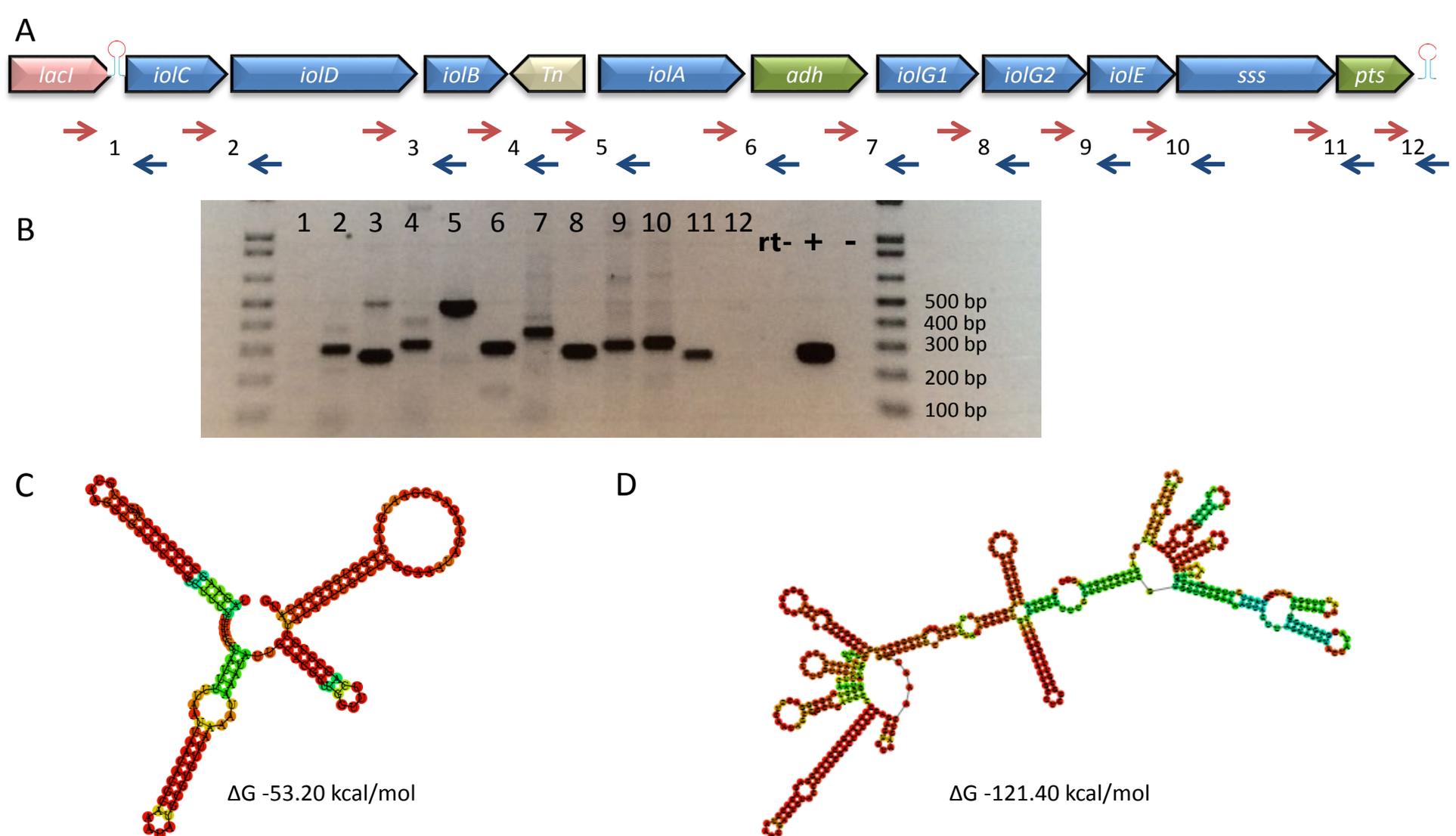
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List of domain hits

+	Name	Accession	Description	Interval	E-value
[+]	PBP1_sugar_binding	cd06307	periplasmic sugar-binding domain of uncharacterized transport systems; Periplasmic ...	60-336	9.86e-63
[+]	RbsB	COG1879	ABC-type sugar transport system, periplasmic component, contains N-terminal xre family HTH ...	8-339	4.19e-45
[+]	Peripla_BP_4	pfam13407	Periplasmic binding protein domain; This domain is found in a variety of bacterial periplasmic ...	62-320	3.14e-44
[+]	HTH_LacI	cd01392	Helix-turn-helix (HTH) DNA binding domain of the LacI family of transcriptional regulators; ...	6-48	3.61e-17
[+]	LacI	pfam00356	Bacterial regulatory proteins, lacI family;	4-48	7.22e-13
[+]	HTH_LACI	smart00354	helix_turn_helix lactose operon repressor;	4-48	2.31e-12
[+]	PRK10014	PRK10014	DNA-binding transcriptional repressor Mall; Provisional	4-173	1.31e-10
[+]	lacI	PRK09526	lac repressor; Reviewed	1-65	7.83e-06

Suppl. Fig. 4: Graphic summary of LacI protein BLAST result.



Suppl. Fig. 5. Structural organization of the *iol* gene cluster in strain *E. faecium* E1504. A: Genomic organization of the *iol* gene cluster, including the *iol* genes indicated in blue, a putative transcriptional repressor (red), a transposase (yellow) and two other genes (green). Also indicated are the predicted transcription terminators (RNAfold), PCR and primer sites for the determination of transcriptional organization. Blue arrows indicate the primers that were used to synthesize cDNA and that were used in combination with the upstream located primer in red for the amplification of the intergenic region. B: PCR results for the 12 PCRs as indicated in panel A. As negative control PCR-2 was also performed on RT- cDNA. As positive control PCR-2 was performed on E1504 genomic DNA. C: downstream *lacI* predicted terminator (RNAfold). D: downstream *pts* predicted terminator (RNAfold).