Development of a diagnostic compatible BCG vaccine against Bovine tuberculosis

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Sup. Fig 1. The cosmid map of pANEE001. The map was generated using Snap gene viewer. In this plasmid, Zeocin antibiotic cassette is flagged with MfeI and Ndel restriction sites at 3' and 5' respectively which enable the user to change the antibiotic cassette as required.



Sup. Fig 2. Confirmation of Δ BCG TK genotype by PCR. (A)PCR products of the expected size were obtained for the mutants with BCG3043_LF_CHK_R and BCG3043_LF_F (PCR1), and using BCG3043_RF_CHK_R and BCG3043_RF_R (PCR2); no products were obtained with wild type (PCRs 1 & 2). PCR over the *BCG3043* gene using BCG3043_LF_F and BCG3043_RF_R further confirms the correct insertion of the Apramycine cassette (PCR3). (B)PCR products of the expected size were obtained for the mutants with MPB70/83_LF_CHK_R and MPB70/83_LF_F (PCR1), and using MPB70/83_RF_CHK_R and MPB70/83_LF_F (PCR2); no products were obtained with wild type (PCRs 1 & 2). PCR

over the *BCG2897/95* gene using MPB70/83_LF_F and MPB70/83_RF_R further confirms the correct insertion of the Hygromycin cassette (PCR3). (C)PCR products of the expected size were obtained for the mutants with BCG3679/80_LF_CHK_R and BCG3679/80_LF_F (PCR1), and using BCG3679/80_RF_CHK_R and BCG3679/80_RF_R (PCR2); no products were obtained with wild type (PCRs 1 & 2). PCR over the *BCG3679/80* gene using BCG3679/80_LF_F and BCG3679/80_RF_R further confirms the correct insertion of the Zeocin cassette (PCR3). All PCR products were run on 1% agarose gel and imaged using a UVP gel doc machine. The schematic representations of genes are not to the scale. The empty spaces around the edges of gel images were trimmed for better representation. The untrimmed version of gel images were given below with exact same order as above images in Sup. Fig 3.



Sup. Fig 3. Confirmation of \triangle BCG TK genotype by PCR. The untrimmed version of gel images of Sup. Fig 2 representing in the exact same order as Sup. Fig. 2.





WT BCG Vaccinated- 24h Pre & Post-Challenge skin Test

WT BCG Vaccinated- 48h pre & post-challenge skin test



Sup. Fig 4: Pre- and Post- *M.bovis* challenge skin test at 24h and 48h against immunodominant antigens. (A, B) The size of erythema against PPD and Fusion antigens in the vaccinate guinea pigs, pre and post *M. bovis* challenge. (C) The size of erythema against PPD and Fusion antigens in the unvaccinated guinea pigs, pre and post *M. bovis* challenge. The error bars indicate standard deviation for each vaccine group.

Skin Test Group	Antigens
Α	PPD-B
B3 (Triple Fusion)	ESAT6-CFP10 Fusion
	MPB70-MPB83 Fusion
	Rv3615c-Rv3020c Fusion
с	ESAT6-CFP10 Fusion
	PBS
D	MPB70-MPB83 Fusion
E3	RV3615c-RV3020c Fusion

Sup.Table1: Details of the skin test antigens. The table list the antigen components for each skin test group

				right flank			left flank	
					Injectio	n Sites		
Vac	cine	Guinea Pig ID	1	2	3	4	5	6
		53587	А	B3	С	D	E3	А
тк/		46349	B3	С	D	E3	А	B3
		22385	С	D	E3	А	B3	С
Vaccine	Group 1	53970	D	E3	A	B3	С	D
(DIMPB	70/83_	73412	E3	A	B3	С	D	E3
ΔRv3615/16_ ΔRv3020)		73656	A	B3	С	D	E3	A
		53286	B3	С	D	E3	A	B3
		46461	С	D	E3	A	B3	С
			right flank					
					Injectio	n Sites		
Vac	cine	Guinea Pig ID	1	2	3	4	5	6
		22603	A	B3	С	D	E3	А
		27113	B3	С	D	E3	А	B3
	h Control/	46267	С	D	E3	А	B3	С
wi Danisi	n Control/	53393	С	D	E3	А	B3	С
vaccine (no.kno	Group 2	73404	С	A	B3	С	D	E3
	ickoutsj	22504	А	B3	С	D	E3	А
		53775	B3	С	D	E3	А	B3
		53446	С	D	E3	А	B3	С
				right flank			left flank	
				right flank	Injectio	on Sites	left flank	
Vac	cine	Guinea Pig ID	1	right flank 2	Injectio 3	on Sites 4	left flank	6
Vac	cine	Guinea Pig ID 23146	1 A	right flank 2 B3	Injectio 3 C	on Sites 4 D	left flank 5 E3	6 A
Vac	cine	Guinea Pig ID 23146 46256	1 A B3	right flank 2 B3 C	Injectio 3 C D	on Sites 4 D E3	left flank 5 E3 A	6 A B3
Vac	cine	Guinea Pig ID 23146 46256 74064	1 A B3 C	right flank 2 B3 C D	Injectio 3 C D E3	n Sites 4 D E3 A	left flank 5 E3 A B3	6 A B3 C
Vac Unvacci Challe	cine	Guinea Pig ID 23146 46256 74064 66922	1 A B3 C D	right flank 2 B3 C D E3	Injectio 3 C D E3 A	D Sites 4 D E3 A B3	left flank 5 E3 A B3 C	6 A B3 C D
Vac Unvacci Challe Gro	cine inated & enged up 3	Guinea Pig ID 23146 46256 74064 66922 53568	1 A B3 C D E3	right flank 2 B3 C D E3 A	Injectic 3 C D E3 A B3	n Sites 4 D E3 A B3 C	left flank 5 E3 A B3 C D	6 A B3 C D E3
Vac Unvacci Challe Gro	cine inated & enged up 3	Guinea Pig ID 23146 46256 74064 66922 53568 73358	1 A B3 C D E3 A	right flank 2 B3 C D E3 A B3	Injectic 3 C D E3 A B3 C	A B B C D C	left flank 5 E3 A B3 C D D E3	6 A B3 C D E3 A
Vac Unvacci Challe Gro	cine inated & enged up 3	Guinea Pig ID 23146 46256 74064 66922 53568 73358 26639	1 A B3 C D E3 A B3	right flank 2 B3 C D E3 A B3 C	Injectio 3 C D E3 A B3 C D	A B B C D E B C D E S	left flank 5 E3 A B3 C D C D E3 A	6 A B 3 C D E 3 A B 3
Vac Unvacci Challe Gro	cine inated & enged up 3	Guinea Pig ID 23146 46256 74064 66922 53568 73358 26639 46990	1 A B3 C D E3 A B3 C	right flank 2 B3 C D E3 A B3 C D	Injectic 3 C D E3 A B3 C D E3	A B B C D E C D E S A	left flank 5 E3 A B3 C D E3 A B3	6 A B3 C D E3 A B3 C
Vac Unvacci Challe Gro	cine inated & enged up 3	Guinea Pig ID 23146 46256 74064 66922 53568 73358 26639 46990	1 A B3 C D E3 A B3 C	right flank 2 B3 C D E3 A B3 C D D	Injectic 3 C D E3 A B3 C D E3	A B B C D E C D E S A	left flank 5 E3 A B3 C D E3 A B3 A B3	6 A B3 C D E3 A B3 C
Vac Unvacci Challe Gro	cine inated & enged up 3	Guinea Pig ID 23146 46256 74064 66922 53568 73358 26639 46990	1	right flank 2 B3 C D E3 A B3 C D right flank	Injectio 3 C D E3 A B3 C D E3	A B B C D E C D E C A	left flank 5 E3 A B3 C D C D E3 A B3 B3 left flank	6 83 C D E3 A B3 C
Unvacci Challe Gro	cine inated & enged up 3	Guinea Pig ID 23146 46256 74064 66922 53568 73358 26639 46990	1 A B3 C D E3 A B3 C	right flank 2 B3 C D E3 A B3 C D right flank	Injectio 3 C D E3 A B3 C D E3	n Sites 4 D E3 A B3 C D E3 A A on Sites	left flank 5 E3 A B3 C D E3 A B3 eft flank	6 A B3 C D E3 A B3 C
Unvacci Challe Groo	cine inated & enged up 3 cine	Guinea Pig ID 23146 46256 74064 66922 53568 73358 26639 46990 Guinea Pig ID	1 A B3 C D E3 A B3 C	right flank 2 B3 C D E3 A B3 C D right flank 2	Injectio 3 C D E3 A B3 C D E3 Injectio 3	n Sites 4 D E3 A B3 C D E3 A A on Sites 4	left flank 5 E3 A B3 C D E3 A B3 B3 left flank	6 A B3 C D E3 A B3 C
Unvacci Challe Groo	cine inated & enged up 3 cine	Guinea Pig ID 23146 46256 74064 66922 53568 73358 26639 46990 Guinea Pig ID 46568	1 A B3 C D E3 A B3 C 1 A	right flank 2 B3 C D E3 A B3 C D right flank 2 B3	Injectio 3 C D E3 A B3 C D E3 Injectio 3 C	n Sites 4 D E3 A B3 C D E3 A E3 A on Sites 4 D	left flank 5 E3 A B3 C D E3 A B3 left flank 5 E3	6 A B3 C D E3 A B3 C
Unvacci Challe Groo	cine inated & enged up 3 cine	Guinea Pig ID 23146 46256 74064 66922 53568 73358 26639 46990 Guinea Pig ID 46568 73776	1 A B3 C D E3 A B3 C 1 A B3 C	right flank 2 B3 C D E3 A B3 C D right flank 2 B3 C B3 C C C C C C C C C C C C C C C C	Injectio 3 C D E3 A B3 C D E3 Injectio 3 C D D	n Sites 4 D E3 A B3 C D E3 A C D E3 A On Sites 4 D C	left flank 5 E3 A B3 C D E3 A B3 left flank 5 E3 A 2 2 2 2 2 2 2 3 2 3 2 2 3 2 3 2 3 2 3 3 3 3 3 3 3 3 3 3 3 3 3	6 A B3 C D E3 A B3 C C
Unvacci Challe Groo Vac	cine inated & enged up 3 cine	Guinea Pig ID 23146 46256 74064 66922 53568 73358 26639 46990 Guinea Pig ID 46568 73776 46761	1 A B3 C D E3 A B3 C 1 A B3 C	right flank 2 B3 C D E3 A B3 C D right flank 2 B3 C B3 C D S C D S C C C C C C C C C C C C C C	Injectio 3 C D E3 A B3 C D E3 Injectio 3 C D E3	on Sites 4 D E3 A B3 C D E3 A O Sites 4 D E3 A C D E3 A	left flank 5 E3 A B3 C D E3 A B3 left flank 5 E3 A B3 C C D C D C C D C C D C C C C C C C C C C C C C	6 A B3 C D E3 A B3 C C 6 A B3 C
Unvacci Challe Gro Vac	cine inated & enged up 3 cine inated & llenged	Guinea Pig ID 23146 46256 74064 66922 53568 73358 26639 46990 Guinea Pig ID 46568 73776 46761 23009	1 A B3 C D E3 A B3 C 1 A B3 C D	right flank 2 B3 C D E3 A B3 C D right flank 2 B3 C B3 C D I I I I I I I I I I I I I I I I I I	Injectio 3 C D E3 A B3 C D E3 Injectio 3 C D E3 C D E3 C C D E3	A B B B C D E C D E C D E C D E S A C D E S C C D E S C C D E S C C D E S C C D E S C C D E S C C D E S C C C D E S C C D E S C C D E S C C D E S C C D E S C C C D E S C C C C C C C C C C C C C C C C C C	left flank	6 A B3 C D E3 A B3 C C 6 A B3 C C D
Unvacci Challe Gro Vac	cine inated & enged up 3 cine inated & llenged up 4	Guinea Pig ID 23146 46256 74064 66922 53568 73358 26639 46990 Guinea Pig ID 46568 73776 46761 23009 46343	1 A B3 C D E3 A B3 C 1 A B3 C D E3 C C C C C C C C C C C C C	right flank 2 B3 C D E3 A B3 C D inght flank 2 B3 C D inght flank 2 B3 C D E3 A A D C C D C C C C C C C C C C C C C C	Injection 3 C D E3 A B3 C D E3 Injection 3 C D E3 A B3 C D E3 C D E3 C C D E3 C C D E3 C C C C C C C C C C C C C	A B B B C D E C D E C C D E S A C C D E S C C C D E S C C C D E S C C D E S C C D E S C C D E S C C D E S C C D E S C C D E S C C D E S C C D E S C C D E S C C D E S C C D E S C C C D E S C C C D E S C C C D E S C C C D E S C C C C C C C C C C C C C C C C C C	left flank	6 A B3 C D E3 A B3 C C 6 A B3 C D E3 C C C C C C C C C C C C C
Unvacci Challe Gro Vac	cine inated & enged up 3 cine cine inated & llenged up 4	Guinea Pig ID 23146 46256 74064 66922 53568 73358 26639 46990 46990 Guinea Pig ID 46568 73776 46761 23009 46343 46999	1 A B3 C D E3 A B3 C 1 A B3 C D E3 A B3 C D E3 A B3 C	right flank 2 B3 C D E3 A B3 C D right flank 2 B3 C D E3 C D E3 A B3 C D E3 A B3	Injection 3 C D E3 A B3 C D E3 Injection 3 C D E3 A B3 C D E3 A B3 C C D E3 C C C D E3 A B3 C C D E3 A B3 C C D E3 A B3 C C D E3 A B3 C C D E3 A B3 C C D E3 A B3 C D E3 A B3 C D E3 A B3 C D E3 A B3 C D E3 A B3 C D E3 A B3 C D E3 A B3 C D E3 A B3 C D E3 A B3 C D E3 A B3 C C D E3 A B3 C C D E3 C C D E3 C C D E3 C C D E3 C C C D E3 C C C D E3 C C C D E3 C C C D C C C D E3 C C C D E3 C C C D E3 C C C D E3 C C C D E3 C C C C C C C C C C C C C	A B B B C D E C D E C D E S A C D E S C C B C C B S C C C C C C C C C C C C	left flank 5 (3) (3) (3) (3) (3) (3) (3) (3)	6 A B3 C D E3 A B3 C C 6 A B3 C C D E3 A
Unvacci Challe Gro Vac Unvacci Unvacci Gro	cine inated & enged up 3 cine cine inated & llenged up 4	Guinea Pig ID 23146 46256 74064 66922 53568 73358 26639 46990 Guinea Pig ID 46568 73776 46761 23009 46343 46999 46428	1 A B3 C D E3 A B3 C 1 A B3 C D E3 A B3 C D E3 A B3 C	right flank 2 B3 C D E3 A B3 C D interpretation 2 B3 C D E3 C D E3 A B3 C D E3 A B3 C C D E3 A B3 C C E3 E3 C E3 E3 E3 E3 E5	Injectic 3 C D E3 A B3 C D E3 Injectic 3 C D E3 A B3 C D E3 A B3 C D D E3 C D D E3 C D D E3 C D C D C D C D C C D C C D C C D C C C C D C C C D C	A B B B B C D E C D E C D E S C C D E S C C D E S C C C C C C C C C C C C C C C C C C	left flank 5 E3 A B3 C D E3 A B3 Image: Comparison of the second	6 A B3 C D E3 A B3 C C 6 A B3 C D E3 A B3 C D E3 A B3 C

Sup. Table2: Pre-challenge & post-challenge skin testing study design (Skin-testing injection Regime). Table shows the injection for each antigen preparations (coded A-E) for each group of animals. Antigen preparation group Key- A: PPD-B, B3: ESAT6-CFP10 fusion+MPB7—83 fusion+Rv3615c-3020 fusion(Triple fusion), C:ESAT6-CFP10 fusion, D: MPB70—83 fusion, E3:RV3615c-Rv3020c fusion

		Latin Square preliminary analys	is
24 hour	24 hour TST reading General Linear model ANOVA (P value)		
		Pre-Challenge	Post-Challenge
Vaccine	Treatment	Position on flank: signal of antigen	Position on flank: signal of antigen
ТК	△BCG TK	0.792	0.323
WT BCG	WT BCG strain	0.339	0.889
Unvaccinated + challenged	Unvaccinated	#	0.362
Unvaccinated + unchallenged	Unvaccinated	#	0.516
		Latin Square preliminary analys	is
48 hour	TST reading	General Linear m	odel ANOVA (P value)
		Pre-Challenge	Post-Challenge
Vaccine	Treatment	Position on flank: signal of antigen	Position on flank: signal of antigen
ТК	△BCG TK	0.762	0.072
WT BCG	WT BCG strain	0.642	0.074
Unvaccinated + challenged	Unvaccinated	#	NA

Sup. Table 3: ANOVA general linear model (Latin square) statistical analysis to determine the effect of skin test flank location. The # symbol denotes the statistical analysis could not generate P value as all responses were 0. NA denotes not done

0.451

Unvaccinated Unvaccinated

+

unchallenged

#

Zeo_casset_F	GAACTCCAATTGATGGCCAAGTTGACCAGTGC
Zeo_casset_R	GAACTCCATATGTCAGTCCTGCTCCTCGGCCAC
pYUB_inv_F	GACATC CAATTGTCACAGCGGACCTCTATTC
pYUB_inv_R	GATCTCCATATGAACTGGCGCAGTTCCTCTGG
BCG3043_RF_F	GATCTCAAGCTTTCCTTCCAATTCGAATC
BCG3043_RF_R	GATCTCACTAGTTGGTGGCGACGAATTTC
BCG3043_LF_F	GATCTCCTTAAGCCAACCACGCCACATAC
BCG3043_LF_R	GATCTCTCTAGATGCTCGGAATGAAAAGG
MPB70/83_RF_F	GATCTCAAGCTTATGCCTCCGGCGTAATC
MPB70/3_RF_R	GATCTCACTAGTGAGCCCTGACCATTTCC
MPB70/83_LF_F	GATCTCCTTAAGGCTCGTCAGCGACGGC
MPB70/83_LF_R	GATCTCTCTAGAACCAGTGATTCGGAGTG
BCG3679/80_RF_F	GATCTCAAGCTTCCTGACCACGTTTGCTGC
BCG3679/80_RF_R	GATCTCACTAGTCGTGCTCTATTAATGCTG
BCG3679/80_LF_F	GATCTCCTTAAGTCTATCAGTAGGCGGCTAG
BCG3645/46_LF_R	GATCTCTCTAGAAACTGCGCTGCGACAATG
BCG3043_RF_CHK_F	GTCGTTGCAGAGTGCGGTGG
BCG3043_LF_CHK_R	CCAATAATGTTGAAACCCAGG

MPB70/83_RF_CHK_F	CCAGCGATTCCTTGTTG
MPB70/83_LF_CHK_R	CAAAACACGAACAAGTGAGG
BCG3679/80_RF_	AAATCGCGTACGTGG
CHK_F	
BCG3679/80_	GAAGTGCACGCAGTTGCC
RF_CHK_R	
BCG3679/80_LF_CHK_	CAAGTTGACCAGTGCCGTTC
F	
BCG3679/80_LF_CHK_	CAATTGAGTCATCCAGCG
R	

Sup. Table4: Primers used in the study