Supplementary Material S1. TREC assay validation

Summary of TREC assay variation during the initial method verification				
	SAMPLES			
	n	**Cal A**	**C1**	**C3**
SD (copies/µL)	28	0.74	0.38	0,44
kit SD expected values		<0.84	<0.65	<0.71
CV (%)	28	85	39	46
kit CV expected values		<101	<73	<81

	n	**Value**
LoD (copies/µL)	20	3.4
LoQ (copies/µL)	20	4.9
External Quality assessment	5	100%
Sensitivity	3006	100%
Specificity	3006	99.9%
Contamination risk	111	0.9%

Suppl. Mat. S1. Abbreviations: CV, coefficient of variation (%); DBS, dry blood spot; LoD, limit of Detection (copies/µL); LoQ, limit of Quantification (copies/µL); SCID, severe combined immunodeficiency; SD, standard deviation (copies/µL); TREC, T-cell receptor excision circle

- Calibrator A (Cal A), Control 1 and Control 2 were from lot 652072.
- Variation was expressed as standard deviations (SD) in the natural logarithmic (ln) scale and as CV% in lognormal scale; both were compared with the kit supplier's SD and CV% for the same range of values.
- LoD and LoQ calculated with a blank sample and very low TRECs sample analyzed during 10 days in duplicate and by calculating the critical value.
- External quality assessment was evaluated as a qualitative method (CDC Program, % of successful results are indicated).
- Sensitivity was evaluated using 6 DBS samples from babies with confirmed SCID
- Contamination risk was evaluated with the C2 control (0 copies/µL) and a blank paper (without sample). A result of >10 copies/µL was considered as contamination.

Supplementary Material S2. SCID criteria defined by Kwan et al (2)

	CD3 T Cells/μL	PHA proliferation	Supporting features
Typical SCID	< 300	< 10% of normal	Detectable maternal T cells in peripheral blood; proven deleterious defect(s) in a known SCID gene

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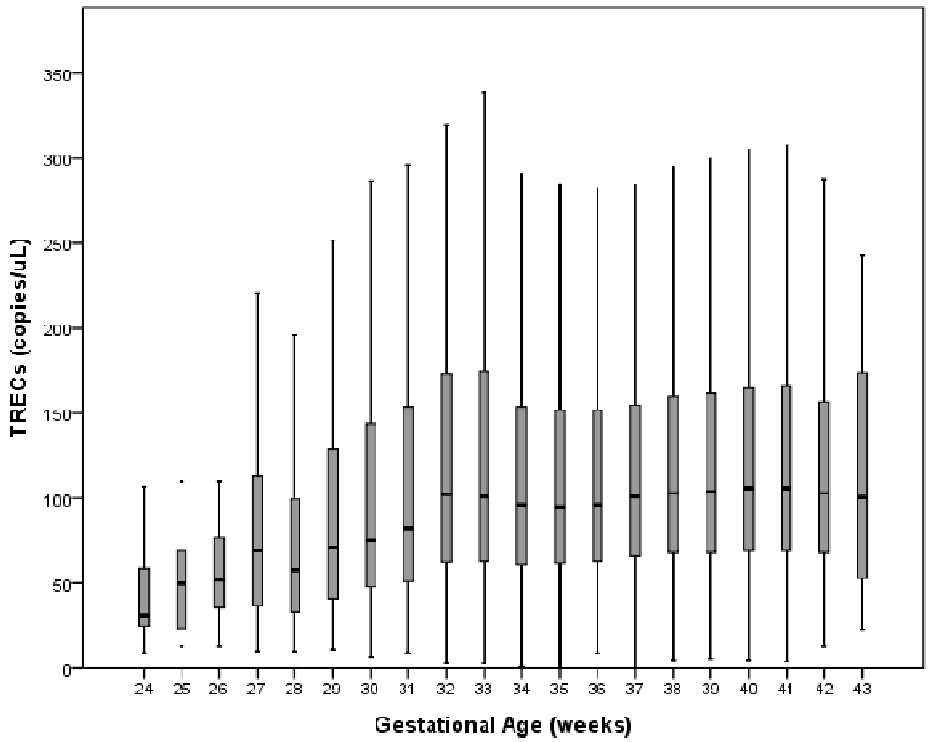
Supplementary Material S3. List of 323 primary immunodeficiency disease genes included in our panel

List of 323 primary immunodeficiency disease genes											
ACP5	*C4BPB*	*CD55*	*CTSC*	*FERMT3*	*IL10RB*	*LAT*	*MVK*	*PIK3R1*	*RNF31*	*TRAF3IP2*	*TAP1*
ACTB	*C5*	*CD59*	*CXCR4*	*FOXN1*	*IL12B*	*LCK*	*MYD88*	*PLCG2*	*RORC*	*TREX1*	*TAP2*
ADA	*C6*	*CD70*	*CYBA*	*FOXP3*	*IL12RB1*	*LIG1*	*MYO5A*	*PMS2*	*RPSA*	*TRNT1*	*TAPBP*
ADAM17	*C4A*	*CD79A*	*CYBB*	*FPR1*	*IL17F*	*LIG4*	*NBN*	*PNP*	*RTEL1*	*TTC37*	*TAZ*
ADAMTS13	*C4B*	*CD79B*	*DCLRE1B*	*G6PC*	*IL17RA*	*LMNA*	*NBS1*	*POLE1*	*SAMHD1*	*TTC7A*	*TBK1*
ADAR	*C4BPA*	*CD81*	*DCLRE1C*	*G6PC3*	*IL17RC*	*LPIN2*	*NCF1*	*PRF1*	*SBDS*	*TYK2*	*TBX1*
AICDA	*C7*	*CD8A*	*DEPTOR*	*G6PD*	*IL18*	*LRBA*	*NCF2*	*PRKCD*	*SERPING1*	*UNC119*	*TCF3*
AIRE	*C8A*	*CEBPE*	*DGKE*	*G6PT1*	*IL1RN*	*LRRC8A*	*NCF4*	*PRKDC*	*SH2D1A*	*UNC13D*	*TCIRG1*
AK2	*C8B*	*CECR1*	*DKC1*	*GATA2*	*IL21*	*LYST*	*NEIL3*	*PSMB8*	*SH3BP2*	*UNC93B1*	*TCN2*
AKT1	*C8G*	*CFB*	*DNMT3B*	*GFI1*	*IL21R*	*MAGT1*	*NFAT5*	*PSTPIP1*	*SKIV2L*	*TERC*	*TNFSF6*
AP3B1	*C9*	*CFD*	*DOCK2*	*GIMAP5*	*IL2RA*	*MALT1*	*NFKB1*	*PTPRC*	*SLC11A1*	*TERT*	*TRAC*
AP3D1	*CARD11*	*CFH*	*DOCK8*	*GP1BA*	*IL2RG*	*MAP3K14*	*NFKB2*	*RAB27A*	*SLC29A3*	*TFRC*	*TRAF3*
AP4E1	*CARD14*	*CFI*	*ELANE*	*HAX1*	*IL36RN*	*MASP1*	*NFKBIA*	*RAC2*	*SLC35C1*	*THBD*	*UNG*
APOL1	*CARD9*	*CFP*	*ELF4*	*ICOS*	*IL7R*	*MASP2*	*NHEJ1*	*RAG1*	*SLC37A4*	*TICAM1*	*USB1*
ARPC1B	*CARMIL2*	*CHD7*	*EPG5*	*IFIH1*	*INO80*	*MBL2*	*NHP2*	*RAG2*	*SLC46A1*	*TINF2*	*VPREB1*
ATM	*CASP10*	*CIITA*	*F12*	*IFNGR1*	*IRAK4*	*MCM10*	*NLRC4*	*RASGRP1*	*SMARCAL1*	*TLR3*	*VPS13B*
BLM	*CASP8*	*CLEC7A*	*FADD*	*IFNGR2*	*IRF7*	*MCM4*	*NLRP12*	*RASGRP2*	*SP110*	*TMC6*	*VPS45*
BLNK	*CD19*	*CLPB*	*FAM105B*	*IGHA1*	*IRF8*	*MEFV*	*NLRP3*	*RBCK1*	*SPINK5*	*TMC8*	*WAS*
BLOC1S6	*CD247*	*COH1*	*FAS*	*IGHG2*	*ISG15*	*MKL1*	*NOD2*	*RECQL4*	*STAT1*	*TMEM173*	*WASF2*
BTK	*CD27*	*COLEC11*	*FASLG*	*IGHM*	*ITCH*	*MLPH*	*NOP10*	*RFX5*	*STAT2*	*TNFAIP3*	*WIPF1*
C1QA	*CD3D*	*COPA*	*FCGR1A*	*IGLL1*	*ITGB2*	*MMACHC*	*NRAS*	*RFXANK*	*STAT3*	*TNFRSF11A*	*WRAP53*
C1QB	*CD3E*	*CORO1A*	*FCGR2A*	*IKBA*	*ITK*	*MPO*	*ORAI1*	*RFXAP*	*STAT5B*	*TNFRSF13B*	*XIAP*
C1QC	*CD3G*	*CR2*	*FCGR2B*	*IKBKB*	*JAGN1*	*MRE11A*	*PARN*	*RMRP*	*STIM1*	*TNFRSF13C*	*XRCC4*
C1R	*CD3Z*	*CSF2RA*	*FCGR3A*	*IKBKG*	*JAK2*	*MS4A1*	*PAX5*	*RNASEH2A*	*STK4*	*TNFRSF1A*	*ZAP70*
C1S	*CD40*	*CSF3R*	*FCGR3B*	*IKZF1*	*JAK3*	*MSH6*	*PGM3*	*RNASEH2B*	*STN1*	*TNFRSF4*	*ZBTB24*
C2	*CD40LG*	*CTLA4*	*FCGRT*	*IL10*	*KRAS*	*MSN*	*PIGA*	*RNASEH2C*	*STX11*	*TNFRSF6*	*ZNF345*
C3	*CD46*	*CTPS1*	*FCN3*	*IL10RA*	*LAMTOR2*	*MTHFD1*	*PIK3CD*	*RNF168*	*STXBP2*	*TNFSF12*	

Supplementary Material S4. Median TREC copy numbers at each gestational week



Suppl. Mat. S5. Error bars indicate 25th and 75th percentile TREC copy numbers at each gestational week. Median TREC levels in our cohort rose significantly between 28 and 32 weeks gestation, in accordance with T-cell maturation in this period, a wider period of time than those reported by other authors (4,18,26).

Abbreviations: TRECs: T-cell receptor excision circles

Supplementary Material S5. Flow cytometry protocols

Peripheral whole blood (50 μL) was incubated with a mix of specific conjugated monoclonal antibodies (mAb) from each panel and gently mixed for 20 min at room temperature (RT) in the dark. The composition of mAb, florochromes, and brands are specified in the following table:

T B NK populations		
Cluster of Differentiation	*Fluorochrome*	*Brand*
CD45	FITC	Beckman Coulter
CD4	RD1	Beckman Coulter
CD8	ECD	Beckman Coulter
CD3	PC5	Beckman Coulter
CD56	RD1	Beckman Coulter
CD19	ECD	Beckman Coulter
CD45 R0/RA		
Cluster of Differentiation	*Fluorochrome*	*Brand*
CD45RA	FITC	Becton Dickinson
CD45R0	PE	Becton Dickinson
CD3	ECD	Beckman Coulter
CD4	PerCP	Becton Dickinson
CD8	PE-Cy7	Beckman Coulter
HLA-DR		
Cluster of Differentiation	*Fluorochrome*	*Brand*
CD3	ECD	Beckman Coulter
CD4	APC	Cytognos
CD8	PE-Cy7	Beckman Coulter
HLA-DR	Pacific Blue	Beckman Coulter

Samples were treated with 1 mL of VersaLyse lysing solution (Beckman Coulter), vortexed, and incubated for 15 min at RT in the dark. Samples were then washed with phosphate-buffered saline (PBS), stored at RT in the dark, and analyzed within 1 h. Samples were acquired with a Navios EX Flow Cytometer (Beckman Coulter), equipped with three lasers: a 405-nm violet laser, a 488-nm blue laser, and a 638-nm red laser. At least 100,000 events were acquired from each sample. Flow cytometry data were analyzed with Kaluza software (Beckman Coulter).