Supplementary Data

Table S1 - NGS EB gene-panels at the Dutch EB Expertise Center.

Next-generation gene-panel epidermolysis bullosa					
Targeted panel sequencing		**Whole-exome sequencing**			
1st version March 2014	2nd version July 2014	1st version October 2017	2nd version March 2020	Last version May 2022	Percentage ≥ 20x coverage
-	-	-	*ATP2A2*	*ATP2A2*	99.88
ATP2C1	*ATP2C1*	*ATP2C1*	*ATP2C1*	*ATP2C1*	99.84
-	-	-	*CAST*	*CAST*	97.31
CD151	*CD151*	*CD151*	*CD151*	*CD151*	99.45
CDSN	*CDSN*	*CDSN*	*CDSN*	*CDSN*	99.94
-	-	-	*CHST8*	*CHST8*	97.87
COL17A1	*COL17A1*	*COL17A1*	*COL17A1*	*COL17A1*	99.17
COL7A1	*COL7A1*	*COL7A1*	*COL7A1*	*COL7A1*	99.84
CSTA	*CSTA*	*CSTA*	*CSTA*	*CSTA*	99.69
-	-	-	-	*CSTB*	100
-	-	-	*DSC1*	*DSC1*	99.92
-	-	-	*DSC3*	*DSC3*	99.37
-	-	-	*DSG1*	*DSG1*	97.62
-	-	-	*DSG3*	*DSG3*	99.2
DSP	*DSP*	*DSP*	*DSP*	*DSP*	100
DST	*DST*	*DST*	*DST*	*DST*	98.9
EXPH5	*EXPH5*	*EXPH5*	*EXPH5*	*EXPH5*	99.7
GJB6	*GJB6*	*GJB6*	-	-	-
FERMT1	*FERMT1*	*FERMT1*	*FERMT1*	*FERMT1*	99.65
-	-	-	*FLG2*	*FLG2*	100
-	-	-	*IKBKG*	*IKBKG*	19.89
ITGA3	*ITGA3*	*ITGA3*	*ITGA3*	*ITGA3*	99.66
ITGA6	*ITGA6*	*ITGA6*	*ITGA6*	*ITGA6*	99.99
ITGB4	*ITGB4*	*ITGB4*	*ITGB4*	*ITGB4*	97.31
JUP	*JUP*	*JUP*	*JUP*	*JUP*	97.69
-	*KLHL24*	-	*KLHL24*	*KLHL24*	100
KRT1	*KRT1*	*KRT1*	*KRT1*	*KRT1*	100
KRT10	*KRT10*	*KRT10*	*KRT10*	*KRT10*	98.48
KRT14	*KRT14*	*KRT14*	*KRT14*	*KRT14*	99.55
KRT16	*KRT16*	*KRT16*	*KRT16*	*KRT16*	82.09
KRT17	*KRT17*	*KRT17*	*KRT17*	*KRT17*	94.16
-	-	-	*KRT2*	*KRT2*	100
KRT5	*KRT5*	*KRT5*	*KRT5*	*KRT5*	100
KRT6A	*KRT6A*	*KRT6A*	*KRT6A*	*KRT6A*	100
KRT6B	*KRT6B*	*KRT6B*	*KRT6B*	*KRT6B*	100
KRT6C	*KRT6C*	*KRT6C*	*KRT6C*	*KRT6C*	98.15
KRT9	*KRT9*	*KRT9*	*KRT9*	*KRT9*	100
LAMA3	*LAMA3*	*LAMA3*	*LAMA3*	*LAMA3*	98.92
LAMB3	*LAMB3*	*LAMB3*	*LAMB3*	*LAMB3*	98.63
LAMC2	*LAMC2*	*LAMC2*	*LAMC2*	*LAMC2*	99.96
PKP1	*PKP1*	*PKP1*	*PKP1*	*PKP1*	99.97
PLEC1	*PLEC1*	*PLEC1*	*PLEC1*	*PLEC1*	99.08
-	-	-	*SERPINB8*	*SERPINB8*	100
SPINK5	*SPINK5*	*SPINK5*	*SPINK5*	*SPINK5*	98.11
TGM5	*TGM5*	*TGM5*	*TGM5*	*TGM5*	99.87

-	-	-	*TP63*	*TP63*	94.05
WNT10A	*WNT10A*	*WNT10A*	-	-	-

Inclusion and coverage of genes as in the latest version (May 2022) of the WES EB gene-panel used in this study. The contents of the NGS EB gene-panels are revisited twice annually and updated based on new genetic findings in the literature. EB, epidermolysis bullosa; NGS, next-generation sequencing; TPS, targeted panel sequencing; WES, whole-exome sequencing.

Table S2 - Diagnostic yield and turnaround times for SS and NGS-based methods per EB subtype performed from 1994–2022.

EB type	EB subtype*		Total cases, *n* (%)			Diagnostic yield, *n* (%)				Turnaround time, *days (IQR)*		
			Total	Solved	Unsolved	SS	NGS			SS	NGS	
							TPS	WES	NGS# 2nd		TPS	WES
EBS	Dominant	Localized	49 (39%)	42 (86%)	7 (14%)	33 (85%)	-	9 (90%)	-	392 (565)	-	38 (8)
		Intermediate	9 (7%)	8 (89%)	1 (11%)	5 (71%)	3 (100%)	-	-	428 (570)	74 (-)	-
		Intermediate *(PLEC1)*	6 (5%)	6 (-)	-	6 (100%)	-	-	-	440 (-)	-	-
		Intermediate (*KLHL24*)	1 (1%)	1 (-)	-	-	-	-	1 (-)	-	-	-
		Mottled pigmentation	7 (6%)	7 (-)	-	6 (100%)	-	1 (100%)	-	179 (279)	-	17 (-)
		Severe	23 (18%)	23 (100%)	-	16 (94%)	1 (100%)	5 (100%)	1 (-)	251 (223)	42 (-)	39 (-)
	Total DEBS		95 (75%)	87 (92%)	8 (8%)	66 (88%)	4 (100%)	15 (94%)	2 (-)	354 (357)	60 (-)	38 (11)
	Recessive	Localized (*DST*)	3 (2%)	3 (-)	-	-	1 (100%)	1 (100%)	1 (-)	-	94 (-)	12 (-)
		Intermediate (*EXPH5*)	4 (3%)	4 (100%)	-	-	-	2 (100%)	2 (-)	-	-	40 (-)
		Intermediate (*PLEC1*)	2 (2%)	2 (-)	-	2 (100%)	-	-	-	1480 (-)	-	-
		Intermediate (*PLEC1α*)	1 (1%)	1 (-)	-	1 (100%)	-	-	-	254 (-)	-	-
		Intermediate/severe	4 (3%)	4 (100%)	-	4 (100%)	-	-	-	167 (227)	-	-
		Suprabasal EBS	18 (14%)	18 (100%)	-	11 (92%)	2 (100%)	3 (100%)	2 (-)	153 (152)	64 (-)	37 (-)
	Total REBS		32 (25%)	32 (100%)	-	19 (83%)	3 (100%)	6 (100%)	4 (-)	162 (238)	90 (-)	36 (18)
Total EBS			127 (41.2%)	119 (94%)	8 (6%)	85 (87%)	7 (100%)	21 (95%)	6 (-)	277 (380)	74 (48)	37 (12)
JEB	Dominant	Localized	1 (1%)	1 (-)	-	1 (100%)	-	-	-	486 (-)	-	-
	Recessive	Localized	15 (22%)	12 (80%)	3 (18%)	10 (67%)	-	-	2 (-)	269 (757)	-	-
		Intermediate	25 (36%)	25 (100%)	-	19 (100%)	3 (100%)	3 (100%)	-	277 (634)	53 (-)	34 (-)
		Severe	25 (36%)	25 (100%)	-	19 (100%)	3 (100%)	3 (100%)	-	149 (314)	28 (-)	34 (-)
		Pyloric atresia	3 (4%)	2 (67%)	1 (33%)	2 (67%)	-	-	-	72 (-)	-	-
Total JEB			69 (22.4%)	65 (94%)	4 (6%)	51 (89%)	6 (100%)	6 (100%)	2 (-)	214 (495)	39 (28)	34 (23)
DEB	Dominant	Localized	26 (24%)	24 (92%)	2 (8%)	20 (100%)	-	4 (67%)	-	128 (325)	-	30 (16)
		Intermediate	17 (16%)	16 (94%)	1 (6%)	12 (92%)	2 (100%)	2 (100%)	-	70 (302)	84 (-)	34 (-)
		Pruriginosa	6 (6%)	6 (-)	-	5 (100%)	1 (100%)	-	-	66 (216)	37	-
		Self-improving	1 (1%)	1 (-)	-	-	-	1 (100%)	-	-	-	24 (-)
		Unknown	3 (3%)	3 (-)	-	-	1 (100%)	2 (100%)	-	-	45	23 (-)
	Total DDEB		53 (49%)	50 (94%)	3 (6%)	37 (97%)	4 (100%)	9 (82%)	-	90 (292)	47 (62)	25 (14)
	Recessive	Localized	6 (6%)	6 (-)	-	2 (50%)	-	2 (100%)	2 (-)	193 (-)	-	119 (-)
		Intermediate	16 (15%)	15 (94%)	1 (6%)	12 (100%)	1 (100%)	2 (67%)	-	67 (158)	56	34 (-)
		Inversa	8 (7%)	8 (100%)	-	7 (100%)	1 (100%)	-	-	1004 (1277)	52 (-)	-

		Severe	24 (22%)	22 (92%)	2 (8%)	19 (90%)	-	3 (100%)	-	302 (917)	-	42 (-)
		Unknown	2 (2%)	2 (-)	-	1 (100%)	1 (100%)	-	-	113 (-)	128 (-)	-
	Total RDEB		56 (51%)	53 (95%)	3 (5%)	41 (91%)	3 (100%)	7 (88%)	2 (-)	202 (941)	56 (-)	42 (17)
Total DEB			109 (35.4%)	103 (94%)	6 (6%)	78 (94%)	7 (100%)	16 (84%)	2 (-)	128 (668)	52 (73)	30 (18)
Total KEB			3 (1.0%)	2 (67%)	1 (33%)	0 (0%)	-	1 (100%)	1 (-)	-	-	46 (-)
Total EB			308	289 (94%)	19 (6%)	214 (89%)	20 (100%)	44 (92%)	11 (50%)	211 (464)	53 (46)	36 (16)

* EB-types are considered as four major EB-types: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB) and Kindler EB (KEB). EB-subtypes are divided in dominant (D) and recessive (R), distinguished between different EBS-intermediate subtypes, both dominant and recessive, according to the latest consensus paper.[1] We included suprabasal EBS-subtypes as these phenotypes were part of the EB classification until 2020 (they are currently classified among the EB-related skin fragility disorders).[15] # EB-cases with pathogenic variant(s) detected via NGS, either TPS or WES, after SS failed to do so. Excluded in the calculation of the diagnostic yield for NGS (only numbers displayed). DEB, dystrophic epidermolysis bullosa; EB, epidermolysis bullosa; EBS, epidermolysis bullosa simplex; IQR, interquartile range; JEB, junctional epidermolysis bullosa; KEB, Kindler epidermolysis bullosa; NGS, next-generation sequencing; IQR, interquartile range; SS, Sanger sequencing; TPS, targeted panel sequencing; WES, whole-exome sequencing.

Table S3 - Distribution and type of additional sequence variants found with SS and NGS in EB patients from 1994–2022.

	Total, *n* (%)	**SS**[*], *n* (%)	**NGS**[#], *n* (%)
Total EB-cases, *n* (%)	308 (-)	240 (-)	90 (-)
VUS, *n* (%)	**33 (-)**	**13 (5%)**	**20 (22%)**
Solved EB-cases, *n* (%)	28 (85%)	12 (82%)	16 (80%)
Candidate EB-gene	13	9	4
Other EB-gene	10	3	7
EB-related gene	3	-	3
EB and EB-related gene	2	-	2
Unsolved EB-cases, *n* (%)	5 (15%)	1 (8%)	4 (20%)
Candidate EB-gene	2	1	1
Other EB-gene	1	-	1
EB-related gene	1	-	1
EB and EB-related gene	1	-	1
AF, *n* (%)	**15 (-)**	**3 (1%)**	**12 (13%)**
Solved EB-cases, *n* (%)	13 (87%)	3 (100%)	10 (83%)
Candidate EB-gene (*LAMA3*)	1	-	1
Other EB-gene (*PLEC1*)	5	2	3
EB-related gene (*CAST*, *KRT6A*, *WNT10A*)	6	-	6
EB (*LAMB3*) and EB-related gene (*WNT10A*)	1	1	-
Unsolved EB-cases, *n* (%)	2 (13%)	-	2 (17%)
Candidate EB-gene	-	-	-
Other EB-gene (*DST*)	1	-	1
EB-related gene (*WNT10A*)	1	-	1

* Number of EB-cases in whom SS was performed as initial diagnostic tier. # Total EB-cases in whom NGS was performed (including EB-cases in whom SS was performed first). AF, additional finding(s); VUS, variant of uncertain clinical significance.

Figure S1

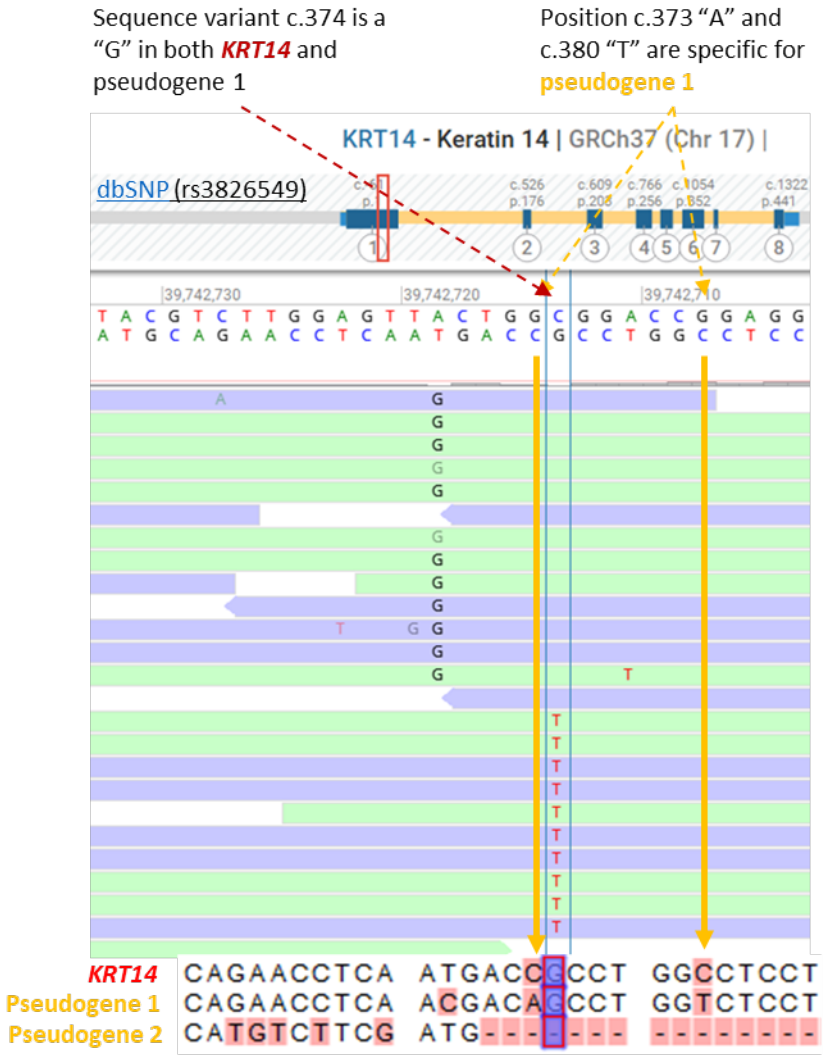

Figure S1 – Discriminating a sequence variant in the functional *KRT14* gene from its pseudogenes. Example of the identification of a c.374G>A, p.(Arg125His) sequence variant in the functional *KRT14* gene. Top, WES sequence reads (blue/green bars) mapped against the *KRT14* reference sequence. Bottom, the reference sequences of a part of the functional *KRT14*-gene and its corresponding pseudogenes. In this example, the software flagged a deviation from the *KRT14* reference sequence: an "A" at position c.374 ("T" in reverse strand). As the sequence of *KRT14* and its 1st pseudogene (KRT16P6) is the same ("G") at this position, a distinction needed to be made whether the G>A transition occurred on the functional *KRT14* gene or one of its pseudogene(s). The 1st pseudogene shows an "A" at position c.373 and a "T" at position c.380, for the 2nd pseudogene (KRT17P5) bases are missing at these positions, while for the functional *KRT14* gene a "C" is present on both positions. These sequence differences of the pseudogenes are not found back in the reads containing the G>A transition (reads with a base "T" shown in red). Therefore, it can be concluded that the G>A transition located on the functional *KRT14* gene.

Figure S2

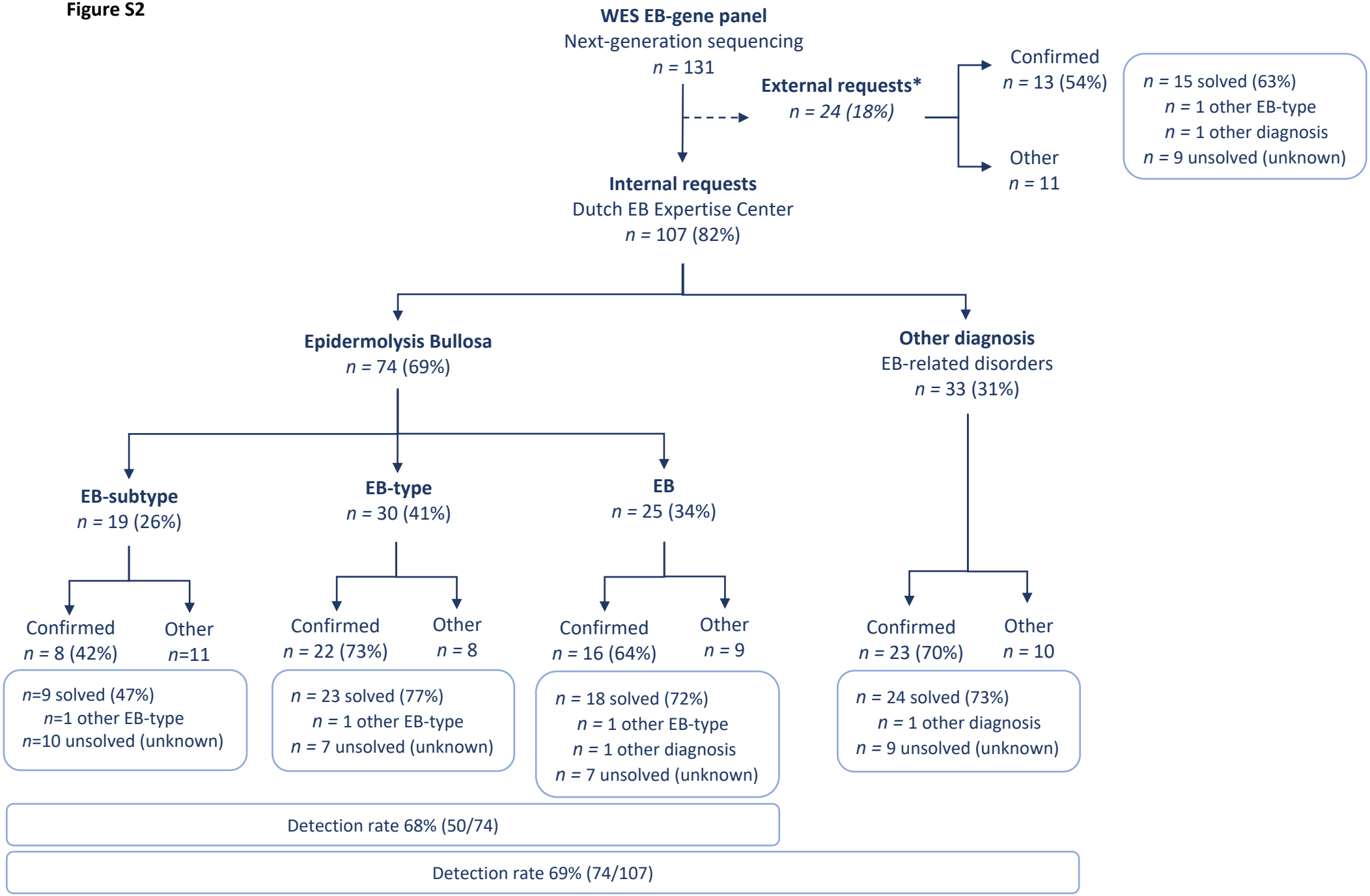
WES EB-gene panel
Next-generation sequencing
n = 131
External requests*
n = 24 (18%)
Confirmed
n = 13 (54%)
Other
n = 11
n = 15 solved (63%)
n = 1 other EB-type
n = 1 other diagnosis
n = 9 unsolved (unknown)
Internal requests
Dutch EB Expertise Center
n = 107 (82%)
Epidermolysis Bullosa
n = 74 (69%)
Other diagnosis
EB-related disorders
n = 33 (31%)
EB-subtype
n = 19 (26%)
EB-type
n = 30 (41%)
EB
n = 25 (34%)
Confirmed
n = 8 (42%)
Other
n=11
Confirmed
n = 22 (73%)
Other
n = 8
Confirmed
n = 16 (64%)
Other
n = 9
Confirmed
n = 23 (70%)
Other
n = 10
n=9 solved (47%)
n=1 other EB-type
n=10 unsolved (unknown)
n = 23 solved (77%)
n = 1 other EB-type
n = 7 unsolved (unknown)
n = 18 solved (72%)
n = 1 other EB-type
n = 1 other diagnosis
n = 7 unsolved (unknown)
n = 24 solved (73%)
n = 1 other diagnosis
n = 9 unsolved (unknown)
Detection rate 68% (50/74)
Detection rate 69% (74/107)

Figure S2 – Detection rate of the WES EB-gene panel from the perspective of the Genome Diagnostics laboratory. Flowchart showing requests received by the Genome Diagnostics laboratory for EB gene-panel WES diagnostics from its implementation in May 2017 until January 2022 (*n* = 131). In total, 74 internal requests were received from specialists of the Dutch EB Expertise Center because of clinical suspicion of EB. Another 33 internal requests were received for EB-related disorders. We analyzed the mutation detection rate per request reason. 'EB' indicates broad clinical suspicion of a type of EB. 'EB-type' indicates clinical suspicion of one of the four major EB-types. 'EB-subtype' indicates clinical suspicion of a specific EB-subtype. 'Other diagnosis' indicates clinical suspicion of an EB-related disorder for which genes are included in the WES EB gene-panel. The overall mutation detection rate was 68% (69% when including requests for EB-related disorders), and the diagnostic accuracy 93%. Interestingly, the mutation detection rate was lowest for the very specific request reason 'EB-subtype', which otherwise implies the highest clinical confidence of a diagnosis of EB. * One EB-case was referred to the Dutch EB Center for further phenotyping and specialized medical care after a positive DNA result. This EB-case is considered external from the Genome Diagnostics laboratory's perspective, but it was included in the 308 EB-cases with a certain clinical diagnosis of EB throughout the rest of the paper. EB, epidermolysis bullosa; WES, whole-exome sequencing.