

TABLE OF CONTENTS / INHALTSÜBERSICHT

PAGE	SUBJECT	DATE
121		
122		
123		
124		
125		
126		
127		
128		
129		
130		
131		
132		
133		
134		
135		
136		
137		
138		
139		
140		
141		
142		
143		
144		
145		
146		
147		
148		
149		
150		
151		
152		
153		
154		
155		
156		

PROJECT: Expression

continued from page

Expression of GST-X-Tag fusion proteins

8.10.19

E coli + Tp15, Tp17, Tp44

Plasmids:

Order No.:	06467376/Koch,Ute	OI11106467376-1-3	Order No.:	06467376/Koch,Ute	OI11106467376-1-1
1	Plasmid Name: pGEX4T3tag// EF030-Tp15 Internal Name: aH88Y-10A-2 Gene Name: Tp15 Gene Size: 435bp Vector Backbone: pGEX4T3tag// EF030 Antibiotic Selection: Ampicillin Cloning: BamHI/Sall Quantity: 1.9µg		1	Plasmid Name: pGEX4T3tag// EF030-Tp17 Internal Name: aH88W-9-3 Gene Name: Tp17 Gene Size: 480bp Vector Backbone: pGEX4T3tag// EF030 Antibiotic Selection: Ampicillin Cloning: BamHI/Sall Quantity: 2µg	
2	Order No. 06467360/Koch,Ute OI11106467360-1-1		2	Order No. 06467376/Koch,Ute OI11106467376-1-2	
3	Plasmid Name: pGEX4T3tag// EF030-Tp44 Internal Name: kH88V-35B-1 Gene Name: Tp44 Gene Size: 1047bp Vector Backbone: pGEX4T3tag// EF030 Antibiotic Selection: Ampicillin Cloning: BamHI/Sall Quantity: 4.6µg		3	Plasmid Name: pGEX4T3tag// EF030-Tp47 Internal Name: kH88X-29ZAB-1 Gene Name: Tp47 Gene Size: 1314bp Vector Backbone: pGEX4T3tag// EF030 Antibiotic Selection: Ampicillin Cloning: BamHI/Sall Quantity: 1.5µg	

Protocol: Expression of GST-X-tag fusion proteins

Cloning is performed virtually and sequences are subcloned into pGEX4T3tag by Eurofins MWG Operon

Day 1:

- Lyophilized Plasmids are resuspended in 20µl RNase and DNase-free water on the bench (lab). Heated up at 60°C for 10min on a shaker, vortexed and centrifuged
- Further dilution:
>5 µg 1:50
<5 µg 1:20

Desired Plasmid: 100ng/ml
conc

Sample	cDNA in µg	Dilution conc (µg/ml)	Plasmid (µl)	H2O (µl)	Date
Tp15	1.9	100	19		
Tp17	2	100	20		
Tp44	4.6	100	46		
	1.5	100			

- Equilibrate LB-Amp plates to RT (stored on the left upper shelf in the 4°C room)
- Let 50µl electrocompetent E. coli BL21 (stored -80°C) thaw on ice. Add 1µl of diluted plasmid (long) and transfer to precooled Trafo-cuvette(drawer "red cross"). Transform with Electroporation-device (Gissmann-lab): leave 5 min

Controls
① plasmid media only

- Turn device on (backside)
- control parameters (written on the machine)
- U = 2,3 kV
- C = 25 µF
- R = 200 Ohm
- Duration 4,7
- Dry the cuvette (shouldn't be wet!)
- Cuvette into carriage (iron should show to me)
- Push carriage into device (click)
- Push simultaneously the two red buttons until it beeps
- Turn device off

- Add immediately 200µl LB (w/o Amp!) and transfer to 1.5ml Eppi, put on ice.
- Incubate 1h at 37°C on a shaker
- Plate our 50µl on LB-Amp plates → 100µl and 200µl applied to plates
- Let dry and incubate in 37°C room ON

continued to page

SIGNATURE

DATE

DISCLOSED TO AND UNDERSTOOD BY

DATE

PROPRIETARY INFORMATION
BELONGING TO

PROJECT:

continued from page

Day 2:

- Pick a single colony and transfer to glas-tube (Measuring-cylinder closet) plus 5 ml LB-Amp (LB in french press room, add 1 tube Amp (stored at -20°C nr. 3) per 1L LB)
- Inc. 6-7 h on a shaker in the 37°C room
- Transfer culture to 1L flask and add 250 ml LB-Amp
- Inc. ON on a shaker in the 37°C room

→ PCR QC

Day 3:

- Prepare Glycerol stocks: 700µl bacterial culture + 700µl 50% Glycerol (final Glycerol concentration: 25%), store at -80°C
- Take QC1: 50ml bacterial culture, centrifuge (5000rpm, 15min), store pellet at -20°C *Midi QC*
- Add ON-culture to 1L LB-Amp in a flask (Fernbachkolben)
- Measure OD: take 1ml bacterial culture into cuvette and measure OD at 600nm against blank LB-Amp

0.6 for blank

Sample	OD ₆₀₀	After Dil	Date
T ₇ 15	0.5517		
T ₇ 17	0.6469	0.5319	
T ₇ 44	0.6399	0.5359	
T ₂ 47	0.6471		

- If OD₆₀₀ is 0.5 induce culture with 500µl IPTG (0.5mM, stored at -20°C)
- Inc. 6h at RT (French press room)
- Centrifuge bacterial culture:
 - Fill 500ml centrifuge-tubes with max 300ml culture, weigh counterparts to avoid imbalance!
 - Centrifuge at 6000 rpm 6-10min at 4°C (Discard supernatant and add left-over culture, centrifuge again)
 - Resuspend Pellets in 10ml PBS or Garcea-buffer (cold!) and transfer to 50ml tubes (on ice), store at -20°C
 - Volume:

Sample	Volume	Date
T ₇ 15	23.4	16.10.19
T ₇ 17	23.2	16.10.19
T ₇ 44	24.5	16.10.19

- Clean centrifuge and Rotor after usage with Antisept
- Waste disposal:
 - Get bucket from kitchen, fill 1 with Sekusept powder, add water and centrifuge tubes. Close with alufoil, write 2.106, date and time on it and bring it to kitchen
 - Liquid waste into the other bucket. Little flasks and glas-tubes: put in autoclave-metal-container
 -
- Precool French press for next day in 4°C room

continued to page

SIGNATURE

DATE

DISCLOSED TO AND UNDERSTOOD BY

DATE

PROPRIETARY INFORMATION
BELONGING TO**dkfz.**

PROJECT:

continued from page

Day 2:

- Pick a single colony and transfer to glas-tube (Measuring-cylinder closet) plus 5 ml LB-Amp (LB in french press room, add 1 tube Amp (stored at -20°C nr. 3) per 1L LB)
- Inc. 6-7 h on a shaker in the 37°C room
- Transfer culture to 1L flask and add 250 ml LB-Amp
- Inc. ON on a shaker in the 37°C room

→ PCR QC

5

10

15

20

25

30

35

40

45

continued to page

SIGNATURE

DATE

DISCLOSED TO AND UNDERSTOOD BY

DATE

PROPRIETARY INFORMATION
BELONGING TO

PROJECT:

continued from page

Day 4:

- Thaw pellets at 37°C and keep proteins afterwards ALWAYS on ICE!
- Add 20µl/10ml DTT (1M, final conc. 2mM(1:500), stored at -20°C)

5

10

15

20

25

30

35

40

45

continued to page

SIGNATURE

DATE

DISCLOSED TO AND UNDERSTOOD BY

DATE

PROPRIETARY INFORMATION
BELONGING TO

Sample	Volume DTT	Date
TPI5	47.2	17.10.19
TPI7	54.4	17.10.19
T2H4	49.0	17.10.19

- Add 500µl Protease-Inhibitor/1L culture (dilute 1 tablet (stored at 4°C) in 1ml H2O)
- Lyse in French Press (See French Press Protocol by Tim)
- After lysis samples in Garcea buffer need additional 20µl ATP (1M, stored at -20°C) and 50µl MgCl2 (1M, stored at Utes bench), inc. 1h at RT on a shaker
- Take QC2: 100µl of crude lysate, store on ice and prepare later 1µg/ml in 100µl volume incl 25µl 4x SDS-buffer
- Centrifuge lysate at 14,000rpm for 1h at 4°C in 50ml centrifuge tubes
- Transfer supernatant to new tube
- Take QC3: 100µl of clear lysate, store on ice and prepare later 1µg/ml in 100µl volume incl 25µl 4x SDS-buffer
- Measure protein concentration with Bradford:
200µl Bradford + 800µl H2O + 0.5µl cleared lysate (Blank H2O). inc. 5min, measure OD at 595nm

Sample	OD	Concentration (μ g/ml) = $OD \times 44.722$	Date
TPI5	0.895	0.086	17.10.19
TPI7	0.893	0.087	17.10.19
T2H4	1.201	0.100	17.10.19
T2H7	1.3529	10.155 (0.5ml)	59.5 30.10.19

- Mix lysates 1:1 with 100% glycerol, store at -20°C
- Final Lysate concentrations:

Sample	Concentration (μ g/ml)	Date
TPI5	0.9	17.10.19
TPI7	0.9	17.10.19
T2H4	13.2	17.10.19
T2H7	26.5	30.10.19

~~TPI5 0.895 0.086 → 0.9~~ ~~TPI7 0.893 0.087 → 0.9~~
~~T2H4 1.201 0.100 → 13.2~~
~~T2H7 1.3529 10.155 (0.5ml) 59.5 → 26.5~~

After lysate is mini prep'd for sequencing.

PROJECT:

continued from page

PCR QC for inserts

10.10.19

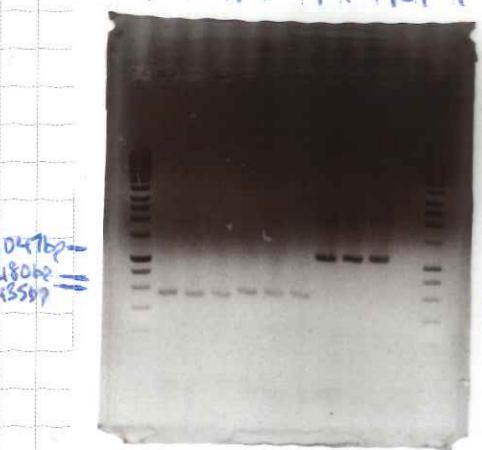
	x1	x5.5	x11	Program: Time Temp
Multiplex MM	12.5	68.75	137.5	Activation 15m 95
Q Soln	2.5	13.75	27.5	Denature 30s 94
Primer F	0.25	1.375	2.75	x4 Anneal 90s 68
Primer R	0.25	1.375	2.75	Elong Extent 90s 72
dd H ₂ O	8.5	46.75	93.5	Final Extent 10m 72

24μl

Sample: Colony PCR - three colonies taken as sample for each plasmid.

Small gel:

500 μl TAE
0.65g Agarose
2.5ml PEG Green



1.5% Agarose + PEG Green
1 kb? Super ladder
Smart

Large Gel

200 μl TAE
2.6g Agarose
10 μl PEG Green

All bands showed for expected sizes in all lanes.

Minimal growth in Ecoli from Day 2 to 3 due to inadequate aeration from bottle used for culturing. Experiment reverted to Day 2 using same colonies.

Midi prep QC

11.10.19

DiaGenic Plasmid Midi Kit (12145) used to isolate plasmids.

Only two midi tips were available, so one max tip was used with appropriate volumes.

50 μl H₂O used to dilute.

Sample ID	User ID	Date	Time	ng/μl	A260	A280	260/280	260/230
Tp15 pGEX E.coli	Default	16.10.2019	17:47	164.17	3.283	1.993	1.65	1.07
Tp17 pGEX E.coli	Default	16.10.2019	17:49	183.39	3.668	1.978	1.85	2.13
Tp44 pGEX E.coli	Default	16.10.2019	17:50	133.23	2.665	1.530	1.74	1.21

SIGNATURE

DATE

DISCLOSED TO AND UNDERSTOOD BY

DATE

PROPRIETARY INFORMATION
BELONGING TO

dkfz.

PROJECT:

continued from page

Restriction Digest

21.10.19

1. Linear digestion with BamH1

Antigen	Construct length (in bp)	Length after digestion (in bp)
Tp15	435	5417
Tp17	480	5462
Tp44	1047	6029
Tp47		

2. Symmetric digestion with BamH1 and SalI

Antigen	Insert Size (in bp)	Vector size (in bp)
Tp15	435	4982
Tp17	480	4982
Tp44	1047	4982
Tp47		

- Total volume of reaction: 20 μl
- Dilute sample to 10ng/20 μl;

	Concentration (Miniprep) (ng/μl)	Volume Sample (μl; for 10ng/20 μl dilution)	Volume H ₂ O (μl)
Tp15	164.17	1.21	18.79
Tp17	183.39	1.09	18.91
Tp44	133.23	1.50	18.50
Tp47			

1. Linear digestion (BamH1):

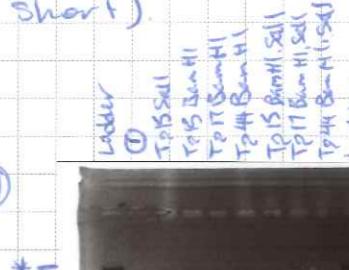
	Volume for reactions (μl)
Buffer 4	1x 2 4 8
DNA-Sample	2
BamH1	1 2 4
H ₂ O (total: 20 μl)	15 30 60

2. Symmetric digestion (with BamH1 and SalI):

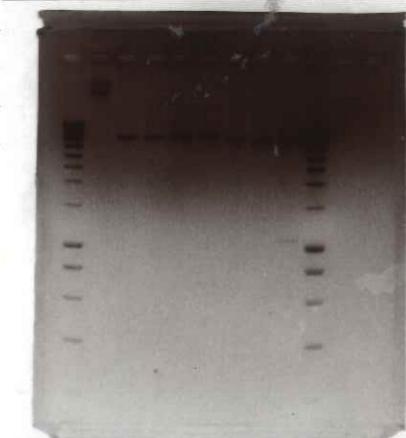
	Volume for reactions (μl)
Buffer 4	1x 2 4 8
DNA-Sample	2
BamH1	0.5 1 2
SalI	0.5 1 2
H ₂ O (total: 20 μl)	15 30 60

- Incubate reactions for at least 1h at 37°C; store them either at 4°C or -20°C or proceed with gel electrophoresis

- Gel electrophoresis: 2% agarose gel at 200V max (do on ice)



2% Agarose + PEG Green
1 kb? Super ladder
same as above



same as above.

continued to page

SIGNATURE

DATE

DISCLOSED TO AND UNDERSTOOD BY

DATE

PROPRIETARY INFORMATION
BELONGING TO

dkfz.

PROJECT:

continued from page

Western Blot

21.10.19

Resolving Gel (13.5%)

Stacking Gel

& Makes 2 gels

H ₂ O	1.6 ml
1M Tris pH 8.8	3.15 ml
Acrylamide 30%	4.5 ml
10% SDS	100 µl
10% APS	50 µl
TEMED	5 µl

H ₂ O	3.675 ml
1M Tris pH 6.8	0.625 ml
Acrylamide 30%	0.665 ml
10% SDS	50 µl
10% APS	25 µl
TEMED	5 µl

SDS Page: Runs ~50 min @ 200 V, 400 mA in running buffer
Bake @ 95°C for 2 mins. Load 10 µl

Blot: Runs ~1 hr @ 100V, 300 mA in EMBL buffer

Primary: Rb & GST 1:10000 in 10% milk, 1 hr
PBS-T

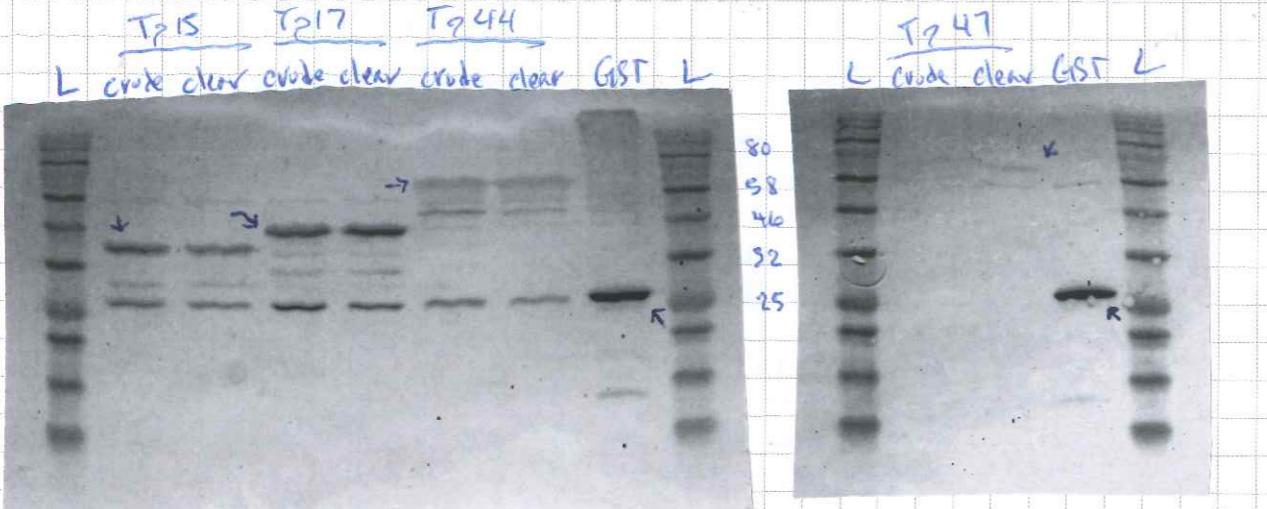
Blocking: 10% milk, 1 hr

Wash: 3x 5 min PBS-T

Secondary: Gt & Rb 1:10000 in 10% milk-PBS-T, 30 min

Wash: 3x 5 min PBS-T

Visualize: Clarity Western ECL substrate (70-5060)



kDa is protein + GST (26.9 kDa)

No GST band present?
Could be hiding like
HSV-1, will redo.

continued to page

SIGNATURE

DATE

DISCLOSED TO AND UNDERSTOOD BY

DATE

PROPRIETARY INFORMATION
BELONGING TO**dkfz.**

PROJECT:

continued from page

Midi Prep - AC - 2

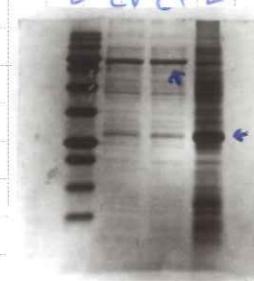
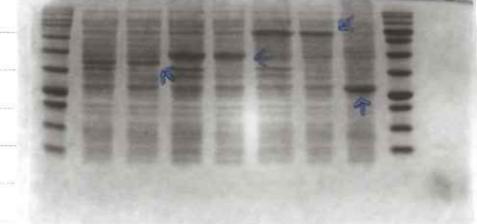
22.10.19

Coomassie Stain

Thermo GelCode Blue Stain Reagent (# 24590)

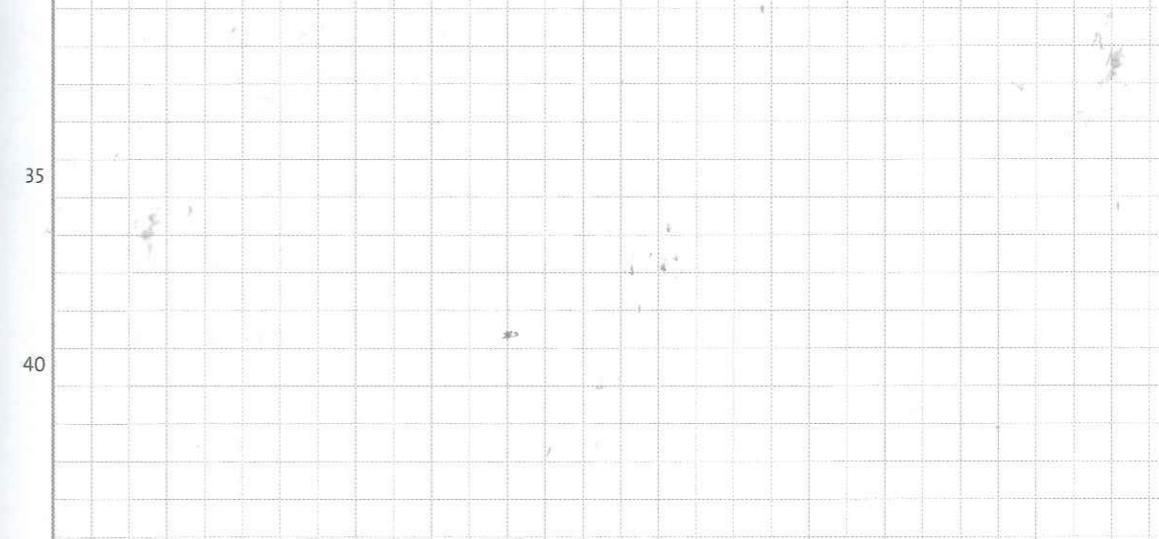
Tp15 Tp17 Tp44

L crv cl crv cl crv cl GST L

crv = crude
cl = clear13.5% SDS Page, NEB
Blue Prestained Protein Ladder (11-245 kDa) same as leftBands for Tp 15, 17, 44 match the western blot.
but Tp 47 doesn't. Coomassie is about expected -
band for Tp47 + GST and a GST band.

Midi Prep

13.5% SDS Page



continued to page

SIGNATURE

DATE

DISCLOSED TO AND UNDERSTOOD BY

DATE

PROPRIETARY INFORMATION
BELONGING TO**dkfz.**

PROJECT:

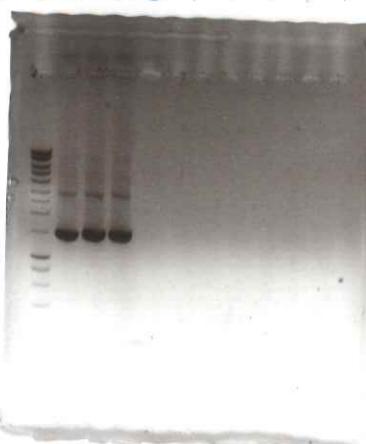
continued from page

PCR QC T7 47

28.10.19

Same procedure as before (Page 4)

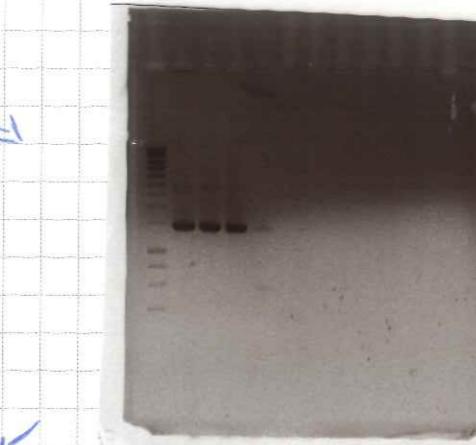
T7 47



1.5% Agarose + gel green
5 μl 1kb Smart ladder
5 μl sample
1 μl NEB 6x purple

Unknown upper band present in all samples

See below

Suspect contamination, so re-run with same colonies:
T7 47

Same as before
Annealing temp increased to 70°C

Potentially could be a chimeric sequence, as second band is 2x length of expected; uses itself as a primer.

Attempted to isolate the bands with a gel extraction kit (ALIEN, 28704), but insufficient

Retried with NEB Q5 Polymerase and bands disappeared.

Q5 PCR

Reagent	Volume (3x; μl)	Step	Temp. (°C)	Time (s)
Reaction Buffer	5	Activation	99	30
High GC Enhancer	5	Denature	98	15
Primer F	1	Anneal	68	30
Primer R	1	Elongation	72	30
dNTPs	0.5	Final Elongation	72	10 min
High Fidelity DNA Polymerase	0.25			
Template	1000 ng			
Final volume (after dilution with ddH ₂ O)	25			

Same as before
Procedure as per below

continued to page

SIGNATURE

DATE

DISCLOSED TO AND UNDERSTOOD BY

PROPRIETARY INFORMATION
BELONGING TO

dkfz.

PROJECT:

continued from page

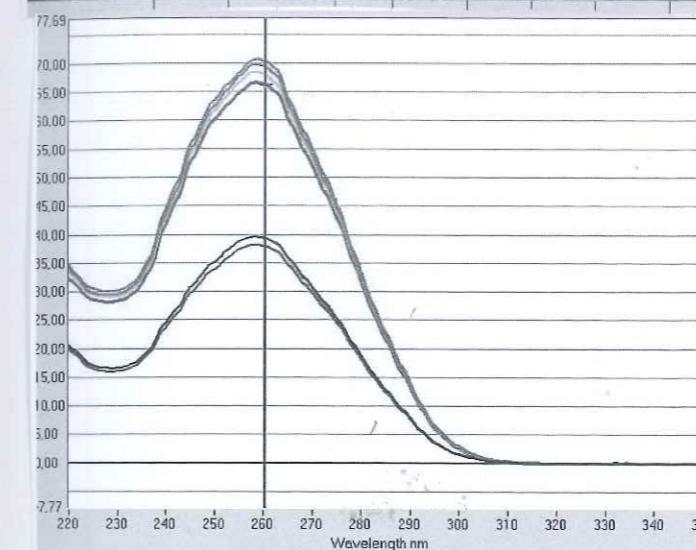
Sequencing

4.11.19

From cell lysates, 200 μl was taken for mini prep (diluted in 1 ml of buffer)

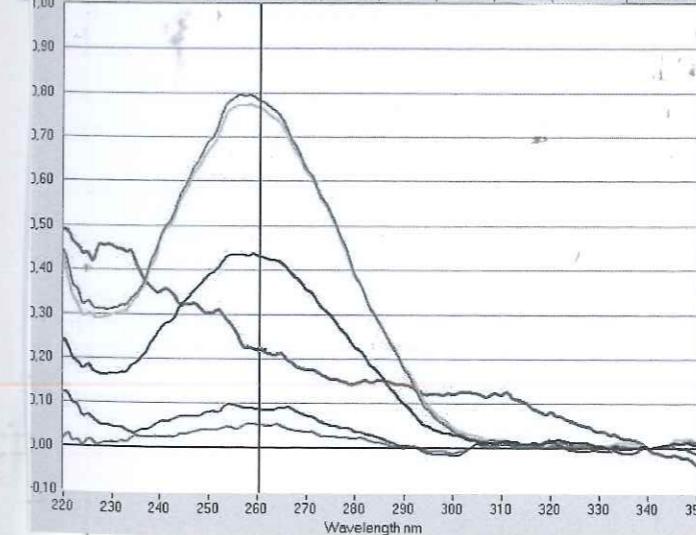
Raw Plasmid

Sample ID	User ID	Date	Time	ng/μl	A260	A280	260/280	260/230
Tp15-pGEX 6811	Default	23.10.2019	14:35	1865.35	39.307	18.800	2.09	2.37
Tp15-pGEX 6812	Default	23.10.2019	14:36	1895.96	37.919	18.235	2.08	2.37
Tp17-pGEX 6809	Default	23.10.2019	14:38	3469.72	69.394	32.964	2.11	2.35
Tp17-pGEX 6810	Default	23.10.2019	14:39	351452	70.290	33.595	2.09	2.33
Tp44-pGEX 6807	Default	23.10.2019	14:40	3403.44	68.069	32.628	2.09	2.34
Tp44-pGEX 6808	Default	23.10.2019	14:41	3316.63	66.333	31.708	2.09	2.33



Diluted Plasmid

Sample ID	User ID	Date	Time	ng/μl	A260	A280	260/280	260/230
Tp15 1:100	Default	23.10.2019	15:04	21.56	0.431	0.225	1.92	2.62
Tp15 1:1000	Default	23.10.2019	15:05	4.32	0.066	0.035	2.45	1.89
Tp17 1:100	Default	23.10.2019	15:06	2.60	0.052	0.021	2.47	5.30
Tp17 1:100	Default	23.10.2019	15:07	39.19	0.784	0.385	2.03	2.51
Tp44 1:100	Default	23.10.2019	15:08	38.31	0.766	0.382	2.00	2.58
Tp44 1:1000	Default	23.10.2019	15:09	11.08	0.222	0.145	1.53	0.49



From Nanodrop,
Found extremely high
concentrations (~800
each plasmid).

Purity levels 260/280
and 260/230 also
very close to ideal,
and absorbance graph
is as expected.

To confirm, 1:100
and 1:1000 dilutions
taken.

1:100 was ~15%
deviation from the
expected concentrations.
1:1000 was below the
concentration range of
the Nanodrop (< 20 ng/μl).

Graph and purity
similar to raw
plasmids.

15 μl of each raw
plasmid was sent
to Eurofins for processing

continued to page

SIGNATURE

DATE

DISCLOSED TO AND UNDERSTOOD BY

DATE

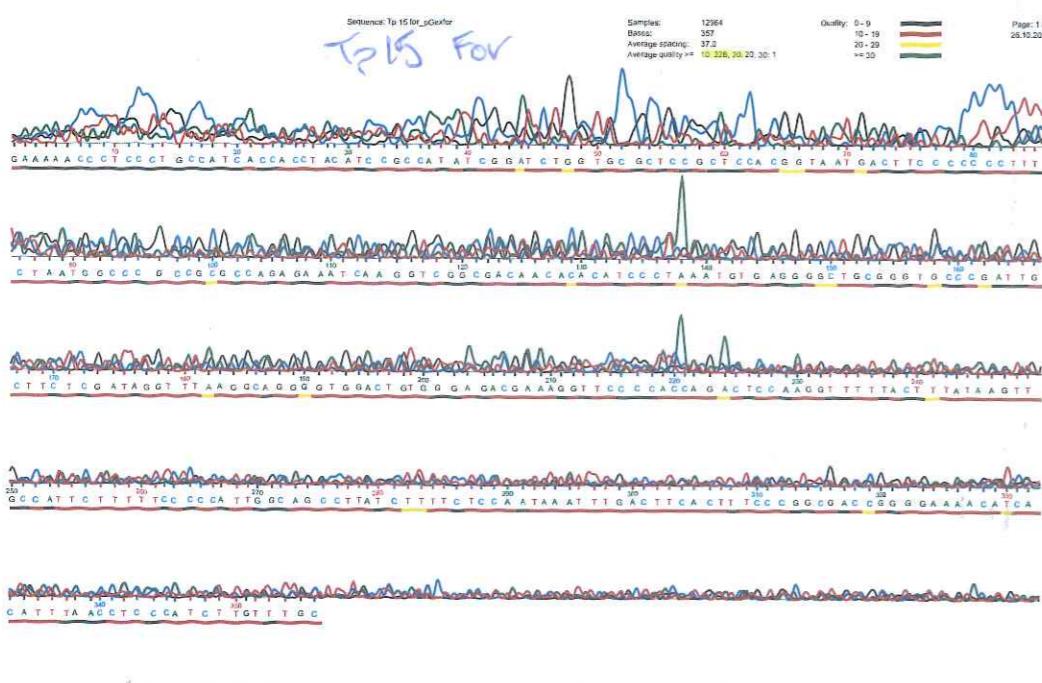
PROPRIETARY INFORMATION
BELONGING TO

dkfz.

PROJECT:

continued from page

From Eurofins:



All other plasmids had similar results.

Quality is terrible - 228b7 < 20 quality.

Suspect high concentration, as Eurofins states 50-100 ng/ml is optimal. Unsure if they dilute samples or not. Will retry with T₂41, once processed.

Sequencing Redo.

PCR product will be used instead. 1 μg template w Q5 gol for 33 cycles.

Eurofins suggests 50-100ng/ml
for sequencing samples

Post-PCR Nanodrop

Sample ID	User ID	Date	Time	ng/ <u>ul</u>	A260	A280	260/280	260/230
Negative Control								
Tp15 PCR with Q5	Default	05.11.2019	09:33	127,17	2,543	1,400	1,82	0,24
Tp17 PCR with Q5	Default	05.11.2019	09:31	57,97	1,159	0,630	1,84	0,20
Tp44 PCR with Q5	Default	05.11.2019	09:34	117,46	2,349	1,208	1,95	0,22
Tp44 PCR with Q5	Default	05.11.2019	09:37	116,09	2,322	1,198	1,94	0,22
Tp47 PCR with Q5	Default	05.11.2019	09:38	119,08	2,382	1,251	1,90	0,22

Pre-PCR clean-up

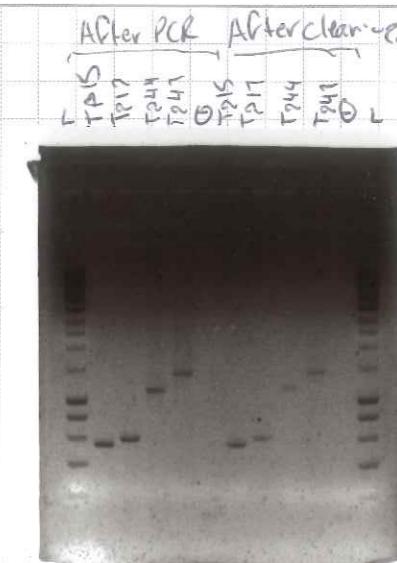
continued to page

PROJECT:

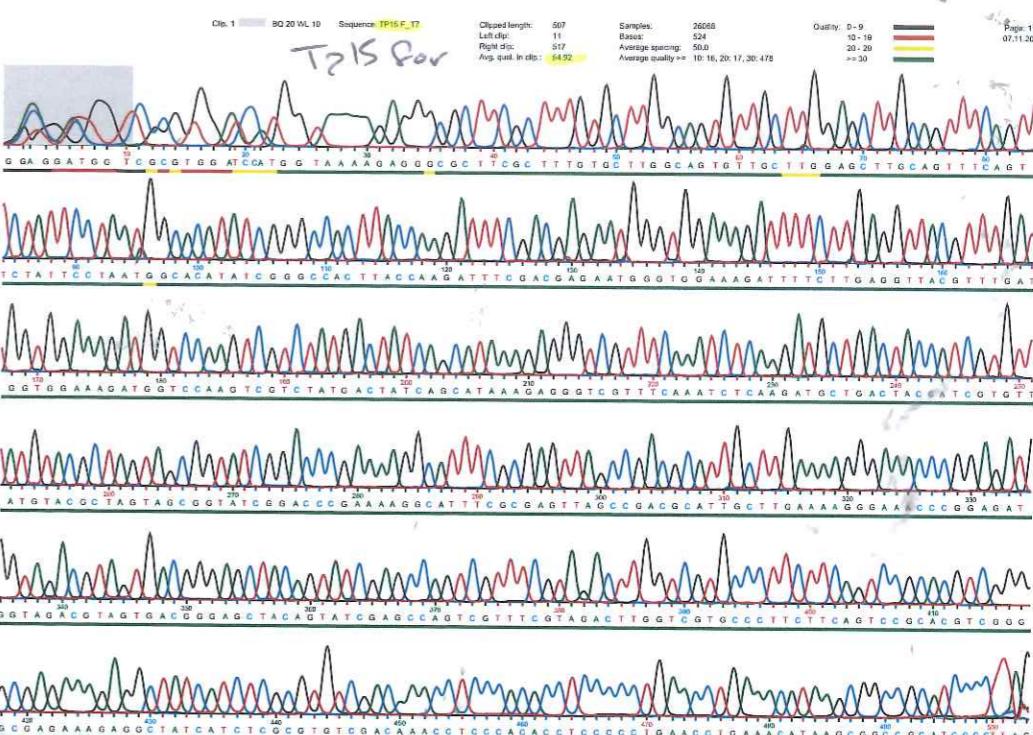
continued from page



Wavelength nm



1.5% AG + pegGREEN



(Good quality across all plasmids
(Aug 75)).

continued to page

SIGNATURE

DAT

DISCLOSED TO AND UNDERSTOOD BY

DATE

PROPRIETARY INFORMATION
BELONGING TO

dkfz

PROJECT:

continued from page

ELISA

4.11.19

Day 1

- Coat Polysorb plate (pre-ventilated in the drawer) with 100µL/well of glutathione casein 1:1000 in ELISA coating buffer (2 ng/µL glutathione-casein in 50 mM carbonate buffer 50 mM Na₂CO₃, 50 mM NaHCO₃ 1:4, pH 9.6). Store overnight at 4°C.
- Prepare BP-Buffer: 2mg/mL Casein in PBS-Tween (0.05%) (dissolves very slowly; Heat and stir) → prepare 200mL

Day 2

- Remove coating buffer from plate
- Add 180µL BP/well and incubate for 1h at 37°C (without rotation)
- In the meantime prepare dilutions: (2µg/µL per sample) (2 × 350µL volume)
 - Use Greiner plate
 - Double measurement for each sample (2 rows per sample)
 - GST-Tag (serology lab) as control

Sample	concentration (µg/µL)	Volumne sample µL	Volume BP µL	total
T215	9.9	70.7	279.3	350
T217	9.9	70.7	279.3	350
T244	13.2	53.0	297.0	350
T247	29.15	23.5	326.5	350
GST	70	10	340	350

- Prepare dilutions in 96-well plate:
 - In columns 2-12: add 200µL/well BP
 - In column 1: add 300µL pre-diluted lysate (2 rows per sample; last 2 rows GST-tag control)
 - Start in column 1 and transfer 100µL to the next column for each sample to have serial dilution; Column 12 stays empty! (blank)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Probe	BP										
B	Probe	BP										
C	BP	BP	BP	BP	BP	BP	BP	BP	BP	BP	BP	BP
D	BP	BP	BP	BP	BP	BP	BP	BP	BP	BP	BP	BP
E	BP	BP	BP	BP	BP	BP	BP	BP	BP	BP	BP	BP
F	BP	BP	BP	BP	BP	BP	BP	BP	BP	BP	BP	BP
G	GST	BP										
H	BP	BP	BP	BP	BP	BP	BP	BP	BP	BP	BP	BP

continued to page

SIGNATURE

DATE

DISCLOSED TO AND UNDERSTOOD BY

DATE

PROPRIETARY INFORMATION
BELONGING TO**PROJECT:**

continued from page

- Remove BP from plate
- Add 100µL of the sample dilutions to the Poly Sorb plate (start with the highest dilution)
- Incubate plate for 1h at RT on the shaker
- Prepare first antibody: per plate 10mL, mouse anti-tag (KT3-Ü) + BP, 1:50
- Remove supernatant and wash the plate 3x with PBS-T (dry plate by knocking it on paper towels)
- Add 100µL/well mouse anti-tag
- Incubate for 1h at RT on the shaker
- Prepare second antibody: 10mL/plate, goat anti-mouse-pox + BP, 1:10000
- Remove supernatant and wash the plate 3x with PBS-T (dry plate by knocking it on paper towels)
- Add 100µL/well goat anti-mouse-pox
- Incubate for 1h at RT on the shaker
- Bring substrate buffer (100 mM NaAc, pH 6) and TMB to room temperature (TMB is light sensitive, keep it in aluminum foil)
- Remove supernatant and wash the plate 3x with PBS-T (from here on the plate should not get dry)
- Finalize substrate buffer (10mL per plate): TMB (100µL/10mL) + H₂O₂ (1.5-2µL/10mL)
- Dry plate by knocking it on paper towel
- Add 100µL/well substrate buffer → Wells should get blue
- Stop reaction after 2-8 min with 5µL/well stop-solution (1M H₂SO₄)
- Measure absorption at 450nm

Elisa Results.

Antigen	Raw											Dilution								Control				
	2 µg/ml	1:3	1:9	1:27	1:81	1:243	1:729	1:2187	1:6561	1:19683	1:59049	1:177147	1:531427	1:1594281	1:4782843	1:14348529	1:44495587	1:133486761						
Tp15	0.120259	0.173032	0.153659	0.141683	0.138409	0.1393	0.095146	0.054887	0.038063	0.029319	0.023186	0.019991	0.0075889	0.013293	0.011476	0.0132524	0.0116659	0.012145	0.011119	0.009348	0.0057629	0.0052883	0.0053832	0.048221
Tp17	0.075889	0.13293	0.11476	0.132524	0.116659	0.12145	0.12145	0.12145	0.12145	0.12145	0.12145	0.12145	0.12145	0.12145	0.12145	0.12145	0.12145	0.12145	0.12145	0.12145	0.12145	0.12145	0.12145	
Tp44	0.1399	0.13475	0.14565	0.13205	0.1602	0.1521	0.12565	0.0933	0.0659	0.0562	0.0562	0.0562	0.0562	0.0562	0.0562	0.0562	0.0562	0.0562	0.0562	0.0562	0.0562	0.0562	0.04991	
Tp47	0.15185	0.1495	0.1539	0.1309	0.15145	0.1347	0.1229	0.09295	0.06595	0.0682	0.0682	0.0682	0.0682	0.0682	0.0682	0.0682	0.0682	0.0682	0.0682	0.0682	0.0682	0.0682	0.04551	
GST	0.1371	0.129	0.1131	0.1088	0.1071	0.1133	0.1172	0.106	0.0827	0.0629	0.0629	0.0629	0.0629	0.0629	0.0629	0.0629	0.0629	0.0629	0.0629	0.0629	0.0629	0.04551		

continued to page

SIGNATURE

DATE

DISCLOSED TO AND UNDERSTOOD BY

DATE

PROPRIETARY INFORMATION
BELONGING TO

PROJECT:

continued from page

Luminex Bead Coupling

6.11.09

1

Protocol: Luminex Wash Assay**Version: 1.1****Date: 09 January 2006****Author: Tim Waterboer****Software: Version 2.3.182****Contents**

- 1. Material**
- 2. Devices**
- 3. Loading of GC-beads with antigen**
- 4. Preincubation of sera**
- 5. Equilibration of wash plates**
- 6. Incubation of beads and sera**
- 7. Incubation with secondary antibody**
- 8. Incubation with Streptavidin-R-Phycoerythrin (Strep-PE)**
- 9. Exemplary calculation**

continued to page

SIGNATURE

DATE

DISCLOSED TO AND UNDERSTOOD BY

DATE

PROPRIETARY INFORMATION
BELONGING TO**dkfz.****PROJECT:**

continued from page

2

1. Material

- GC-beads
- bacterial lysates, sera
- 1.5 ml Eppendorf tubes (e.g., Starlab)
- blocking buffer (BP): PBS, 1 mg/ml Casein pH 7.4 ± 0.1
- storage buffer (LP): BP, 0.05 % sodium azide
- detection system (e.g. goat-α-human IgG-Biotin & Streptavidin-PE)
- 96 well wash plates (Millipore)
- 96 well polystyrene plates
- Casein, Polyvinylalcohol, Polyvinylpyrrolidone, CBS-K

2. Devices

- Luminex 100, XY Platform, Sheath Delivery System
- vortexer
- table centrifuge
- ultrasonic bath
- shaker
- vacuum manifold

3. Loading of GC-beads with antigen

Dilute the bacterial lysates to 1 mg/ml in BP in Starlab Eppendorf tubes. 1 ml of the dilution is sufficient for up to 3 million beads. Centrifuge the GC-beads for 2 min at 13,000 rpm and thoroughly vortex them. Pipet the necessary amount of beads (see exemplary calculation) directly in the lysate dilution (one bead sort per antigen). Incubation takes place on a shaker (200 rpm) for 1 h at RT in the dark. Afterwards, centrifuge the beads for 2 min at 13,000 rpm, discard the supernatant, add 1 ml of BP, sonicate for 1 min and vortex until the beads are resuspended. Repeat this washing procedure twice (three times total). After the third time, remove the supernatant thoroughly and add e.g. 200 µl of BP (LP if beads are loaded one day in advance and stored o/n at 4 °C).

Keep wet

continued to page

SIGNATURE

DATE

DISCLOSED TO AND UNDERSTOOD BY

DATE

PROPRIETARY INFORMATION
BELONGING TO**dkfz.**

PROJECT:

continued from page

5

3

4. Preincubation of sera

Sera are preincubated in BP containing 2 mg/ml GST tag lysate, 0.5 % Polyvinylalcohol, 0.8 % Polyvinylpyrrolidone. Per serum 50 μ l dilution buffer is required. The serum dilution has to be prepared twice as concentrated as finally needed. Usually, each 100 μ l of serum in a dilution of 1:50 (finally 1:100) is prepared in a 96 well polystyrene plate. Incubate the serum dilution on a shaker (200 rpm) for 1 h at RT in the dark.

GST mouse IgG completed

20

5. Equilibration of wash plates

Incubate the wash plates with 100 μ l BP per well for 10 min at RT. Afterwards, remove the buffer with the vacuum manifold and dry the plate using the hammer (2-3 times).

25

6. Incubation of beads and sera

Resuspend the antigen loaded and washed beads extremely (!) thoroughly by alternating sonification (1 min) and vortexing until the suspension is completely homogeneous (3-6 times depending on number of beads and antigen).

Combine bead suspensions in an appropriate vessel. In order not to lose the remaining beads, add adequate volume (e.g. 500 μ l) of BP to the Eppendorf tubes, vortex, and transfer the buffer to the vessel with the multiplex mix. Add BP to the needed volume (see exemplary calculation). Vortex the bead mix and fill each well of the equilibrated wash plates with 50 μ l bead mix. Transfer each 50 μ l of the serum dilutions from the polystyrene to the wash plates. Incubate the plate on a shaker (200 rpm) for 1 h at RT in the dark.

As a control, crude KT-3 supernatant (mouse anti-SV40 tag) is used as a loading control diluted 1:25 in BP (secondary antibody is goat anti-mouse 1:1000)

30

35

40

45

continued to page

SIGNATURE

DATE

DISCLOSED TO AND UNDERSTOOD BY

DATE

PROPRIETARY INFORMATION
BELONGING TO**PROJECT:**

continued from page

5

4

7. Incubation with secondary antibody

Remove serum from the wash plates using the vacuum manifold. Wash the plates three times with 100 μ l BP per well. Afterwards, dry the plate using the hammer. Add secondary antibody (e.g. goat- α -human IgG-Biotin 1:1000 in BP) and incubate the plate on a shaker (200 rpm) for 1 h at RT in the dark. Do not add secondary antibody to the KT-3 well! Here, goat anti-mouse is needed!

10

15

20

25

30

35

40

45

8. Incubation with Streptavidin-R-Phycoerythrin (Strep-PE)

Remove the secondary antibody using the vacuum manifold. Wash the plates three times with 100 μ l BP per well. Afterwards, dry the plate using the hammer. Add Strep-PE (light sensitive!) diluted 1:1000 in BP and incubate the plate on a shaker (200 rpm) for 1 h at RT in the dark. After this incubation, the plate is washed again three times and dried. Add 100 μ l of BP per well (LP if plates are stored o/n at 4 °C and read the next day), shake for 5 min at RT in the dark to resuspend the beads and perform measurement in the Luminex analyzer (see corresponding protocol).

continued to page

SIGNATURE

DATE

DISCLOSED TO AND UNDERSTOOD BY

DATE

PROPRIETARY INFORMATION
BELONGING TO

PROJECT:

continued from page

5

5

9. Exemplary calculation

The reactivity of 120 sera against 5 different antigens plus GST-Tag is to be determined. Per data point 2,000-3,000 beads are needed (depending on the desired measuring time). Therefore, using 2,500 beads per data point, each 300,000 beads from 6 different bead sorts are needed.

Loading of GC-beads with antigen: The concentration of beads after the coupling reaction with GC has been determined. A typical value is 15,000 beads per μl . For 300,000 beads ($300,000 / 15,000 =$) 20 μl of bead suspension have to be loaded with antigen.

Incubation of beads and sera: After washing the antigen, 200 μl loaded beads per bead sort are at hand. For 120 sera ($120 \times 50 =$) 6,000 μl bead mix are required. The empty Eppendorf tubes are washed with each 500 μl BP, the final mix looks therefore like this:

$$\begin{array}{ll} 6 \times 200 \mu\text{l} \text{ bead suspension} & = 1.2 \text{ ml} \\ 6 \times 500 \mu\text{l} \text{ BP wash volume} & = 3.0 \text{ ml} \\ \text{add BP} & 1.8 \text{ ml} \\ & 6.0 \text{ ml} \end{array}$$

Beads
360 samples \rightarrow

continued to page

SIGNATURE

DATE

DISCLOSED TO AND UNDERSTOOD BY

DATE

PROPRIETARY INFORMATION
BELONGING TO**dkfz.**

PROJECT:

continued from page

Multiplier Serology.

7.11.19

5

300

200

100

0

20

15

10

5

0

25

20

15

10

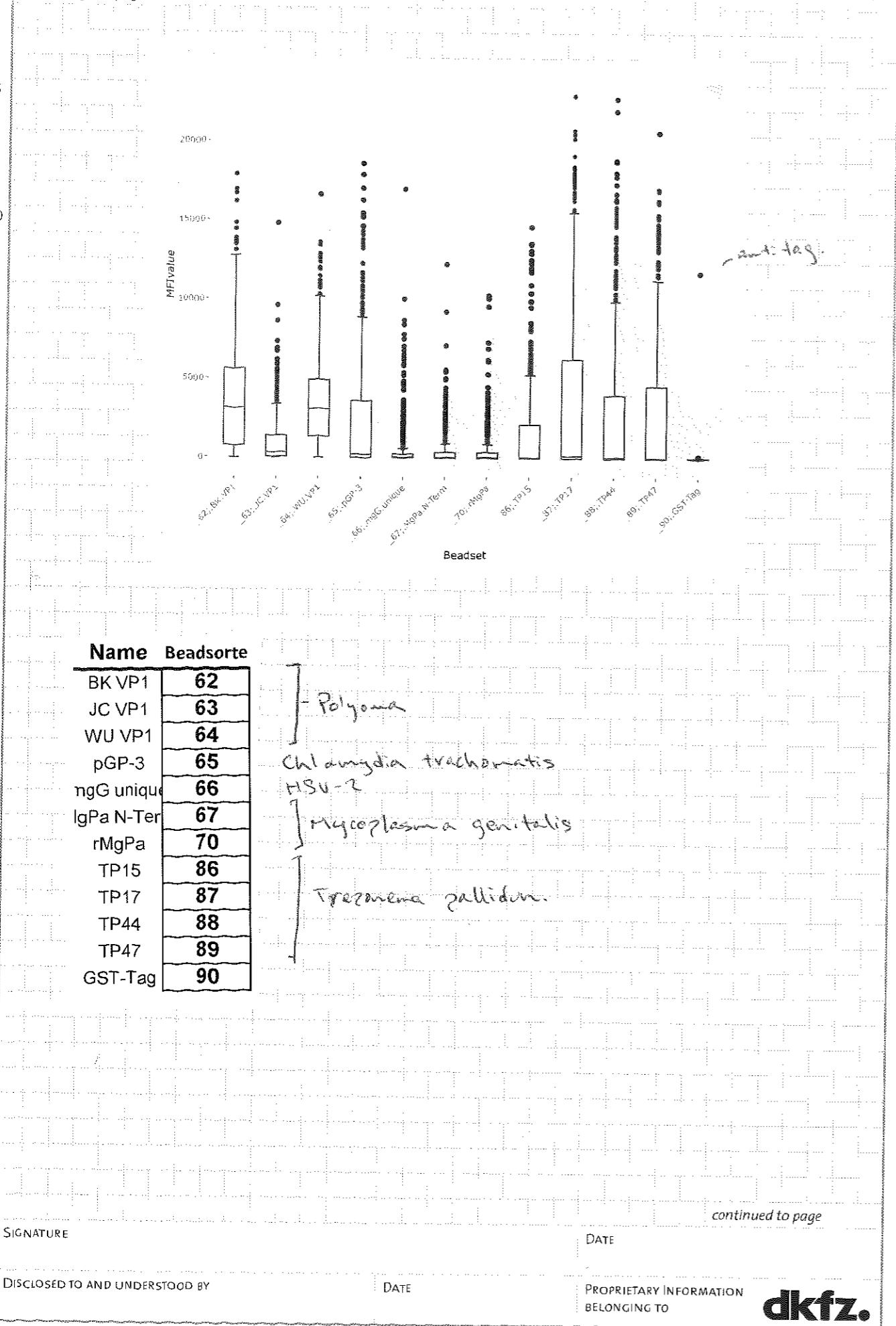
5

0

G2: BK VP1 G3: JC VP1 G4: WU VP1 G5: PGP-3 G6: mgC unique G7: NgrA N-Term G8: rMgP G9: TP1 G10: TP2 G11: TP3 G12: TP4 G13: TP5 G14: TP6 G15: TP7 G16: TP8 G17: TP9 G18: TP10 G19: TP11 G20: TP12 G21: TP13 G22: TP14 G23: TP15 G24: TP16 G25: TP17 G26: TP18 G27: TP19 G28: TP20 G29: TP21 G30: TP22 G31: TP23 G32: TP24 G33: TP25 G34: TP26 G35: TP27 G36: TP28 G37: TP29 G38: TP30 G39: TP31 G40: TP32 G41: TP33 G42: TP34 G43: TP35 G44: TP36 G45: TP37 G46: TP38 G47: TP39 G48: TP40 G49: TP41 G50: TP42 G51: TP43 G52: TP44 G53: TP45 G54: TP46 G55: TP47 G56: TP48 G57: TP49 G58: TP50 G59: TP51 G60: TP52 G61: TP53 G62: TP54 G63: TP55 G64: TP56 G65: TP57 G66: TP58 G67: TP59 G68: TP60 G69: TP61 G70: TP62 G71: TP63 G72: TP64 G73: TP65 G74: TP66 G75: TP67 G76: TP68 G77: TP69 G78: TP70 G79: TP71 G80: TP72 G81: TP73 G82: TP74 G83: TP75 G84: TP76 G85: TP77 G86: TP78 G87: TP79 G88: TP80 G89: TP81 G90: TP82 G91: TP83 G92: TP84 G93: TP85 G94: TP86 G95: TP87 G96: TP88 G97: TP89 G98: TP90 G99: TP91 G100: TP92 G101: TP93 G102: TP94 G103: TP95 G104: TP96 G105: TP97 G106: TP98 G107: TP99 G108: TP100 G109: TP101 G110: TP102 G111: TP103 G112: TP104 G113: TP105 G114: TP106 G115: TP107 G116: TP108 G117: TP109 G118: TP110 G119: TP111 G120: TP112 G121: TP113 G122: TP114 G123: TP115 G124: TP116 G125: TP117 G126: TP118 G127: TP119 G128: TP120 G129: TP121 G130: TP122 G131: TP123 G132: TP124 G133: TP125 G134: TP126 G135: TP127 G136: TP128 G137: TP129 G138: TP130 G139: TP131 G140: TP132 G141: TP133 G142: TP134 G143: TP135 G144: TP136 G145: TP137 G146: TP138 G147: TP139 G148: TP140 G149: TP141 G150: TP142 G151: TP143 G152: TP144 G153: TP145 G154: TP146 G155: TP147 G156: TP148 G157: TP149 G158: TP150 G159: TP151 G160: TP152 G161: TP153 G162: TP154 G163: TP155 G164: TP156 G165: TP157 G166: TP158 G167: TP159 G168: TP160 G169: TP161 G170: TP162 G171: TP163 G172: TP164 G173: TP165 G174: TP166 G175: TP167 G176: TP168 G177: TP169 G178: TP170 G179: TP171 G180: TP172 G181: TP173 G182: TP174 G183: TP175 G184: TP176 G185: TP177 G186: TP178 G187: TP179 G188: TP180 G189: TP181 G190: TP182 G191: TP183 G192: TP184 G193: TP185 G194: TP186 G195: TP187 G196: TP188 G197: TP189 G198: TP190 G199: TP191 G200: TP192 G201: TP193 G202: TP194 G203: TP195 G204: TP196 G205: TP197 G206: TP198 G207: TP199 G208: TP200 G209: TP210 G210: TP211 G211: TP212 G212: TP213 G213: TP214 G214: TP215 G215: TP216 G216: TP217 G217: TP218 G218: TP219 G219: TP220 G220: TP221 G221: TP222 G222: TP223 G223: TP224 G224: TP225 G225: TP226 G226: TP227 G227: TP228 G228: TP229 G229: TP230 G230: TP231 G231: TP232 G232: TP233 G233: TP234 G234: TP235 G235: TP236 G236: TP237 G237: TP238 G238: TP239 G239: TP240 G240: TP241 G241: TP242 G242: TP243 G243: TP244 G244: TP245 G245: TP246 G246: TP247 G247: TP248 G248: TP249 G249: TP250 G250: TP251 G251: TP252 G252: TP253 G253: TP254 G254: TP255 G255: TP256 G256: TP257 G257: TP258 G258: TP259 G259: TP260 G260: TP261 G261: TP262 G262: TP263 G263: TP264 G264: TP265 G265: TP266 G266: TP267 G267: TP268 G268: TP269 G269: TP270 G270: TP271 G271: TP272 G272: TP273 G273: TP274 G274: TP275 G275: TP276 G276: TP277 G277: TP278 G278: TP279 G279: TP280 G280: TP281 G281: TP282 G282: TP283 G283: TP284 G284: TP285 G285: TP286 G286: TP287 G287: TP288 G288: TP289 G289: TP290 G290: TP291 G291: TP292 G292: TP293 G293: TP294 G294: TP295 G295: TP296 G296: TP297 G297: TP298 G298: TP299 G299: TP300 G300: TP301 G301: TP302 G302: TP303 G303: TP304 G304: TP305 G305: TP306 G306: TP307 G307: TP308 G308: TP309 G309: TP310 G310: TP311 G311: TP312 G312: TP313 G313: TP314 G314: TP315 G315: TP316 G316: TP317 G317: TP318 G318: TP319 G319: TP320 G320: TP321 G321: TP322 G322: TP323 G323: TP324 G324: TP325 G325: TP326 G326: TP327 G327: TP328 G328: TP329 G329: TP330 G330: TP331 G331: TP332 G332: TP333 G333: TP334 G334: TP335 G335: TP336 G336: TP337 G337: TP338 G338: TP339 G339: TP340 G340: TP341 G341: TP342 G342: TP343 G343: TP344 G344: TP345 G345: TP346 G346: TP347 G347: TP348 G348: TP349 G349: TP350 G350: TP351 G351: TP352 G352: TP353 G353: TP354 G354: TP355 G355: TP356 G356: TP357 G357: TP358 G358: TP359 G359: TP360 G360: TP361 G361: TP362 G362: TP363 G363: TP364 G364: TP365 G365: TP366 G366: TP367 G367: TP368 G368: TP369 G369: TP370 G370: TP371 G371: TP372 G372: TP373 G373: TP374 G374: TP375 G375: TP376 G376: TP377 G377: TP378 G378: TP379 G379: TP380 G380: TP381 G381: TP382 G382: TP383 G383: TP384 G384: TP385 G385: TP386 G386: TP387 G387: TP388 G388: TP389 G389: TP390 G390: TP391 G391: TP392 G392: TP393 G393: TP394 G394: TP395 G395: TP396 G396: TP397 G397: TP398 G398: TP399 G399: TP400 G400: TP401 G401: TP402 G402: TP403 G403: TP404 G404: TP405 G405: TP406 G406: TP407 G407: TP408 G408: TP409 G409: TP410 G410: TP411 G411: TP412 G412: TP413 G413: TP414 G414: TP415 G415: TP416 G416: TP417 G417: TP418 G418: TP419 G419: TP420 G420: TP421 G421: TP422 G422: TP423 G423: TP424 G424: TP425 G425: TP426 G426: TP427 G427: TP428 G428: TP429 G429: TP430 G430: TP431 G431: TP432 G432: TP433 G433: TP434 G434: TP435 G435: TP436 G436: TP437 G437: TP438 G438: TP439 G439: TP440 G440: TP441 G441: TP442 G442: TP443 G443: TP444 G444: TP445 G445: TP446 G446: TP447 G447: TP448 G448: TP449 G449: TP450 G450: TP451 G451: TP452 G452: TP453 G453: TP454 G454: TP455 G455: TP456 G456: TP457 G457: TP458 G458: TP459 G459: TP460 G460: TP461 G461: TP462 G462: TP463 G463: TP464 G464: TP465 G465: TP466 G466: TP467 G467: TP468 G468: TP469 G469: TP470 G470: TP471 G471: TP472 G472: TP473 G473: TP474 G474: TP475 G475: TP476 G476: TP477 G477: TP478 G478: TP479 G479: TP480 G480: TP481 G481: TP482 G482: TP483 G483: TP484 G484: TP485 G485: TP486 G486: TP487 G487: TP488 G488: TP489 G489: TP490 G490: TP491 G491: TP492 G492: TP493 G493: TP494 G494: TP495 G495: TP496 G496: TP497 G497: TP498 G498: TP499 G499: TP500 G500: TP501 G501: TP502 G502: TP503 G503: TP504 G504: TP505 G505: TP506 G506: TP507 G507: TP508 G508: TP509 G509: TP510 G510: TP511 G511: TP512 G512: TP513 G513: TP514 G514: TP515 G515: TP516 G516: TP517 G517: TP518 G518: TP519 G519: TP520 G520: TP521 G521: TP522 G522: TP523 G523: TP524 G524: TP525 G525: TP526 G526: TP527 G527: TP528 G528: TP529 G529: TP530 G530: TP531 G531: TP532 G532: TP533 G533: TP534 G534: TP535 G535: TP536 G536: TP537 G537: TP538 G538: TP539 G539: TP540 G540: TP541 G541: TP542 G542: TP543 G543: TP544 G544: TP545 G545: TP546 G546: TP547 G547: TP548 G548: TP549 G549: TP550 G550: TP551 G551: TP552 G552: TP553 G553: TP554 G554: TP555 G555: TP556 G556: TP557 G557: TP558 G558: TP559 G559: TP560 G560: TP561 G561: TP562 G562: TP563 G563: TP564 G564: TP565 G565: TP566 G566: TP567 G567: TP568 G568: TP569 G569: TP570 G570: TP571 G571: TP572 G572: TP573 G573: TP574 G574: TP575 G575: TP576 G576: TP577 G577: TP578 G578: TP579 G579: TP580 G580: TP581 G581: TP582 G582: TP583 G583: TP584 G584: TP585 G585: TP586 G586: TP587 G587: TP588 G588: TP589 G589: TP590 G590: TP591 G591: TP592 G592: TP593 G593: TP594 G594: TP595 G595: TP596 G596: TP597 G597: TP598 G598: TP599 G599: TP600 G600: TP601 G601: TP602 G602: TP603 G603: TP604 G604: TP605 G605: TP606 G606: TP607 G607: TP608 G608: TP609 G609: TP610 G610: TP611 G611: TP612 G612: TP613 G613: TP614 G614: TP615 G615: TP616 G616: TP617 G617: TP618 G618: TP619 G619: TP620 G620: TP621 G621: TP622 G622: TP623 G623: TP624 G624: TP625 G625: TP626 G626: TP627 G627: TP628 G628: TP629 G629: TP630 G630: TP631 G631: TP632 G632: TP633 G633: TP634 G634: TP635 G635: TP636 G636: TP637 G637: TP638 G638: TP639 G639: TP640 G640: TP641 G641: TP642 G642: TP643 G643: TP644 G644: TP645 G645: TP646 G646: TP647 G647: TP648 G648: TP649 G649: TP650 G650: TP651 G651: TP652 G652: TP653 G653: TP654 G654: TP655 G655: TP656 G656: TP657 G657: TP658 G658: TP659 G659: TP660 G660: TP661 G661: TP662 G662: TP663 G663: TP664 G664: TP665 G665: TP666 G666: TP667 G667: TP668 G668: TP669 G669: TP670 G670: TP671 G671: TP672 G672: TP673 G673: TP674 G674: TP675 G675: TP676 G676: TP677 G677: TP678 G678: TP679 G679: TP680 G680: TP681 G681: TP682 G682: TP683 G683: TP684 G684: TP685 G685: TP686 G686: TP687 G687: TP688 G688: TP689 G689: TP690 G690: TP691 G691: TP692 G692: TP693 G693: TP694 G

PROJECT:

continued from page



PROJECT:

continued from page

FortNr	plateN	Loca	ID	Dilution	rowMea	rowSum	Warning	Status	62	63	64	65	66	67	70	86	87	88	89	90
2	1	B1	SE7263	100	2106	25274	TRUE	4090	282	1265	1209	751	67	272	3812	5369	4816	5462	16	
3	1	C1	SE7274	100	3345	40136	TRUE	267	5027	3839	1	1	145	711	8569	7064	8584	8737	94	
4	1	D1	SE7136	100	2487	29845	TRUE	1672	2026	1514	4437	1	2555	3638	4222	3953	3792	4568	21	
5	1	E1	SE7136	100	2719	32627	TRUE	4677	116	4422	1869	1	59	296	3333	8198	5604	6419	58	
6	1	F1	SE7137	100	4175	50095	TRUE	4366	980	7942	1	4305	2463	11	5230	14070	7205	7453	75	
7	1	G1	SE7138	100	5387	64649	TRUE	11177	37	1434	167	492	1973	6190	6575	15637	11332	15282	51	
8	1	H1	SE7140	100	5794	69523	TRUE	6668	958	6596	195	1	3608	2182	12515	15610	15219	14178	46	
9	1	A2	SE5192	100	1959	23504	TRUE	3871	245	2391	1	1	140	1	4422	4542	5002	4766	24	
10	1	B2	SE6016	100	2769	33232	TRUE	152	46	429	6060	4326	243	1012	4802	6227	6496	6315	20	
11	1	C2	SE5085	100	1935	23218	TRUE	563	3917	4756	655	1	51	85	1390	5941	1145	6358	42	
12	1	D2	SE7258	100	1712	20543	TRUE	4331	21	4030	3545	2164	270	242	993	3807	338	2501	12	
13	1	E2	SE8265	100	2943	35313	TRUE	4612	340	3695	905	1491	452	257	7214	5888	6813	6544	35	
14	1	F2	SE8270	100	3440	41281	TRUE	5861	1031	703	4922	1	4136	1563	6105	6943	7239	6249	37	
15	1	G2	SE8271	100	1838	22054	TRUE	6762	63	1	1	506	11	34	890	8665	4850	2100	15	
16	1	H2	SE8288	100	2327	27919	TRUE	3463	1070	3600	3715	1	1364	376	2678	4751	4240	4304	78	
17	1	A3	SE8270	100	894	10731	FALSE	3387	62	5746	7	465	1412	202	10	46	7	105	12	
18	1	B3	SE8270	100	639	7672	FALSE	2685	2731	2070	161	1	170	44	1	62	72	4	21	
19	1	C3	SE8270	100	853	10237	FALSE	1195	3761	4844	756	1	1	185	3	35	154	1	10	
20	1	D3	SE8270	100	292	3508	FALSE	676	209	326	14	18	340	22	601	37	1	19		
21	1	E3	SE8270	100	200	2396	FALSE	585	209	326	14	18	340	22	601	37	1	19		
22	1	F3	SE8271	100	804	9644	FALSE	2339	127	3474	3656	1	23	204	18	56	1	3	43	
23	1	G3	SE8271	100	764	9173	FALSE	4844	472	3655	20	1	190	405	4	177	1	1	10	
24	1	H3	SE8271	100	1071	12851	FALSE	3920	3962	5990	2	3	14	1	2	32	13	2	1	
25	1	A4	SE8283	100	948	11381	FALSE	4302	2981	3604	2	4	44	1	1	81	90	961	20	
26	1	B4	SE8283	100	1744	20924	FALSE	6720	5348	6965	462	1	178	446	14	36	2011	1	53	
27	1	C4	SE8283	100	122	1459	FALSE	188	3	1125	13	1	9	1	3	12	1	1	4	
28	1	D4	SE8283	100	355	4259	FALSE	139	601	3188	1	42	52	1	8	1	1	36		
29	1	E4	SE8285	100	990	11878	FALSE	3916	2265	4320	1	1	19	1918	1	58	1	1	36	
30	1	F4	SE8285	100	1853	22239	FALSE	10909	158	6190	5756	1	394	436	1	64	1	1	31	
31	1	G4	SE8285	100	675	8095	FALSE	5383	255	1408	671	1	1	1	529	1	1	1	46	
32	1	H4	SE8285	100	1282	15388	FALSE	5366	4171	6119	8	1	6	540	2	59	161	7	24	
33	1	A5	SE6218	100	5419	65032	TRUE	4685	119	5083	14346	1	3223	1409	8298	9629	11383	12408	63	
34	1	B5	SE6224	100	824	9885	TRUE	45	65	556	1	1	51	1	327	6663	428	1833	64	
35	1	C5	SE6072	100	1698	20375	TRUE	5286	935	3810	5445	1	100	76	430	3456	421	1927	26	
36	1	D5	SE6073	100	1535	18415	TRUE	2798	1099	1789	94	1	547	38	2766	4223	3598	2814	24	
37	1	E5	SE6074	100	1986	23829	TRUE	4066	353	750	2231	624	53	105	2105	7459	3761	4232	22	
38	1	F5	SE6089	100	2195	26245	TRUE	2450	2258	473	1	1	461	9	3384	7186	6795	5661	9	
39	1	G5	SE6091	100	1591	19088	TRUE	2972	391	1319	1731	1043	63	1058	2116	3213	2941	3754	15	
40	1	H5	SE6096	100	1214	14567	TRUE	737	341	345	66	30	427	26	3347	3667	2617	3840	31	
41	1	A6	SE8137	100	1848	22172	TRUE	544	20	557	4882	2732	673	1794	908	4394	3645	3673	30	
42	1	B6	SE8150	100	1360	16318	TRUE	202	372	4817	1	1	129	1						