

Additional data file 1 for:

A benchmarked protein microarray-based platform for the identification of novel low affinity extracellular protein interactions.

Yi Sun, Marcus Gallagher-Jones, Colin Barker and Gavin J. Wright

Design and construction of a bespoke loading apparatus.

All recombinant proteins for our binding studies are produced as secreted recombinant proteins using a mammalian expression system. Proteins are expressed by transient transfection resulting in the expression of 6His-tagged proteins in spent tissue culture in a volume of 50 ml. To purify a large number of His-tagged proteins in parallel, we used a commercially available 96-well microtitre plate containing Ni²⁺-NTA resin (His MultiTrap 96-well filter plate, GE Healthcare). However, because the holding volume of each well is limited to only ~ 500 microlitres, this makes loading 50 ml of supernatant through each well challenging. To address this, we designed and built a bespoke loading press.

The press was designed to be operated on a normal laboratory bench using pneumatics and consists of a frame constructed of 40x40 aluminium speed frame (Rose & Krieger), a pneumatic piston (we used a C(D)Q2, Compact Cylinder, Double Acting, Single Rod (SMC Pneumatics, UK)) attached to an ~ 25 mm thick aluminium plate and a holding base to contain the 96-well filter plate (Fig. S1A). The pneumatics system is required to have sufficient force to compress 96 fully-loaded 50ml syringes at a controlled speed of approx 1 ml min⁻¹, which is the standard flow rate for protein purification using this resin. To achieve the required speed, high grade precision needle valves (SMC Pneumatics, UK) were used as the regulators. The movement of the pressure plate is relatively slow when in operation, so a set of digital vernier calipers were attached between the pressure plate and the main frame to enable visual indication and quantification of the movement rate. The transfection supernatants were loaded into 50 ml disposable plastic syringes and sited into metal loading bars (Fig. S1B, S1C) that were removable and therefore allowed sequential and convenient loading of all 96 syringes into the frame.

The filter plate assembly and fittings are made of aluminium and consist of a lower block with a drain outlet and a top plate held in position using four locator pins (Fig. S1D, S1E). The Ni²⁺-NTA filter plate is fixed between these two and bounded by two silicon membranes to ensure a tight seal and prevent well-to-well leakage (Fig. S1F). The top plate was drilled to make 96

tapped holes at a spacing that corresponded to the wells in the filter plate (Fig. S1G, S1H, S1I). Tubing and fittings were purchased from PTFE Parts Ltd, Cambridgeshire, UK: 1.6mm outer diameter, 1.0 mm microbore PTFE tubing were fixed to the top plate with acetal fittings and cone seals (Cat. Nos. 4-SPAN-001 and 4-PP-1012B). The other end of the tubing had a luer connection made using a microbore tubing adapter, end connector and fitting (Cat. Nos. 2-PP-0012-TUB, 4-PP-1056, 4-VP-BSFTLL-6) that could be attached directly to the syringes. Each connection was colour-coded and numbered to facilitate correct positioning.

Fig S1 A

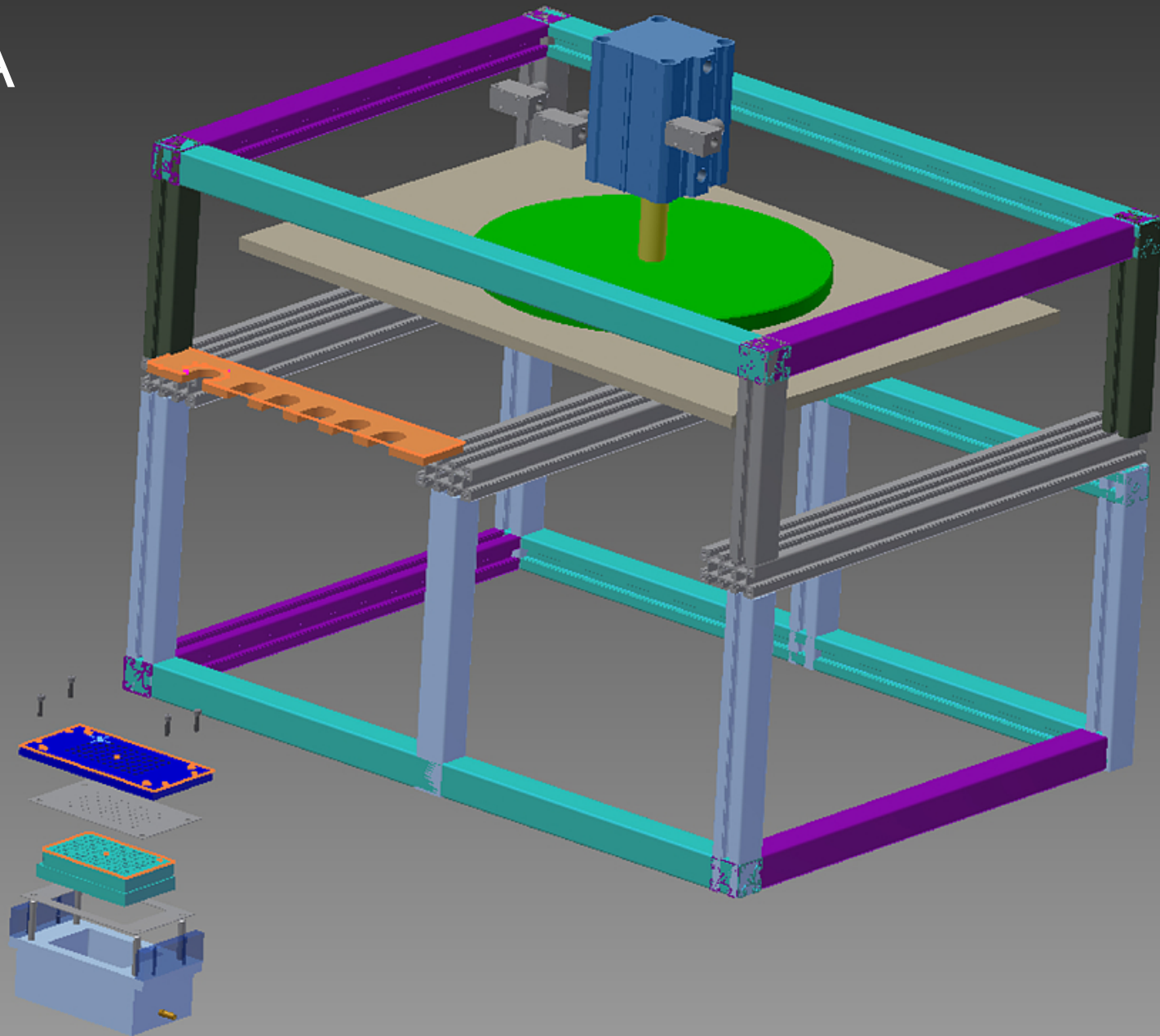
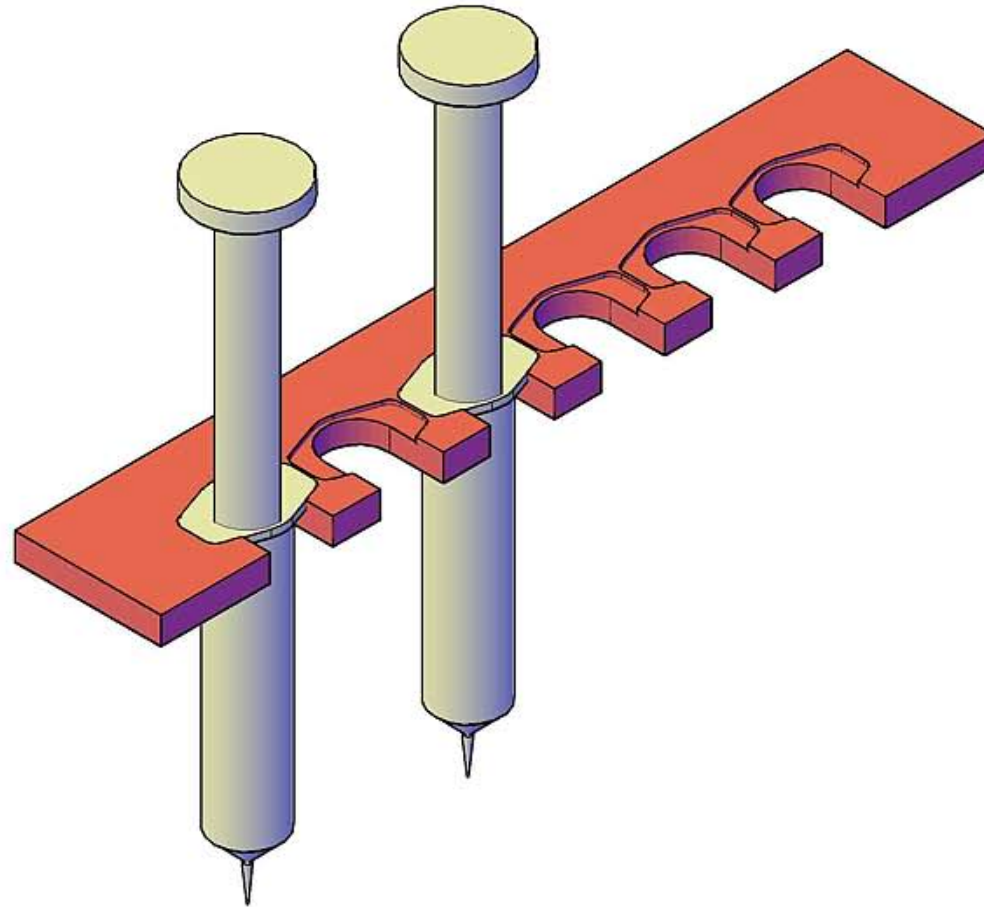


Fig S1 B



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Scale	NTS
Drawn	Date 16/06/09
Chkd	Date CB
Appd	Date

Tolerance unless othwise stated
 Angular ± 0.25 deg
 X.XX ± 0.05
 X.X ± 0.1
 X ± 0.5

MATERIAL	AL/AL
FINISH	Clean

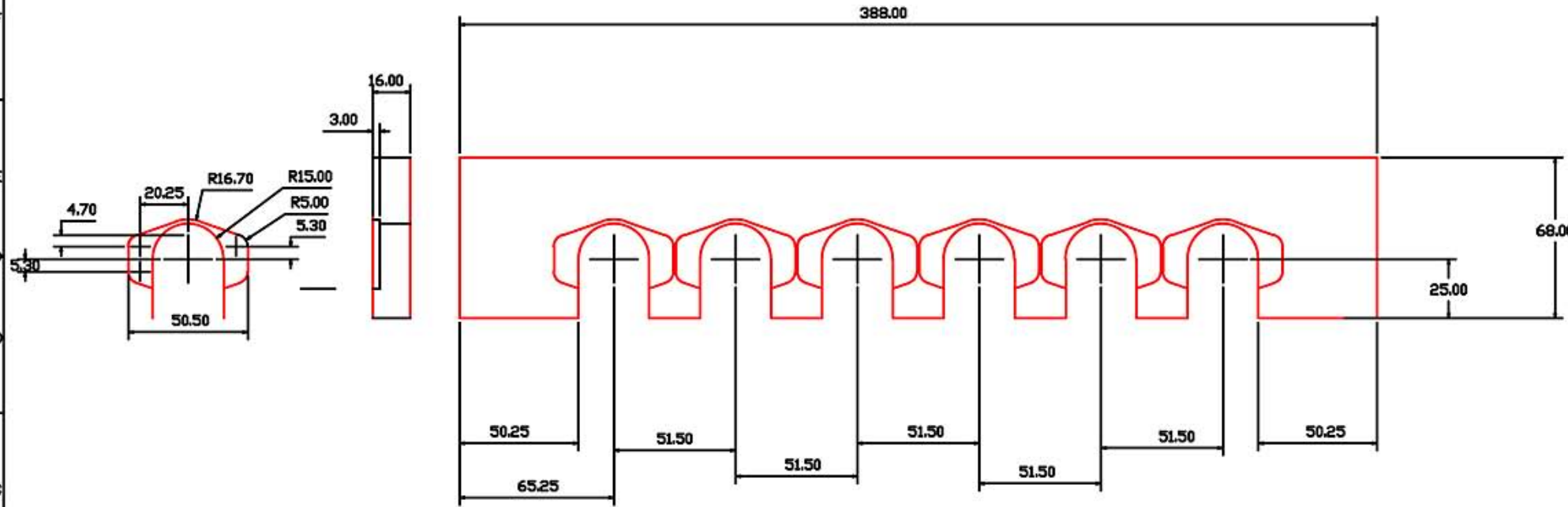
PROJECT No
 WS-10-001

TITLE
 SYRINGE HOLDER X 6

DRAWING No
 GW -1-Assy

REV No	A
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Fig S1 C



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Scale	NTS
Drawn	Date 18/06/09
Chkd	Date CB
Appd	Date

Tolerance unless othwise stated
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 X.X ± 0.1
 X ± 0.5

MATERIAL	Tooling Plate
FINISH	Clean

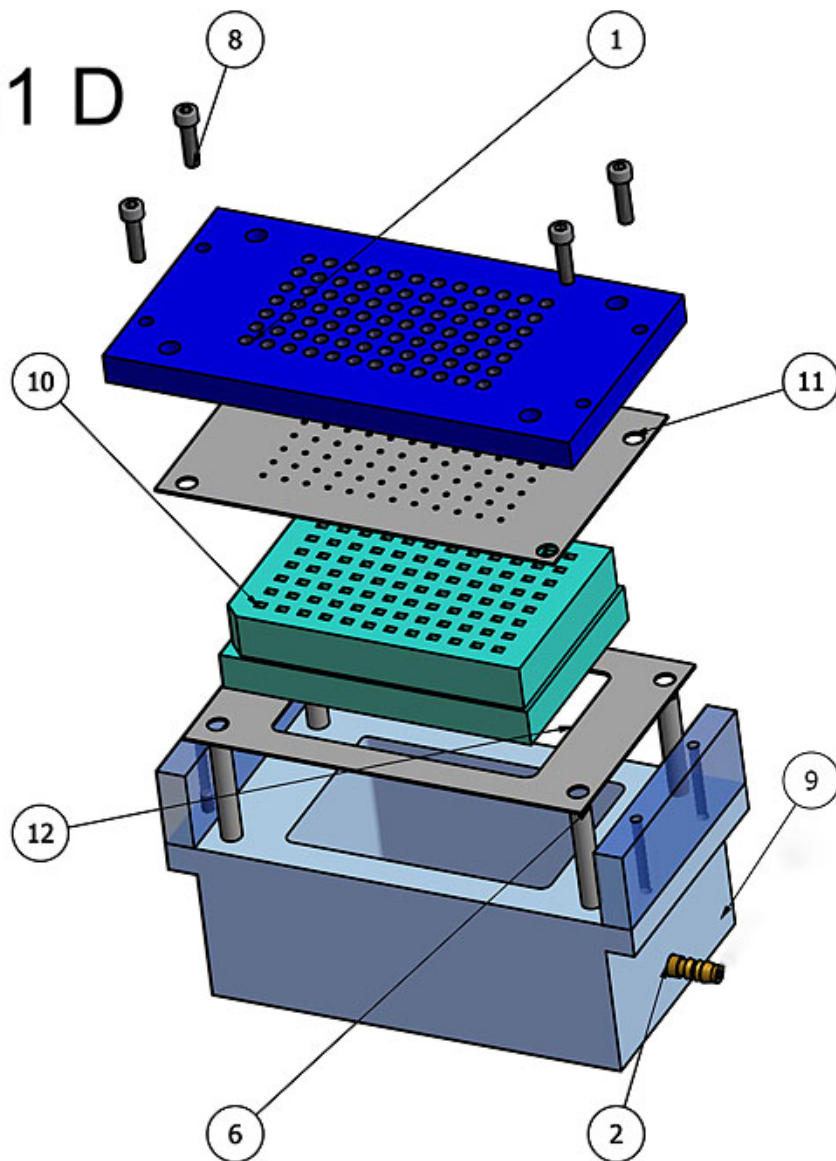
PROJECT No
 WS-2010-01

TITLE
 Syringe Holder

DRAWING No
 2010-01

REV No
 A

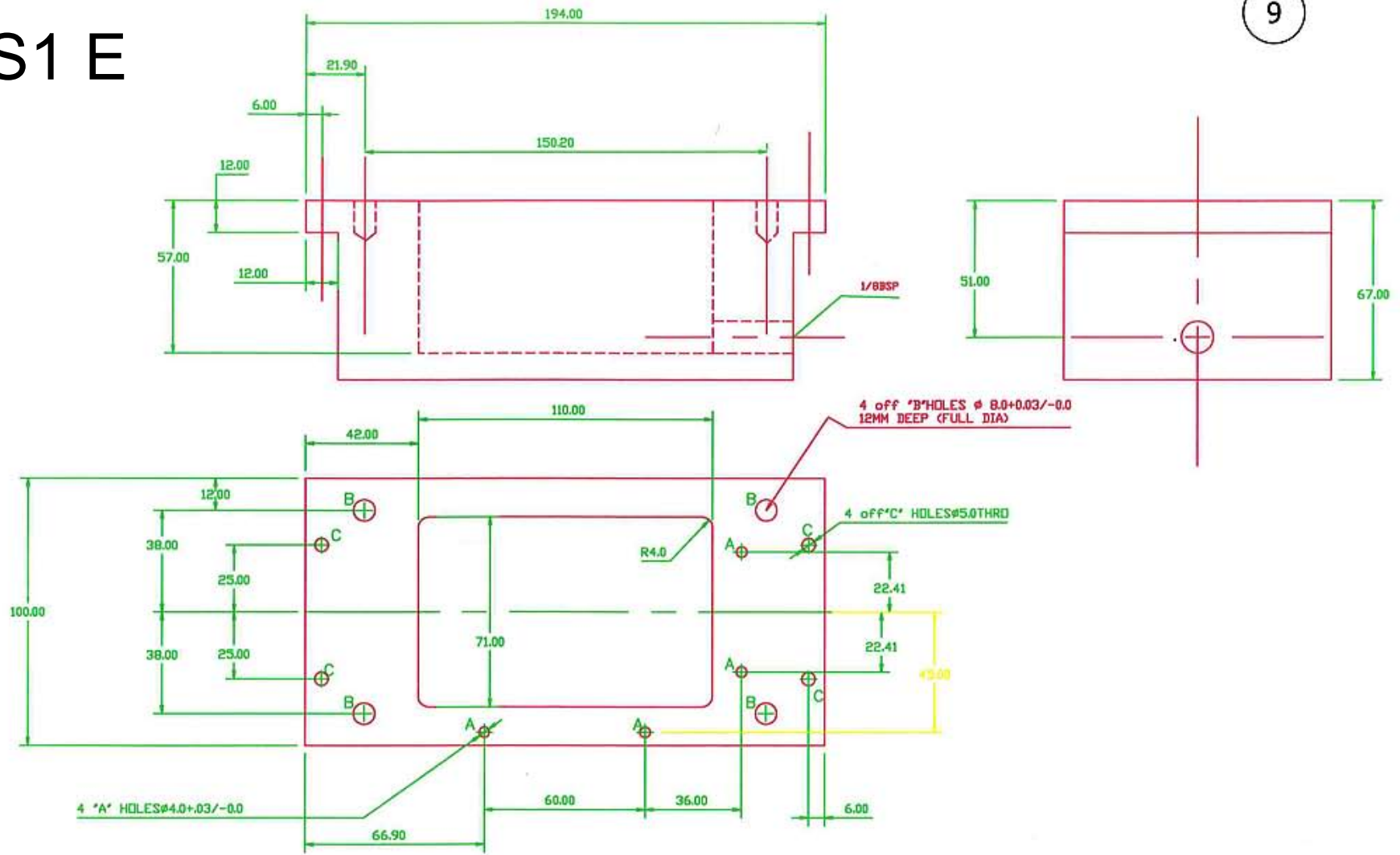
Fig S1 D



PARTS LIST			
ITEM	QTY	PART NUMBER	DESCRIPTION
1	1	Top plate	
2	1	Barb fitting	
3	2	Side spacers	
4	1	Location Dowels 60lg	
5	1	Location Dowels 60lg_CPY	
6	1	Location Dowels 60lg_CPY1	
7	1	Location Dowels 60lg_CPY_CPY	
8	6	ANSI B18.3.1M - M5x0.8 x 20	Forged Socket Head Cap Screw - Metric
9	1	Lower plate	
10	1	Target Filter Plate	
11	1	Top seal	
12	1	Bottom seal	

Designed by cb4	Checked by	Approved by	Date	Date 16/08/2011
		Edition		Sheet 1 / 1

Fig S1 E



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Scale	NTS
Drawn	Date 14/08/08
Chkd	Date 14/08/08
Appd	Date 14/08/08

Tolerance unless otherwise stated
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 X.XX ± 0.05
 X.X ± 0.1
 X ± 0.5

MATERIAL
 Aluminium Alloy 6082
 FINISH
 Anodise

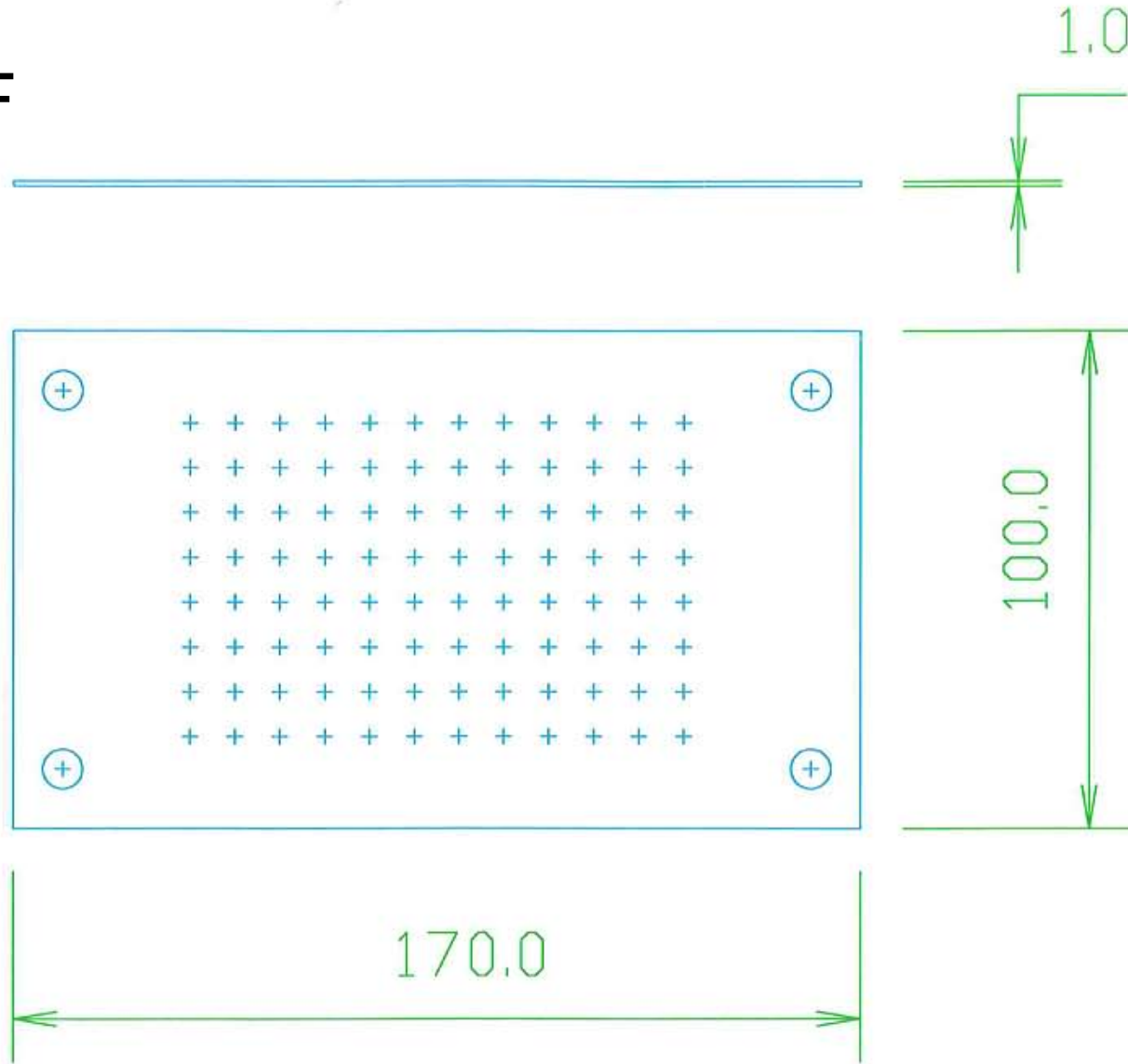
PROJECT No
 WS-08001

TITLE
 Lower vac plate

DRAWING No
 0002

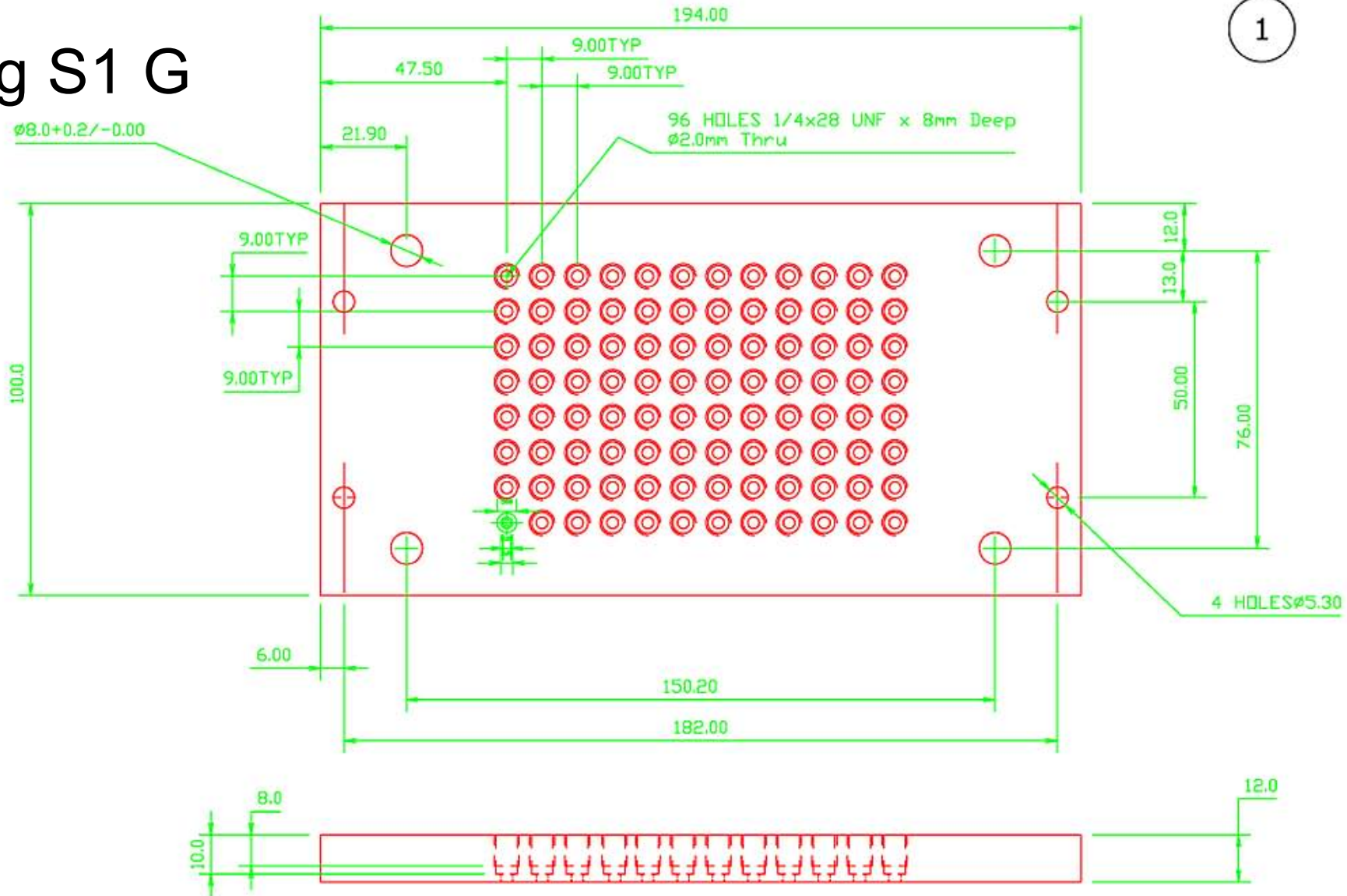
REV No
 A

Fig S1 F



The Sanger Institute Genome Campus Hinxton Cambs, BC6 1SA Tel No 01223 834244 Fax No 01223494919	Scale	NTS	Tolerance unless otherwise stated Angular +/- 0.25 deg X.XX +/- 0.05 X.X +/- 0.1 X +/- 0.5	MATERIAL	PROJECT No	TITLE	
	Drawn	Date		SILICONE SEAL	WS-08001	TOP GASKET	
	Chkd	Date		FINISH	CLEAN	DRAWING No	REV No
	Appd	Date				GASKET 1	

Fig S1 G



1

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Scale	NTS	
Drawn	Date	14/08/08
Chkd	Date	14/08/08
Appd	Date	14/08/08

Tolerance unless otherwise stated
 Angular ± 0.25 deg
 X.XX ± 0.05
 X.X ± 0.1
 X ± 0.5

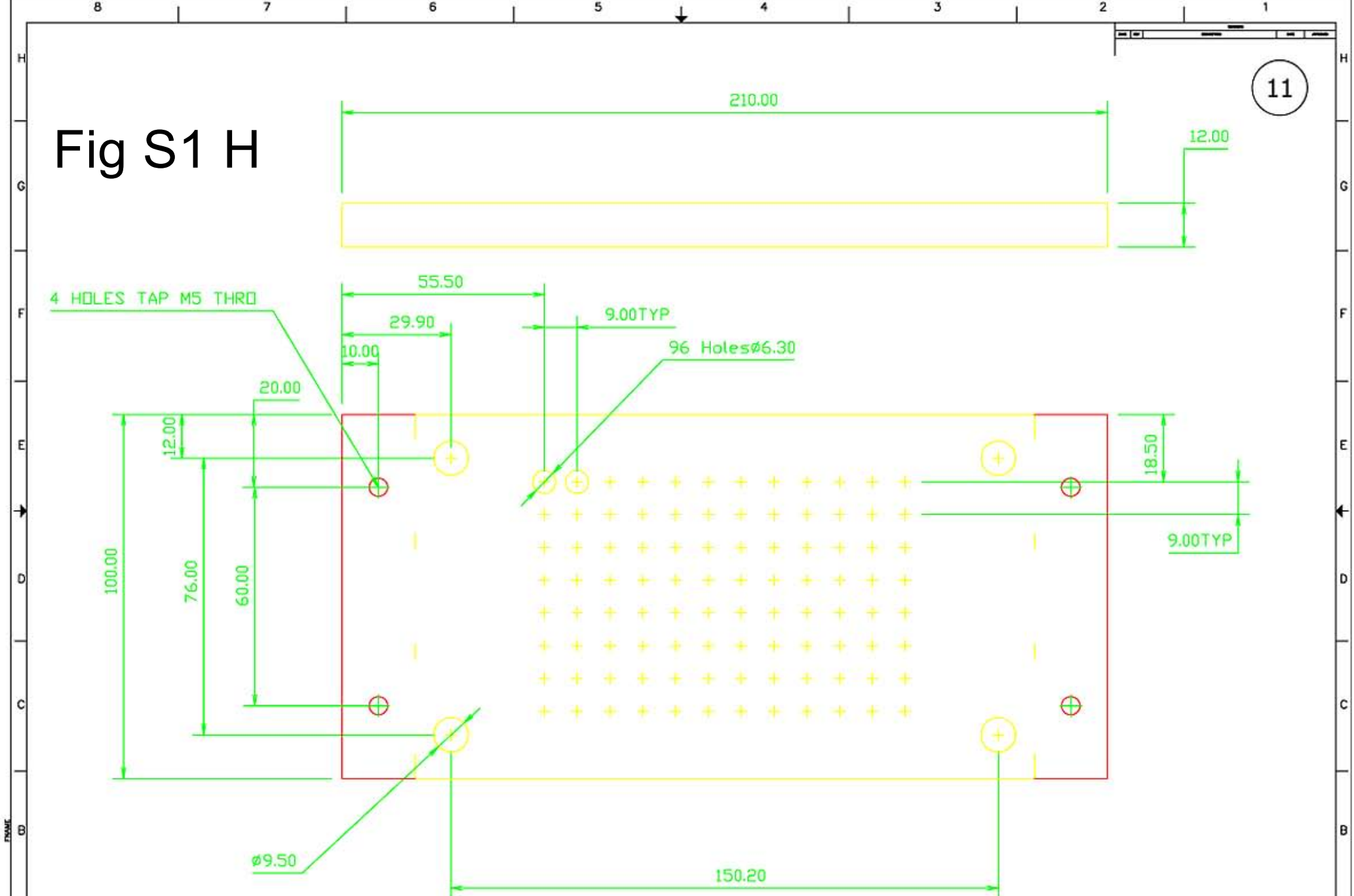
MATERIAL	Aluminium Alloy 6082
FINISH	Anodise

PROJECT No	WS-08001
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TITLE	Top connection plate	
DRAWING No	0001	REV No A
		A

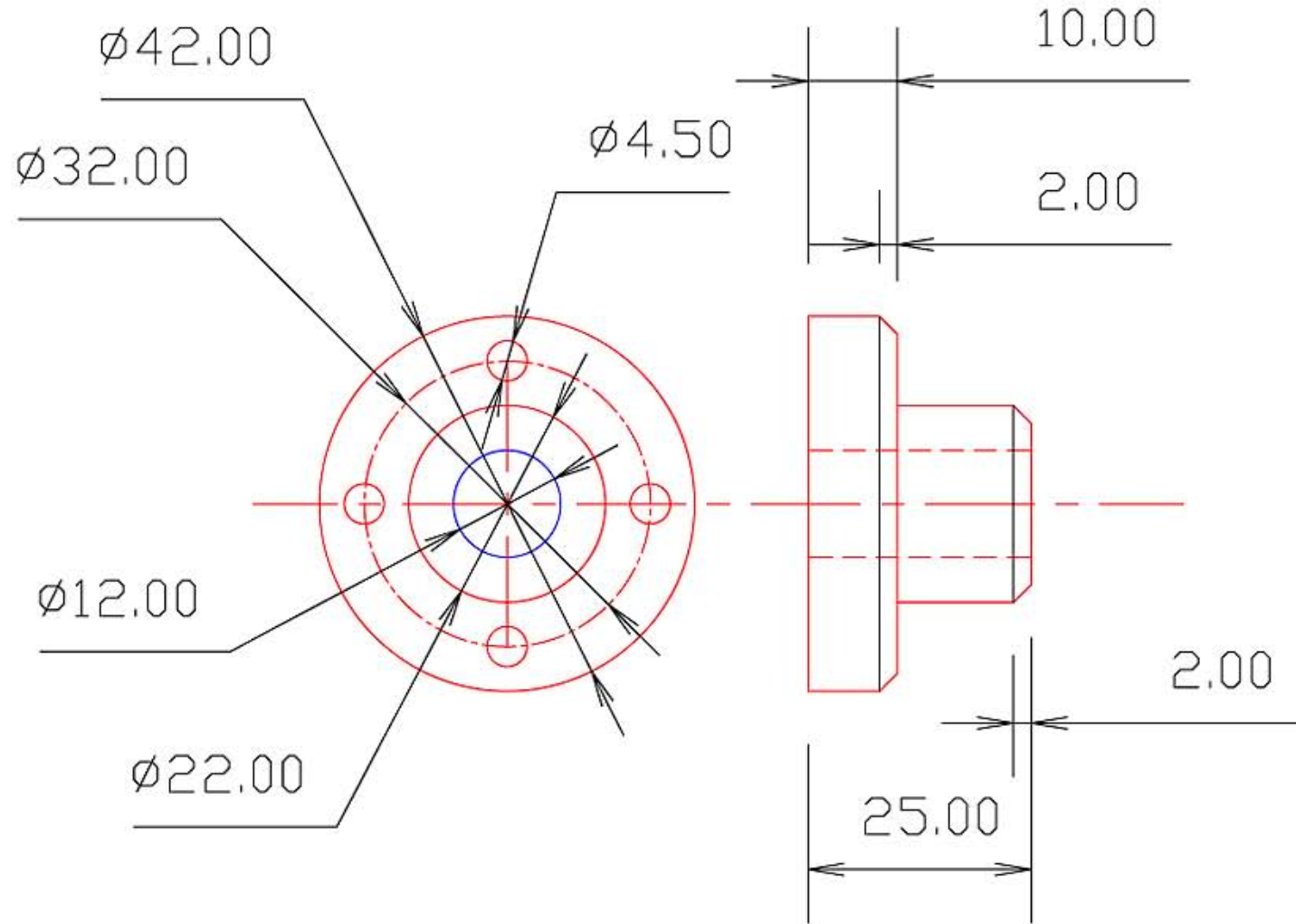
Fig S1 H

11



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	Drawn	Date		ALUMINIUM ALLOY 6082		TOP TEMPLATE	
	Chkd	Date		FINISH	CLEAN	DRAWING No	REV No
	Appd	Date				0004	

Fig S1 I



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Scale	NTS
Drawn	Date 01/12/08
Chkd	Date 01/12/08
Appd	Date 01/12/08

Tolerance unless othwise stated
 Angular ± 0.25 deg
 X.XX ± 0.05
 X.X ± 0.1
 X ± 0.5

MATERIAL	AL/AL
FINISH	CLEAN

PROJECT No	WS-2010-01
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TITLE	Guide Rod Holders
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DRAWING No	10-004
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REV No	A
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