Next-generation high-density self-assembling functional protein arrays

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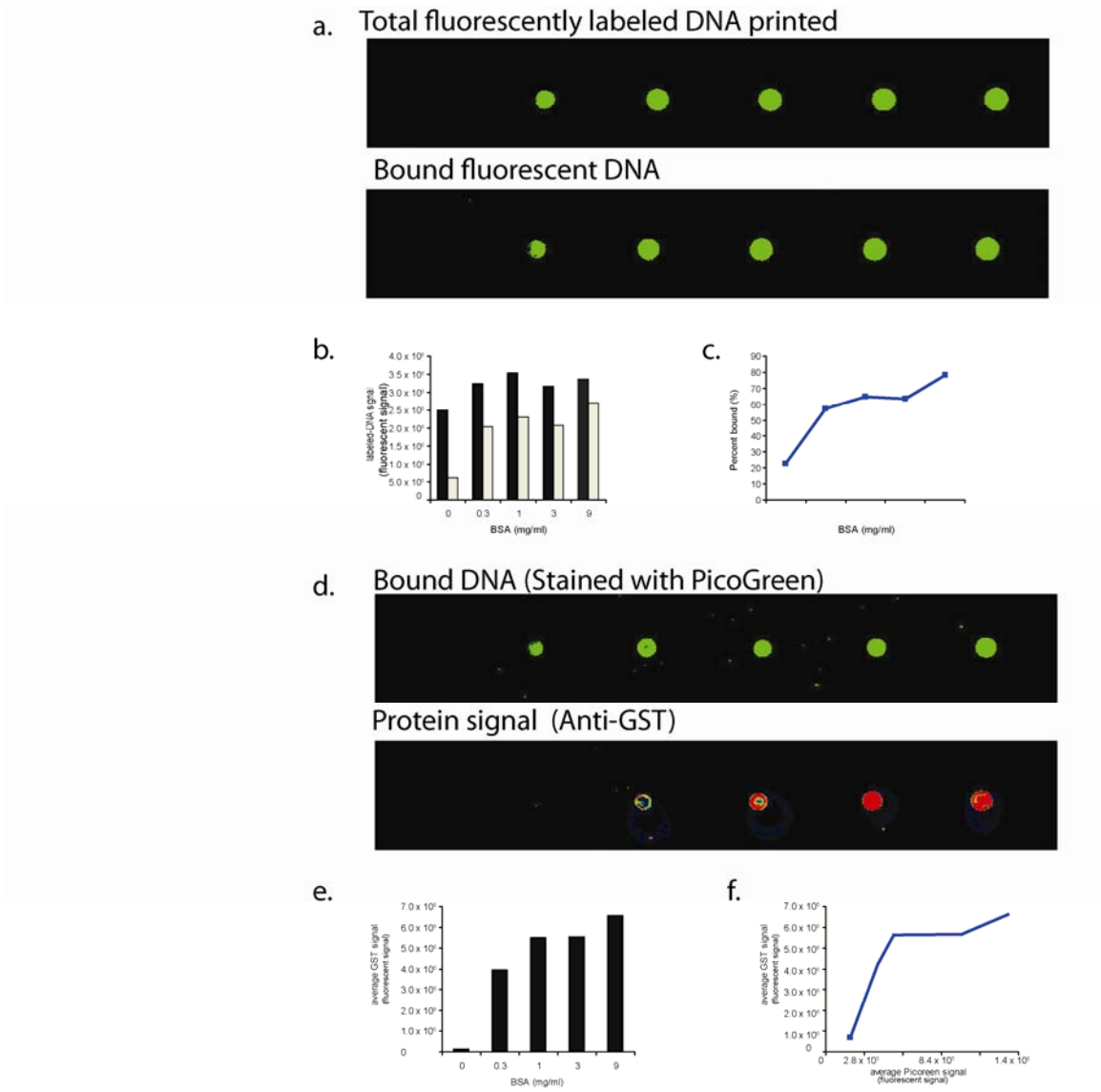
Supplementary figures and text:

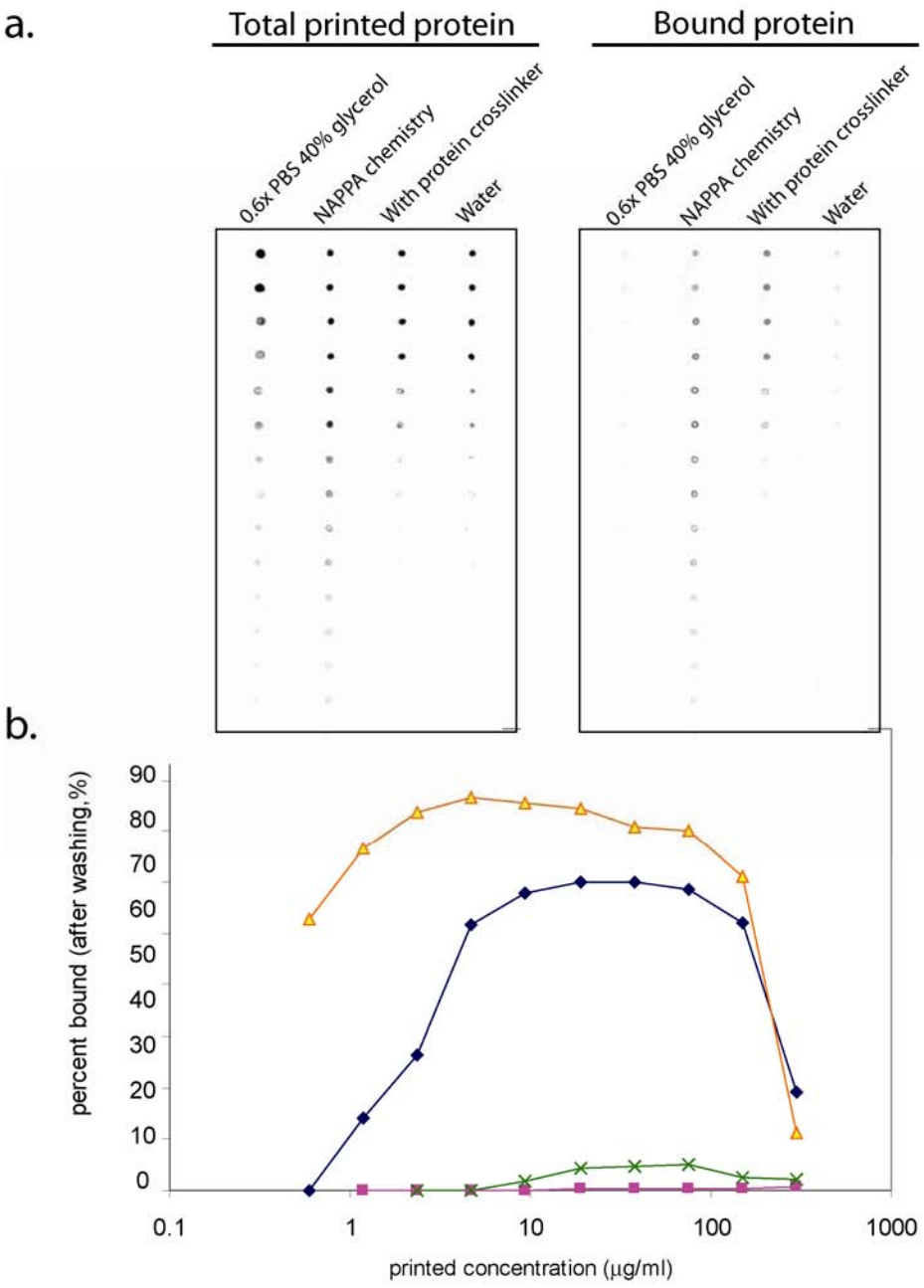
Supplementary Figure 1. Optimization of DNA binding



Supplementary Figure 1. Optimization of DNA binding. (**a**) To estimate the amount of DNA captured onto the array surface, 30 µg of plasmid DNA was incubated with 20 µL of PicoGreen dye. The DNA was precipitated and washed with 80% ethanol to remove unincorporated PicoGreen dye. DNA was dissolved to a final concentration of 1.5 µg/µL and supplemented with the capture antibody (final 50 µg/mL), protein crosslinker (final 2 mM) and varying concentrations of BSA (0-9 mg/mL). The sample was printed onto amine coated glass slides, and the printed samples were imaged. The slides were washed with 1xPBS for 1 hr at RT and the slides were imaged again to measure the bound fraction. (**b**) Total fluorescent DNA is indicated by black bar and bound DNA is indicated by the grey bar. (**c**) Amount of protein signal obtained with respect to the amount of DNA printed**.** (**d**) Sample containing unlabelled DNA (final 1.5 µg/µL), capture antibody (final 50 µg/mL), protein crosslinker (final 2 mM) and varying

concentrations of BSA (09 mg/mL) were printed on to the amino coated array surface. The arrays were stained with PicoGreen dye to measure the DNA bound. To detect protein signal, the arrays were activated with the cell free lysate and stained with anti-GST antibody. The colors ranging from black, blue, green, yellow, to red represent low to high signal, respectively. (**e**) Amount of protein signal attained with increasing amounts of BSA. (**f**) Amount of protein signal obtained with respect to the amount of DNA bound**.**

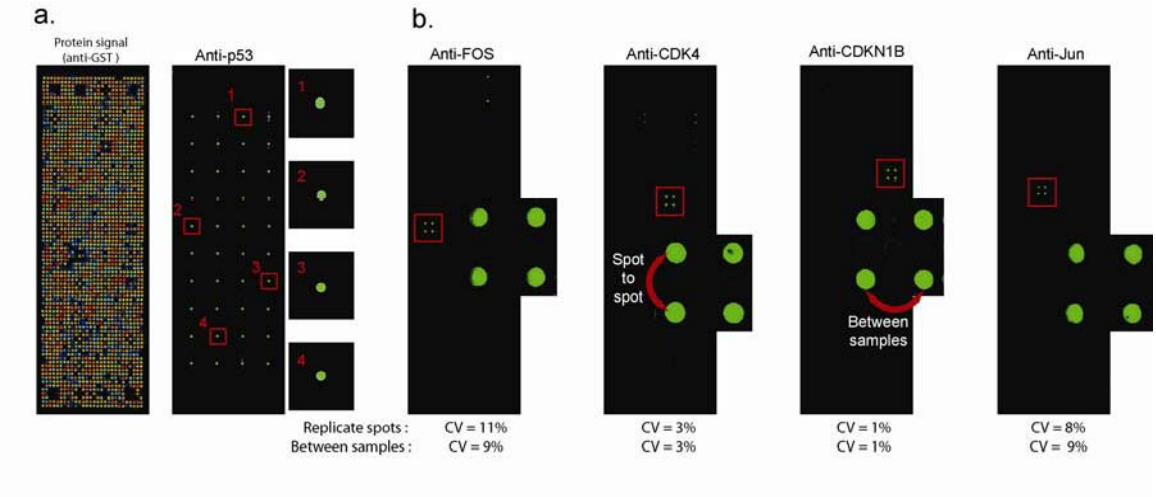
Supplementary figure 2 Purified protein spotting



Supplementary Figure 2. Purified protein spotting. (**a**) Purified Cy5 labeled anti-mouse antibody was printed at various concentrations (0-300 µg/mL) in four different buffers: 0.6x PBS with 40% glycerol; NAPPA chemistry (BSA (3 mg/mL), crosslinker (2 mM)); crosslinker only (2

mM); and water. The arrays were imaged to determine total signal from the printed protein and then washed in 1x PBS for 1 hour and re-imaged to determine signal from the bound protein. (**b**) The binding efficiency of the protein under various spotting conditions was determined by dividing the bound signal by the total signal.

Supplementary figure 3 Signal variation



Supplementary Figure 3. Signal variation**.** (**a**) To assess the zone variation due to array processing, a single sample preparation of the *p53* gene was aliquoted into multiple wells and printed throughout the high density array (left panel) in a 4 x 10 pattern. The *p53* protein signal from all 40 *p53* features was detected using an anti-*p53* antibody (CV for 40 spots = 7%). Crosstalk of the p53 signal into the neighboring spots was evaluated by comparing the signal intensity of the spots surrounding the *p53* against a group of spots that were at least 4 spots or 2572 microns away from the nearest *p53* signal. The average signal neighboring *p53* was 1.9% of the *p53* signal (compared with 0.7% for control features). (**b**) To assess the variation in sample preparation prior to printing, we independently processed duplicate samples for 48 genes. The samples were printed and their protein signal was measured using protein specific antibodies when available. Between sample CV was calculated based on the 4 spots (duplicates of the two independently processed samples).

Supplementary Methods

Expression plasmids were transformed into *E.coli* DH5alpha and grown overnight at 37°C in 1.5 mL terrific broth and ampicillin (100 μg/mL). Cultures were pelleted by centrifugation at 5000 rcf for 15 mins. DNA was purified using the NucleoPrepII anion exchange resin (Macherey Nagel). Bacterial pellets were resuspended by vortex in 200 μL Buffer 1 (50 mM tris, 10 mM EDTA, 100 μg/mL RNAse A). Cells were lysed by adding 200 μL Buffer 2 (200 mM NaOH, 1% SDS), mixing by block inversion and incubating for 5mins. The preparation was neutralized by adding 200 μL Buffer 3 (2.8 M KOAc, pH to 5.3 with glacial acetic acid) and mixing by block inversion. The resulting lysate from the alkaline lysis preparation was cleared by centrifugation at 5000 rcf for 15 mins. The supernatant was loaded directly onto 80 mg of equilibrated (Buffer N2: 100 mM tris, 900 mM KCl, 15% EtOH, 0.15% Triton X-100, pH to 6.3 with phosphoric acid) NucleoPrep II anion exchange resin using a Biomek FX (Beckman Coulter) automated laboratory workstation. The column was washed with 2 mL of wash Buffer N3 (100 mM tris, 1.15 M KCl, 15% EtOH, pH to 6.3 with phosphoric acid) over vacuum, dried by centrifugation and eluted with 300 μL Elution Buffer N5 (100 mM tris, 1 M KCl, 15% EtOH, pH to 8.5 with phosphoric acid). Automated addition of all solutions was accomplished using a WellMate (Matrix) rapid bulk liquid-dispensing instrument.

Purified DNA was precipitated by addition of 0.6 volumes isopropanol, followed by centrifugation at 5000 rcf for 30 mins. The DNA pellet was washed with 200 μL of 80% ethanol, centrifuged at 5000 rcf for 15 mins and dried. The dried DNA pellet was dissloved in 23 μL printing solution containing 50 ng/μL of capture antibody (Amersham), 3.6 mg/mL bovine serum albumin (Sigma) and 2 mM BS3 (Bis[sulfosuccinimidyl] suberate) (Pierce). The

printing solution was transferred to a 384-well printing plate (Genetix) and arrayed onto aminosilane coated glass slides. The printing was performed using a Genetix QArray2 with 300 μm solid tungsten pins and the slides were stored dry at room temperature.

The slides were blocked in Superblock (Pierce) for 1 hour at room temperature, rinsed with double deionized water, and dried using house air. An incubation chamber (Grace Bio.) was applied to the array surface and ~130 μL of the cell-free transcription and translation mix (T7-TNT system, Promega) prepared according to manufacturers instructions were added to the slides. The slides were incubated in a chilling oven (Torrey Pines) at 30°C for 1.5 hours and 0.5 hours at 15°C. For protein interactions, the cell free lysate was supplemented with 100-300 ng of query DNA (pANT7_HA,[27]) and incubated in the chilling incubator at 30°C for 1.5 hours and 2 hours at 15°C. Following activation with cell free lysate, the slides were blocked with Blocking Buffer (5% milk in phosphate buffered saline supplemented with 0.2% Tween 20) for 1 hour. For detecting protein expression universally on the array, the slides were incubated with 2 mL of primary monoclonal antibody (10 μg/mL, mouse anti-GST antibody, Cell Signaling Technologies) and a HRP linked secondary antibody (10 μg/mL anti-mouse IgG, Amersham) diluted in Blocking Buffer. For detection of specific proteins, the slides were incubated with 2 mL of primary monoclonal antibody (Santa Cruz mouse anti-p53 (D01), Santa Cruz rabbit anti-c-Jun (N), Cell Signaling rabbit anti-c-Fos, Sigma mouse antiCDK4, Sigma mouse anti-p27-KIP1) diluted 1:200 in Blocking Buffer followed by a HRP linked secondary antibody (Jackson goat anti-mouse IgG, Santa Cruz goat anti-rabbit IgG) diluted 1:500 or 1:200 respectively in Blocking Buffer. The incubation with the detection antibodies was carried out using a hybridization chamber (Corning) mixing for 16 hours with the primary antibody at 4°C and 1

hour at room temperature for the secondary. The slides were rinsed with the Blocking Buffer between the two incubations with the antibodies and finally rinsed with PBS prior to applying the developing solution. The arrays were developed by adding 600 μL of the tyramide signal amplification reagent (Perkin Elmer) for 10 mins using a cover slip (Lifterslips, Erie). The slides were rinsed with de-ionized water, dried using house air and scanned with a ProScanArray HT scanner (PerkinElmer). The array images were quantified using the MicroVigene software, version 2.9.9.2 (VigeneTech).

Human kinase list was assembled by mining gene functional and structural annotations in public databases[13,28]. Human Transcription Factors (TF) were assembled by mining the literature about well-studied TF[29] and the literature of genome-scale TF search by sequence similarity[30]. This list was also supplemented by the TFs identified by mining Gene Ontology and other databases such as Swissprot and Genatlas. TM was predicted using TMHMM[31, 32] and Sosui[33].

Supplementary Protocols

1. DNA Minipreps

Material/Equipment	**Amount for one 96-well block**
TB culture medium (KPI+Ampicillin)	1.5 mL
96-pin device (Boekel 140500)	1
Solution 1	200 uL/well
Solution 2	200 uL/well
Solution 3	200 uL/well
Isopropanol	600 uL/well
Solution N2	200 uL/well
Solution N3	2000 uL/well
Solution N5	300 uL/well
800 uL glass fiber MBPP 25 micron filter plate (Whatman 13503-040)	1
Deep-well block	2
Gas permeable plate seal	1
Aluminum plate seal	2
ATR Multitron shaker (37oC)	1
Centrifuge, Eppendorf 5810	1
Eppendorf Thermomixer	1
Omni plate (Nunc 242811)	
LB	
Agar	
Sorvall RC12 centrifuge	
Sorvall Legend RT centrifuge	
350 uL 96-well plate (Greiner 651201) for alkaline lysis DNA prep	
800 uL 96-well block (Abgene AB-0859) for Nucleobond prep	
Nucleobond resin (Machery-Nagel custom order)	

1) Antibiotic concentrations – Ampicillin (100ug/ml), Chloramphenicol (34 ug/ml), Kanamycin (50ug/ml).

2) Create an overnight culture using either LB/Agar or LB liquid cultures.

LB/Agar culture

a. Spot 3ul from the glycerol stock onto a pre-warmed agar plate. Incubate overnight at 37°C.

b. Sterilize the 96-pin device using 80% ethanol and a flame. Let it cool. Inoculate the blocks (1.5mL of TB) and culture at 37°C with vigorous shaking for 24-26 hours.

3)**Pellet cultures.** Spin blocks for 15 min at 4000rpm /5300 rcf on the Sorvall RC12 centrifuge. After spinning, decant the media from each block into a large bucket or beaker. Blot the decanted blocks, upside-down, on paper-towels spread on the bench-top to remove excess media. Seal each block using an aluminum plate seal and store the blocks at –20°C until needed for DNA purification. The decanted media should be bleached at a final concentration of 5% bleach for 20-30 mins in the fume hood before being discarded.

4) Prepare solutions 1, 2, and 3 according to the following recipes (also available at the end):

Soln 1: TE Resuspension Buffer
50 mM Tris pH 8.0 10 mM EDTA (8.0)
0.1 mg/mL RNAse (2ml of Sigma RNAse/1L of solution 1)
Store at 4°C

Soln 2: NaOH/SDS Lysis Buffer
0.2 M NaOH
1% SDS

Soln 3: KOAC Neutralization Buffer
2.8 M KOAc
Glacial Acetic Acid: added until pH is 5.1
Store at 4°C

5)**Add 200 uL of soln 1** and resuspend by vortexing vigorously. Make sure that no wells contain clumps of bacteria as they will result in low plasmid yield. If unable to disperse by vortexing, then pipette using a P1000.

6)**Add 200 uL of soln 2**, seal the plate with an aluminum seal and gently mix the plate by inverting 4 or 5 times. Carefully time this step from the beginning of soln 2 addition so as not to exceed 5 minutes. Do not shake the plate vigorously as this will result in the undesired shearing of bacterial genomic DNA.

7)**Add 200uL of soln 3**, seal the plate with an aluminum seal and mix the plate by inverting 4 or 5 times. The seal will be loose due to the lysis/neutralization buffers so use caution when inverting.

8)**Spin the block for 20 minutes at 4000rpm/5300 rcf** on the Sorvall RC12 centrifuge to pellet the lysate.

For Nucleobond anion exchange DNA preparation

9) Prepare solutions N2, N3, and N5 according to the following recipes (also available at end of protocol):

Soln N2: Equilibration Buffer

100 mM Tris 15% EtOH 900 mM KCl 0.15% Triton X-100 Phosphoric Acid: added until pH is 6.3

*To prepare **anion exchange slurry**, add 200ml of N2 buffer to every 100ml of beads.*

Soln N3: Wash Buffer

100 mM Tris 15% EtOH
1.15 M KCl
Phosphoric Acid: added until pH is 6.3

Soln N5: Elution Buffer

100 mM Tris 15% EtOH 1 M KCl Phosphoric Acid: added until pH is 8.5

10) **Prepare anion exchange resin plate**. Transfer 400ul of the slurry into each well 25 um Whatman MBPP filter plate. When done, centrifuge at 500 rpm for 5 minutes using the table-top centrifuges.

11) **Transfer lysate supernatant to the resin plate**

12) **Spin the stacked plates for 10 mins at 300 rpm**.

13) **Wash column step**. Place stack plate onto the WellMate and add **500 uL of wash buffer N3** to each well. Alternatively, a 1 mL multi-channel pipette may be used to add the wash buffer. Transfer the resin plate to vacuum manifold to remove wash buffer**. Repeat wash steps 4x**. On the last wash make sure all wells are properly emptied. **Spin the stack plate at 500rpm/750 rcf for 3 mins** using any centrifuge to remove residual wash buffer.

14) **Elution**. Place resin plate onto a clean 800 uL collection plate. Place the stacked plates onto the WellMate and add **300 uL of elution buffer N5** to each well. Alternatively, a 1 mL multi-channel pipette may be used to add the elution buffer. Let **sit at RT for ~30 minutes** then **spin the stacked plates for 10 mins at 300 rpm, then 2 mins at 1000 rpm**.

15) Quantitate DNA using UV or fluorescence.

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2. Aminosilane slide coating

Material/Equipment	**Amount (for 30 slides)**
Glass slides (VWR 48300-047)	30
Acetone 99.9%	300 mL
Aminosilane (Pierce 80370)	6 mL
Metal 30-slide rack (Wheaton 900234) with no handles	1
Glass box (Wheaton 900201)	1
Lock & Lock 1.5 cup boxes (ZHPL810)	1
Rocking shaker	

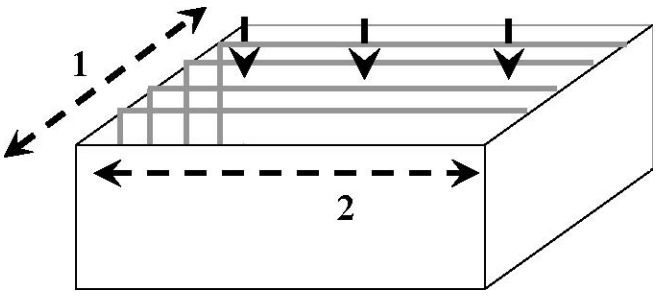
1) Put 30 slides in each metal rack (remember to fill one extra rack).

2) Prepare 300 mL of 2% aminosilane coating solution in glass troughs and cover with saran wrap (6 mL aminosilane in 300 mL acetone – use plastic pipette for silane). *This solution can be used 3-4 times.*

3) Treat glass slides in aminosilane coating solution for 15 minutes. Rinse off with acetone in another trough, and then dip in a third trough containing distilled water.

4) Dry with filtered compressed air in chemical hood. *Drying pattern as depicted below:*

3 3 3



1 Longitudinal, along slides
2 Along the width of the slides
3 Down the edges of the slides

5) Store at room temperature in rack in Lock & Lock box. Use within one week.

3. Array printing

Material/Equipment	**Amount/well**
Plasmid DNA (from NAPPA DNA prep protocol)	
Sodium acetate (3M, pH 5.5)	
Isopropanol	
Ethanol	
384 well plate for arraying, Genetix x7020	
Polyclonal anti-GST antibody (GE Healthcare/Amersham 27457701)	
Polyclonal anti-FLAG antibody (Sigma F7425)	
BS_3 Linker (Pierce 21580)	
Purified GST protein (Sigma G5663)/Flag protein ()	
Whole mouse IgG antibody (Pierce 31204)	
Centrifuge, Eppendorf 5810	
QArray2	
Silica packets (VWR 100489-246)	
Genetix Bioassay dish dividers (x6026 divider only; x6027 with dish)	
Corning deep bioassay dish (431111)	
WellMate	
Eppendorf Thermomixer	

1) Take out the DNA plates to be printed from the -20°C freezer and allow them to come to room temperature.

2) Precipitate the DNA by **adding 200 uL of isopropanol to each well**. Cover the plate with an aluminum seal and mix by inverting a few times.

3) **Centrifuge at 4000rpm for 30 minutes**. Discard the supernatant.

4) **Add 400-500 uL of 75% ethanol to each well** using WellMate.

5) Centrifuge at 4000rpm for 15 mins at 20°C. Discard the supernatant.

6) Dry the plate, uncovered for 10-15 mins. You should not see any alcohol at bottom of well. Seal and centrifuge at 1000 rpm for 2 minutes to bring any pellets down.

Array sample preparation:

7) **Prepare master mix**. For one 96-well plate prepare approximately 3 mL of master mix. Master mix contains polyclonal antibody (final: 1:100 dilution or 50 ug/mL), BSA (final: 3.6 mg/mL) and BS^3 linker (final: 1.25 mg/mL or 2 mM).

For GST arrays:

Number of 96 well plates	1	4	8	24
Volume needed	3ml	10ml	20ml	50ml
BSA (66mg/ml)	166.5	555	1110	2775
BS3 linker	75	250	500	1250
anti-GST (5mg/ml)	30	100	200	500
AC mQ H2O	2728.5	9095	18190	45475
Total	3000	10000	20000	50000

8) Transfer 20ul to each well of the dry DNA pellet. Spin down and shake at 200 rpm for 30-60 minutes.

9) **Transfer all 20uL to 384 array plate**.

10) Spin the plate down briefly (1500 rpm for 1 minute – to get rid of bubbles).

11) **Array** *(see below)* using the appropriate array setup and humidity control approximately 60%.

Arrayer Setup

12) If the 384 well plates for printing were frozen, take them out and allow to come to room temperature.

13) **Put blank slides on arrayer, start vacuum and check for no leaks.** If all is good, start humidifier.

14) Spin down your 384 well plates at ~1500rpm for 1 minute. Remove foil and place on arrayer deck so that the A1 position is closest to you – bottom right. Check the parameters of the program, and then start it.

15) **When arraying is done, place slide labels on the bottom** (non-arrayed) side of each slide. Maintain the slides order on the deck in numerical order.

16) **Place the printed slides back in the metal rack**, then place in lock-and-lock boxes along with 1-2 silica packs.

4. Detection of the DNA on NAPPA slides

Material/Equipment	**Amount (for 4 slides)**
PicoGreen (Invitrogen P11495)	
PicoGreen stock solution	33 uL
SuperBlock	50 mL
PBS (pH 7.4)	150 mL
Coverslips, 24 x 60 mm	4
Lifterslips, 24 x 65 mm	4
Rocking shaker	
Scanner, PerkinElmer ProScanArray	

1) Block the slides with SuperBlock on a rocking shaker for 30-60 minutes.

2) If necessary, prepare PicoGreen stock solution: To the 100 uL/vial that comes, add 200 uL TE buffer, then do a 1:600 dilution in SuperBlock (i.e. for 4 slides, add 33 uL PicoGreen stock solution to 20 mL SuperBlock).

3) For a single slide, small array: apply 150 uL PicoGreen mix, and apply coverslip. Let sit for 5 minutes at room temperature. For 4 slides, add 20 mL in a box and shake on rocking shaker for 5 minutes.

4) Wash with 1xPBS (pH 7.4) 3 times, ~ 5 min each. Quickly rinse with Milli-Q water.

5) Dry with filtered compressed air.

6) Scan

5. Expression of the NAPPA slides

Material/Equipment	**Amount (for 3 slides)**
HybriWell gaskets (Grace HBW2160-1LA)	3
Cell free expression system i.e. rabbit reticulocyte lysate (Promega L4610)	1 tube
RNaseOUT (Invitrogen 10777-019)	8 uL
DEPC water (Ambion 9906)	160 uL
SuperBlock (Pierce 37535)	~30 mL
Blocking solution: 5% Milk in PBS with 0.2% Tween20	~120 mL
PBS	
Programmable chilling incubator, with leveling shelves	
Rocking shaker	
Genetix Bioassay dish dividers (x6026 divider only; x6027 with dish)	
Corning deep bioassay dish (431111)	

1) Block slides in 30-50ml Superblock for 30-60 minutes.

2) Pre-heat the incubator to be used for IVT at 30°C.

3)**Rinse with Milli-Q water**. Dry with filtered compressed air.

4)**Apply HybriWell gasket** to each slide (align at the top of the slides). Use the wooden stick to rub the areas where the adhesive is to make sure it is stuck to the slide all around.
Do not press down too hard, otherwise it will be difficult for the retic to go in.

5)**Prepare IVT**. Each slide will require **130 uL of IVT lysate mix**. Each tube after component addition will contain 400 uL of lysate mix. Since the lysate tubes cannot be re-frozen, always try to express slides in batches of some multiple of three.

e.g. 1 tube = 3 slides = 400 uL -16 uL TNT buffer -8 uL T7 polymerase -4 uL of –Met -4 uL of –Leu or –Cys -168 uL of DEPC water -200 uL of reticulocyte lysate

6)**Add IVT mix from the non-label or non-specimen end**. Place the tip against the bottom of the entry port (nearest to the spots) and dispense the IVT quickly into the hybriwell. Gently massage the HybriWell to get the IVT mix to spread down along the slides first then down the center of the slide. When done, wipe the portals dry with gloved hand (not tissue) and apply seal to each portal. Push any bubbles to the edges of the hybriwell.

7)**Place the slides on a bioassay dish** with divider on top of the leveling shelf inside the incubator. Incubate for 1.5 hr at 30°C for protein expression (30 is key; 28 or 32 give reduced yield), followed by 30 min at 15°C for the query protein to bind to the immobilized protein.

8) Remove the HybriWell and rinse twice with PBS.

9)**Immerse each slide in milk immediately**; wash with milk 3 times, 5 minutes each, in a pipette box. Use about 30 mL milk per wash.

10) **Block with milk** on rocking shaker at room temperature for an additional 30-45 minutes.

6. Detection of expression on NAPPA

Material/Equipment	**Amount (for 1 slide)**
Primary AB, mouse anti-GST (Cell Signal #2624)	150 uL of stock solution
Primary AB, mouse anti-HA (12CA5, ordered from DFCI)	150 uL of stock solution
Primary AB, mouse anti-Flag (Sigma #)	
Secondary AB, HRP-conjugated anti-mouse (Amersham NA931)	150 uL of stock solution
Secondary AB, HRP-conjugated anti-mouse (Jackson Lab Cat #515-035-062)	
TSA reagent (PerkinElmer SAT704B001EA)	150 uL of stock solution
Milk (5% Milk in PBS with 0.2% Tween20)	90 mL for 4 slides at once
PBS (pH 7.4)	90 mL for 4 slides at once
Coverslips, 24 x 60 mm (VWR 48393-106)	3
Lifterslips, 24 x 65 mm (Erie 25X65I-2-5251-001-LS)	3
Pipette boxes	1
Scanner, PerkinElmer ProScanArray	

If needed, prepare antibody solutions in 5% milk/PBST:

Antigen (antibody against)	Dilution factor
GST	300
HA	1000
Mouse IgG (secondary)	500

1)**Apply primary AB** (mouse anti-GST, mouse anti-HA or mouse anti-Flag): ***Work quickly but do one slide at a time.*** Take a slide out of the milk block and tap against a paper towel to get rid of excess milk. Add 600 uL of the primary antibody and **incubate for 1 hr at RT; wash 3x 5 minutes with milk on a rocking shaker**.

2)**Apply secondary AB**: ***Work quickly but do one slide at a time.*** Take a slide out of the milk block and tap against a paper towel to get rid of excess milk. Add 600 uL of the secondary antibody and **incubate for 1 hr at RT; wash 3x 5 minutes with PBS on a rocking shaker**.

3)**Dilute the appropriate amount of 50x TSA into the diluent.** The dried TSA tube should be resuspended in 150ul DMSO and vortexed vigorously. This is now the 50x TSA. For each slide, you will need 500ul of 1x TSA (so 10ul of 50x TSA and 500ul of diluent).

4)After the last PBS wash, rinse the slides quickly with water and apply 500ul of 1x TSA. Use the lifterslip to spread the TSA across the slide. **Incubate for 10 minutes at room temperature**. Rinse in Milli-Q water; dry with filtered compressed air

5) Scan.

Supplementary Table 1. List of genes printed on array in Fig 1

GeneID	Gene Symbol	Reference GI
207	AKT1	190827
226	ALDOA	16877048
332	BIRC5	21707886
347	APOD	4502162
388	RHOB	4757763
389	RHOC	4885066
573	BAG1	1143475
578	BAK1	595923
595	CCND1	19264142
596	BCL2	179370
598	BCL2L1	17939633
638	BIK	929654
813	CALU	6005991
891	CCNB1	15082288
894	CCND2	179999
960	CD44	180129
1017	CDK2	180177
1019	CDK4	16936531
1026	CDKN1A	11386202
1027	CDKN1B	9652559
1029	CDKN2A	862412
1326	MAP3K8	31244
1440	CSF3	31693
1903	EDG3	38173856
1977	EIF4E	306486
2113	ETS1	29881
2237	FEN1	19718776
2252	FGF7	15147344
2255	FGF10	4758359
2353	FOS	6552332
2574	GAGE2	4503878
2576	GAGE4	4503882
2697	GJA1	4755136
2810	SFN	12803037
2896	GRN	12653114
3265	HRAS	4885424
3479	IGF1	32991
3486	IGFBP3	17511987

3489	IGFBP6	13097569
3490	IGFBP7	861520
3600	IL15	26787985
3606	IL18	4504652
3725	JUN	186624
3939	LDHA	5031856
3945	LDHB	14286301
4070	TACSTD2	1524102
4072	TACSTD1	181132
4102	MAGEA3	13543626
4102	MAGEA3	16877053
4105	MAGEA6	27371333
4312	MMP1	15530200
4582	MUC1	4204966
4609	MYC	12962934
4700	NDUFA6	12803858
5008	OSM	15079522
5111	PCNA	38383149
5155	PDGFB	4505680
5245	PHB	6031190
5329	PLAUR	37604
5468	PPARG	20336230
5603	MAPK13	13325217
5606	MAP2K3	21618348
5888	RAD51	285976
5915	RARB	5616236
5947	RBP1	8400726
6164	RPL34	12804692
6275	S100A4	179916
6278	S100A7	9845518
6280	S100A9	34783534
6623	SNCG	15559464
7031	TFF1	4507450
7157	TP53	339815
7163	TPD52	17390256
8031	NCOA4	16306752
8290	HIST3H3	871259
8519	IFITM1	12654158
8614	STC2	33875707
8650	NUMB	20070355
8712	PAGE1	33988857

8823	FGF16	4503690
8900	CCNA1	23271352
9077	DIRAS3	4757771
9133	CCNB2	10938017
10000	AKT3	5804885
10983	CCNI	38197480
11031	RAB31	1388194
11200	CHEK2	3982839
25803	SPDEF	18204265
79444	BIRC7	15680240
723790	HIST2H2AA4	12804444

Supplementary Table 2. List of genes printed on array in Fig 2

Current GeneID	Current symbol	Final GI
18	ABAT	15990486
50	ACO2	15559447
55	ACPP	14250149
81	ACTN4	13477150
95	ACY1	12653544
100	ADA	14043372
161	AP2A2	13544040
163	AP2B1	13623210
203	AK1	12654562
205	AK3L1	16740594
207	AKT1	190827
224	ALDH3A2	12803234
226	ALDOA	16877048
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25796	PGLS	15559292
25814	ATXN10	13960134
25873	RPL36	12804380
25913	POT1	12804138
25936	NSL1	13937915
25937	WWTR1	15559367
26003	GORASP2	14043582
26061	HACL1	12804440
26225	ARL5A	12654822
26271	FBXO5	17511910
26277	TINF2	13477144
26284	ERAL1	17512226
26291	FGF21	22450793
26499	PLEK2	12654766
26517	TIMM13	14250352
26574	AATF	12653626
26762	HAVCR1	15426446
26986	PABPC1	16358989
26999	CYFIP2	15079948
27044	SND1	16877908
27067	STAU2	14249966
27092	CACNG4	21961609
27122	DKK3	14043329
27129	HSPB7	13623438
27229	76P	15215402
27235	COQ2	14250675
27243	CHMP2A	12803364
27301	APEX2	12804202
27316	RBMX	13938568
27352	RUTBC3	14165548

28232	SLCO3A1	12653614
28511	NKIRAS2	13938596
28969	BZW2	12804386
28976	ACAD9	14044101
29079	MED4	13528773
29089	UBE2T	13278752
29097	CNIH4	12653594
29100	HSPC171	13111781
29127	RACGAP1	21595804
29763	PACSIN3	14043957
29894	CPSF1	16878040
29902	C12orf24	18088080
29940	DSE	24659665
29959	NRBP1	12654756
29967	LRP12	21594265
29970	SCHIP1	23306469
29995	LMCD1	12653722
29997	GLTSCR2	13623422
30012	TLX3	16878170
30844	EHD4	13623376
30968	STOML2	12803254
50615	IL21R	13279298
50814	NSDHL	12652968
50853	VILL	12652964
50855	PARD6A	15990483
51004	COQ6	15559648
51006	SLC35C2	18089293
51010	EXOSC3	12803246
51067	YARS2	15990481
51121	RPL26L1	16878335
51130	ASB3	13623716
51131	PHF11	16877992
51132	RNF12	15426503
51155	HN1	12655134
51177	PLEKHO1	14603393
51192	CKLF	13325133
51207	DUSP13	14602534
51226	COPZ2	16198486
51268	PIPOX	14286317
51271	UBAP1	18088060
51284	TLR7	21708074

51361	HOOK1	15079604
51372	CCDC72	12654536
51393	TRPV2	17511937
51400	PPME1	12804370
51429	SNX9	13477130
51447	IHPK2	18043110
51465	UBE2J1	15559236
51477	ISYNA1	17511981
51501	C11orf73	12804532
51504	HSPC152	16877889
51506	UFC1	13528770
51510	CHMP5	13937758
51512	GTSE1	13623450
51522	TMEM14C	12803354
51523	CXXC5	12803342
51534	VTA1	13937779
51538	ZCCHC17	13938588
51540	SCLY	14043900
51547	SIRT7	16878202
51566	ARMCX3	13528785
51616	TAF9B	16306982
51645	PPIL1	12804374
51657	STYXL1	33869206
51678	MPP6	33879924
51684	SUFU	15342037
51703	ACSL5	14124925
51728	POLR3K	15080354
51808	RNUXA	18089303
53371	NUP54	15214834
53405	CLIC5	23273551
53822	FXYD7	17391356
53831	GPR84	18089044
54205	CYCS	13529022
54328	GPR173	14602675
54407	SLC38A2	25955654
54472	TOLLIP	17390640
54606	DDX56	12654784
54738	FEV	33871725
54852	PAQR5	24657740
54971	BANP	14495633
55034	MOCOS	15082341

55038	CDCA4	15079878
55041	PLEKHB2	12655146
55081	IFT57	15080266
55146	ZDHHC4	12654792
55211	DPPA4	23271562
55269	PSPC1	15559655
55326	AGPAT5	33879276
55336	FBXL8	15680141
55486	PARL	15559381
55559	UCHL5IP	14198167
55577	NAGK	12654406
55662	HIF1AN	14043455
55760	DHX32	12803310
55768	NGLY1	13938210
55802	DCP1A	13938576
55846	ITFG2	16306861
55863	TMEM126B	15082318
55929	DMAP1	12804006
55968	NSFL1C	12803908
56159	TEX11	23271197
56172	ANKH	15778895
56256	SERTAD4	15082345
56606	SLC2A9	17511905
56616	DIABLO	15080296
56670	SUCNR1	21410927
56848	SPHK2	13544054
56852	RAD18	12654912
56897	WRNIP1	17511929
56903	PAPOLB	22477166
56923	NMUR2	16877376
56940	DUSP22	33872098
56941	C3orf37	14603027
56944	OLFML3	14602834
56949	XAB2	13938178
56993	TOMM22	14424694
57003	CCDC47	14286217
57007	CXCR7	22477172
57048	PLSCR3	15079875
57062	DDX24	14250755
57085	AGTRAP	16878259
57095	C1orf128	16877980

57109	REXO4	14424508
57147	SCYL3	15779206
57175	CORO1B	13623648
57215	THAP11	15082546
57325	CSRP2BP	14043102
57338	JPH3	22328099
57549	IGSF9	33879604
57617	VPS18	16306677
57801	HES4	15214448
57819	LSM2	14327935
58505	DC2	16740922
59307	SIGIRR	13097794
59338	PLEKHA1	12654600
59348	ZNF350	14602837
60370	AVPI1	12654124
60386	SLC25A19	12654490
60436	TGIF2	33870164
60491	NIF3L1	14043316
60678	EEFSEC	14044000
60681	FKBP10	16741259
63943	FKBPL	15080434
64093	SMOC1	14250354
64172	OSGEPL1	15080281
64231	MS4A6A	18605535
64422	ATG3	33872933
64518	TEKT3	21618791
64710	NUCKS1	12654010
64754	SMYD3	21410973
64805	P2RY12	17389766
64840	PORCN	17512192
64849	SLC13A3	23243409
64969	MRPS5	15559627
65108	MARCKSL1	14043933
65987	KCTD14	12654468
78988	MRP63	23273804
79058	ASPSCR1	17511731
79070	KDELC1	12654902
79074	C2orf49	12654926
79075	DCC1	12654936
79080	CCDC86	12655056
79102	RNF26	14043098

79147	FKRP	12803560
79412	KREMEN2	13097629
79414	LRFN3	13097761
79444	BIRC7	15680240
79603	LASS4	14602620
79663	HSPBAP1	15080263
79666	PLEKHF2	15080048
79706	PRKRIP1	15679979
79723	SUV39H2	14043540
79727	LIN28	33872076
79796	ALG9	14328091
79814	AGMAT	13477244
79817	MOBKL2B	21542523
79934	ADCK4	20071711
80011	NIP30	18088224
80142	PTGES2	15079582
80146	UXS1	14602606
80218	NAT13	15215283
80306	MED28	15080366
81285	OR51E2	18088468
81472	OR2C3	21315036
81488	GRINL1A	16306672
81533	ITFG1	18848294
81579	PLA2G12A	16878004
81607	PVRL4	14714573
81621	KAZALD1	14043549
81631	MAP1LC3B	17391392
81786	TRIM7	15079462
83442	SH3BGRL3	33879575
83463	MXD3	12653904
83540	NUF2	14250143
83640	FAM103A1	13177690
83743	GRWD1	12803252
83746	L3MBTL2	16877934
83755	KRTAP4-12	13278824
83758	RBP5	20810176
83939	EIF2A	15080228
83998	REG4	16877696
84056	KATNAL1	12653658
84153	RNASEH2C	33874513
84419	C15orf48	18088945

84517	ARPM1	13938318
84522	JAGN1	33874712
84527	ZNF559	13623632
84690	SPATA22	20810259
84725	PLEKHA8	12803978
84749	USP30	13436088
84795	C10orf33	13543987
84811	BUD13	13623490
84812	PLCD4	13623500
84824	FCRLA	13676371
84838	ZNF496	13938273
84859	LRCH3	13960125
84932	RAB2B	18088785
84957	RELT	16878143
84958	SYTL1	23274197
84962	JUB	14043184
89884	LHX4	15079939
90780	PYGO2	33991480
90952	ESAM	16877212
90993	CREB3L1	15559461
91039	DPP9	33991522
91304	C19orf6	14286311
91603	CCDC16	15079508
92283	ZNF461	20306350
92745	SLC38A5	17512591
92856	IMP4	14603152
92922	CCDC102A	14249826
93107	KCNG4	14286334
93627	MGC16169	14327966
94031	HTRA3	23273037
94240	EPSTI1	33878618
112464	PRKCDBP	15079511
112479	EXOD1	14714720
112942	CCDC104	14603077
112950	MED8	14714788
113130	CDCA5	15012189
113878	DTX2	14250773
114897	C1QTNF1	18204860
114898	C1QTNF2	15079785
115330	GPR146	15559780
116448	OLIG1	23271032

117246	FTSJ3	23331071
118425	GDEP	18088729
118426	LOH12CR1	15489113
122011	CSNK1A1L	20380987
122769	PPIL5	33988785
124540	MSI2	16306701
126321	C19orf28	33991499
127435	PODN	22418048
131566	DCBLD2	20988614
138065	RNF183	15991881
138151	BTBD14A	15990514
140576	S100A16	14714784
140578	CHODL	14495622
140625	ACTRT2	20809587
140767	NRSN1	33871592
142679	DUSP19	23273914
143684	FAM76B	19683969
143689	PIWIL4	21410560
146395	GSG1L	19116249
150590	C2orf15	18204634
154791	C7orf55	14250109
159163	RBMY1F	20987384
170384	FUT11	23271235
192286	HIGD2A	12653618
196383	MGC7036	15278388
199720	GGN	23271255
200420	LOC200420	15680270
203260	CCDC107	17511815
220202	ATOH7	21618609
220965	FAM13C1	22209064
221079	ARL5B	18848192
222662	LHFPL5	20306955
246243	RNASEH1	12804228
283748	PLA2G4D	21961640
387032	ZKSCAN4	15559335
548596	CKMT1A	12804946
653145	ANXA8	13325125
654817	NCF1C	12803938
723790	HIST2H2AA4	12804444
751867	SNHG3-RCC1	13938340

Supplementary Table 3. List of genes printed on array in Fig 3

GeneID	Gene Symbol	Reference GI
33	ACADL	24660233
55	ACPP	14250149
58	ACTA1	15214922
60	ACTB	12654910
72	ACTG2	15214974
117	ADCYAP1R1	34398688
128	ADH5	15779215
146	ADRA1D	148277040
148	ADRA1A	111118991
151	ADRA2B	110227861
175	AGA	15214538
186	AGTR2	6715584
203	AK1	4502010
207	AKT1	190827
215	ABCD1	15930220
221	ALDH3B1	15488910
226	ALDOA	16877048
279	AMY2A	13937900
293	SLC25A6	15928607
302	ANXA2	16306977
308	ANXA5	33876205
311	ANXA11	14043152
322	APBB1	14790012
332	BIRC5	21707886
347	APOD	4502162
354	KLK3	22208990
355	FAS	15214691
359	AQP2	27769001
360	AQP3	9257193
362	AQP5	21432082
366	AQP9	11038652
388	RHOB	4757763
389	RHOC	13938242
389	RHOC	4885066
392	ARHGAP1	17390259
397	ARHGDIB	14327951
432	ASGR1	33879712
496	ATP4B	20809654

573	BAG1	1143475
577	BAI3	4502358
578	BAK1	595923
595	CCND1	19264142
596	BCL2	179370
596	BCL2	20072667
597	BCL2A1	16740835
598	BCL2L1	17939633
627	BDNF	20987591
638	BIK	929654
694	BTG1	33869459
771	CA12	18645128
805	CALM2	17391485
813	CALU	6005991
831	CAST	15488897
873	CBR1	33991545
874	CBR3	33990900
881	CCIN	17512603
891	CCNB1	15082288
894	CCND2	179999
901	CCNG2	21619119
931	MS4A1	12803920
947	CD34	24657612
960	CD44	180129
966	CD59	37589019
967	CD63	33876593
969	CD69	13937862
977	CD151	33870216
997	CDC34	17390317
1017	CDK2	180177
1019	CDK4	16936531
1026	CDKN1A	11386202
1027	CDKN1B	9652559
1029	CDKN2A	862412
1048	CEACAM5	21961633
1054	CEBPG	14043188
1066	CES1	15214584
1068	CETN1	20809601
1102	RCBTB2	20810514
1131	CHRM3	54792120
1132	CHRM4	52426747

1140	CHRNB1	15030221
1155	TBCB	13543641
1191	CLU	14714740
1192	CLIC1	14251208
1234	CCR5	154091329
1237	CCR8	13929430
1269	CNR2	4502928
1317	SLC31A1	15488971
1326	MAP3K8	31244
1327	COX4I1	14250513
1329	COX5B	38197026
1440	CSF3	31693
1460	CSNK2B	21428316
1466	CSRP2	12803035
1471	CST3	15341821
1490	CTGF	37622417
1497	CTNS	4826681
1511	CTSG	4503148
1537	CYC1	18088252
1638	DCT	20271406
1791	DNTT	15277812
1841	DTYMK	38114725
1854	DUT	21708113
1890	ECGF1	17390354
1901	EDG1	17391431
1903	EDG3	38173856
1906	EDN1	16307263
1910	EDNRB	4557546
1915	EEF1A1	16307286
1977	EIF4E	306486
2023	ENO1	15029813
2029	ENSA	13325294
2040	STOM	38016910
2113	ETS1	29881
2114	ETS2	20127471
2117	ETV3	18605788
2123	EVI2A	23272679
2146	EZH2	14790028
2150	F2RL1	17390291
2152	F3	15029642
2168	FABP1	21618453

2170	FABP3	13937836
2173	FABP7	15126753
2182	ACSL4	23273826
2209	FCGR1A	21619685
2237	FEN1	19718776
2252	FGF7	15147344
2255	FGF10	4758359
2286	FKBP2	13097251
2319	FLOT2	16878172
2348	FOLR1	33877393
2353	FOS	33872858
2353	FOS	6552332
2354	FOSB	5803016
2357	FPR1	36951095
2358	FPRL1	54112386
2444	FRK	15277743
2543	GAGE1	23273582
2550	GABBR1	10835014
2553	GABPB2	23273576
2565	GABRG1	21411385
2574	GAGE2	4503878
2576	GAGE4	4503882
2669	GEM	18314424
2676	GFRA3	23274188
2692	GHRHR	58530850
2693	GHSR	4758433
2697	GJA1	4755136
2705	GJB1	12803916
2706	GJB2	6980947
2764	GMFB	31795542
2791	GNG11	16307238
2810	SFN	12803037
2827	GPR3	21618432
2828	GPR4	4885334
2846	GPR23	4885310
2848	GPR25	38194223
2852	GPER	91106388
2865	FFAR3	23272748
2867	FFAR2	4885332
2896	GRN	12653114
2922	GRP	4504158

2968	GTF2H4	13436277
2987	GUK1	1513314
3005	H1F0	12652786
3026	HABP2	21618648
3043	HBB	13937928
3046	HBE1	15930211
3104	ZBTB48	15488885
3142	HLX	13938328
3156	HMGCR	21707181
3162	HMOX1	4504436
3174	HNF4G	33096751
3200	HOXA3	15929499
3202	HOXA5	9506790
3265	HRAS	4885424
3269	HRH1	149158707
3280	HES1	8400709
3337	DNAJB1	38197192
3351	HTR1B	4504532
3357	HTR2B	141801728
3397	ID1	15214588
3479	IGF1	32991
3486	IGFBP3	17511987
3489	IGFBP6	13097569
3490	IGFBP7	861520
3543	IGLL1	15126737
3569	IL6	15930148
3570	IL6R	4504672
3577	IL8RA	4504680
3579	IL8RB	29171680
3586	IL10	10835140
3598	IL13RA2	34783375
3600	IL15	26787985
3606	IL18	4504652
3624	INHBA	14043814
3705	ITPK1	17390428
3725	JUN	186624
3753	KCNE1	4557686
3903	LAIR1	20379722
3906	LALBA	4504946
3934	LCN2	5031852
3939	LDHA	5031856

3945	LDHB	14286301
3957	LGALS2	37590555
3958	LGALS3	1196441
4007	LMO6	16877180
4049	LTA	21961576
4056	LTC4S	20809585
4068	SH2D1A	18088433
4070	TACSTD2	1524102
4072	TACSTD1	181132
4084	MXD1	4505068
4102	MAGEA3	13543626
4102	MAGEA3	16877053
4105	MAGEA6	27371333
4111	MAGEA12	13097311
4115	MAGEB4	29171715
4151	MB	15778932
4158	MC2R	66346710
4160	MC4R	119508432
4176	MCM7	15426527
4184	SMCP	15779037
4312	MMP1	15530200
4357	MPST	16876912
4507	MTAP	6006025
4582	MUC1	4204966
4602	MYB	4028593
4605	MYBL2	14043193
4609	MYC	12962934
4633	MYL2	21411328
4675	NAP1L3	23270931
4700	NDUFA6	12803858
4705	NDUFA10	13097332
4709	NDUFB3	17390401
4733	DRG1	4758795
4792	NFKBIA	10092618
4801	NFYB	13529070
4879	NPPB	19343959
4887	NPY2R	27552771
4888	NPY6R	5453797
4987	OPRL1	23468340
5008	OSM	15079522
5010	CLDN11	15488893

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5080	PAX6	15080396
5105	PCK1	18645160
5111	PCNA	38383149
5128	PCTK2	21542570
5155	PDGFB	4505680
5193	PEX12	22658424
5245	PHB	6031190
5264	PHYH	20809598
5266	PI3	4505786
5320	PLA2G2A	13543520
5327	PLAT	13938220
5329	PLAUR	37604
5336	PLCG2	14043154
5341	PLEK	17391305
5345	SERPINF2	21594845
5375	PMP2	23271223
5423	POLB	4505930
5439	POLR2J	18848195
5441	POLR2L	13543491
5468	PPARG	20336230
5500	PPP1CB	33877033
5540	PPYR1	57527791
5549	PRELP	21618472
5552	SRGN	15930160
5553	PRG2	13543541
5603	MAPK13	13325217
5606	MAP2K3	21618348
5606	MAP2K3	21619100
5608	MAP2K6	15080539
5621	PRNP	34335269
5624	PROC	21707770
5671	PSG3	13543532
5682	PSMA1	15929868
5696	PSMB8	4758969
5720	PSME1	33988241
5721	PSME2	4506236
5732	PTGER2	31881629
5739	PTGIR	39995095
5763	PTMS	16877543

5805	PTS	16307195
5817	PVR	15930222
5834	PYGB	16877585
5869	RAB5B	4506370
5872	RAB13	4506362
5873	RAB27A	34485705
5884	RAD17	4506382
5888	RAD51	285976
5915	RARB	5616236
5919	RARRES2	8051632
5920	RARRES3	16307177
5947	RBP1	8400726
5987	TRIM27	15488900
5992	RFX4	21040408
5997	RGS2	13937881
6000	RGS7	18314629
6013	RLN1	13543608
6046	BRD2	39645316
6049	RNF6	34193514
6097	RORC	21594879
6134	RPL10	20070800
6137	RPL13	15341811
6141	RPL18	16307236
6146	RPL22	48255919
6164	RPL34	12804692
6165	RPL35A	38541168
6181	RPLP2	38383132
6208	RPS14	13097293
6231	RPS26	12803548
6242	RTKN	17389369
6274	S100A3	15277621
6275	S100A4	179916
6278	S100A7	9845518
6280	S100A9	34783534
6288	SAA1	40316911
6291	SAA4	10835094
6344	SCTR	114205382
6376	CX3CL1	4506856
6418	SET	4506890
6441	SFTPD	34766
6449	SGTA	12804260

6456	SH3GL2	4506930
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6522	SLC4A2	14495651
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6734	SRPR	15488907
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27306	PGDS	7657456

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64231	MS4A6A	18605535
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79290	OR13A1	51468040
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120775	OR2D3	51470918
123887	ZG16	20810120
125875	CLDND2	20810284
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127066	OR5BF1	50054469
127069	OR2T10	17437031
127074	OR2T4	17437058
128367	OR10X1	51458906
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129563	DIS3L2	23271316
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134864	TAAR1	21264323
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151306	GPBAR1	116284380
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155368	WBSCR27	34783307
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344561	GPR148	46409359
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387748	OR56B1	21928889
389090	OR6B2	21928600
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