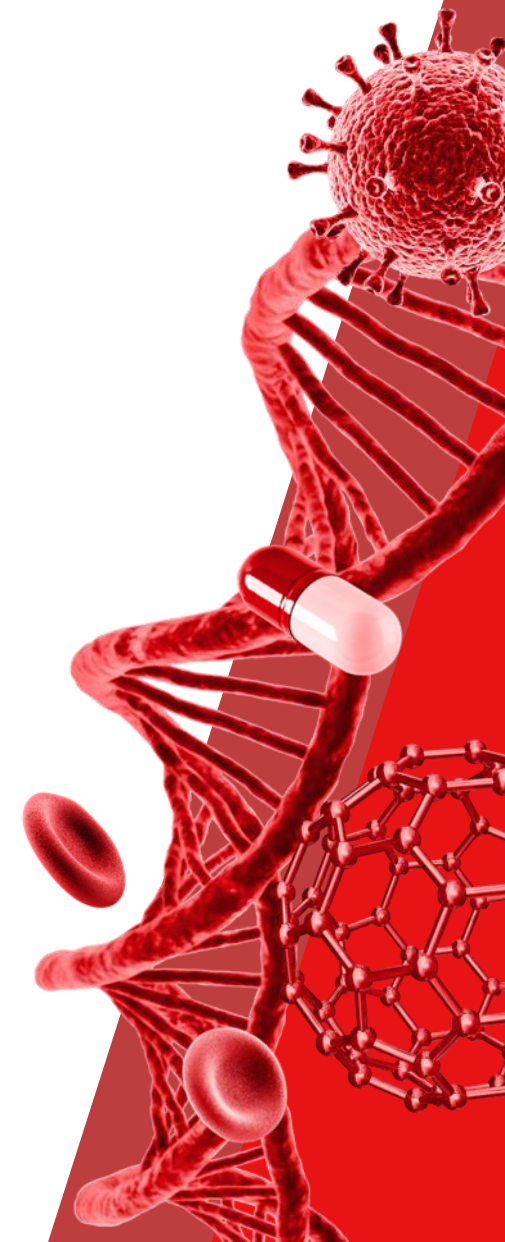


マイクロアレイによる網羅的遺伝子発現解析

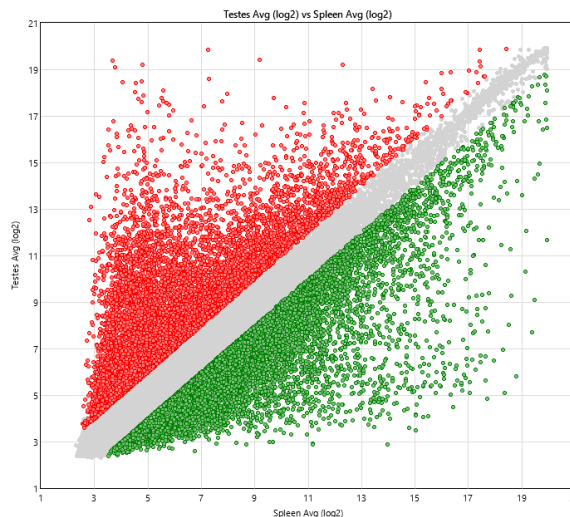
サーモフィッシャーサイエンティフィック
ライフテクノロジーズジャパン株式会社
テクニカルサポート

 The world leader in serving science



- **マイクロアレイ技術の概要 ～ マイクロアレイ解析とは**
- 遺伝子発現解析用ツールの紹介と使用例について
- データ解析方法について

マイクロアレイ技術による発現解析



網羅的に遺伝子の発現量の増減を検出

- ⇒多数の遺伝子の発現量を比較したい
- ⇒ある生命現象に関わる遺伝子群の候補を見つけたい
- ⇒全遺伝子の発現プロファイルを調べたい

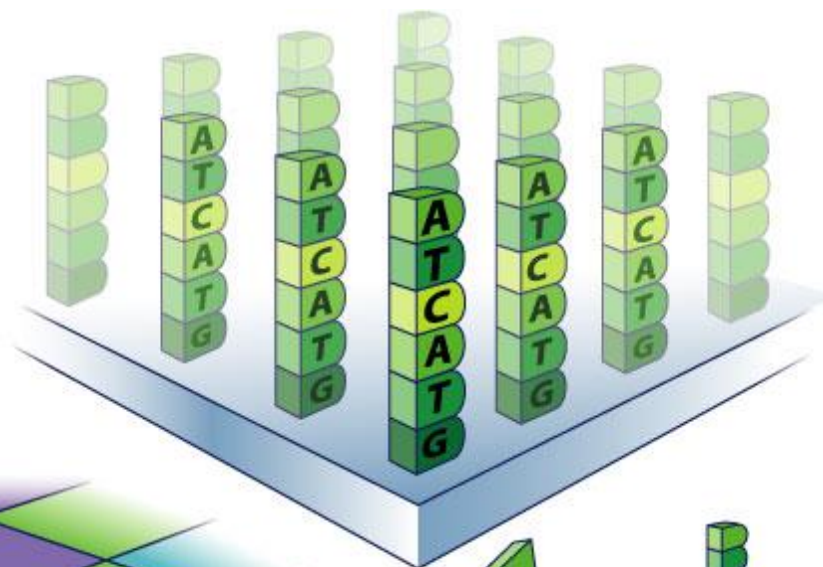
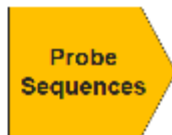
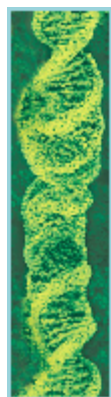


スクリーニングツールとして利用して遺伝子を絞り込んでいく

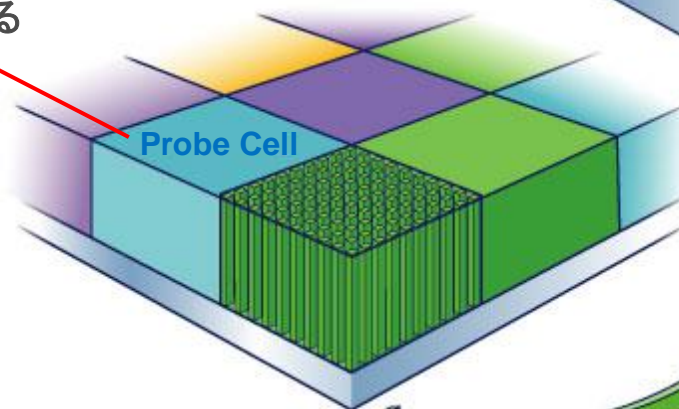
マイクロアレイ (GeneChip Array) の構造

フォトリソグラフィによる製造

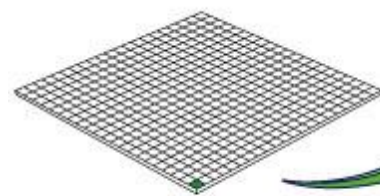
公共データベース
の配列情報等



オリゴヌクレオチドプローブが
 $10^5 \sim 10^6$ コピー配置されている

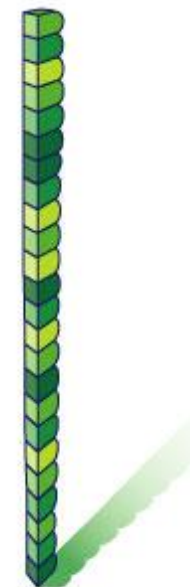


1.28cm



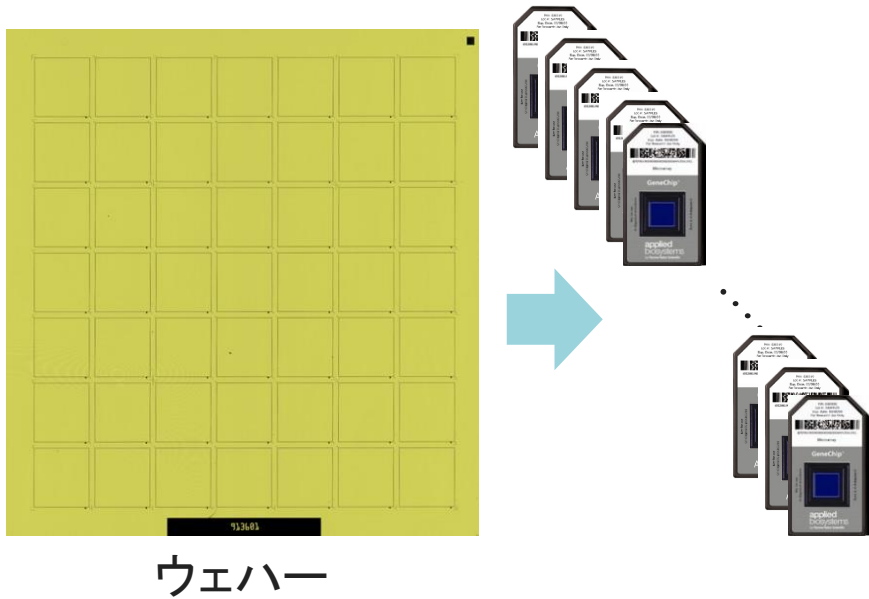
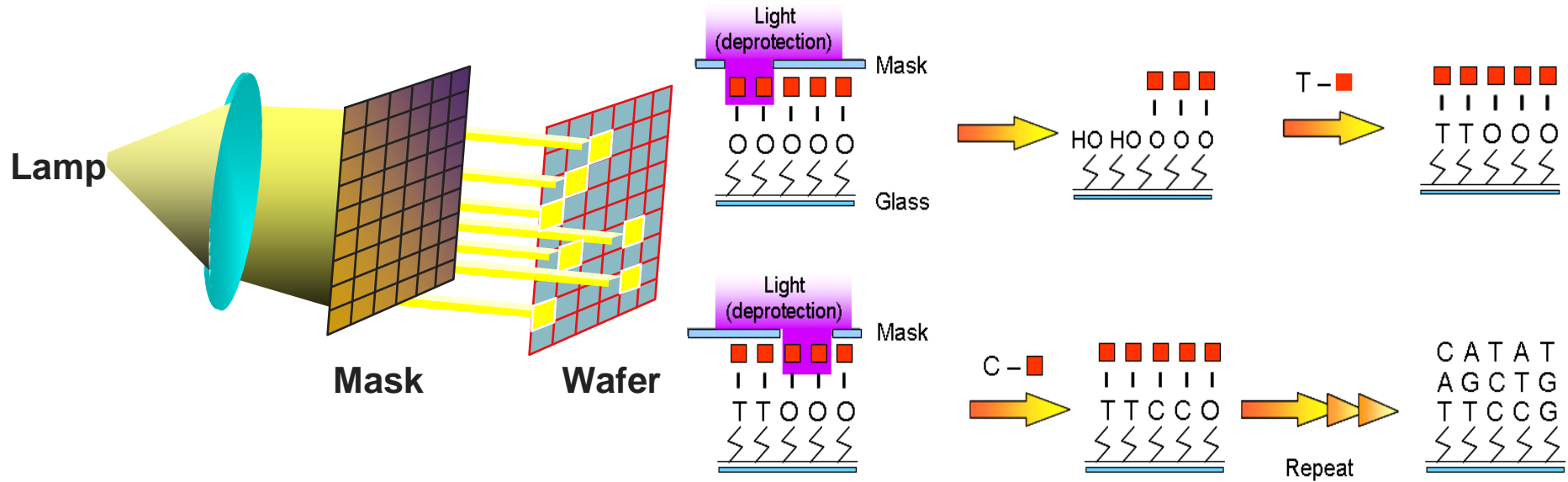
600,000 cells on each GeneChip™ array

Millions of DNA strands built up in each cell

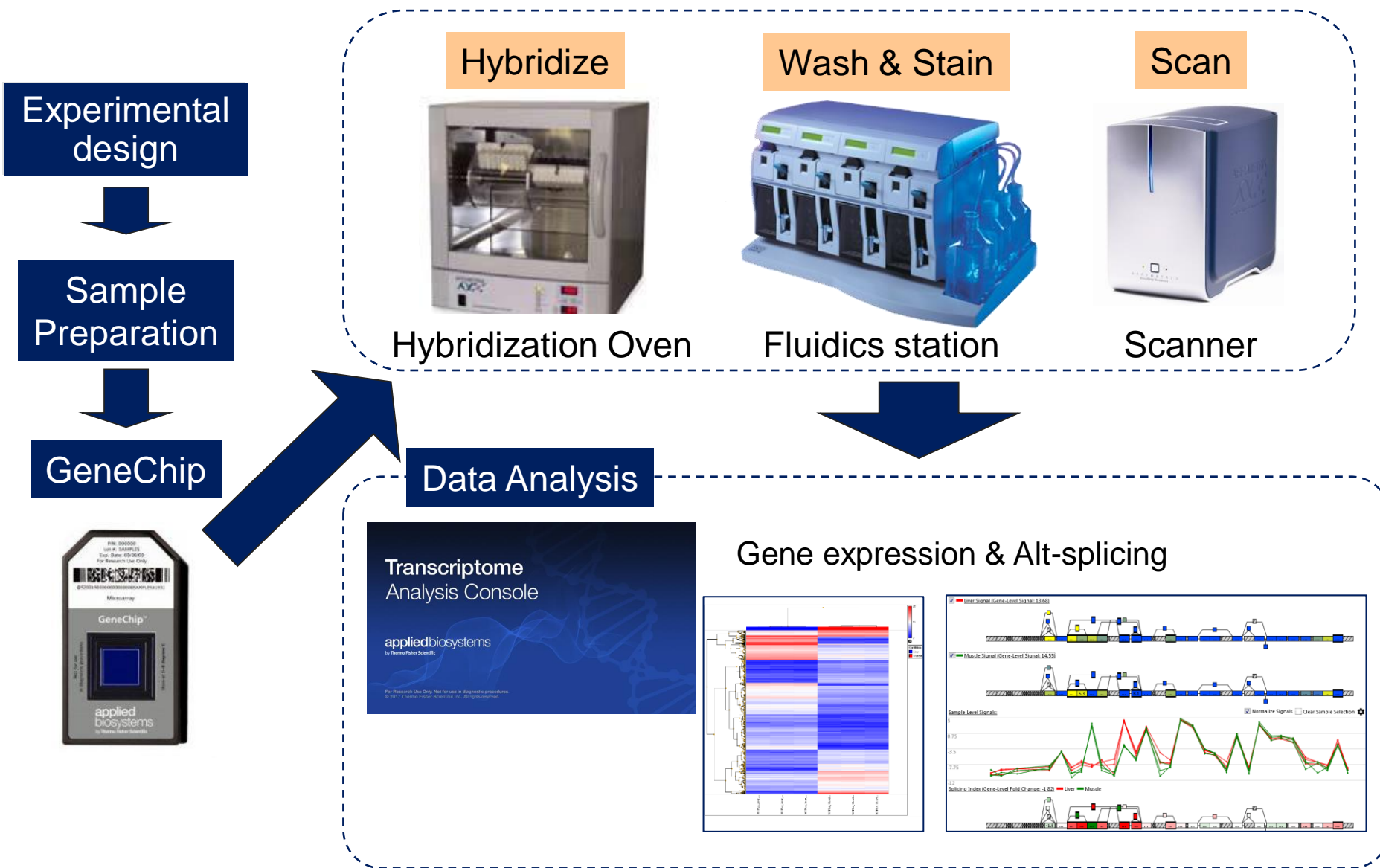


Actual strand = 25 base pairs

マイクロアレイ製造工程 (フォトリソグラフィによる製造)



マイクロアレイを用いた実験の流れ



- マイクロアレイ技術の概要 ～ マイクロアレイ解析とは
- 遺伝子発現解析用ツールの紹介と使用例について
- データ解析方法について

遺伝子発現解析アレイのラインナップ

Type	Array	発売年	特徴1	特徴2
3' IVT	3' IVT	2003	<ul style="list-style-type: none"> 多くの文献報告があり、データベース登録されている Gene-level解析用 	<ul style="list-style-type: none"> ヒト・マウス・ラット以外の生物種にも対応
WT	Exon ST	2004	<ul style="list-style-type: none"> Gene & Exon-level解析用 	<ul style="list-style-type: none"> Exon予測した領域もプローブをデザイン
WT	Gene ST	1.0:2006 2.0:2012	<ul style="list-style-type: none"> Exon ST の簡易版 2.0でlncRNA用のプローブを搭載 Gene-level解析用 	<ul style="list-style-type: none"> ヒト・マウス・ラット以外の生物種にも対応 一部のArrayはlncRNA解析にも対応
WT	HTA2.0 MTA1.0 RTA1.0	2013	<ul style="list-style-type: none"> Exon-Exonのjunction部分にプローブをデザイン Gene & Exon-level解析用 	<ul style="list-style-type: none"> Alternative splicing解析に適する lncRNA解析も可能
WT	Clariom™ D	2016	<ul style="list-style-type: none"> HTA2.0の進化系 MTA、RTAは製品名変更のみ Gene & Exon-level解析用 	<ul style="list-style-type: none"> デザインに使用したデータベースが新しい Alternative splicing解析に適する lncRNA解析も可能
WT	Clariom™ S	2016	<ul style="list-style-type: none"> Clariom Dの簡易版 Gene-level解析用 	<ul style="list-style-type: none"> デザインに使用したデータベースが新しい 安価

過去に取得したデータと比較解析が必要な場合

新規実験におすすめ

次世代型マイクロアレイ～Clariom Array～

トランスクリプトームレベルの発現解析アレイ

Clariom™ D Array

- 600万を超えるプローブを搭載。マイクロアレイ最高峰の性能を実現
- 遺伝子発現はもちろん、選択的スプライシングによるバリエーション解析や、低発現の non-coding RNA 解析にも対応
- 7億リードの RNA-Seq に匹敵する高精度な解析を達成

遺伝子レベルの発現解析アレイ

Clariom™ S Array

- 遺伝子発現解析に特化することで驚異的な低価格を実現
- アノテーション済みの代表的な 2 万以上の遺伝子が解析可能
- 1000万～1億リードの RNA-Seq 相当の発現解析性能

用途に応じて 2 種類のフォーマットが選択可能



- 既知の転写産物の網羅的な発現解析を行いたい
- Long non-coding RNA の発現や選択的スプライシングの違いも見たい

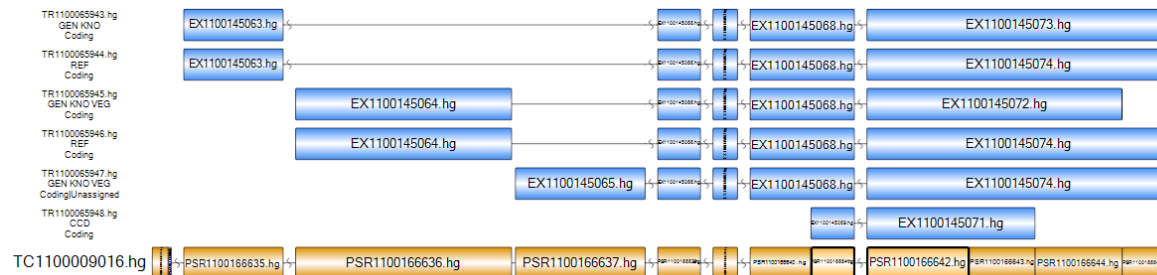
- コストを抑えて既知の遺伝子のみの発現を解析したい
- Long non-coding RNA まで解析対象ではない

Content summary	Human
Genes	>134,700
Transcripts	>542,500
Exons	>948,300
Exon-exon splice junctions	>484,900
Total probes	>6,765,500
Probes targeting exons	>4,781,200
Probes targeting exon-exon splice junctions	>1,984,300

Content summary	Human
Genes	>20,800
Transcripts	>337,100
Total probes	>211,300
Probes targeting genes	>205,800

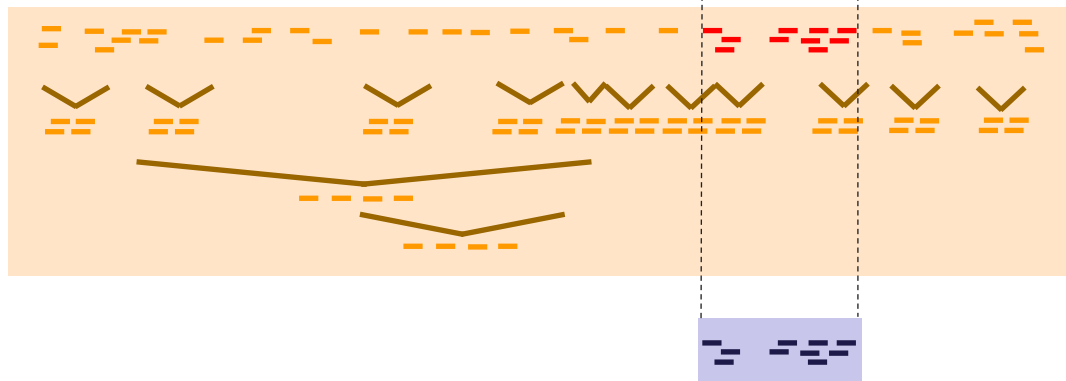
Clariom Array シリーズは、ヒト・マウス・ラットの解析に対応！

アレイデザインの比較



Example: COCLA2
Colorectal Cancer Associated 2 Gene

Clariom Array 転写産物全領域に渡って特異的な
プローブを複数デザイン



Clariom D

全転写領域に渡ってエクソンごとにプローブ
をデザイン
エクソンジャンクション部にもプローブがある
ため、選択的スプライシングの解析が可能

Clariom S

転写領域の重なりが最も
多い部分をClariom Dの
プローブの中から選択。

これまでのMicroarray

3'末端にプローブを配置しているため、解析できない
転写産物アイソフォームもある



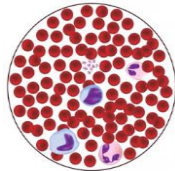
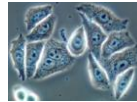
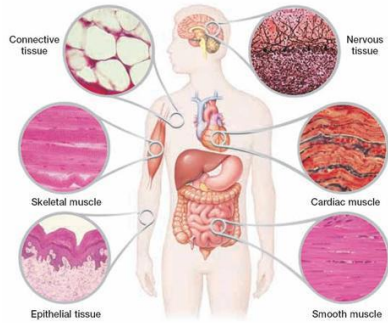
3' IVT

3'末端側より600ntの範囲で特異的
なプローブを複数デザイン



一般的な
マイクロアレイ

Assay kits for sample preparation



WT assay kit	Target	RNA amount
GeneChip® WT PLUS Reagent Kit	FF	50-500 ng
GeneChip® WT Pico Reagent Kit GeneChip® Pico Reagent Kit	微量RNA FFPE	100 pg -10 ng 500 pg -50 ng

- total RNAから事前にrRNAやGlobin mRNAの除去を行う必要はない
⇒ Plus、Pico
- 100 pgのtotal RNAからアッセイが可能
⇒ Pico
- 核酸の分解が進行しているFFPE由来サンプルでもアッセイが可能
⇒ Pico

WT Pico Kit assay workflow

Incubation Time

Day 1
First-Strand cDNA Synthesis

1 hr 5 min

Clean-up Excess Primers

40 min

3' Adaptor Synthesis

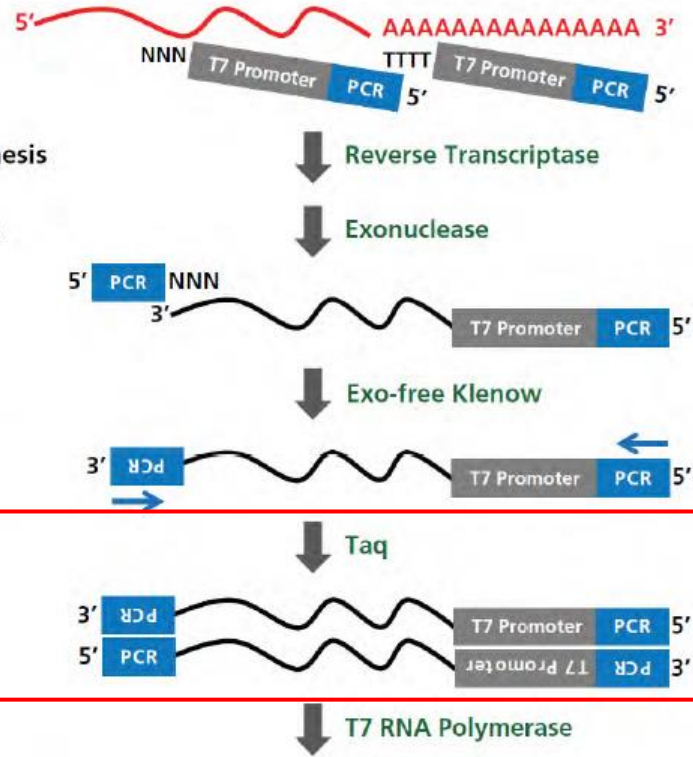
40 min

Pre-IVT Amplification

< 1.5 hr

cRNA Amplification

14 hr



Day 2
cRNA Purification & Quantitation

2nd-Cycle ss-cDNA Synthesis

2 hr

Template RNA Removal

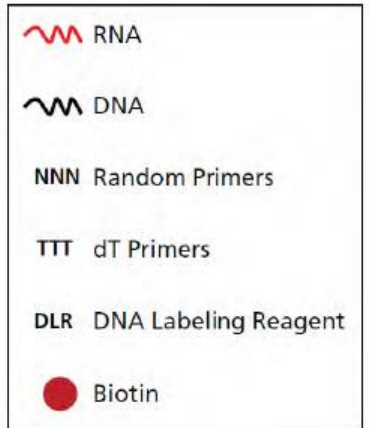
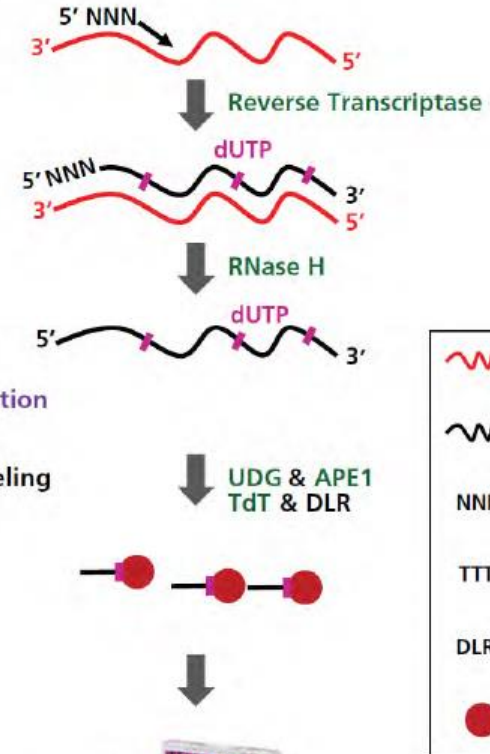
50 min

ss-cDNA Purification & Quantification

Fragmentation and Terminal Labeling

1 hr

Hybridization to WT Array



Software: Transcriptome Analysis Console (TAC)



Comparison: KCHNP2 vs non_target

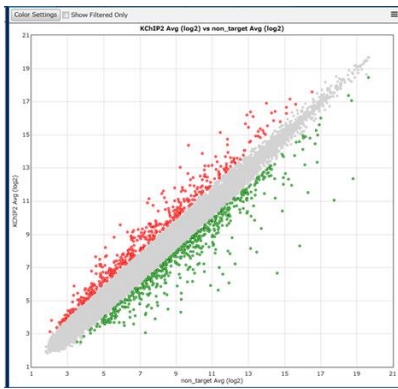
ID	KCHNP2 Avg (log2)	non_target Avg (log2)	Fold Change	P-val	FDR P-val	Gene Symbol	Description	Group
TC1400001510	6.47	14.6	-243.44	7.12E-12	1.01E-07	Ccrl8	chemokine (C-X-C motif)	Coding
TC1030000275	12.25	18.8	-47.26	9.04E-12	1.01E-07	Lipocalin 2	lipocalin 2	Coding
TC1030000206	6.09	11.95	-16.49	1.20E-11	1.01E-07	Grsf5	chemokine (C-C motif)	Coding
TC1030000208	5.94	10.89	-30.91	4.08E-11	2.27E-07	Pipen	prostaglandin-E synthase	Coding
TC1100000591	7.99	13.44	-45.05	4.89E-11	2.27E-07	Serpina2	serpin peptidase inhibitor	Coding
TC1400001511	7.23	12.25	-29.26	2.49E-10	9.61E-07	Ccrl1	chemokine (C-X-C motif)	Coding
TC1040001136	11.52	16.28	-27.95	3.58E-10	1.19E-06	Hsp90	regenerating islet-derived	Coding
TC1020001076	1.63	11.22	-12.41	6.10E-10	1.77E-06	C2-CD9	complement component	Coding
TC1010000294	8.31	15.83	-184.01	9.39E-10	2.24E-06	Hmx	homeobox	Coding
TC1080000402	13.14	11.44	12.96	8.86E-10	2.24E-06	Mmp12	matrix metalloproteinase	Coding
TC1400001304	7.84	11.98	-23.62	1.12E-09	2.24E-06	Ccrl1	chemokine (C-X-C motif)	Coding
TC1100000301	7.02	11.38	-20.58	1.14E-09	2.24E-06	Chn1	chitinase 3-like 1 (cartila	Coding
TC1040000001	8.36	13.71	-20.38	1.40E-09	2.20E-06	Iti5	interleukin 5	Coding
TC1020001026	14.38	16.01	-18.22	1.80E-09	2.88E-06	Car3	carbonic anhydrase 3	Coding
TC1020001466	10.41	14.87	-22.08	1.93E-09	2.89E-06	Shp2	secreted phosphatase-related	Coding
TC1400000252	13.96	16.81	-18.95	2.63E-09	2.81E-06	Ccrl10	chemokine (C-X-C motif)	Coding
TC1010000658	3.91	7.82	-15.1	3.33E-09	4.54E-06	Iti11	interleukin 11	Coding
TC1020001401	4.89	7.94	-8.29	3.69E-09	4.75E-06	Serpina1	serpin peptidase inhibitor	Coding
TC1010001714	31.87	18.45	18.72	3.95E-09	4.82E-06	Iti2	interleukin 2	Coding
TC1030004717	4.1	9.25	-30.95	4.20E-09	4.87E-06	Shp	secretory leukocyte peptid	Coding
TC140001127	13.04	9.21	18.56	4.48E-09	4.19E-06	Serp1	secreted phosphatase-related	Coding
TC1010001341	8.15	11.1	-18.91	5.11E-09	5.10E-06	Iti9p1	interleukin 9	Coding
TC1500010260	6.18	8.89	-11.11	6.11E-09	6.16E-06	Hsp1	immunoresponsive gene 1	Coding
TC1040001066	4.14	7.1	-8.9	6.48E-09	6.62E-06	Hes6	heparan sulfate proteoglycan	Coding
TC1020002456	4.5	8.54	-16.4	7.78E-09	7.95E-06	Pipenr	prostaglandin-E receptor	Coding
TC1080002466	11.61	8.28	30.85	8.17E-09	7.95E-06	Iti1	immunoglobulin superfa	Coding
TC100001127	13.21	16.18	-7.96	8.21E-09	7.95E-06	G0T7	chemokine (C-C motif)	Coding
TC1090001001	8.01	11.85	-14.97	8.68E-09	7.19E-06	Col20	chemokine (C-C motif)	Coding
TC1020001011	88.4	13.11	8.77	1.17E-08	9.34E-06	CD	CDKN2D/INK4 cellular cycl	Coding
TC1120001365	11.97	13.38	-10.27	1.19E-08	9.41E-06	Chad1	D-5-epigallocatechin gall	Coding

Primary Analysis

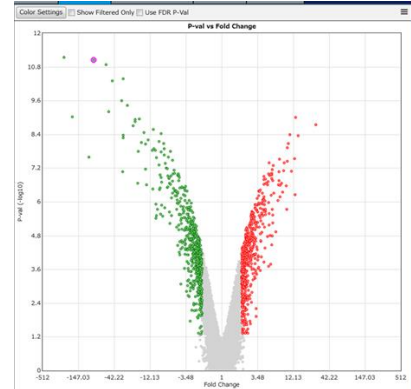
- ・プローブセットのシグナル強度
- ・データQCの確認

Secondary Analysis

- ・遺伝子発現変動の検出
- ・選択的スプライシング解析



Scatter Plot



Volcano Plot

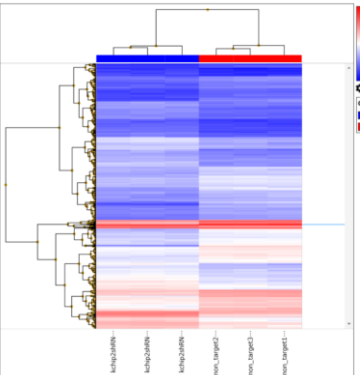
PC
スペック

Recommended

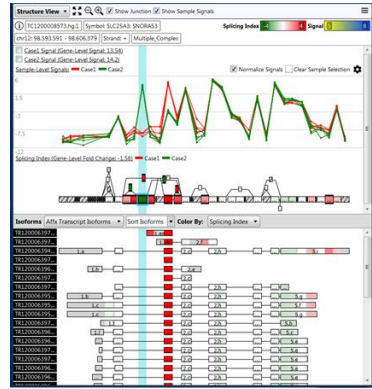
Operating System	CPU	Memory (RAM)	Hard Drive Space	Browser
Microsoft Windows® 11 (64 bit) Professional	Intel Pentium 4X 2.83 GHz (Quad Core processor)	16 GB	150 GB HD + Data storage	Microsoft Edge

Minimum

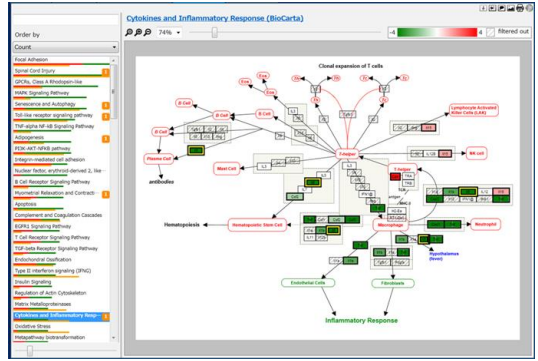
Operating System	CPU	Memory (RAM)	Hard Drive Space	Browser
Microsoft Windows® 10 (64 bit) Professional	Intel Pentium 4X 2.83 GHz (Quad Core processor)	8 GB	150 GB HD + Data storage	Microsoft Edge



Hierarchical Clustering



Alternative Splicing



Pathways

RNA-Seq and Expression Arrays: Selection Guidelines for Genome-Wide Expression Profiling

	<i>Value</i>	<i>Pros</i>	<i>Cons</i>
<i>Arrays</i>	<ul style="list-style-type: none"> - Accurate and reproducible expression data - Established, reliable platform 	<ul style="list-style-type: none"> - Standardized workflows and user-friendly software available for data processing and analysis - Fast turnaround times, esp. for small studies 	<ul style="list-style-type: none"> - Limited dynamic range for fold change measurements - Require known sequence info for array fabrication - Limited detection of very low abundance transcripts
<i>RNA-Seq</i>	<ul style="list-style-type: none"> - Accurate and reproducible expression data - Transcript sequence information included 	<ul style="list-style-type: none"> - Extended dynamic range - Ability to detect rare transcripts with deep sequencing - Ability to identify novel transcripts and sequence variation 	<ul style="list-style-type: none"> - Complex data analysis w/ no 'gold-standard' pipeline - Requires mRNA selection or abundant transcript removal to avoid deep sequencing costs

Fig. 3 Performance and use considerations for gene expression profiling with microarrays versus RNA-Seq

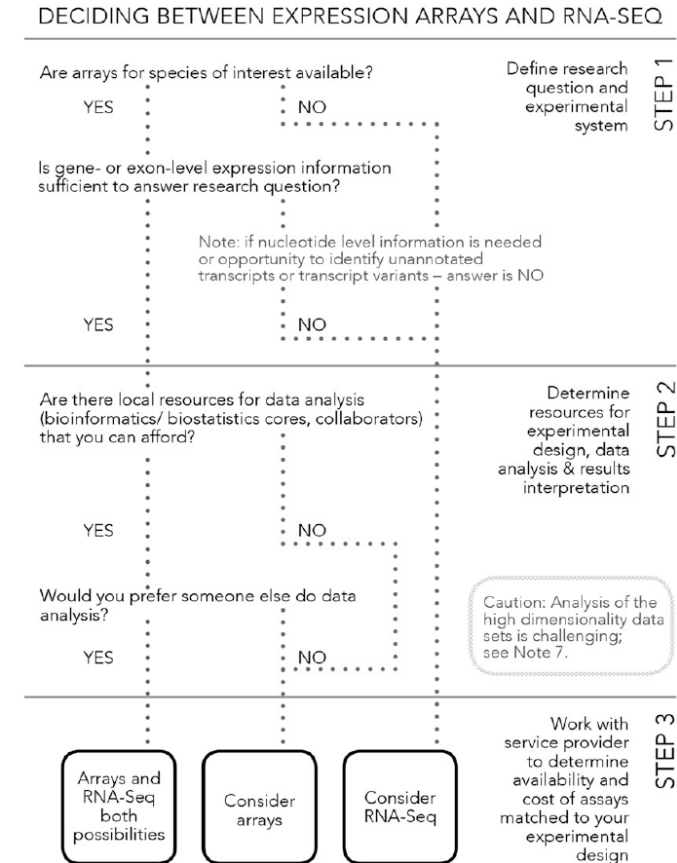


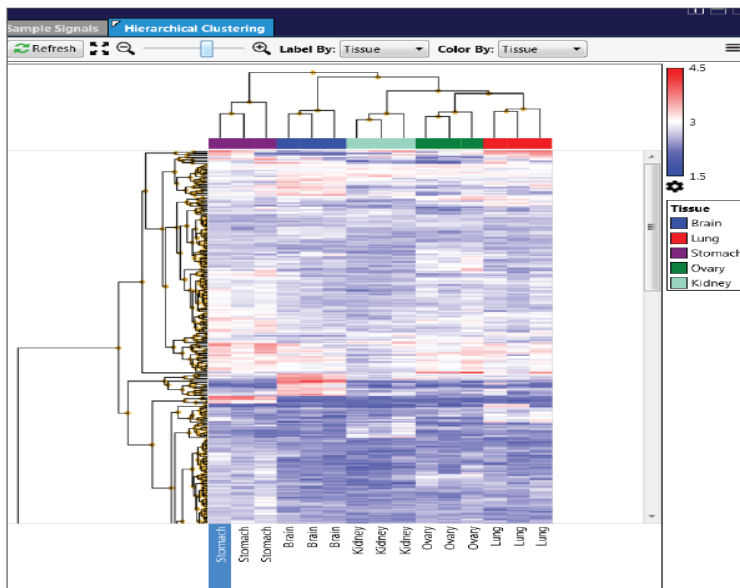
Fig. 4 Technology platform selection process for gene expression profiling

アプリケーションや解析目的に応じてMicroarrayとNGSを使い分けることが相乗的に効果的

Minnier J., et al., *Gene Expression Analysis, Methods and Protocols*, 2018, 7

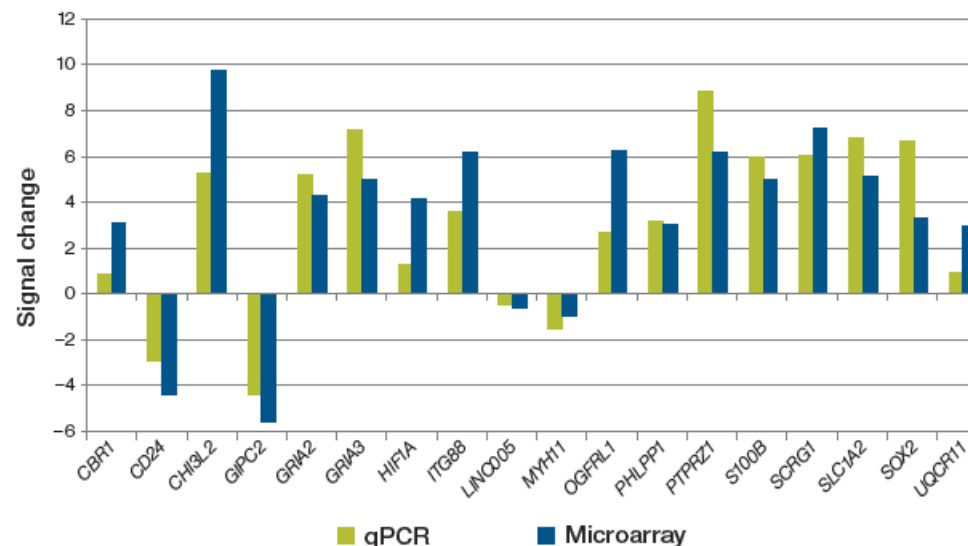
FFPEサンプルの場合、Microarrayでは専用のアッセイキットを使用してtotal RNAからデータ取得できるが、NGSではtotal RNAの前処理と量が必要になり、解析にも工夫が必要

Clariom D Arrayを用いたFFPEサンプルの解析



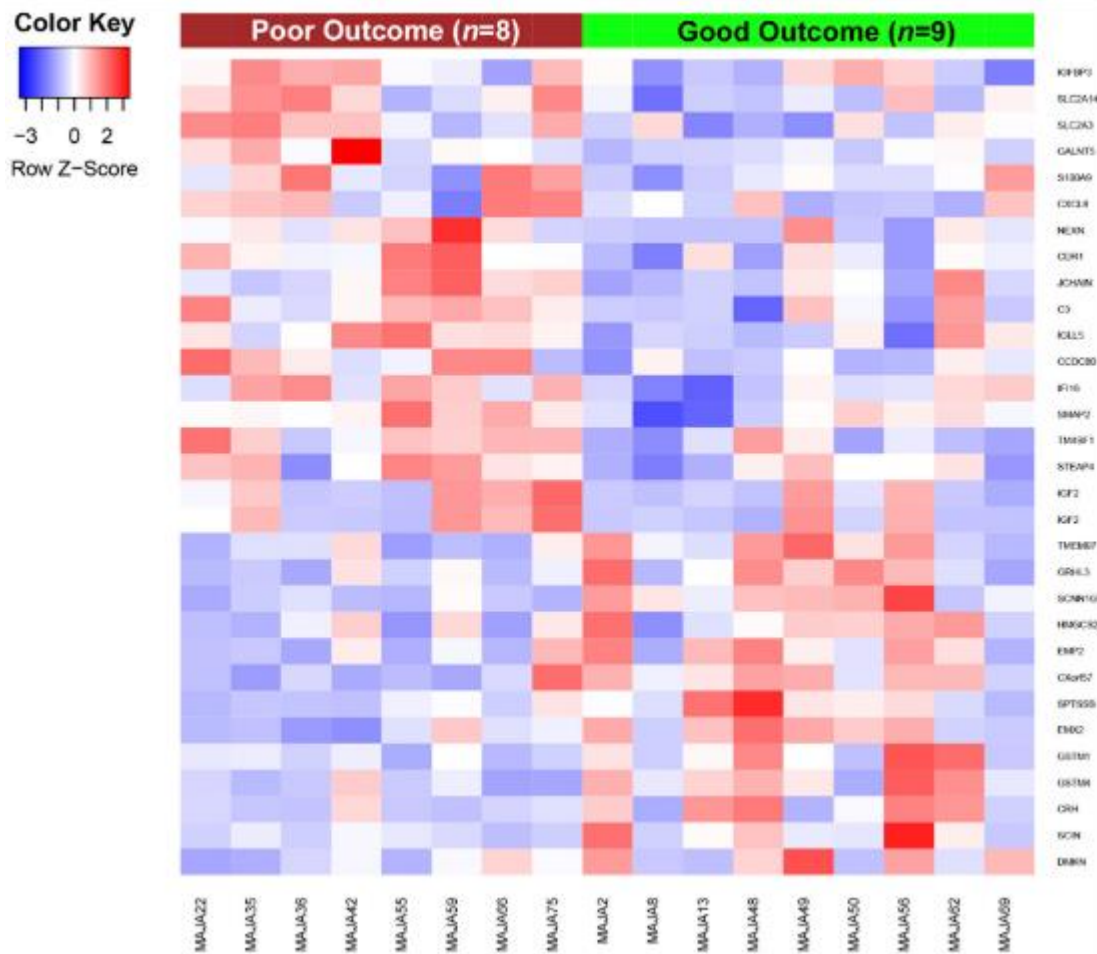
- ・脳、腎臓、肺、卵巣、胃の腫瘍生検 (FFPE) 由来 total RNA を Clariom D, human で解析
⇒ 各組織ごとにクラスター化され、同様の発現レベルを示す
- ・各組織や各腫瘍での特異的な発現パターンが Human Protein Atlas の公開データと一致
⇒ LIPF, GIF, GKN1, PGA4: 胃腫瘍検体で高発現
SLC1A2, GFAP: 脳腫瘍検体で高発現
lncRNA の発現プロファイルにおいても公開されたデータと一致性がある
- ・脳腫瘍 vs 腎臓腫瘍で発現差の高い 18 遺伝子を TaqMan アッセイ (best coverage) で検証
⇒ 2つのプラットフォーム間での相関性が認められる

Gene symbol	Brain average (log ₂)	Kidney average (log ₂)	Lung average (log ₂)	Ovary average (log ₂)	Stomach average (log ₂)
Tissue-specific genes					
LIPF	4.22	4.44	4.62	4.14	13.43
GIF	4.52	4.54	4.15	4.5	7.06
GKN1	4.19	4.03	3.98	4.02	6.74
PGA4	4.55	4.54	4.68	4.46	8.61
SLC1A2	10.23	5.09	5.77	5.57	5.53
GFAP	6.86	3.72	3.7	4.06	4
GIPC2	4.21	9.84	4.57	5.15	11.78
SLC16A4	6.96	9.45	6.59	6.7	6.9
IGHG4	4.74	5.5	8.05	4.52	5.71
SCGB3A2	5.26	5.38	6.23	5.33	4.23
Tumor-specific genes					
PVRL1	4.58	4.65	7	4.5	4.97
VTCN1	3.96	4.25	5.15	8.62	4.62
THSD4	6.11	6.58	6.73	7.25	5.98
SLC1A2	10.23	5.09	5.77	5.57	5.53
SLC12A2	4.88	4.89	4.6	5.15	11.49
TMEM91	4.33	5.84	4.04	4.25	4.43
MLLT3	6.03	5.96	7.47	5.87	6.89
Long noncoding RNA					
LINC00260	4.45	4.3	4.34	5.4	5.02
LINC00461; MIR9-2	13.45	5.8	6.14	6.17	5.96
LINC01381	4.75	3.83	4.27	4.13	4



Application Note: Whole-transcriptome analysis of FFPE samples using Clariom D Assays

進行性尿路上皮癌(aUCC)におけるビンフルニン耐性メカニズム解析 (Clariom S, human)



|FC| > 1.5、p ≤ 0.05 でFiltering

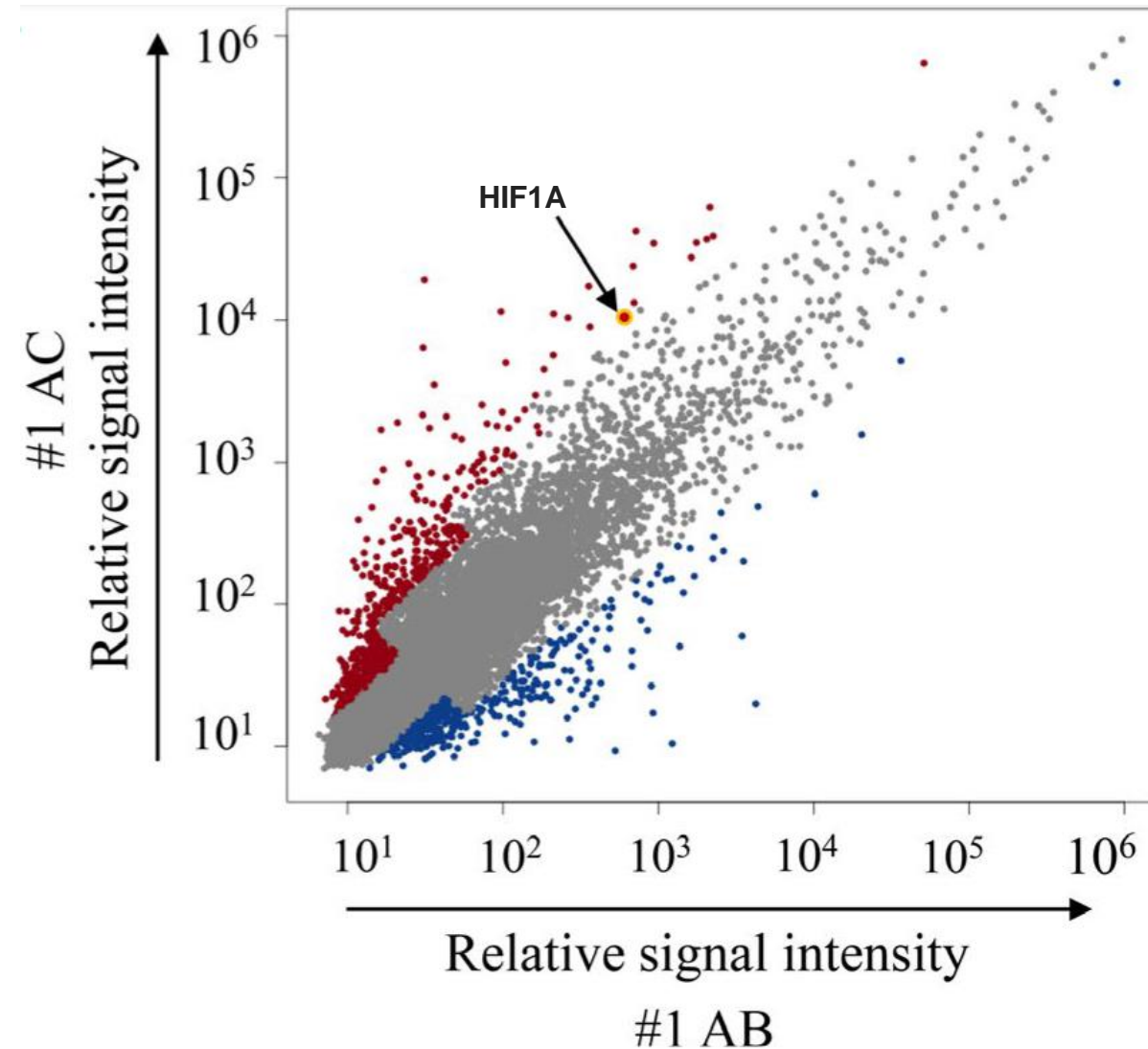
Gene	Gene Description	Chromosome	FC *	p-Value *
Genes downregulated in patients with good outcome to vinflunine treatment				
C3+C18F2B3:D19	Complement component 3	chr19	-2.0511	0.0066
CDR1	Cerebellar degeneration related protein 1	chrX	-2.0409	0.0019
IGFBP3	Insulin like growth factor binding protein 3	chr7	-1.9304	0.0109
IGF2	Insulin-like growth factor 2	chr11	-1.9108	0.0343
CCDC80	Coiled-coil domain containing 80	chr3	-1.8935	0.0043
JCHAIN	Joining chain of multimeric IgA and IgM	chr4	-1.8610	0.0230
CXCL8	Chemokine (C-X-C motif) ligand 8	chr4	-1.8393	0.0399
S100A9	S100 calcium binding protein A9	chr1	-1.7811	0.0460
TM4SF1	Transmembrane 4 L six family member 1	chr3	-1.7805	0.0070
IGLL5	Immunoglobulin lambda-like polypeptide 5	chr22	-1.6382	0.0185
Genes upregulated in patients with good outcome to vinflunine treatment				
GRHL3	Grainyhead-like transcription factor 3	chr1	1.5581	0.0011
SCIN	Scinderin	chr7	1.5654	0.0120
CXorf57	Chromosome X open reading frame 57	chrX	1.5952	0.0066
GSTM1	Glutathione S-transferase mu 1	chr1	1.5981	0.0105
SCNN1G	Sodium channel non-voltage gated 1 gamma subunit	chr16	1.6793	0.0007
EMX2	Empty spiracles homeobox 2	chr10	1.6874	0.0014
DMKN	Dermokine	chr19	1.6882	0.0261
TMEM97	Transmembrane protein 97	chr17	1.7270	0.0052
CRH	Corticotropin releasing hormone	chr8	1.8411	0.0394
SPTSSB	Serine palmitoyltransferase small subunit B	chr3	1.8950	0.0144

* FC (fold change) and p-value shown for the comparison between patients with good and poor outcome to vinflunine treatment.

- ・aUCC: プラチナベースの化学療法 ⇒ ビンフルニンによる維持療法 ⇒ ビンフルニン耐性を示すケースがあるが、メカニズムは不明
- ・予後良好: 9、予後不良: 8のFFPEサンプルをClariom S, humanで解析 ⇒ 予後良好~downregulate: 18、upregulate: 13
- ・GSEAより、予後良好において上皮間葉転換(EMT)とIL6/JAK/STAT3 pathway がdownregulate ⇒ 細胞をクルクミン処理でEMTを抑制することでビンフルニンに感作

マイクロアレイ解析で予後良好・不良検体間で発現差のある遺伝子を特定 ⇒ ビンフルニン耐性メカニズムと対処療法の可能性を示唆

悪性転換に関するバイオマーカー探索 (Clariom D, human)



Gene symbol	Z score	Fold changes #1 AB vs #1 AC	Gene name
PRDM1	4.579540676	102.8520686	PR domain containing 1, with ZNF domain
TSC22D3	3.40930138	34.41627375	TSC22 domain family, member 3
HIF1A	2.026398792	17.01183663	Hypoxia-inducible factor 1, alpha subunit
RCAN1	3.586628078	14.59752625	Regulator of calcineurin 1
MEIS2	3.218579318	11.278482	Meis homeobox 2
TFEC	3.001384157	9.68589256	Transcription factor EC
BHLHE41	1.559432856	9.652883719	Basic helix-loop-helix family, member e41
TSC22D1	1.535772787	9.382549523	TSC22 domain family, member 1
KLF5	2.940451309	9.280953297	Kruppel-like factor 5 (intestinal)
ZEB1	1.947696648	8.768748792	Zinc finger E-box binding homeobox 1

- ・エナメル上皮癌 (AC) はエナメル上皮腫 (AB) の悪性型
- ・ABの悪性化に関連するメカニズムをClariom D, humanで解析
- ・ACでupregulateしている中からGO termsを使用して、低酸素誘導因子サブユニットHIF1Aとジンクフィンガー転写因子ZEB1に着目
- ・免疫組織化学解析においてもHIF1AとZEB1はACで有意にupregulate
- ・低酸素状態で誘導されるHIF1AとZEB1でTGF- β が誘導され、上皮間葉転換 (EMT) を介したABの悪性転換に関与

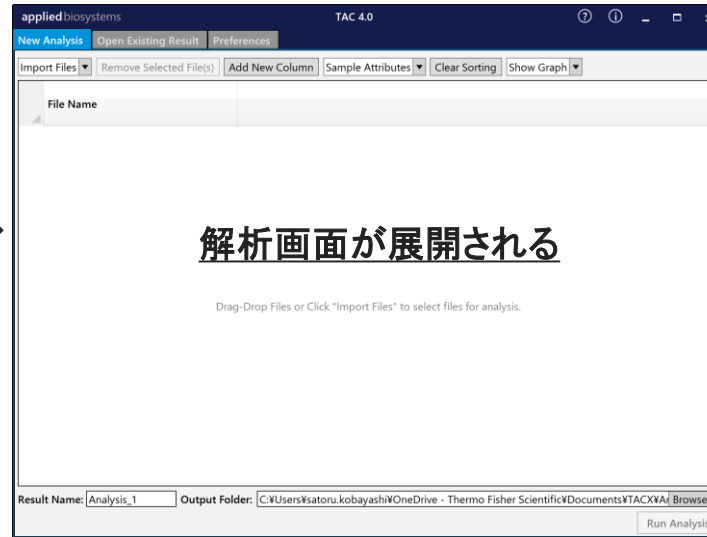
マイクロアレイ解析で悪性転換前後検体で発現差のある遺伝子を特定 ⇒ 該当遺伝子群から悪性転換に関わる経路を示唆

- マイクロアレイ技術の概要 ～ マイクロアレイ解析とは
- 遺伝子発現解析用ツールの紹介と使用例について
- データ解析方法について

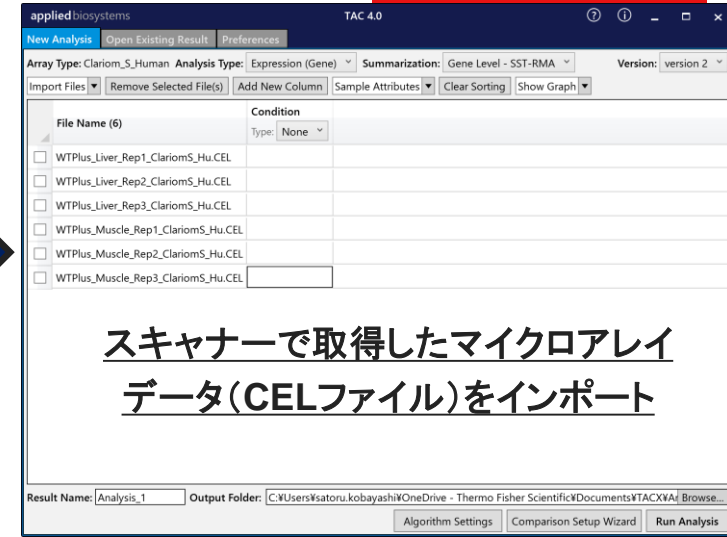
TACを使用したデータ解析の流れ



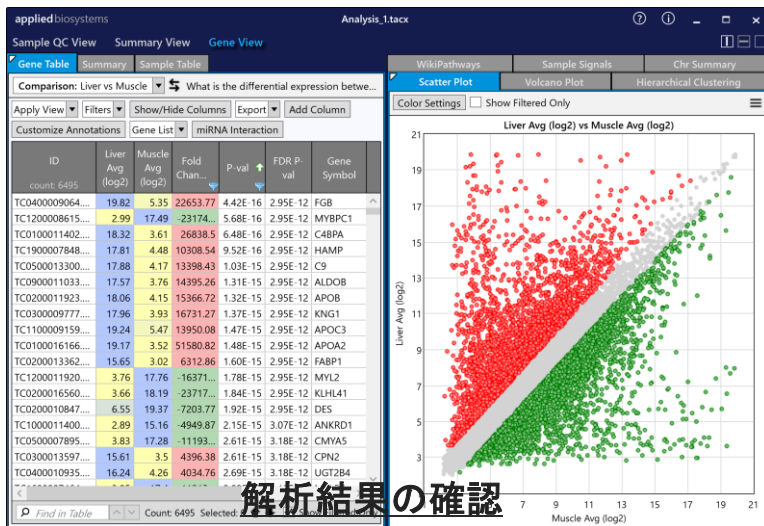
PCにインストールしたTACを起動



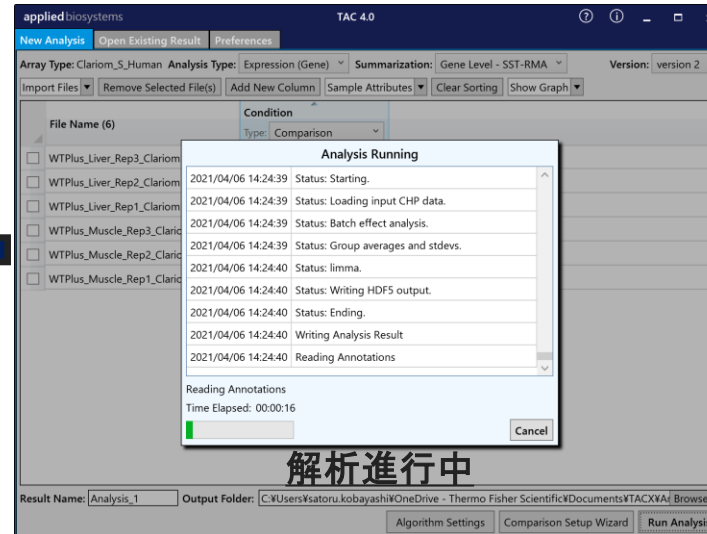
解析画面が展開される



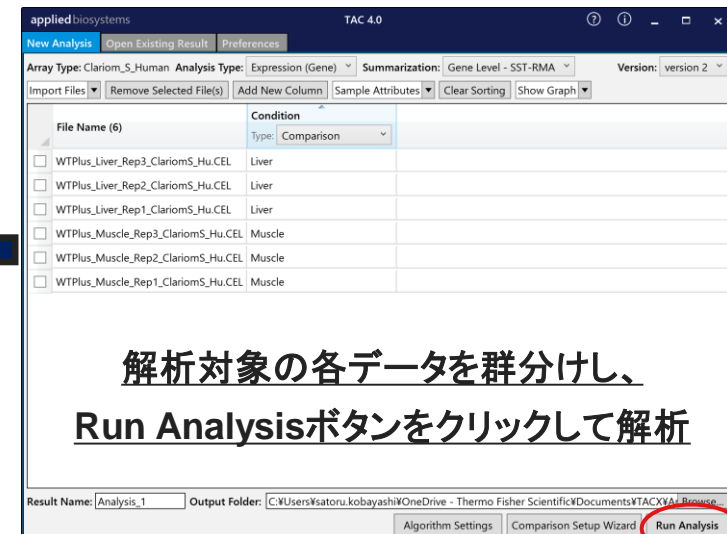
スキャナーで取得したマイクロアレイデータ(CELファイル)をインポート



解析結果の確認



解析進行中



解析対象の各データを群分けし、Run Analysisボタンをクリックして解析

一連の操作は単純で、簡単に結果を導くことが可能

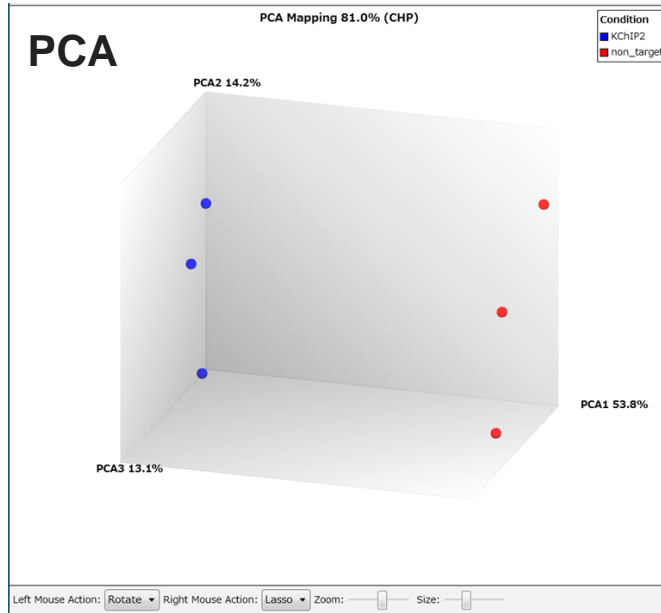
疑問点:

- ハイブリダイズは成功しているか？
- それぞれのサンプルのクオリティはSecondary Analysisを始めるに際して、実験群に含めても問題ないか？

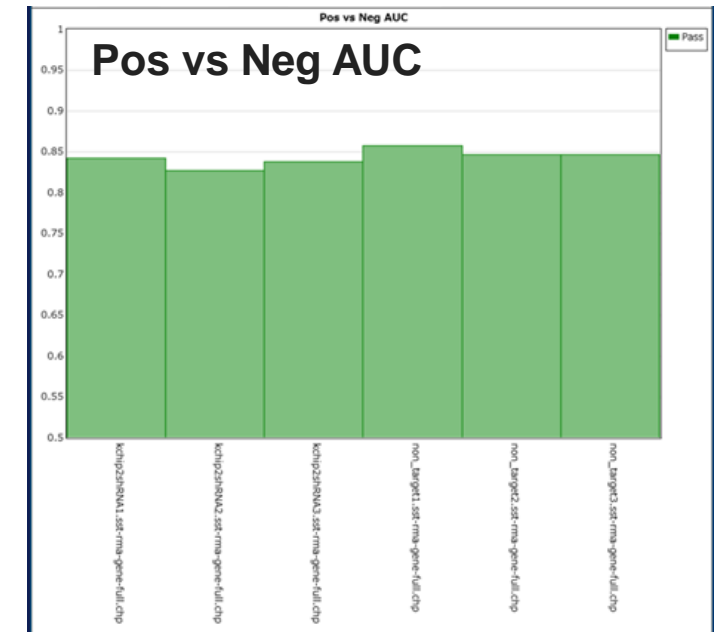
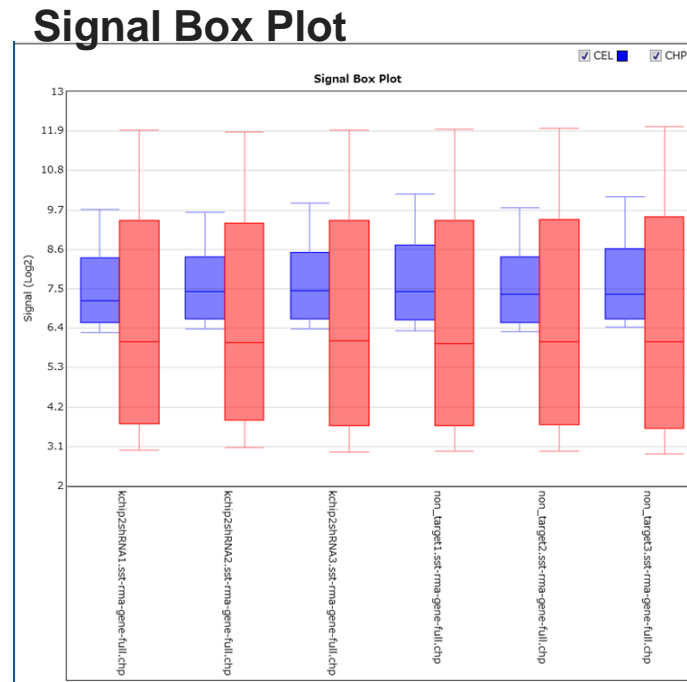
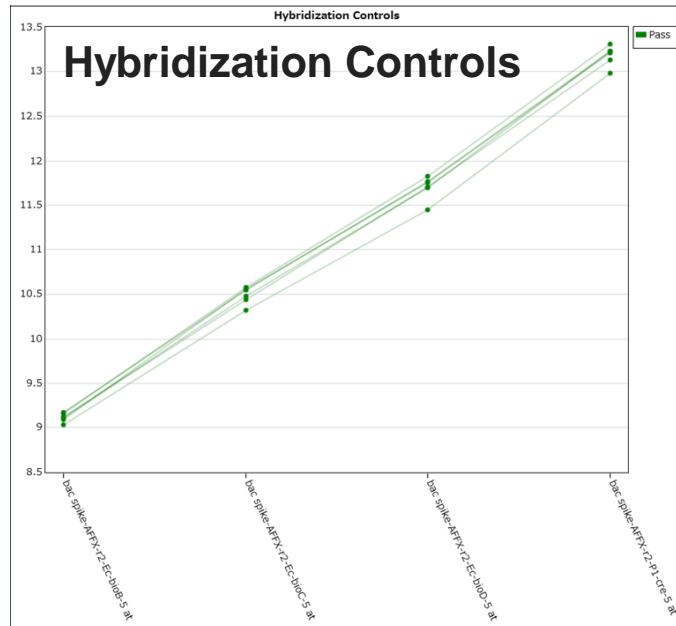
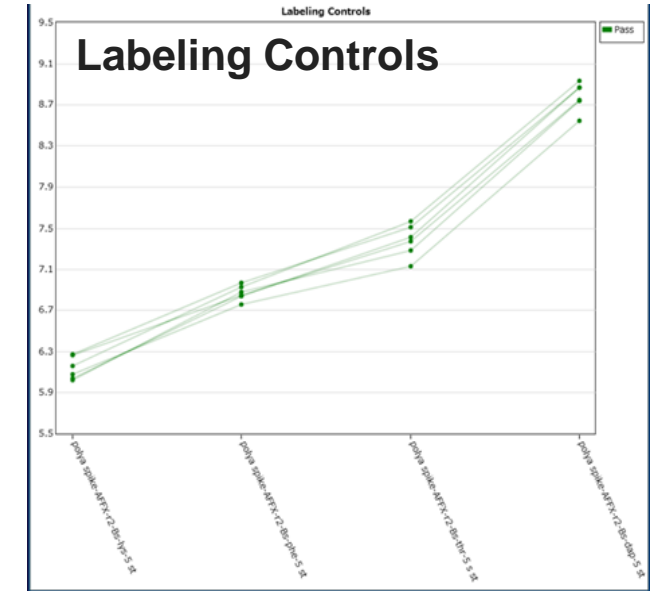
解決法:

- Primary Analysisによって、サンプル調製実験の状況を明らかにし、はずれサンプルOutlierを見つける
 - 同条件サンプルとの間に同傾向があるかどうか
 - Secondary Analysisの群間解析等に進めるデータかどうかを判断

Primary Analysis: Quality control of expression data



File Name	Labeling Controls Threshold	Hybridization Controls Threshold	Pos vs Neg AUC Threshold	Condition
count: 6				
kchip2shRNA1.sst-rma-gene-full.c...	Pass	Pass	Pass	kchip2
kchip2shRNA2.sst-rma-gene-full.c...	Pass	Pass	Pass	kchip2
kchip2shRNA3.sst-rma-gene-full.c...	Pass	Pass	Pass	kchip2
non_target1.sst-rma-gene-full.chp	Pass	Pass	Pass	non-target
non_target2.sst-rma-gene-full.chp	Pass	Pass	Pass	non-target
non_target3.sst-rma-gene-full.chp	Pass	Pass	Pass	non-target



Summary View

appliedbiosystems

20180205_ClariomS_Rat.tacx

Sample QC View **Summary View** Gene View

Analysis Summary Gene Lists and Venn Diagram

20180205 ClariomS Rat

Data Created: 2018/02/05 13:42:42

Array Type: Clariom_S_Rat

Analysis Type: Expression (Gene)

Analysis Version: version 1

Summarization Method: Gene Level - SST-RMA

Pos vs Neg AUC Threshold: 0.7

Genome Version: rn6 (Rattus norvegicus)

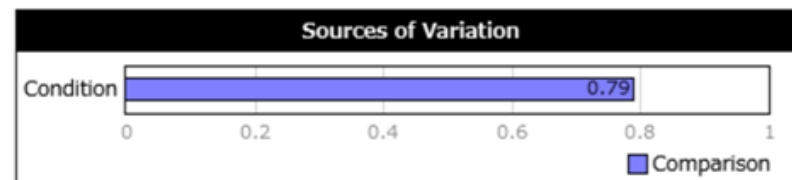
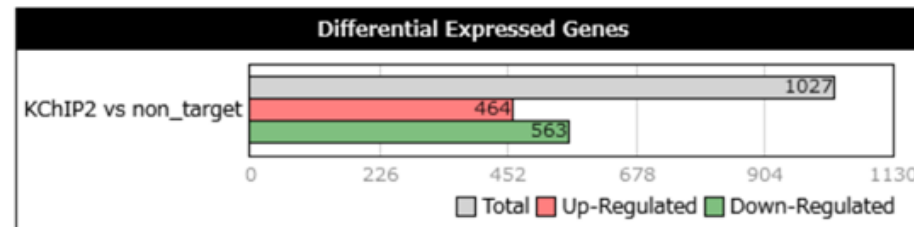
Annotation: Clariom_S_Rat.na36.rn6.transcript.csv

Condition (Comparison): KChIP2; non_target

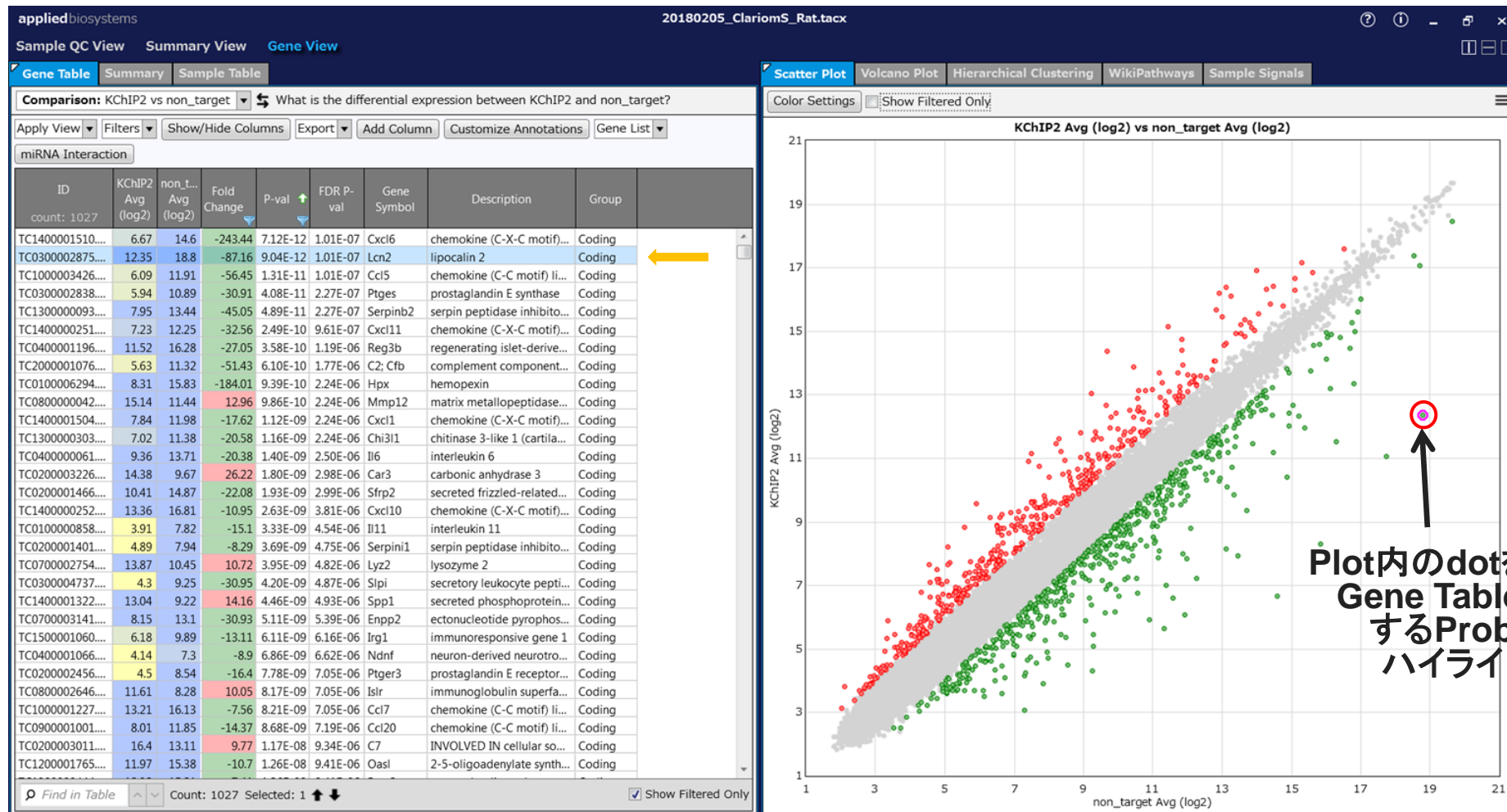
Expression Analysis Settings:

- Gene-Level Fold Change < -2 or > 2
- Gene-Level P-Value < 0.05
- Anova Method: ebayes
- A Probeset (Gene/Exon) is considered expressed if \geq 50% samples have DABG values below DABG Threshold.
- DABG < 0.05

Comparison	Group 1	Group 2	Count 1	Count 2	Up	Down
KChIP2 vs non_target	KChIP2	non_target	3	3	464	563



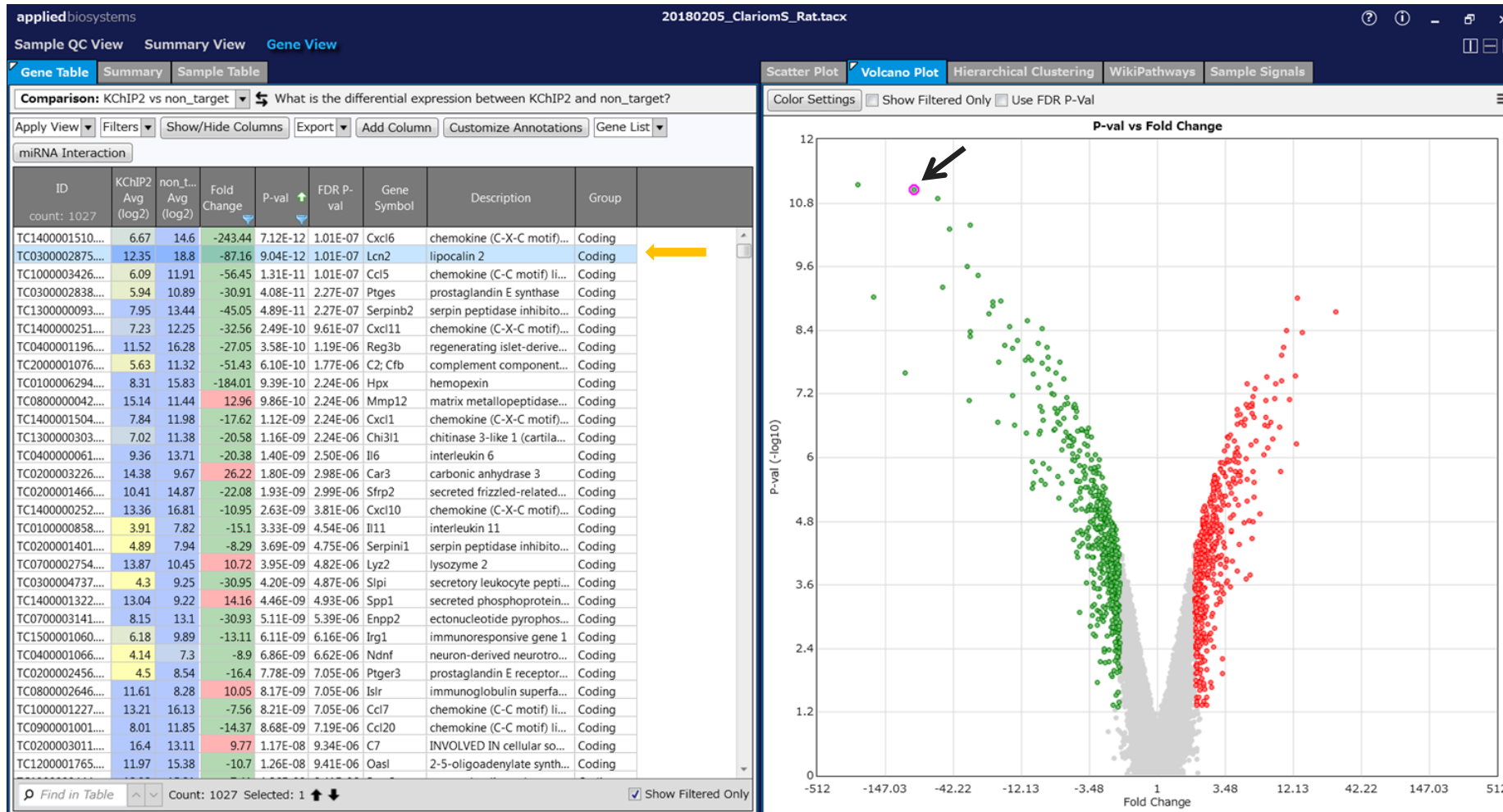
Gene View – Scatter Plot



Txtファイルでexport可能

PNG formatでexport可能

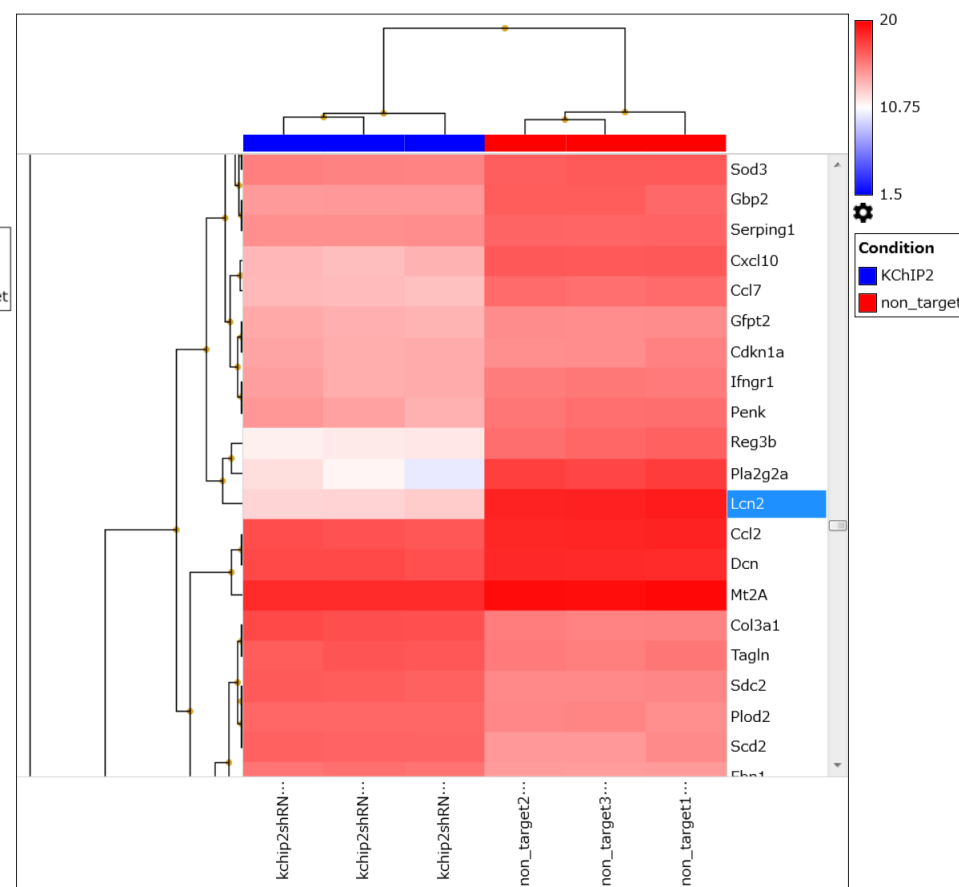
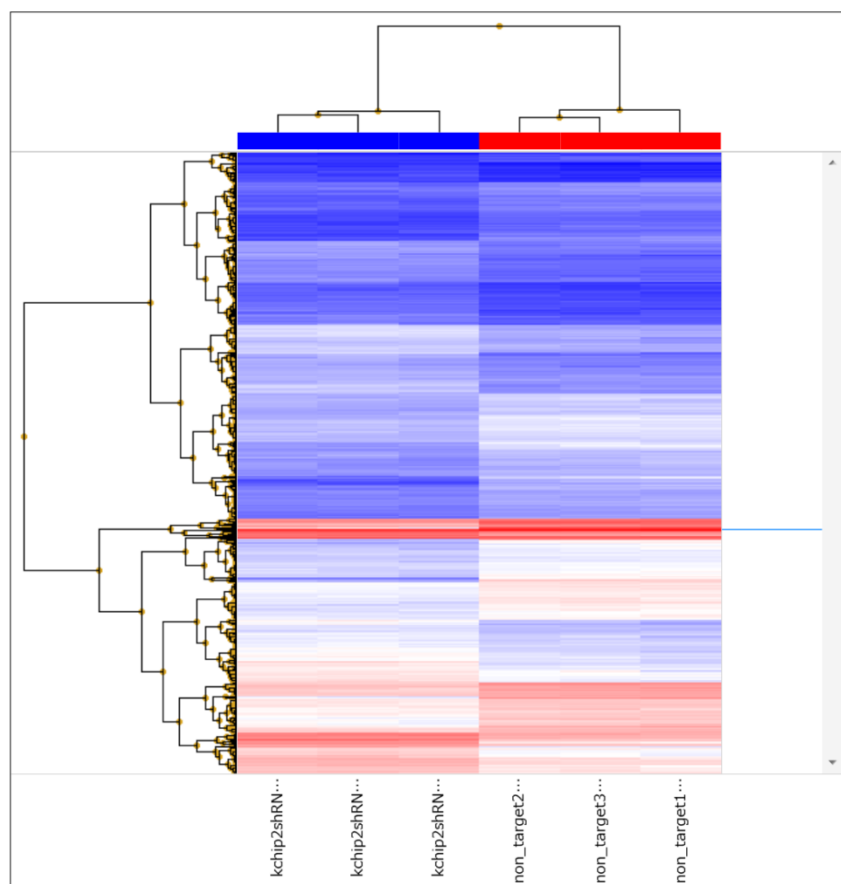
Gene View – Volcano Plot



↳ Txtファイルでexport可能

↳ PNG formatでexport可能

Gene View – Hierarchical Clustering



PNG formatでexport可能

ズームイン

表示は $\leq 5,000$ genesに絞り込む必要がある \Rightarrow Fold Changeやp-valueのフィルタリング条件変更

Gene View – WikiPathways

appliedbiosystems 20180205_ClariomS_Rat.tacx

Sample QC View Summary View Gene View

Gene Table Summary Sample Table

Comparison: KChIP2 vs non_target What is the differential e...

Apply View Filters Show/Hide Columns Export

Add Column Customize Annotations Gene List

miRNA Interaction

ID	KChIP2 Avg (log2)	non_t... Avg (log2)	Fold Change	P-val	FDR P-val	Gene Symbol
TC1400001510...	6.67	14.6	-243.44	7.12E-12	1.01E-07	Cxcl6
TC0300002875...	12.35	18.8	-87.16	9.04E-12	1.01E-07	Lcn2
TC1000003426...	6.09	11.91	-56.45	1.31E-11	1.01E-07	Ccl5
TC0300002838...	5.94	10.89	-30.91	4.08E-11	2.27E-07	Ptges
TC1300000093...	7.95	13.44	-45.05	4.89E-11	2.27E-07	Serp1nb2
TC1400000251...	7.23	12.25	-32.56	2.49E-10	9.61E-07	Cxcl11
TC0400001196...	11.52	16.28	-27.05	3.58E-10	1.19E-06	Reg3b
TC2000001076...	5.63	11.32	-51.43	6.10E-10	1.77E-06	C2; Cfb
TC0100006294...	8.31	15.83	-184.01	9.39E-10	2.24E-06	Hpx
TC0800000042...	15.14	11.44	12.96	9.86E-10	2.24E-06	Mmp12
TC1400001504...	7.84	11.98	-17.62	1.12E-09	2.24E-06	Cxcl1
TC1300000303...	7.02	11.38	-20.58	1.16E-09	2.24E-06	Chi3l1
TC0400000061...	9.36	13.71	-20.38	1.40E-09	2.50E-06	Il6
TC0200003226...	14.38	9.67	26.22	1.80E-09	2.98E-06	Car3
TC0200001466...	10.41	14.87	-22.08	1.93E-09	2.99E-06	Sfrp2
TC1400000252...	13.36	16.81	-10.95	2.63E-09	3.81E-06	Cxcl10
TC0100000858...	3.91	7.82	-15.1	3.33E-09	4.54E-06	Il11
TC0200001401...	4.89	7.94	-8.29	3.69E-09	4.75E-06	Serp1n1
TC0700002754...	13.87	10.45	10.72	3.95E-09	4.82E-06	Lyz2
TC0300004737...	4.3	9.25	-30.95	4.20E-09	4.87E-06	Slpi
TC1400001322...	13.04	9.22	14.16	4.46E-09	4.93E-06	Spp1
TC0700003141...	8.15	13.1	-30.93	5.11E-09	5.39E-06	Enpp2
TC1500001060...	6.18	9.89	-13.11	6.11E-09	6.16E-06	Irg1
TC0400001066...	4.14	7.3	-8.9	6.86E-09	6.62E-06	Ndnf
TC0200002456...	4.5	8.54	-16.4	7.78E-09	7.05E-06	Ptger3
TC0800002646...	11.61	8.28	10.05	8.17E-09	7.05E-06	Islr
TC1000001227...	13.21	16.13	-7.56	8.21E-09	7.05E-06	Ccl7
TC0900001001...	8.01	11.85	-14.37	8.68E-09	7.19E-06	Ccl20

Order by Count

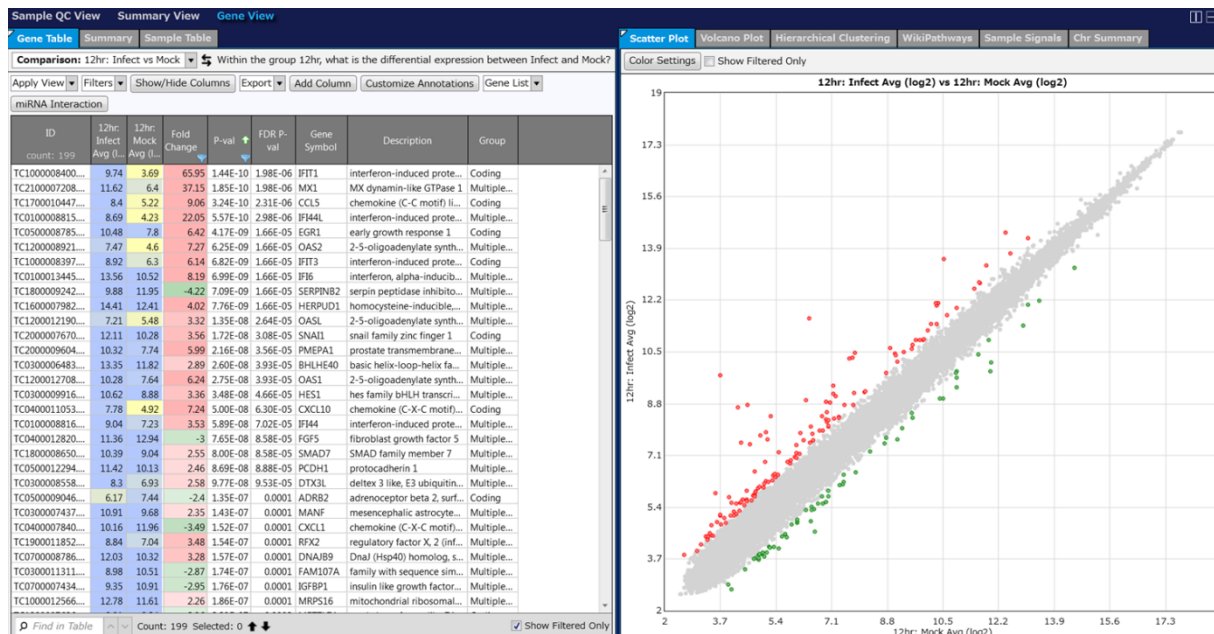
Cytokines and Inflammatory Response (BioCarta)

74% -4 4 filtered out

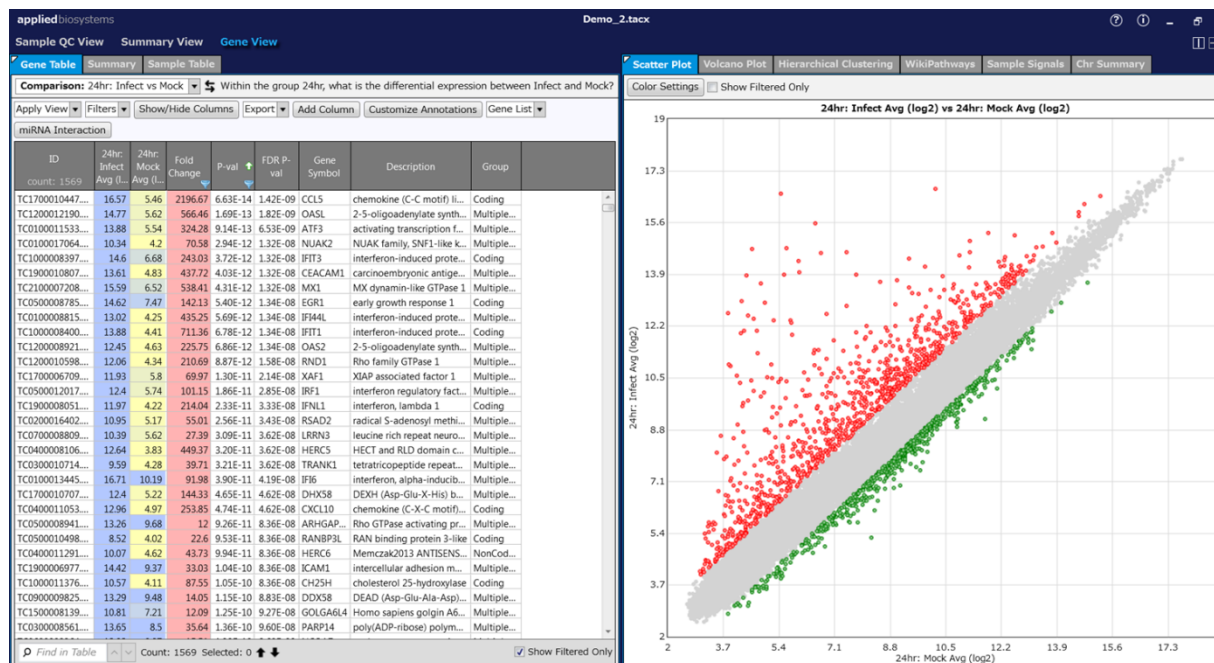
PNG formatでexport可能

Communityによって構築されているパスウェイデータベースとリンク
(インターネット接続が必要)

2つの解析結果から共通の発現変動遺伝子を抽出 (Venn Diagramの利用)

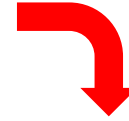
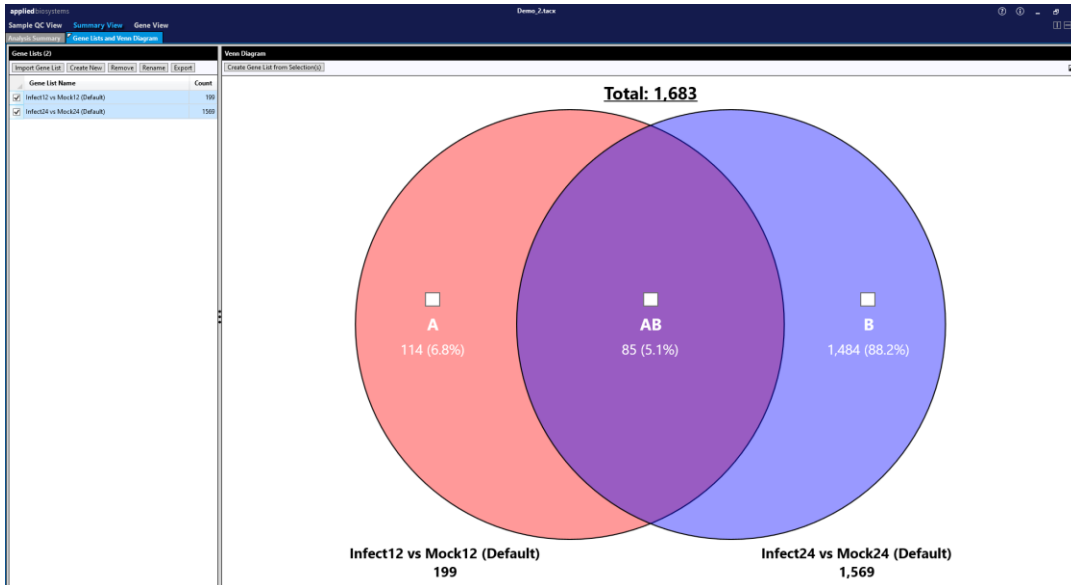


199 genes

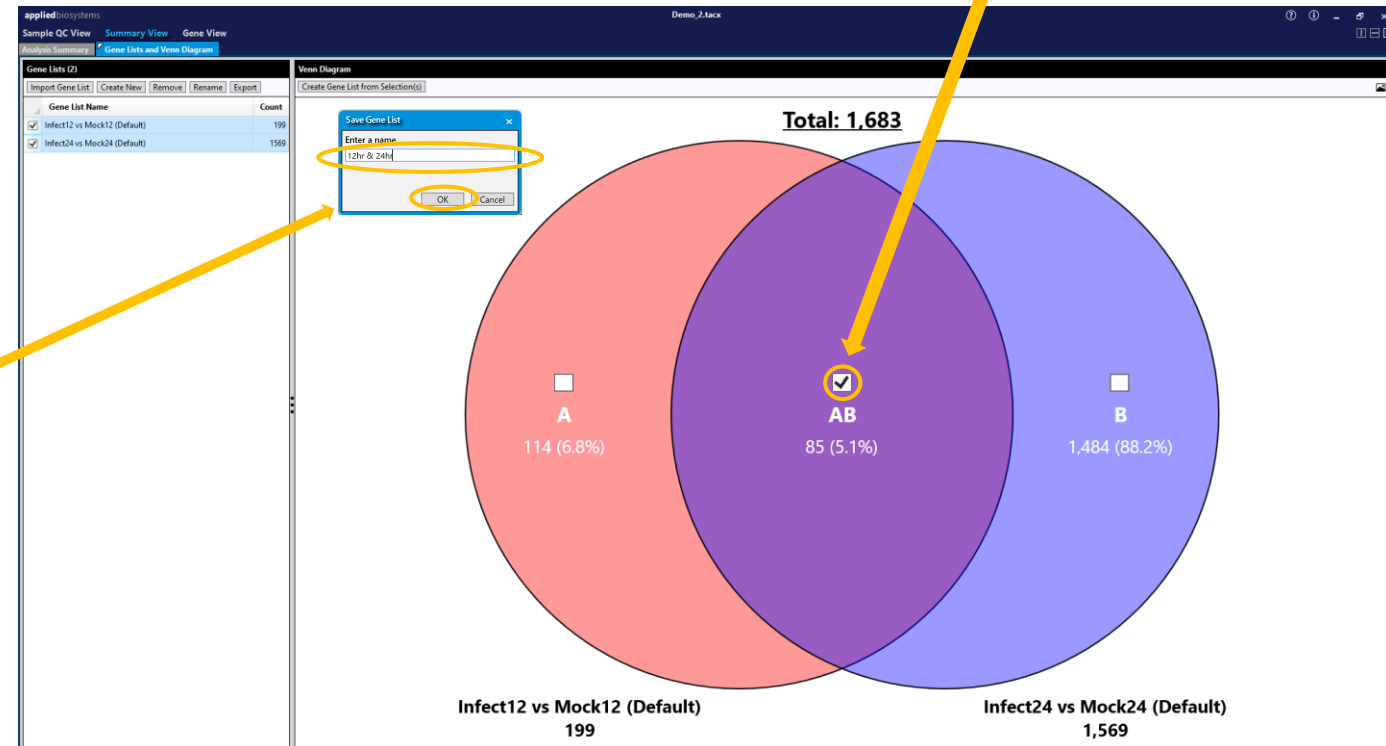


1,569 genes

2つの解析結果から共通の発現変動遺伝子を抽出 (Venn Diagramの利用)



②: Nameを入力後、OKをクリック



2つの解析結果から共通の発現変動遺伝子を抽出 (Venn Diagramの利用)

applied biosystems Demo_2.tacx

Sample QC View Summary View Gene View

Analysis Summary **Gene Lists and Venn Diagram**

Gene Lists (3)

12hr & 24hr

Import Gene List Create New Remove Rename Export

Gene List Name Count

- Infect12 vs Mock12 (Default) 199
- Infect24 vs Mock24 (Default) 1569
- 12hr & 24hr 84

Show/Hide Columns Export Add Column Add Selected Row(s) to Gene List Remove Selected Row(s) from Gene List

ID	Gene Symbol	Description	Chro...	Group	Start	Stop
TC0100008120.h...	HPDL	4-hydroxyphenylpyruvat...	chr1	Coding	45326873	45328674
TC0100008815.h...	IFI44L	interferon-induced protei...	chr1	Multiple...	78619922	78646145
TC0100008816.h...	IFI44	interferon-induced protei...	chr1	Multiple...	78649792	78664078
TC0100011533.h...	ATF3	activating transcription f...	chr1	Multiple...	212565334	212620777
TC0100013445.h...	IFI6	interferon, alpha-inducib...	chr1	Multiple...	27666061	27672218
TC0100014855.h...	GBP4	guanylate binding protei...	chr1	Multiple...	89181148	89198950
TC0100015752.h...	CTSS	cathepsin 5	chr1	Multiple...	150730196	150765957
TC0100016214.h...	CCDC190	coiled-coil domain conta...	chr1	Multiple...	162824458	162868815
TC0100016715.h...	PTGS2	prostaglandin-endopero...	chr1	Multiple...	186671791	186680427
TC0200006891.h...	RHOB	ras homolog family mem...	chr2	Coding	20447071	20449445
TC0200009829.h...	GCA	grancalcin, EF-hand calci...	chr2	Multiple...	162318840	162371595
TC0200010980.h...	CCL20	chemokine (C-C motif) li...	chr2	Multiple...	227813842	227817564
TC0200014772.h...	IFIH1	interferon induced, with...	chr2	Multiple...	162267079	162318708
TC0200016402.h...	RSAD2	radical S-adenosyl methi...	chr2	Multiple...	6877665	6898239
TC0300007432.h...	MAPKAP...	mitogen-activated protei...	chr3	Multiple...	50611520	50649297
TC0300007437.h...	MANF	mesencephalic astrocyte...	chr3	Multiple...	51385047	51389397
TC0300008558.h...	DTX3L	deltex 3 like, E3 ubiquitin...	chr3	Multiple...	122564238	122575203
TC0300008561.h...	PARP14	poly(ADP-ribose) polym...	chr3	Multiple...	122680618	122730840
TC0300009916.h...	HES1	hes family bHLH transcri...	chr3	Multiple...	194136142	194138732
TC0300011311.h...	FAM107A	family with sequence sim...	chr3	Multiple...	58564112	58627610
TC0300011391.h...	ADAMT59	ADAM metallopeptidase...	chr3	Multiple...	64515654	64688000
TC0300012191.h...	PARP9	poly(ADP-ribose) polym...	chr3	Multiple...	122527911	122564676
TC0400008106.h...	HERC5	HECT and RLD domain c...	chr4	Multiple...	88457117	88506168
TC0400009431.h...	TENM3	teneurin transmembrane...	chr4	Multiple...	182143987	182803024
TC0400011014.h...	CXCL5	chemokine (C-X-C motif...	chr4	Multiple...	73995642	73998779
TC0400011052.h...	CXCL9	chemokine (C-X-C motif...	chr4	Coding	76001275	76007523
TC0400011053.h...	CXCL10	chemokine (C-X-C motif...	chr4	Coding	76021116	76023536
TC0400011054.h...	CXCL11	chemokine (C-X-C motif...	chr4	Coding	76033682	76041415
TC0400012818.h...	CENPG2	cyclin G2	chr4	Multiple...	77157151	77179142
TC0400012820.h...	FGF5	fibroblast growth factor 5	chr4	Multiple...	80266598	80291017
TC0400012828.h...	BANK1	B-cell scaffold protein wi...	chr4	Multiple...	101790607	102074812
TC0500006436.h...	PLEKHG4B	Zhang2013 ALT_ACCEPT...	chr5	NonCo...	173548	173767
TC0500007376.h...	FST	follistatin	chr5	Multiple...	53480409	53487134
TC0500008785.h...	EGR1	early growth response 1	chr5	Coding	138465490	138469315
TC0500009046.h...	ADRB2	adrenoceptor beta 2, surf...	chr5	Coding	148825245	148828687
TC0500011334.h...	TMEM16...	Transcript Identified by A...	chr5	Unassig...	83059099	83059250
TC0600007384.h...	HIST1H2...	histone cluster 1, H2bo	chr6	Coding	27893425	27893891
TC0600009333.h...	SMPDL3A	sphingomyelin phospho...	chr6	Multiple...	122788826	122809720
TC0600009843.h...	PLEKHG1	pleckstrin homology do...	chr6	Multiple...	150599863	150843665
TC0700007434.h...	IGFBP1	insulin like growth factor...	chr7	Multiple...	45888357	45893669
TC0700008786.h...	DNAJB9	DnaJ (Hsp40) homolog, s...	chr7	Multiple...	108569568	108574850
TC0700008809.h...	LRRN3	leucine rich repeat neuro...	chr7	Multiple...	111091006	111125454
TC0700008868.h...	TES	testin LIM domain protein	chr7	Multiple...	116210493	116258783
TC0800009891.h...	STC1	stanniocalcin 1	chr8	Multiple...	23841913	23854807
TC0900009688.h...	IFNB1	interferon, beta 1, fibrob...	chr9	Coding	21077105	21077963
TC0900011192.h...	LPAR1	lysophosphatidic acid rec...	chr9	Multiple...	110873263	111038458
TC0X00007310.h...	TSPYL2	TSPY-like 2	chrX	Multiple...	53082360	53088546
TC0X00010355.h...	TMM8A	translocase of inner mito...	chrX	Multiple...	101345656	101349196

Find in Table Count: 84 Selected: 0

Clariom D Array: Alt-Splice View

applied biosystems Case1vs2.tacx

Sample QC View Summary View Gene View **Alt-Splice View**

Table Summary Sample Table

Comparison: Case1 vs Case2 What is the differential expression between Case1 and Case2?

Gene Table

ID	Fold Change	Exp... in Bot...	Gene Symbol	Description	Group	Exon Splicing Index	Exon P-val	Exon FDR P-val	Exon Event Name	Exon Event Score	Exo...
TC1200008573...	-1.58	T	SLC25A3...	solute carrier family 25 (...)	Multipl...	-3642.84	1.14E-10	5.97E-07	Cassette Exon	0.9	
TC1700010230...	-8.38	T	TIAF1; M...	TGFB1-induced anti-apo...	Multipl...	-3.04	0.0050	0.0294	Complex Event	0.83	
TC0100013134...	1.09	T	CAPZB	capping protein (actin fil...	Multipl...	-223.28	2.45E-08	6.57E-06	Cassette Exon	0.83	
TC0100015899...	-11.54	T	TPM3	tropomyosin 3	Multipl...	-177.89	4.28E-10	9.61E-07	Cassette Exon	0.82	
TC2100006852...	-1.56	T	USP16	ubiquitin specific peptid...	Multipl...	-164.66	1.96E-08	5.81E-06	Cassette Exon	0.82	
TC0300013996...	2.71	T	TMEM11...	TMEM110-MUSTN1 read...	Multipl...	-749.01	3.84E-07	3.43E-05	Alternative Last...	0.8	
TC0200016603...	1.2	T	LRRFP1	leucine rich repeat (in FLI...	Multipl...	-306.21	4.38E-09	2.74E-06	Cassette Exon	0.8	
TC0400012940...	3.43	T	SEC31A	SEC31 homolog A, COPII...	Multipl...	62.65	7.97E-08	1.31E-05	Cassette Exon	0.8	
TC1500010869...	2.24	T	CCPG1...	cell cycle progression 1...	Multipl...	-130.26	3.26E-07	3.09E-05	Cassette Exon	0.78	
TC1200010518...	-3.23	T	SLC38A2	solute carrier family 38...	Multipl...	158.45	2.12E-07	2.35E-05	Intron Retention	0.77	

PSRs/JUCs Table - TC1200008573.hg.1

PSR/JUC ID	Case1 Avg (log2)	Case2 Avg (log2)	Splicing Index	P-val	FDR P-val	Event Name	Event Score	Expr... in At leas...
PSR120016295...	4.99	17.66	-3642.84	1.14E-10	5.97E-07	Cassette Exon	0.9	T
PSR120016295...	17.65	11.14	140.08	2.51E-09	2.12E-06	Cassette Exon	0.69	T
PSR120016295...	5.81	4.58	3.63	0.0010	0.0091	Intron Retention	0.43	T
PSR120016295...	4.4	3.9	2.11	0.0035	0.0229	Intron Retention	0.43	T
PSR120016295...	9.04	8.17	3.15	0.0012	0.0104	Complex Event	0.42	T
PSR120016295...	6.19	5.25	3.35	0.0074	0.0392	Alternative 5' D...	0.25	T
PSR120016296...	6.09	5.51	2.27	0.0004	0.0045	Intron Retention	0.21	T
PSR120016298...	5.88	5.21	2.5	0.0016	0.0130			T
JUC1200056046...	14.74	8.46	134.55	1.18E-08	4.45E-06			T
JUC1200056047...	13.16	8.68	37.52	5.88E-07	4.52E-05			T
JUC1200056053...	3.26	10.75	-119.01	2.23E-08	6.23E-06			T

Alt-Splice Visualization 3D Line Plot

Structure View Show Junction Show Sample Signals

TC1200008573.hg.1 Symbol: SLC25A3; SNORA53 Splicing Index -4 Signal 5

chr12: 98,593,591 - 98,606,379 Strand: + Multiple_Complex

Case1_Signal (Gene-Level Signal: 13.54)
Case2_Signal (Gene-Level Signal: 14.2)

Sample-Level Signals: Case1 Case2

Normalize Signals Clear Sample Selection

Splicing Index (Gene-Level Fold Change: -1.58) Case1 Case2

Isoforms Affx Transcript Isoforms Sort Isoforms Color By: Splicing Index

TR120006397... 1.a 1.a.a 1.h 2.f 2.g 2.h 5.i

TR120006397... 1.h 2.e

TR120006396... 2.d 2.h

TR120006397... 2.g 2.h

TR120006395... 1.b 2.g 2.h 5.g

TR120006395... 1.c 2.g 2.h 5.f

TR120006395... 1.c 2.g 2.h 5.g

TR120006397... 1.t 2.g 2.h 5.b

TR120006396... 1.l 2.g 2.h 5.c

TR120006396... 2.g 2.h 5.e

TR120006397... 2.g 2.h 5.e

TR120006397... 2.g 2.h 5.e

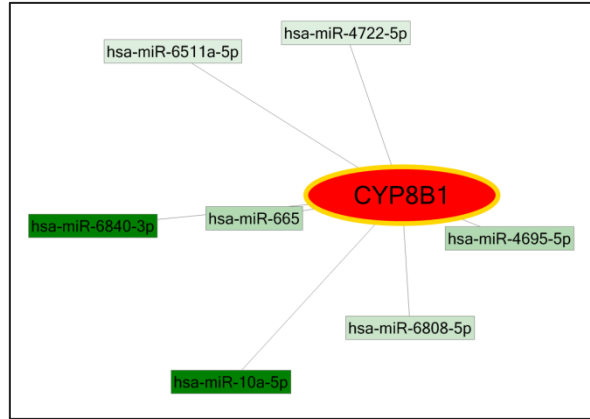
TR120006396... 2.g 2.h 5.e

TR120006396... 2.g 2.h 5.e

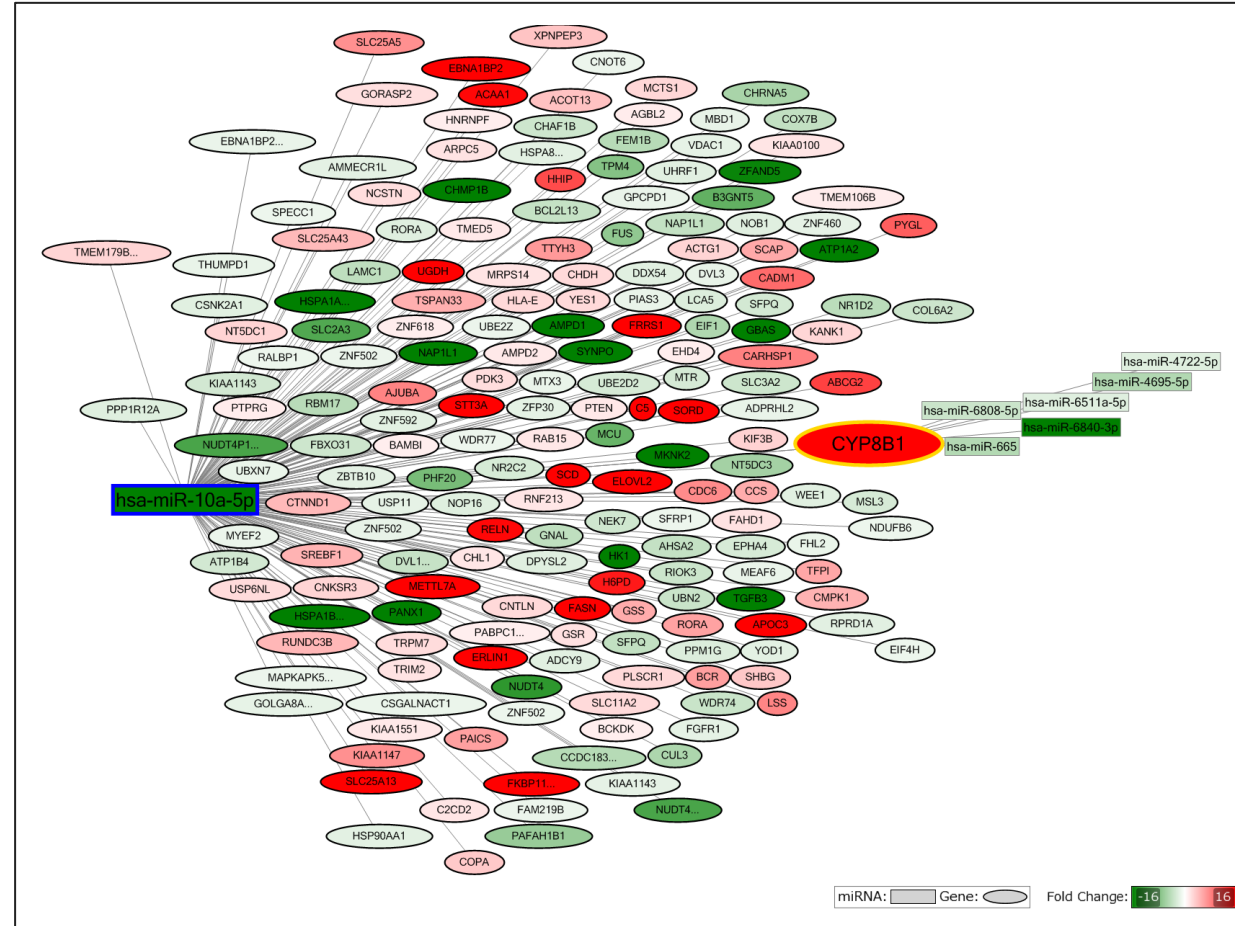
5:25 PM 8/16/2017

PNG formatでexport可能

Interaction Network Graph



同じRNAサンプルを使ったmRNA解析データと
miRNA解析データを統合



PNG formatでexport可能

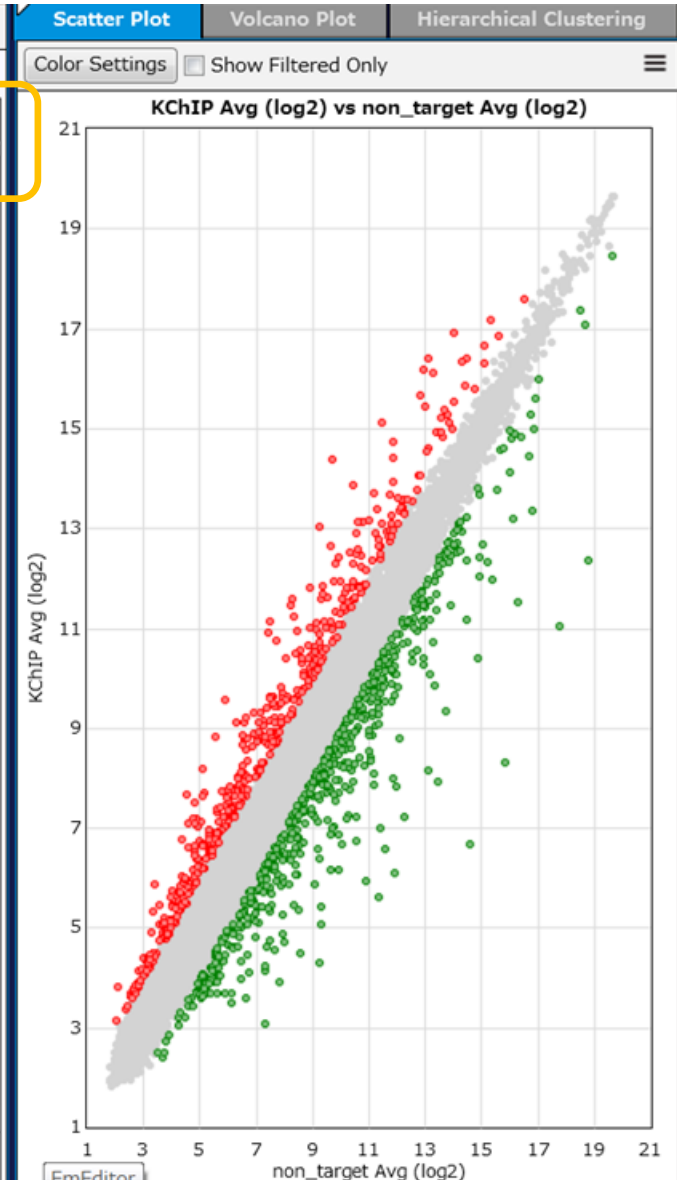
TaqMan AssayとClariom Arrayとのマッピング

Comparison: KChIP vs non_target What is the differential expression between KChIP and non_target?

Apply View Filters Show/Hide Columns Export Add Column Customize Annotations Gene List miRNA Interaction

ID	KChIP Avg (log2)	non_t... Avg (log2)	Fold Change	P-val	FDR P-val	Gene Symbol	Description	Group	Best_Coverage_TaqMan_...	Best_Coverage_TaqMan_Assay_HTM
TC1400001510.m.2	6.67	14.6	-243.44	7.12E-12	1.01E-07	Cxcl6	chemokine (C-X-C motif)...	Coding	Rn00573587_g1	http://www.thermofisher.com/order/geno...
TC0300002875.m.2	12.35	18.8	-87.16	9.04E-12	1.01E-07	Lcn2	lipocalin 2	Coding	Rn01439062_g1	http://www.thermofisher.com/order/geno...
TC1000003426.m.2	6.09	11.91	-56.45	1.31E-11	1.01E-07	Ccl5	chemokine (C-C motif) li...	Coding	Rn00579590_m1	http://www.thermofisher.com/order/geno...
TC0300002838.m.2	5.94	10.89	-30.91	4.08E-11	2.27E-07	Ptges	prostaglandin E synthase	Coding	Rn00572047_m1	http://www.thermofisher.com/order/geno...
TC1300000093.m.2	7.95	13.44	-45.05	4.89E-11	2.27E-07	Serpib2	serpin peptidase inhibito...	Coding	Rn00572553_m1	http://www.thermofisher.com/order/geno...
TC1400000251.m.2	7.23	12.25	-32.56	2.49E-10	9.61E-07	Cxcl11	chemokine (C-X-C motif)...	Coding	Rn00788261_g1	http://www.thermofisher.com/order/geno...
TC0400001196.m.2	11.52	16.28	-27.05	3.58E-10	1.19E-06	Reg3b	regenerating islet-derive...	Coding	Rn00583920_m1	http://www.thermofisher.com/order/geno...
TC2000001076.m.2	5.63	11.32	-51.43	6.10E-10	1.77E-06	C2; Cfb	complement component...	Coding	Rn00597178_g1	http://www.thermofisher.com/order/geno...
TC0100006294.m.2	8.31	15.83	-184.01	9.39E-10	2.24E-06	Hpx	hemopexin	Coding	Rn00670220_g1	http://www.thermofisher.com/order/geno...
TC0800000042.m.2	15.14	11.44	12.96	9.86E-10	2.24E-06	Mmp12	matrix metalloproteinase...	Coding	Rn00588640_m1	http://www.thermofisher.com/order/geno...
TC1400001504.m.2	7.84	11.98	-17.62	1.12E-09	2.24E-06	Cxcl1	chemokine (C-X-C motif)...	Coding	Rn00578225_m1	http://www.thermofisher.com/order/geno...
TC1300000303.m.2	7.02	11.38	-20.58	1.16E-09	2.24E-06	Chi3l1	chitinase 3-like 1 (cartila...	Coding	Rn01490606_m1	http://www.thermofisher.com/order/geno...
TC0400000061.m.2	9.36	13.71	-20.38	1.40E-09	2.50E-06	Il6	interleukin 6	Coding	Rn00561420_m1	http://www.thermofisher.com/order/geno...
TC0200003226.m.2	14.38	9.67	26.22	1.80E-09	2.98E-06	Car3	carbonic anhydrase 3	Coding	Rn00695939_m1	http://www.thermofisher.com/order/geno...
TC0200001466.m.2	10.41	14.87	-22.08	1.93E-09	2.99E-06	Sfrp2	secreted frizzled-related...	Coding	Rn01458836_m1	http://www.thermofisher.com/order/geno...
TC1400000252.m.2	13.36	16.81	-10.95	2.63E-09	3.81E-06	Cxcl10	chemokine (C-X-C motif)...	Coding	Rn00594648_m1	http://www.thermofisher.com/order/geno...
TC0100000858.m.2	3.91	7.82	-15.1	3.33E-09	4.54E-06	Il11	interleukin 11	Coding	Rn00591721_m1	http://www.thermofisher.com/order/geno...
TC0200001401.m.2	4.89	7.94	-8.29	3.69E-09	4.75E-06	Serpini1	serpin peptidase inhibito...	Coding	Rn00587242_m1	http://www.thermofisher.com/order/geno...
TC0700002754.m.2	13.87	10.45	10.72	3.95E-09	4.82E-06	Lyz2	lysozyme 2	Coding	Rn00562794_m1	http://www.thermofisher.com/order/geno...
TC0300004737.m.2	4.3	9.25	-30.95	4.20E-09	4.87E-06	Slpi	secretory leukocyte pepti...	Coding	Rn00670378_m1	http://www.thermofisher.com/order/geno...
TC1400001322.m.2	13.04	9.22	14.16	4.46E-09	4.93E-06	Spp1	secreted phosphoprotein...	Coding	Rn00681029_m1	http://www.thermofisher.com/order/geno...
TC0700003141.m.2	8.15	13.1	-30.93	5.11E-09	5.39E-06	Enpp2	ectonucleotide pyrophos...	Coding	Rn00678654_m1	http://www.thermofisher.com/order/geno...
TC1500001060.m.2	6.18	9.89	-13.11	6.11E-09	6.16E-06	Irg1	immunoresponsive gene 1	Coding	Rn01467901_m1	http://www.thermofisher.com/order/geno...
TC0400001066.m.2	4.14	7.3	-8.9	6.86E-09	6.62E-06	Ndnf	neuron-derived neurotro...	Coding	Rn06420062_s1	http://www.thermofisher.com/order/geno...
TC0200002456.m.2	4.5	8.54	-16.4	7.78E-09	7.05E-06	Ptger3	prostaglandin E receptor...	Coding	Rn01636929_m1	http://www.thermofisher.com/order/geno...
TC0800002646.m.2	11.61	8.28	10.05	8.17E-09	7.05E-06	Islr	immunoglobulin superfa...	Coding	Rn01766566_g1	http://www.thermofisher.com/order/geno...
TC1000001227.m.2	13.21	16.13	-7.56	8.21E-09	7.05E-06	Ccl7	chemokine (C-C motif) li...	Coding	Rn01467286_m1	http://www.thermofisher.com/order/geno...
TC0900001001.m.2	8.01	11.85	-14.37	8.68E-09	7.19E-06	Ccl20	chemokine (C-C motif) li...	Coding	Rn01400117_g1	http://www.thermofisher.com/order/geno...
TC0200003011.m.2	16.4	13.11	9.77	1.17E-08	9.34E-06	C7	INVOLVED IN cellular so...	Coding	Rn01402702_m1	http://www.thermofisher.com/order/geno...
TC1200001765.m.2	11.97	15.38	-10.7	1.26E-08	9.41E-06	Oasl	2-5-oligoadenylate synth...	Coding	Rn01481416_m1	http://www.thermofisher.com/order/geno...
TC1300000444.m.2	12.32	15.21	-7.41	1.26E-08	9.41E-06	Ptas2	prostaalandin-endopero...	Coding	Rn00682542_g1	http://www.thermofisher.com/order/geno...

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TaqMan AssayとClariom Arrayとのマッピング

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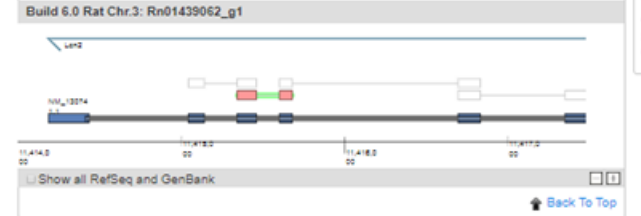
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Gene Expression 901680

お問い合わせ サインイン

Genomic Map



Assay Details

Gene Symbol: Lcn2					
Interrogated Sequence	Translated Protein	Exon Boundary	Assay Location	IMAGE Clone ID	Amplicon Length
RefSeq cf NM_130741.1	cf NP_570097.1	3 - 4	399		69
GenBank cf BC089053.1	-	3 - 4	481	7310185	69
cf X13295.1	-	3 - 4	399		69

More Information

Updated Mapping Information [▶ This assay targets the same location as RTA transcript cluster\(s\) TC0300002875.m.1](#)

Set Membership: [▶ Ambion siRNA available](#) [▶ Amplicon length less than or equal to 70](#) [▶ Mammalian Gene Collection](#) [▶ Detect Genomic DNA](#) [▶ Rattus norvegicus](#) [▶ Probe spans exons](#)

Matching Ambion siRNAs: [▶ Click here to find siRNAs for Gene Lcn2](#)

Panther Classification:
Molecular Function - [▶ isomerase](#) [▶ transfer/carrier protein](#)

MicroArray Probes: [Affymetrix](#)

Array Name	Probe IDs	Targets	Target Type
Affymetrix Rat Clariom S Assay	PSR0300022176.m.1	TC0300002875.m.1	RTA TC

TACの使い方の動画リンク(YouTube)

TACの使い方：第1回データ解析の準備と手順

https://www.youtube.com/watch?v=nruX6bil_L4

TACの使い方：第2回QC結果の確認

<https://www.youtube.com/watch?v=r9QvATvPgCE>

TACの使い方：第3回遺伝子発現解析の手順

https://www.youtube.com/watch?v=_9eDxzllsYI

TACの使い方：第4回TACの便利な機能

<https://www.youtube.com/watch?v=5ZbL0XZ7oCg>

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