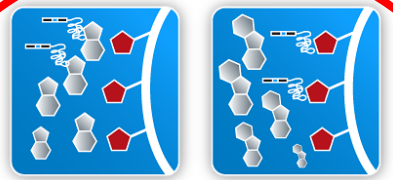
DiscoveRx

KINOMEscan

KINOMEscan— Robust Experimental Approach for Best-in-class Kinase Inhibitors

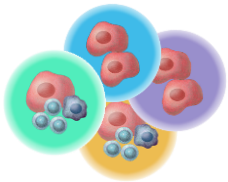
崔瑞廷, Tim
細胞影像分析工程師

DiscoveRx Technologies Platform



Quantitative Binding

Enzyme Complementation



Primary Human Cell Profiling

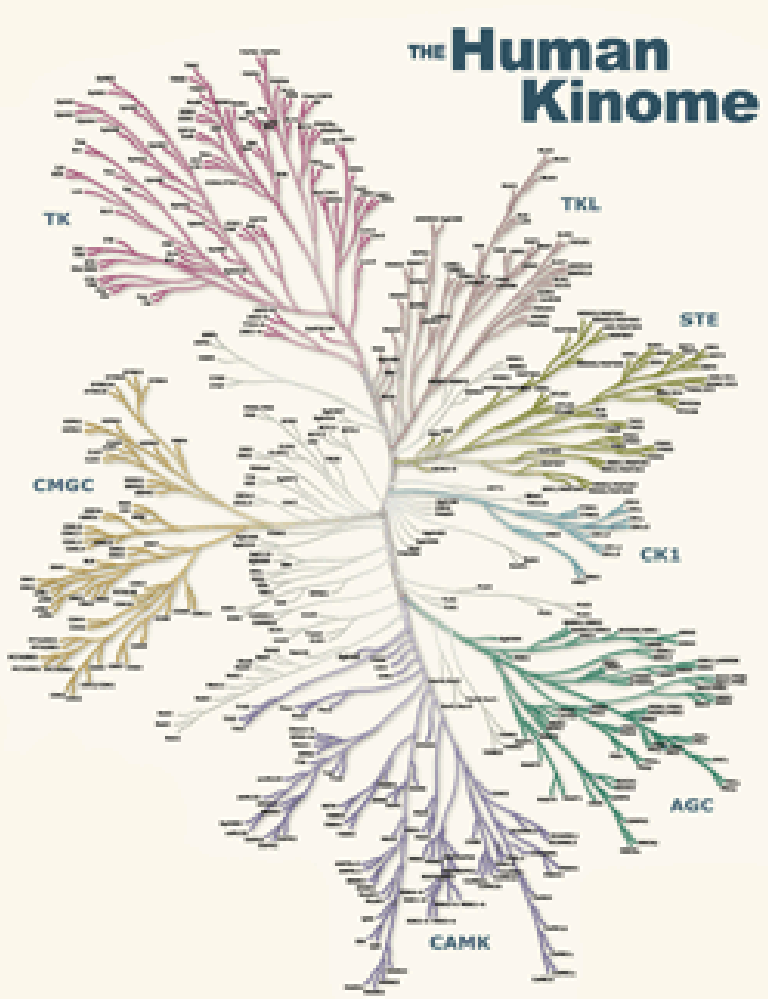
Products & Services Portfolio

Kits and Cell Lines

LeadHunter Services

Assay Development

Kinome



518 kinases have been identified divided into many groups

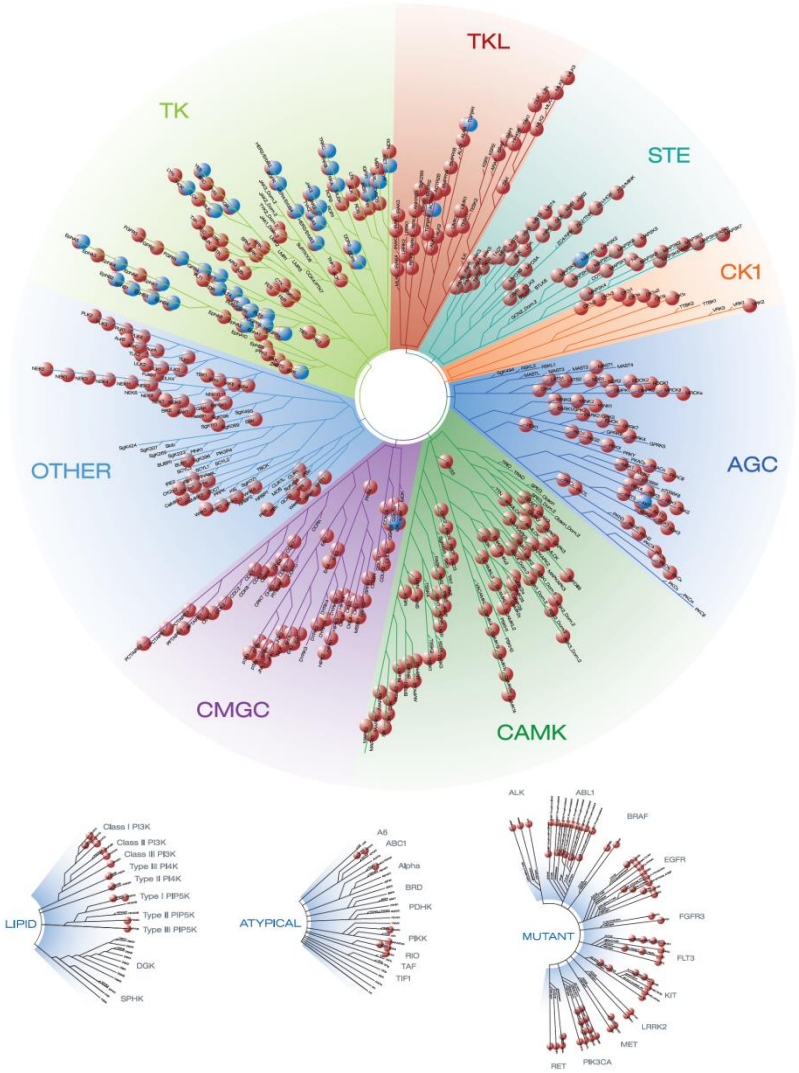
1.Serine Threonine Kinases
2.Tyrosine Kinase
3.Tyrosine Kinase-Like
4.CAMK (CALMADULIN dependent)
5.CMGC (Cyclin dependent)
6.AGC
7.CK1 (casein Kinase 1)
8.Atypical
9.Other

The Protein Kinase Complement of the Human Genome
G. Manning, D. B. Whyte, R. Martinez, T. Hunter, S. Sudarsanam
SCIENCE 2002 VOL 298

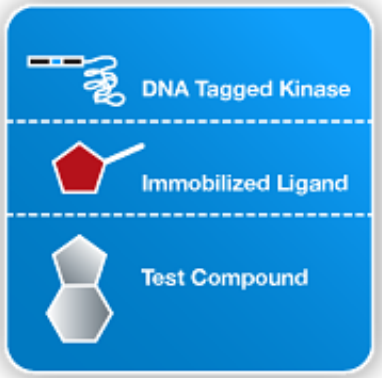
KINOME*scan*™ - World's Largest Kinase Panel

469 Kinase Assays (>90% Coverage)

- World's Largest Kinase Assay Panel
- 389 of the 518 distinct kinases
- 54 clinically relevant mutants
- 132 tyrosine kinase assays
- 20 lipid kinase assays
- >120 unique assays
- ***New Assays***
  - **SGK2 NEK10**
  - **NIK ALK(C1156Y) ALK(L1196M)**
- Custom assay development



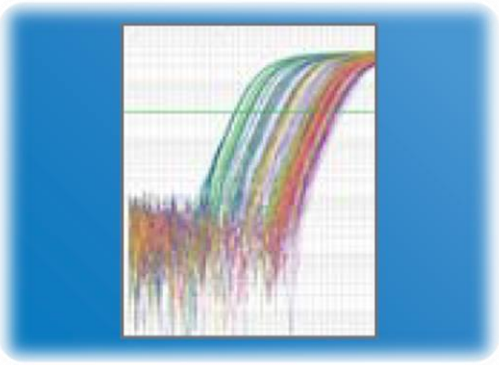
KINOME*scan* Technology: Active-Site Competition Binding Assays



Measure amount of kinase bound to immobilized ligand in the presence and absence of test compound

Competition

No Competition



Quantitate (RT-PCR)

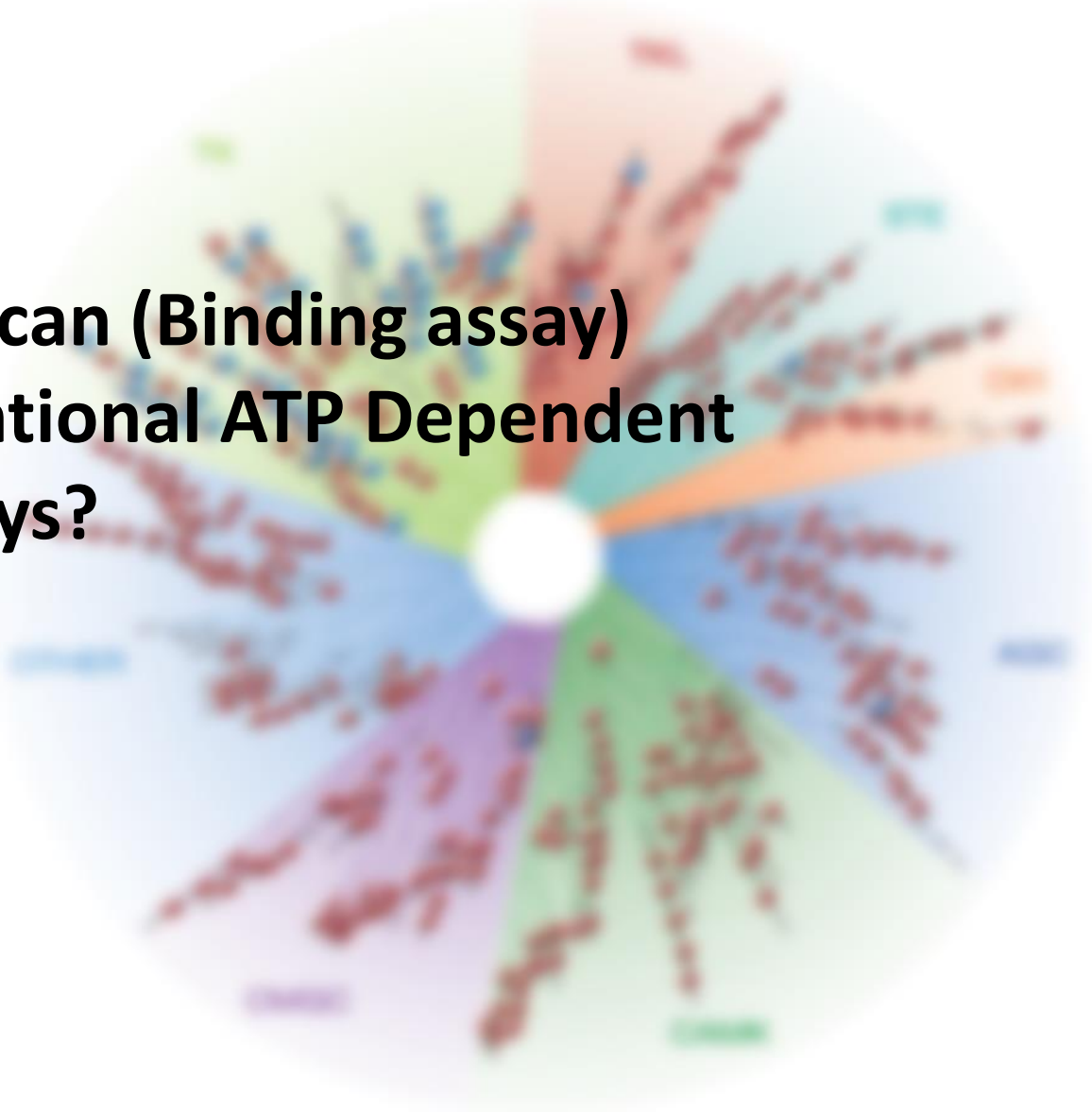
- Test Compound

+ Test Compound

Features and Benefits of KINOME*scan* Service

Features	Benefits
ATP-independent assay	Provides true binding constants & reduces variability
Measures thermodynamic K_d values as opposed to IC_{50}s	Enables inter-kinase inhibitor SAR analysis Robust interpretation of structural data
Unprecedented dynamic range (pM to mM)	Accurate potency rank ordering for high affinity inhibitors
No assay interference from fluorescent or colored compounds	Reliable screening of diversity decks and fragment libraries
Equally measure Type I and Type II inhibitors	Detect Type I and Type II inhibitors
Get structural insights from biochemistry	Structural classification of inhibitor binding mode without crystal structures Understand inhibitor binding kinetics

How Does Kinomescan (Binding assay) Compare to Conventional ATP Dependent Activity Based Assays?



Activity Assay Considerations

IC50 VS Kd Values

Feature	Binding Assay (Kd Values)	Activity Assay (IC50 Values)
Assays performed under the same conditions	Yes	No
Assay is independent of choice of substrate and ATP concentration	Yes	No
Detection of inactive and low activity kinases	Yes	No
Screening of activated and non-activated assay pairs; understand how phosphorylation state affects inhibitor affinity	Yes	No
Sensitivity & dynamic range	100 pM to > than 10 uM	No discrimination b/t cpds of different affinities below ~1 nM
Immune to assay interference from fluorescent or colored compounds	Yes	No

Relationship Between KINOMEscan

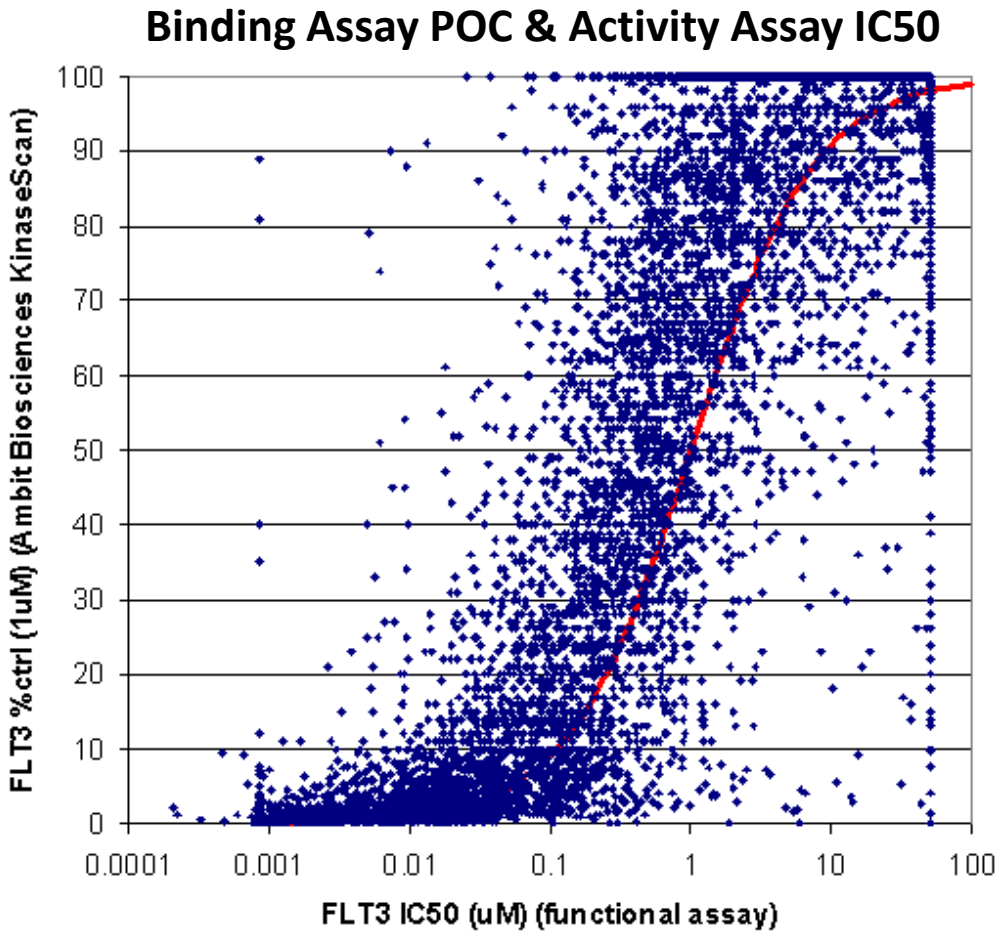


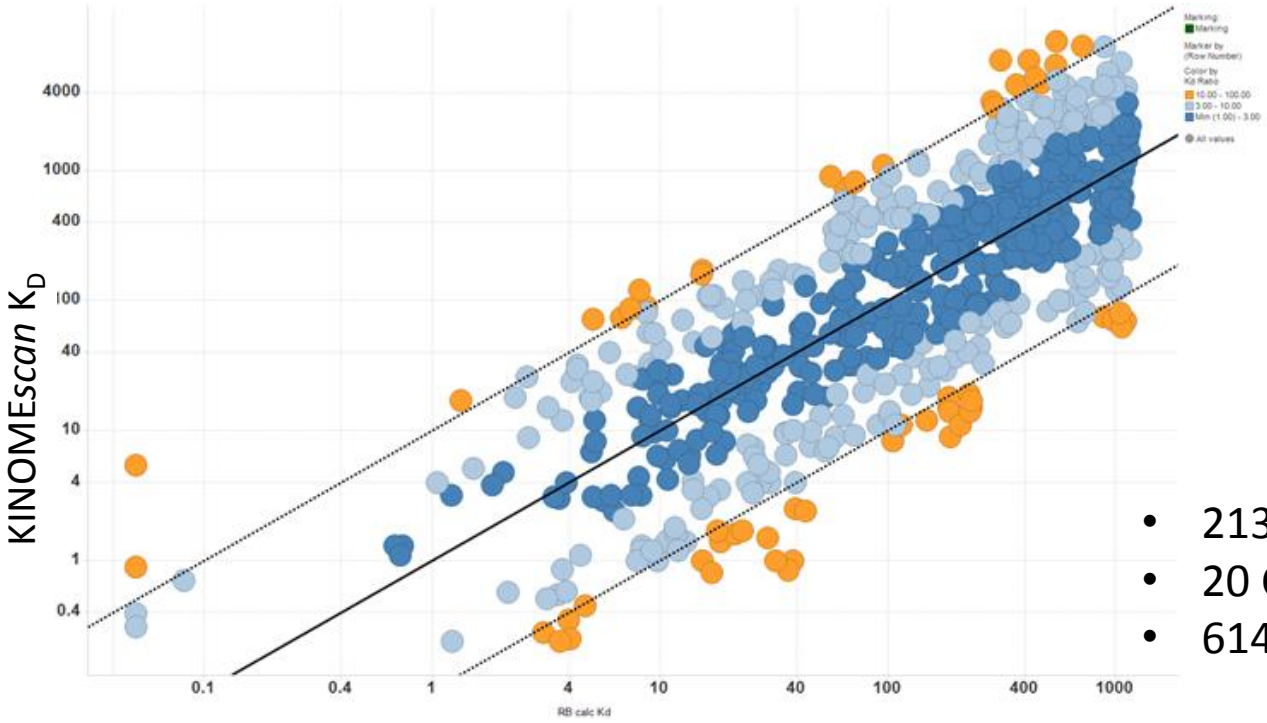
Figure 1, J. Med. Chem. 2011, 54, 54–66

Bristol-Myers Squibb

- BMS Project - 30,000 compounds against *scan*MAX panel (@ 1µM)
- POC and IC50 relationship for FLT3

Consistency Between Binding Assay & Activity Assays

KINOME*scan* K_D vs Reaction Biology calculated K_D values

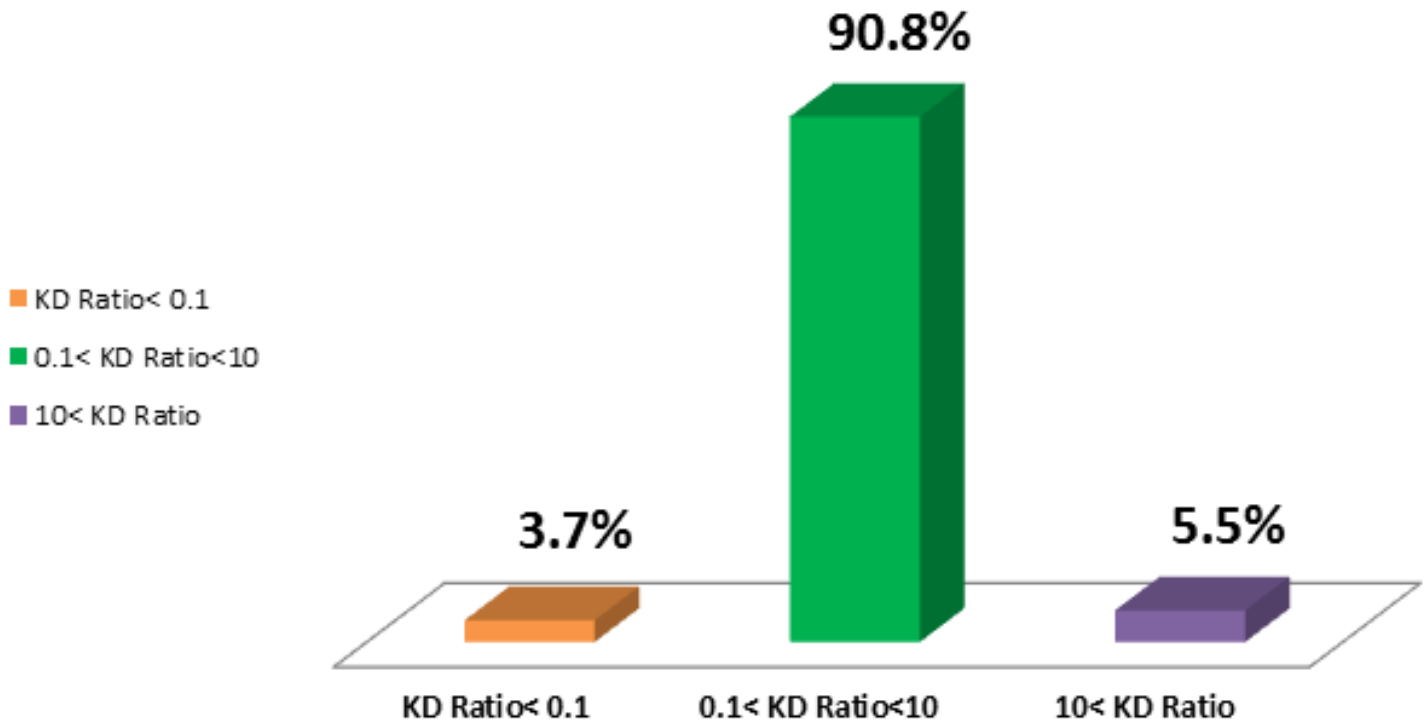


Reaction Biology calculated K_D

- 213 Kinases
- 20 Compounds
- 614 interactions

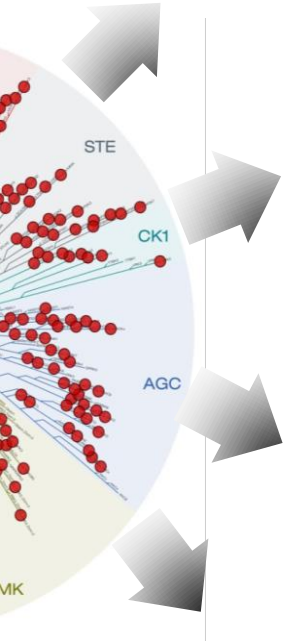
Consistency Between Binding & Activity Assays

Binned KINOME*scan* K_D Values *vs.* Reaction Biology calculated K_D.



Reaction Biology (activity assay) Kds (calculated from percent of control in a 500nM compound screen) were compared to KINOME*scan* (competition assay) measured KD data for >600 common interactions across >200 kinases, and the KD ratios are binned and presented. KDs are within 10-fold between assay formats for >90% of the interactions.
Note: Only Reaction Biology data for PoC<70 and KINOME*scan* KDs <40uM were used.

Advantages of KINOMEscan Service



Highly Validated

- Highly impactful papers with over 200 citations
- 250+ customer publications using KINOME*scan*

Quality

- Accurate, precise, reproducible
- Reference compounds and data

Quantity

- Largest target menu (468 kinase assays)
- Continuous expansion of assay panel
- High throughput capabilities

Flexibility

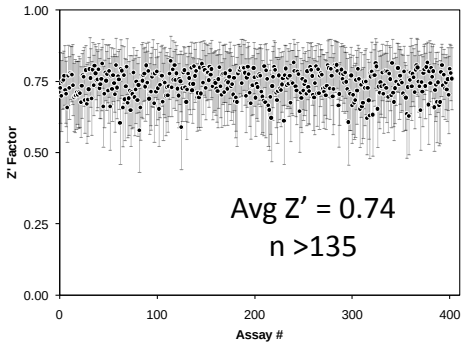
- Custom or pre-defined panels
- Custom assay development
- Flexible business models

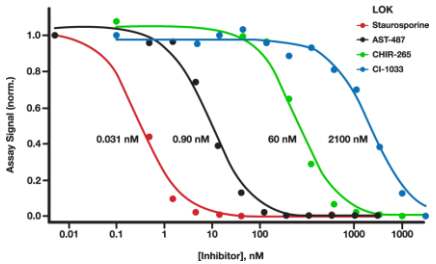
Speed

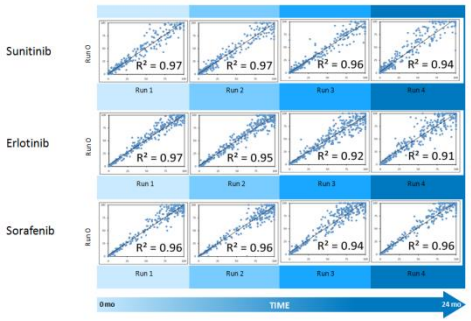
- Rapid turnaround (≤ 10 business days)

Expertise

- Direct access to drug discovery scientists







Beyond Selectivity & Potency
Tools to analyze mode of action

- Activated/non-activated assay pairs to elucidate compound binding mode

scanMODE™

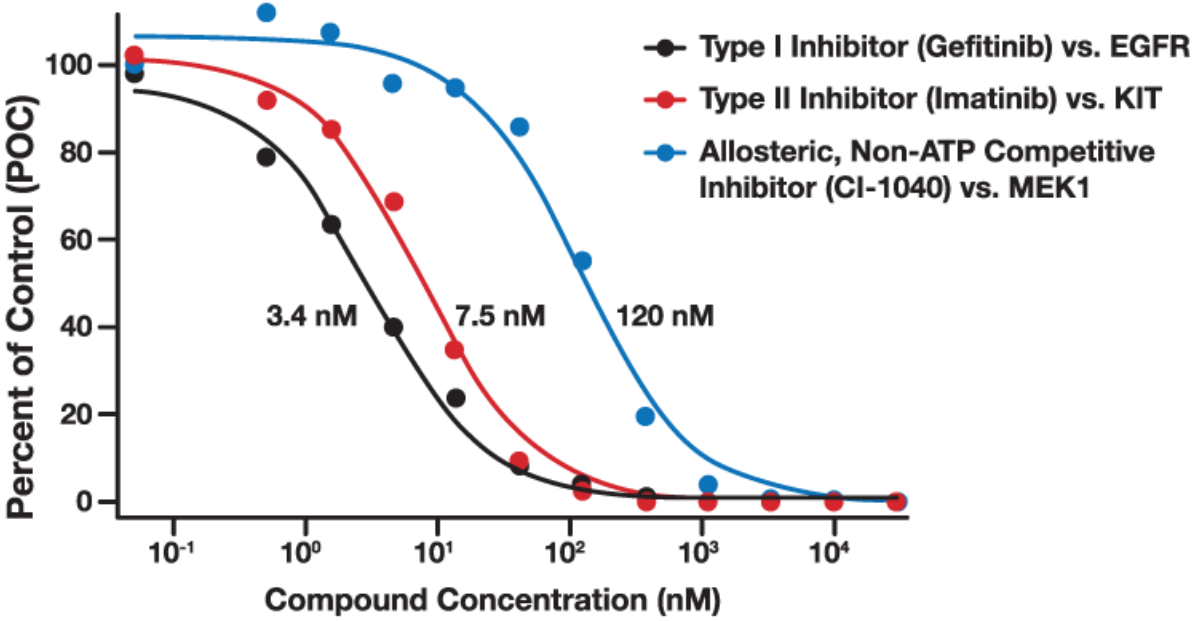
- Classify inhibitors as having:
  - "rapid" kinetics (equilibration in < 30 minutes)
  - "slow" kinetics (equilibration in > 30 minutes)
  - "irreversible" dissociation kinetics

scanKINETIC

The Importance of Understanding Inhibitor Binding Kinetics

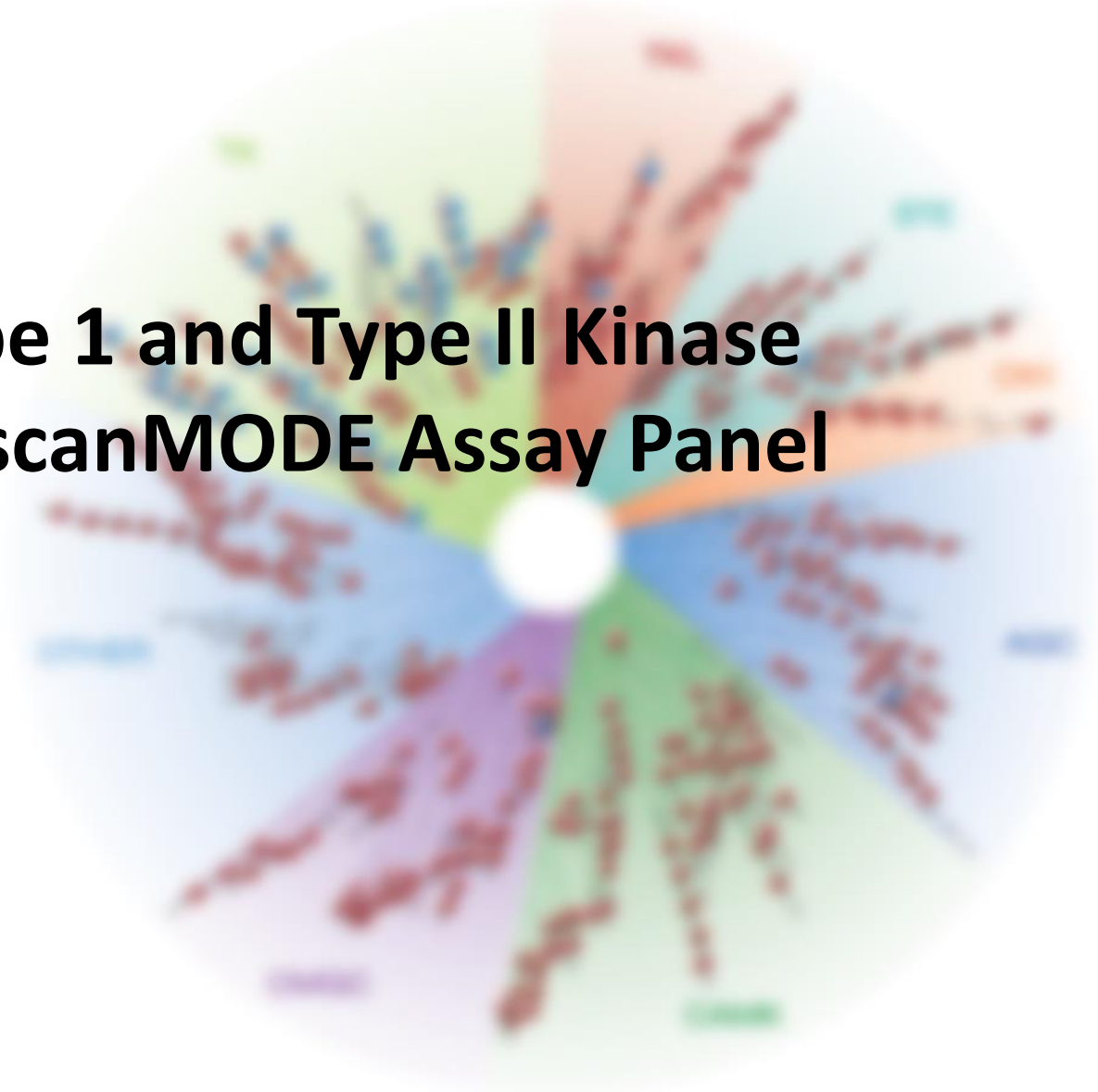
- **Inhibitors with slow binding kinetics can give misleading results in vitro & in vivo**
  - in vitro potency & selectivity
  - Cellular potency
  - Pharmacodynamics
- **Slow association kinetics can suggest non-Type I binding mode**
- **Classify inhibitors as having:**
  - “rapid” kinetics (equilibration in < 30 minutes)
  - “slow” kinetics (equilibration in > 30 minutes)
  - “irreversible” dissociation kinetics

Detect Multiple Inhibitor Types



- ATP competitive
  - Type I & II inhibitors
- Non-ATP competitive
  - Bind allosteric pocket within the kinase domain, distal to ATP site
  - Competitive with binding of protein/peptide substrate with allosteric effect on active-site conformation

Differentiate Type 1 and Type II Kinase Inhibitors using scanMODE Assay Panel



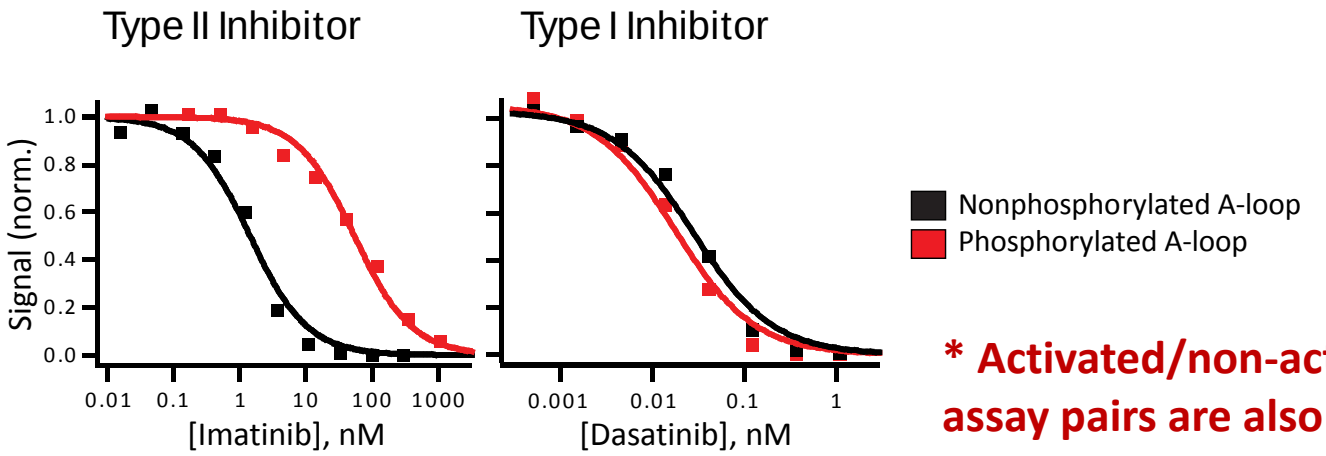
Binding Mode / Activation State

- **Type I Inhibitors**
  - Bind primarily within ATP site
  - Generally insensitive to kinase conformation/activation state
  - Kinome-wide selectivity difficult to optimize
  - Rapid target association/dissociation kinetics
  - Examples: dasatinib, sunitinib, erlotinib

- **Type II Inhibitors**
  - Bind within ATP site and access a distal "allosteric" pocket
  - Sensitive to kinase conformation/activation state: bind to "DFG-out" inactive enzyme conformation
  - Generally more selective than type I inhibitors
  - Slow target association/dissociation kinetics
  - Examples: imatinib, sorafenib, AC220

Mechanism of Action
*scan*MODE: Binding Classification

- **Activated/non-activated ABL assay pairs***
  - + or - phosphorylation of activation loop (A-loop)
- **Key Observations**
  - Type II inhibitor binding is activation state-dependent
  - Inhibitor binding mode is generally conserved across kinases
  - Many inhibitors have sufficient ABL affinity to qualify for scanMODE screens



*** Activated/non-activated ABL assay pairs are also in scanMAX**

*scan*MODE: Activation State-dependent Inhibitor Binding

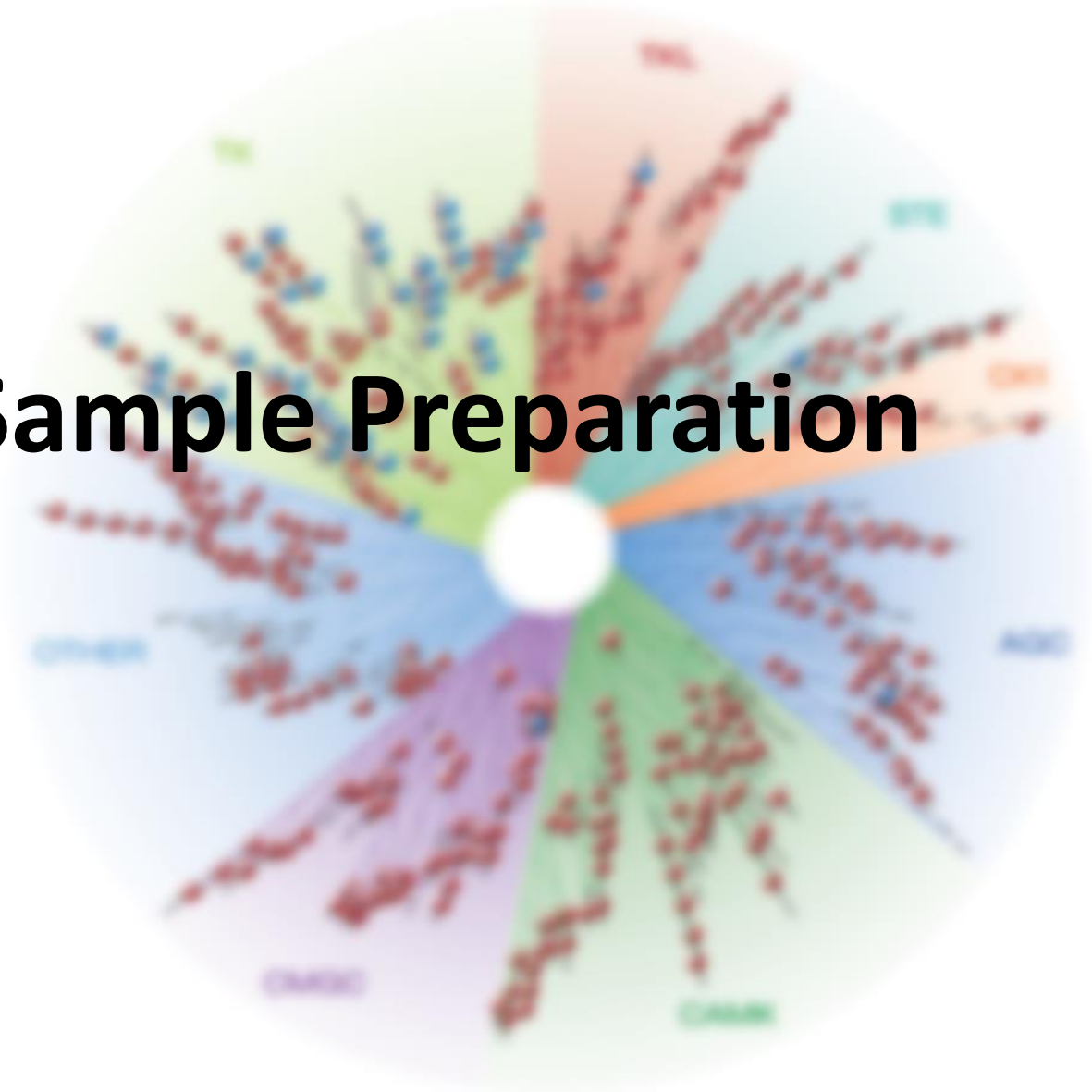
Kinase Target	PLX-4032 AC016623		
KINOMEscan Gene Symbol	%Ctrl @ 100nM	%Ctrl @ 1000nM	%Ctrl @ 10000nM
AAK1	86	89	86
ABL1(E255K)-phosphorylated	75	72	69
ABL1(F317I)-nonphosphorylated	70	80	26
ABL1(F317I)-phosphorylated	100	100	100
ABL1(F317L)-nonphosphorylated	100	100	41
ABL1(F317L)-phosphorylated	100	100	85
ABL1(H396P)-nonphosphorylated	59	49	7
ABL1(H396P)-phosphorylated	100	100	75
ABL1(M351T)-phosphorylated	100	100	68
ABL1(Q252H)-nonphosphorylated	85	46	7.8
ABL1(Q252H)-phosphorylated	100	100	76
ABL1(T315I)-nonphosphorylated	96	100	90
ABL1(T315I)-phosphorylated	100	100	100
ABL1(Y253F)-phosphorylated	100	100	54
ABL1-nonphosphorylated	100	76	10
ABL1-phosphorylated	100	100	71
CSF1R	77	61	5.4
CSF1R-JMplus	100	100	100
FLT3	64	14	1.6
FLT3-JMplus	100	100	100
KIT	49	8.7	0.1
KIT-JMplus	100	62	26

ABL1 pairs

RTKs

- *scan*MAX screens contain all of the assays that comprise our *scan*MODE offering
  - ABL1 phosphorylated and non-phosphorylated – Type I vs Type II inhibitors
  - Autoinhibited/non-autoinhibited PDGFR family RTKs – activation state-dependent inhibition

Report and Sample Preparation



KINOME*scan* Report

1. Percent Control (%Ctrl)

$$\%Ctrl = \left(\frac{\text{test compound signal - positive control signal}}{\text{negative control signal - positive control signal}} \right) \times 100$$

Kinase Target	Gleevec	GW-2016	SU-11248
KINOMEscan Gene Symbol	%Ctrl @ 10000nM	%Ctrl @ 10000nM	%Ctrl @ 10000nM
AAK1	34	75	0.85
ABL1	1.4	62	8.6
ABL1(E255K)	3.4	87	25
ABL1(H396P)	1.5	80	8
ABL1(M351T)	1.4	75	6.5
ABL1(Q252H)	1	84	12
ABL1(T315I)	34	79	0.1
ABL1(Y253F)	2.2	80	9.4
ABL2	0.4	77	20

KINOME*scan* Report

2. Selectivity Score (S-scores)

S = Number of hits / Number of assays

S(35) = (number of non-mutant kinases with %Ctrl <35)/(number of non-mutant kinases tested)

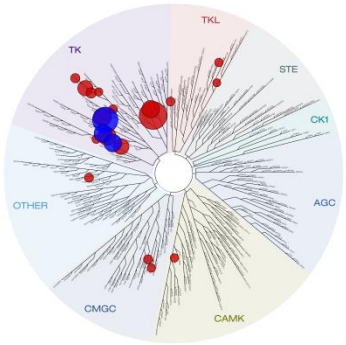
S(10) = (number of non-mutant kinases with %Ctrl <10)/(number of non-mutant kinases tested)

S(1) = (number of non-mutant kinases with %Ctrl <1)/(number of non-mutant kinases tested)

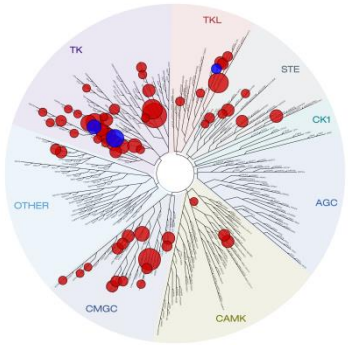
Compound Name	Selectivity Score Type	Number of Hits	Number of Non-Mutant Kinases	Screening Concentration (nM)	Selectivity Score
Gleevec	S(35)	41	290	10000	0.141
Gleevec	S(10)	19	290	10000	0.066
Gleevec	S(1)	7	290	10000	0.024
GW-2016	S(35)	6	290	10000	0.021
GW-2016	S(10)	3	290	10000	0.01
GW-2016	S(1)	2	290	10000	0.007
SU-11248	S(35)	182	290	10000	0.628
SU-11248	S(10)	140	290	10000	0.483
SU-11248	S(1)	82	290	10000	0.283

KINOME*scan* Report

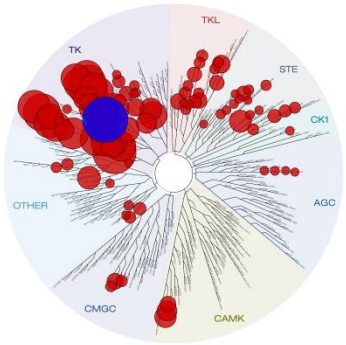
3. TREE*spot*™ *Interaction Maps*



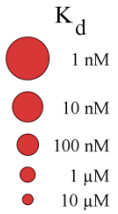
Imatinib
(Gleevec®)
Approved: ABL, KIT, PDGFR

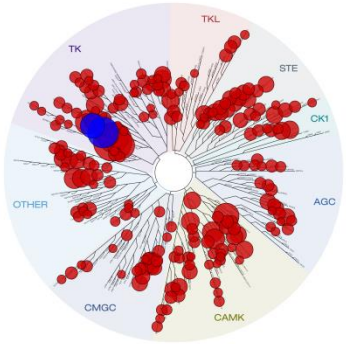


Sorafenib
(Nexavar®)
Approved: VEGFR2
Clinical: FLT3, BRAF

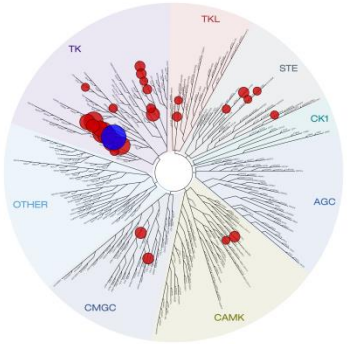


Dasatinib
(Sprycel®)
Approved: ABL





Sunitinib
Approved: VEGFR2, KIT



AC220
Clinical: FLT3

- Most potent & selective FLT3 inhibitor described to date
- Optimized “start to finish” using KINOMEscan™ technology
- Potency & selectivity assessed for all compounds during lead optimization

Various service panel

scanMAX™	Panel screen of world's largest kinase collection
scanEDGE™	Panel screen of 97 selected kinases
scanELECT™	Choose your kinases or build custom panels
k_dELECT™	Quantitative binding constants for any kinase
scanMODE™	Elucidate compound binding mode
scanKINETIC	Characterize compound binding kinetics

KINOME*scan sample preparation*

Complete Service Request Form (SRF)

1. **For Solids Complete:**
   - **µL of DMSO to add to make a 1000X stock solution: Enter microliters (µL) of DMSO required to make a 1000X stock solution for each Compound**
   - **Compound Weight: Enter Weight in milligrams (mgs) for each Compound - (optional)**

2. **For Liquids Complete:**
   - **Stock Concentration: For Liquids enter Stock Concentration (mM) for each Compound**
   - **Stock Volume: For Liquids enter Stock Volume (µL) for each Compound**

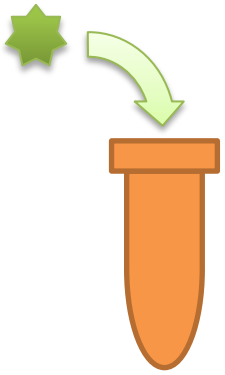
3. **For Plated Liquids Complete:**
   - **Well Location: Enter Well Location of each Compound**
   - **Plate ID #: Enter Plate ID Number for each compound**

KINOME*scan sample preparation*

Complete Service Request Form (SRF)

1. Solid

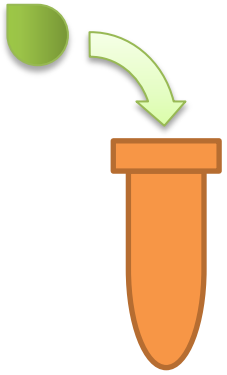
1- 2 mg



Inform:
µL of DMSO to get 10mM

1. Liquid

50 µL/ 10mM

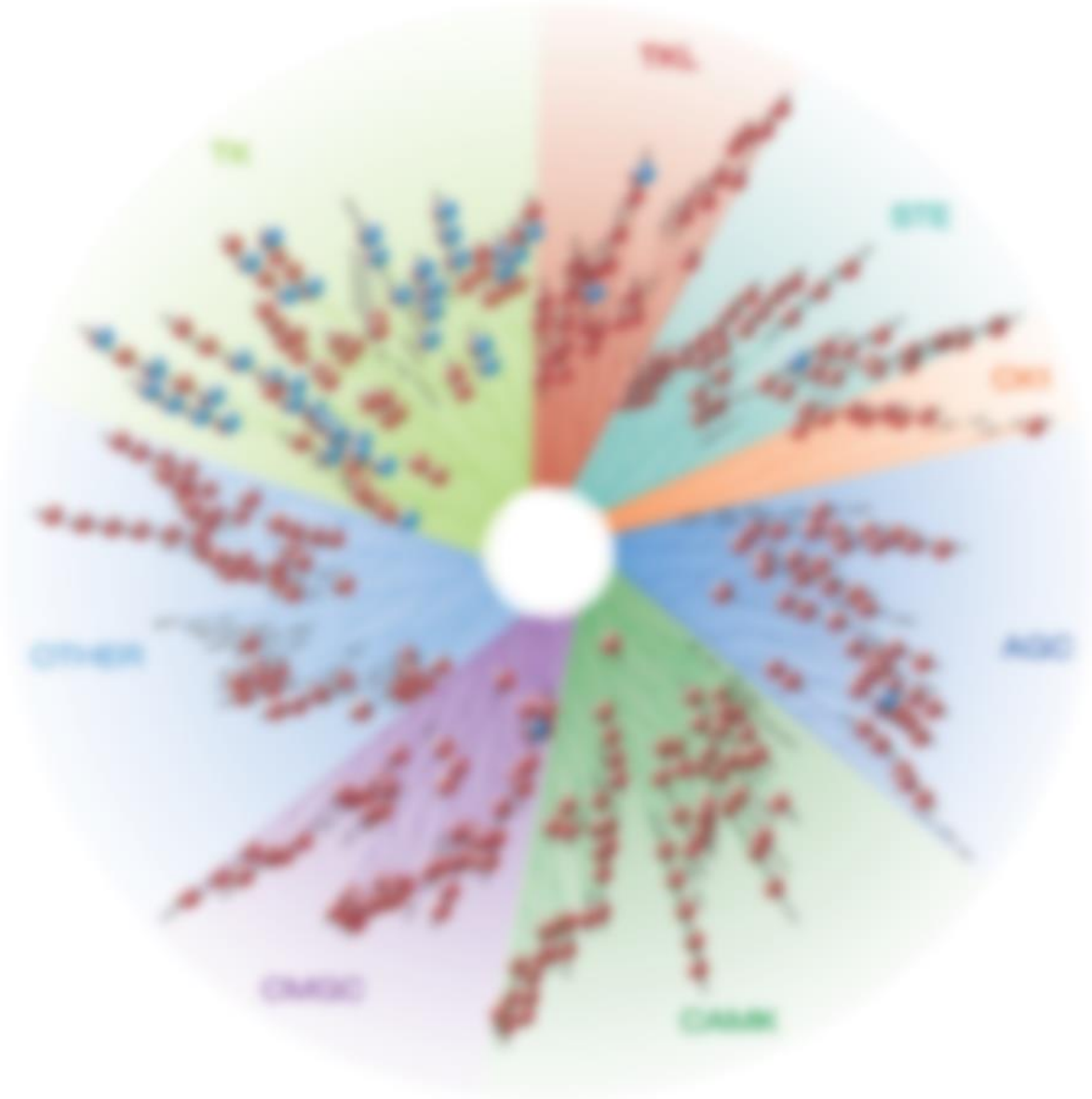


1. Plate Liquid

50 µL/ 10mM

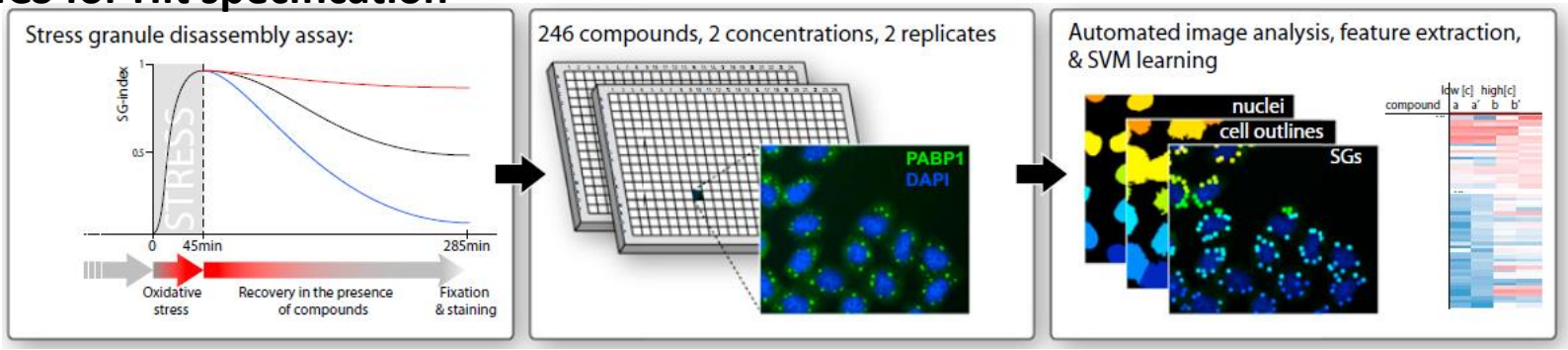
10 business day to turn around data after received compound

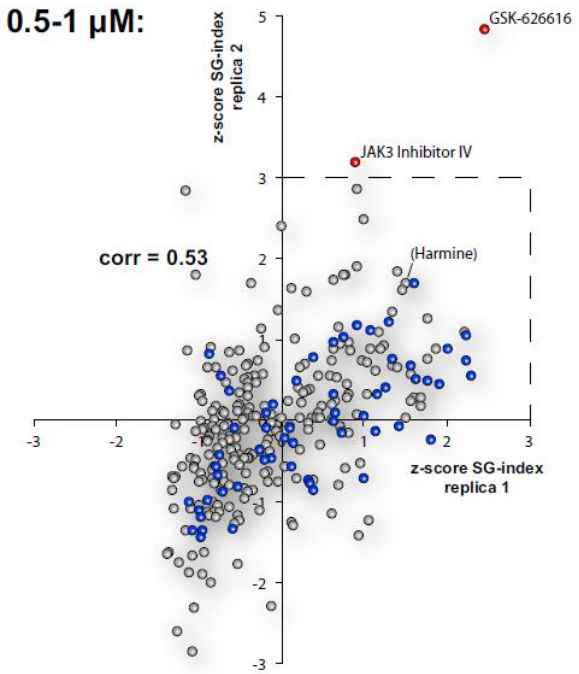
Case Study

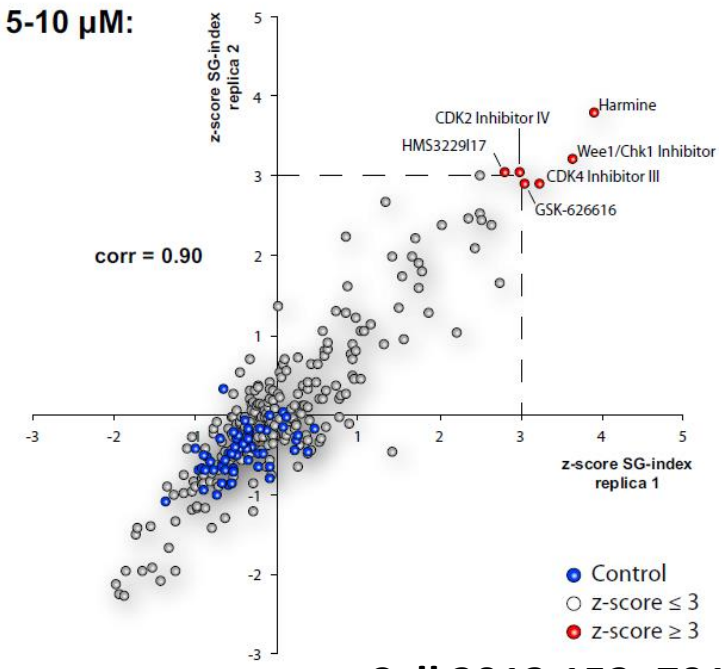


Drug Screening assay

HCS for Hit specification





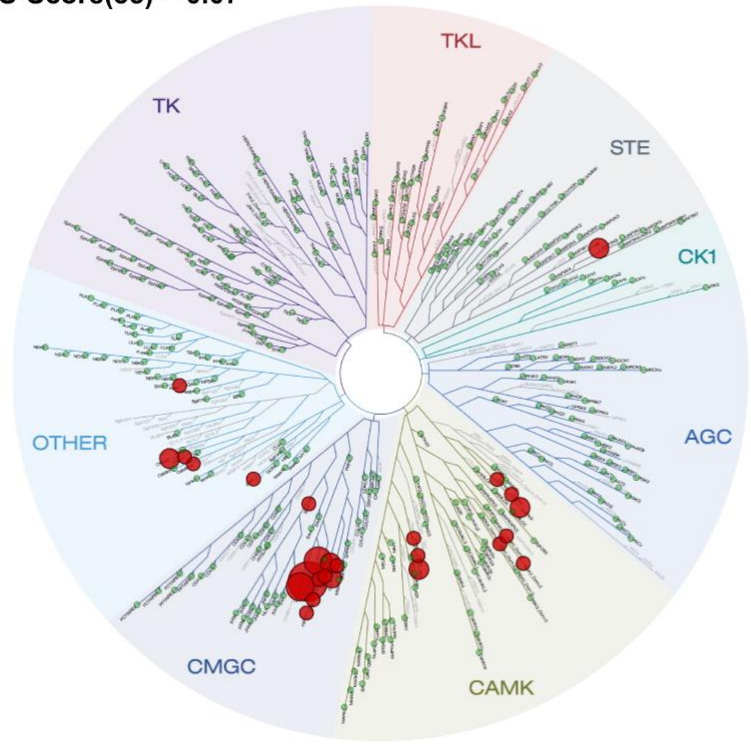


Cell 2013 152; 791–805

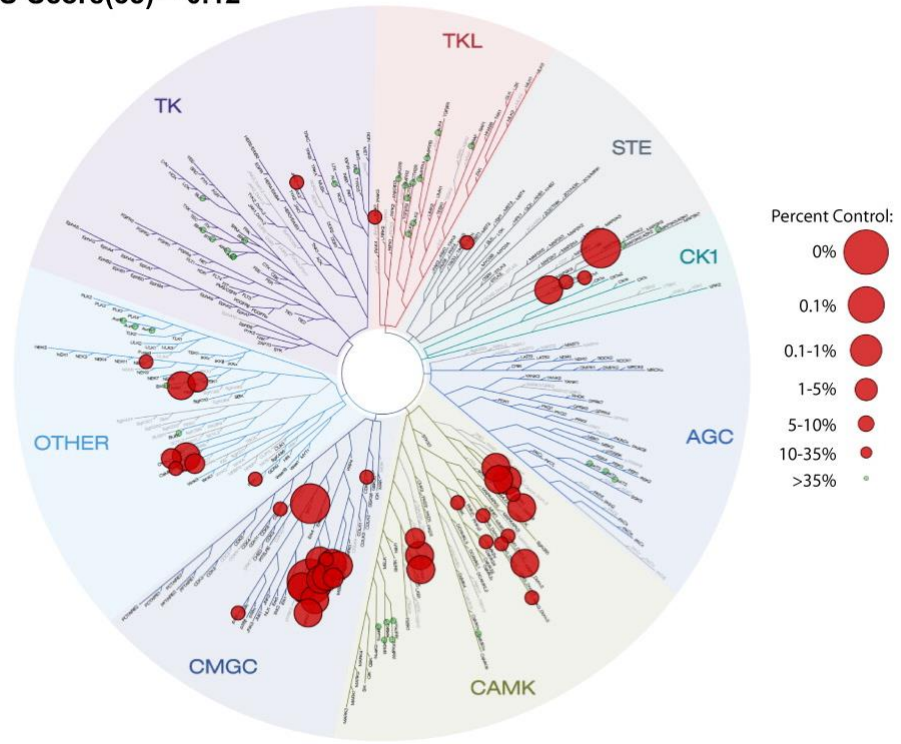
Drug Screening assay

KINOMEscan for target specification

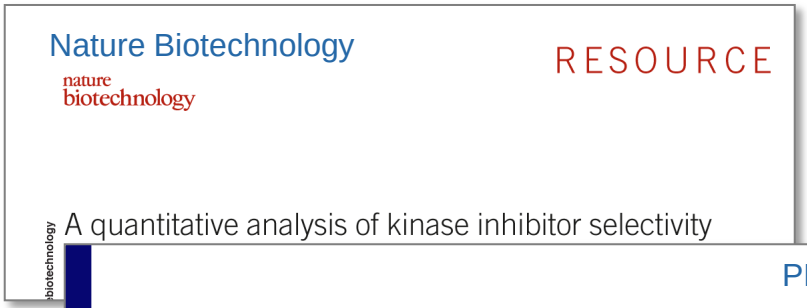
GSK-626616 0.1 μM
451 Kinase Assays Tested
S-Score(35) = 0.07

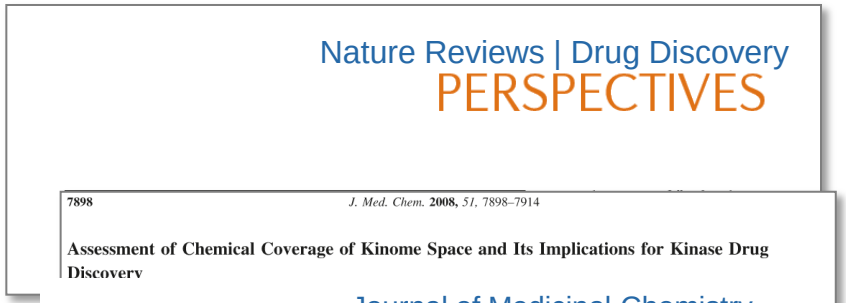


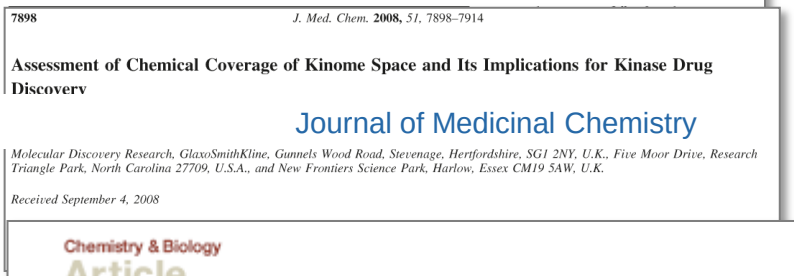
GSK-626616 1 μM
451 Kinase Assays Tested
S-Score(35) = 0.12

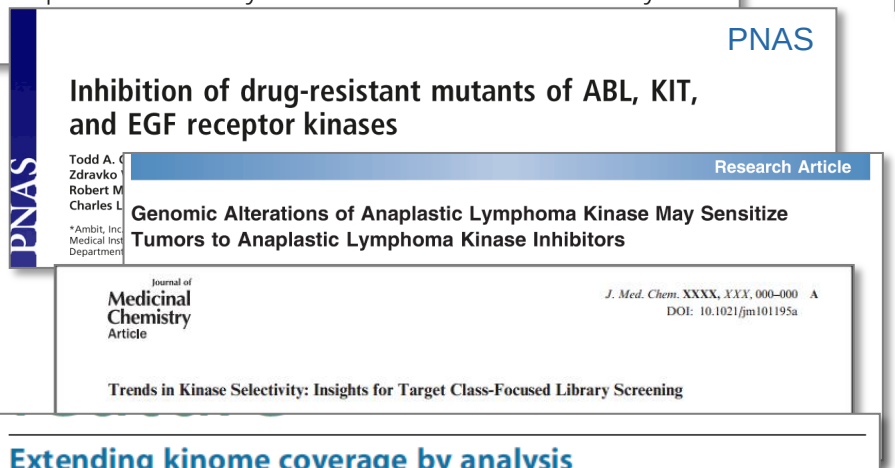


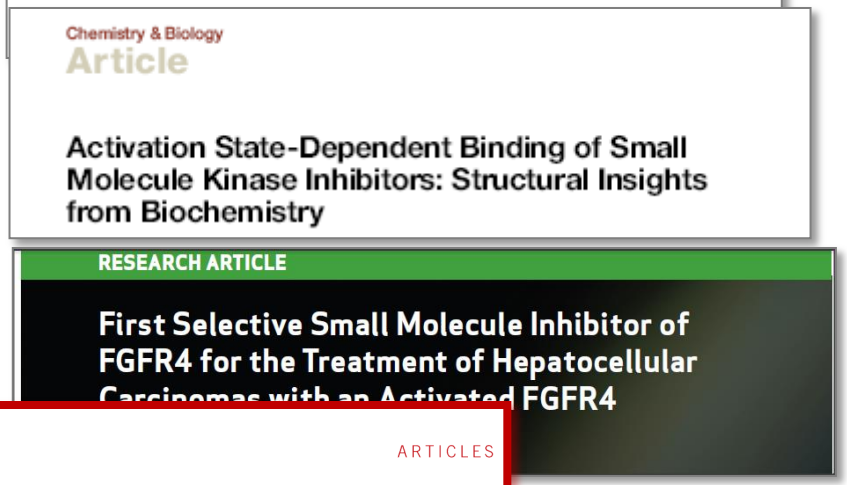
Publications

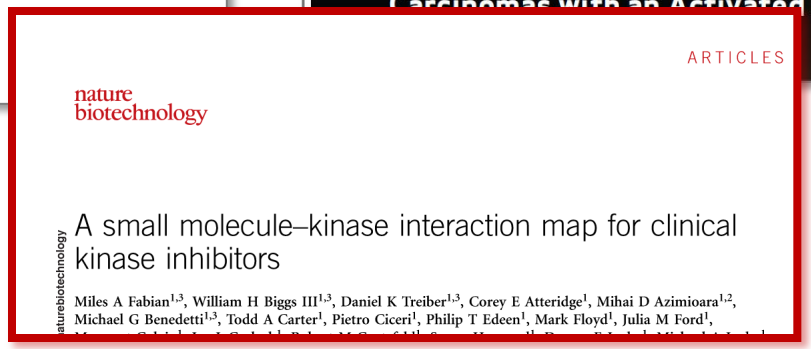












"…past 5 years' most highly cited research articles…"

Nature Biotechnology Volume 29 number 3 march 2012

Partner Publications

BMS

- 30,000 compound screen
- "…results support a scaffold-oriented approach for building compound collections to screen kinase targets."
- "…a reasonable relationship between single-concentration binding data and IC50 values from functional assays…"

Keyword: BMS pub

Janssen

- 3,300 compound screen
- Starting points for 6 disease area projects
- Confirmed previously identified HTS hits for 3 projects

Keyword: Janssen pub

Ambit

- Exclusively supported by KINOME*scan* (Technology validation)
- AC220 (Quirzartinib) in phase 3 clinical trials
- Acquisition in 2014 by Daiichi Sankyo

Keyword: KINOME*scan* pub

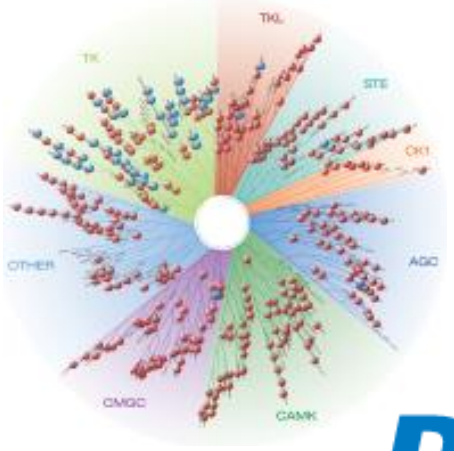
Blueprint Medicines

- "…a novel irreversible kinase inhibitor that specifically targets FGFR4 while sparing all other FGFR paralogs and demonstrates exquisite kinome selectivity…"

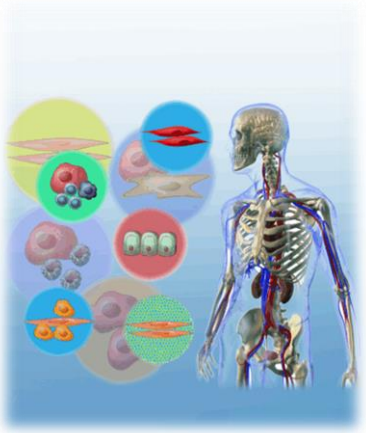
Keyword: blueprint pub

Total solution to in vitro drug development

KINOMEscan

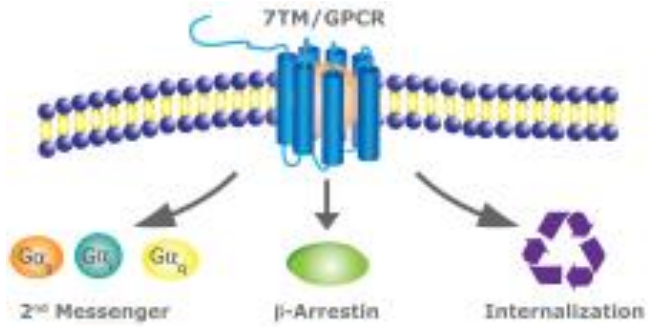


BioMAP

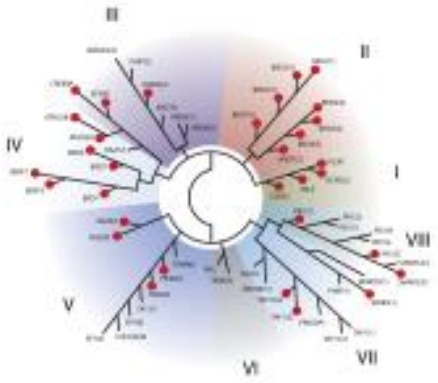


DiscoveRx

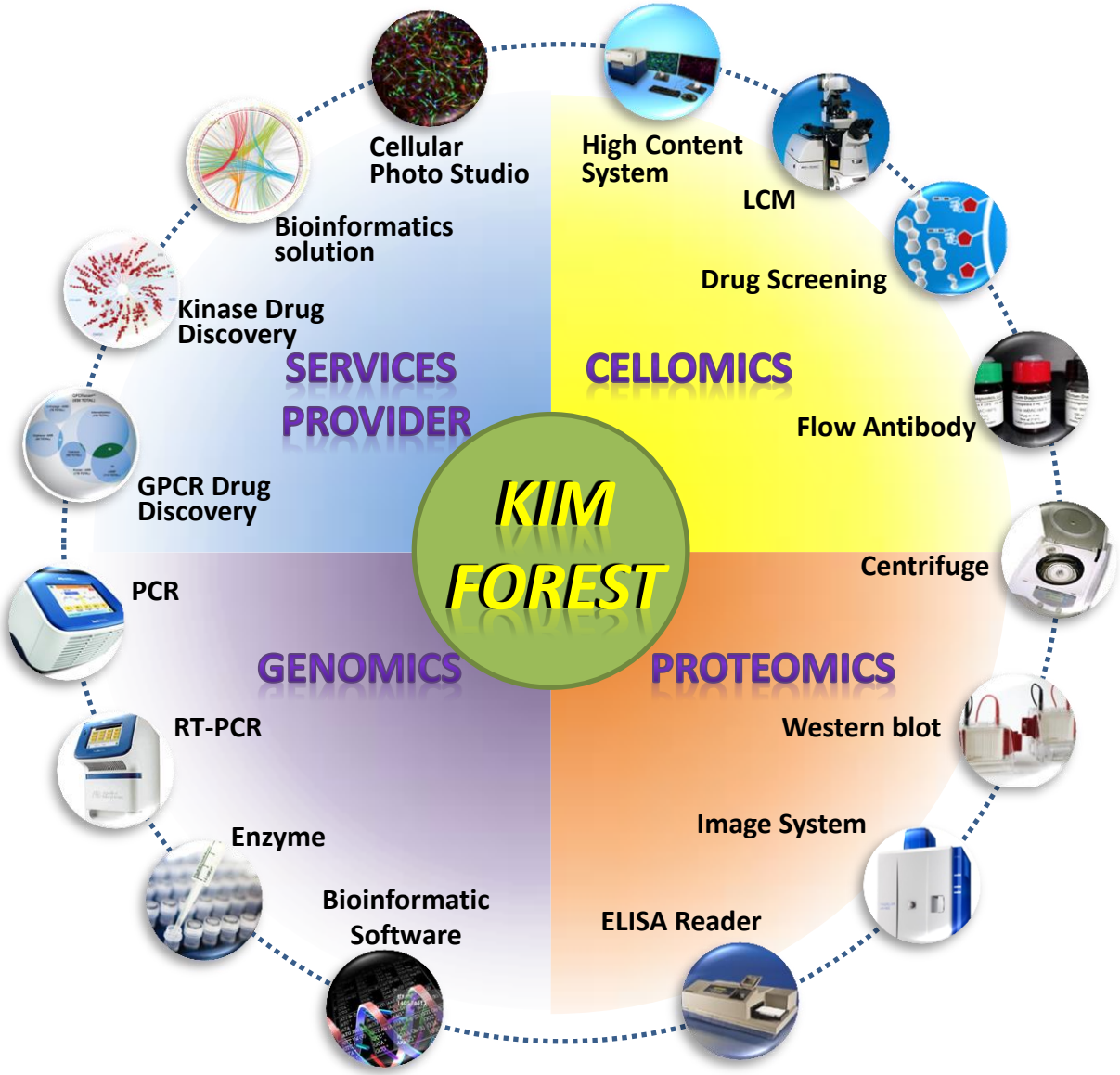
GPCRscan



BROMOscan



Our Company- Kim Forest



Thanks for your attention!

Contact

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DiscoveRx

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