NCGM
國家基因體醫學研究中心
National Center for Genome Medicine

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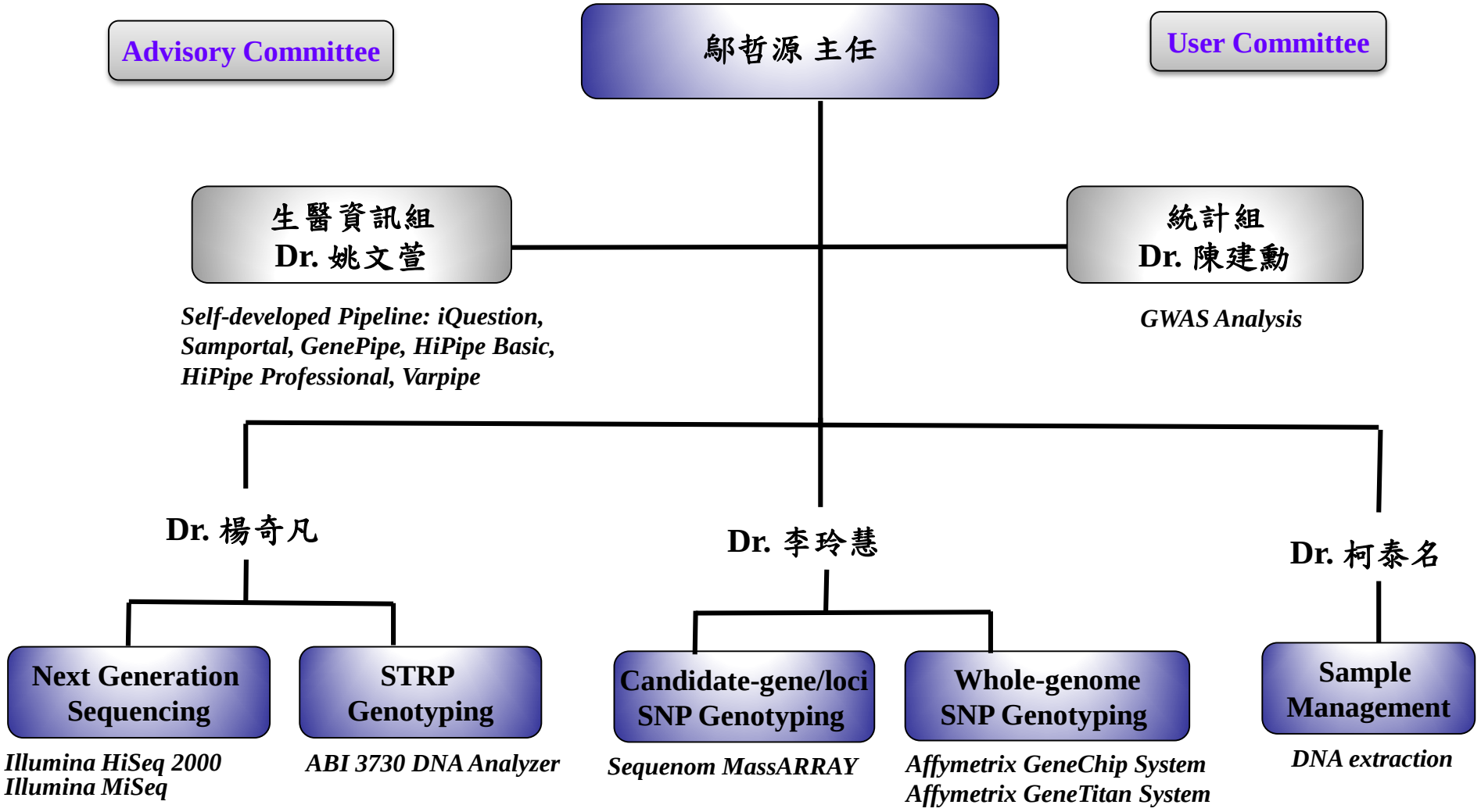
基因體醫學研究服務介紹

演講者：Dr. 楊奇凡

源起與目標

「基因型鑑定」與「基因體定序」是全面搜尋人類致病基因的重要分析技術。在科技部規劃的核心設施平台維運計畫之補助下，國家基因體醫學研究中心秉持著「提供與世界同步的高通量基因型鑑定及基因體定序服務」的理念，希望藉由SNP (Single Nucleotide Polymorphism)、STRP (Short Tandem Repeat Polymorphism)、Next Generation Sequencing及自行研發之高效能 NGS分析與註解平台，提供基因醫學研究相關領域的研究人員完整、大量、迅速、品管嚴格的基因體研究服務。期能從浩瀚的人類基因體內辨認出與致病及藥物感受性相關之基因，促進研究開發新穎診斷方法及治病新藥，達到「個人化醫療」、「轉譯醫學」及「精準醫學」，提高預防醫學及增強國人的健康保護。

組織架構


Advisory Committee
鄔哲源 主任
User Committee
生醫資訊組
Dr. 姚文萱
統計組
Dr. 陳建勳
Self-developed Pipeline: iQuestion, Samportal, GenePipe, HiPipe Basic, HiPipe Professional, Varpipe
GWAS Analysis
Dr. 楊奇凡
Dr. 李玲慧
Dr. 柯泰名
Next Generation Sequencing
STRP Genotyping
Candidate-gene/loci SNP Genotyping
Whole-genome SNP Genotyping
Sample Management
Illumina HiSeq 2000
Illumina MiSeq
ABI 3730 DNA Analyzer
Sequenom MassARRAY
Affymetrix GeneChip System
Affymetrix GeneTitan System
DNA extraction

ISO/IEC 17025認證實驗室

- 本中心於2012年8月14日通過全國認證基金會（TAF）ISO/IEC 17025實驗室認證。
- 獲得認證之服務項目：ENU Mouse Gene Mapping Panel (C2-7), iPLEX SNP Genotyping (C2-10), Mouse Speed Congenics Panel (C2-12), Affymetrix Genome-Wide Human SNP 6.0 (C2-14), PCR Product STRP Genotyping (C2-4)。
- TAF為ILAC (the International Laboratory Accreditation Cooperation, 國際實驗室認證聯盟) 相互承認協議之簽屬會員，ILAC同意TAF使用相互承認協議標記 (ILAC-MRA MARK)，本中心亦在TAF審核同意下，可使用此標記於檢測報告上，代表本中心的檢測能力已達到國際水準，並與全球 60個國家、73個認證機構之認可實驗室具有技術同等性。
- 本中心秉持「技術卓越、品質穩定、迅速精確、客戶至上」之品質政策，提供完整、大量、迅速、品管嚴格的基因體研究服務，使基因體醫學研究相關領域的研究人員達到最大的研究效益，並獲得具有公信力的檢測報告，以供申請專利、新藥或新醫材等的重要資料及依據。

Technology & Platform

- Sample Management
  - Samportal
  - DNA extraction
- SNP Genotyping
  - Fine mapping
    Sequenom MassARRAY System
  - Human whole-genome scan
    Affymetrix GeneChip
    Affymetrix GeneTitan
- STRP Genotyping
- Next Generation Sequencing
  - illumina HiSeq 2000
  - illumina MiSeq
- BioIT Services: iQuestion, GenePipe, HiPipe Basic, HiPipe Professional, Varpipe
- Statistical Analysis: GWAS

Human Whole-Genome Genotyping using Affymetrix GeneChip System / Affymetrix GeneTitan System



Affymetrix GeneChip System



Affymetrix GeneTitan System

Affymetrix Human Whole-Genome Genotyping Arrays (Statistical analysis included)

	ASI	CHB	TWB	SNP 6.0
Technology platform	Affymetrix GeneTitan	Affymetrix GeneTitan	Affymetrix GeneTitan	Affymetrix GeneChip
Population	Asian	Han Chinese in Beijing, China	Han Chinese	all
SNP probes	600,307	642,832	653,291	906,600
DNA required	300 ng (15 ng/uL*20 uL)	300 ng (15 ng/uL*20 uL)	300 ng (15 ng/uL*20 uL)	500 ng (50 ng/uL*10 uL)
Average call rate	99.63%	99.54%	99.69%	98.80%
Method	Ligation	Ligation	Ligation	Hybridization
CNV probes	none	none	none	946,000
Throughput (samples/week)	285	285	285	36
DNA source	cell lines, blood, and saliva	cell lines, blood, and saliva	cell lines, blood, and saliva	cell lines and blood
Service code	C2-15	C2-16	C2-42	C2-14

APPLICATIONS

SNP Genotyping using Human-Whole Genome Genotyping Arrays

- Linkage analysis
- Whole-genome association study
- Population genetics
- Chromosomal copy number analysis
- Cancer genetics
- Linkage disequilibrium

Candidate gene/loci SNP Genotyping using Sequenom MassARRAY System



- **MALDI-TOF (Matrix-Assisted Laser Desorption / Ionization Time-Of-Flight) Mass Spectrometry based platform**
- **Multiplex assay design: cost effective solution**

APPLICATIONS

SNP Genotyping using Sequenom MassARRAY System

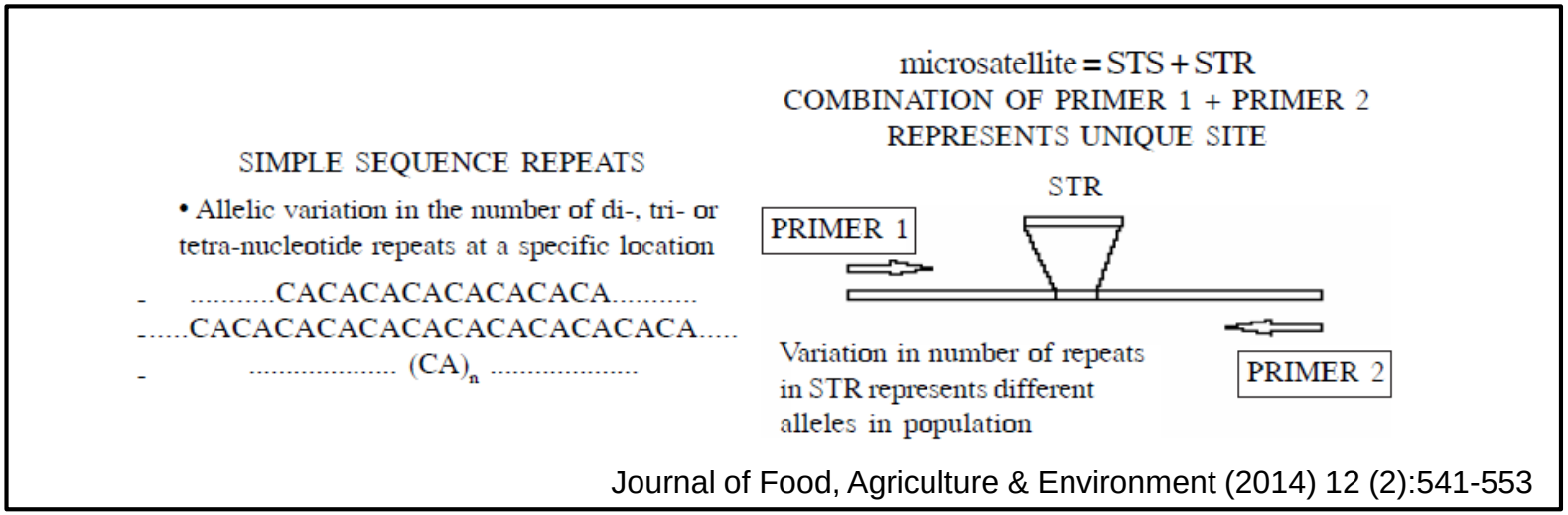
- High-throughput genotyping of customer selected SNPs.
- Fine mapping of SNPs in candidate genes or pre-localized regions for both human and mouse samples.
- Whole-genome SNP genotyping for disease gene mapping in ENU study and speed congenics mouse model establishment.

ABI 3730 DNA Analyzer



APPLICATIONS

STRP Genotyping



Journal of Food, Agriculture & Environment (2014) 12 (2):541-553

- Whole genome linkage analysis
- PCR fragment analysis
- Kinship analysis: most commonly in paternity testing
- Forensic identification
- Genetic improvement (strain improvement)
- Animal breeding
- Examination of genetic diversity and phylogeny
- Conservation of biodiversity

Next Generation Sequencing

(提供 16種定型化分析流程)





	illumina HiSeq 2000	illumina MiSeq				
Sequencing Chemistry	Sequencing by synthesis (Reversible terminator)					
Amplification	Bridge amplification					
Read Length	100 bp x 2 (Paired-end)	1x50 bp	2x150 bp*	2x250 bp**	2x300 bp	150 bp
No. of Reads/ Flow Cell	175-200 million / lane (8 lanes/Flow Cell)	12~15 million			20~25 million	
Output/run	480-600 Gb / 2 Flow cells	600~750 Mb / FC	3.5~4.5 Gb / FC	6~7.5 Gb / FC	12~15 Gb / FC	3~3.8 Gb / FC
Multiplex	12 barcodes	~ 96 barcodes				
Instrument Run Time/run	13 days	1 day	1 day	2 days	3 days	1 day
Data Processing /run	2.5 - 4 days (depends on project)	2.5 - 4 days (depends on project)				

* 2x150 bp Nano: 0.8~1 million (240~300 Mb / FC); Micro: 3~4 million (1~1.2 Gb)

** 2x250 bp Nano: 0.8~1 million (400~500 Mb / FC)

APPLICATIONS
Next Generation Sequencing

illumina HiSeq 2000



- Genome re-sequencing
- *de novo* sequencing
  for small genome
- Transcriptome sequencing
  - mRNA, stranded
  - rRNA-depleted, stranded
- Exome sequencing
- ChIP sequencing
- RNA IP sequencing
- Ribosome profiling
- Small RNA sequencing
- Methylation sequencing (coming soon)

illumina MiSeq



- *de novo* sequencing for small genome
- Whole genome re-sequencing for small genome
- Metagenomics (16s rRNA sequencing)
- Targeted re-sequencing (two-step PCR amplification, gene panels, Nextera XT tagmentation)
- Small RNA sequencing

Next Generation RNA Sequencing

Application	Transcriptome	Small RNA	RNA IP
Sample source	Total RNA	Total RNA	RNA IP
Sample requirement-Amount and Conc.	① Agilent Bioanalyzer Amount: 100ng ~ 2μg Conc.>50 ng/μL RNA Integrity Number (RIN) > 8	① Agilent Bioanalyzer Amount: >3μg Conc.>100 ng/μL RNA Integrity Number (RIN) > 8	① Agilent Bioanalyzer Amount:>10ng Size> 100 nt
Sample requirement-OD ratio	② NanoDrop OD260/280: 1.8~2.0 OD260/230: 1.8~2.2		
Sample requirement-others	③ DNase treated ④ No contaminants such as organic solvent, inhibitors, etc. ⑤ In RNase-Free Water (DEPC Water)		
Service Code for library prep	1) C2-36 mRNA sequencing sample preparation 2) C2-37 rRNA-Depleted RNA sequencing sample preparation	C2-45 Small RNA sequencing sample preparation	C2-36 mRNA sequencing sample preparation
Platform	HiSeq	HiSeq or MiSeq	HiSeq or (MiSeq)

Next Generation DNA Sequencing

Application	Genome Resequencing	Exome Sequencing	CHIP sequencing	Targeted Resequencing	
Sample source	Genomic DNA	Genomic DNA	CHIP DNA	Amplicon	Amplicon
Sample requirement-Amount and Conc.	① Qubit Amount: 100ng~1μg Conc.>100 ng/μL	① Qubit Amount: > 3 μg Conc.>100 ng/μL	① Qubit & Agilent Bioanalyzer Amount: > 10 ng Size: 150~500 bp	① Qubit Amount: 100ng~1μg Conc.>100 ng/μL	① Qubit Amount: > 10 ng Conc.>15 ng/μL
Sample requirement-OD ratio	② NanoDrop OD260/280: 1.8~2.0 OD260/230: >1.7				
Sample requirement-others	③ RNase treated ④ No degradation of gDNA (examined by 1% agarose gel electrophoresis) ⑤ No contaminants such as organic solvent, inhibitors, etc. ⑥ In molecular grade water or 10 mM Tris-HCl (pH 7.5–8.5)				
Service Code for library prep	1) C2-27 Human whole genome sequencing, 30X 2) C2-29 DNA sequencing sample preparation	C2-48 SureSelect Exome Enrichment (Human/Mouse)	C2-50 ChIP sequencing sample preparation	C2-29 DNA sequencing sample preparation	C2-51 DNA library sample preparation by Nextra XT
Platform	HiSeq or MiSeq	HiSeq	HiSeq	MiSeq	MiSeq

Next Generation Sequencing

(提供 16種定型化分析流程)

Transcriptome	Small RNA	RNA IP	Genome Resequencing	Exome Sequencing	Targeted Resequencing	CHIP sequencing
Differential Expression Analysis Gene fusion detection variant detection De novo assembly	Index trimming, QC, Grouping (read-count format), Alignment, Filter (rRNA, tRNA, mRNA, sRNA, genome), Expression Level, isomiR identification, Novel miRNA identification, Differential Expression	peak calling peak annotation	DNA basic analysis: mapping, Variant calling, annotation of SNVs and indels (Gene information, location in coding, UTR, or intron regions, splice site change, nonconservative/ conservative amino acid substitution, related known disease, affected protein domain, predicted splicing regulation), novel SNVs/indels detection / annotation, and exome CNV detection. Advanced analysis: customized grouping analysis. LOH analysis Amplicon and whole genome CNV detection Low frequency mutation detection			peak calling motif search peak annotation

HiPipe Professional

http://hipipe.ncgm.sinica.edu.tw/workspace



- 於 2015年 11月上線開放測試
- 具帳號與權限管理功能
- 提供 7種常用的 NGS分析流程
- 可進行多樣本分析
- 可與合作夥伴進行資料分享
- 結合軟硬體提供雲端與 Onsite分析解決方案

NGS分析服務量

分析項目		樣本數
DNA	全基因體變異點偵測	147
	Exome & Panel變異點偵測	2011
	CNV、LOH偵測	702
	低頻度變異點偵測	287
RNA	基因表現量差異分析	213
	基因融合與 miRNA分析	65
	變異點偵測	45
其他	*de novo*序列組裝	9
	ChIP, Aptamer分析等	187
總和 (2011/10 ~ 2016/11)		3666

Quality Control Measures



- **SNP**：每樣本進行 8 個standard assay 測試，成功率須達95%。
- **STRP**：樣本由用戶自行準備 (PCR)並且測試。
- **AFFY**：
  ① 使用NanoDrop® ND-1000/ND-8000 Spectrophotometer 測量樣本濃度、OD 260/280 與 OD 260/230。
  ② 進行DNA電泳確定樣本無高度降解之情形。
- **NGS**：
  ① DNA樣本使用NanoDrop® ND-1000 Spectrophotometer 測量樣本OD 260/280與 OD 260/230，以Qubit 測量dsDNA 濃度，進行DNA電泳確定樣本無高度降解之情形。
  ② RNA樣本以Agilent Bioanalyzer估算樣本濃度, RIN值 與 28S/18S rRNA比值，並確認RNA無高度降解之情形。

- **線上服務系統**

協助用戶線上申請NCGM各項基因體鑑定服務、追蹤實驗進度、下載實驗結果，隨時隨地掌握最新相關資訊！

- **設置無塵室**

樣品及Pre-PCR於無塵室操作，避免交互污染。

- **實驗室環境監控**
- **平台穩定性及儀器性能監控**

- **SNP**：協助用戶使用primer比對軟體，以剔除不適合的assay ，提高成功率

- Hardy-Wienberg equilibrium test
- 計畫整體成功率計算
- 個別assay成功率計算
- 低成功率樣本篩選
- 低成功率樣本品質鑑定
  以釐清樣本是否有污染問題
- 低成功率assay篩選
- NGS: Q30%和 Error rate

- **SNP**：每實驗盤放置positive 及 negative control 各一。
- **STRP**：每實驗盤放置兩個positive control。
- **AFFY**：
  SNP 6.0：
  ① PCR反應及實驗過程放置negative control，監控過程是否有污染。
  ② 以spiked hybridization control oligonucleotide 監控晶片與樣本雜交反應的效率
  ③ Shared SNP probes可監控實驗過程中是否發生樣本交叉污染或樣本互換。
  Axiom: 每盤放置reference Genomic DNA，監控實驗流程及實驗結果。
- **NGS**:
  ① 每個 lane 中放入1% control (Φ X174 DNA library) ，此spike-in control可推估每個sample的error rate，監控定序品質。
  ② 當樣本無法使用spike-in control 時， 可由同時進行定序的Control lane確認定序流程與品質

整合性之「一站式」基因體研究服務

DNA Samportal

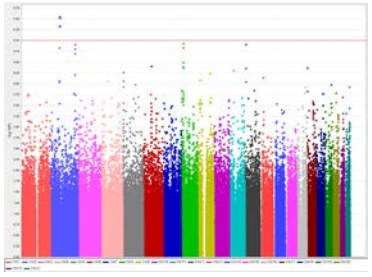
問卷 iQuestion

BioIT: data processing, storage & backup；STAT: data QA & QC

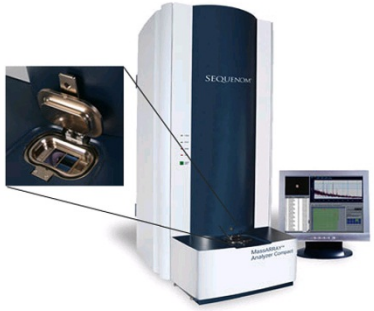
Human whole genome genotyping



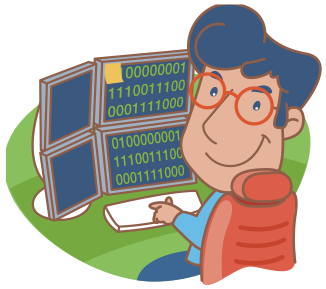
GWAS analysis



Validation Fine mapping Replication

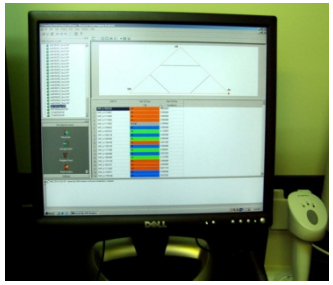


Joint analysis



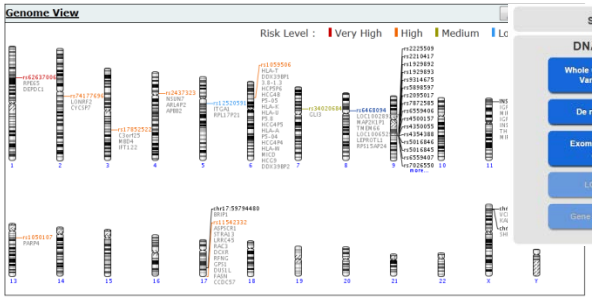
SCIENCE nature genetics The NEW ENGLAND JOURNAL of MEDICINE

Causal variant analysis



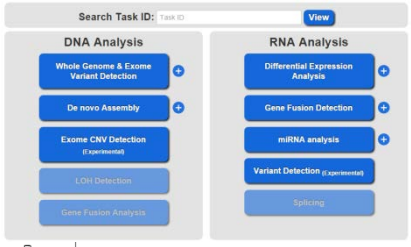
Variants and genes interpretation

GenePipe V3.0



NGS data analysis

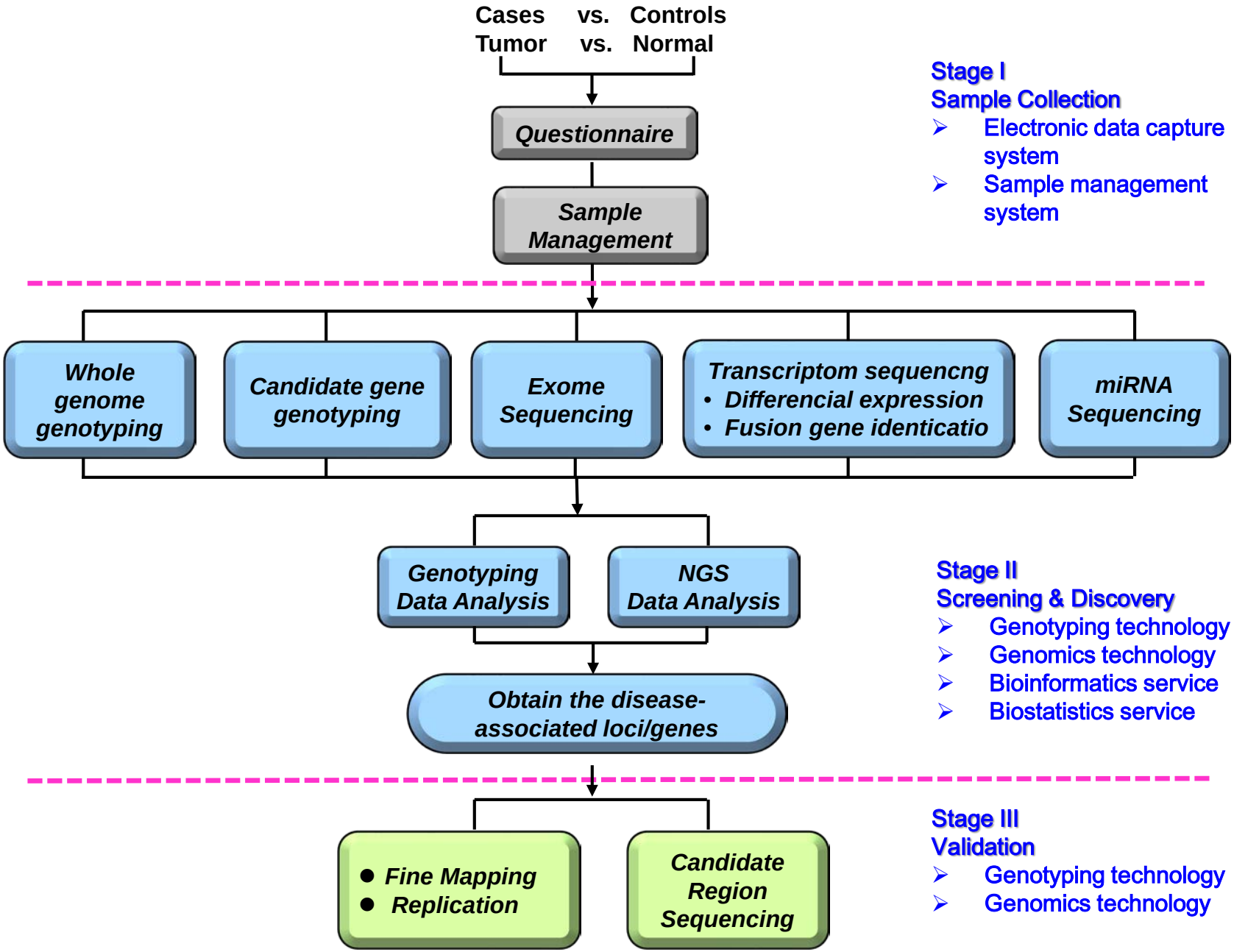
HiPipe



High-throughput candidate gene sequencing



整合性之「一站式」基因體研究服務



Stage I
Sample Collection
- Electronic data capture system
- Sample management system

Stage II
Screening & Discovery
- Genotyping technology
- Genomics technology
- Bioinformatics service
- Biostatistics service

Stage III
Validation
- Genotyping technology
- Genomics technology

NCGM官網首頁



下載「線上服務申請及點數申購流程」說明書

技術平台原理、樣本需求、服務項目及收費標準、收費試算

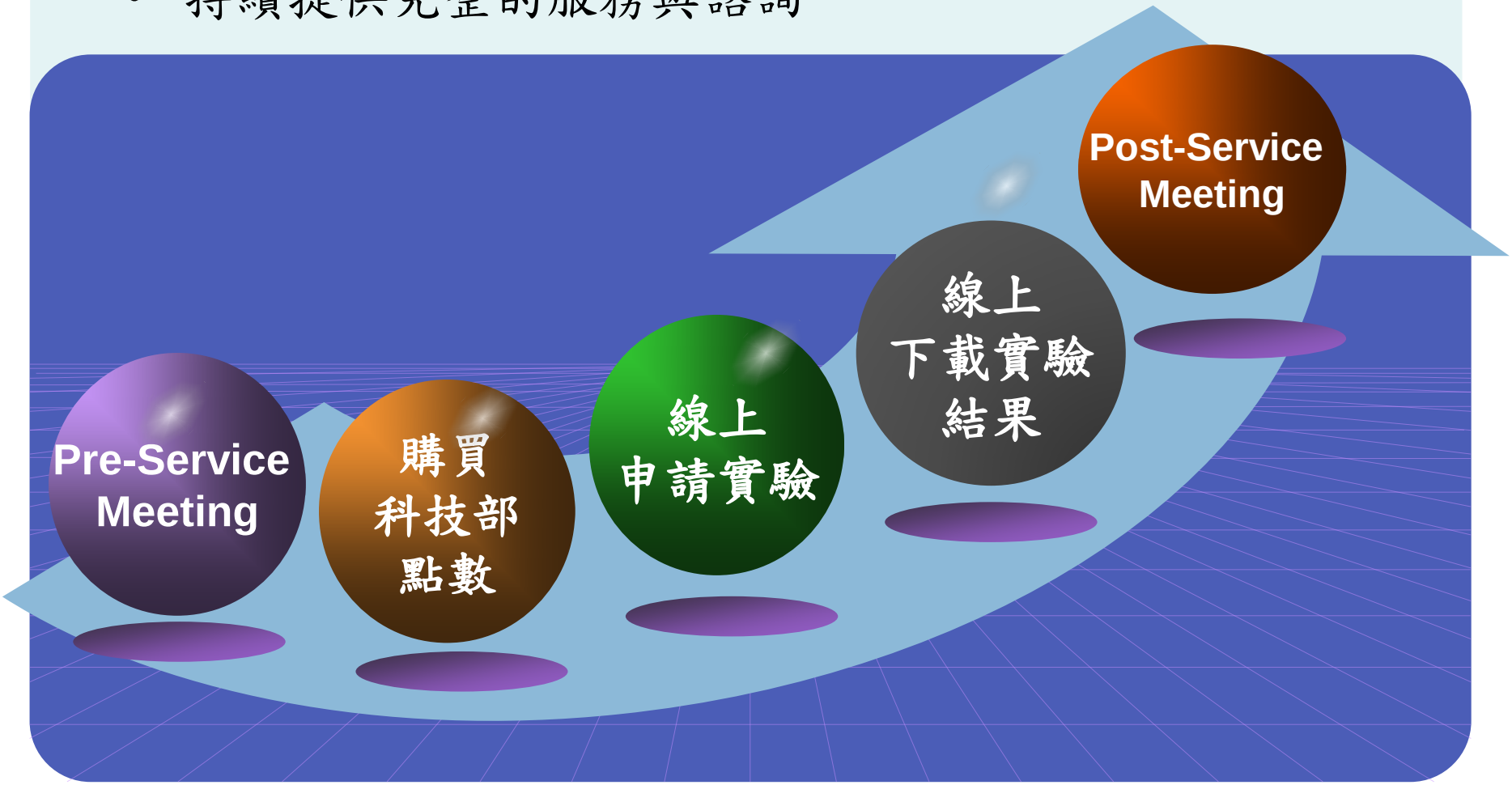
HiPipe Basic:
無須帳號登入，立即免費進行NGS資料分析
HiPipe Professional:
多樣本NGS分析、儲存、分享與管理系統

線上申請NCGM各項基因體鑑定與NGS服務、追蹤實驗進度、下載實驗結果，隨時隨地掌握最新相關資訊！

服務流程

服務滿意度調查
提昇NCGM服務品質!

- 持續提供完整的服務與諮詢



致謝範例與成果回報

- 依科技部規定，凡使用者使用核心設施服務之實驗結果發表研究成果（含學術演講、論文、著作、專利...等），請務必於「Acknowledgments」或「Methods」中列入本核心設施 -「國家基因體醫學研究中心, National Center for Genome Medicine」，以呈現本核心設施之貢獻，並將成果項目回報本核心設施以利統計使用者論文發表。
- 致謝內容範例：We thank the National Center for Genome Medicine for the technical/bioinformatics support.
- 使用者可隨時透過E-Mail或填寫調查表之方式回報成果

1) E-Mail: ncgm@ncgm.sinica.edu.tw
2) 使用者之成果調查表（每半年email給使用者填寫）

Total solutions for genotyping, NGS and NGS/GWAS data analysis!

THANK YOU!

聯絡我們：

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