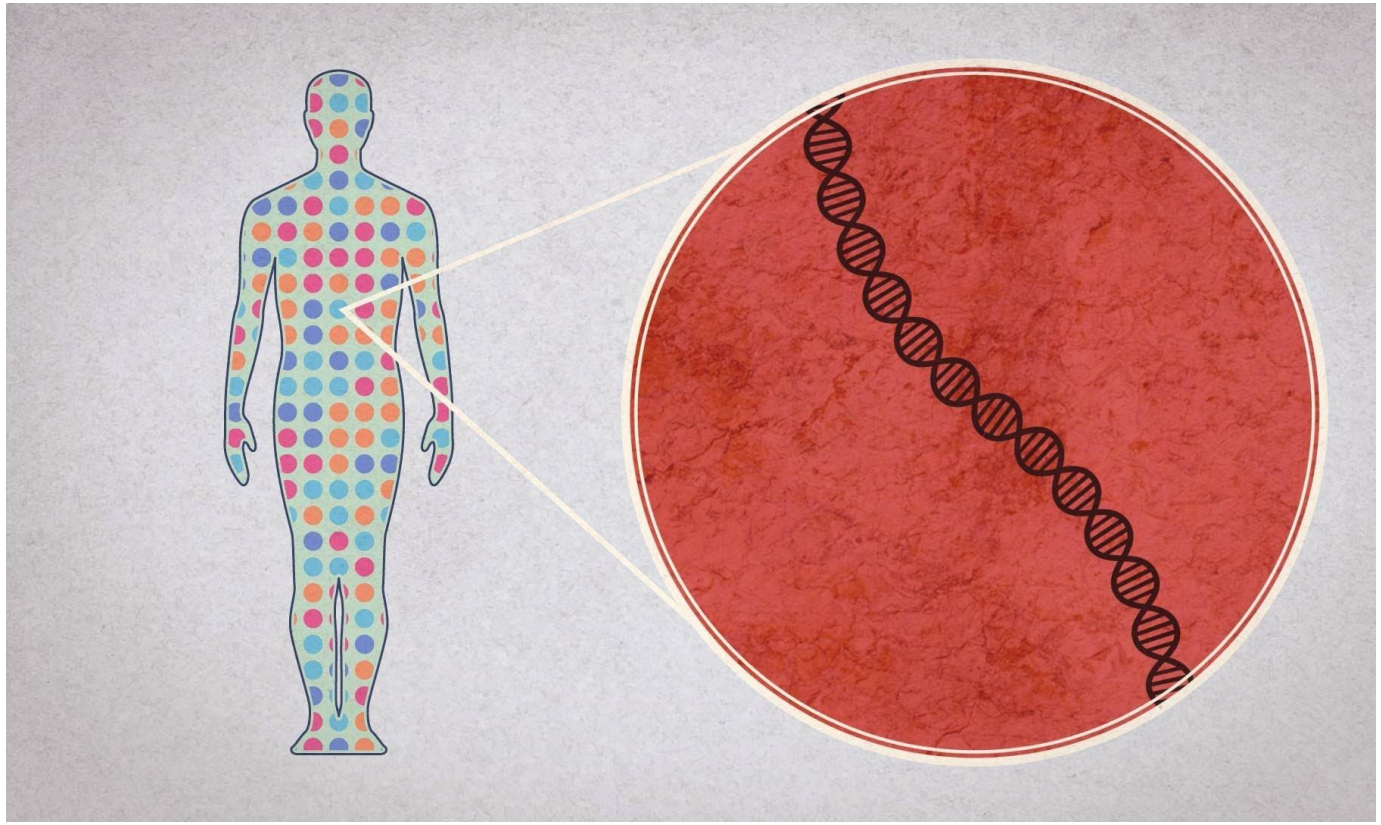
運用「高速次世代基因診斷輔助系統」快速確診精準治療

胡務亮

臺大醫院小兒部暨基因醫學部

The human genome contains 3 billion base pair DNA

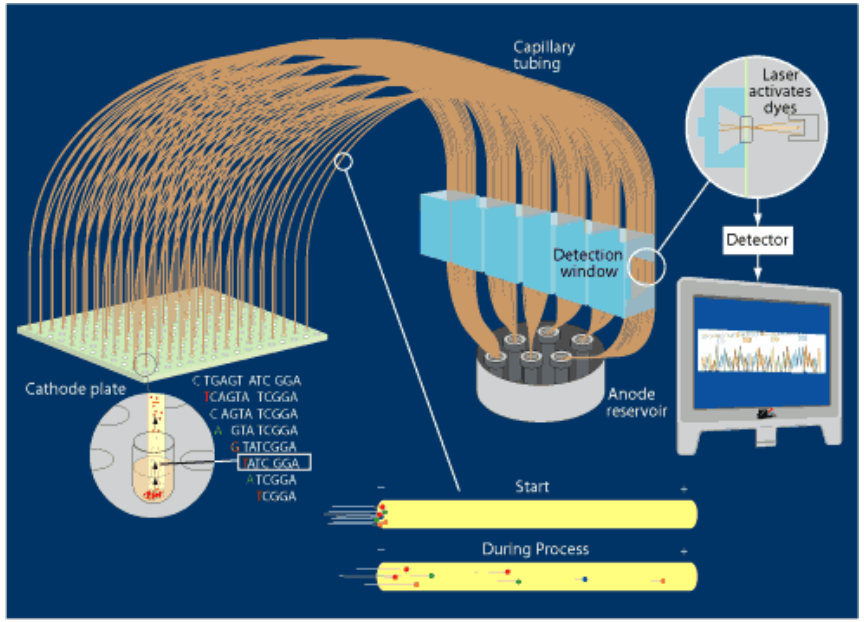


- 43,162 genes
  - 21,306 protein-coding
  - 21,856 noncoding
  - 323,824 transcripts
  - (Pertea, M. *et al*. 2018) https://doi.org/10.1101/332825

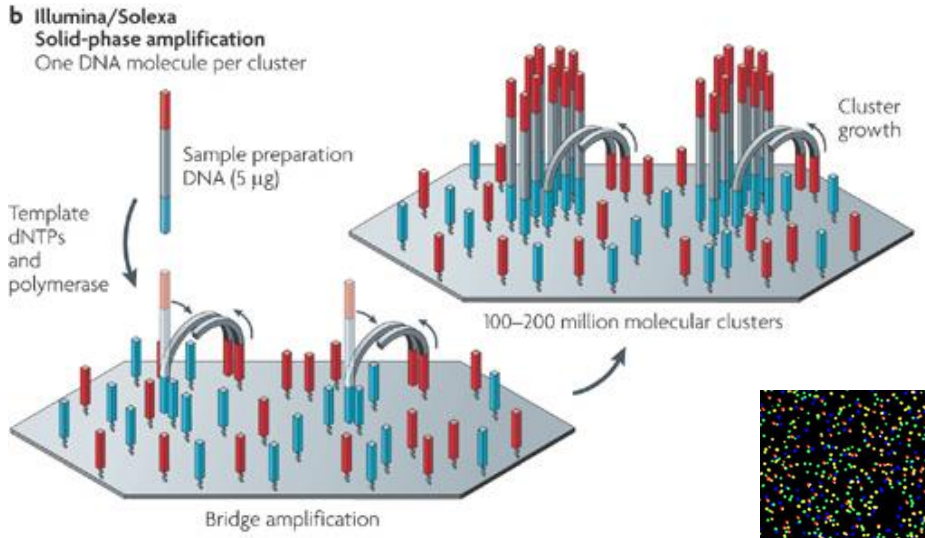
https://www.youtube.com/watch?v=MvuYATh7Y74; https://www.ncbi.nlm.nih.gov/genome/guide/human/

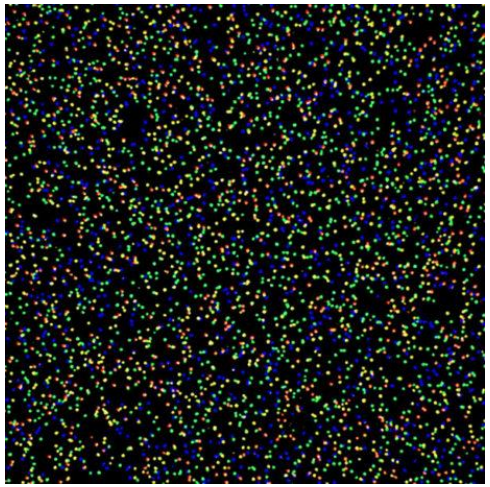
基因序列分析

Sanger sequencing



Next-generation sequencing (NGS)



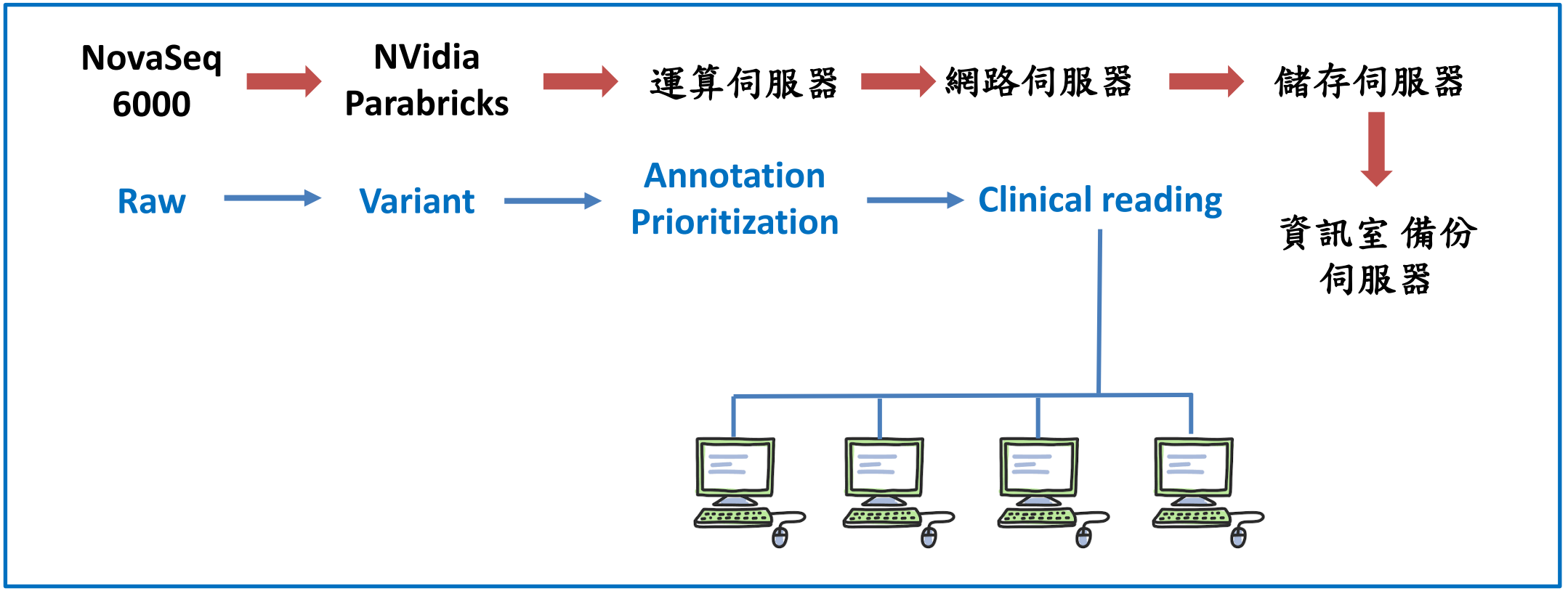


NovaSeq 6000

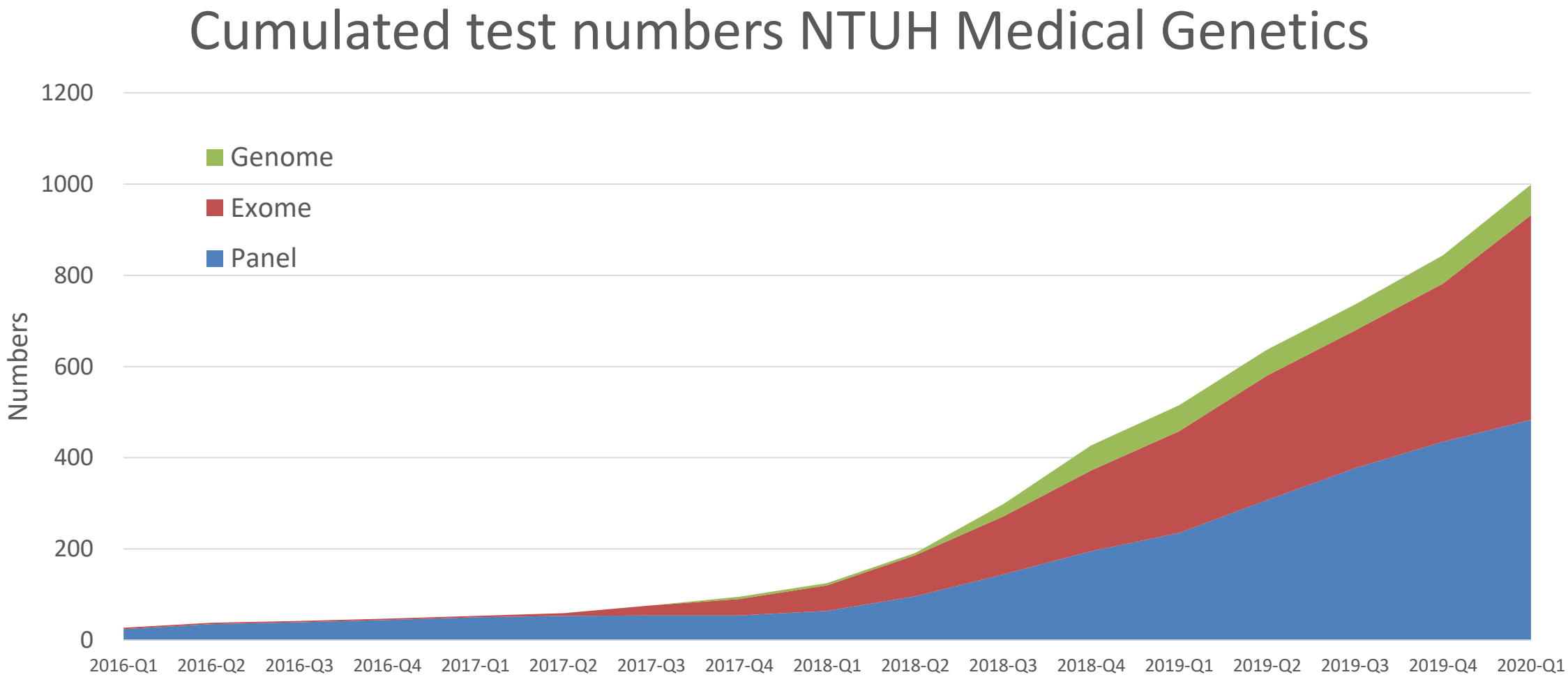
Flow Cell	Cycles	Read Options	# Lanes	Reads/ Lane (M)	GB/Lane	RNA-Seq samples @ 20 M	Human Exome @ 40X (50 Mb)	Human Genome @ 30X
SP	100	1 X 100 SR 2 X 50 PE	2	375	37.5	18	9	N/A
SP	300	2 X 150 PE	2	375	112.5	18	28	1
SP	500	2 X 250 PE	2	375	187.5	18	46	2
S1	100	1 X 100 SR 2 X 50 PE	2	750	75	37	18	N/A
S1	300	2 X 150 PE	2	750	225	37	56	2.5
S2	100	1 X 100 SR 2 X 50 PE	2	1,800	180	80	45	N/A
S2	300	2 X 150 PE	2	1,800	540	80	135	5.33
S4	300	2 X 150 PE	4	2,250	675	112	168	7.5

臺大醫院 全外顯子定序 基因診斷

兒童醫院: 小兒部遺傳科/基因醫學部



NGS的使用快速增加


Cumulated test numbers NTUH Medical Genetics
Numbers
1200
1000
800
600
400
200
0
Genome
Exome
Panel
2016-Q1
2016-Q2
2016-Q3
2016-Q4
2017-Q1
2017-Q2
2017-Q3
2017-Q4
2018-Q1
2018-Q2
2018-Q3
2018-Q4
2019-Q1
2019-Q2
2019-Q3
2019-Q4
2020-Q1

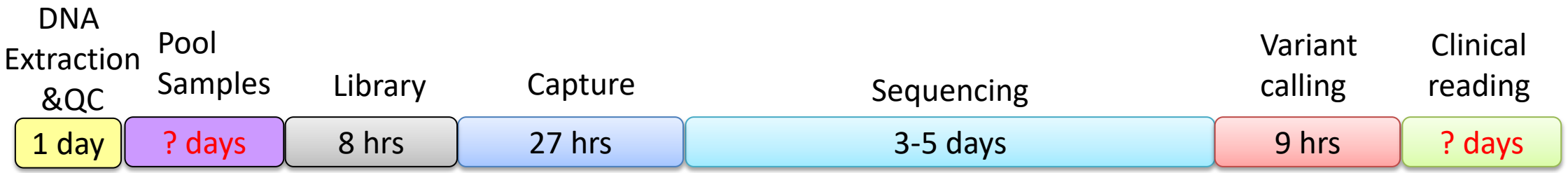
兒童及新生兒急重症之高速次世代基因診斷

Diagnosis in the genomics era 0

科技部計畫(2017-2020)，臺大醫院 胡務亮醫師

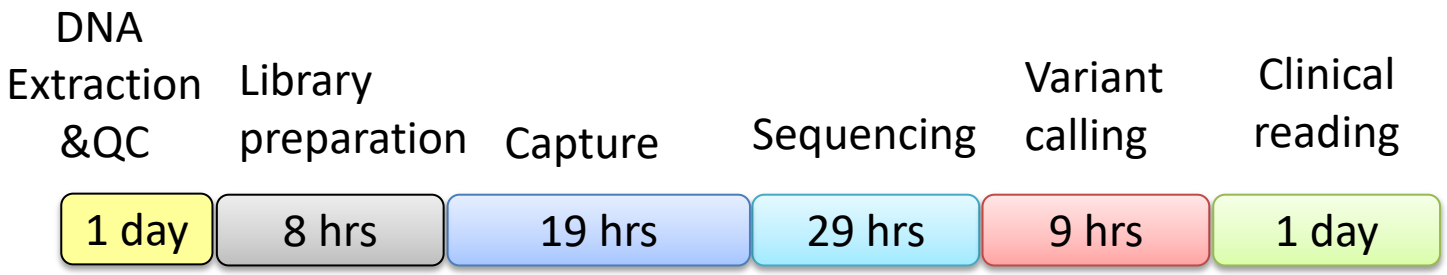
Previous turnaround time of WES

3 months



Rapid WES trio analysis

7 days

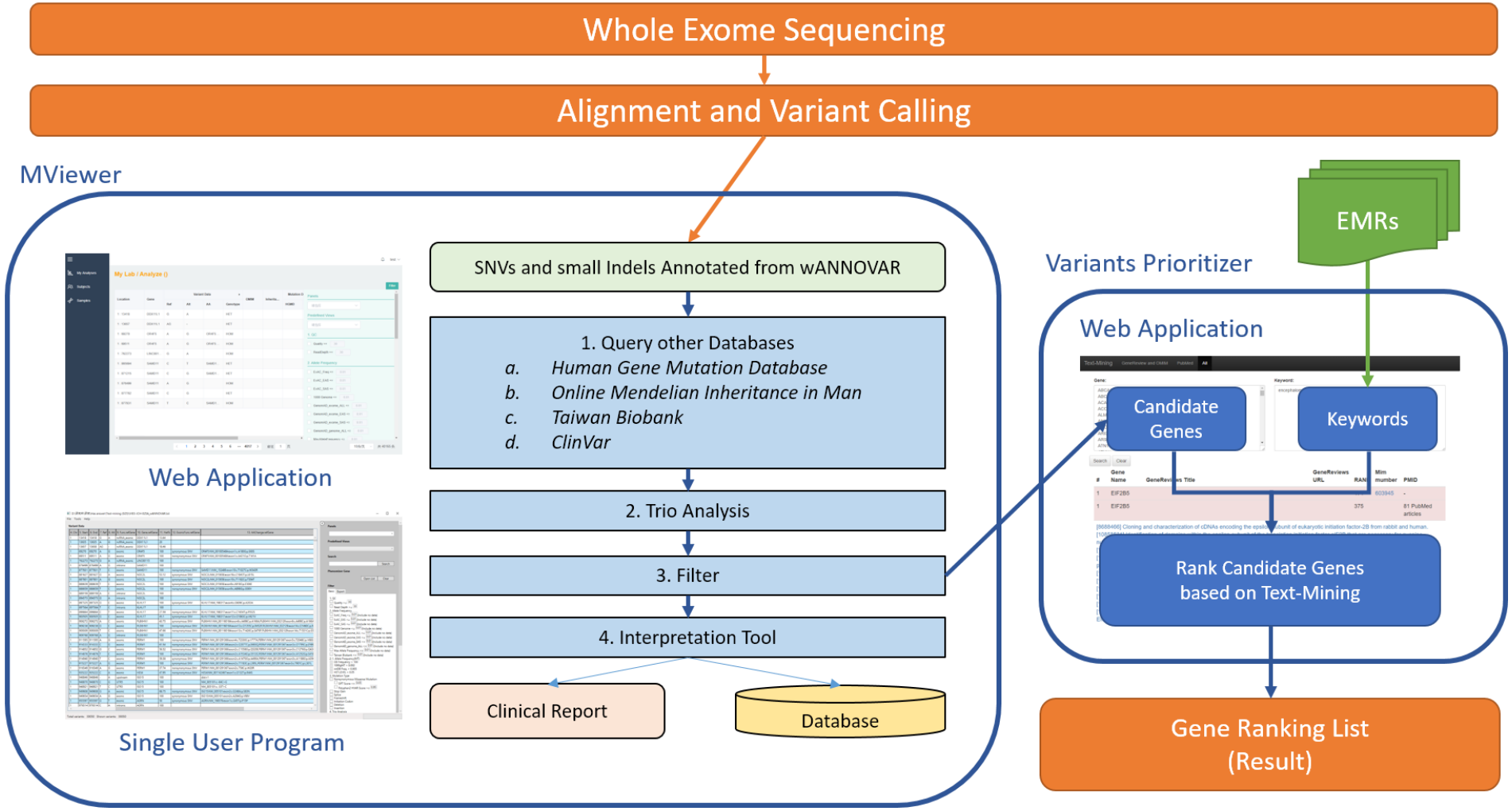


More than 40,000 variants arise from WES

40,000-100,000 variants

1-2 variants in 1 gene

The MViewer software: Developed by NTU/NTUH



Case enrollment

- Enrollment criteria
  - Pediatric patients
    - Admitted to ICU
    - Positive newborn screen
    - Patients need transplants
    - Undiagnosed disease
- Trio analysis
  - 94 trios

送件醫院	個案數
台大	50
馬偕	8
成大	5
高長	4
高榮	4
奇美	3
國泰	3
中國	2
北榮	2
慈濟	2
新竹台大	2
彰基	2
中山	1
林長	1
花慈	1
高醫	1
新竹馬偕	1
義大	1

Diagnostic yield: 51.1% (48/94)

	Total (n=94)	Positive (n=48)
Mean age±SD (months)	17±2.4	15±2.7
Sex (M/F)	53/41	25/23
Turn around time (working days)		
Mean±SD	5.9±1.1	**5.7±1.0**
Median (Range)	4.0-9.9	4.1-9.1

45.8% (22/48) of the cases are first identified in Taiwan

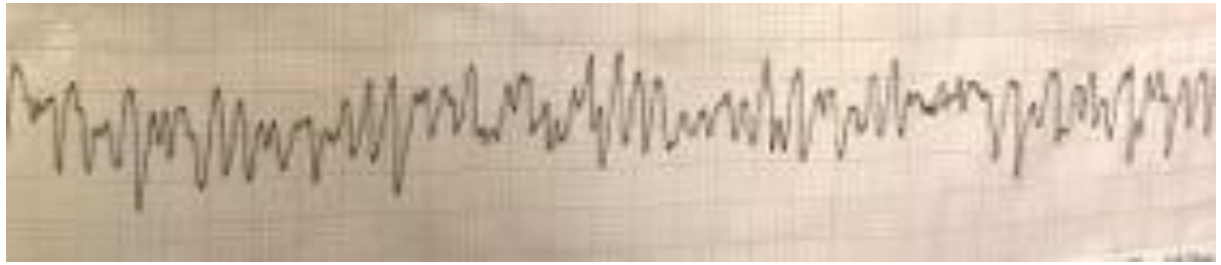
Benefits from molecular diagnosis

System involved	Positive cases	Specific treatment (%)	Total cases	Redirection of treatment (%)
Nervous system	11	6 (55%)	22	8 (36%)
Cardiovascular system	3	3 (100%)	13	2 (15%)
Metabolism/homeostasis	10	8 (80%)	16	6 (38%)
Immune system	3	3 (100%)	8	3 (38%)
Liver	3	3 (100%)	5	3 (60%)
Genitourinary system	2	2 (100%)	2	2 (100%)
Musculature	2	0 (0%)	2	2 (100%)
Multiple congenital anomaly	3	0 (0%)	3	1 (33%)

Wu ET, et al. Pediatr Crit Care Med. 2019 Jul 1

A child with recurrent ventricular arrhythmia

- 4y4m girl, Acute gasotroenteritis for 1 day then loss of conscious, EKG showed ventricular fibrillation
  - CPCR, bosmin 0.1mg, DC shock 70J once, loading amiodarone and intubation, transfer to PICU
- CPCR for several times due to refractory ventricular arrhythmia
- Exome result: RYR2 NM_001035.2
  - c.6737C>T (p.Ser2246Leu)
- Diagnosis: Catecholaminergic Polymorphic Ventricular Tachycardia
- Action
  - beta-blockers
  - Implantable cardioverter defibrillator



A child with recurrent ventricular arrhythmia

2018 智慧醫療獎 金獎

2018未來科技展


2018
未來科技
Future Tech
高速次世代基因診斷輔助系統
A system to facilitate next generation sequencing analysis
生技與新藥 A01
BIO-TECH & NEW DRUGS
國立台灣大學醫學院附設醫院 National Taiwan University Hospital
技術簡介 | Technology Introduction
科學突破 | Scientific Innovation
產業應用 | Industrial Application

未來科技展: 未來科技突破獎

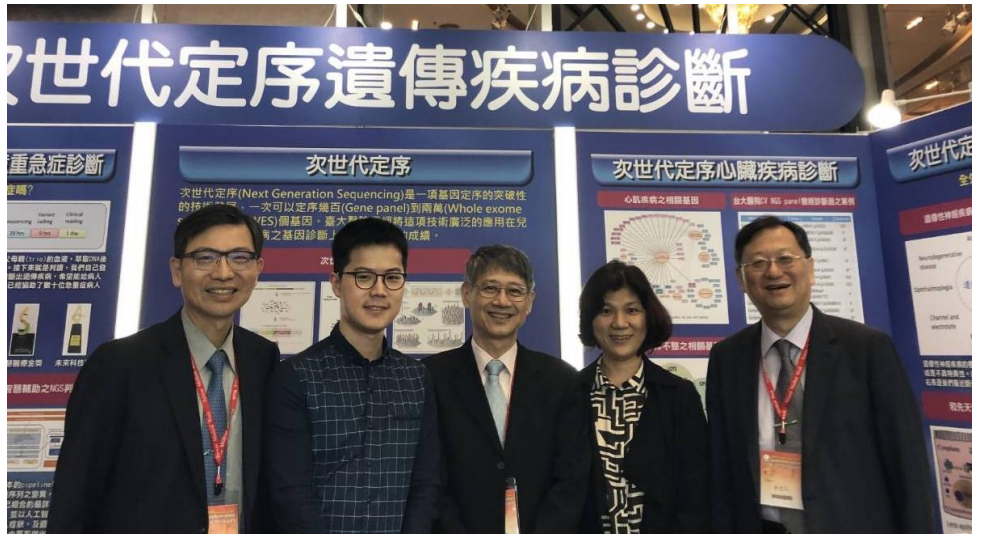
2019 台灣國際醫療及健康照護展

2019 服務特殊優異團隊獎 第一名

高速次世代基因診斷團隊: 李妮鍾醫師(左二)代表領獎

2019 台灣醫學會

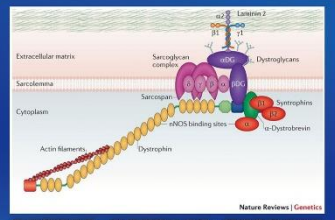

台大醫院
NTUH
次世代定序遺傳疾病診斷
快速次世代定序兒童重急症診斷
次世代定序
次世代定序心臟疾病診斷
次世代定序神經及眼科疾病診斷
紀念品兌換處
攜出資料合併方式範例一


次世代定序遺傳疾病診斷
次世代定序
次世代定序心臟疾病診斷

2019 台灣醫學會

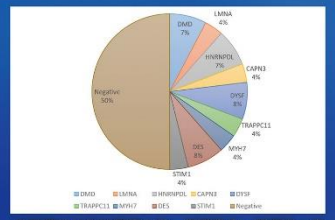
次世代定序骨骼肌肉疾病診斷

肌肉萎縮症 (congenital muscular dystrophy)



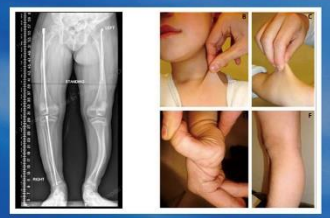
肌肉萎縮症是一群很複雜的疾病，臨床表現卻可能十分的類似。以較常見的裘馨氏肌肉萎縮症為例，疾病基因所製造的dystrophin蛋白會和細胞膜的一群蛋白一起工作，因此任何一個蛋白功能異常都可能引起肌肉萎縮症。

成果(2017)



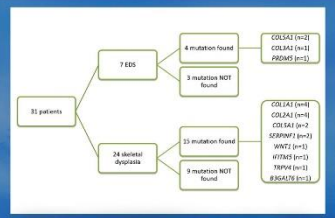
在我們2017年26個病人的一份報告中，我們在一半的病人可以找到致病性的突變。有發現病人的基因包括DYSF, DES, DMD, LMNA, CAPN3, TRAPPC11, MYH7, STIM1。

成骨不全症(Osteogenesis imperfect, OI) and Ehlers-Danlos syndrome (EDS)



成骨不全症又俗稱玻璃娃娃，以往都是說是膠原纖維基因突變所引起。EDS主要的表現是皮膚或關節的鬆弛，臨床表現差異很大。

成果

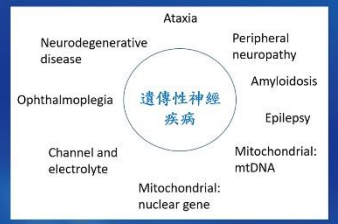


在7個EDS病人中有3個找到突變(COL5A1, COL3A1, PRDM5)。在24個OI病人中有15個找到突變(COL1A1, COL2A1, COL5A1, etc)

次世代定序神經及眼科疾病診斷

全外顯子定序是診斷複雜疾病的利器

遺傳性神經疾病種類繁多

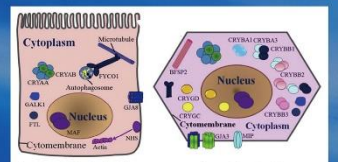


遺傳性神經疾病的種類非常的多，臨床症狀常常重疊或是不具特異性。所以WES是診斷這些疾病的利器。右表是我們最近斷過的一些疾病。

遺傳性神經疾病的基因診斷

Molecular diagnosis	Gene	Variant	Classification
Alzheimer disease 9, susceptibility to	ABCA7	c.2933T>C (p.I978T)	VUS
Alzheimer disease, type 3	PSEN1	c.1141C>T (p.L381F)	LP
Amyloidosis, hereditary, transthyretin-related	TTR	c.G349T (p.A117S)	P
Andersen syndrome	KCNJ2	c.212A>G (p.D71G)	LP
Ataxia telangiectasia	ATM	c.1369C>T (p.R457X) c.7878_7882del (p.A2626Fs)	P/P
Ataxia telangiectasia	ATM	c.46920delT (p.P15664Ls*34) c.6679C>T (p.R2227C)	P/LP
Charcot-Marie-Tooth disease, axonal, type 2CC	NEFH	c.G559T (p.D187Y)	VUS
Epileptic encephalopathy, infantile or early childhood, 3	ATP6V1A	c.745C>T (p.P249S)	LP
Fahr's syndrome	PDGFB	c.365C>T (p.P122L)	P
Hyperekplexia 1	GLRA1	c.1324C>T (p.R442C)	VUS
Mitochondrial complex I deficiency, nuclear type 16	NDUFAF5	c.836T>G (p.M279R) c.836T>G (p.M279R)	LP
Niemann-Pick disease, type C1	NPC1	c.3044G>T (p.G1015V)	LP

和先天性白內障相關的基因



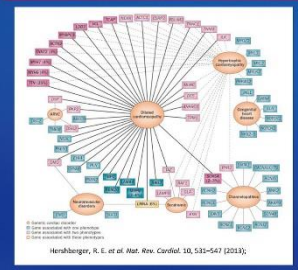
Eur J Med Genet. 2014 Feb;57(2-3):113-22

眼科疾病的基因診斷

Molecular diagnosis	Gene	Variant 1	Classification
Brittle cornea syndrome 2	PRDM5	c.106G>A (p.G36R) c.106G>A (p.G36R)	LP
Cataract 1, multiple types	GJA8	c.434G>T (p.G145V)	LP
Cataract 10, multiple types	CRYBA1	c.215+1G>A	LP
Cataract 15, multiple types	MIP	c.513dupG (p.H172Afs*28)	P
Cataract 17, multiple types	CRYBB1	c.667C>T (p.Q223X)	P
Leber congenital amaurosis 1	GUCY2D	c.935C>T (p.T312M) c.935C>T (p.T312M)	LP
Microcephaly and chorioretinopathy, autosomal recessive, 1	TUBGCP5	c.1601G>A (p.R534Q) c.4374+6T>A	VUS/VUS

次世代定序心臟疾病診斷

心肌疾病之相關基因

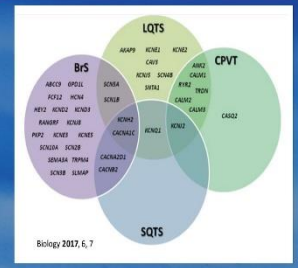


Hershberger, R. E. *et al. Nat. Rev. Cardiol.* 10, 531–547 (2013);

台大醫院CV NGS panel曾經診斷過之案例

Molecular diagnosis	Gene	Variant	Classification
Cardiomyopathy, dilated, 1C	LDB3	c.92G>A (p.R31Q)	VUS
Cardiomyopathy, dilated, 1G	TTN	c.49870C>T (p.R16624X)	P
Cardiomyopathy, dilated, 1S	MYH7	c.1922G>C (p.G641A)	LP
Cardiomyopathy, familial hypertrophic, 9	TTN	c.64291G>A (p.V21431M)	LP
Cardiomyopathy, familial restrictive, 3	TNNT2	c.280C>T (p.R94C)	LP
Cardiomyopathy, hypertrophic, 1	MYH7	c.1358G>A (p.R453H)	LP
Cardiomyopathy, hypertrophic, 12	CSRP3	c.191G>A (p.R64H)	VUS
Cardiomyopathy, hypertrophic, 17	JPH2	c.463G>A (p.V155M)	VUS
Cardiomyopathy, hypertrophic, 4	MYBPC3	c.1084dupA (p.S362Ifs*27)	P
Cardiomyopathy, hypertrophic, 4	MYBPC3	c.2065C>T (p.Gln689Ter)	P
Cardiomyopathy, hypertrophic, 4	MYBPC3	c.3097C>T (p.R1033W)	LP
Cardiomyopathy, hypertrophic, 7	TNNI3	c.370G>C (p.E124Q)	LP

遺傳性心律不整之相關基因



Biology 2017, 6, 7

台大醫院CV panel及WES曾經診斷過之案例

Molecular diagnosis	Gene	Variant 1	Classification
CV panel			
Arrhythmogenic right ventricular dysplasia 2	RYR2	c.8291C>G (p.S2764C)	LP
Arrhythmogenic right ventricular dysplasia 8	DSP	c.1034A>G (p.D345G)	VUS
Brugada syndrome 3	CACNA1C	c.1613C>T (p.T538M)	VUS
Long QT syndrome 2	KCNH2	c.1707C>A (p.Tyr569Ter)	P
Long QT syndrome 3	SCN5A	c.1231G>A (p.V411M)	P
Long QT syndrome 3	SCN5A	c.3391-7T>C	VUS
Long QT syndrome 13	KCNJ5	c.10G>A (p.D4N)	VUS
WES			
Arrhythmogenic right ventricular dysplasia 8	DSP	c.7964C>G (p.A2655G)	VUS
Atrial and ventricular conduction disturbance	NOTCH1	c.1753G>A (p.A585T)	LP
Atrial and ventricular conduction disturbance	NOTCH1	c.1753G>A (p.A585T)	LP
Long QT syndrome 3	SCN5A	c.4299G>T (p.G1433C)	VUS

2019台灣醫療科技展

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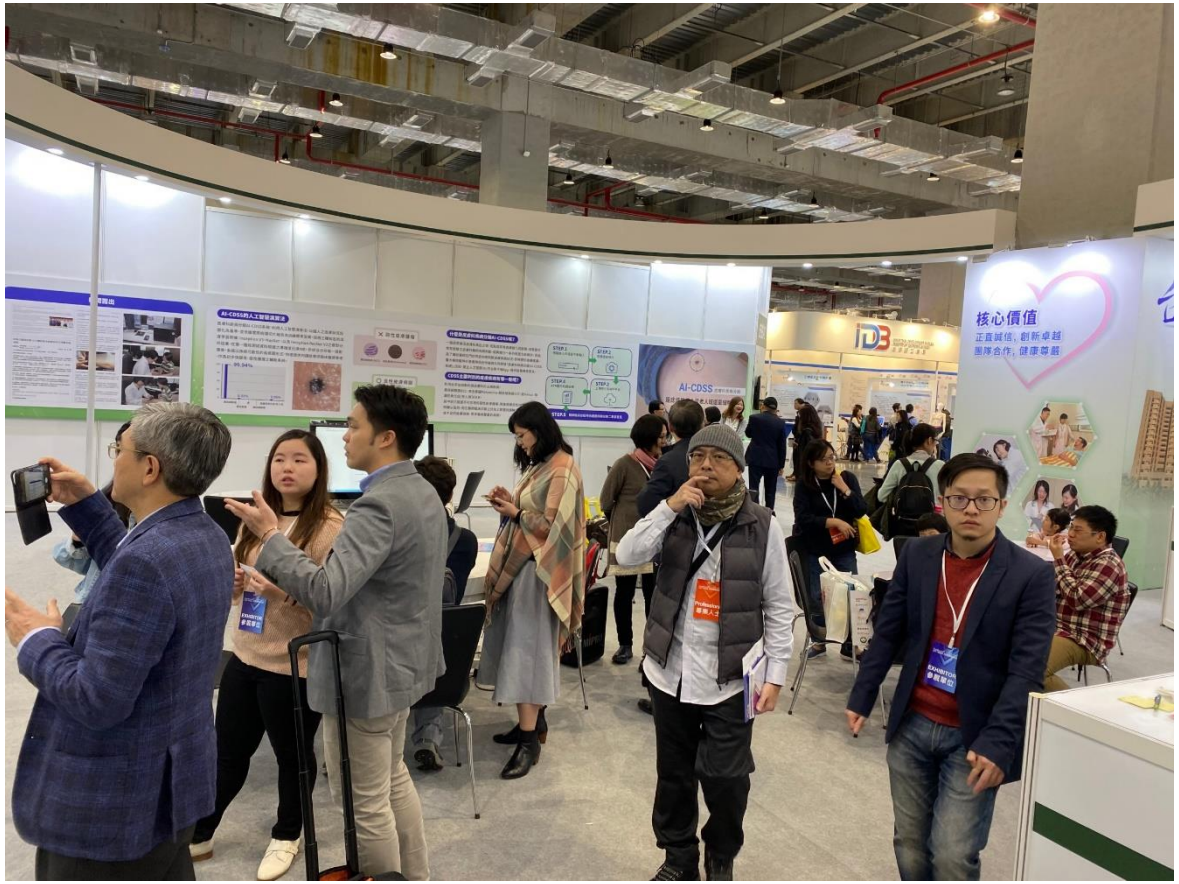
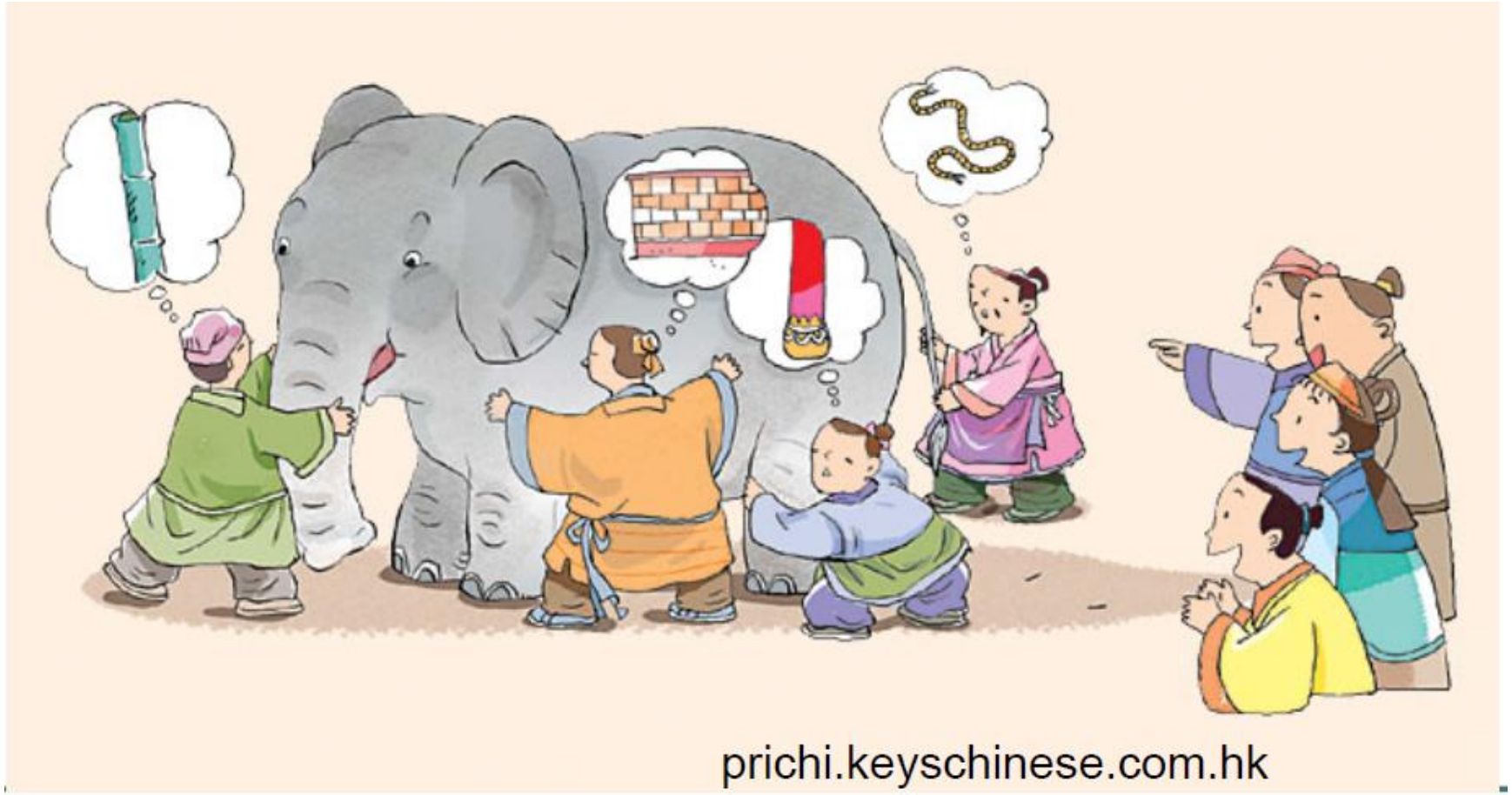
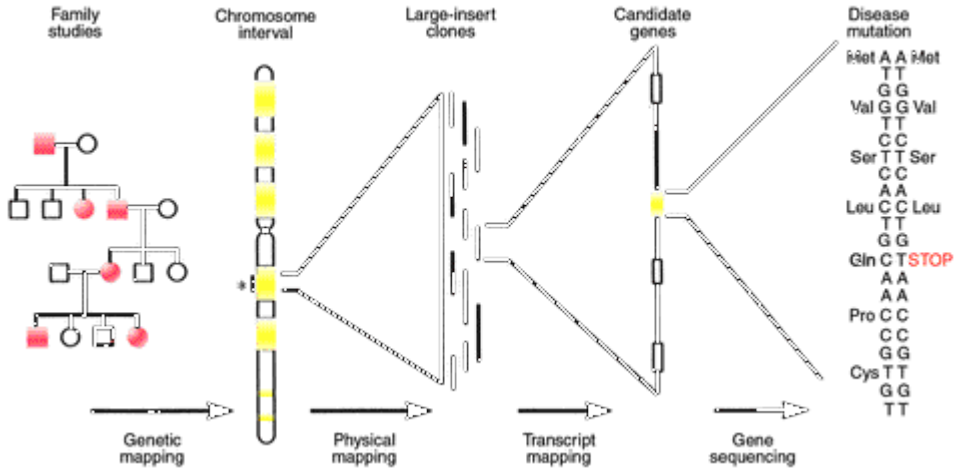


Sometime you just don’t have an idea about the diagnosis



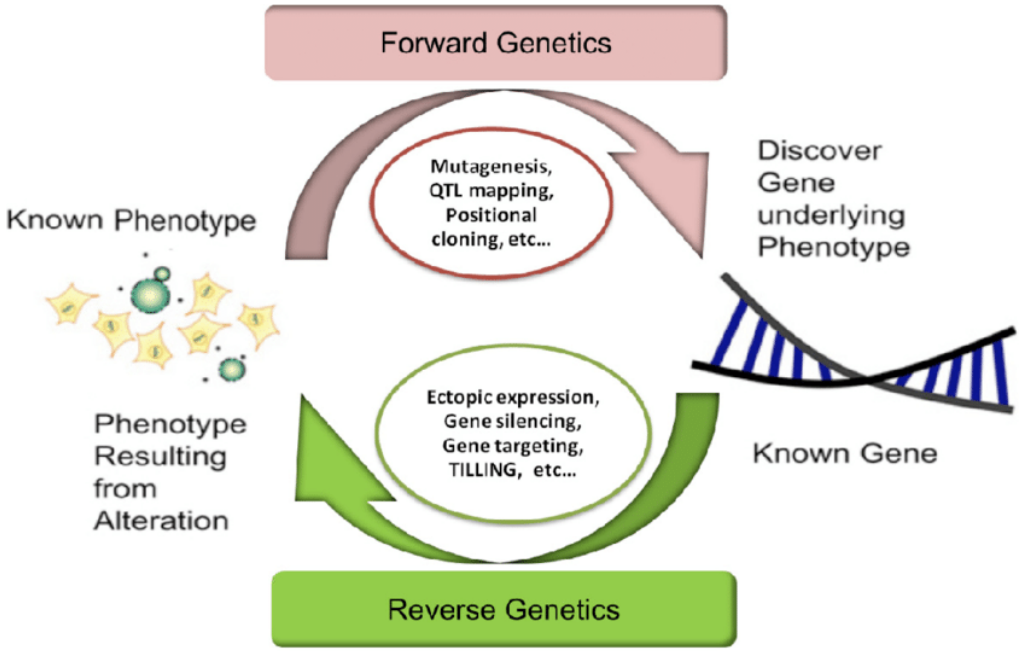
Reverse Genetics

Position cloning



Jul 19, 1999, NADIA HALIM

Current genomics



Current Genomics 17(999):1-1 · May 2016

感謝

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  - 李建南主任
- 小兒部
  - 黃立民主任
- 小兒遺傳團隊
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  - 簡穎秀
- 臨床合作夥伴
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- 資訊室
  - 熊漢昌、黃鐘磊、孫行人
- 醫學研究部
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- 科技部旗艦計畫
- 科技部人工智慧計畫
- 病患補助
  - 臺大醫院社會工作室
  - 罕見疾病基金會
  - 兒童心臟基金會
- 廣達電腦及協力廠商