Clinical Whole Exome Sequencing: For the Evaluation of Genetic Disorders

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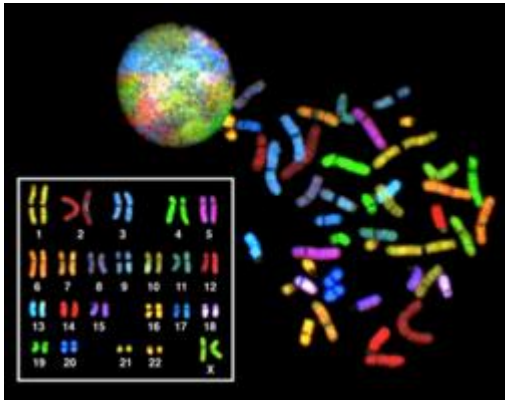
The patient with a suspected genetic disorder poses a challenge to diagnosis

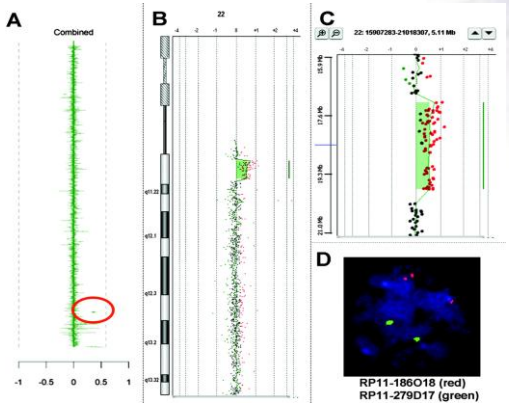
- Thousands of genetic disorders
- Rare
  - Cystic fibrosis 1:2500
  - Hunter syndrome 1:150,000
- Clinical heterogeneity
- Specialized testing needed to confirm diagnosis
  - 3.5 geneticists per 1 million population
- Many patients do not have a diagnosis

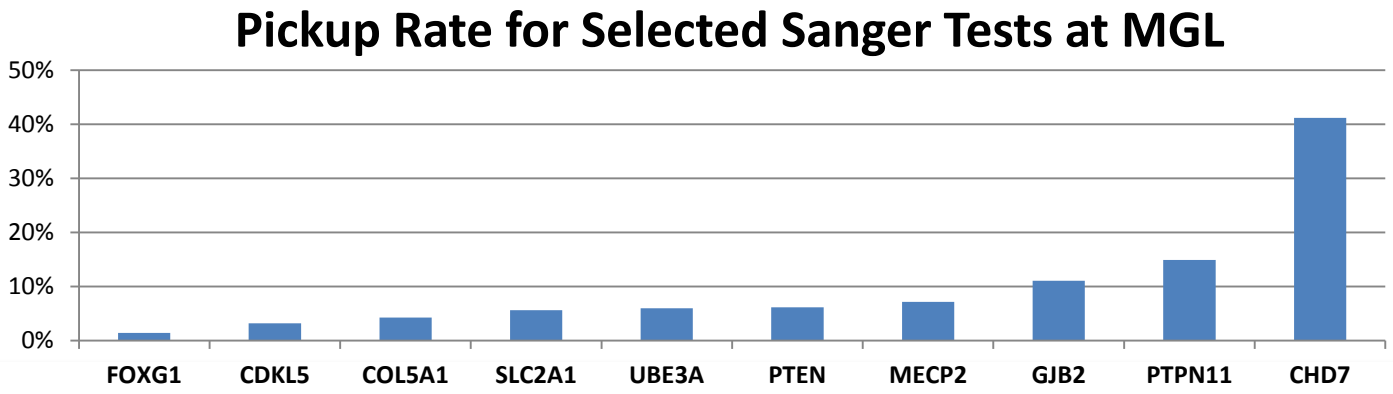
Diagnostic Yield of Common Genetic Tests

Karyotype 5 -15%



Array-CGH – 15-20%





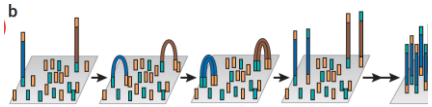
Prior Studies of Exome Approaches to Cohorts of Undiagnosed Patients

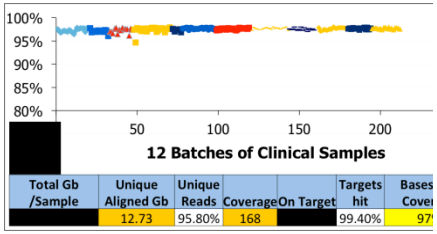
- Gahl, et al, Genet in Med 14:52, 2012
  - Selected patients for undiagnosed genetic disorder
  - Battery of diagnostic approaches
  - 24% diagnostic rate
- deLigt, et al, NEJM 367:1921, 2012
  - Selected cohort of severe intellectual disability
  - Molecular diagnostic rate through exome of 16%

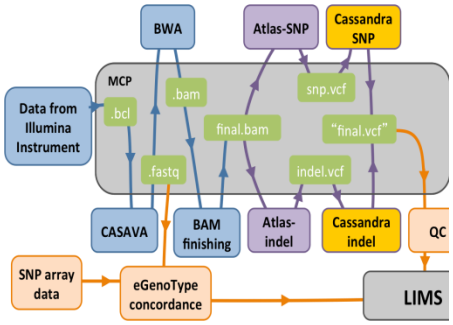
Whole Exome Sequencing:

CLINICAL UTILITY IN A CLINICAL DIAGNOSTIC LAB

HGSC
HUMAN GENOME SEQUENCING CENTER







WGL
WHOLE GENOME LABORATORY

Baylor College of Medicine

MGL
MEDICAL GENETICS LABORATORIES

Dept of Molecular and Human Genetics

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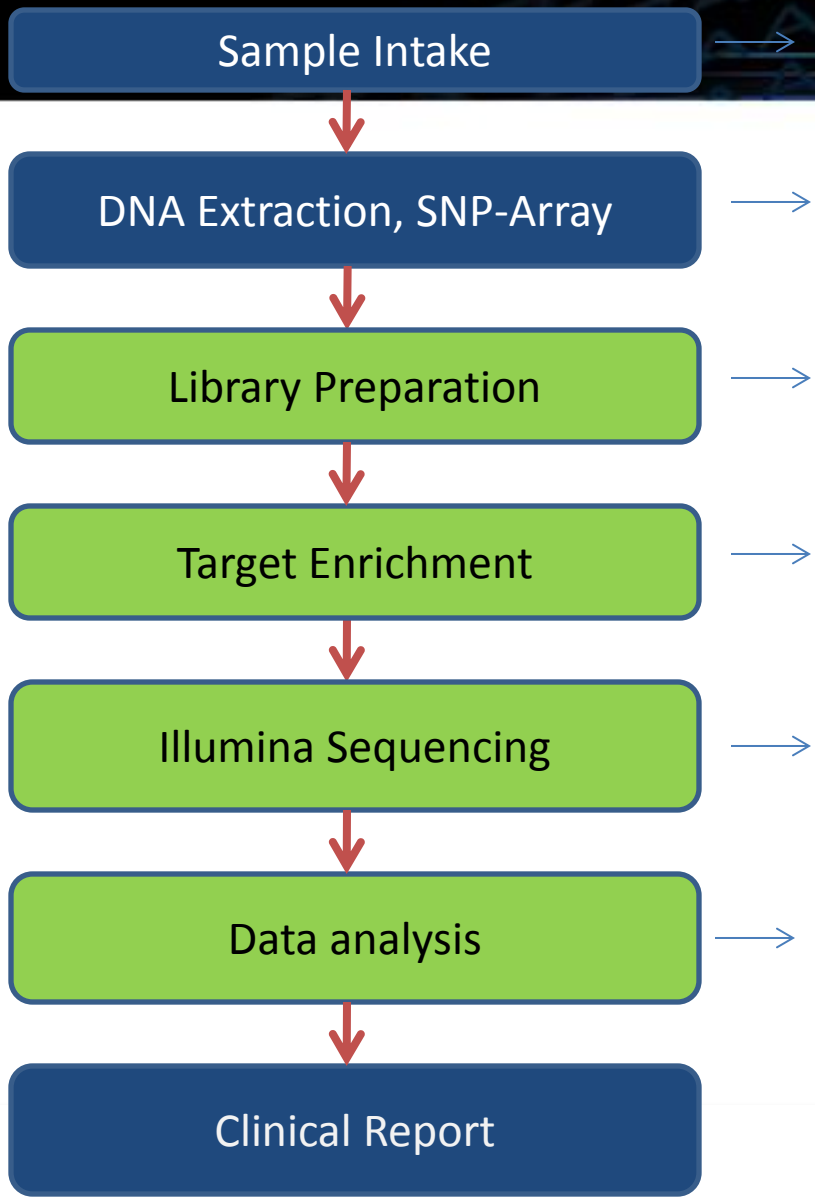
CMS
CENTERS for MEDICARE & MEDICAID SERVICES

AMERICAN BOARD OF MEDICAL GENETICS

Whole Exome Sequencing Workflow

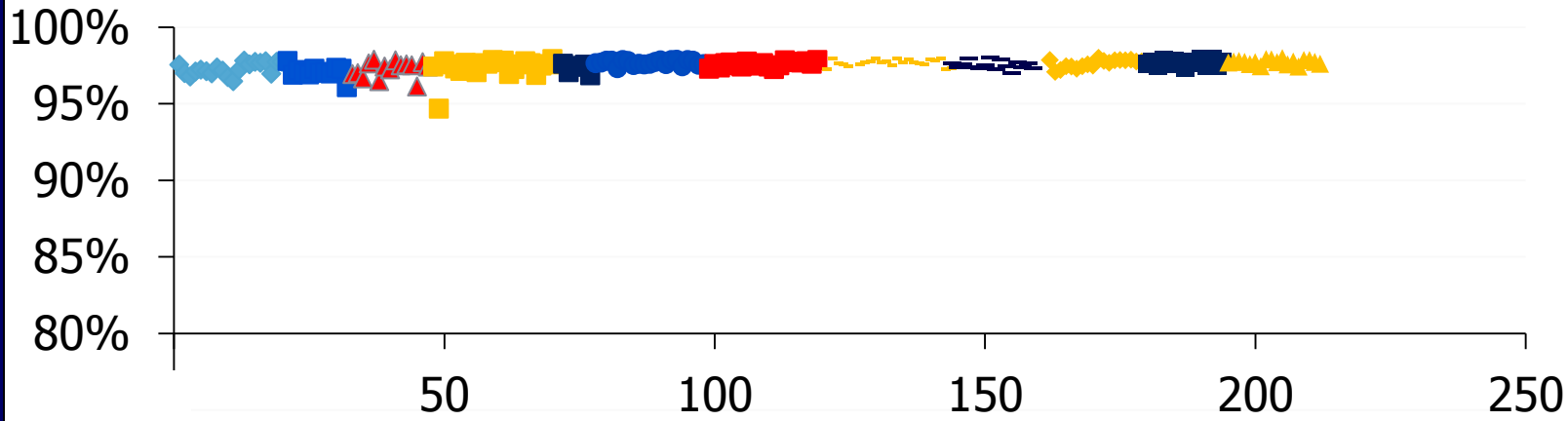


QC steps at each stage

√ QC:

1) **Mapping: <4% error rate, >90% unique reads**
2) **Data analysis>10 Gb data, >95% target bases >20X, >85% target bases >40X, mean coverage >140X**
3) **SNP concordance to genotype array: >99.8%**

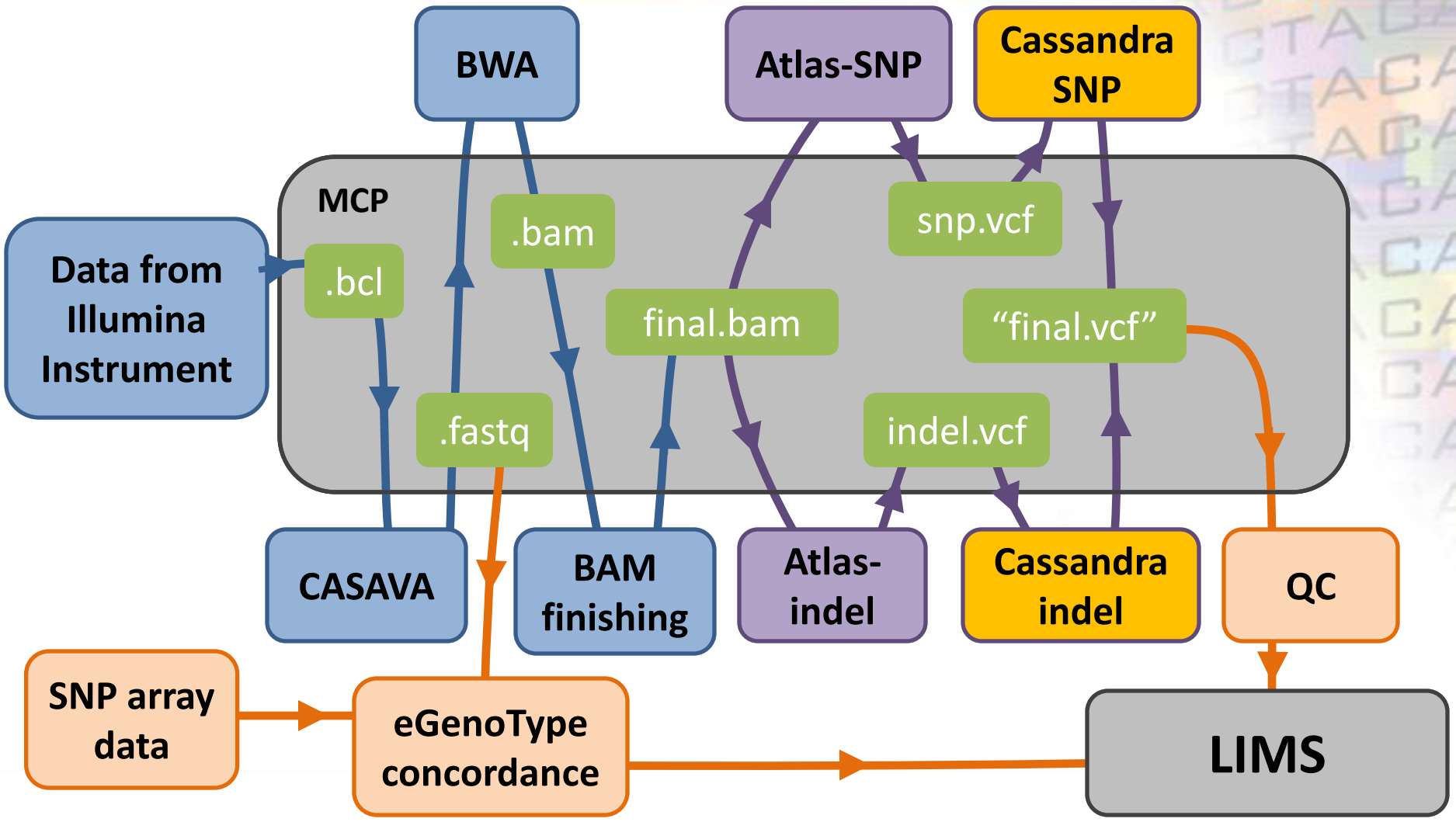
Baylor WES: 97% of Target Covered at 20+ Times



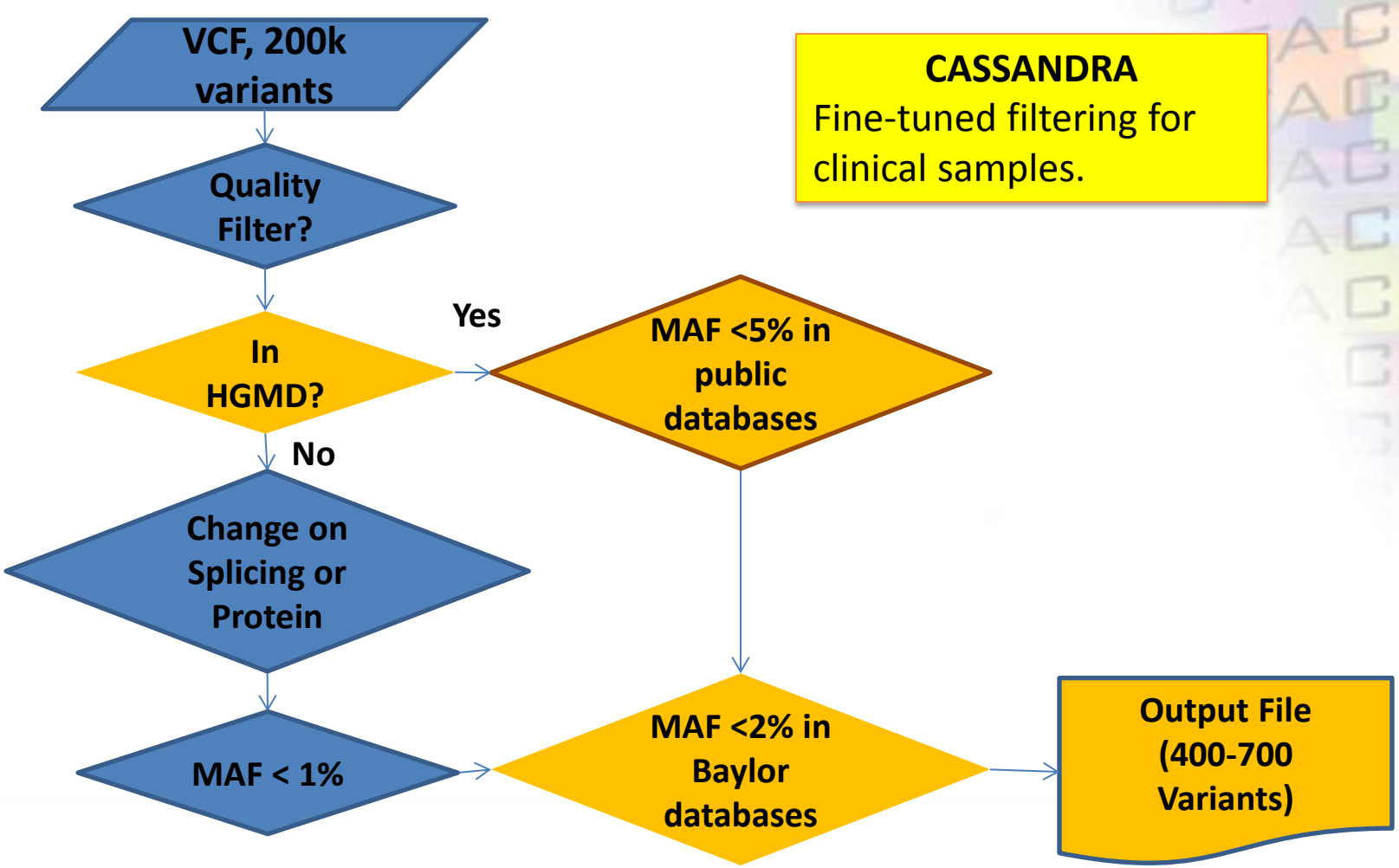
Total Gb /Sample	Unique Aligned Gb	Unique Reads	Coverage	On Target	Targets hit	Bases 20+ Coverage
13.48	12.73	95.80%	168	76%	99.40%	97%

Robust lab performance to deliver high-standard consistency.

Analysis Pipeline (Mercury v1.0)



Variant Filtering for Clinical WES



CASSANDRA
Fine-tuned filtering for clinical samples.

Variant Interpretation

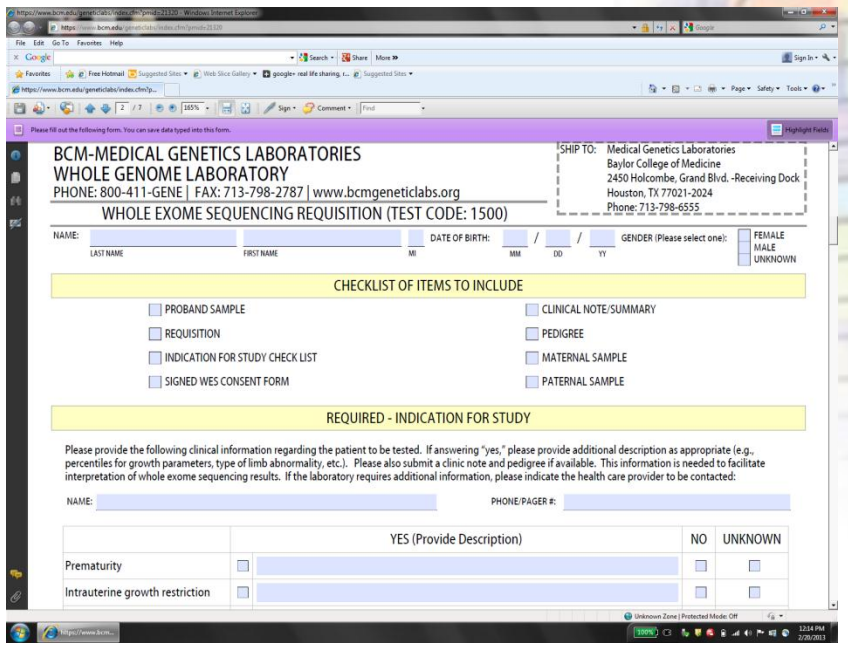
- Correlation with patient phenotype
- Public databases
  - ESP (NHLBI GO Exome Sequencing Project), TG
  - OMIM, HGMD, GeneTests, LSDB
- Internal knowledge base from 800 Clinical Exomes
  - Curated lists of variant classifications
    - Internally annotated mutations/VUS lists
    - Common variants
  - New gene list updated by WGL weekly

Clinical Reporting of Whole Exome

- Sign out team of ABMG-certified laboratory directors, medical directors, clinicians, genetic counselors
- Three levels of review
  - Disease-gene association, functional prediction, in silico prediction
- Focused and expanded report
- Components of WES
  - Sequence result
  - Sanger confirmation, parental inheritance of significant findings
  - Mitochondrial genome

Disease Phenotype

- Detailed phenotype informs analysis
- Questionnaire by organ system
- Request clinic note



BCM-MEDICAL GENETICS LABORATORIES
WHOLE GENOME LABORATORY
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Baylor College of Medicine
2450 Holcombe, Grand Blvd. -Receiving Dock
Houston, TX 77021-2024
Phone: 713-798-6555

WHOLE EXOME SEQUENCING REQUISITION (TEST CODE: 1500)

NAME: LAST NAME FIRST NAME MI DATE OF BIRTH: MM / DD / YY GENDER (Please select one): FEMALE MALE UNKNOWN

CHECKLIST OF ITEMS TO INCLUDE

- PROBAND SAMPLE
- REQUISITION
- INDICATION FOR STUDY CHECK LIST
- SIGNED WES CONSENT FORM
- CLINICAL NOTE/SUMMARY
- PEDIGREE
- MATERNAL SAMPLE
- PATERNAL SAMPLE

REQUIRED - INDICATION FOR STUDY

Please provide the following clinical information regarding the patient to be tested. If answering "yes," please provide additional description as appropriate (e.g., percentiles for growth parameters, type of limb abnormality, etc.). Please also submit a clinic note and pedigree if available. This information is needed to facilitate interpretation of whole exome sequencing results. If the laboratory requires additional information, please indicate the health care provider to be contacted:

NAME: PHONE/PAGER #:

	YES (Provide Description)	NO	UNKNOWN
Prematurity			
Intrauterine growth restriction			

Focused Report: Based on Disease Phenotype

- Deleterious mutations in disease genes related to clinical phenotype
- Variants of unknown clinical significance in genes related to phenotype
- Immediately "medically actionable" mutations
  - Marfan, NF1, VHL, MEN2A
- Autosomal recessive carrier status
- Pharmacogenetic loci

Medically Actionable Definition

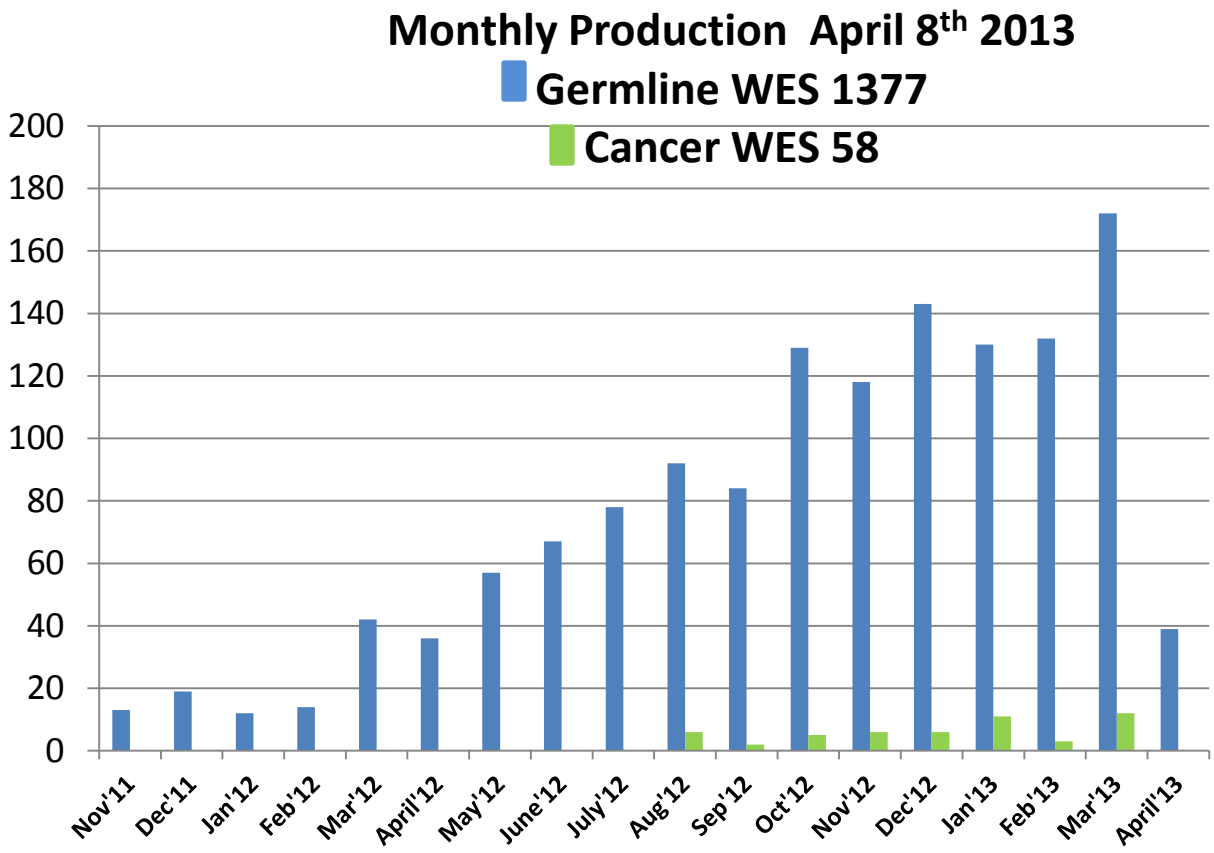
- Finding with direct clinical utility based on established guidelines and/or medical literature
- Availability of treatment or established guidelines for disease prevention
- Unrecognized secondary diagnosis: Marfan, NF1, NF2
- Preventable disease: HNPCC, BRCA1,2

Whole Exome Report: Expanded Report

- Deleterious mutations in genes apparently unrelated to phenotype
- Variants of unknown significance
  - For AR, a deleterious mutation in same gene must also be present
- Predicted clearly deleterious mutations in genes with no current association with disease

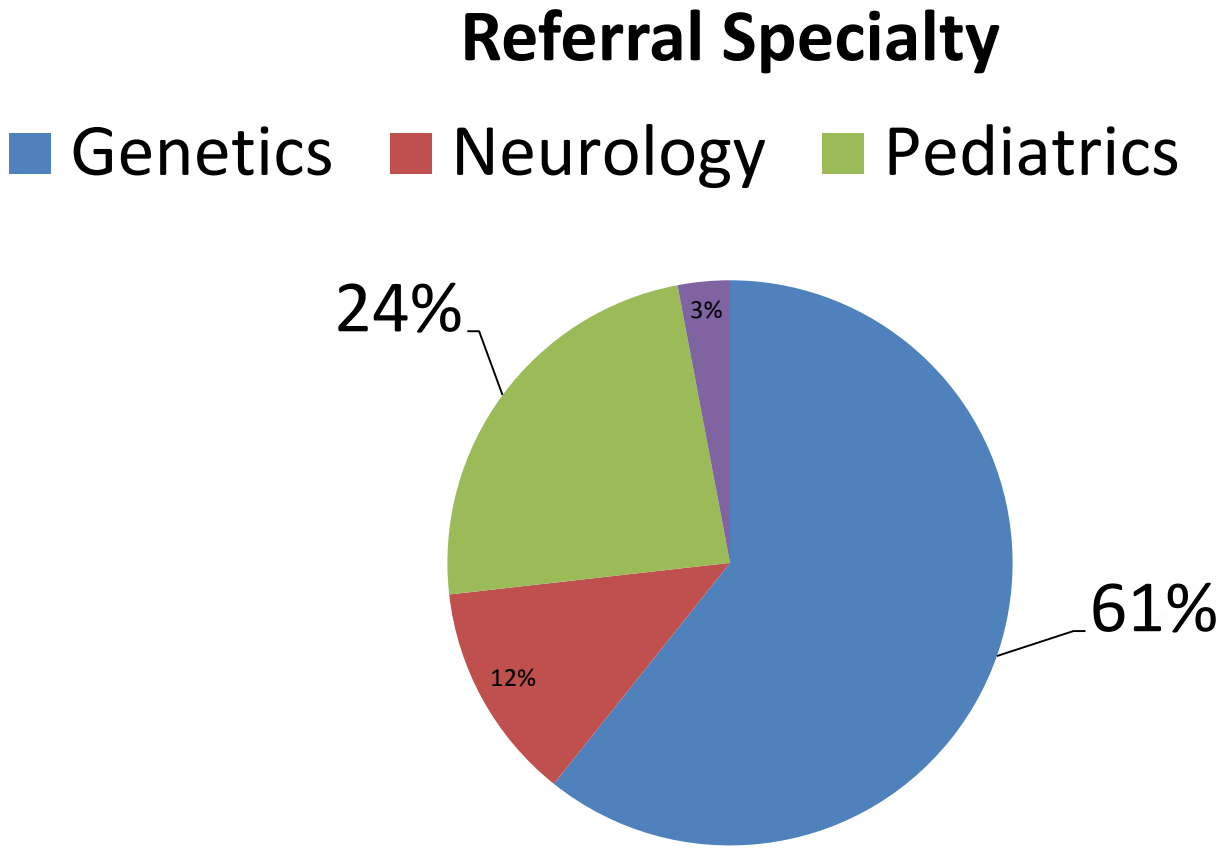
Baylor Experience with Clinical Exome Sequencing

BCM WGL Launches Whole Exome Sequencing Oct 2011



- ~1500 samples
- 85% peds; 15% adult
- Mostly neurologic
- In addition: skeletal disorders, pulmonary artery hypertension, cardiovascular dz
- Variety of referral sources – academic medical centers

Samples Referred by Specialty



WES Sample Positive Rate

- Of over 1,200 samples received since November 2011, 760 samples have been finalized

- Causative deleterious mutations related to patient phenotype have been identified in a minimum of 25% (190) patients

  - 52% (99) of the positive cases are AD disorders
  - 33% (62) of the positive cases are AR disorders
  - 12% (22) of the positive cases are X-linked disorders
  - 4% (7) of the positive cases have two molecular disorders

Molecular Diagnoses in Mendelian Disorders
Positive Rate: 62/250, ~25%

Inherit.	Genes with mutant alleles (times observed)	de novo mutants (%)	Novel variants (%)
AD	ANKRD11 (2), ARID1B (2)*, ATL1 (2), KRAS (2) ¶; ABCC9, ARID1A, CBL¶, CHD7, COL3A1, CREBBP, CRYGD, DYRK1A, EP300, FGFR1, HDAC8§, ITPR1, KANSL1, KAT6B, KIF1A, MLL2, NIPBL§, PTEN, PTPN11¶, SCN2A, SCN8A, SETBP1, SHANK3, SMARCB1*, SPAST, SRCAP, SYNGAP1, ZEB2	27/32 (84%) (4 unknown)	24/36 (67%)
AR	SACS (2), C5orf42, CLCN1, COL7A1, FBNL5, GAN, GLB1, HIBCH, KIF7, NDUFV1, PEX1, PNPO, POMT2, PRKRA, RAPSN, SLC19A3, STRC, TREX1, WDR19	40 alleles 6 HMZ 14 cmpnd HTZ	20/40 (50%)
XL	ATRX (2), OFD1 (2), CASK, MECP2, MTM1, PHEX, RBM10, SMC1A§	5/10=50% 1 mosaic	4/10 (40%)

§ 3 different genes for Cornelia de Lange; ¶ 3 different genes for Noonan

*3 SWI/SNF complex genes for MR

Cases with Two Molecular Diagnoses 7/760

Cases	Genes	Diseases	Inheritance
1	*SETBP1*	Schinzel-Giedion midface retraction syndrome	AD
	CLCN1	Myotonia congenita	AR
2	*TREX1*	Aicardi-Goutieres syndrome	AR
	PHEX	Hypophosphatemic rickets, X-linked dominant	X-linked
3	*RAPSN*	Congenital myasthenic syndrome	AR
	ABCC9	Cardiomyopathy dilated type 1O	AD
4	*POMT2*	Muscular dystrophy-dystroglycanopathy	AR
	SCN2A	Seizures	AD
5	*SMARCA2*	ID, Coffin Siris	AD
	SCN1A	Seizures	AD
6	*ATM*	Ataxia telangiectasia (AT)	AR
	AP4M1	Spastic paraplegia	AR
7	*NF1*	Neurofibromatosis, type 1	AD
	MEGF8	Carpenter syndrome 2	AR

Medically Actionable Mutations Reported

- Strong evidence for pathogenicity and altering management
- Examples of medically actionable mutations
  - Seven patients with FBN1 mutations (Marfan Syndrome)
  - Four patients with mutations in hereditary cancer genes: APC, BRCA2, CDH1, MSH6
  - Three male patients with G6PD mutations
  - Other patients carry mutations mostly in cardiovascular disease genes

Use of WES in Different Clinical Scenarios

- Pediatric
- Adult
- Prenatal
  - DOK7 mutations in case of fetal akinesia
  - NIPBL mutation in case of multiple congenital anomalies
- Pre-conception in case of two previous affected children

WES Diagnoses: Impact on Medical Management

Cases	Genes	Diseases
1	*SLC19A3*	Biotin- or thiamine-responsive encephalopathy type 2
2	*PHOX2B*	Central hypoventilation syndrome, congenital, with or without Hirschsprung disease (CCHS)
3	*ENPP1*	Arterial calcification of infancy, generalized, type 1 (GACI1)
4	*RAPSN*	Congenital Myasthenic Syndrome
5	*DOLK*	Congenital Myasthenic Syndrome
6	*CHRNE*	Congenital Myasthenic Syndrome
7	*SLC25A38*	Anemia, sideroblastic, pyridoxine-refractory, autosomal recessive
8	*TTC37*	Trichohepatoenteric syndrome 1

Statistics of WES Reports

Focused Report				
Related Disease-causing	Related VUS	Medically Actionable	*Carrier Status	Pharmaco-genetics
0-2	4-9	0-1	0-1	0-4

Expanded Report (ordered by 1/3 clients)		
Unrelated Disease-Causing	Unrelated VUS (AD, 1 hit; AR, 2 hits)	Molecularly deleterious Clinically Unknown
1-3	17-41	17-25

Unrelated VUS (AR, 1 hit)	Molecularly Unclassified, Clinically Unknown
26-64	300-600

Prior Diagnostic Evaluation

- Most cases had extensive prior genetic testing
- CMA, metabolic studies, single gene tests, panels, biopsies
- Suggestion that early use of WES may have cost savings but formal cost-effectiveness studies need to be performed

Example of Previous Evaluation

- 1 – metabolic screening, karyotype, PWS, brain MRI
- 2 –VLCFA, muscle bx, respiratory chain, mito DNA, mito depletion panel, PHOX2B, myotonic dystrophy, congenital disorders glycosylation next-gen panel,
- 3 – CSF neurotransmitters, Cr/guanidinoacetate, urine purines/pyr, NCL, DNA testing for 7 genes – ARX, CDKL5, MECP2

Case Studies

WHOLE EXOME SEQUENCING

Case 1

- 9.5 yo caucasian M
- H/o several episodes of extreme weakness, spells of apnea requiring intubation, increased respiratory secretions, ptosis, dysphagia, all usually when suffering from a febrile illness. At 8 mo was diagnosed with cardiomyopathy (note says has not recurred)
- FH: Younger sister died when 20 mo (developed febrile illness and stopped breathing), other siblings normal

- PHE: Wt 30th%, Ht 5th%, Ptosis, café au lait spot on right trunk, several small telangiectasias. Normal otherwise.
- Testing: muscle bx: ↑ type I fibers (slow), DNA studies: ETF-A, ETF-B, ETFDH (glutaric aciduria II), Acid Maltase (Pompe's) all normal. Fibroblast enzyme assays: PDH complex, CPT1, CPT2, CACT, CAT, SCAD, MCAD, LCHAD, VLCAD: all normal

Case #1 Compound heterozygous mutation/variant identified in *RAPSN*

- Gene: *RAPSN (RECEPTOR-ASSOCIATED PROTEIN OF THE SYNAPSE)*, 11p11.2
- Mutation: c.457G>A, p.A153T – mother is het

  c.872G>A, p.G291D – father is het

  – Both confirmed by Sanger sequencing
- Congenital Myasthenic Syndrome (CMS), AR
  - Symptoms include bilateral ptosis, weakness of limb, etc. Affects skeletal muscle
  - Frequent exacerbations with respiratory insufficiency provoked by illness/fever/stress
  - Treatment available

Case 1 Possible Second Diagnosis

- *ABCC9 (ATP-BINDING CASSETTE, SUBFAMILY C, MEMBER 9)*, 12p12.1
  - c.4570_4572delTTA_insAAAT, p.V1524fs
  - Mother (age 33) is heterozygous
  - Confirmed by Sanger sequencing
- Cardiomyopathy Dilated Type 10 (CMD10), AD
  - Severely dilated hearts with compromised contractile function and rhythm disturbances

Case 2

- 38 month-old female with static encephalopathy, hypotonia, and seizures
- On Keppra for seizures
- Receives speech, PT and OT
- Physical exam
  - Non-dysmorphic facial features
  - FOC <3rd, length 34th, weight 25th
- Previously evaluated in genetics at 23 and 27 months of age for hypotonia and motor delays

Case 2: Previous work-up

- Ophthalmology
- Neurology x 3
- EEG and a brain MRI (unremarkable)
- Labs (all normal)
  - Thyroid function studies
  - CK
  - Lactate
  - Aldolase
  - Plasma amino acids
  - Acylcarnitine profile
  - Urine organic acids
  - N-glycan and transferrin
  - Very long-chain fatty acids
  - Plasma creatine and guanidinoacetate determination
- CMA- paternally inherited 0.27Mb loss at Xp22.11
- WES was ordered at her third genetics visit

Case 2: WES Results

- De novo heterozygous c.376C>T (p.R126C) mutation in SLC2A1 associated with GLUT1 deficiency syndrome
- Glucose transporter type 1 deficiency syndrome (OMIM #606777)
  - Characterized by infantile-onset seizures, delayed neurologic development, acquired microcephaly, and complex movement disorders, low-normal/low CSF lactate, normal blood glucose, and low CSF glucose
  - Inherited in an AD manner
  - Ketogenic diet is highly effective in controlling seizures and improving the movement disorder and alertness

Case 2: Results Follow-up

- Family was counseled on new diagnosis
- Recurrence risk for future siblings of this patient is low, germline mosaicism cannot be excluded
- AD inheritance reviewed with parents for patient's future children
- Patient was referred to epilepsy clinic to start ketogenic diet

Case 2: Update

- She's been on ketogenic diet for ~3 months and is tolerating it well
- Taken off of Keppra, no seizures to date
- Family reports improvement with development
  - More active and alert
  - No longer naps during the day
  - Balance has improved – now rarely falls and is able to make quick turns without falling
  - Improvement in fine motor skills and speech

Summary for WES

- Strong, growing interest in whole exome testing
- Diagnose rare conditions and common conditions
  - Positive rate of 25% in unselected clinical samples
- Early evidence of clinical utility and cost-effectiveness
- Reporting of non-phenotype findings can be challenging
- Expand phenotypic spectrum of many disorders

BCM Team

Arthur Beaudet	Richard Gibbs	Jim Lupski
Christine Eng	Donna Muzny	Yaping Yang
Sharon Plon	Jennifer Scull	Jeffrey Reid
Will Parsons	Peter Pham	Alecia Willis
Jeff Mize	Michelle Rives	Alicia Braxton
Yan Ding Brandon Perthius	Joke Beuten Eric Burgess	Sean Kim Fan Xia
Mark Scheel Matthew Hardison	Neal Niu Robert Pace	Pat Ward Amy McGuire
Nehad Saada William Craigan	Doreen Ng Megan Landswerk	Mir Reza Bekheirnia Magalie Leduc
Wendy Liu	Richard Person	Alicia Hawes

Clinical Whole Exome Sequencing (WES) Sign-Out Conference

http://www.bcm.edu/geneticlabs/index.cfm?PMID=21319

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