Insights from the Lab: Tips for Clinical Genetic Counselors

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1

Disclaimer

I am a full-time employee of Sema4 and own Sema4 stock.

2

Learning Objectives

- Identify examples of genes that can be difficult to evaluate with NGS
- Examine strategies that laboratories can take to ensure high detection of "difficult genes"

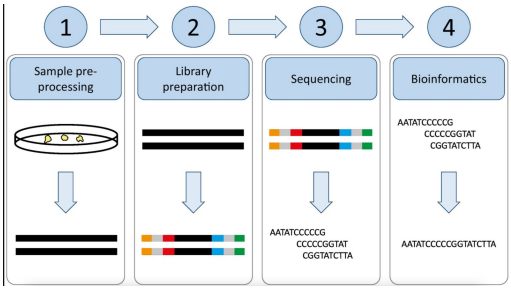
3

Genetic Technologies

4

Next-Generation Sequencing

- Allows for sequencing of multiple genes (*even the whole genome!*) simultaneously
- Cost-effective and time-efficient
- Known challenges
  - Trinucleotide repeats
  - Copy number variants
  - Insertions
  - Higher incidence of VUS results
  - Mosaicism
  - Low coverage regions



PMID: 32199980

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5

Pseudogenes

- What are pseudogenes?
  - "Fossil" copies of functional genes
  - *PKD1* has six pseudogenes
  - *NF1* has 7 pseudogenes
- A gene may have more than one pseudogene
- Pseudogenes have higher mutation rates than their functional gene counterparts
  - This can lead to variant detection misidentified to functional gene
- Degree of homology between pseudogene and gene of interest impacts next generation sequencing

Claes KBM, Rosseel T, De Leeneer K. Dealing with Pseudogenes in Molecular Diagnostics in the Next Generation Sequencing Era. Methods Mol Biol. 2021;2324:363-381. doi: 10.1007/978-1-0716-1503-4_22. PMID: 34165726.

6

Long range PCR

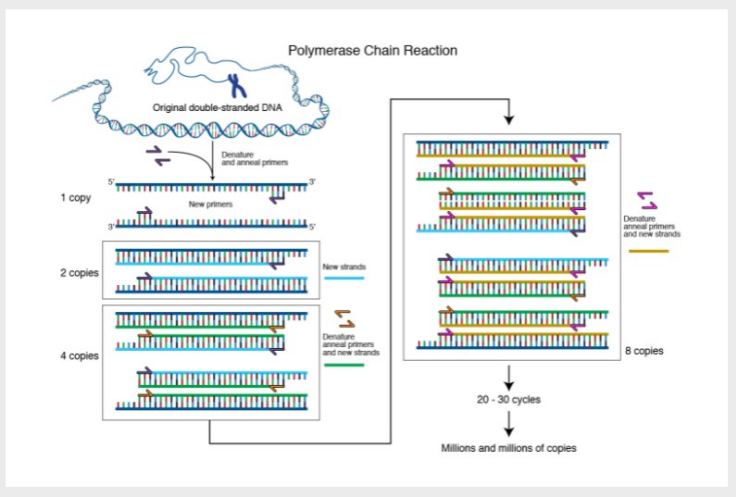

Figure from https://www.genome.gov/genetics-glossary/Polymerase-Chain-Reaction

7

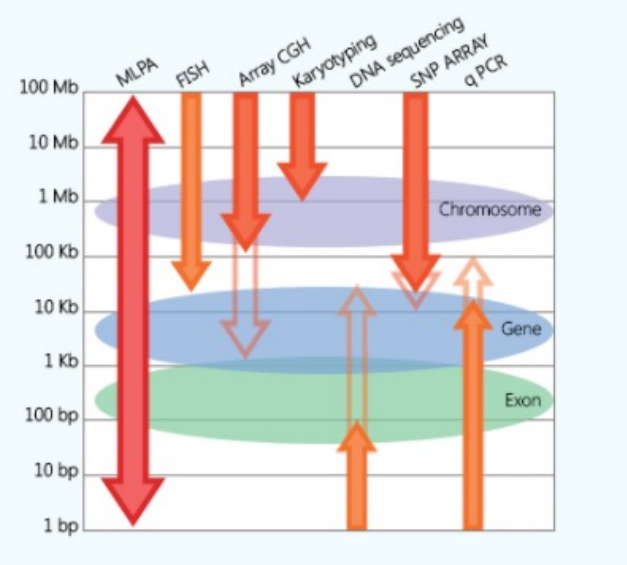

Figure from https://www.mrcholland.com/technology/mlpa

MLPA (Multiplex Ligation-dependent Probe Amplification)

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Technical Challenges: Hereditary Cancer

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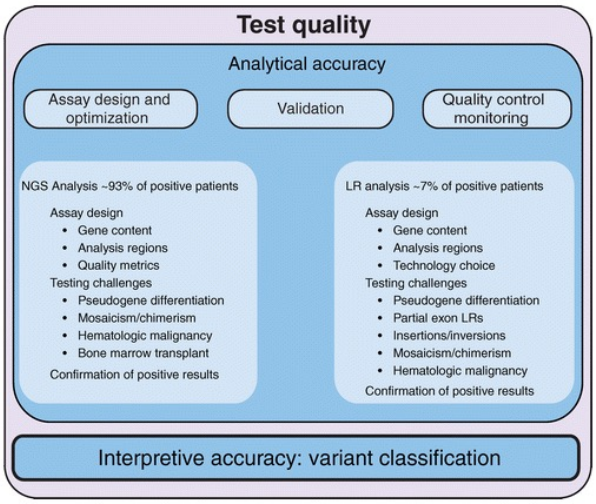
Hereditary Cancer Testing Challenges



PMID: 30113232

10

Lynch syndrome

- Most common hereditary colorectal cancer syndrome
  - Accounts for ~3% of colorectal cancers and ~3% of endometrial cancers[1]
- Characterized by increased risks for colorectal and endometrial cancers, as well as cancers of the ovary, stomach, small bowel, urinary tract, biliary tract, brain, skin, pancreas and prostate
- Caused by variants in mismatch repair genes
  - *MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*
- Multiple screening approaches
  - Microsatellite instability (may not be readily available or cost-saving)
  - Immunohistochemistry (variation in staining; may be less reliable with small sample; some variants will not result in absence of protein product)

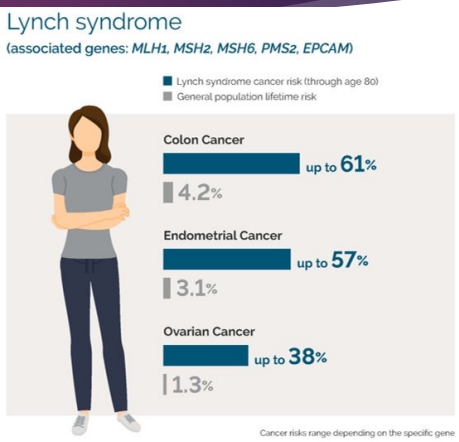


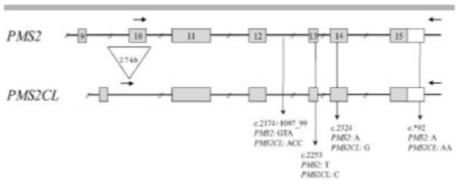
Figure from Sema4

[1]Weissman SM, Burt R, Church J, Erdman S, Hampel H, Holter S, Jasperson K, Kalady MF, Haidle JL, Lynch HT, Palaniappan S, Wise PE, Senter L. Identification of individuals at risk for Lynch syndrome using targeted evaluations and genetic testing: National Society of Genetic Counselors and the Collaborative Group of the Americas on Inherited Colorectal Cancer joint practice guideline. J Genet Couns. 2012 Aug;21(4):484-93. doi: 10.1007/s10897-011-9465-7. Epub 2011 Dec 14. PMID: 22167527.

11

PMS2 – The Challenge of Pseudogenes

- *PMS2* has more than 15 pseudogenes
- Long range amplification can be utilized to avoid amplification of pseudogenes
- Deletion/duplications in *PMS2* require MLPA, which requires identification of unique sequences
- Next-generation sequencing with long range PCR and MLPA improves reliability oof results and may resolve the *PMS2* pseudogene challenge



PMID: 21618646

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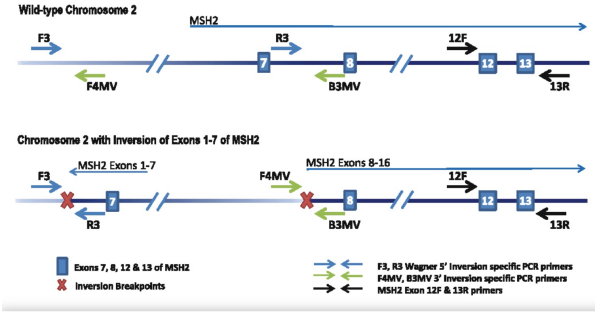
12

MSH2 - The Boland Inversion

- Inversion of exons 1-7 of *MSH2*[1]
- Reported in multiple studies[1,2]
- Labs utilize differing methods
  - PCR and electrophoresis[3]
  - MLPA[4]



PMID: 24114314

[1]Rhees J, Arnold M, Boland CR. Inversion of exons 1-7 of the MSH2 gene is a frequent cause of unexplained Lynch syndrome in one local population. Fam Cancer. 2014 Jun;13(2):219-25. doi: 10.1007/s10689-013-9688-x. PMID: 24114314; PMCID: PMC3984383. [2]Wagner A, van der Klift H, Franken P, Wijnen J, Breukel C, Bezrookove V, Smits R, Kinarsky Y, Barrows A, Franklin B, Lynch J, Lynch H, Fodde R. A 10-Mb paracentric inversion of chromosome arm 2p inactivates MSH2 and is responsible for hereditary nonpolyposis colorectal cancer in a North-American kindred. Genes Chromosomes Cancer. 2002 Sep;35(1):49-57. doi: 10.1002/gcc.10094. PMID: 12203789. [3]https://www.genedx.com/Resources/TIS-Files/TIS-J006.pdf
[4]https://www.ambrygen.com/file/material/view/1420/CancerNext%20v5.5_Negative_07_14_2020.pdf

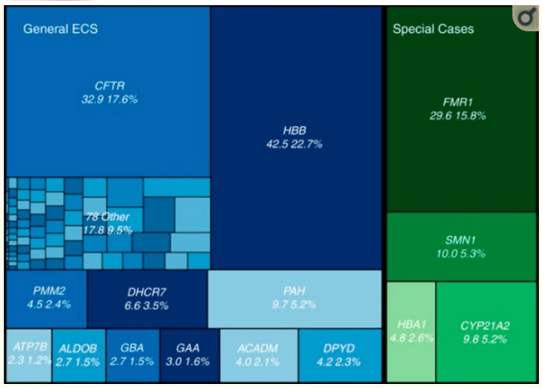
13

Technical Challenges: Carrier Screening

14

Carrier screening

- Expanded carrier screening panels possible due to NGS[1]
- ACMG recently proposed criteria to standardize screening[2]
- High prevalence genes that fit criteria for carrier screening may have known technical challenges[1]



PMID: 28640244

[1]Beauchamp KA, Muzzey D, Wong KK, Hogan GJ, Karimi K, Candille SI, Mehta N, Mar-Heyming R, Kaseniit KE, Kang HP, Evans EA, Goldberg JD, Lazarin GA, Haque IS. Systematic design and comparison of expanded carrier screening panels. Genet Med. 2018 Jan;20(1):55-63. doi: 10.1038/gim.2017.69. Epub 2017 Jun 22. PMID: 28640244; PMCID: PMC5763154. [2]Gregg, A.R., Aarabi, M., Klugman, S. *et al.* Screening for autosomal recessive and X-linked conditions during pregnancy and preconception: a practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genet Med* 23, 1793–1806 (2021).

15

CYP21A2 – Pseudogene + Gene Complexities

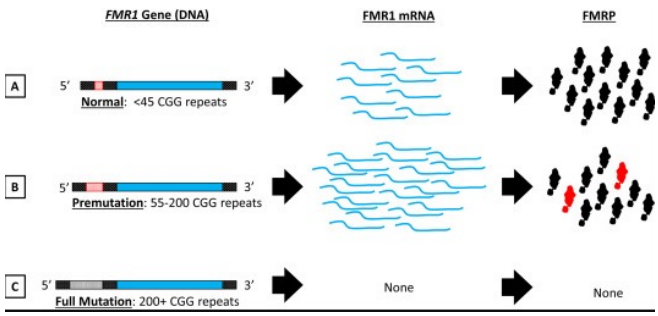
- Enzyme defects of 21-hydroxylase accounts for 90-95% of patients with congenital adrenal hyperplasia
- *CYP21A1P* is a functionally inactive pseudogene near *CYP21A2*
- Targeted sequencing for 10 common variants performed initially
  - Expensive and labor intensive
- MLPA for deletions and duplications
- Long range PCR amplification followed by sequencing

Carvalho B, Marques CJ, Santos-Silva R, Fontoura M, Carvalho D, Carvalho F. Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency: An Update on Genetic Analysis of CYP21A2 Gene. Exp Clin Endocrinol Diabetes. 2021 Jul;129(7):477-481. doi: 10.1055/a-1108-1419. Epub 2020 Mar 4. PMID: 32131114.

16

Fragile X syndrome – Trinucleotide Repeats & Carrier Screening

- NGS cannot identify trinucleotide repeat expansions
- PCR for shorter repeat expansions
- Southern blot for longer expansions
- AGG interruption studies



PMID: 31927143

Salcedo-Arellano MJ, Dufour B, McLennan Y, Martinez-Cerdeno V, Hagerman R. Fragile X syndrome and associated disorders: Clinical aspects and pathology. Neurobiol Dis. 2020 Mar;136:104740. doi: 10.1016/j.nbd.2020.104740. Epub 2020 Jan 10. PMID: 31927143; PMCID: PMC7027994.

17

FMR1 AGG Studies

Figure 3. Reclassification of fragile X risk transmission based on AGG interruption status

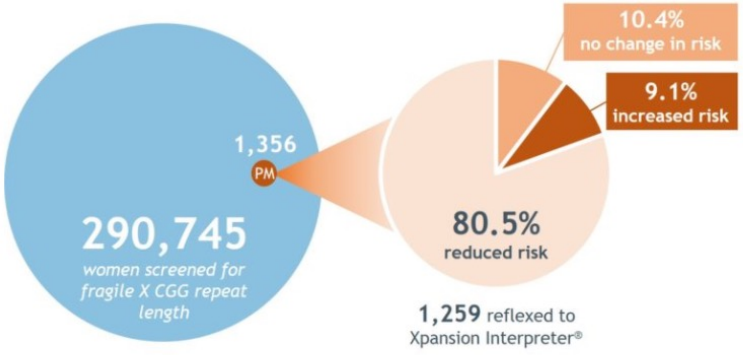


Figure from https://asuragen.com/portfolio/genetics/xpansion-interpreter/

18

NGS and VUS

19

VUS rates

- The more sequencing information, the greater the chance of novel or rare variants
- Variant interpretation guidelines exist, but no single source of truth
  - In one study, 71% consensus on variant classification[1]
- VUS rates impacted by knowledge of carrier frequency in population

Disease (Inheritance)	Gene	Ethnicity	Carrier Frequency	Detection Rate	Residual Risk	Analytical Detection Rate
Tay-Sachs Disease (AR)	*HEXA* *	Worldwide	1 in 111	88%	1 in 910	99%
NM_000520.4		African	1 in 147	63%	1 in 400	
		Ashkenazi Jewish	1 in 31	99%	1 in 3,000	
		East Asian	1 in 191	93%	1 in 2,700	
		Finnish	1 in 401	99%	1 in 40,000	
		European (Non-Finnish)	1 in 87	94%	1 in 1,400	
		Native American	1 in 219	79%	1 in 1,000	
		South Asian	1 in 251	37%	1 in 400	

Residual Risk Table from Sema4.com

[1] Amendola LM, Jarvik GP, Leo MC, McLaughlin HM, Akkari Y, Amaral MD, Berg JS, Biswas S, Bowling KM, Conlin LK, Cooper GM, Dorschner MO, Dulik MC, Ghazani AA, Ghosh R, Green RC, Hart R, Horton C, Johnston JJ, Lebo MS, Milosavljevic A, Ou J, Pak CM, Patel RY, Punj S, Richards CS, Salama J, Strande NT, Yang Y, Plon SE, Biesecker LG, Rehm HL. Performance of ACMG-AMP Variant-Interpretation Guidelines among Nine Laboratories in the Clinical Sequencing Exploratory Research Consortium. Am J Hum Genet. 2016 Jun 2;98(6):1067-1076. doi: 10.1016/j.ajhg.2016.03.024. Epub 2016 May 12. Erratum in: Am J Hum Genet. 2016 Jul 7;99(1):247. PMID: 27181684; PMCID: PMC4908185. Yohe S, Thyagarajan B. Review of Clinical Next-Generation Sequencing. Arch Pathol Lab Med. 2017 Nov;141(11):1544-1557. doi: 10.5858/arpa.2016-0501-RA. Epub 2017 Aug 7. PMID: 28782984.

20

Final Considerations

21

Considerations for Clinical GCs

- Does the lab use supplemental technologies?
- What testing are you ordering? Do you anticipate there being "difficult" genes?
- Is the lab transparent about their methods and limitations?
  - Check sample reports – often in the methods section
- How do we ensure equitable care across populations?

22

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25