All-in-one solution for single gene disorders with low sequencing quality

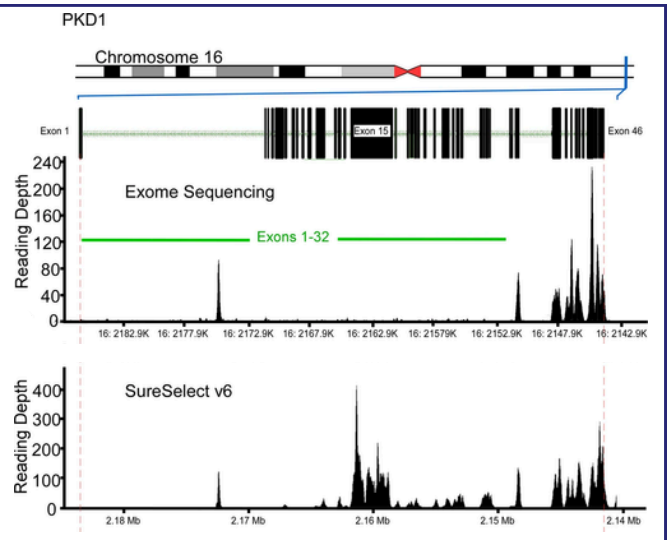
Autosomal Dominant Polycystic Kidney Disease

Background

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited cause of end-stage kidney disease (ESKD) worldwide. Symptoms typically appear in adulthood, though rare cases occur before age 15. Diagnosis is typically based on family history, imaging, and clinical evaluation. Genetic testing is required when imaging and family history cannot provide a diagnosis, particularly in young patients. Notably, around 10-15% of ADPKD cases occur without a prior family history [1].

While *PKD1* and *PKD2* variants account for 78% and 15% of cases, respectively [1], standard methods struggle to resolve the full spectrum of *PKD1* variants. NGS often performs poorly for *PKD1*, particularly in capture-based assays, due to:

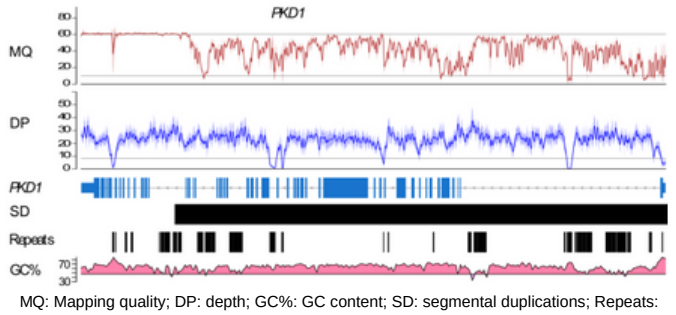
- Coverage and mapping challenges: *PKD1* is a large gene with extreme GC content in exon 1 and shares 97.7% sequence homology with 6 pseudogenes
- Structural variant detection: Cannot reliably identify duplications/deletions
- Phasing limitations: Struggles to distinguish *cis*/*trans* variants



WES coverage map of *PKD1* using commercial probes (TruSeq v3, SureSelect v4/v6) showed low coverage for exons 1–32, with 7.14% sensitivity for variant detection. While SureSelect v6 improved exon coverage, exons 1-14 remained poorly covered. [2]

Consequently, WES sensitivity for *PKD1* may be as low as 50% [2], and even non-capture WGS still struggle due to poor alignment with short-reads [3]. Although LR-PCR–based NGS targeting *PKD1* can significantly improve detection, it requires high sequencing depth, with coverage varying from <8× to >30,000× [3].

Genotype	Phenotype
PKD1 vs *PKD2*	*PKD1* variants are associated with a more severe phenotype
Homozygous vs Heterozygous	Homozygous mutations often result in embryonic lethality
Truncating vs Non-truncating	Truncating variants are associated with a more severe phenotype
Biallelic vs Monoallelic	Biallelic variants result in a more severe phenotype



MQ: Mapping quality; DP: depth; GC%: GC content; SD: segmental duplications; Repeats: repetitive elements

PKD1 gene NGS inaccessible and unresolvable regions with available WGS dataset show dips in read depth and mapping quality, especially where repeats and high GC content overlap. [3]

Comprehensive Analysis of *PKD1* and *PKD2*

Comprehensive Analysis of *PKD1* and *PKD2* (CAPKD)

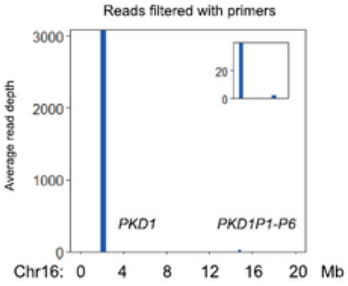
improves the resolution of ADPKD genetic bias

Leveraging SMRT technology, CAPKD overcomes the "dark regions" of the *PKD1* gene. It can distinguish the functional *PKD1* gene from its pseudogenes, ensuring reliable variant detection.

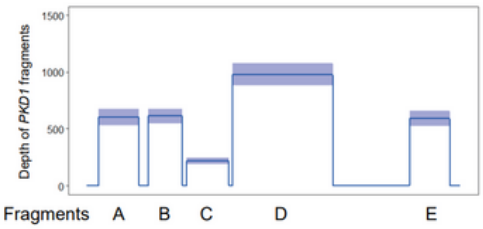
Technology: Single molecule real-time (SMRT) sequencing
Platform: PacBio Vega, Sequel II, and Sequel IIe system
Sample type: Blood, DBS, gDNA, and buccal swab
Turnaround time: 17 working days
(starting from the date of sample arrival at the testing laboratory)
Testing Scope: Detects ADPKD-associated variants in the *PKD1* and *PKD2* genes, including: P, LP and some VUS SNVs/InDels, and exon deletions/duplications.

Uniform *PKD1* coverage with a low noise ratio (0.05%) to pseudogenes

Average depth of CCS reads aligned to hg38 of clinical samples for *PKD1* fragments and *PKD1P1-P6*.[4]



Average sequencing depth of *PKD1* fragments for all samples. The blue regions showed a 95% confidence interval of depth. The average CCS read depth 602× (range: 38×–3318×).[4]



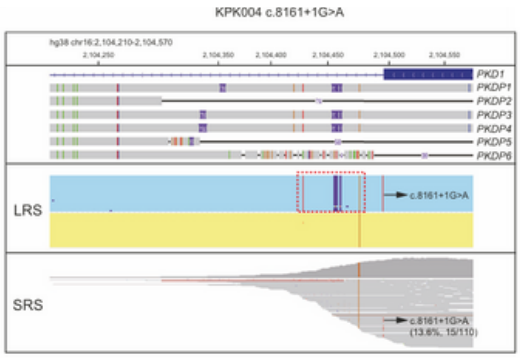
E: exon 1; D :exons 2-16; C: exons 17-26; B: exons 27-34; A: exons 35-46

vs. Capture-Based NGS [5]

Increased detection yield by solving 31% of cases that genetically unexplained

CAPKD additionally identified 10 P/LP *PKD1* variants in 40 patients without full genetic characterization, including four patients with micro–gene conversion events between *PKD1* and its pseudogenes *PKD1P1-P6*. CAPKD also identified 22% more *PKD1* CNVs than NGS.

IGV plot of CAPKD (LRS) CCS reads and NGS(SRS) reads showing *PKD1:c.8161+1G>A*. CAPKD additionally reported a microgene conversion variant.



vs. Combined Strategy (LR-PCR NGS + MLPA) [4]

Identified variants in 16% of ADPKD patients that previously returned negative findings

CAPKD identified a total of 160 P/LP/VUS variants in 170 unrelated patients, while NGS missed 2 *PKD1* InDels (c.78_96dup and c.10729_10732dup) and miscalled a *PKD2* InDels (c.810_811del). Additionally, 11 patients had 2 or more variants and the *cis*/*trans* configuration is identified by CAPKD.

More precise CNVs identifation than MLPA

CAPKD accurately detected 7 deletions/duplications. Compared to MLPA, discrepancies occurred in 2 out of 6 deletions.

	MLPA	CAPKD	
Gene	Nucleotide change	Nucleotide change	Region
PKD1	Del(Exon 1–38)	Del(chr16:2092764–2133011)	Exon 1–38
PKD1	Del(Exon 22–31)	Del(chr16:2097378–2102046)	Exon 22-E33
PKD1	Del(Exon 27–31)	Del(chr16:2098463–2101235)	Exon 27–30
PKD1	Del(Exon 22)	Del(chr16:2104373–2104922)	Exon 22
PKD1	Del(Exon6)	c.1251_1385 + 198del	Exon 6
PKD2	Del(Exon2)	Del(chr4:88015500–88022890)	Exon 2

References:[1] Orphanet: Autosomal dominant polycystic kidney disease. *Orpha.net*. Published 2020. https://www.orpha.net/en/disease/detail/730 [2] Ali H, Al-Mulla F, Hussain N, et al. *PKD1* Duplicated regions limit clinical Utility of Whole Exome Sequencing for Genetic Diagnosis of Autosomal Dominant Polycystic Kidney Disease. *Sci Rep*. 2019;9(1):4141. [3] Borràs DM, Vossen RHAM, Liem M, et al. Detecting *PKD1* variants in polycystic kidney disease patients by single-molecule long-read sequencing. *Hum Mutat*. 2017;38(7):870-879. [4] Xu D, Mao A, Chen L, Wu L, Ma Y, Mei C. Comprehensive Analysis of *PKD1* and *PKD2* by Long-Read Sequencing in Autosomal Dominant Polycystic Kidney Disease. *Clin Chem*. 2024;70(6):841-854. [5] Sun Q, Xu P, Mao A, et al. Targeted long-read sequencing enables higher diagnostic yield of ADPKD by accurate PKD1 genetic analysis. *NPJ Genom Med*. 2025;10(1):22.
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