OncoDNA®
THE CANCER THERANOSTIC COMPANY

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Managing challenging NGS Samples

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Managing Challenging NGS Samples

NGS-based **Comprehensive Genomic Profiling (CGP)** panels are powerful, but they're tricky to do well because challenges pile up at *every step*—from the sample to the final interpretation. It's not just sequencing; it's an end-to-end system challenge.

Sample quality (biggest practical headache)

- **FFPE samples** (common in oncology) often have:
  - DNA fragmentation
  - Chemical damage (e.g., deamination → false C>T variants)
- Low tumor content → mutations get diluted by normal DNA
- Small biopsies → very limited input material

👉 Result: noisy data + risk of false negatives

Tumor heterogeneity

•Tumors aren’t uniform—different regions can have different mutations
•Subclonal variants may exist at very low allele frequencies

👉 You need **high sensitivity** without increasing false positives

Breadth vs depth trade-off

•CGP panels cover many genes
•But:
- More breadth → less sequencing depth per region (if cost constrained)
- Less depth → harder to detect low-frequency variants

👉 Balancing coverage vs sensitivity is non-trivial

Detecting multiple variant types
CGP isn't just SNVs:

•SNVs / small indels
•Copy number alterations (CNAs)
•Structural variants / fusions
•MSI status
•Tumor mutational burden (TMB)
Each requires **different algorithms and assumptions**:
•CNAs → need normalization + baseline
•Fusions → need RNA or sophisticated DNA inference
•TMB → sensitive to panel size and filtering

👉 One pipeline rarely handles all perfectly

Bioinformatics complexity

•Alignment errors (especially in repetitive regions)
•Variant calling thresholds (sensitivity vs specificity trade-off)
•Artifact filtering (FFPE, PCR duplicates, sequencing errors)

👉 Small parameter changes can significantly alter results

Lack of matched normal samples

•Many clinical CGP tests are **tumor-only**
•Hard to distinguish:
- Somatic mutations
- Germline variants
- Clonal hematopoiesis (CHIP)

👉 Leads to:
•False somatic calls
•Missed clinically relevant germline findings

Copy number & ploidy estimation

•Tumor purity and ploidy affect signal interpretation
•Same read depth can mean different things depending on purity

👉 CNA calling becomes **mathematically underdetermined**

Real World Experience

Managing Challenging NGS Samples

AQUARIUS NPG Cancer Genomic Laboratory

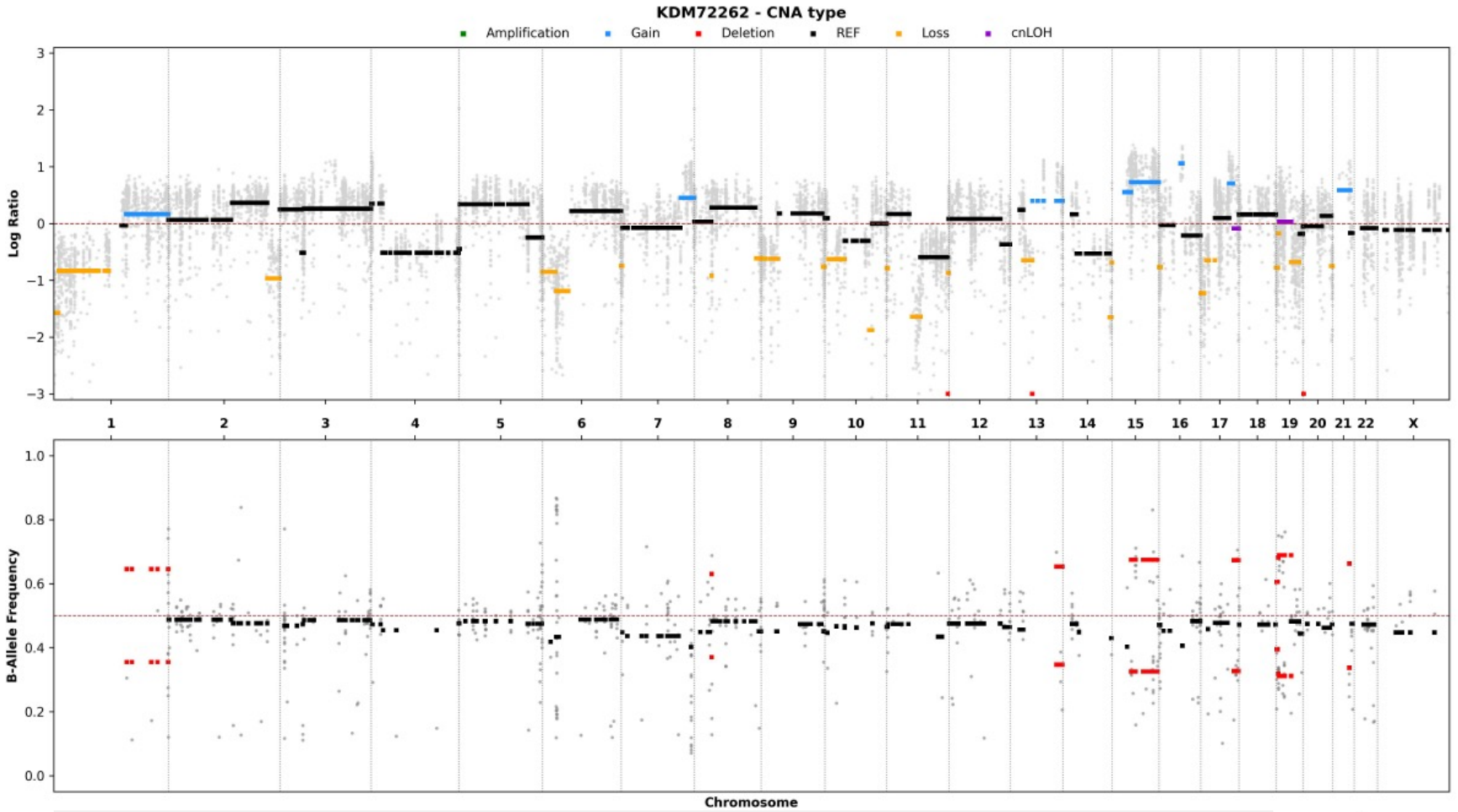
HRD scores negative and BRCA1/2 mutation negative, but copy-number analysis shows multiple LOHs and terminal (telomeric) losses consistent with HRD (KDM66093; KDM70391;KDM72262)

- What are the possible explanations for this “discrepancy”? Technical, genomic and/or cellular?

Sample ID	**69036**
Provider	npg-turkey
Provider ID	KDM72262
Project code	NPGTURKEY1
Product	OncoDEEP KIT
Cancer	Leiomyosarcoma
KIT	-
External Id	8126M195-D_8126M195-R
Due for	2026-03-25
NGS monitoring	-

Sample ID	**67165**
Provider	npg-turkey
Provider ID	KDM70391
Project code	NPGTURKEY1
Product	OncoDEEP KIT
Cancer	Head and neck cancer
KIT	-
External Id	8126M120-D_8126M120-R
Due for	2026-03-02
NGS monitoring	-

Sample ID	**62867**
Provider	npg-turkey
Provider ID	KDM66093
Project code	NPGTURKEY1
Product	OncoDEEP KIT
Cancer	Colorectal Cancer
KIT	-
External Id	8125M1026-D
Due for	2025-12-10
NGS monitoring	-



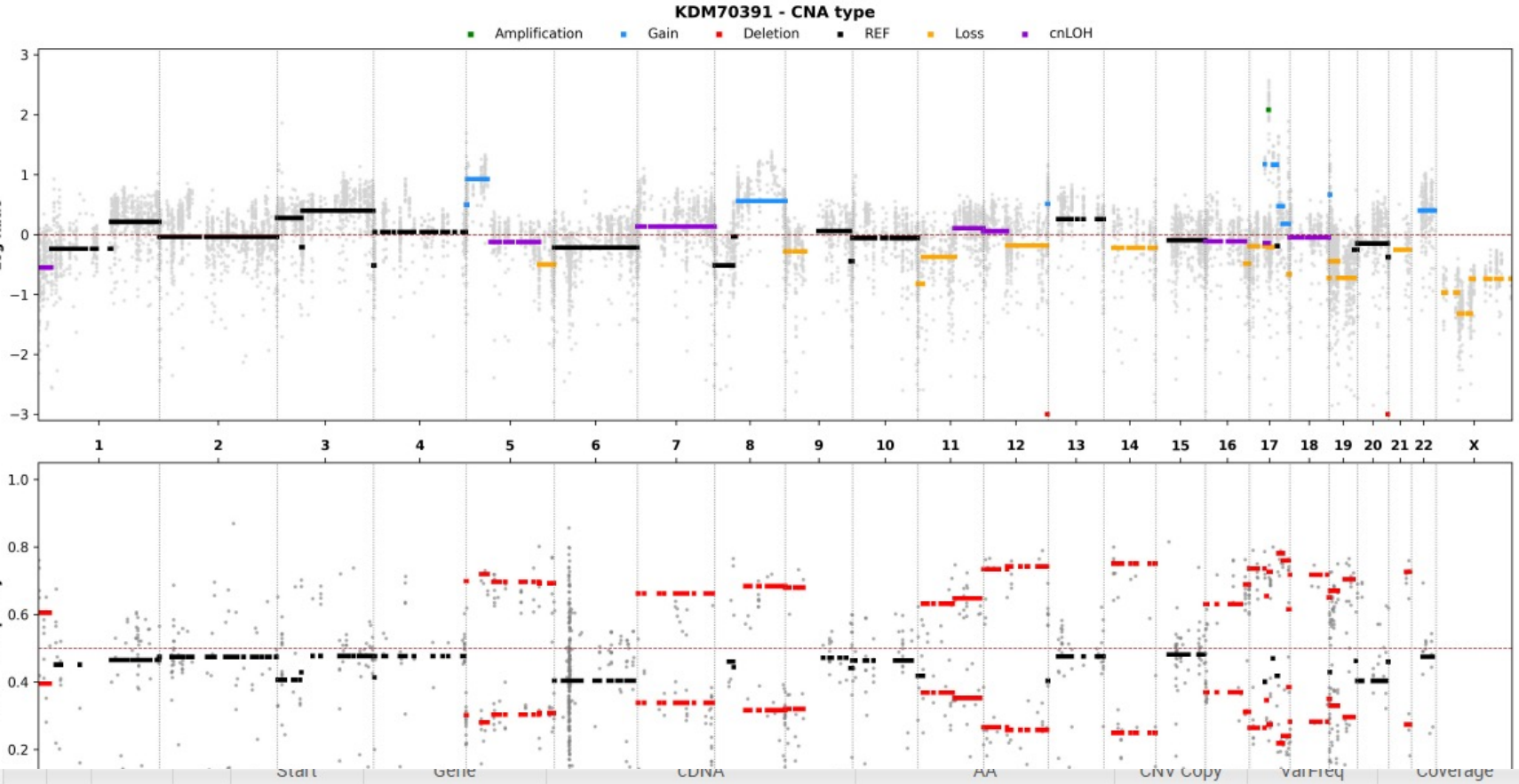
KDM72262 Leimyosarcoma

HRD Negative 30,8

Show 1000 entries

			Start	Gene	cDNA	AA	CNV Copy	VarFreq	Coverage	Bio Impact	Tiering system	Thera Lvl
LOH	chr4	152320543	FBXW7			1	-	?	Damaging	Likely Pathogenic	Level III
LOH	chr6	35452337	FANCE			1	-	?	Potentially Damaging	Likely Pathogenic	Level III
LOH	chr9	21967751	CDKN2A			1	-	?	Potentially Damaging	Likely Pathogenic	Level III
LOH	chr10	87863624	PTEN			1	-	?	Damaging	Likely Pathogenic	Level III
LOH	chr11	108223066	ATM			1	-	?	Potentially Damaging	Likely Pathogenic	Level III
LOH	chr13	32315507	BRCA2			1	-	?	Potentially Damaging	Likely Pathogenic	Level III
CNV_LOSS	chr13	48303750	RB1			0	-	?	Damaging	Likely Pathogenic	Level III
LOH	chr14	67819778	RAD51B			1	-	?	Potentially Damaging	Likely Pathogenic	Level III
LOH	chr17	7668420	TP53			1	-	?	Potentially Damaging	Likely Pathogenic	Level III
SNV	chr17	7674231	TP53	c.731G>A	p.(G244D)	0.85	0.9408	304	Potentially Damaging	Likely Pathogenic	Level III
SNV	chrx	71119478	MED12	c.204+2T>G		3.02	0.5389	501	Potentially Damaging	Likely Pathogenic	Level III
SNV	chrx	77652354	ATRX	c.4318-2A>G		2.89	0.9385	130	Potentially Damaging	Likely Pathogenic	Level III

cellularity	ploidy	SLPP	Line_Number
0.35	2.05	0.0416498505671855	1



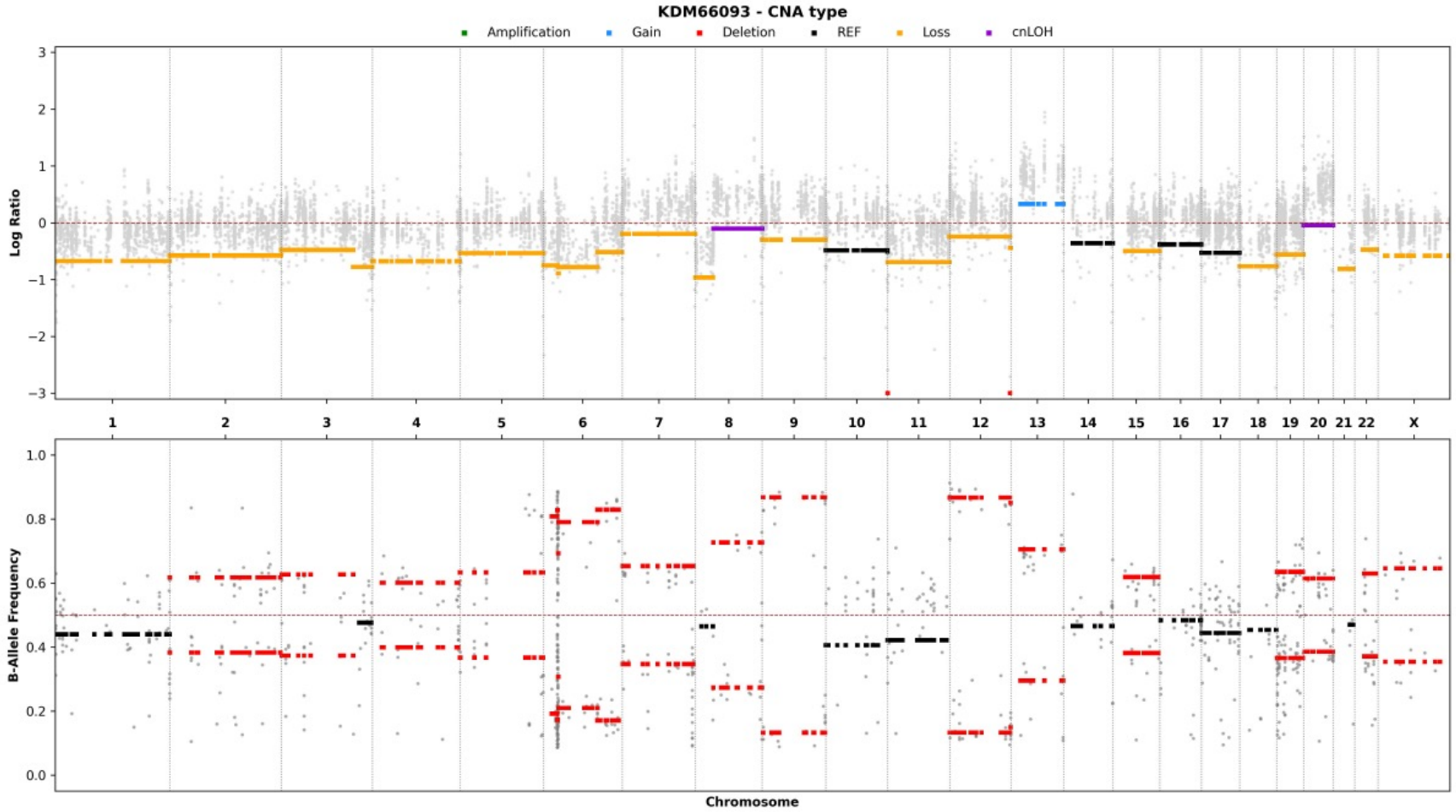
KDM70391 Head and Neck Cancer

HRD Negative 34,8

cellularity	ploidy	SLPP	Line_Number
0.4	2.85	0.135939681021217	1

		Start	Gene	cDNA	AA	CNV Copy	VarFreq	Coverage	Bio Impact	Tiering system	Thera Lvl
LOH	chr3	52401007	BAP1			1	-	?	Potentially Damaging	Likely Pathogenic	Level III
LOH	chr6	35452337	FANCE			1	-	?	Potentially Damaging	Likely Pathogenic	Level III
LOH	chr9	21967751	CDKN2A			1	-	?	Potentially Damaging	Likely Pathogenic	Level III
LOH	chr9	22002902	CDKN2B			1	-	?	Potentially Damaging	Likely Pathogenic	Level III
LOH	chr9	35073838	FANCG			1	-	?	Potentially Damaging	Likely Pathogenic	Level III
LOH	chr17	7668420	TP53			1	-	?	Potentially Damaging	Likely Pathogenic	Level III

		Start	Gene	cDNA	AA	CNV Copy	VarFreq	Coverage	Bio Impact	Tiering system	Thera Lvl
CNV_AMPL	chr17	39688093	ERBB2			8.41	-	?	Damaging	Pathogenic	Level II C



KDM66093 Colorectal Cancer

HRD Negative 34,4

cellularity	ploidy	SLPP	Line_Number
0.6	2.75	0.00441300971076029	1

		Start	Gene	cDNA	AA	CNV Copy	VarFreq	Coverage	Bio Impact	Tiering system	Thera Lvl
SNV	chr12	25245346	KRAS	c.38G>A	p.(G13D)	2.46	0.6695	956	Damaging	Pathogenic	Level I A
SNV	chr17	7673775	TP53	c.844C>T	p.(R282W)	1.66	0.2227	1006	Damaging	Pathogenic	Level III
LOH	chr6	35452337	FANCE			1	-	?	Potentially Damaging	Likely Pathogenic	Level III
SNV	chr17	7675130	TP53	c.481G>A	p.(A161T)	1.66	0.3667	908	Potentially Damaging	Likely Pathogenic	Level III
LOH	chr17	43044294	BRCA1			1	-	?	Potentially Damaging	Likely Pathogenic	Level III

Sample / cellular factors

a) Tumor purity
Low tumor content:
• Dilutes allelic imbalance signals
• Reduces amplitude of CN changes
→ Leads to **underestimation of HRD score** despite visible patterns.

b) Tumor heterogeneity / subclonality
If HRD is present only in a subclone:
• CN scars may be visible
• But not strong enough globally
→ Score falls below threshold.

c) Ploidy / whole-genome doubling
High ploidy tumors:
• Can “mask” LOH signals
• Alter how events are counted
→ Leads to **discordant interpretation vs. score**.

Chromosomal instability not related to HRD

Some tumors (e.g. TP53-driven, aneuploid tumors) show:
• Widespread CN changes
• LOH and telomeric events
→ But driven by **general chromosomal instability (CIN)**, not true HRD

Algorithm / scoring limitations

Event size and definition matter
Not all LOHs contribute equally:
• Only **LOH above certain Mb size ranges** are counted
• Telomeric events must meet strict criteria
→ IGV/CN plots can look “HRD-like,” but the **algorithm excludes some segments**.
They may be **below size cutoffs**

Most likely combined explanation

In that cases, the most common reality is a **combination** of:

- **Borderline HRD genomic scars**
- **Algorithm thresholds excluding some events**
- ± **purity or ploidy effects**
- **CIN (**LOH patterns driven by non-HRD genomic instability)**and tumors driven by other even (e.g. TP53, KRAS, ERBB2,…)**

Managing Challenging NGS Samples take away Mgs

- Poor sample quality (FFPE damage, low DNA, low tumor content)
- Tumor heterogeneity (low-frequency subclonal variants)
- Trade-off between panel breadth and sequencing depth
- Need to detect multiple variant types (SNVs, indels, CNAs, fusions, TMB, MSI)
- Complex and sensitive bioinformatics pipelines
- Lack of matched normal → hard to separate somatic vs germline
- Difficult copy number analysis (affected by purity and ploidy)
- Challenging clinical interpretation (many variants of unknown significance)
- Strict validation and regulatory requirements
- Pressure for fast turnaround despite complexity

Managing difficult NGS samples is not about eliminating uncertainty — it's about **making it visible, controlled, and clinically interpretable**.

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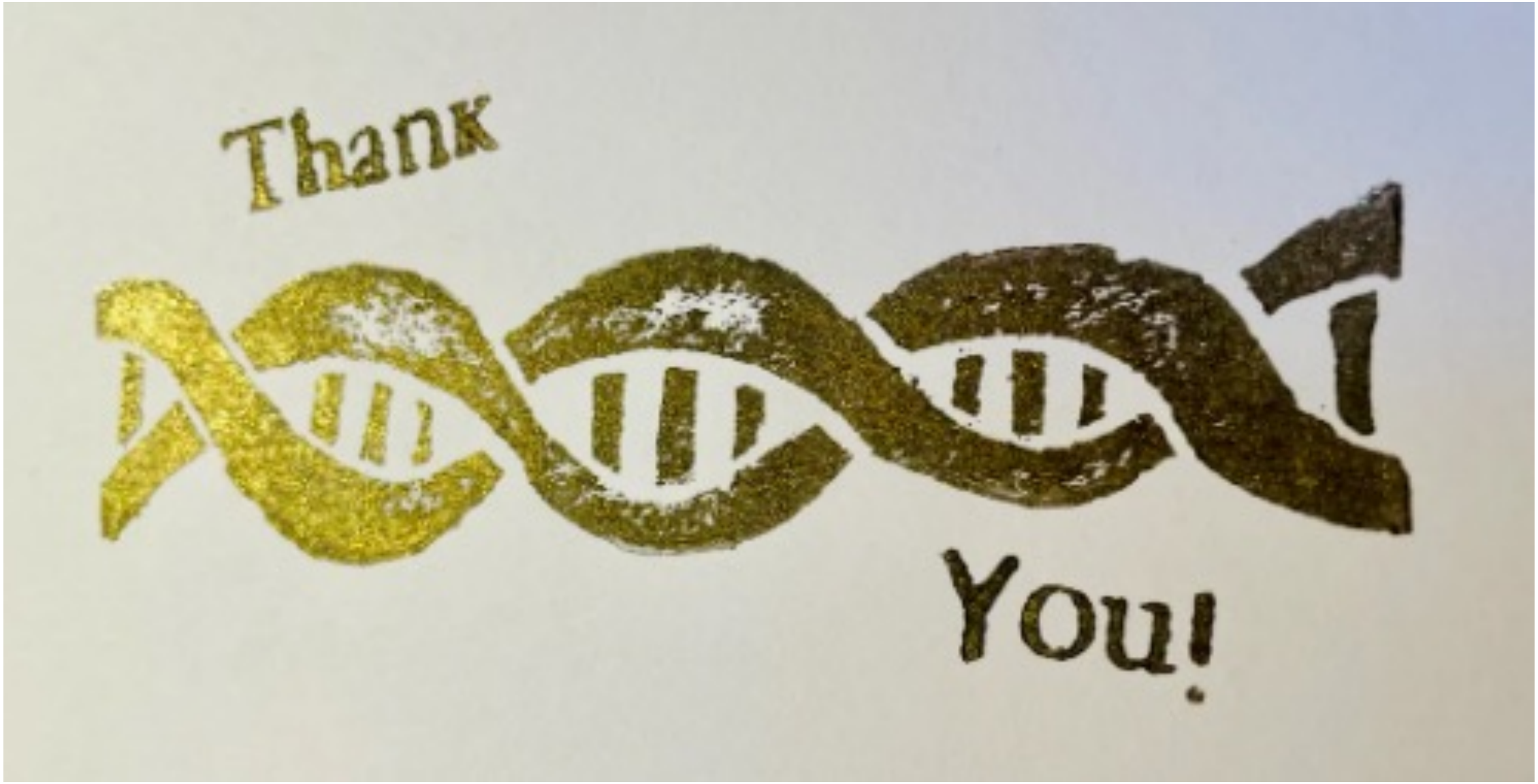
Thank
You!