Genome-Wide Germline Genotypes from Normal Tissue Stored in FFPE Samples

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Thousands of FFPE blocks have been stored in Utah since 1960s

Stacks of trays containing formalin fixed paraffin embedded (FFPE) tissue blocks are easily stored at room temperature for years.

A tray contains hundreds of tissue blocks with easy-to-see de-identified numbers.

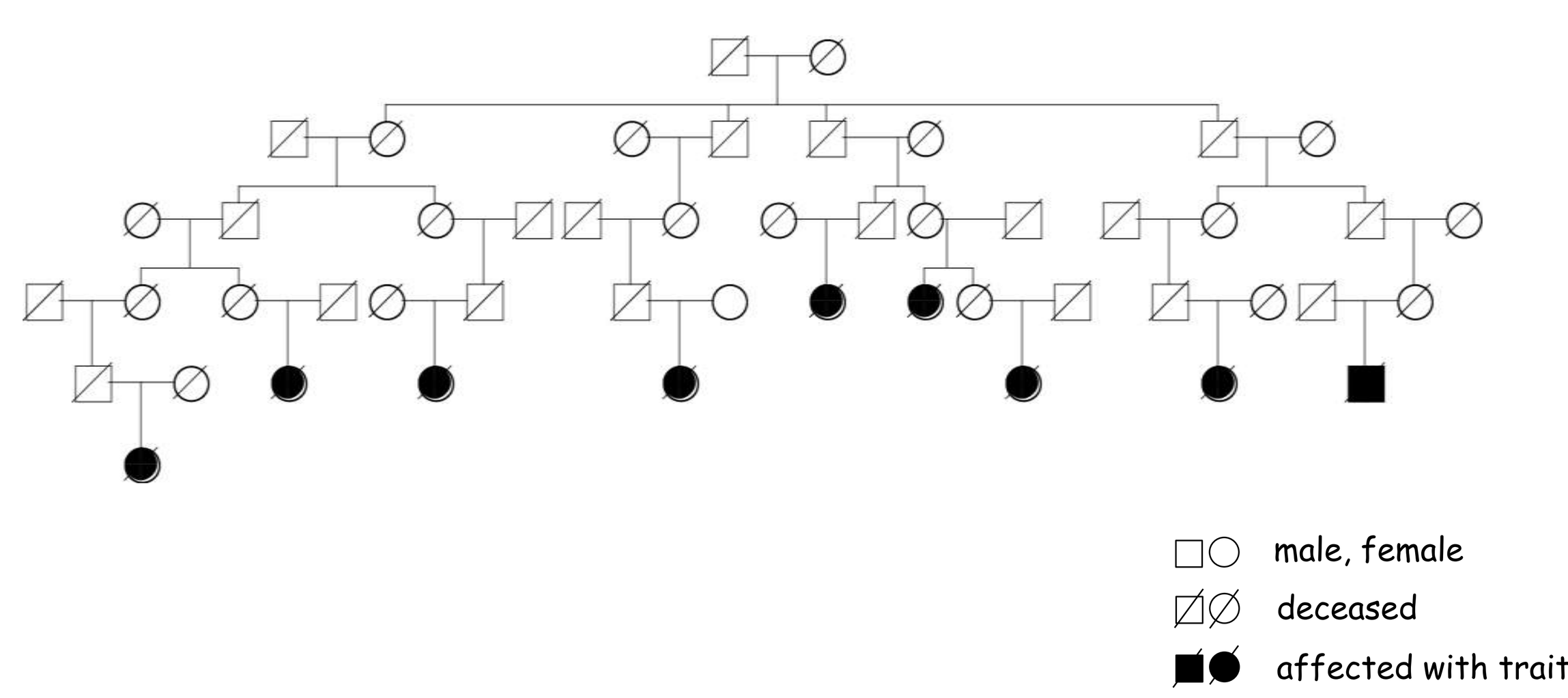
A single individual may have several tissue blocks associated with that tissue collection.

FFPE blocks as a germline DNA source?

Normal tissue harvested from FFPE blocks could provide germline genomic DNA from individuals no longer available for sampling

This could enable retrospective linkage analysis studies on certain diseases:

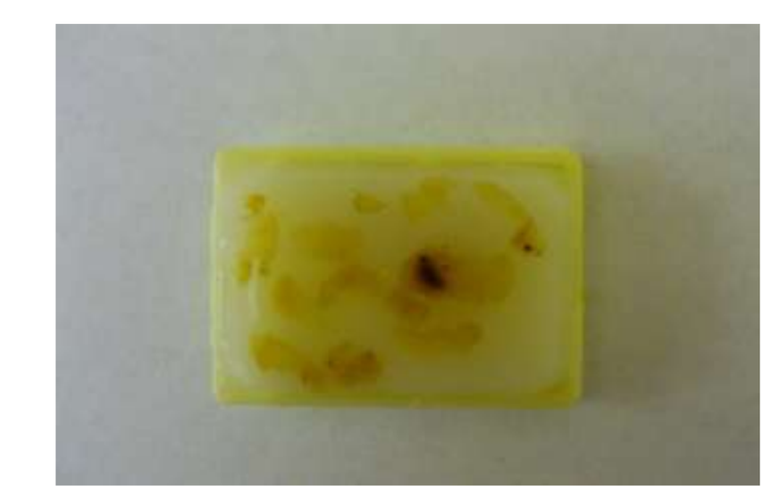
- Diseases with short survival times
- Diseases no longer commonly diagnosed (for example, pandemic influenza)



For example, this colorectal cancer pedigree shows several generations of affected individuals. Most individuals are deceased.

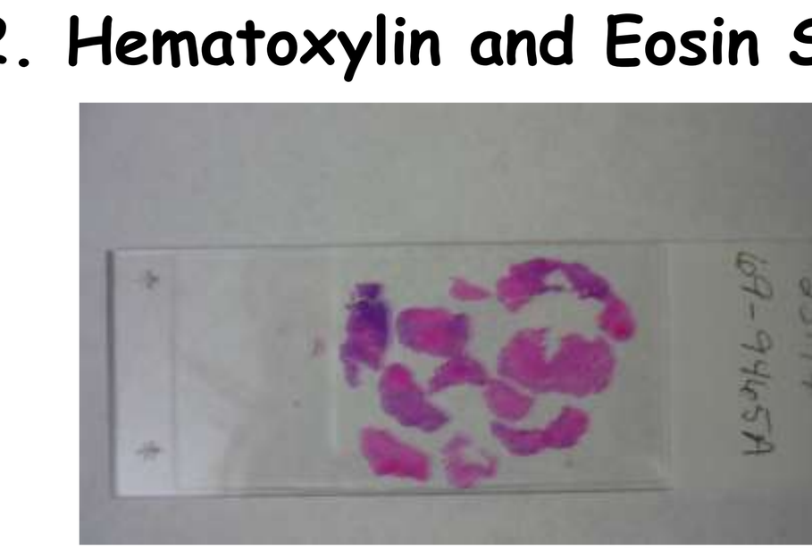
Method

1. FFPE Blocks



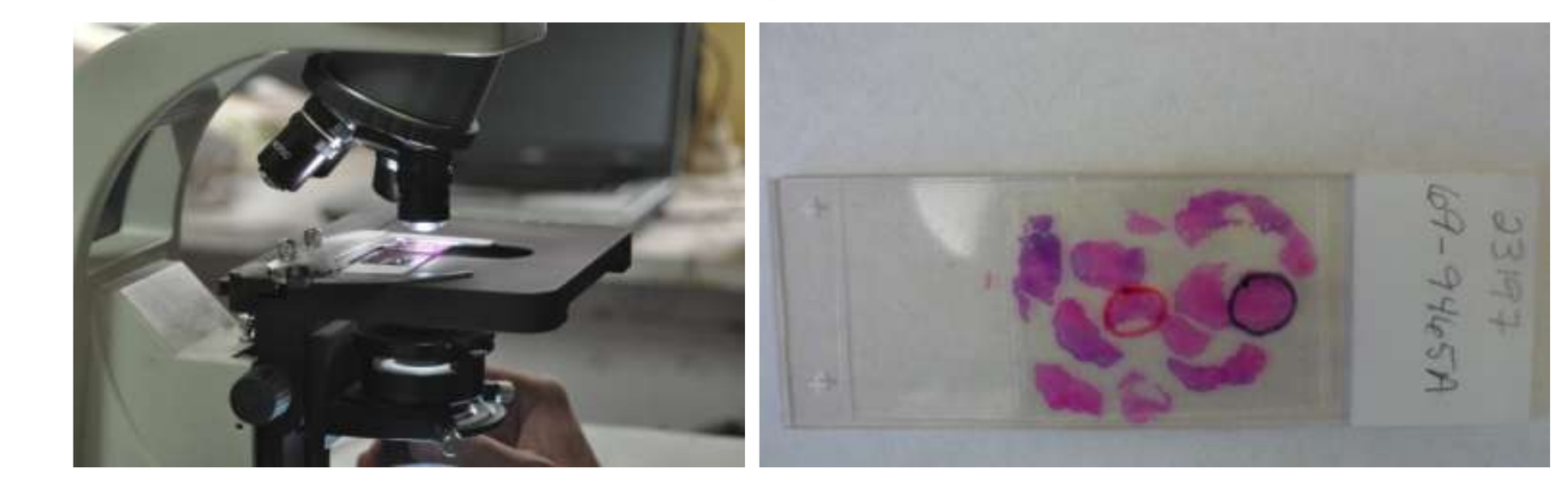
Identify the FFPE tissue block(s) for each individual to be genotyped.

2. Hematoxylin and Eosin Stain



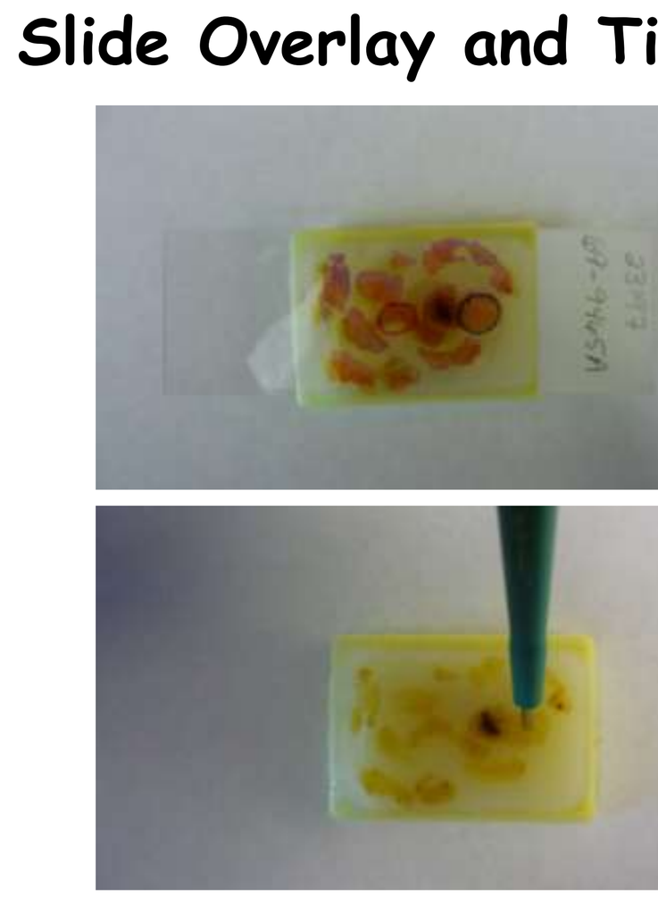
Section a 6 μm cut from each block and stain using standard histological techniques.

3. Pathology Review



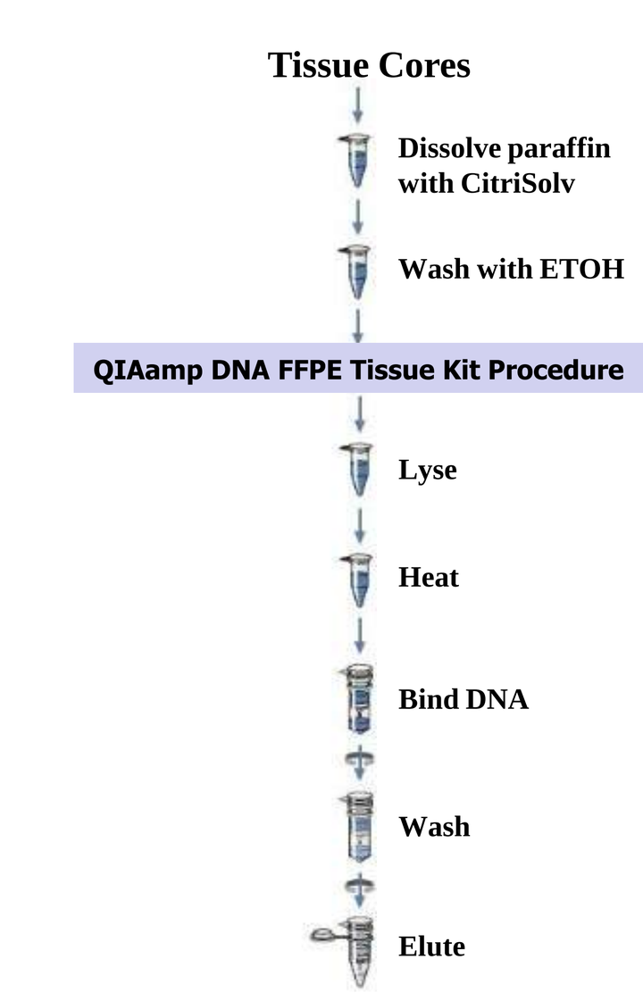
A pathologist reviews the H&E slide and circles tumor-rich regions in red and uninvolved "normal tissue" regions in black.

4. Slide Overlay and Tissue Punches



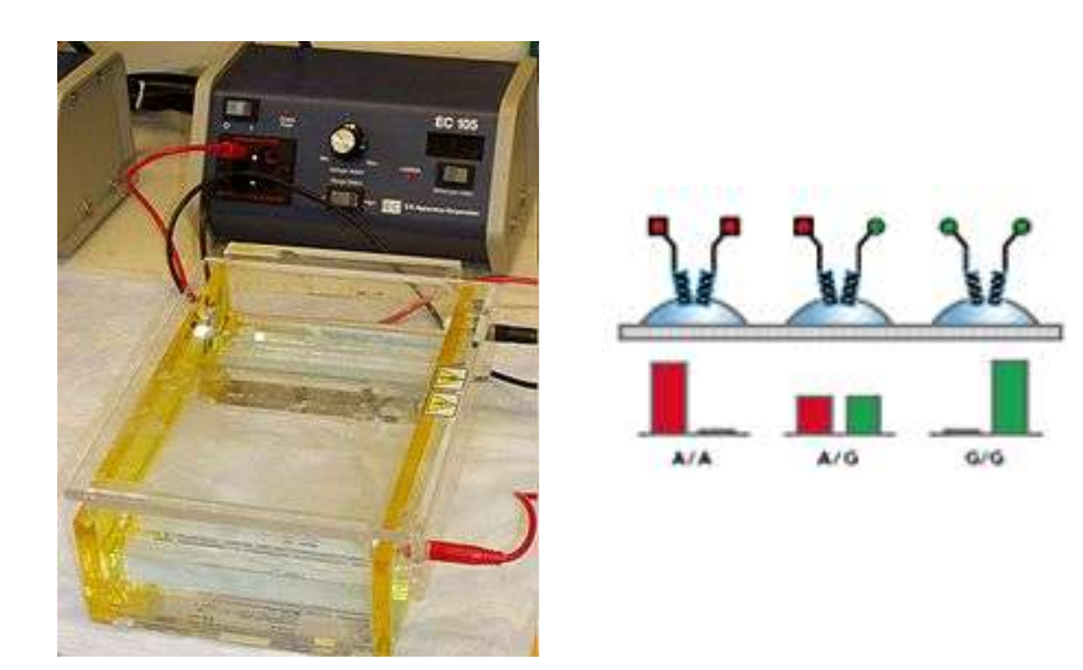
Overlay the marked H&E onto the original FFPE block for orientation. Take tissue punches from the uninvolved areas containing normal tissue.

5. Genomic DNA Extraction



Extract genomic DNA using a modified Qiagen protocol.

6. DNA Integrity and Genome-wide Genotyping Yield Evaluation



Assess integrity of the isolated genomic DNA by agarose gel electrophoresis. Evaluate genotyping results by % call rates using the Illumina 610Q platform.

Experimental Results

Table 1. Five case samples were chosen spanning a range of FFPE tissue block qualities, quantities, and age. DNA extraction from normal FFPE tissue yielded 5-48 μg DNA of good purity.

Table 1. DNA Extraction Results

Case Sample	Year	# blocks/slides	Yield (μg)	Abs 260/280	Abs 260/230
423197	1969	[illegible]	tissue	NA	NA
525593	1984	[illegible]	48	1.82	1.84
620691	1985	[illegible]	6	1.82	2.45
723932	[illegible]	[illegible]	12	1.77	2.12
826190	[illegible]	[illegible]	[illegible]	1.87	[illegible]

Table 2. GW Genotyping Results

Case Sample	Year	SNP call rate (%)	Reproducibility (%)
423197	1969	NA	NA
525593	1984	89.1	NA
620691	1985	97.1	NA
723932	[illegible]	98.9	99.9[1]
826190	[illegible]	87.3	99.3[2]

[1]Conc[illegible] whole blood
[2]Concordan[illegible]ous genotyping results

Figure 1. To evaluate DNA integrity of normal tissue extracted from FFPE, 100 ng of genomic DNA was resolved on a 0.8% agarose gel. All four samples showed [illegible] with most fragments 5 kb or smaller.

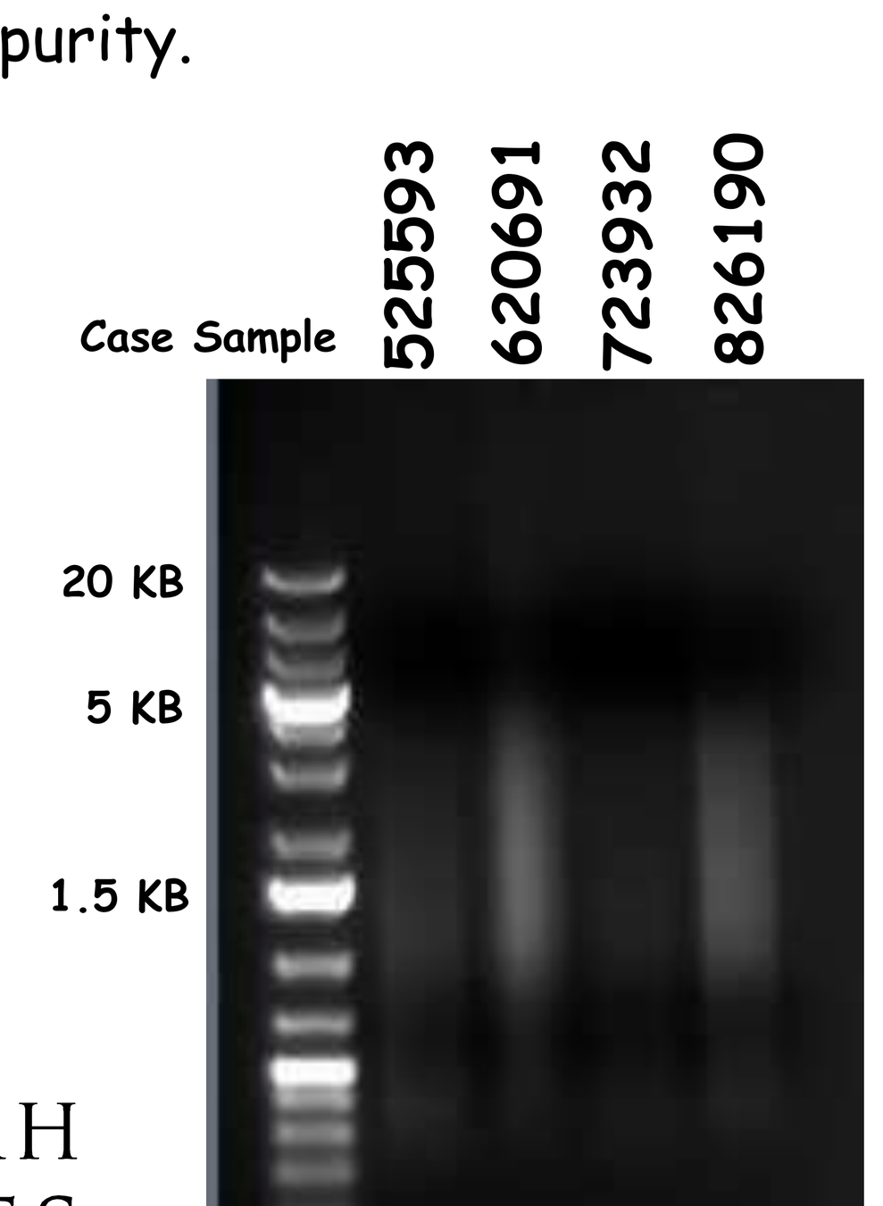


Table 2. Genome-wide genotyping perfomance and reproducibility using the Illumina 610Q platform was indicated by % call rate. DNA extracted from normal tissue stored in FFPE produced excellent genotyping results with call rates ranging from 87.3-98.9%. These results were reproducible using DNA from whole blood and were in concordance with previous genotyping results.

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Conclusions

- "Normal" tissue from archived FFPE samples, even decades old, is an excellent source of germline DNA from individuals no longer available for sampling.
- Both high quality and high yield whole-genome genotyping data can be obtained from DNA extracted from normal tissue stored in FFPE samples, even when samples are stored long term.
- Genome-wide genotyping data from DNA extracted from normal tissue from FFPE samples is more than adequate to allow genome-wide linkage analysis and may be used for association studies.

Future Directions

- Use this method to identify genes for pancreas cancer predisposition under a 2011 Pancreatic Cancer Action Network-AACR Innovative Grant was awarded to L. C-A.
- Develop a quantitative DNA integrity assay to establish quality guidelines for use of FFPE-derived DNA in various downstream applications.

Acknowledgments

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