



Development of a Protocol for Obtaining Biological Samples for Genetic Testing from Remote Individuals

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Background

Sequencing for pharmacogenomics requires a DNA sample. Blood works well but requires a clinical visit and is stable at ambient temperatures for the length required for mailing samples. We are testing various other samples for DNA quantity and quality.



Picture 1. Samples that will be tested include saliva, swabs, dried blood spot cards, and buccal swabs.

Methods

Swab and saliva kits from Mauwi, Zymo, Gentueri, and DNA Genotek have been purchased for testing. Filter paper for DBS collections has been purchased from Qiagen and Cytiva. Forty different DNA extraction kits from various companies have been obtained. Extractions up to this point were performed using the Beckman Coulter GenFind V3 kit.

Results

Completion of dried blood spot cards required more lancet punctures than anticipated, with a median of 3 punctures (range 2-11) per card. Out of 17 completed cards, only 3 of them required more than 4 punctures. Yields per tissue were as follows: 400 µl of buffy coat - 5.6-24 µg; 200 µl from Mauwi swab kits – 2.5-3.7 µg; 400 µl from DNA Genotek swab kits – 2.4-4.4 µg; 400 µl from DNA Genotek saliva kits – 1.9-2.1 µg; One square inch Qiagen FTA transfer card – 350-400 ng; One square inch Cytiva Whatman filter paper – 600 ng.

Saliva and buccal swab samples are easier to collect than blood, but produce lower DNA yields

Results

Blood		Dried Blood Spots	
Pros	Cons	Pros	Cons
High yield (>6 µg)	Requires a venipuncture from a clinical visit	Minimal contamination	Requires finger stick
High purity		Stable at ambient temperatures	Induces pain
Inexpensive	Induces pain	No shipping restrictions	Risk of infection
Gold standard tissue	Risk of infection	Stable for years	Low yield (<1 µg)
High average molecular weight	Sample instability at ambient temperatures	Inexpensive	Unknown average molecular weight

Saliva		Buccal Swab	
Pros	Cons	Pros	Cons
Painless to collect	Difficult for the elderly	Painless to collect	Difficult for the elderly
Stable at ambient temperatures	High levels of microbial contamination	Stable at ambient temperatures	Medium levels of microbial contamination
No shipping restrictions	Low yield (~2 µg)	No shipping restrictions	Low yield (~3-4 µg)
Stable for years	Lower average molecular weight	Stable for years	Lower average molecular weight
	Relatively expensive	Less contamination than saliva	Relatively expensive

Tables 1-4. Pros and Cons. These tables list the identified pros and cons of using each sample type being tested.

Company	Product	Cat. Number
Beckman Coulter	GenFind V3 Reagent Kit-50	C34880
Beckman Coulter	DNAAdvance	A48705
Biomiga	Biomiga EZgene Tissue DNA Kit 50 Preps	N/A
cytiva life sciences	Tissue and cells genomicPrep Mini Spin Kit	28904275
cytiva life sciences	Nucleon BACC Genomic DNA Extraction Kits	RPN8501
cytiva life sciences	Sera-Xtracta HMW DNA kit	29429140
cytiva life sciences	Blood genomicPrep Mini Spin Kit	28904264
Fortis Life Sciences	Pure Tissue DNA Kit	EB-TDK-50
New England BioLabs	Mondarch Genomic DNA Purification kit	T3010S
New England BioLabs	Monarch HMW DNA Extraction Kit for Blood and Cells	T3050S
Omega Bio Tek	E.Z.N.A. Tissue DNA Kit	D3396-01
Omega Bio Tek	Mag-Bind® Blood & Tissue DNA HDQ 96 Kit	M6399-00
Omega Bio Tek	E.Z.N.A.® Blood DNA Mini Kit	D3392-00
Omega Bio Tek	E.Z.N.A.® SQ Blood DNA Kit	D5032-00
Perkin Elmer	Chemagic DNA Cyte Pure Kit	CMG-196
Promega	Wizard Genomic DNA Purification Kit	A1120
Promega	Wizard SV Genomic DNA Purification System	A2360
Promega	MagaZorb DNA Mini-Prep Kit	MB1004
Promega	ReliaPrep gDNA Tissue Miniprep System	A2051
Promega	Wizard HMW DNA Extraction Kit	A2920
Promega	ReliaPrep™ Blood gDNA Miniprep System	A5081
Qiagen	DNEasy Blood and Tissue Kit	69504
Qiagen	QIAamp Fast DNA Tissue Kit	51404
Qiagen	Puregene Tissue Kit	158063
Qiagen	QIAGEN Genomic-tips 20/G	10223
Qiagen	MagAttract HMW DNA Kit	67563
Qiagen	QIAamp DNA Blood Mini Kit	51104
Qiagen	Blood & Cell Culture DNA Mini Kit (25)	13323
Sigma-Aldrich	Extract-N-AMP Tissue PCR Kit	XNAT2-1KT
Sigma-Aldrich	GenElute Blood Genomic DNA Kit	NA2010-1KT
Takara	NucleoSpin Tissue	740952.5
Takara	NucleoMag Tissue	744300.1
Takara	NucleoSpin® 96 DNA RapidLyse	740110.1
Takara	NucleoBond HMW DNA	740160.2
Takara	NucleoMag DNA Swab	744601.1
Takara	NucleoSpin® Blood	740951.5
Takara	NucleoMag® Blood 200 µL	744501.1
Thermo	DNA Extract All Reagents Kit	4403319
Thermo	MagMax DNA Multi-Sample Kit	4413020
Thermo	JetFlex Genomic DNA Purification Kit	A30700
Thermo	GeneJet Genomic DNA Purification Kit	K0721
Thermo	GeneJet Genomic DNA Purification Kit	K0721
Thermo	PureLink Genomic DNA Mini Kit	K182001
Thermo	PureLink Genomic DNA Mini Kit	K182001
Thermo	ChargeSwitch gDNA Mini Tissue Kit	CS11204
Zymo	Quick-DNA Miniprep	D3024
Zymo	Quick-DNA miniprep plus	D4068
Zymo	Quick-DNA Magbead Plus Kit	D4081
Zymo	Quick-DNA HMW MagBead Kit	D6060

Table 5. Kits being tested. The DNA extraction kit names, production companies, and catalog numbers are listed here.

Yields from GenFind V.3

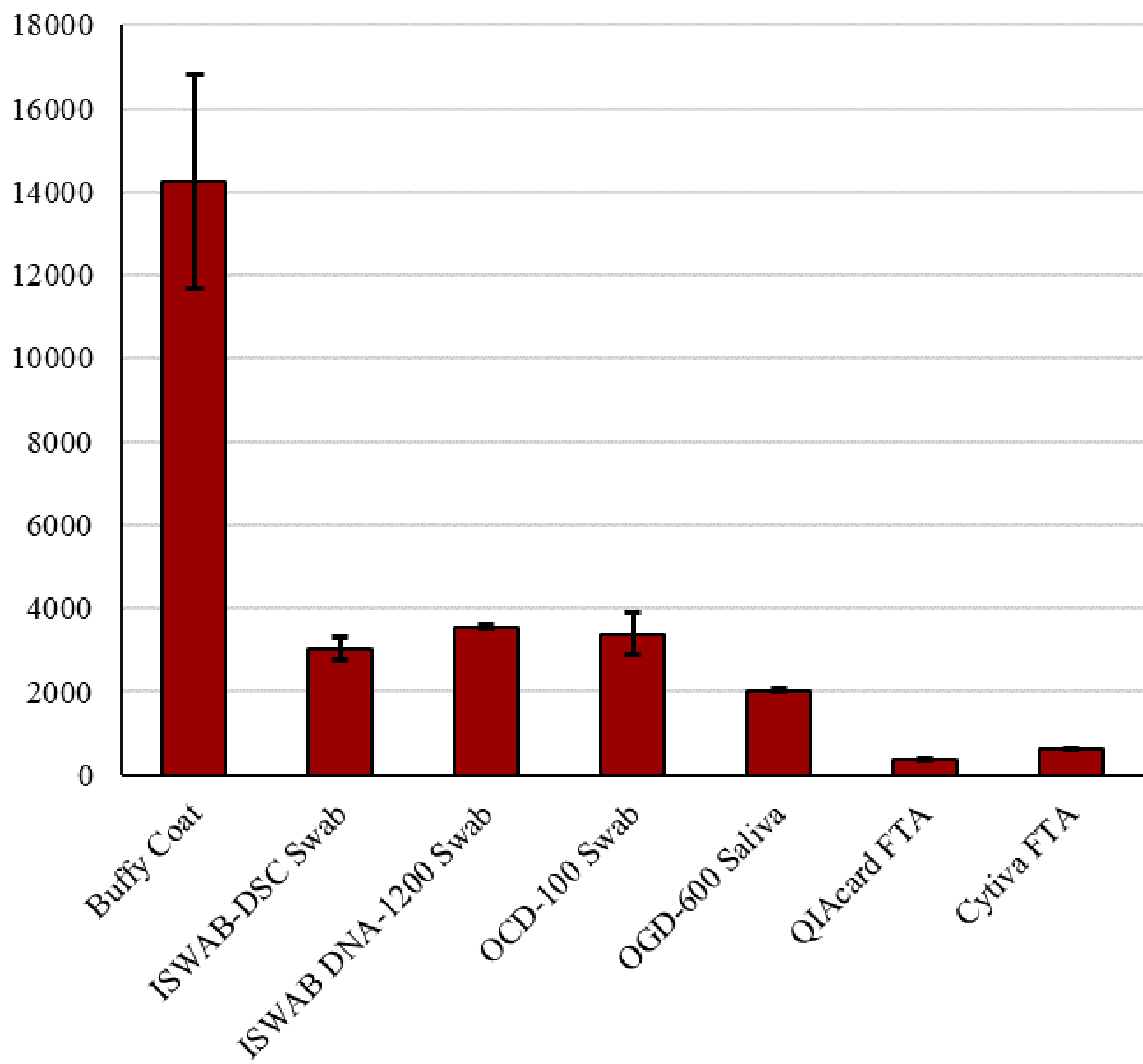


Figure 1. DNA extraction Yields. DNA extraction yields using the Beckman Coulter GenFind V3 (Cat# C34880) are far higher for buccal swabs and saliva than for 1-inch diameter blood spots, but not nearly as high as for blood.

Conclusion

Preliminary results indicate that the yield from swabs are greater than from saliva, but not as high as buffy coat. Yields from dried blood spots were significantly lower than from other tissues. Sample size and purity have not yet been evaluated.

