

BECKMAN COULTER GENOMIC SAMPLE PREPARATION

SPRI Automated gDNA Extraction from iSWABs

Jan 2014



iSwab from Mawi DNA Technologies

- Buccal swabs
- Comb in the cap of the collection tube, so cells are scraped off into in a preservative
- No need to squeeze or clip swabs

– <http://mawidna.com/>



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SPRI Extraction of iSwabs

- Two Beckman Coulter gDNA extraction kits tested
 - DNAdvance (designed for mammalian tissue)
 - Genfind v2 (designed for whole blood)
- Biomek 4000 (B4k) automated methods used for both
- Only part not automatable is aliquoting sample to plates from the iSwab tube.
 - The comb necessitates manual pipetting with a p200 to get most of the sample
 - Everything else is straight forward



DNAdvance

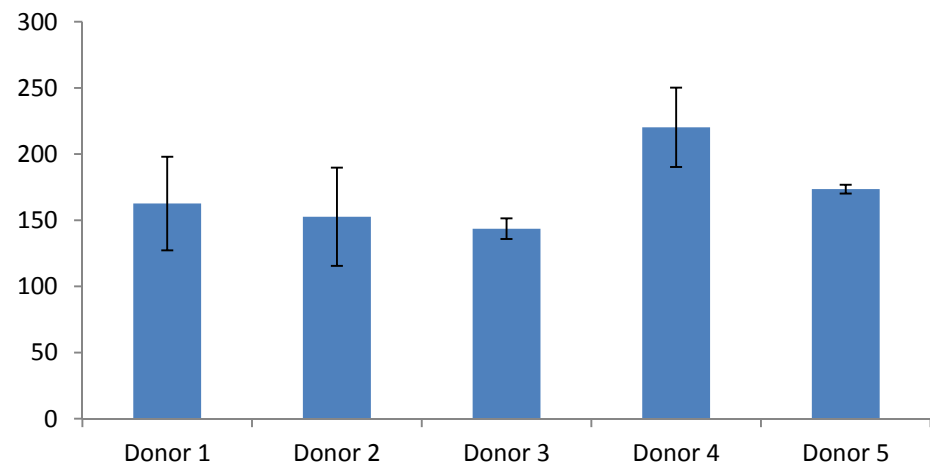
- Automated 96MC method adapted for higher input volume to accommodate liquid sample (A48708)
- Per well:
 - 300uL Sample + 193uL Lysis + 7uL Proteinase K
- Digest at 37C for 1hr
- Run Settings

Step 0: Lysate Vol:	500				
Step 1: Bind Buf Vol:	200	Shake Sec:	60		
	Bead Vol:	340	Shake Sec:	90	Inc Min: 1
					Settle Min: 10
Step 2: Ethanol Vol:	700	Shake Sec:	120	Settle Min:	3
				Cycles:	3
Step 3: Elution Vol:	50	Shake Sec:	60	Settle Min:	5
				Transfer Vol:	45

DNAdvance Results

- Modified run settings and DNAdvance 96MC method produces significant yields
- Single plate 1 hour

iSwab DNAdvance Yields (ng/uL)



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Genfind v2

- 300uL input
- Default Span8 Method (A42569)
 - Elution in 50uL

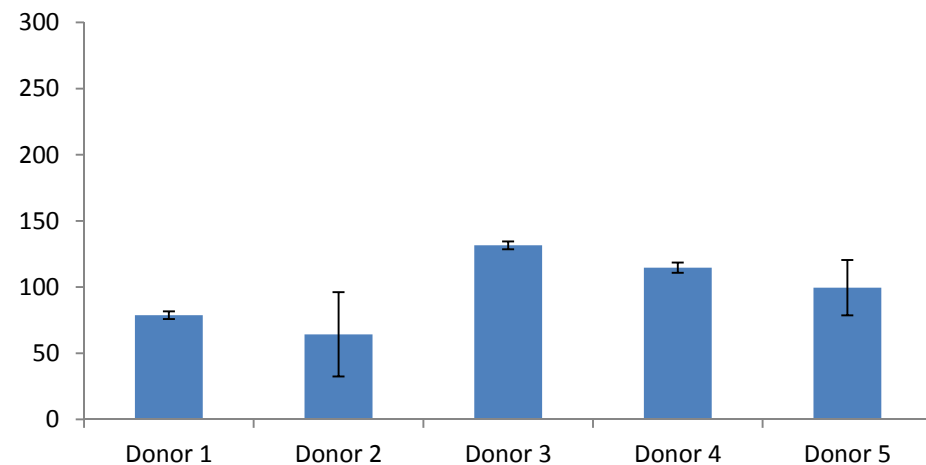
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Genfind v2 Results

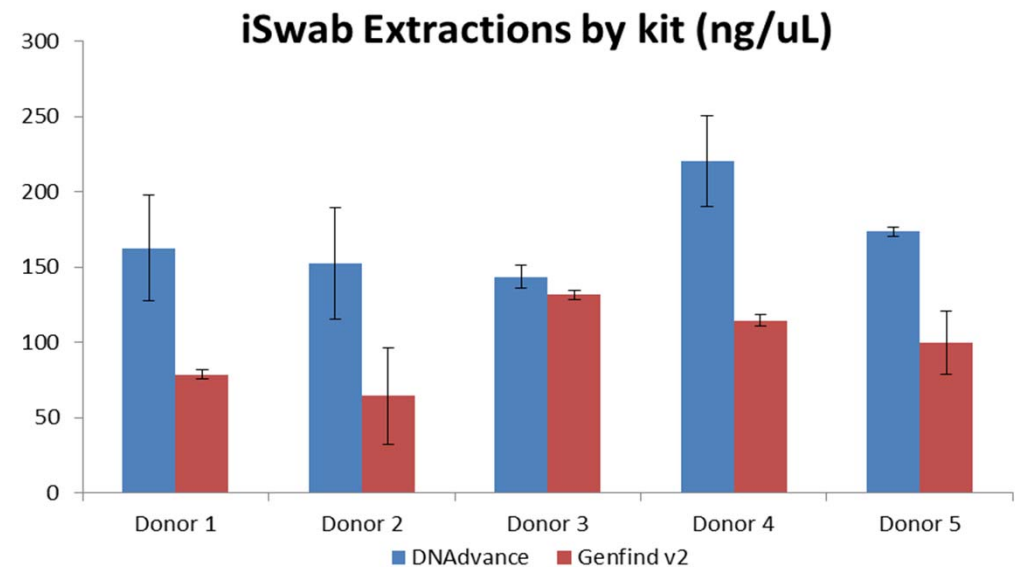
- Genfind v2 and default Span8 method successfully extract gDNA
- Lower Yields than DNAdvance, likely due to shorter lysis at Room Temperature

iSwab Genfind Yields (ng/uL)



Comparison of the two Kits

- Both DNAdvance and Genfind v2 successfully extract gDNA from iSWABs
- Higher yield from DNAdvance likely due to lysis:
 - 37C 1hr vs Room Temperature 30min on deck



Ordering Information

Product	Part Number
DNAdvance	A48705 (384 preps), A48706 (9600 preps)
Genfind v2	A41499 (50 preps), A41497 (384 preps), A83078 (4800 preps)
Biomek 4000 Liquid Handler	B22867

www.beckmancoulter.com

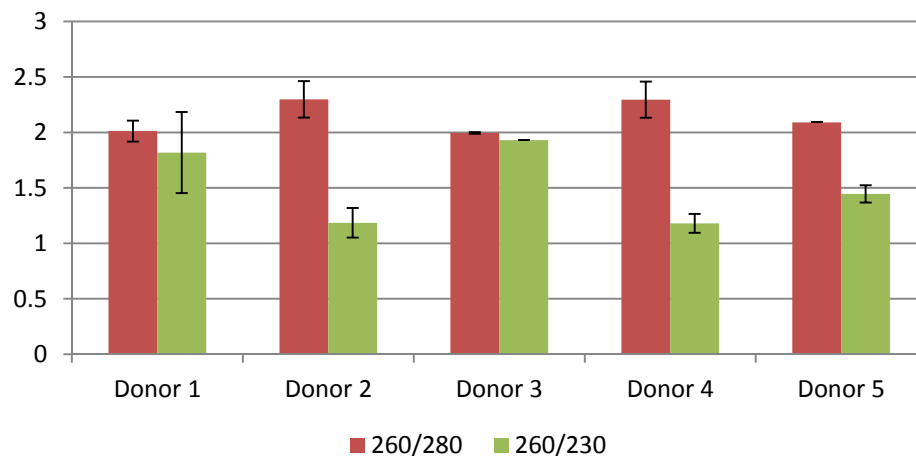
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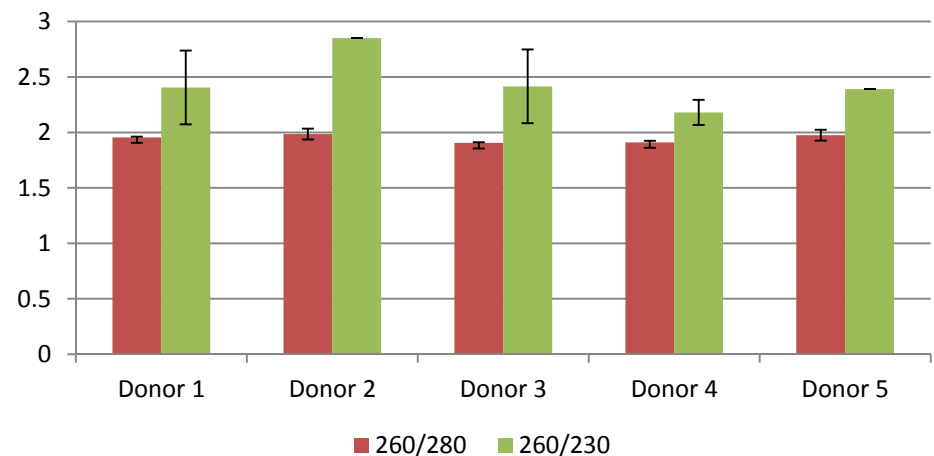
More Data – 260/280 and 260/230 ratios

- 260/280 consistently ~ 2.0
- 260/230 varies mainly by donor

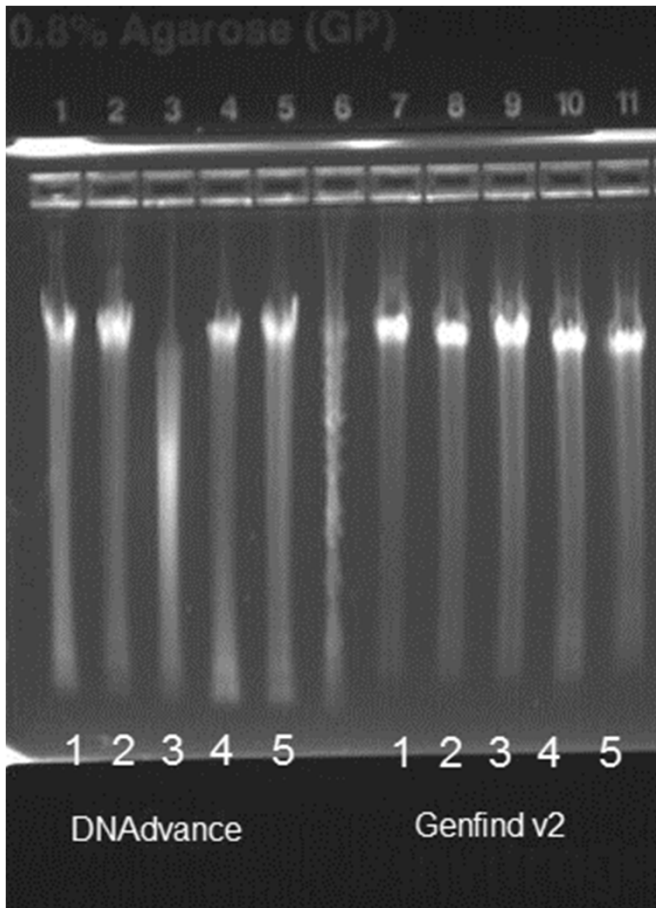
Purity - DNAdvance



Purity - Genfind v2



Gel analysis– gDNA Size / Stability



- Full length gDNA
- gDNA smears at up to 40kb
- Samples from each of 5 donors
 - Eluted in DNAadvance/ or Genfind v2 elution buffer
 - Extracted gDNA was Stored at 4°C for 3 weeks
 - Run on 0.8% agarose gel

