

## Column Concentrator Comparison for Bone Extraction

### Objective:

Centricon YM-30 concentrators used in the "Demineralization Extraction for Skeletal Remains" SOP at the UNT Center for Human Identification have been discontinued. The purpose of this study is to compare two different manufacturing brands of concentrators to the Centricon YM-30 to determine if either or both brands are a suitable replacement.

### Null Hypothesis:

There is no significant difference between results obtained from the Demineralization Extraction for Skeletal Remains using Amicon Ultra-4 (10K, 30K, and 50K pore sizes), Vivacon (30K, 50K, and 100K), and Centricon YM-30K concentrators.

### Materials and Methods:

Four bone samples were extracted in quadruplicate using 1g each of homogenized bone powder from each sample. Samples were decalcified and digested using 4.5ml of demineralization buffer and 300 $\mu$ L ProK (20mg/ml). Samples were incubated at 56°C on an orbital shaker for a minimum of 16 hours. Samples were extracted with an equal volume of phenol chloroform isoamyl alcohol and centrifuged for 3minutes at 3,100xg. The aqueous phase of the quadruplicate extracts for each sample were combined and evenly divided across the seven tested columns. All columns were spun at 2,000xg until the majority of liquid had passed through the respective filters. Two milliliters of TE<sup>-4</sup> were added to each column. Columns were spun at 2,000xg until all but approximately 50ul of TE<sup>-4</sup> had passed through the filters. Final extract volumes were recovered by adding an additional volume of TE<sup>-4</sup> to each filter for a combined total of 100ul. Filters were rinsed with the 100ul volume by pipetting up and down. Final extracts were then transferred to 1.5ml tubes.

After initial quantification and amplification, samples were processed with Qiagen QIAquick spin columns according to current laboratory procedure. Approximately 60ul of each of the twenty-eight extracts (4 samples tested across 7 columns) were filtered through the columns to remove inhibitors. Samples were recollected in 60ul of elution buffer.

Samples were quantified in duplicate using Quantifiler® Human DNA Quantification Kit according to UNTCHI's Quantifiler SOP before and after DNA purification via Qiagen's QiaQuick Columns. Samples were amplified in duplicate using AmpF $\ell$ STR® Profiler Plus® ID PCR Amplification Kit following the UNTCHI's STR Amplification SOP. A target of 6ng DNA was used for amplification at 28 cycles. Less than 1ng DNA was amplified at 32 cycles. Bone samples 1 & 3 were amplified in duplicate using AmpF $\ell$ STR® MiniFiler® PCR Amplification Kit following the UNTCHI's STR Amplification SOP. DNA input ranged from 0.2-0.6ng amplified for 30 cycles.

Samples were electrophoresed on the 3130 Genetic Analyzer according to UNTCHI's SOP. For the reported results, GeneMapper ID was used as an expert system. Loci with Processing Quality Values less than 0.75 were flagged as low quality. Those exceeding

0.75 were deemed passing. The Peak Height Ratio threshold was set at 70%. The number of loci with PHR flags does not include loci in which the flag was fired due to an extraneous peak being called. It represents the number of loci which displayed heterozygote imbalance between the two major (true) allele peaks. The minimum heterozygote intensity was set at 100 RFU and the minimum homozygote intensity was set at 200 RFU. The detection threshold was set at 100 RFU for all colors in the dye set.

**Results:**

Quantitation results for samples before purification with QiaQuick Columns are listed in Table 1. Sample that obtained partial profiles using Profiler Plus ID are shown in bold. All other samples failed. Internal Positive Control (IPC) critical threshold values were >30 in ~60% of the samples, indicating the presence of PCR inhibitors in the samples.

	Sample 1	Sample 2	Sample 3	Sample 4
<b>Centricon 30K</b>	5.16E-02	3.76E-03	4.79E-02	<b>3.24E-01</b>
<b>Vivacon 30K</b>	4.20E-02	1.39E-02	5.68E-02	2.55E-01
<b>Vivacon 50K</b>	4.19E-02	2.70E-02	4.88E-02	3.09E-01
<b>Vivacon 100K</b>	<b>3.08E-02</b>	<b>1.14E+00</b>	6.29E-02	<b>1.62E-01</b>
<b>Amicon Ultra-4 10K</b>	4.29E-02	6.95E-04	4.99E-02	2.42E-01
<b>Amicon Ultra-4 30K</b>	4.60E-02	3.34E-01	5.57E-02	2.53E-01
<b>Amicon Ultra-4 50K</b>	3.51E-02	<b>1.59E+00</b>	4.98E-02	<b>1.19E-01</b>

**Table 1: Quantitation Results of Samples without QiaQuick Purification.** Quantitation results are given for averages in ng/μL of duplicate bone samples 1-4 for each of the different column concentrators. Partial profiles were obtained for samples in bold type.

	Sample 1	Sample 2	Sample 3	Sample 4
<b>Centricon 30K</b>	2.88E-02	2.15E+00	4.60E-02	1.24E-01
<b>Vivacon 30K</b>	3.17E-02	1.76E+00	5.60E-02	2.25E-01
<b>Vivacon 50K</b>	2.41E-02	1.53E+00	5.95E-02	1.89E-01
<b>Vivacon 100K</b>	1.82E-02	1.47E+00	4.60E-02	9.86E-02
<b>Amicon Ultra-4 10K</b>	2.78E-02	1.79E+00	5.51E-02	1.76E-01
<b>Amicon Ultra-4 30K</b>	3.39E-02	1.48E+00	5.09E-02	1.82E-01
<b>Amicon Ultra-4 50K</b>	3.05E-02	1.35E+00	5.32E-02	7.58E-02

**Table 2: Quantitation Results of Samples after QiaQuick Purification.** Quantitation results are given for averages in ng/μL of duplicate bone samples 1-4 for each of the different column concentrators.

Overall greatest quantitation yields based on Rank Sum Test:

1. Vivacon 30K
2. Vivacon 50K
3. Amicon Ultra-4 (10K and 30K)
4. Centricon 30K
5. Amicon Ultra-4 50K
6. Vivacon 100K

GeneMapper ID was used as an expert system to evaluate the data. Loci which did not meet pre-determined criteria for acceptable, good quality data would result in rule firings

requiring analyst intervention. Those less than 0.75 were flagged as low quality. The Peak Height Ratio (PHR) threshold was set at 70%. PHR flags represent the number of loci which displayed heterozygote imbalance between the two major (true) allele peaks. The minimum heterozygote intensity was set at 100 RFU and the minimum homozygote intensity was set at 200 RFU. The detection threshold was set at 100 RFU for all colors in the dye set. Results for Profiler Plus ID and Minifiler amplifications are summarized in Tables 3 & 4, respectively.

	Low Quality	PHR	Loci D.O.	Allelic D.O
<b>Centricon 30K</b>	41.67%	26.92%	15.83%	13.21%
<b>Vivacon 30K</b>	<b>33.33%</b>	22.78%	<b>15.00%</b>	<b>12.74%</b>
<b>Vivacon 50K</b>	<b>33.33%</b>	21.05%	18.33%	15.57%
<b>Vivacon 100K</b>	39.09%	<b>20.97%</b>	22.73%	19.49%
<b>Amicon Ultra-4 10K</b>	43.33%	31.17%	18.33%	16.98%
<b>Amicon Ultra-4 30K</b>	35.00%	21.79%	18.33%	15.57%
<b>Amicon Ultra-4 50K</b>	40.83%	30.67%	20.00%	15.09%

**Table 3: Data Evaluation via GeneMapper ID as Expert System for Profiler Plus ID System.** Percentage of Loci exhibiting flagged data due to low quality genotype, imbalance in peak height ratio (PHR), or locus/allelic dropout are listed for each of the different column concentrators. Lowest percentage of flagged loci per rule violated is represented in bold.

	Low Quality	PHR	Dropout
<b>Centricon 30K</b>	<b>16.67%</b>	<b>20.00%</b>	0.00%
<b>Vivacon 30K</b>	33.33%	40.00%	0.00%
<b>Vivacon 50K</b>	22.22%	26.67%	0.00%
<b>Vivacon 100K</b>	27.78%	33.33%	0.00%
<b>Amicon Ultra-4 10K</b>	22.22%	26.67%	0.00%
<b>Amicon Ultra-4 30K</b>	22.22%	26.67%	0.00%
<b>Amicon Ultra-4 50K</b>	22.22%	26.67%	0.00%

**Table 4: Data Evaluation via GeneMapper ID as Expert System for Minifiler System.** Percentage of loci exhibiting flagged data due to low quality genotype, imbalance in peak height ratio (PHR), or locus/allelic dropout are listed for each of the different column concentrators. Lowest percentage of flagged loci per rule violated is represented in bold. Full profiles were obtained for samples with each concentrator.

### Conclusions:

These data suggest the following:

- A. Samples must be purified (e.g., QiaQuick) following concentration, as PCR inhibitors are still present
- B. No significant differences were detected between the seven concentrators tested in DNA yield or genotype quality.
- C. Minor differences were seen in DNA yields based on Rank Sum Test:  
Vivacon 30/50K > Amicon 10/30K > Centricon 30K
- D. Minor differences were seen in genotype quality based on number of Low Quality Flags fired for analyst review:  
Vivacon 30/50K < Amicon 30K < Centricon 30K
- E. Minor differences were seen in genotype quality based on number of allelic and locus dropout:  
Vivacon 30K < Centricon 30K < Amicon 30K

Vivacon 30K faired best in each test; however, all columns tested (Amicon Ultra-4 10K, 30K, & 50K; Vivacon 30K, 50K, & 100K) perform comparably to the Centricon YM-30 concentrators. Any of the tested columns would be a suitable replacement for the Centricons.