QIAGEN Supplementary Protocol:

Scalable purification of archive-quality DNA from buffy coat using the Gentra® Puregene® Blood Kit

This protocol provides information about scaling of reagents required for purification of DNA from buffy coat prepared from 0.3–15 ml samples of whole blood using the Gentra Puregene Blood Kit.

The Gentra Puregene Blood Kit enables convenient, scalable purification of DNA from buffy coat. Reagent volumes are scaled proportionately according to the amount of starting material. Tables 1 and 2 show the volumes of reagents required for DNA purification from different sample sizes. The information provided in Tables 1 and 2 is intended to supplement the information given in "Protocol: DNA Purification from Buffy Coat Using the Gentra Puregene Blood Kit" in the *Gentra Puregene Handbook*.

IMPORTANT: Please read the *Gentra Puregene Handbook*, paying careful attention to the safety information, before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, consult the appropriate material safety data sheets (MSDSs), available from the product supplier. The Gentra Puregene Blood Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

Table 1. Purification of DNA from buffy coat prepared from 0.3–3 ml samples of whole blood

	Number of white blood cells*			
	2.1×10^6	**1.05×10^7**	**1.75×10^7**	**2.1×10^7**
Volume of blood used to prepare buffy coat (ml)	0.3	1.5	2.5	3
Tube size (ml)	1.5	15	15	15
Volume of RBC Lysis Solution	3 volumes†			
Volume of Cell Lysis Solution (µl)	300	1500	2500	3000
Volume of RNase A Solution (µl)	1.5	7.5	12.5	15
Volume of Protein Precipitation Solution (µl)	100	500	835	1000
Volume of 100% isopropanol (µl)	300	1500	2500	3000
Volume of 70 % ethanol (µl)	300	1500	2500	3000
Volume of DNA Hydration Solution (µl)	30	150	250	300
Typical DNA yield (µg)	5–15	25–75	40–125	50–150

* Cell number estimates assume an average of 7×10^6 white blood cells per milliliter whole blood.

† To lyse residual red blood cells in the buffy coat sample, use 3 volumes of RBC Lysis Solution for every volume of buffy coat. For example, 3 ml RBC Lysis Solution should be used to lyse red blood cells in 1 ml of buffy coat.

Note: To process samples in 1.5 ml microcentrifuge tubes, adapt the centrifugation steps in "Protocol: DNA Purification from Buffy Coat Using the Gentra Puregene Blood Kit" as follows:

- Centrifuge at 13,000–16,000 x *g* for 20 s for the first centrifugation step.
- Centrifuge at 13,000–16,000 x *g* for 3 min for the second and third centrifugation steps.
- Centrifuge at 13,000–16,000 x *g* for 1 min for the fourth centrifugation step.

Table 2. Purification of DNA from buffy coat prepared from 7–15 ml samples of whole blood

	Number of white blood cells*			
	4.9 x 10^7	**5.6 x 10^7**	**8.4 x 10^7**	**1.05 x 10^8**
Volume of blood used to prepare buffy coat (ml)	7	8	12	15
Tube size (ml)	50	50	50	50
Volume of RBC Lysis Solution	3 volumes†			
Volume of Cell Lysis Solution (ml)	7	8	12	15
Volume of RNase A Solution (µl)	35	40	60	75
Volume of Protein Precipitation Solution (ml)	2.33	2.67	4	5
Volume of 100% isopropanol (ml)	7	8	12	15
Volume of 70 % ethanol (ml)	7	8	12	15
Volume of DNA Hydration Solution (µl)	700	800	1000	1000
Typical DNA yield (µg)	120–350	140–400	200–600	250–750

* Cell number estimates assume an average of 7 x 10^6 white blood cells per milliliter whole blood.

† To lyse residual red blood cells in the buffy coat sample, use 3 volumes of RBC Lysis Solution for every volume of buffy coat. For example, 3 ml RBC Lysis Solution should be used to lyse red blood cells in 1 ml of buffy coat.

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