QIAGEN Supplementary Protocol:

Scalable purification of archive-quality DNA from 1 x 10^6 – 3 x 10^7 fixed cells using the Gentra® Puregene® Tissue Kit

This protocol provides information about scaling of reagents required for purification of DNA from 1 x 10^6 – 3 x 10^7 fixed cells using the Gentra Puregene Tissue Kit.

The Gentra Puregene Tissue Kit enables convenient, scalable purification of DNA from a wide variety of tissue types as well as paraffin-embedded tissues and fixed cells. Reagent volumes are scaled proportionately according to the amount of starting material. Table 1 shows the volumes of reagents required for DNA purification from 1–30 x 10^6 fixed cells. The information provided in Table 1 is intended to supplement the information given in "Protocol: DNA Purification from Fixed Cells Using the Gentra Puregene Tissue 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 Tissue 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 1 x 10^6 – 3 x 10^7 fixed cells

	Number of cells			
	1–2 x 10^6	**3–5 x 10^6**	**1–2 x 10^7**	**3 x 10^7**
Tube size (ml)	1.5	1.5	15	15
Volume of Cell Lysis Solution (ml)	0.3	0.6	3	4.5
Volume of Puregene Proteinase K (µl)	1.5	3	15	22.5
Volume of RNase A Solution (µl)	1.5	3	15	22.5
Volume of Protein Precipitation Solution (ml)	0.1	0.2	1	1.5
Volume of 100% isopropanol (ml)	0.3	0.6	3	4.5
Volume of Glycogen Solution (µl)	0.5	1	–	–
Volume of 70% ethanol (ml)	0.3	0.6	3	4.5
Volume of DNA Hydration Solution (µl)	50	100	100	300

See note on other side.

Note: To process samples in 15 ml centrifuge tubes, adapt the centrifugation steps in "Protocol: DNA Purification from Fixed Cells Using the Gentra Puregene Tissue Kit" as follows:

- Centrifuge at 2000 x *g* for 3 min for the first centrifugation step.
- Centrifuge at 2000 x *g* for 10 min for the second centrifugation step.
- Centrifuge at 2000 x *g* for 3 min for the third and fourth centrifugation steps.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature/handbooks/default.aspx. Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp.

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