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Till now, we have been able to generate heavy methylated libraries from 10mL of human urine sample. We have also tried to construct heavy methylated libraries of larger volumes of urine (80mL), but the quantity of

extracted DNA was not sufficient to generate adequate heavy methylated library. We were able to generate a single library containing approximately 5000 CpG-free fragments from approximately 890mL of human urine sample. The protocol used in this study takes nearly 24h, but we believe that we can significantly improve the protocol and make it more efficient to construct multiple libraries from a single urine sample. The main reason for that is that current amount of input DNA was not sufficient to construct sufficient number of fragments for sequencing with the current protocol. We believe that we can use a stronger denaturing condition, or leave the DNA for longer time in the MspI digestion at 37C, or even omit the time of RT steps. Sequence analyzed in this study: The synthetic DNA library from the pGL4.10-Sasaki-SS-v1 vector was cloned into the CpG-free pcpgf-promlc vector by the use of the homology arms. Subsequently, the Lucia CDS followed by the SV40 polyA tail was inserted in the CpG-free vectors. All the oligos used for the vector construction are listed in Supplementary Table 1. The CpG-free vectors were compared with the pGL4.10-EF1-SS vector (a) containing the triple hemaglutinin (3HA) tag. The Bs 6651 pdf file is available here: Bs 6651 Pdf Free Download. The PCR products of pSasIII was carried out using forward and reverse primers containing a Universal M13 tail and addded a CpG-free tag (pMT-upstream) with 24 bp as homology arm with AgeI and XmaI restriction enzymes. After adding 1 mM of CpG-free oligo to the PCR reaction, the CpG-free PCR products was cloned into the CpG-free pcpgf-promlc vector. The CpG-free EFS vector (pcpgf-promlc-EF) was digested with AgeI and XmaI and gel-purified before ligation. The CpG-free EFS vector (pcpgf-promlc-EF) containing the reporter gene for this study was cloned into the pcpgf-promlc-Lucia vector with SalI restriction enzyme. Luciferase reporter vectors were validated with restriction enzymes (New England BioLabs). The restriction enzyme digested vectors with normalized electrophoretic mobility of 5kDa were confirmed by gel electrophoresis. Gel purified DNA was quantified using the Qubit fluorometer. Subsequently, 150 ng of the DNA was sent for sequence analysis (Agilent Technologies) with the primer sequence listed below (in the Bs 6651 pdf file) using the FP and RP primer pairs for their corresponding cloning sites. 5ec8ef588b

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