METODE RISET UJI HALAL PADA BIOMATERIAL KEDOKTERAN GIGI BERBASIS GENOMIK

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Isolasi DNA

Graft DBM ditimbang seberat 300 mg, didekalsifikasi dalam 10 ml *Ethylene Diamine Tetra-acetic Acid* (EDTA 0,5 M, pH 8,0) selama 12 jam pada suhu 4° C. Dilakukan sentrifugasi (4000 rpm) selama 5 menit, supernatan dibuang dan setelah langkah ini akan diperoleh pelet. 300 µl *cell lysis buffer*, 30 µl *proteinase K* dan 5 µl DTT (*dithiothreitol*) ditambahkan ke pellet dan diinkubasi selama 3 jam pada 56° C. 100 µl *Nucleus Lysis Buffer* ditambahkan dan diinkubasi selama 30 menit pada 70° C. Larutan di sentrifugasi selama 5 menit pada 4.000 rpm menggunakan *centrifuge*. Supernatan dipindahkan ke tabung *polypropylene* 2 ml. sodium acetate (5 M) 0,1 volume larutan dan etanol (100%) 1,5 volume larutan ditambahkan dan diistirahatkan pada -20 ° C selama minimal 3 jam. Larutan disentrifugasi pada 14000 rpm selama 10 menit dan seluruh supernatan dibuang. 170 µl larutan *Chelex* (5%) dan larutan tris (10mM) sebanyak 0,1 volume larutan ditambahkan. Sampel diinkubasi pada 56° C selama 1 jam. Supernatan dipindahkan ke microtube 1,5 ml dan ditambahkan 100 µl etanol (100%), disentrifugasi pada 14000 rpm selama 10 menit. Larutan etanol dibuang dan ditambahkan 30 ml *deionized water*. Pada tahap ini, sampel siap untuk dilakukan amplifikasi PCR. (Mohammadi *et al*.,2017. p.6011)_

Uji Kadar dan Kemurnian DNA

Uji kadar dan kemurnian hasil isolasi DNA di ukur melalui *spectrophotometer* (*Thermo Scientific,* USA). DNA sebanyak 10µl dipipet, dilarutkan dalam 1 ml aqua, kemudian divorteks. Dibaca dengan spektrofmeter pada λ 260 nm dan 280 nm.

Kadar DNA dianalisis menggunakan UV *Spectrophotometer* yang dibaca pada panjang gelombang 260 nm, dihitung dengan rumus:

Kadar DNA = Hasil OD 260 x pengenceran x 50 ng/mL

Kemurnian DNA dianalisis menggunakan UV *Spectrophotometer* yang dibaca pada panjang gelombang 260 nm dan 280 nm, dihitung dengan rumus:

Kemurnian DNA = OD 260 : OD 280

Polymerase Chain Reaction (PCR)

DNA digandakan dengan metode PCR untuk dapat dianalisis berat molekulnya. Amplifikasi PCR dilakukan pada tabung plypropilene 1,5 ml sejumlah 25µL *reaction volume* yang terdiri dari 1 µg DNA, 1 µM primer, 2 µM Mgcl2, 200 µM dNTP, 2.5 µL10X PCR buffer dan 1 unit Taq DNA polymerase. (Doosti *et a.l,* 2014, p.149).

Reaksi amplifikasi segmen DNA yang terdiri dari denaturasi awal dengan pemanasan pada suhu 94 ^{o}C, selama 2 menit. Dilanjutkan pengulangan denaturasi 94 °C selama 30 detik, sebanyak 35 putaran. *Anealing* pada suhu 60 °C untuk primer babi dan 60°C untuk primer sapi, masing-masing selama 30 detik. *Extention* dengan katalis enzim DNA polymerase pada suhu 72°C selama 30 detik, *Final extention* pada 72 °C selama 5 menit (Nikzad *et al.,* 2017 p.3). Amplifikasi PCR dilakukan menggunakan mesin PCR Thermal Cycler (*Master Cycler Gradient, Eppendrof, Germany*).

Electrophoresis

Gel agarose 2% seberat 5 gr ditambah 200µl TE Buffer dan cairkan gel agarose dengan pemanasan tabung pada 70ºC selama 10 menit. Tuang agarose cair pada *comb electrophoresis.* Di aplikasikan 5µl DNA dicampur dengan 1 µl Loading buffer, dialirkan medan listrik bermuatan positif ke arah medan listrik bermuatan negatif. Hasil amplifikasi PCR produk PCR dan 100 bp DNA *ladder* atau DNA Marker, dijalankan pada gel agarosa setebal 2 mm. Larutan diseparasi secara *horizontal discontinue type electrophoresis*. Pergerakan molekul dalam medan listrik dipengaruhi oleh bentuk, ukuran, besar muatan dan sifat kimia dari molekul DNA.

Hasil elektroforesis ditampilkan dengan menggunakan gel agarosa dengan pengecatan *Ethidium Bromide.* Setelah electroforesis, visualisasi dengan sinar ultra violet gelombang panjang.

Pengecatan *Ethidium Bromide*

Gel hasil elektroforesis direndam selama 10 menit dalam *Ethidium Bromide* dalam wadah tertutup, terbuat dari bahan non logam. Pengecatan dilakukan pada suhu kamar, operator mengunakan sarung tangan karet karena Ethidium Bromide bersifat kariogenik. Gel dicuci dengan *deionized water* untuk mengeliminasi bahan cat yang berlebih. Gel hasil pengecatan dengan *Ethidium Bromide* difoto dengan *UV illumination* (Imager UV Light Base, Thermo Fisher Scientific, cat 4466602). Selanjutnya dilakukan dokumentasi menggunakan kamera digital.

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