DNA Assay in engineered tissue

Purpose:

To determine DNA content in engineered tissue samples. Originally developed to be compatible with GAG assay

Safety:

1. **PPE:** Wear protective clothing, including latex gloves (for tissue culture), lab coat, sleeve and shoe covers. Wear safety goggles when adjusting pH and when handling sodium hydroxide solutions (NaOH).
2. **Engineering controls:** Work with unfixed human cells only in a biological safety cabinet. Refer to Blood-borne Pathogen Exposure Control Plan for other details of working with human cells.
3. **Work practice controls:** Medium and all fluids which have been in contact with human cells must be combined with undiluted bleach, overnight, before being disposed of. All plasticware in contact with human cells or with fluids from human cells are to be disposed of in the red sharps receptacle and non-sharps in designated bio-hazard containers.

Materials:

Sterile #15 scalpel blade to cut up big bits

Sterile 100mm plate to cut up big bits in

Balance if DNA/mass needed

1.5 ml microfuge tubes

Micropipettor and tips

Water Bath set to 65 °C

Vortex

96 multi-well plates

Calf thymus DNA standard (Sigma D-0805 5 units or similar. Validate against previous standards, this stuff can be very inconsistent batch-to-batch)

Fluorimeter

0.2µm Nalgene filter

Papain Buffer:

papain: 25 µg/ml, 2 mM cysteine; 50 mM sodium phosphate; 2 mM EDTA; pH 6.5, store -20°C

For 150 ml:

EDTA (disodium dihydrate; FW 372.2): 111.66 mg

Sodium phosphate (disodium anhydrous; FW 141.96): 1.06 g

Dissolve in 150 ml nuclease-free water, adjust pH to 6.5, and filter sterilize with a 0.2µm Nalgene filter.

**** Add Papain and Cysteine on the day of experiment:

Papain: 3.755 mg

Cysteine: 47.28 mg

Neutralizing Solution: 4 M NaCl; 100mM Na_2HPO_4 (pH7.2); 0.1 N HCl, store RT

For 500 ml:

Prepare 400 ml of 5M NaCl

(116.88 g NaCl in 350 ml H_2O; heat to dissolve salt, Cool down and bring to 400 ml)

Prepare 50 ml of 1 M Na_2HPO_4

(disodium anhydrous; FW 141.96, 7.098 g Na_2HPO_4 in 50 ml in H_2O)

Mix 400 ml of NaCl, and 50 ml of 1 M Na_2HPO_4

Adjust pH to 7.2*

Add 50 mL of 1 N HCl

*(critical)

Hoechst 33258 dye stock solution: 1 mg/ml in H_2O; Store at 4°C. Solution is light sensitive

Hoechst dye working solution: Dilute dye stock solution 1:1500 in H_2O. Solution is light sensitive

Procedure:

1. Tissue samples may be stored frozen (-20 or -80) until ready. For example all time points of an experiment might be assayed with a single standard curve for simplicity. Thaw material at room temperature. Volumes given are for typical aggregate culture pellets. Adjust as needed, but remember to adjust calculations appropriately.
2. If DNA/mass is desired, lightly blot tissue on #1 filter paper and then weigh; record mass.
3. Add 200 µl of papain buffer per tube.
4. Place the tubes in water bath at 65°C; check every 30 minutes until digested. If needed triturate using hand-held epoxy pestle.
5. Add 400 µl 0.1N NaOH to each of the digested material and standards, vortex briefly.
6. Incubate at room temperature for 20-30 minutes.
7. Add 400 µl Neutralizing solution and vortex briefly
    a. If needed this can be an overnight hold point. Store @ 4°C.
8. Transfer lysate to 96-well plate (100 µl/well; 4 replicates/sample)
9. Add Calf thymus DNA to papain buffer standards for the standard curve.

0 µl Calf thymus DNA	+	200 µl papain buffer + 400 µl 0.1N NaOH + 400 µl neutralizing buffer
4.0 µl Calf thymus DNA (400 ng)	+	200 µl papain buffer + 400 µl 0.1N NaOH + 400 µl neutralizing buffer
8.0 µl Calf thymus DNA (800 ng)	+	200 µl papain buffer + 400 µl 0.1N NaOH + 400 µl neutralizing buffer
12.0 µl Calf thymus DNA (1200 ng)	+	200 µl papain buffer + 400 µl 0.1N NaOH + 400 µl neutralizing buffer
16.0 µl Calf thymus DNA (1600 ng)	+	200 µl papain buffer + 400 µl 0.1N NaOH + 400 µl neutralizing buffer

10. Add 100 µl of each of the 5 different concentrations of DNA for a standard curve (3 replicates/sample).
11. Make Hoechst working dye solution
12. Add 100 µl of the working dye to each well.
13. Read in fluorimeter (XFluor4GENiosPro) with cover off.