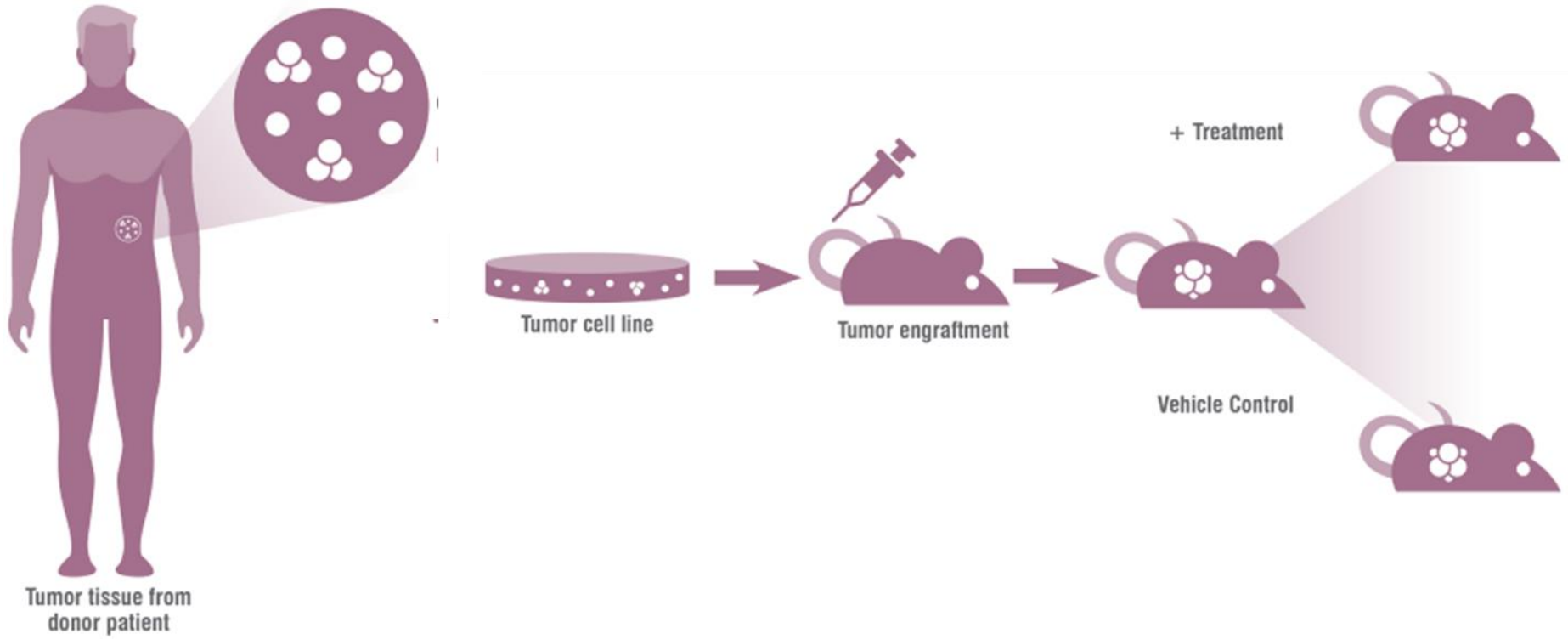
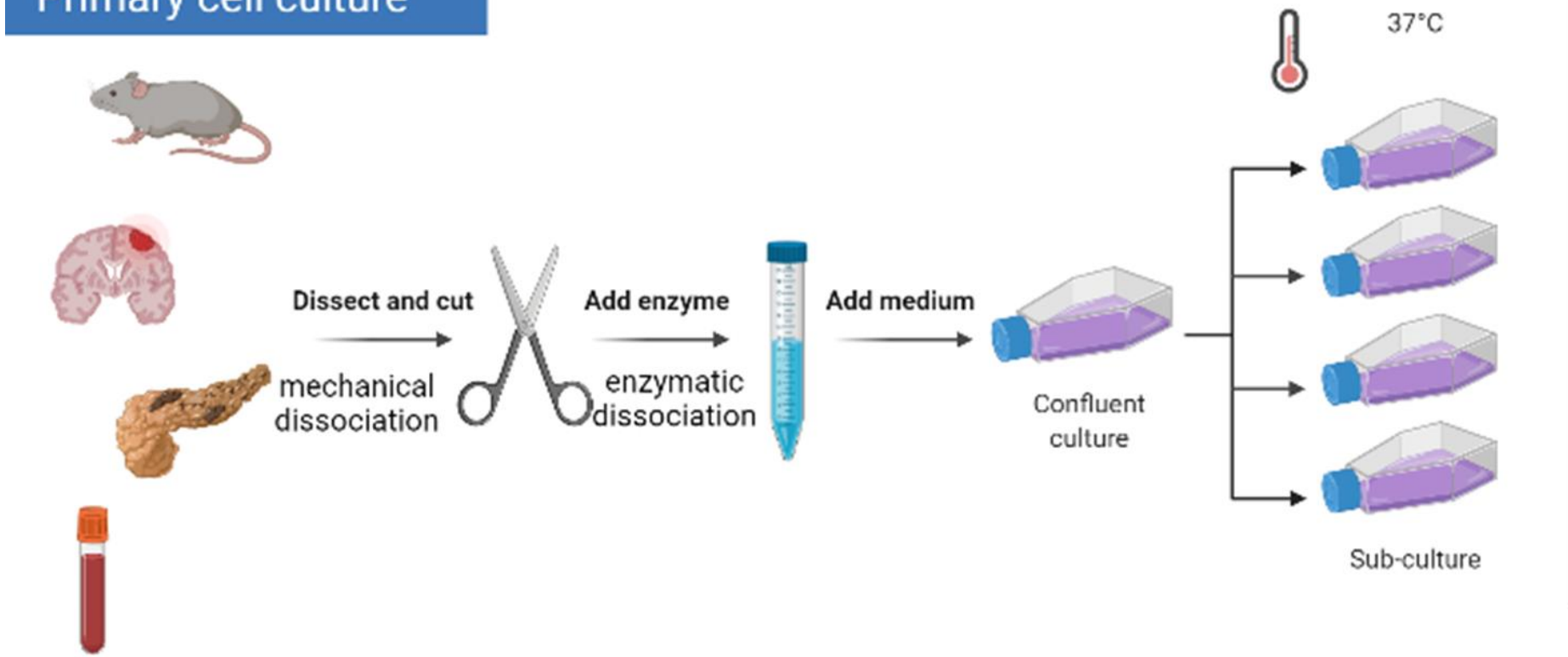
PDX Model Studies

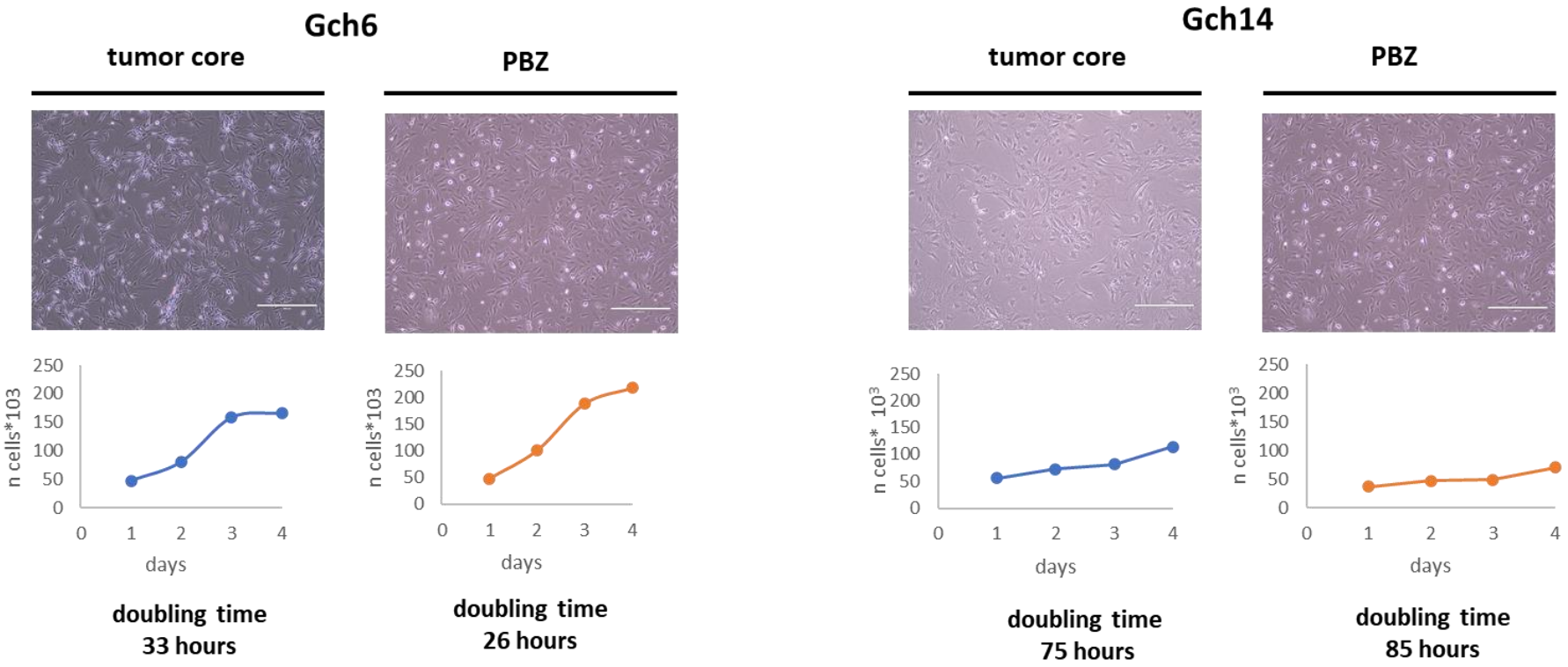


Primary cell culture



Primary culture of glioblastoma (GBM) cell lines

Establishment and characterization of stable primary GBM cell lines



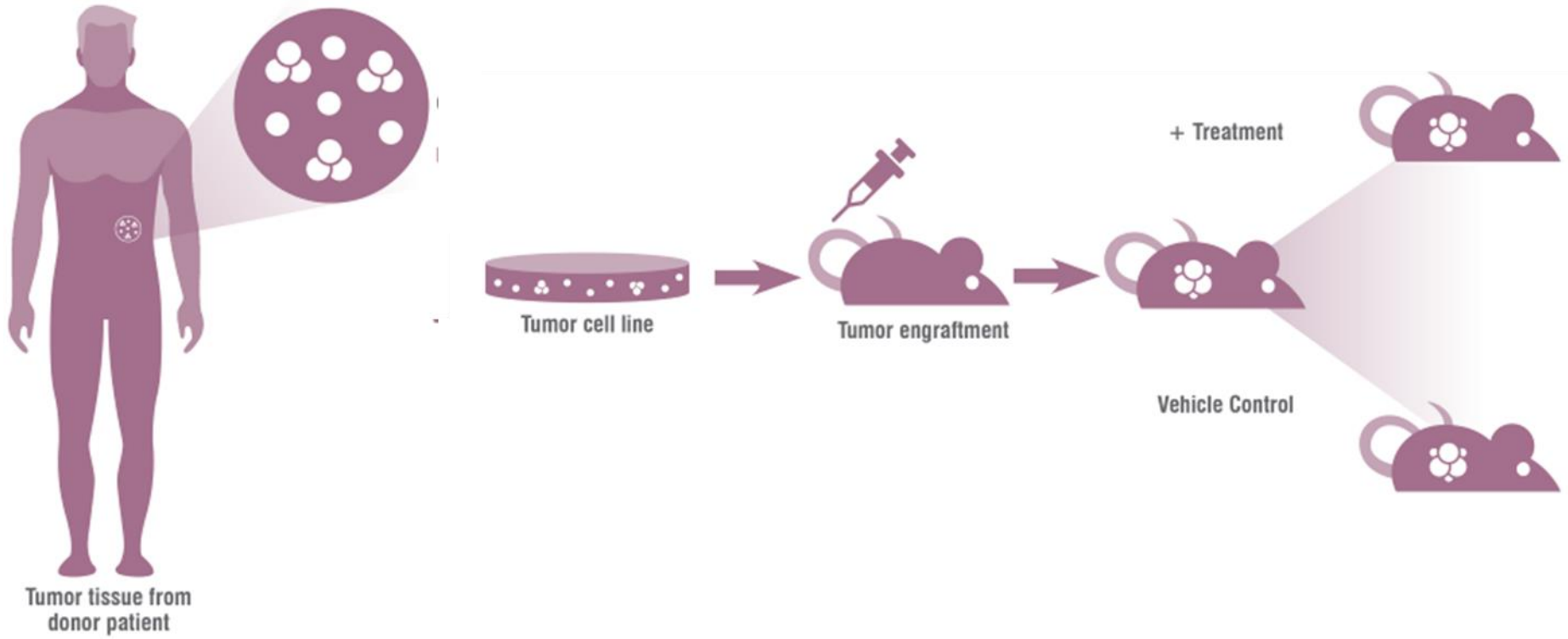
Primary Cell Cultures

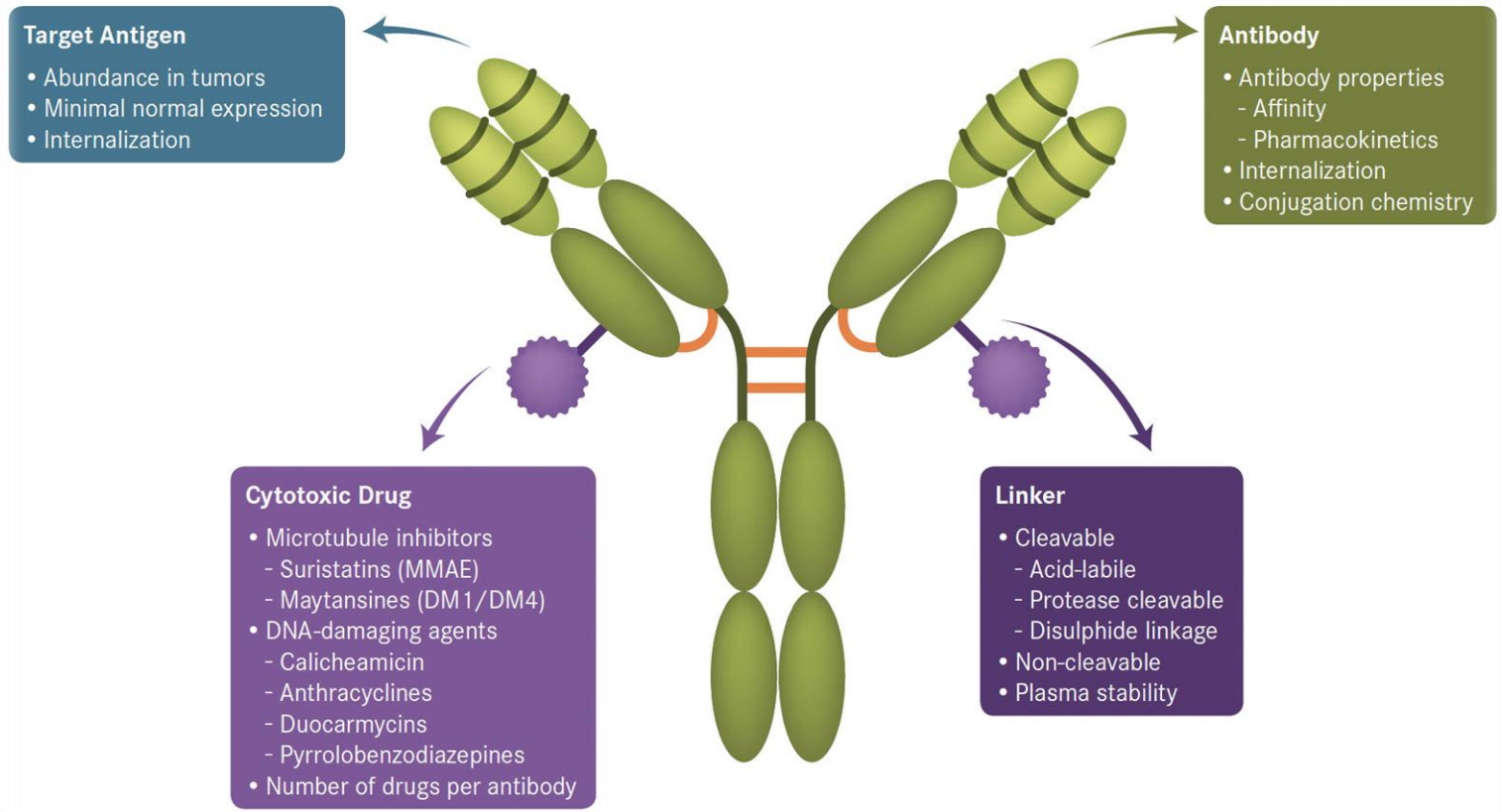
Cancer Research: to studying the tumor biology, for identifying novel biomarkers, and for testing new compounds.

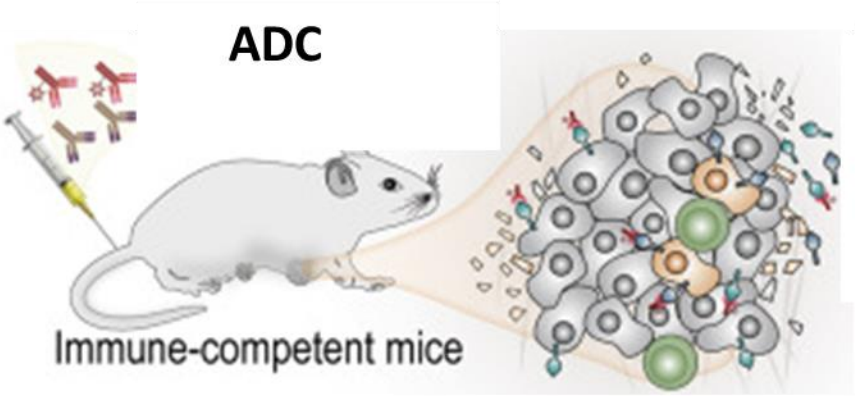
Drug Screening and Toxicity Testing: to study the cytotoxicity of new drugs (to study the effect and safe dosage) and/or drug carriers (nanoparticles).

Vaccine Production: Primary animal cells are used in the production of viruses and these viruses are used to produce vaccines (such as vaccines, for deadly diseases like polio, rabies, chicken pox, measles and hepatitis B are produced using animal cell culture)

PDX Model Studies




Target Antigen
• Abundance in tumors
• Minimal normal expression
• Internalization
Antibody
• Antibody properties
- Affinity
- Pharmacokinetics
• Internalization
• Conjugation chemistry
Cytotoxic Drug
• Microtubule inhibitors
- Suristatins (MMAE)
- Maytansines (DM1/DM4)
• DNA-damaging agents
- Calicheamicin
- Anthracyclines
- Duocarmycins
- Pyrrolobenzodiazepines
• Number of drugs per antibody
Linker
• Cleavable
- Acid-labile
- Protease cleavable
- Disulphide linkage
• Non-cleavable
• Plasma stability



INHIBITION TUMOR GROWTH

PROLONGED ANIMAL SURVIVAL

TOXICITY

Altamente espressa nei tessuti neoplastici, MMP9 correla con l'aggressività del tumore cerebrale

Diversi studi hanno mostrato che un'elevata espressione di alcuni membri della famiglia MMP è legata a invasione tumorale, metastasi e, dunque, ad una cattiva prognosi.

Studiare I profili di espressione delle MMPs In diversi tessuti e vederne la modulazione in ctrl vs condizione patologia e/o farmaco trattata

ZIMOGRAFIA

La zimografia su substrato di gelatina rappresenta il gold standard per la determinazione qualitativa e semi-quantitativa delle isoforme di MMP-2 e -9.

Nella tecnica della zimografia le proteine sono separate mediante elettroforesi in condizioni denaturanti [(sodio dodesil fosfato (SDS)] ma non riducenti. La separazione viene eseguita con gel di poliacrilamide co-polimerizzato con uno specifico substrato.

ZIMOGRAFIA

Dopo la migrazione elettroforetica, il gel viene incubato in detergente non ionico (Triton® X-100) per eliminare l'SDS.

Successivamente il gel è incubato in appropriato refolding buffer che consente alle MMPs di recuperare struttura e funzioni.

Durante l'incubazione, le gelatinasi attivate digeriscono la gelatina che è convertita in peptidi a basso peso molecolare che vengono eliminati dal gel mediante lavaggi.

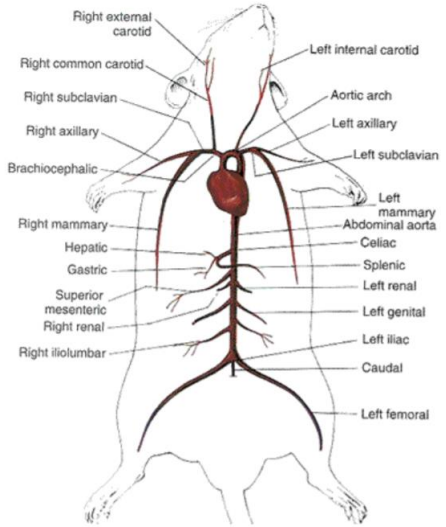
ZIMOGRAFIA

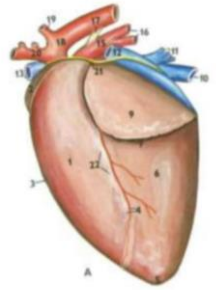
Il gel viene quindi colorato con Coomassie® blue e le gelatinasi evidenziate come bande bianche contro lo sfondo blu del substrato non degradato ed identificate in base al peso molecolare, paragonando le bande con uno standard di proteine pre-colorato e con gli enzimi purificati.

Successivamente l'entità della gelatina digerita può essere quantificata usando software di analisi di immagini

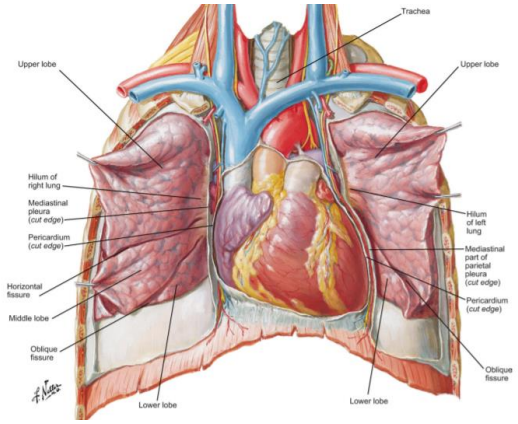
Enzima	MMP	Cromosoma umano	Peso Molecolare (Latente/Attiva)	Substrati Collageni	Substrati addizionali
Matrilisine					
Matrilisina 1	MMP-7	11q21-q22	28.000/19.000	IV, X	Aggrecan, Elastina, Fibronectina, Gelatina, Laminina, MMP-1, -2, -9
Matrilisina 2 (endometasi)	MMP-26	11p15	28.000/19.000	IV	Fibrinogeno, Fibronectina, Gelatina
Collagenasi					
Collagenasi 1 (interstiziale)	MMP-1	11q22-q23	55.000/45.000	I, II, III,VII,VIII, X	Aggrecan, Gelatina, MMP-2, -9
Collagenasi 2 (neutrofila)	MMP-8	11q21-q22	75.000/58.000	I, II, III,V, VII,VIII, X	Aggrecan, Elastina, Fibronectina, Gelatina, Laminina
Collagenasi 3	MMP-13	11q22.3	60.000/48.000	I, II, III, IV, IX, X, XIV	Aggregan, Gelatina
Collagenasi 4 (*Xenopus*)	MMP-18	No Umano	70.000/53.000		
Stromelisina					
Stromelisina 1	MMP-3	11q23	57.000/45.000	II, III, IV, IX, X, XI	Aggrecan, Elastina, Fibronectina, Gelatina, Laminina, MMP-7, -8, -13
Stromelisina 2	MMP-10	11q22.3-q23	57.000/44.000	III, IV, V	Aggrecan, Elastina, Fibronectina, Gelatina, Laminina, MMP-1, -8
Stromelisina 3	MMP-11	22q11.2	51.000/44.000	IV	Aggrecan, Fibronectina, Laminina
Gelatinasi					
Gelatinasi A	MMP-2	16q13	72.000/66.000	I, II, III, IV, V, VII, X, XI	Aggrecan, Elastina, Fibronectina, Gelatina, Laminina, MMP-9, -13
Gelatinasi B	MMP-9	20q11.2-q13.1	92.000/86.000	IV, V, VII, X, XIV	Aggrecan, Elastina, Fibronectina, Gelatina
Altre					
Macrofagi elastasi (metalloelastatsi)	MMP-12	11q22.2-q22.3	54.000/45.000	IV	Elastina, Fibronectina, Gelatina, Laminina
---	MMP-19	12q14	54.000/45.000	IV	Aggrecan, Fibronectina, Gelatina, Laminina
Enamelisina	MMP-20	11q22.3	54.000/22.000		Aggrecan, Amelogenina
	MMP-27	11q24			----
Epilesina	MMP-28	17q21.1			------

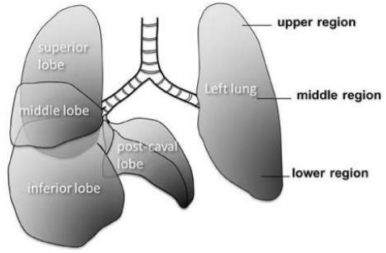
Tabella 2. Componenti della famiglia delle MMPs secrete.



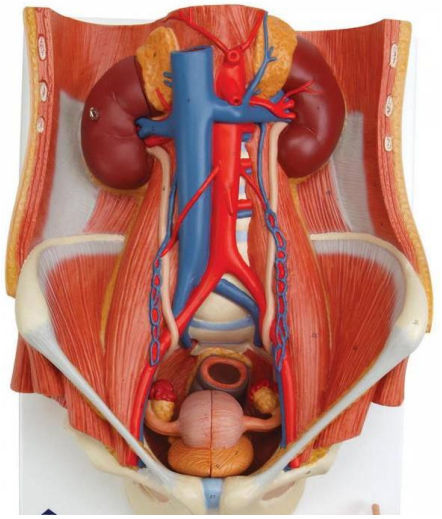


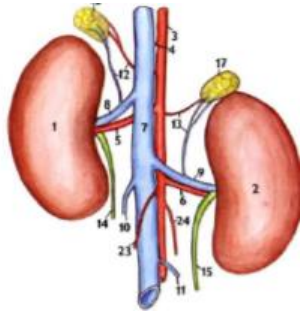
heart



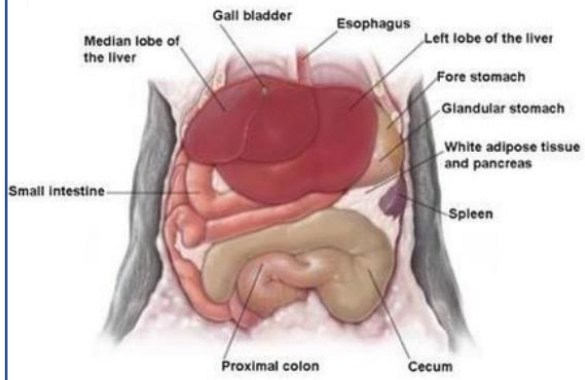


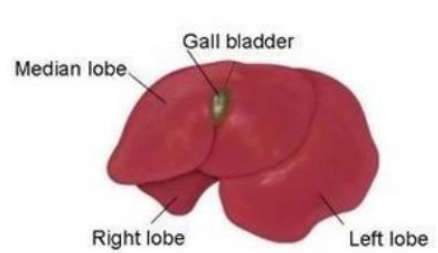
lungs





kidney





liver

Sample preparation

- Prepare tissue by rapidly removing the organ and snap freezing in liquid nitrogen (LN_2) and storing at − 80 °C.
- It provides excellent protein integrity and conserves functional activity

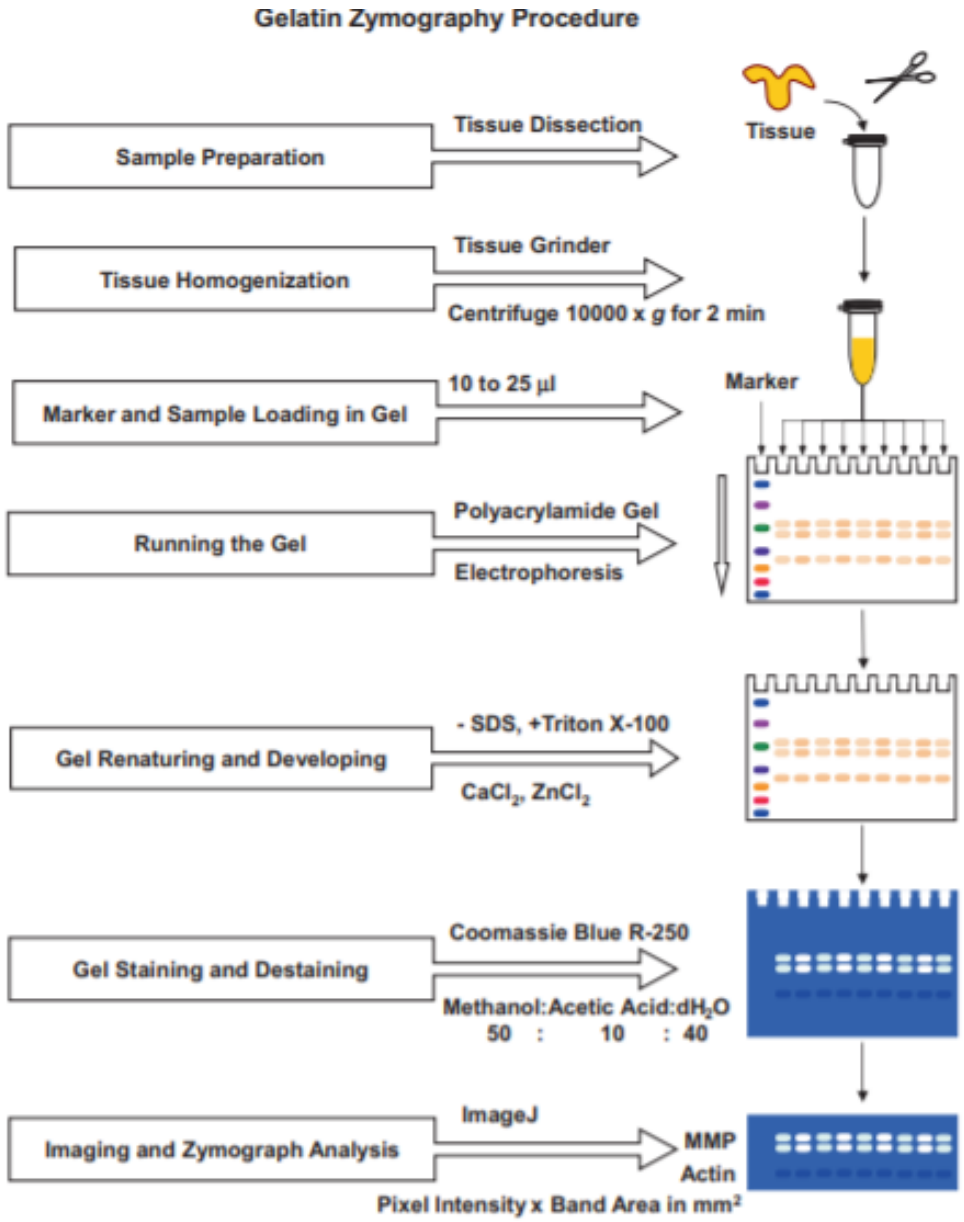


Fig. 2 Flow chart of gelatin zymography procedure

Weigh approximately 50 mg for each sample

Cut the tissue into small pieces in a small weighing boat on ice

Transfer the tissue to a tissue grinder or mortar (Kimble) on ice

Add cold homogenization buffer with anti-protease cocktail

50 mg tissue add 300 µL homogenization buffer 20’ on ice

Centrifuge the homogenate at 10,000 × g for 5’ min at 4 °C. (x2)

Save the supernatant, discard the pellet, and measure the protein concentration in the supernatant using Bradford protein assay

Use or store at −80 °C for maximum 1 month

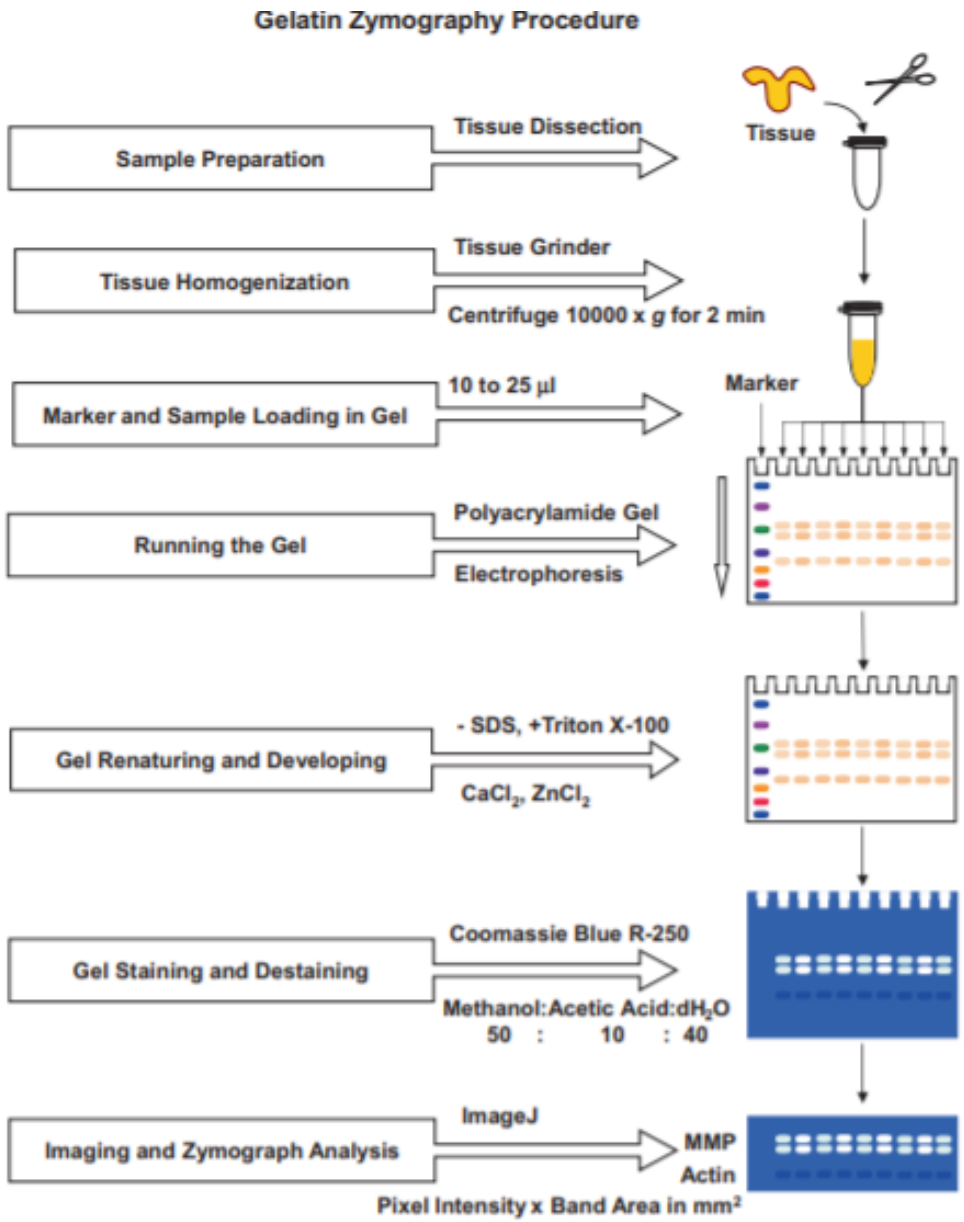


Fig. 2 Flow chart of gelatin zymography procedure

According to the results of protein measurement, sample loading mass and volume should be calculated.

From a tissue homogenate, 50 µg protein per lane should be loaded.

CTRL+ Metalloproteinase Prepare human recombinant MMP-2 or MMP-9 (0.13 µg/mL).
CTRL- Inhibitor Metalloproteinase

Sample heart, lungs, kidneys and livers

Run the gel in apparatus using gel electrophoresis at the standard running conditions (125 V, constant voltage) and until the bromophenol blue tracking dye reaches the bottom of the gel

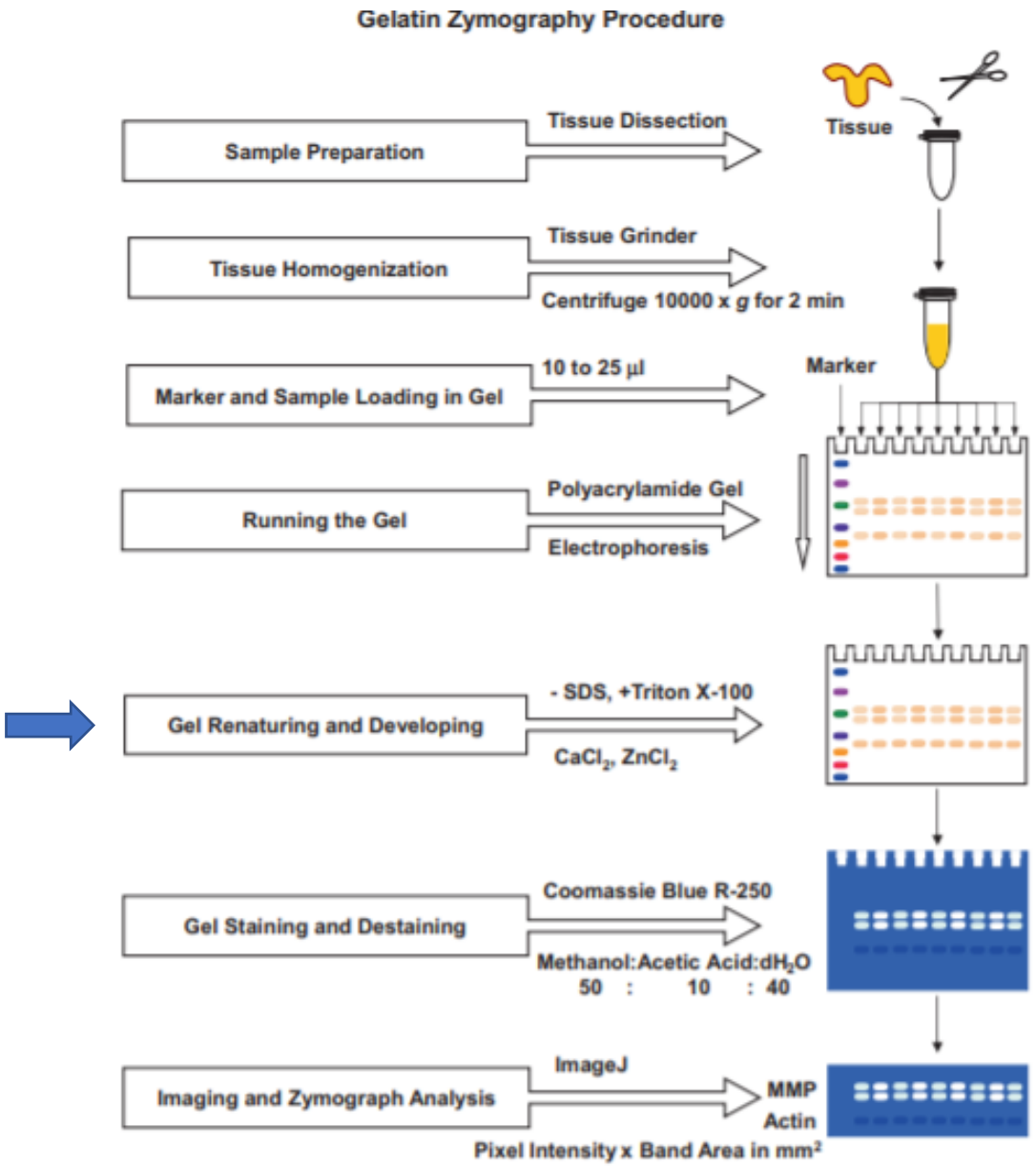


Fig. 2 Flow chart of gelatin zymography procedure

Incubate the gel for 30 min at room temperature with gentle shaking in order to remove SDS which causes MMPs to denature and become inactive.

Decant gel with 50 mL of fresh 1× developing buffer and incubate the gel at 37 °C overnight for ~18 h for maximum sensitivity

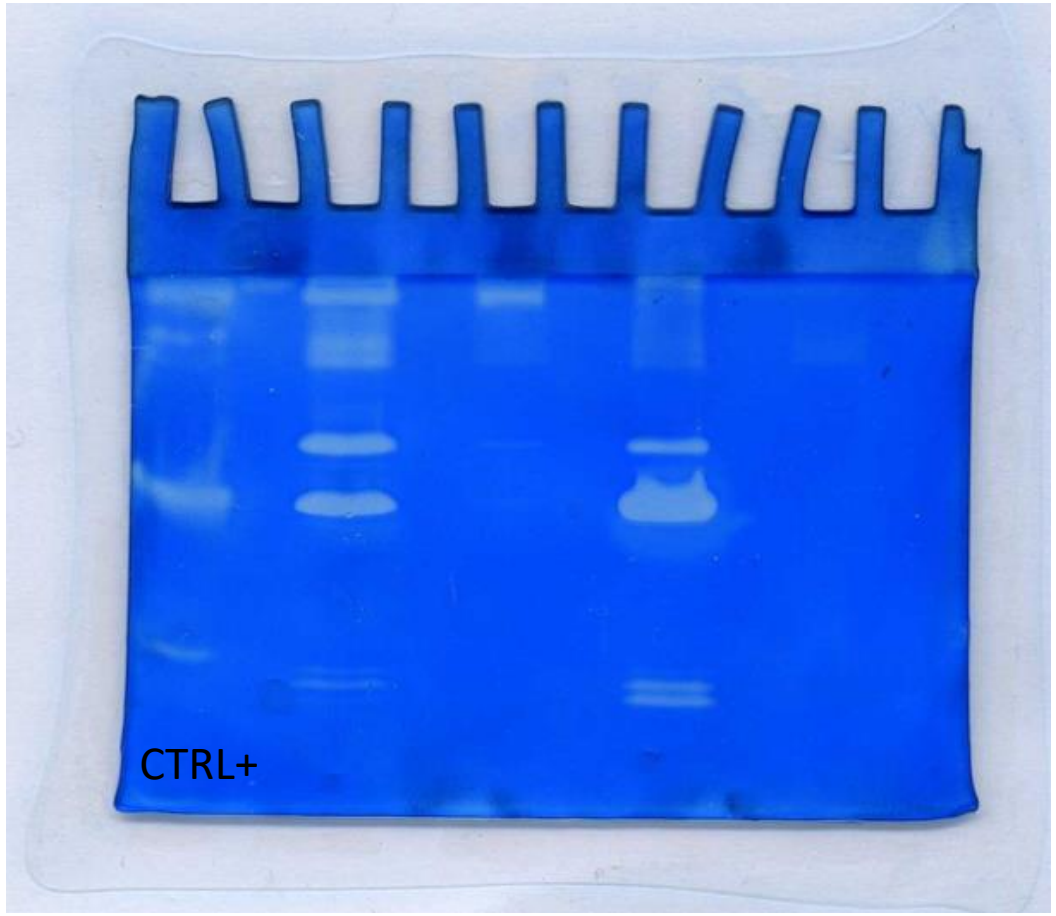
Decant the developing buffer and stain the gel with Commassie blue R-250 staining solution for at least 30 min until the gel is uniformly dark blue

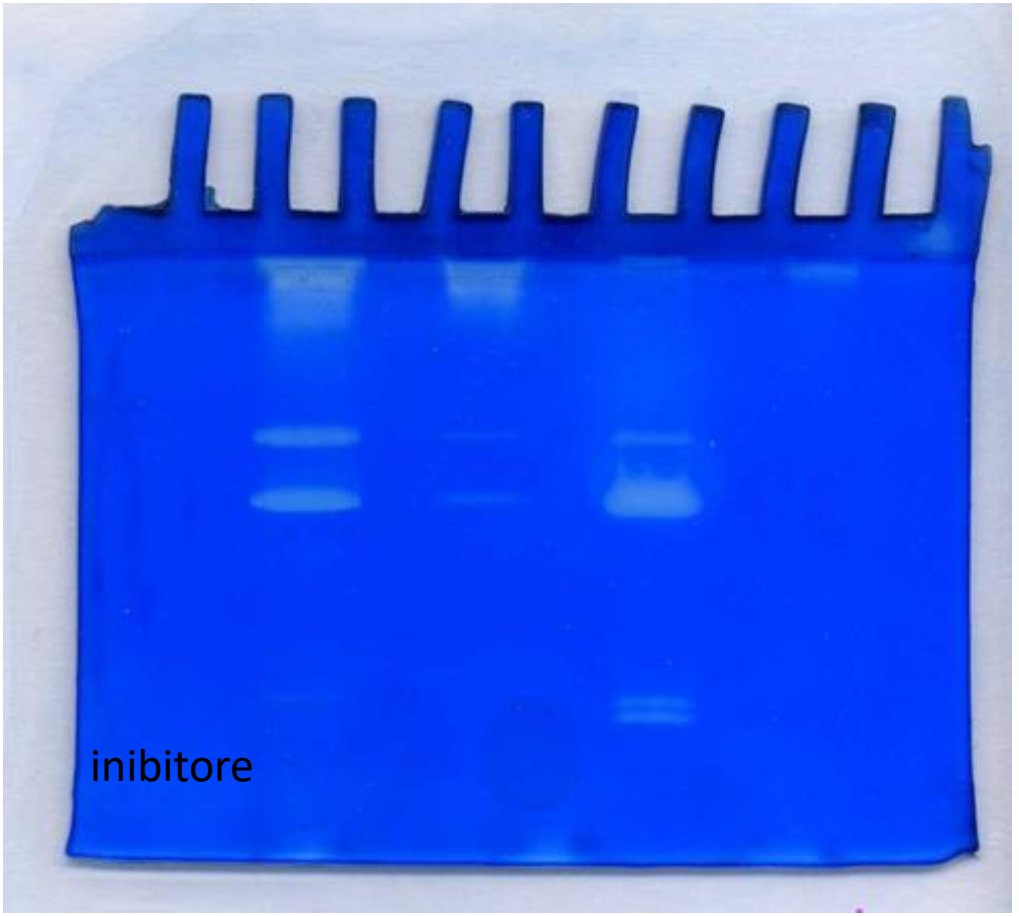
Destain the gel with destaining solution until areas of gelatinolytic activity appear as clear sharp bands against dark blue background.

Comparison of the location of the gelatinolytic with molecular weight standards run simultaneously on the same gel should help identify the specific MMP involved

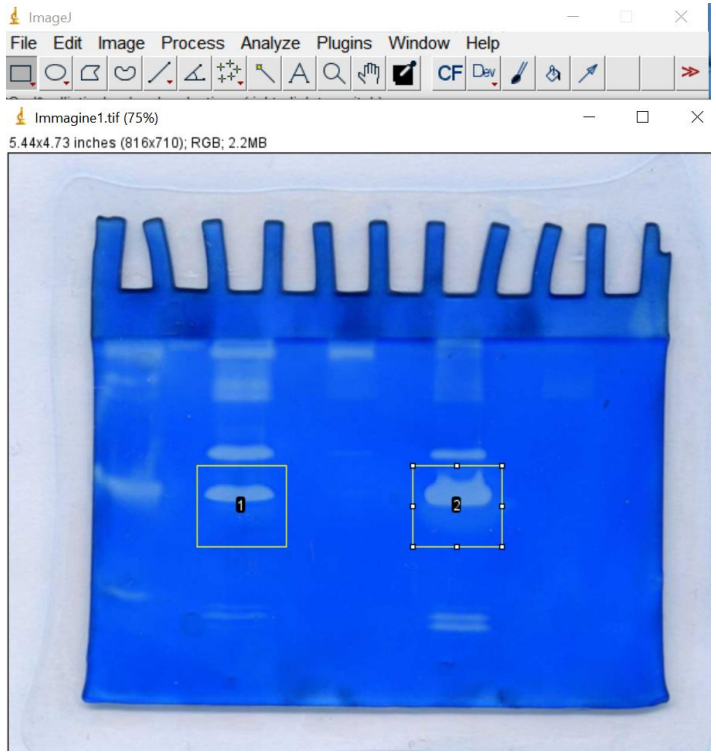
The bands in the gel are quantified using ImageJ software

Analisi risultati zimografia





Dal zimografia misura l'attività enzimatica dopo denaturazione e rinaturazione degli enzimi


ImageJ
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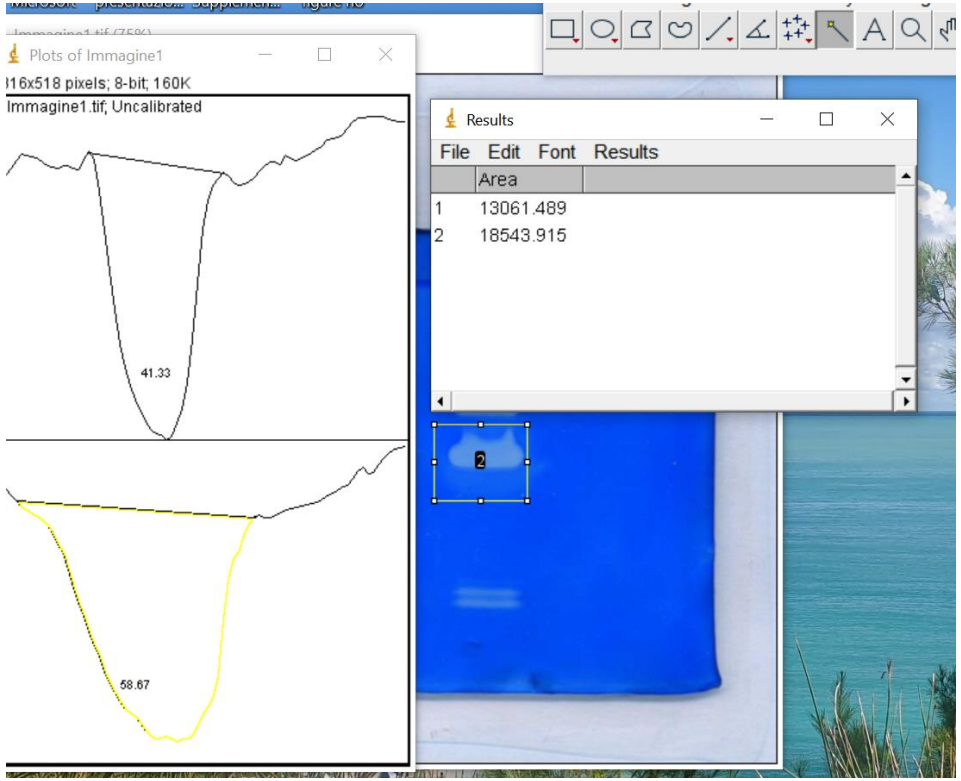
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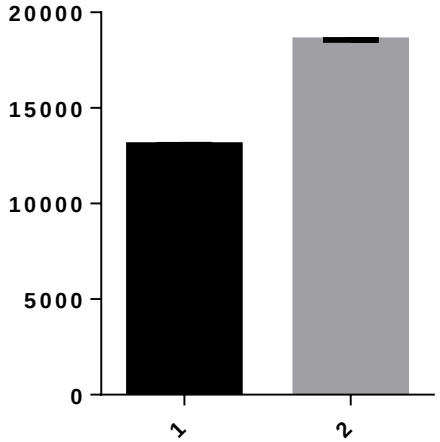
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