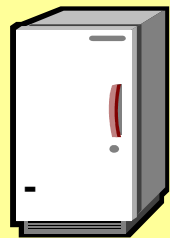


Biobanking av fryst vävnad - logistik, provkvalitet, organisation



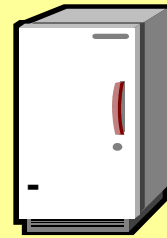
Johan Botling
Patologen, Akademiska, Uppsala

Gamla tumörbanker på patologen



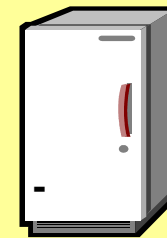
-80°C

Hematopat
4.400



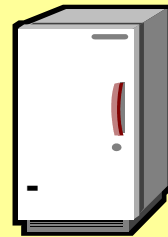
-80°C

Neuropat
1.800



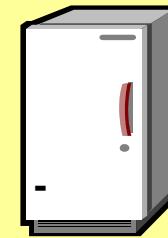
-80°C

Urologi
1.800



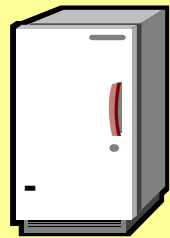
-80°C

Transplantation
2.500



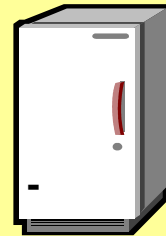
-80°C

Hud
1.300



-80°C

Bröst ca
5.400



-80°C

Blandade tumörer
4.000

Traditionella frysbanker

Representationsproblem

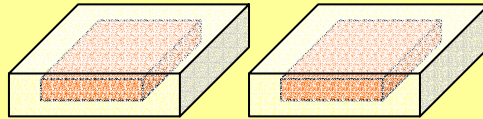
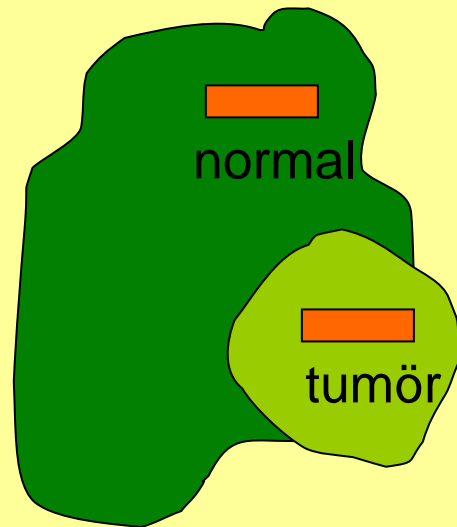
1) Selektion - fall fattas i behandlade patientserier

Stora tumörer bankas - Små tumörer bankas ej
Frysbankning ett diagnostiskt riskmoment

2) Vad är bankat egentligen?

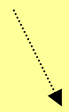
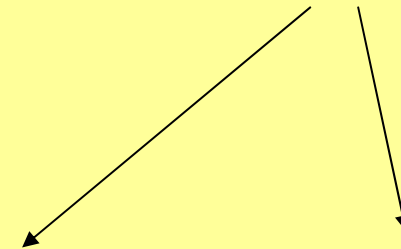
Tumör, normalvävnad, stroma, inflammation, nekros?

Ny frysbankningsrutin



15 x 10 x 5 mm
kryogel - OCT
isopentane + torr-is

fryssnitt

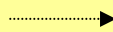
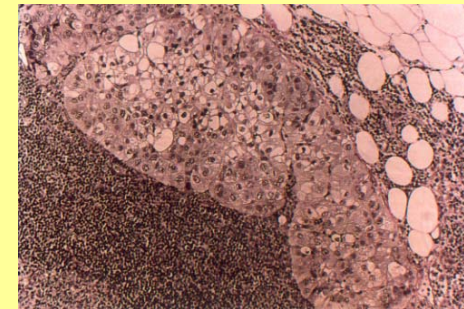


formalinfixering
paraffinkossar

diagnostik



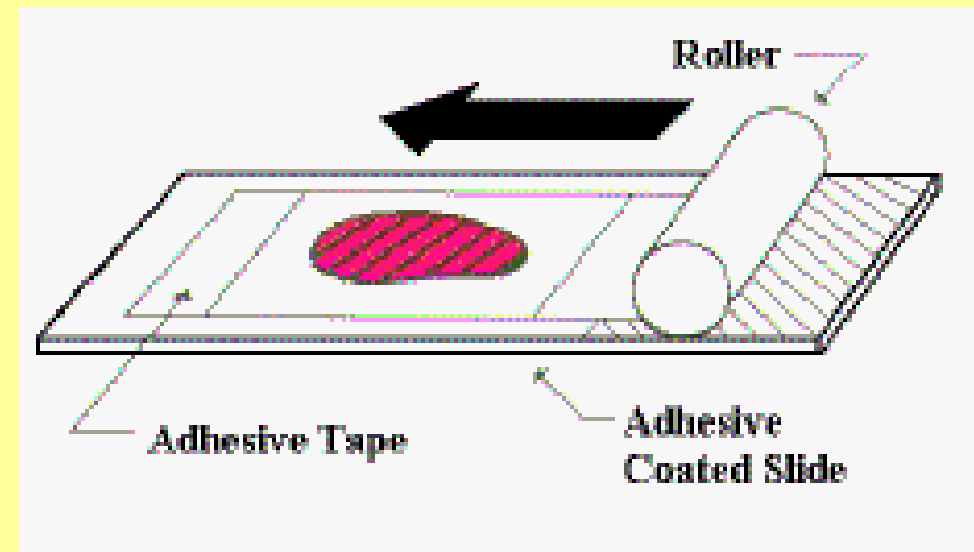
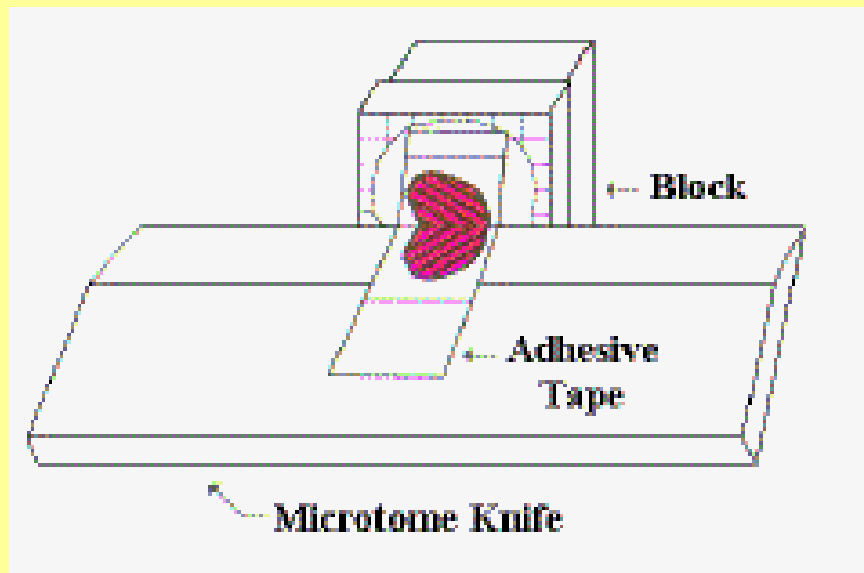
databas

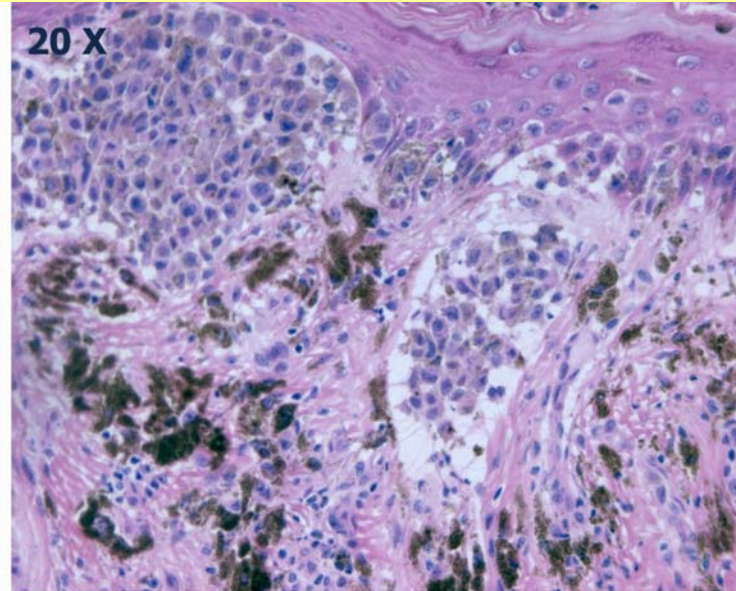
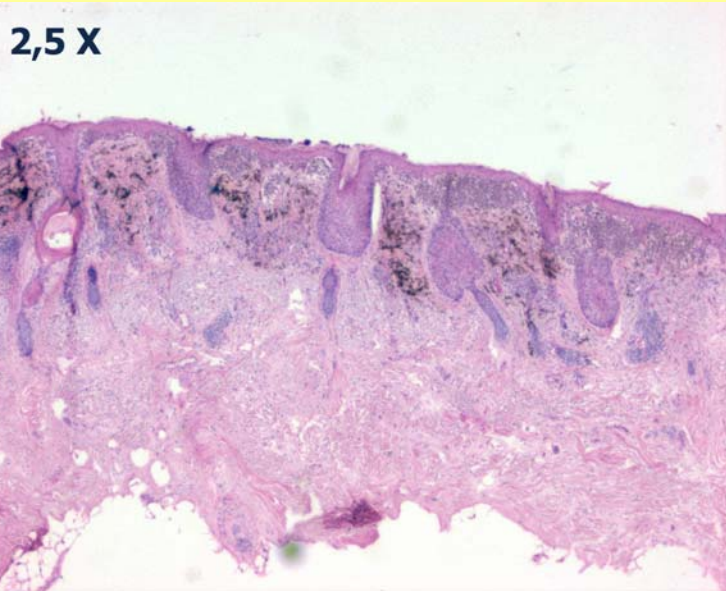


CryoJane

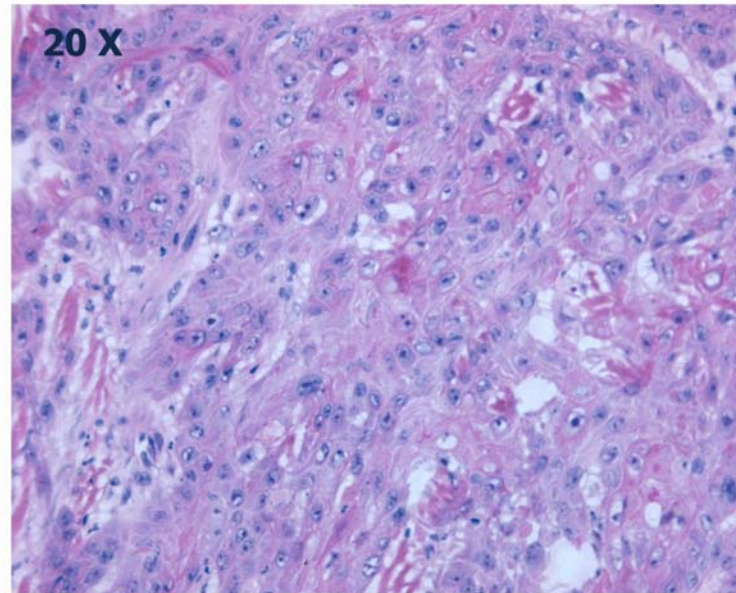
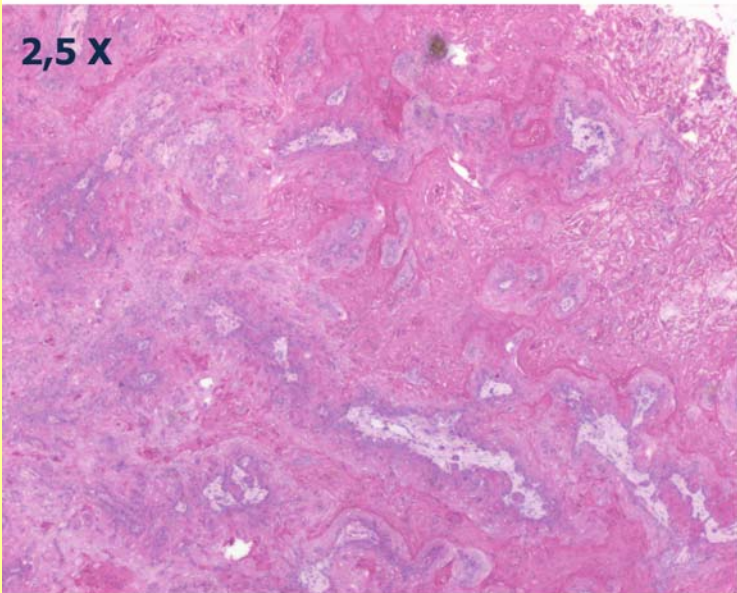
Tape Transfer System

för fryssnitt





Lentigo maligna melanom, Clark II

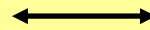


Högt differentierad skivepitelcancer

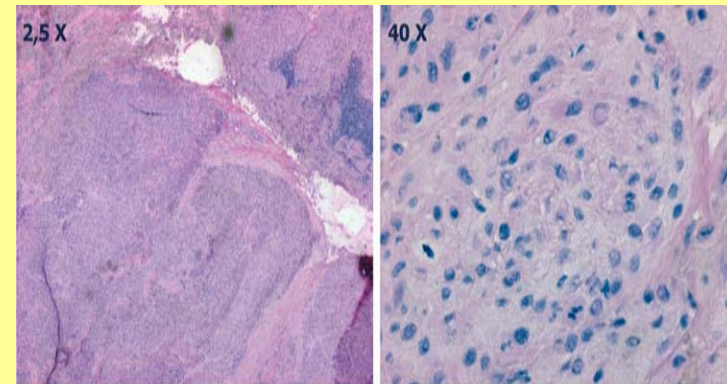
Databas

case data

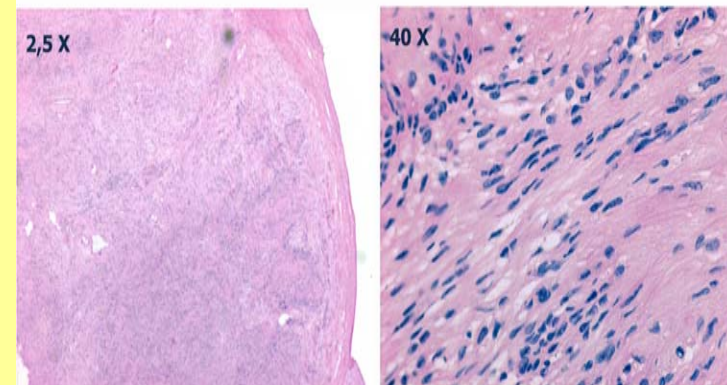
biobank number					
case number					
"person nr"					
diagnosis					
SNOMED code (T/M)					
position i freezer					
comments...					



digital image



Subkutan melanom metastas

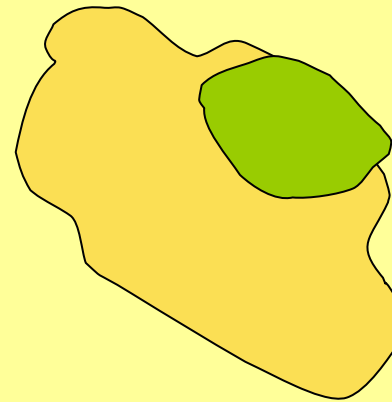


Kutant schwanom

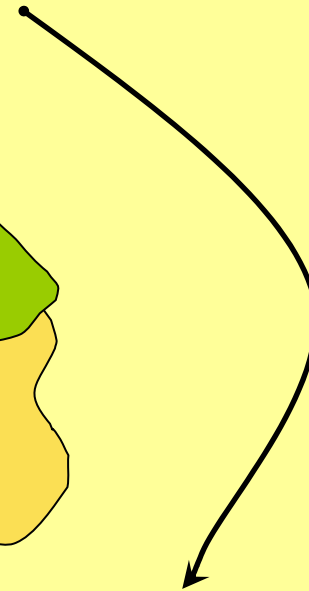
transporttid? transportmedium?



kirurgi



biobank



Rörpost



15 min

Jourhavande BMA Vaktmästare



30 min

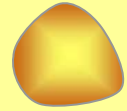


1/2-3 h

...till infrysning

Test av transportkinetik/transportmedier

Tonsillvävnad/
Normal colon

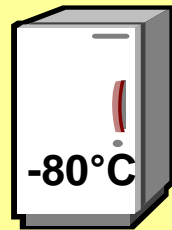


ice

RT

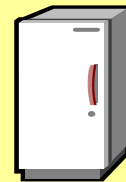
0.9% NaCl

RNAlater

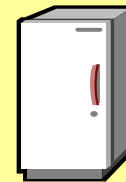


time point 0 h

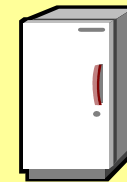
0.5 h



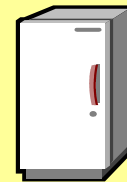
1 h



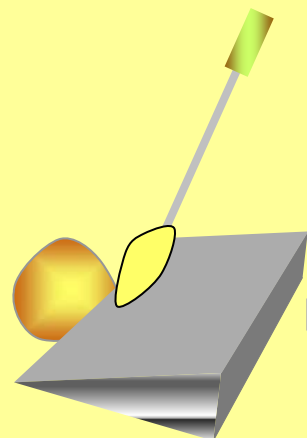
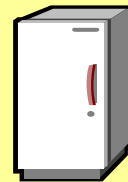
3 h



6 h



16h



cryosections

RNA extraction

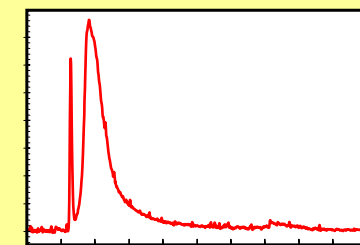
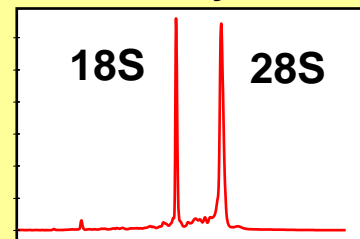
Qiagen



Trizol

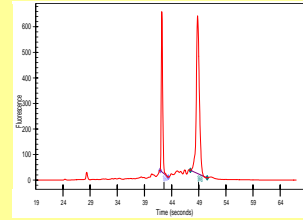


Bioanalyzer



(Micke et al., Lab Invest, in press)

direct frozen
time point 0h



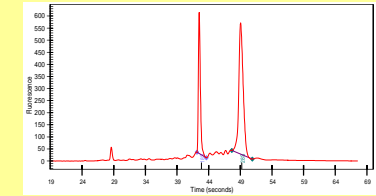
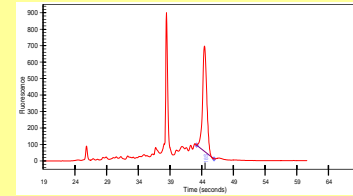
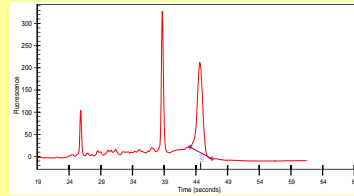
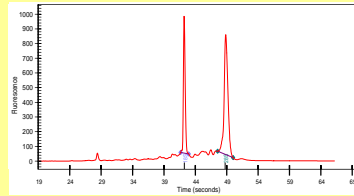
room temperature

ice

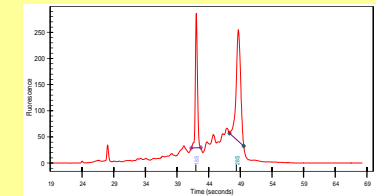
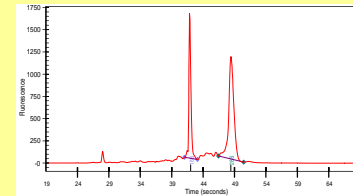
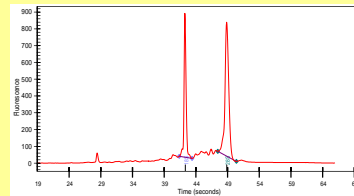
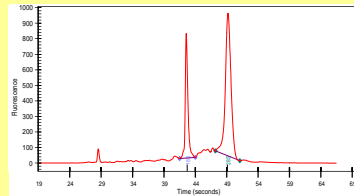
0.9 % NaCl

RNAlater

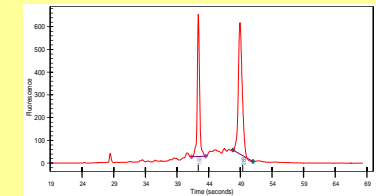
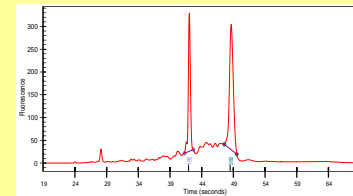
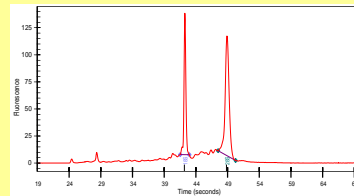
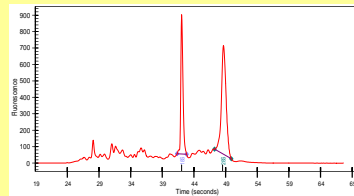
0.5 h



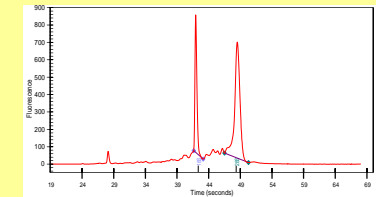
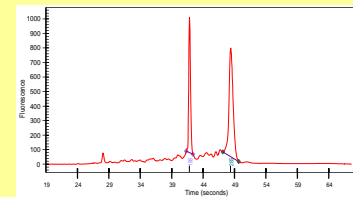
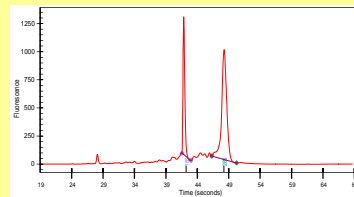
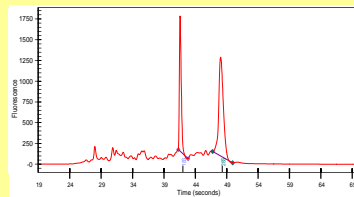
1.0 h



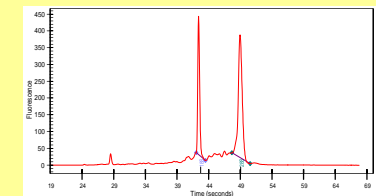
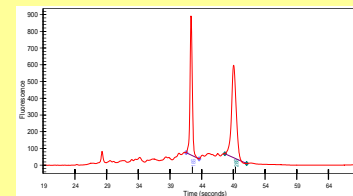
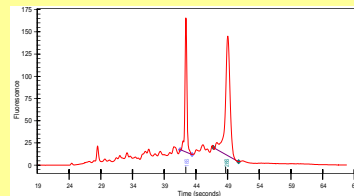
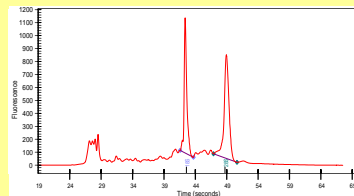
3 h

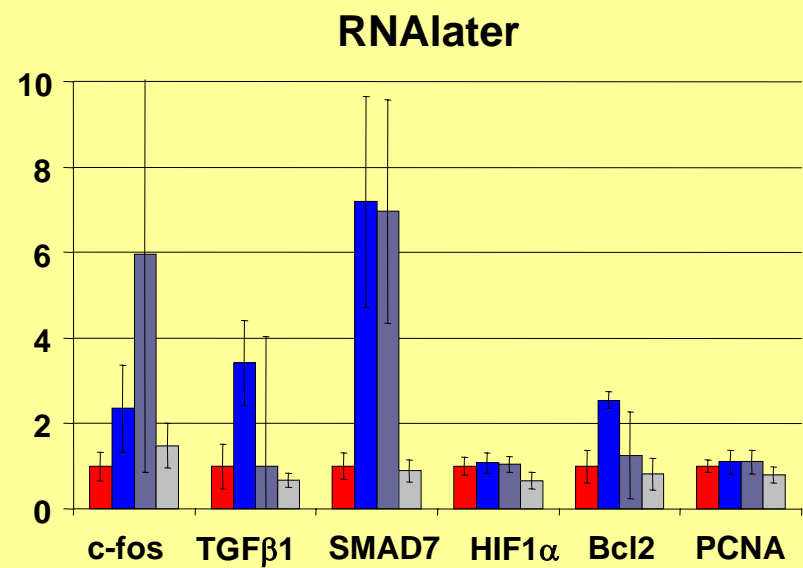
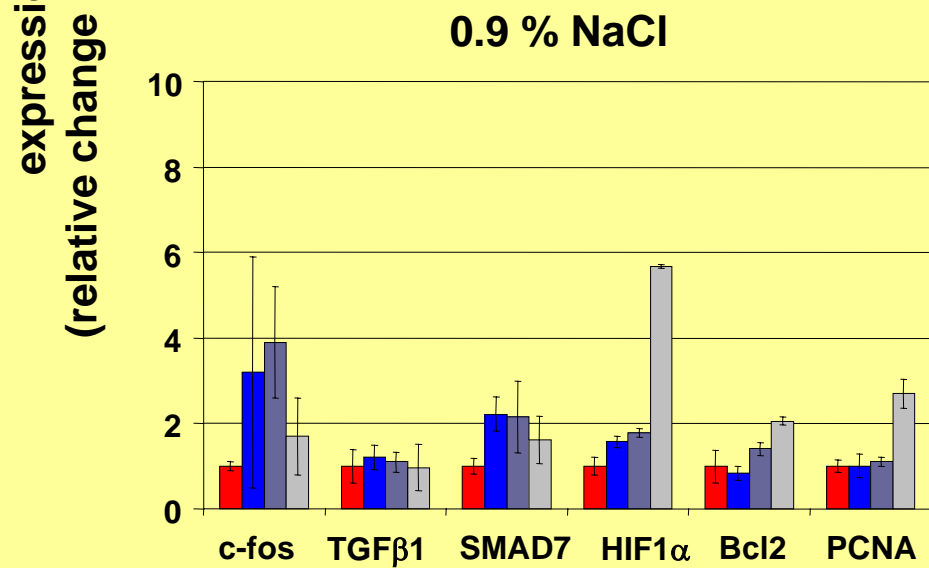
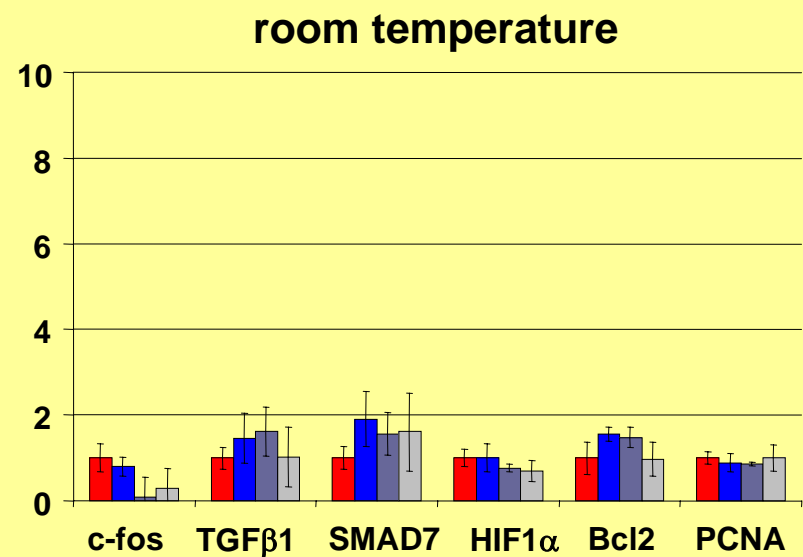
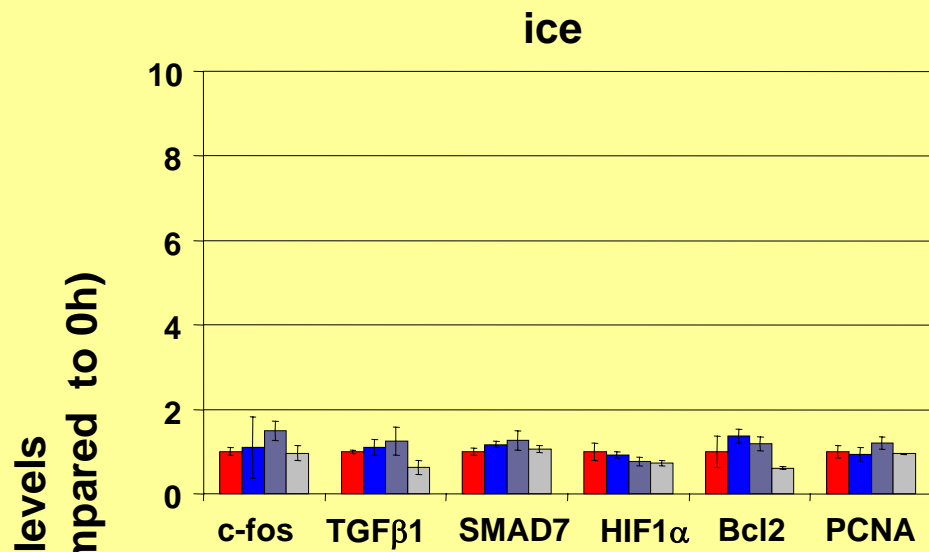


6 h



16 h





■ 0h
 ■ 0.5h
 ■ 6h
 ■ 16h

DNA kvalitet

Fryssnitt (10-20 μm)

1000-50000 celler

i 100 μl PCR-buffert + proteinase K

Digerering, inaktivering

5 μl till PCR-reaktion (50 celler)

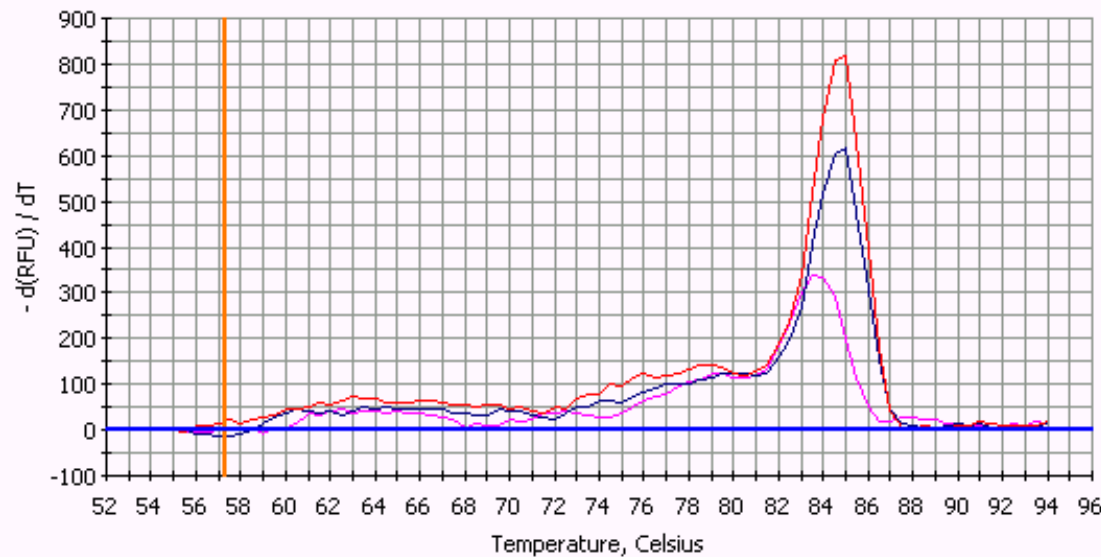
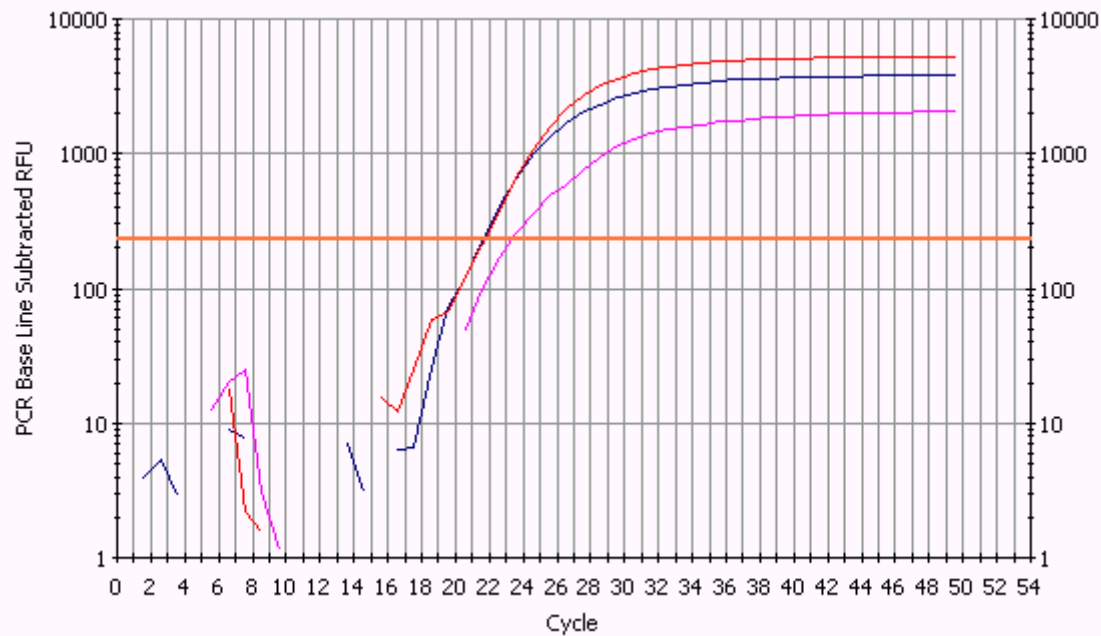
Real-time PCR + melting point analysis

Amplicon:

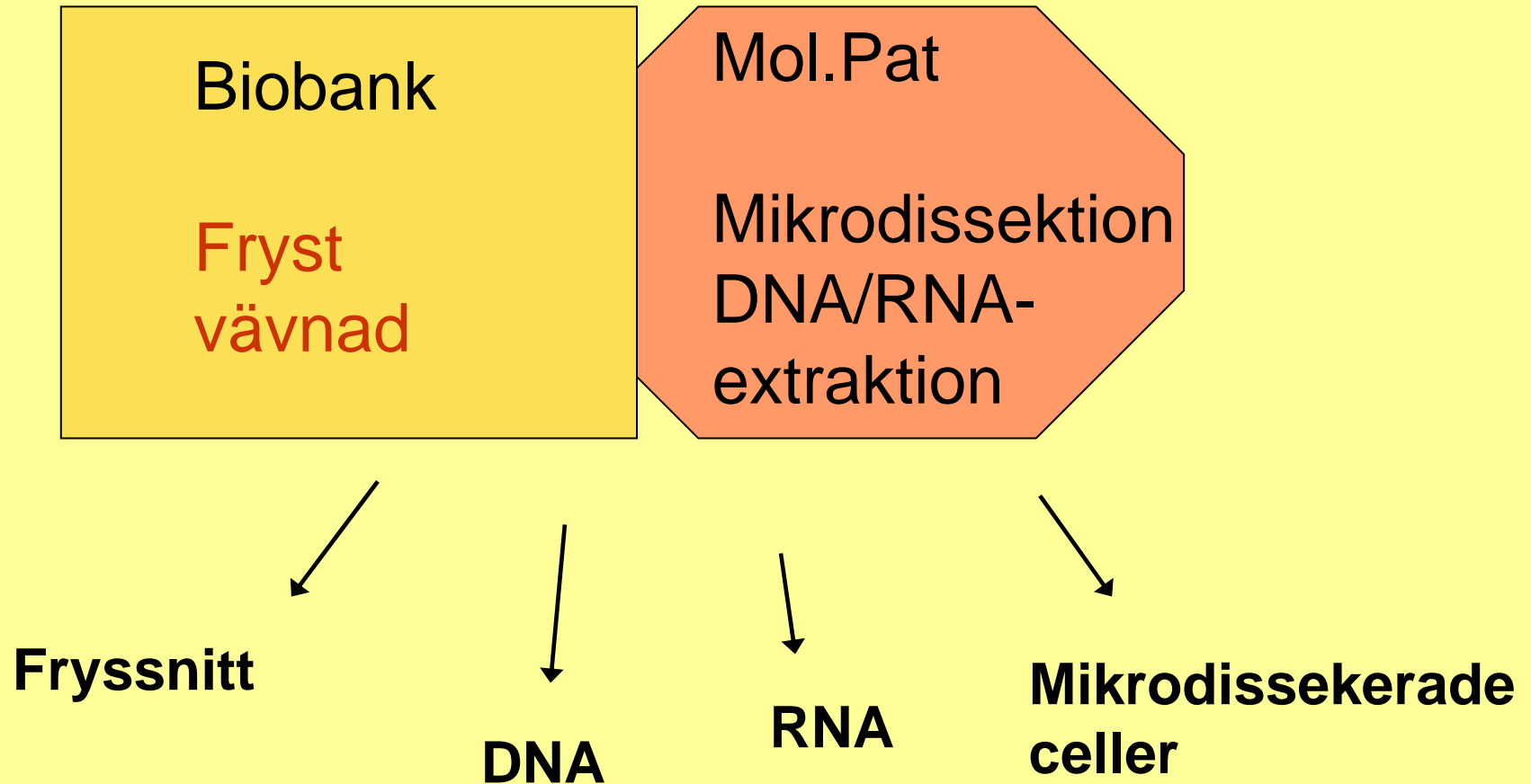
800 bp beta-2-mikroglobulin

njurcancerfall

frysta för ca
10 år sedan



Leverans av prover
Tina aldrig...Spara prov...Kolla provkvalitet



Slutsatser

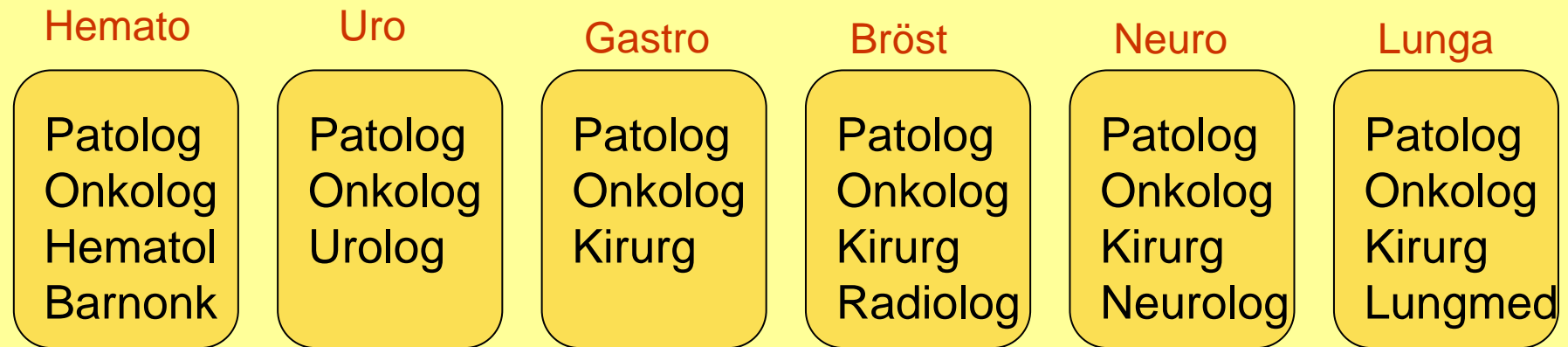
RNA är stabilt i flera timmar under transport av färska kirurgiska preparat
Transport på is att föredra i rutinsammanhang - stabil genexpression
Inget behov av avancerad logistik -
Kan klaras inom rutinpatologi för en rimlig kostnad
Ingen anledning att skära sönder preparat på operationsavd för frysbankning

Det lönar sig att kvalitetstesta gamla frysta prover
RNA- och DNA-kvaliten bättre än väntat

Morfologisk karakterisering av fryst material den viktigaste kvalitetshöjande faktorn
Fryssnittskarakterisering av bankat material
Komplett frysbankning av hela behandlade patientkohorter möjlig utan att riskera korrekt diagnos

Tina aldrig prover – provmaterial kan delas ut som fryssnitt
En frysbank måste ha histoteknisk kompetens

Organisation dispositionsrätt, finansiering



Tvärprofessionella team ansvariga för diagnostik och forskning

”Styr” dispositionen av frysta prover

Är ansvariga för finansieringen!

Patologavdelningen kan inte bära denna kostnad själv

Biobanking in the 21st century

Uppsala 18-20 januari
Institutionen för Genetik och Patologi
Rudbeckslaboratoriet

Mer info på www.biobanks.se (klicka på "courses")

VÄLKOMNA!