KUPNÍ SMLOUVA

1. Smluvní strany

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bankovní spojení: Česká národní banka, č. ú.: 20001-87535621/0710
(dále jen „kupující“)

a

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bankovní spojení: Československá obchodní banka a.s., č. ú.: 195092776/0300
společnost zapsaná v obchodním rejstříku vedeném Městským soudem v Praze, spisová značka B 9836
(dále jen „prodávající“)

uzavírají níže uvedeného dne, měsíce a roku, v souladu s § 2079 a násl. zákona č. 89/2012 Sb., občanský zákoník, ve znění pozdějších předpisů (dále jen „občanský zákoník“), v návaznosti na zadávací řízení k veřejné zakázce s názvem **„Izolátor nukleových kyselin pro OPATOL [2023]“** (evidenční číslo veřejné zakázky: Z2023-048225) (dále jen „veřejná zakázka“) tuto kupní smlouvu (dále jen „smlouva“).

2. Předmět smlouvy

2.1. Prodávající se zavazuje dodat kupujícímu spotřební materiál dle specifikace a v množství uvedeném v příloze č. 1 smlouvy (dále jen „zboží“).

2.2. Kupující se zavazuje řádně a včas dodané zboží převzít a zaplatit za něj cenu dle smlouvy.

2.3. Množství zboží je určeno přibližně. Ke stanovení přesného množství je oprávněn kupující, přičemž je oprávněn odebrat zboží v množství až o 50 % vyšším a až o 70 % nižším.

3. Kvalita zboží

3.1. Prodávající se zavazuje dodávat výhradně zboží, které splňuje veškeré technické, hygienické, veterinární, bezpečnostní a další standardy dle předpisů platných v EU a odpovídá požadavkům stanoveným právními předpisy v České republice, harmonizovanými českými technickými normami a ostatními ČSN a EN, které se vztahují k předmětu plnění, zejména, že splňují podmínky dle zákona č. 22/1997 Sb., o technických požadavcích na výrobky, ve znění pozdějších předpisů a zákona č. 268/2014 Sb., o zdravotnických prostředcích, ve znění pozdějších předpisů.

3.2. Jakost, úprava balení a značení zboží musí odpovídat příslušným platným právním předpisům a technickým normám.

3.3. Zboží musí být označeno šarží na vnějším i vnitřním obalu. Zboží s dobou použitelnosti (exspirační dobou) musí být opatřeno také údajem o exspiraci.

3.4. V případě, že je v rámci jedné dodávky dodáno zboží různých šarží, je prodávající povinen uvádět na dodacích listech počty kusů zboží s každou šarží samostatně.

3.5. Exspirační doba dodávaného zboží nesmí být při dodání kratší než 3 měsíce (nedohodnou-li se smluvní strany v některých případech jinak).

3.6. Záruční doba k dodávanému zboží se sjednává v délce do konce exspirační doby.

3.7. Poskytnutá záruka za jakost znamená, že zboží bude po dobu záruky za jakost v souladu s právními předpisy a se smlouvou.

4. Způsob, doba a místo dodání

4.1. Zboží bude dodáno formou průběžných dílčích plnění. Množství zboží a dobu dodání dílčího plnění stanovuje kupující výzvou k dílčímu plnění (dále jen „výzva“), přičemž poslední výzvu musí prodávajícímu doručit tak, aby poslední dílčí plnění mohlo být realizováno do 8 let od nabytí účinnosti smlouvy. K výzvám zaslaným později se nepřihlíží.

4.2. Kupující je oprávněn zaslat výzvu na níže uvedené kontaktní údaje:
E-mailová adresa [redacted]

Poštovní adresa [redacted]

4.3. Kupující je oprávněn zaslat výzvu prodávajícímu kdykoliv. V případě, že je výzva prodávajícímu doručena mimo pracovní dny od 8.00 do 15.00 h, považuje se za okamžik doručení 8.00 h nejbližšího pracovního dne (po doručení výzvy).

4.4. Minimální (finanční) objem jednotlivých dílčích plnění není stanoven.

4.5. Prodávající se zavazuje zboží dodávané v rámci dílčího plnění dodat do 15 pracovních dní (pokud kupující ve výzvě neuvede dobu delší) od okamžiku, kdy byla výzva prodávajícímu doručena.

4.6. Předání a převzetí zboží v místě dodání lze provést v pracovních dnech od 7.00 h do 15.00 h.

4.7. Místem dodání zboží je Ústavní lékárna kupujícího ve Švejdově pavilonu na adrese sídla kupujícího.

4.8. Při předání a převzetí zboží obdrží kupující dodací list, který potvrdí. Prodávající je povinen předat fakturu kupujícímu buď při předání a převzetí zboží společně s dodacím listem, nebo ji zašle v elektronické podobě na adresu [redacted] nejpozději v den předání a převzetí zboží.

4.9. K převzetí zboží a potvrzení dodacího listu v místě dodání zboží jsou oprávněni pracovníci Ústavní lékárny kupujícího.

4.10. Kupující je oprávněn odmítnout převzetí zboží zejména v následujících případech:

- **a)** prodávající (resp. jím pověřený přepravce) při dodávání zboží v místě dodání nepředá kupujícímu dodací list obsahující minimálně číslo výzvy a datum jejího odeslání, množství zboží s uvedením druhů zboží a ceny za množstevní jednotku, exspirační dobu a šarži dodávaného zboží;
- **b)** množství zboží uvedené na dodacím listě neodpovídá množství skutečně dodávaného zboží;
- **c)** neodpovídá-li kvalita dodávky (teplota uchovávaných reagencií, jakost obalového souboru atp.) požadavkům pro transport dle doporučení výrobce.

4.11. Náklady na případný odvoz zboží (včetně balného), které kupující v souladu se smlouvou nepřevzal, nese prodávající.

4.12. Kupující je oprávněn převzít i takové zboží, která vykazuje vady, které nebrání jeho řádnému užívání. Tyto vady se vyznačí v protokolu při přejímacím řízení. Prodávající je povinen tyto vady bezodkladně odstranit.

5. Přechod vlastnického práva a nebezpečí škody

5.1. Kupující nabývá vlastnické právo ke zboží okamžikem jeho převzetí od prodávajícího.

5.2. Nebezpečí škody na zboží přechází na kupujícího okamžikem převzetí zboží od prodávajícího.

6. Další práva a povinnosti smluvních stran

6.1. Smluvní strany prohlašují, že splňují náležitosti vyžadované právními předpisy pro nakládání se zbožím, a dále prohlašují, že se tyto právní předpisy zavazují v souvislosti s plněním smlouvy dodržovat. Prodávající se zavazuje kupujícímu tyto skutečnosti na výzvu kupujícího prokázat.

6.2. Prodávající se zavazuje dodávat pouze zboží splňující požadavky stanovené pro zboží tohoto druhu v České republice včetně požadavků na nakládání s takovým zbožím.

6.3. Prodávající není oprávněn postoupit jakoukoli pohledávku ze smlouvy na třetí osobu bez předchozího písemného souhlasu kupujícího.

7. Kupní cena a platební podmínky

7.1. Kupní cenou se rozumí cena 1 jednotky zboží uvedená v příloze č. 1 smlouvy v Kč bez DPH (dále jen „kupní cena“). Kupní cena zahrnuje veškeré náklady kupujícího na pořízení zboží (přirážky distributorů, celní poplatky, dopravné, balné, apod.). Smluvní strany se dohodly, že kupní cena je cenou nepřekročitelnou, zároveň, že je prodávající oprávněn snížit kupní cenu i jednostranně, a to na základě písemného oznámení doručeného kupujícímu.

7.2. DPH bude dopočítána a spolu s kupní cenou uhrazena ve výši dle právních předpisů účinných ke dni uskutečnění zdanitelného plnění.

7.3. Kupní cena za zboží dodané v rámci dílčího plnění je splatná do 30 kalendářních dnů ode dne doručení faktury (s náležitostmi daňového dokladu) kupujícímu.

7.4. V případě, že faktura nebude obsahovat náležitosti dle platných právních předpisů, popř. bude obsahovat jiné chyby či nedostatky, je kupující oprávněn v době splatnosti takovou fakturu vrátit, přičemž nová doba splatnosti počíná běžet dnem doručení opravené faktury kupujícímu. Za den úhrady se považuje den odeslání celé fakturované částky z účtu kupujícího na účet prodávajícího.

7.5. Veškeré platby mezi smluvními stranami se uskutečňují prostřednictvím bankovního spojení uvedeného v záhlaví smlouvy.

7.6. Bude-li k datu uskutečnění zdanitelného plnění nebo k datu poskytnutí úplaty za takové plnění prodávající nespolehlivým plátcem ve smyslu § 106a zákona č. 235/2004 Sb., o dani z přidané hodnoty, ve znění pozdějších předpisů (dále jen „ZoDPH“), nebo bude-li na daňovém dokladu uveden bankovní účet nezveřejněný v souladu s § 109 odst. 2 písm. c) ZoDPH, je kupující oprávněn postupovat dle § 109a ZoDPH, tj. uhradit část kupní ceny odpovídající výši vypočtené daně z přidané hodnoty přímo na bankovní účet příslušného správce daně (jako úhradu daně za poskytovatele zdanitelného plnění z takového zdanitelného plnění), přičemž se tímto považuje daná část kupní ceny za uhrazenou.

7.7. Prodávající je každoročně (počínaje rokem 2025) oprávněn zvýšit kupní cenu s účinností od 1. dubna dotčeného kalendářního roku, nikoliv však dříve, než ode dne doručení písemného oznámení o zvýšení kupní ceny kupujícímu, o přírůstek průměrného ročního indexu spotřebitelských cen (dále jen „míra inflace“) vyhlášený Českým statistickým úřadem za předcházející kalendářní rok, vždy však nejvýše o 5 %. V případě záporné míry inflace se kupní cena nesnižuje. Oznámení musí obsahovat míru inflace, aktualizovaný ceník (ve struktuře dle přílohy č. 1 smlouvy) a podrobnosti výpočtu zvýšení kupní ceny.

8. Reklamace vadného zboží

8.1. Vady zboží projevující se tím, že zboží neodpovídá smluvené kvalitě a projeví se v době použitelnosti (exspirace), je kupující oprávněn uplatnit u prodávajícího nejpozději poslední den exspirační doby.

8.2. Kupující je v případě vady zboží povinen tuto vadu nahlásit (reklamovat) prodávajícímu telefonicky na [redacted] nebo e-mailem na [redacted] Vadu nahlášenou telefonicky kupující potvrdí nahlášením vady e-mailem. Reklamace musí obsahovat stručný popis toho, jak se vada projevuje.

8.3. V případě uplatnění záruky za jakost může kupující:
- **a)** požadovat bezplatné dodání nového bezvadného zboží,
- **b)** požadovat poskytnutí slevy z kupní ceny, nebo
- **c)** odstoupit od smlouvy v případě, že se jedná o opakující se vady stejného druhu nebo pokud kupující v souladu se smlouvou požadoval nové bezvadné zboží a toto mu nebylo dodáno ani ve lhůtě do 60 dnů ode dne uplatnění tohoto požadavku.

8.4. V případě uplatnění nároku na dodání nového bezplatného zboží je prodávající povinen zboží dodat nejpozději ve lhůtě do 5 pracovních dnů počítaných ode dne reklamace zboží kupujícím.

9. Smluvní sankce

9.1. V případě prodlení kupujícího s platbou kupní ceny je prodávající oprávněn po kupujícím žádat uhrazení úroku z prodlení ve výši dle nařízení vlády č. 351/2013 Sb., kterým se určuje výše úroků z prodlení a nákladů spojených s uplatněním pohledávky, určuje odměna likvidátora, likvidačního správce a člena orgánu právnické osoby jmenovaného soudem a upravují některé otázky Obchodního věstníku a veřejných rejstříků právnických a fyzických osob.

9.2. V případě prodlení prodávajícího s dodáním zboží se prodávající zavazuje uhradit kupujícímu smluvní pokutu ve výši 500 Kč za každý započatý den prodlení.

9.3. V případě prodlení prodávajícího s vyřízením reklamace se prodávající zavazuje uhradit kupujícímu smluvní pokutu ve výši 500 Kč za každý započatý den prodlení.

9.4. Prodávající se zavazuje uhradit smluvní pokutu kupujícímu ve lhůtě do 10 dnů ode dne doručení výzvy k jejímu zaplacení.

9.5. Zaplacením smluvní pokuty není dotčeno právo kupujícího na náhradu škody v plné výši.

10. Platnost a účinnost smlouvy, změny smlouvy, ukončení smlouvy

10.1. Smlouva nabývá platnosti dnem podpisu oběma smluvními stranami a účinnosti uveřejněním v Registru smluv (smlouvy.gov.cz).

10.2. Plnění předmětu smlouvy před účinností smlouvy se považuje za plnění podle smlouvy a práva a povinnosti z něj vzniklé se řídí smlouvou.

10.3. Smlouvu lze změnit výhradně dohodou smluvních stran v písemné formě podepsanou oběma smluvními stranami, přednostně prostřednictvím vzestupně číslovaných dodatků. Výjimkou je změna adresy sídla a kontaktních údajů, v takovém případě postačuje oznámení dotčené smluvní strany doručené v písemné formě druhé smluvní straně, v případě změny adresy sídla spolu s doklady prokazujícími oznamovanou změnu; ke změně smlouvy dochází dnem doručení oznámení druhé smluvní straně.

10.4. Prodávající je oprávněn převést svoje práva a povinnosti ze smlouvy vyplývající na jinou osobu pouze s písemným souhlasem kupujícího.

10.5. Smluvní strany se nad rámec § 576 občanského zákoníku pro případ neplatnosti některého z ustanovení smlouvy či celé smlouvy zavazují, že si poskytnou potřebnou součinnost k uzavření dohody, kterou by dotčené ustanovení, případně celou smlouvu, nahradily tak, aby obsah a účel smlouvy zůstal v nejvyšší možné míře zachován.

10.6. Každá ze smluvních stran je oprávněna od smlouvy odstoupit v případě podstatného porušení smlouvy druhou smluvní stranou. Na straně kupujícího se za podstatné porušení smlouvy považuje jeho prodlení s úhradou kupní ceny přesahující 60 dnů. Odstoupením od smlouvy se smlouva rozvazuje dnem doručení písemného odstoupení druhé smluvní straně.

10.7. Každá ze smluvních stran je oprávněna smlouvu vypovědět písemnou výpovědí i bez udání důvodů, a to i jen ve vztahu k jednomu či více typů zboží uvedenému v příloze č. 1 smlouvy.

Smlouva je v takovém případě ukončena posledním dnem 6 měsíce následujícího po měsíci, ve kterém je písemná výpověď doručena druhé smluvní straně, v případě prodávajícího však nejdříve uplynutím 4 let od nabytí účinnosti smlouvy.

Kupující v takovém případě není povinen odebrat zboží ve sjednaném množství a současně mu zaniká nárok na dodání zboží, ke kterému prodávajícího do konce účinnosti smlouvy nevyzval. Práva smluvních stran na uplatnění nároků na smluvní sankce a na náhradu škody tím zůstávají nedotčena.

11. Závěrečná ustanovení

11.1. Smlouva je vyhotovena ve dvou stejnopisech, přičemž každá smluvní strana obdrží jeden stejnopis v případě, že bude podepsána v listinné podobě. Pokud je smlouva podepisována elektronicky, je vyhotovena v jednom stejnopise podepsaném elektronicky oběma smluvními stranami.

11.2. V otázkách výslovně neupravených smlouvou se závazky smluvních stran řídí ustanoveními příslušných právních předpisů, zejména § 2079 a násl. občanského zákoníku upravujícími kupní smlouvu.

11.3. Smluvní strany souhlasí se zveřejněním smlouvy v úplném znění, stejně jako s uveřejněním úplného znění případných dohod (dodatků), kterými se smlouva doplňuje, mění, nahrazuje nebo ruší, a to zejména prostřednictvím Registru smluv (**smlouvy.gov.cz**) v souladu se zákonem č. 340/2015 Sb., o registru smluv, ve znění pozdějších předpisů. Výjimku tvoří osobní údaje, vlastnoruční podpisy a razítka smluvních stran. Prokázal-li prodávající před uzavřením smlouvy kupujícímu, že jednotkové ceny zboží, případně množství zboží, mají objektivně povahu obchodního tajemství, zveřejní se smlouva bez těchto údajů. Kupující se do 14 dnů od uzavření smlouvy zavazuje smlouvu uveřejnit, prodávající se zavazuje v rozmezí 15.–30. dne ode dne uzavření smlouvy uveřejnění smlouvy v registru smluv ověřit a v případě, že smlouva v registru smluv nebude uveřejněna, ji sám uveřejnit; obdobně se postupuje v případě dodatků ke smlouvě.

11.4. Prodávající si je vědom toho, že v souladu s § 2 písm. e) zákona č. 320/2001 Sb., o finanční kontrole ve veřejné správě, ve znění pozdějších předpisů, je osobou povinnou spolupůsobit při výkonu finanční kontroly. Prodávající se zavazuje poskytnout kontrolním orgánům při provádění kontroly maximální součinnost. Prodávající je zároveň povinen zavázat své poddodavatele, aby tito spolupůsobili při provádění kontroly a poskytovali kontrolním orgánům při provádění kontroly maximální součinnost.

11.5. Prodávající se zavazuje zajistit dodržování pracovněprávních předpisů, zejména zákona č. 262/2006 Sb., zákoník práce, ve znění pozdějších předpisů (se zvláštním zřetelem na regulaci odměňování, pracovní doby, doby odpočinku mezi směnami, atp.), zákona č. 435/2004 Sb., o zaměstnanosti, ve znění pozdějších předpisů (se zvláštním zřetelem na regulaci zaměstnávání cizinců), a to vůči všem osobám, které se na plnění zakázky podílejí a bez ohledu na to, zda jsou práce na předmětu plnění prováděny bezprostředně prodávajícím či jeho poddodavateli. Nedodržení tohoto závazku je podstatným porušením smlouvy.

11.6. Smluvní strany se v souladu s § 89a zákona č. 99/1963 Sb., občanský soudní řád, ve znění pozdějších předpisů, dohodly, že místně příslušným soudem je Městský soud v Brně. Smluvní strany dále sjednávají, že smlouva a veškeré nároky nebo spory vzniklé na jejím základě nebo v souvislosti s ní (včetně mimosmluvních sporů a nároků) se budou řídit českým právem a budou vykládány v souladu s právními předpisy České republiky.

11.7. Pokud jakékoliv ustanovení smlouvy netvořící její podstatnou náležitost je nebo se stane neplatným nebo nevymahatelným jako celek nebo jeho část, je plně oddělitelným od ostatních ustanovení smlouvy a taková neplatnost nebo nevymahatelnost nebude mít žádný vliv na platnost a vymahatelnost jakýchkoliv ostatních ustanovení ze smlouvy, strany se zavazují v rámci smlouvy nahradit prostřednictvím dodatku ke smlouvě toto neplatné nebo nevymahatelné oddělené ustanovení takovým novým platným a vymahatelným ustanovením, jehož předmět bude v nejvyšší možné míře odpovídat předmětu původního odděleného ustanovení. Pokud však jakékoliv ustanovení smlouvy tvořící její podstatnou náležitost je nebo se stane neplatným nebo nevymahatelným jako celek nebo jeho část, strany nahradí neplatné nebo nevymahatelné ustanovení v rámci nové smlouvy takovým novým platným a vymahatelným ustanovením, jehož předmět bude v nejvyšší možné míře odpovídat předmětu původního ustanovení obsaženému ve smlouvě.

11.8. Součástí smlouvy je její **příloha č. 1 – Specifikace a ceník zboží**.

11.9. Smluvní strany prohlašují, že si smlouvu před jejím podpisem přečetly a že s jejím obsahem souhlasí, na důkaz výše uvedeného připojují své podpisy.

V Brně dne 11-01-2024

V Praze dne 02-01-2024

[redacted]

za kupujícího:
prof. MUDr. Marek Svoboda, Ph.D.
ředitel Masarykova onkologického ústavu

za prodávajícího:
RNDr. Petr Kvapil
předseda představenstva

Příloha č. 1
SPECIFIKACE A CENÍK ZBOŽÍ

SPOTŘEBNÍ MATERIÁL							
Konkrétní požadavky na spotřební materiál	**Počet izolací (vzorků) za 8 let**	**Cena za jednu izolaci (vzorek)**	**Cena celkem (Kč bez DPH)**	**Počet izolací (vzorků) na 1 kit**	**Cena kit (Kč bez DPH)**	**Množství kitů**	**Splňuje (ANO / NE)**
Kit pro izolaci DNA Ionic G2 FFPE to DNA Kit (90164)	2 304,00	310,40 Kč	715 161,60 Kč	48,00	14 899,20 Kč	48,00	ANO
Kit pro izolaci RNA Ionic G2 FFPE to RNA Kit (90167)	2 304,00	310,40 Kč	715 161,60 Kč	48,00	14 899,20 Kč	48,00	ANO
Kit pro kompletní izolaci DNA i RNA Ionic G2 FFPE Complete Kit (90170)	2 304,00	560,40 Kč	1 291 161,60 Kč	48,00	26 899,20 Kč	48,00	ANO

Příloha 1a - Ionic G2 FFPE to Pure DNA Protocol (pdf)
Příloha 1b - Ionic G2 FFPE to Pure RNA Protocol (pdf)
Příloha 1c - Ionic G2 FFPE Complete Protocol (pdf)

Ionic G2 FFPE to DNA Kit Protocol

DOCUMENT NUMBER:
CG-00036

DOCUMENT REVISION:
B

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Legal Notice

For Research Use Only. Not for use in diagnostic procedures.

Revision History

REVISION	NOTES
A	Initial release.

Safety Recommendations

For all procedures in this document, the use of appropriate personal protective equipment (PPE) is strongly recommended.

- Disposable gloves should always be worn when handling samples, reagents, fluidic chips, and any other materials that may encounter samples.
- Gloves should be changed immediately after any contact with the sample.

Inappropriate use of an Ionic® Purification System may cause personal injury or irreparable damage to the instrument.

- Only trained personnel should operate the Ionic system following published methods.
- All operators should review and be familiar with the Ionic Purification System User Guide.
- Only Bionano qualified service engineers should service the Ionic system.

Damage to the Ionic system caused by inappropriate use, neglect to perform required maintenance, or performing inappropriate maintenance may void warranty or require services not covered by standard service contract terms.

- Do not move the instrument while it is in operation.
- Do not unplug the instrument while it is in operation.
- Do not spill liquids on any area of the instrument.
- Do not use with flammable materials or in the presence of toxic fumes.
- Do not use excessive force to open or close the system cover.
- Use only with Ionic Fluidic Chips and associated kits and protocols.

Laboratory supervisors and/or facility managers must take the necessary precautions to ensure a safe workplace and appropriate training of personnel.

- All laboratory activities should be in accordance with all national, state, and local health and safety regulations.
- Follow all applicable SDS (or MSDS) recommendations for proper handling and disposal of chemicals and reagents.
- Follow all safety guidelines for use of personal protective equipment, laboratory devices, and labware established for the laboratory where the instrument is used.

Kit Contents

Table 1: Contents provided in the Ionic® FFPE to DNA Kit

Container	Item Name	Description	Volume	Quantity per Kit
-20ºC Reagents Box	Lysis Buffer 1	Lysis Buffer 1	1.35 mL	6
-20ºC Reagents Box	RNase	RNase A Reagent	120 µL	1
-20ºC Reagents Box	Proteinase K	Proteinase K Reagent	810 µL	1
-20ºC Reagents Box	Sample Buffer	Blank Sample Buffer	1.6 mL	1
RT Reagents Box	Lysis Buffer 2	Lysis Buffer 2	3.8 mL	1
RT Reagents Box	1 - Extraction Buffer	Extraction Buffer	12 mL	1
RT Reagents Box	2 - Anodic Buffer	Anodic Buffer	12 mL	1
RT Reagents Box	3 - Separation Buffer	Separation Buffer	18 mL	1
RT Reagents Box	4 - Neutralization Buffer	Neutralization Buffer	12 mL	1
RT Reagents Box	5 - Cathodic Buffer	Cathodic Buffer	12 mL	1
RT Reagents Box	Mineral Oil	Mineral Oil	15 mL	1
Fluidic Chip Set Box	Ionic Fluidic Chip	Fluidic Chips	N/A	6

Table 2: Reagents provided by the user.

User-supplied Reagents	For Lysate Preparation	For Purification
FFPE sections or scrolls	✓	

Table 3: Equipment provided by the user.

User-supplied Equipment (or equivalent)	For Lysate Preparation	For Purification
Razors/scalpels (if using slides)	✓	
12-column reservoir (Agilent 204365-100)		✓
P200 multichannel pipette		✓
P200 single channel pipette	✓	✓
P20 single channel pipette		✓
Microcentrifuge	✓	
Programmable ThermoMixer	✓	
Vortex mixer (adjustable speed)	✓	✓

Table 4: Labware provided by the user.

User-supplied Labware (or equivalent)	For Lysate Preparation	For Purification
DNA LoBind Tube, 1.5 mL (Eppendorf 22431021)	✓	✓
Optional: DNA LoBind Tube, 2.0 mL (Eppendorf 22431048)	✓	
Optional: DNA LoBind Plate, 96-well (Eppendorf 951032000)		✓

Protocol at a Glance

Lysate Preparation

1. Centrifuge FFPE tissue sample tubes for 2 minutes.

2. Add 300 µL of mineral oil.

3. Add 165 µL of Lysis Mix 1.

4. Incubate samples on ThermoMixer:
   - 65°C for 5 mins at 1000 rpm
   - 56°C for 1 hour at 1000 rpm
   - 70°C for 8 hours at 1000 rpm
   - Hold at 8°C

5. Centrifuge lysate tubes for 5 minutes.

6. Transfer 155 µL of lysate.

7. Add 65 µL of Lysis Mix 2.

DNA Purification

1. Prepare Purification Buffer Reservoir.

2. Place Ionic Fluidic Chip onto instrument.

3. Load Purification Buffers and prime Ionic Fluidic Chip.

4. Add 200 µL of each lysate to the chip.

5. Start purification run (~70 minutes).

6. Collect ~50 µL of each extract.

Sample Requirements

This protocol is intended for the extraction and purification of DNA from up to eight Formalin-Fixed Paraffin-Embedded (FFPE) tissue samples in parallel and is optimized to recover the maximum amount of DNA from a minimal amount of tissue. Bionano recommends a single 10 µm section of FFPE tissue (scroll or slide-mounted) with an area of 50–300 mm^2 for most downstream applications. For this protocol, a 10 µm section of FFPE tissue with dimensions of 1 inch x 1 inch (25.4 mm x 25.4 mm) and a mass of < 12 mg is typical.

The optimal amount of starting material will be informed by the FFPE tissue thickness, cross-sectional area, and cellularity. If a 10 µm section yields <1 µg of DNA with traditional kits (bead- or column-based), increasing the input FFPE tissue amount for DNA purification on the Ionic Purification System is acceptable. In such cases, this protocol can accommodate FFPE tissue sections totaling 30 µm in thickness (e.g., 3 x 10 µm or 6 x 5 µm). Care must be taken to avoid overloading since adding too much input FFPE tissue can lead to lower than expected DNA recovery (see **Figure 1**). A small pilot study is helpful in defining optimal input conditions as some FFPE tissue samples present challenges to extraction and purification requiring optimization beyond the standard protocol. Contact support@bionano.com for guidance on processing samples that do not meet the above requirements.

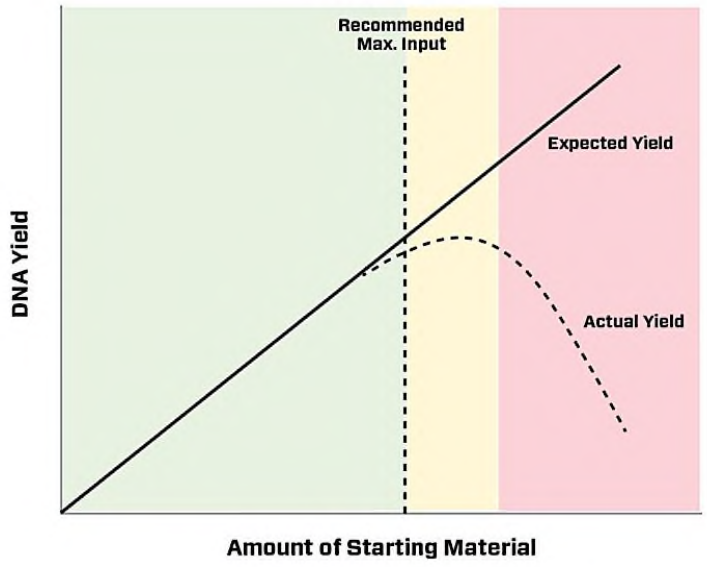

Figure 1. Impact of overloading

Lysis Procedure

Preparation of Lysates

1. Transfer each FFPE tissue section into a 1.5 mL LoBind Eppendorf tube.
2. Centrifuge tubes containing FFPE tissue sections at maximum speed (>10,000x g) for 2 minutes to move tissue to the bottom of the tube. Hold at room temperature.
3. Place one tube of Lysis Buffer 1 on the ThermoMixer and incubate at 56°C for 10 minutes at 1000 rpm. If a precipitate is present after incubation, place tube back on ThermoMixer until buffer is clear.
4. Vortex the Lysis Buffer 1 tube for 3 seconds, pulse spin and immediately proceed to the next step. Hold at room temperature.
5. Remove Proteinase K from the freezer, flick three times and pulse spin. Hold on ice.
6. To prepare Lysis Mix 1, add 135 µL of Proteinase K directly to the Lysis Buffer 1 tube. Vortex for 3 seconds, and pulse spin. Hold at room temperature.
7. Add 300 µL of mineral oil to each sample tube from Step 2.
8. Using a P200 pipette, add 165 µL of prepared Lysis Mix 1 to each sample tube.

   NOTE: If a precipitate is present in Lysis Mix 1, heat at 56°C until the solution is clear before adding to tubes. Do not vortex sample tube containing mineral oil and Lysis Mix 1.
9. Place sample tubes into a ThermoMixer and incubate using a program with the following steps:
   - Incubate at 65°C for 5 minutes at 1000 rpm.
   - Incubate at 56°C for 1 hour at 1000 rpm.
   - Incubate at 70°C for 8 hours at 1000 rpm.
   - Hold at 8°C.
10. After the program completes, remove tubes from the ThermoMixer and hold on the benchtop for 5 minutes. Set ThermoMixer to 20°C in preparation of the RNase treatment below.

    NOTE: If upper mineral oil layer is solidified after incubation in Step 10, place at 20°C or higher until mineral oil is no longer solid.
11. Centrifuge tubes at maximum speed (>10,000x g) for 5 minutes.

    NOTE: The mixture will separate into two phases with the nucleic acid-containing lysate in the lower phase.
12. Using a P200 pipette with the tip touching the bottom of the tube, slowly aspirate and transfer 155 µL of lysate from the lower phase of each tube into new microtubes avoiding any pelleted material that may be present. Hold lysate tubes on ice.

    NOTE: A minimal amount (~5-10 µL) of mineral oil may be aspirated during transfer of the lysate but will not impact the purification process. To preserve DNA integrity, proceed immediately to the next step.

    Alternatively, store lysate tubes at -20°C for up to seven days. Frozen lysates should be thawed on ice prior to proceeding to RNase treatment.

RNase Treatment

1. Remove RNase from the freezer, flick three times and pulse spin. Hold on ice.
2. To prepare Lysis Mix 2, combine 630 µL of Lysis Buffer 2 and 20 µL of RNase in a 1.5 mL tube. Invert Lysis Mix 2 ten times to mix and pulse spin. Hold on ice.
3. Add 65 µL of Lysis Mix 2 to each lysate tube from Step 12 of the Preparation of Lysates section above while on ice.
4. Invert lysate tubes ten times, pulse spin and incubate on a ThermoMixer at 20°C for 10 minutes at 300 rpm.
5. Vortex lysate tubes for 10 full seconds and pulse spin. Hold on ice for at least 5 minutes and proceed directly to Purification.

Purification

Prepare Buffer Reservoir

1. Label a 12-channel reservoir as shown in **Figure 2** skipping every other column to prevent purification buffer cross-contamination.

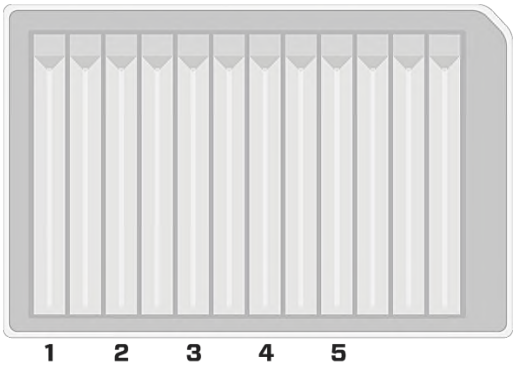

Figure 2. Reservoir column labels

2. Add purification buffers to each column according to **Table 5**.

Table 5. Reservoir buffers and volumes

Reservoir Column	Buffer from Kit	Volume
1	1 - Extraction Buffer	2.0 mL
2	2 - Anodic Buffer	2.0 mL
3	3 - Separation Buffer	3.0 mL
4	4 - Neutralization Buffer	2.0 mL
5	5 - Cathodic Buffer	2.0 mL

Setup Ionic Purification Run

1. From the Ionic Purification System start screen, press **Start** then select a User profile for the run.
2. Press the **G2 FFPE to DNA** button. The instrument cover will open.
3. When prompted, remove a fluidic chip from its packaging, handle it only by the side skirting, and place it on the instrument stage with the barcode on the right as shown in **Figure 3**. Gently apply pressure to all four corners of the fluidic chip simultaneously to confirm that the chip is fully seated on the stage. Press the **Arrow** on the right side of the screen to continue.

   NOTE: Remove fluidic chip from its packaging and place chip directly on the instrument stage to minimize accumulation of static electricity. Avoid contact with the top and bottom surfaces of the fluidics chip.

Figure 3. Proper handling and placement orientation of an Ionic fluidic chip.

4. Enter Run Name, Chip ID, and Reagent Lot and press the **Arrow** on the right side of the screen to continue.

   NOTES:

   - Chip ID is located on the label on the top surface of the Ionic fluidic chip. Include both the P/N and L/N for the chip:

   - Reagent Lot number is located on the G2 FFPE to DNA Kit room temperature box label.
   - Barcodes can be scanned into the software using a handheld barcode reader connected to the instrument USB port.

5. Using a P200 multichannel pipette and the proper pipetting technique shown in **Figure 4**, add the appropriate volume of each purification buffer as shown on the screen (also see **Figure 5**), working left to right (1 to 5), to the fluidic chip**.**

   NOTE: Do not remove the plastic film from the sample wells at this point as the fluidic chip will not prime correctly without these wells being covered.

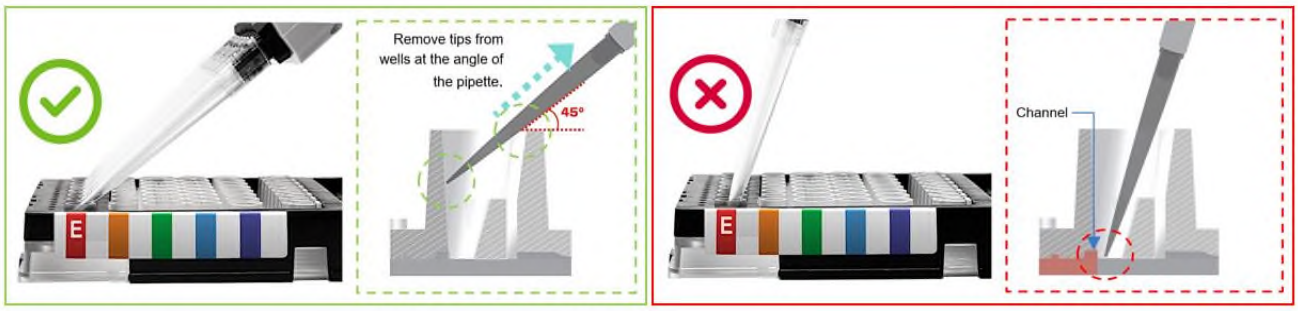

Figure 4. Proper (left) and improper (right) pipetting techniques for fluidic chip buffer wells.

   NOTE: Correct technique is important to ensure the buffers prime correctly into the fluidic chip.

   - Visually inspect tips to ensure each contains the same volume prior to adding to the fluidic chip.
   - Rest pipette tips on the top right of the wells and dispense against the left wall no more than halfway into the well with the pipette at an angle of ~45° relative to the chip surface.
   - Smoothly dispense at a steady speed to the first stop only then remove tips from the well by dragging the tips against the top right wall of the wells.

- Always avoid contact with the bottom of the wells when dispensing.
- Change tips after each dispense.

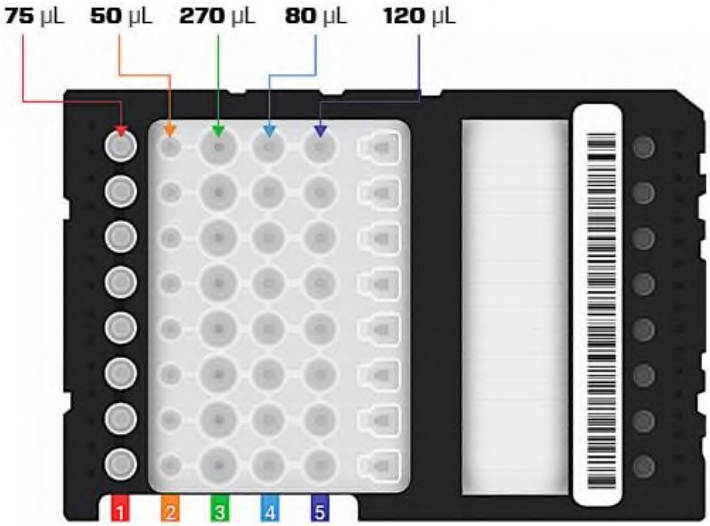

Figure 5. G2 FFPE To DNA purification buffer dispense volumes.

NOTE: The Separation Buffer should be added using two pipette transfers with fresh tips for each transfer.

6. After loading purification buffers press the **Arrow** on the right side of the screen to continue. The instrument cover will close and the fluidic chip will prime.

   NOTE: Priming takes approximately 4 minutes.

   IMPORTANT: Samples should be loaded within 10 minutes of priming completion.

7. During fluidic chip priming, vortex lysates for 5 seconds and pulse spin for 10 seconds. Hold on ice.
8. When priming is complete press the **Arrow** on the right side of the screen to continue. Press **OK** to confirm samples have been vortexed. The instrument cover will open.
9. Enter sample naming information (See Ionic User Manual for additional information on sample naming options) and press the **Arrow** on the right side of the screen to continue. Confirm run information is correct and press the **Arrow** on the right side of the screen to continue.
10. While firmly holding the chip by the side skirt, carefully remove the plastic film from the sample wells using the pull tab and press **OK** to continue (**Figure 6**).

Figure 6. Remove the plastic film from the sample wells.

11. Using a P200 pipette and keeping the tip in contact with the bottom of the tube, slowly aspirate and transfer 200 µL of lysate to each sample well.

    NOTE: Insert tip no more than halfway into the well at the position noted in **Figure 7** and smoothly dispense at a steady speed to the first stop only.

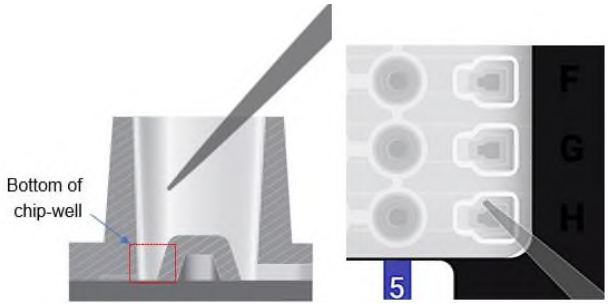

Figure 7. Proper Sample Well lysate loading positioning.

12. Press **Begin Run**. The instrument cover will close and the purification process will begin.

    NOTE: The G2 FFPE to DNA purification run will proceed for approximately 70 minutes and the instrument will indicate when the run has completed.

    IMPORTANT: extracts should be collected within one hour upon completion.

Collect DNA Extracts

1. Once the purification run has completed, click the **Arrow** on the right side of the screen to continue. The instrument cover will open.
2. Using a P200 pipette set to 60 µL, aspirate each purified DNA extract (~50 µL) by placing the tip at the *bottom* of the left wall of each Extraction Buffer well and transfer to a LoBind microcentrifuge tube or LoBind 96-well microplate.

    NOTE: As demonstrated in **Figure 7**, the Extraction Buffer well has a small ledge. Be sure to navigate the pipette tip to the left of the small ledge to reach the well bottom.

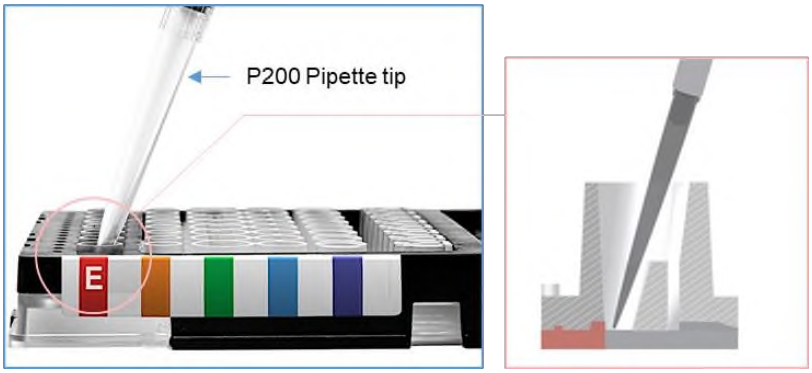

Figure 7. Aspirating the DNA extract from the Extraction Well

3. Using a P20 pipette set to 10 µL, aspirate any remaining DNA extract from the microchannel on the right side of the Extraction Buffer well and combine with the extract from Step 2. See **Figure 8**.

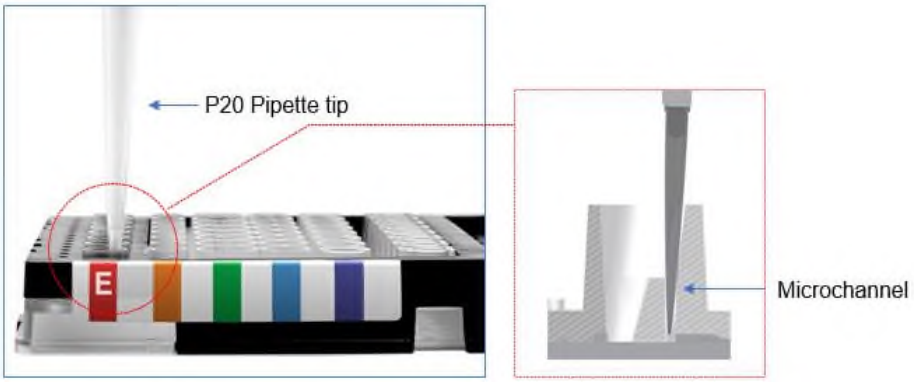

Figure 8. Recovery of any remaining DNA from the Extraction Well microchannel

4. Press the **Arrow** on the right side of the screen and confirm the extracts have been collected by clicking **OK**.
5. Remove the chip from the instrument stage and click the **Arrow** on the right side of the screen to continue. The instrument cover will close.
6. Press **Finish** to set up another purification run or return to the **Home** screen.
7. Vortex DNA extracts and pulse spin before using in downstream assays.

   NOTE: Store DNA extracts on ice for same-day use and -20°C for long term storage.

Troubleshooting

Ionic Purification System Feedback

The Ionic Purification System provides post-purification feedback for each sample lane as described in **Table 6**.

Table 6. Run-status icon description.

Run-status Icon	Description
	A green checkmark indicates a successful purification run for that lane. Collect samples by continuing to Step 3 below.
	A yellow warning indicates that a purification run abnormality has been detected for that lane. • Please collect the sample from the Extraction Buffer Well • If additional information is needed, please Save System Logs as described below and contact support@bionano.com
	An orange recycle icon indicates that the purification run for that lane did not initiate. • Please recover the sample from the Sample Input well as described below. • This sample is not lost and can be run on a new chip.

Recovery of Non-Initiated Lysate

If the Ionic system displays an orange recycle icon (shown above) upon completion of a purification run, follow the steps below to recover the non-initiated sample so it can be run on a new Ionic fluidic chip.

1. Before collecting non-initiated lysate(s), collect all purified extracts as described above in the Collect DNA Extracts section.
2. Remove the Ionic fluidic chip from the instrument stage and place on a level surface. Cover columns 1–5 with a plastic adhesive film leaving the Sample Well column uncovered as shown in **Figure 9**. Ensure a tight seal for each well.

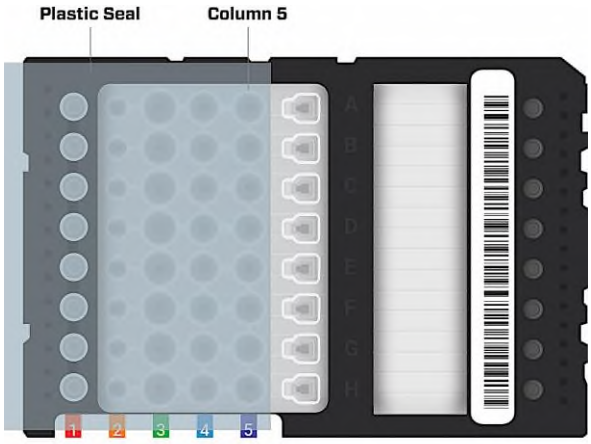

Figure 9. Proper plastic seal application for sample recovery

3. Set a 200 µL pipette to 200 µL. While using the thumb/forefinger to reinforce the seal on the well in Column 5, aspirate slowly from the bottom right side of the sample well of the non-initiated sample where the well and channel meet (see **Figure 10**) and transfer to a new microtube.

   NOTES: Typically, between 75–125 µL is expected to be recoverable from a non-initiated sample.

   - Aspirating from the bottom right side of the sample well allows lysate to be collected from the sample well, and potentially to the right, the sample channel.
   - Aspiration of fluid from the channel between the Cathodic Buffer (5) and the Sample Well should be avoided.
   - Ensuring a proper seal on the Cathodic Buffer (5) well will minimize the amount of buffer that is aspirated from the channel to the left of the sample well.

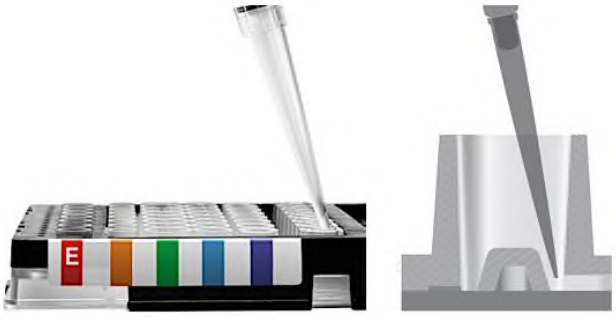

Figure 10. Sample recovery technique

4. Add a sufficient volume of FFPE Sample Buffer to the sample to bring the total volume to ~210 µL.
5. Store recovered samples at -20°C until they are ready to be re-run. Then, thaw recovered samples, vortex, spin down, place samples on ice and proceed directly to purification. When re-running the sample, always use the same protocol from the original Run.

If errors are encountered while running the instrument, follow the steps in Save System Logs and then email the log file to support@bionano.com. Support will be in contact within 48 hours to follow up.

Save System Logs

The **Save System Logs** maintenance screen is used to save system log files to a USB flash drive. Bionano Support can use the system log files to diagnose problems with the instrument.

NOTE: The USB flash drive must be in a FAT32 format. It is recommended to have a minimum of 1 GB of available space on the flash drive.

Cases where this function should be used:

- If the Run results are not as expected
- If the self-test fails
- If Support personnel request a system log

1. Press the **Save System Logs** button to save system log files to a USB device, as shown in **Figure 11**.

Figure 11. Save System Logs screen - Step 1

2. Insert a USB drive into the USB slot located at the bottom-right on the front of the instrument.
3. Once a valid USB drive is detected, the next screen is displayed, shown in **Figure 12**. The definitions in **Table 7** are helpful here. Press **Write** to begin the transfer.

Figure 12. Save System Logs screen - Step 2

Table 7. Save System Logs screen.

Call Out	Screen Component	Definition
1	Write button	A button to copy the system log to the USB drive inserted in the USB slot in the font of the instrument. The system log file is copied to the root directory of the USB drive
2	Help icon	Loads the Help screen

4. A status bar is displayed on the touchscreen. The status bar may seem inactive for larger log files. Wait for the system log to be saved to the USB flash drive.
5. After the system log file is saved to the USB flash drive, press the button on the left side to return to the **Maintenance & Service** screen.

Technical Assistance

For technical assistance, contact Bionano Genomics Technical Support.

You can retrieve documentation on Bionano products, SDS's, certificates of analysis, frequently asked questions, and other related documents from the Support website or by request through e-mail and telephone.

TYPE	CONTACT
Email	support@bionano.com
Phone	Hours of Operation: Monday through Friday, 9:00 a.m. to 5:00 p.m., PST US: +1 (858) 888-7663
Website	www.bionano.com/support
Address	Bionano Genomics, Inc. 9540 Towne Centre Drive, Suite 100 San Diego, CA 92121

Ionic G2 FFPE to RNA Kit Protocol

DOCUMENT NUMBER:
CG-00038

DOCUMENT REVISION:
B

Table of Contents

Legal Notice

For Research Use Only. Not for use in diagnostic procedures.

Revision History

REVISION	NOTES
A	Initial release.

Safety Recommendations

For all procedures in this document, the use of appropriate personal protective equipment (PPE) is strongly recommended.

- Disposable gloves should always be worn when handling samples, reagents, fluidic chips, and any other materials that may come in contact with samples.
- Gloves should be changed immediately after any contact with the sample.

Inappropriate use of an Ionic® Purification System may cause personal injury or irreparable damage to the instrument.

- Only trained personnel should operate the Ionic system following published methods.
- All operators should review and be familiar with the Ionic Purification System User Guide.
- Only Bionano qualified service engineers should service the Ionic system.

Damage to the Ionic system caused by inappropriate use, neglect to perform required maintenance, or performing inappropriate maintenance may void warranty or require services not covered by standard service contract terms.

- Do not move the instrument while it is in operation.
- Do not unplug the instrument while it is in operation.
- Do not spill liquids on any area of the instrument.
- Do not use with flammable materials or in the presence of toxic fumes.
- Do not use excessive force to open or close the system cover.
- Use only with Ionic Fluidic Chips and associated kits and protocols.

Laboratory supervisors and/or facility managers must take the necessary precautions to ensure a safe workplace and appropriate training of personnel.

- All laboratory activities should be in accordance with all national, state, and local health and safety regulations.
- Follow all applicable SDS (or MSDS) recommendations for proper handling and disposal of chemicals and reagents.
- Follow all safety guidelines for use of personal protective equipment, laboratory devices, and labware established for the laboratory where the instrument is used.

Kit Contents

Table 1: Contents provided in the Ionic® FFPE to Pure RNA Kit

Container	Item Name	Description	Volume	Quantity per Kit
-20ºC Reagents Box	Lysis Buffer 1	Lysis Buffer 1	1.35 mL	6
-20ºC Reagents Box	DNase I	DNase I Reagent	120 µL	1
-20ºC Reagents Box	Proteinase K	Proteinase K Reagent	810 µL	1
-20ºC Reagents Box	Sample Buffer	Blank Sample Buffer	1.6 mL	1
RT Reagents Box	Lysis Buffer 2	Lysis Buffer 2	3.8 mL	1
RT Reagents Box	1 - Extraction Buffer	Extraction Buffer	12 mL	1
RT Reagents Box	2 - Anodic Buffer	Anodic Buffer	12 mL	1
RT Reagents Box	3 - Separation Buffer	Separation Buffer	18 mL	1
RT Reagents Box	4 - Neutralization Buffer	Neutralization Buffer	12 mL	1
RT Reagents Box	5 - Cathodic Buffer	Cathodic Buffer	12 mL	1
RT Reagents Box	Mineral Oil	Mineral Oil	15 mL	1
Fluidic Chip Set Box	Ionic Fluidic Chip	Fluidic Chips	N/A	6

Table 2: Reagents provided by the user

User-supplied Reagents	For Lysate Preparation	For Purification
FFPE sections or scrolls	✓	

Table 3: Equipment provided by the user

User-supplied Equipment (or equivalent)	For Lysate Preparation	For Purification
Razors/scalpels (if using slides)	✓	
12-column reservoir (Agilent 204365-100)		✓
P200 multichannel pipette		✓
P200 single channel pipette	✓	✓
P20 single channel pipette		✓
Microcentrifuge	✓	
Programmable ThermoMixer	✓	
Vortex mixer (adjustable speed)	✓	✓

Table 4: Labware provided by the user

User-supplied Labware (or equivalent)	For Lysate Preparation	For Purification
DNA LoBind Tube, 1.5 mL (Eppendorf 22431021)	✓	✓
Optional: DNA LoBind Tube, 2.0 mL (Eppendorf 22431048)	✓	
Optional: DNA LoBind Plate, 96-well (Eppendorf 951032000)		✓

Protocol at a Glance

Lysate Preparation

1. Centrifuge FFPE tissue sample tubes for 2 minutes.
2. Add 300 µL of mineral oil.
3. Add 165 µL of Lysis Mix 1.
4. Incubate samples on ThermoMixer:
   - 65°C for 5 mins at 1000 rpm
   - 60°C for 1 hour at 500 rpm
   - 70°C for 1 hour at 0 rpm
5. Centrifuge lysate tubes for 5 minutes.
6. Transfer 155 µL of lysate.
7. Add 65 µL of Lysis Mix 2.

RNA Purification

1. Prepare Purification Buffer Reservoir.
2. Place Ionic Fluidic Chip onto instrument.
3. Load Purification Buffers and prime Ionic Fluidic Chip.
4. Add 200 µL of each lysate to the chip.
5. Start purification run (~70 minutes).
6. Collect ~50 µL of each extract.

Sample Requirements

This protocol is intended for the extraction and purification of RNA from up to eight Formalin-Fixed Paraffin-Embedded (FFPE) tissue samples in parallel and is optimized to recover the maximum amount of RNA from a minimal amount of tissue. Bionano recommends a single 10 µm section of FFPE tissue (scroll or slide-mounted) with an area of 50–300 mm2 for most downstream applications. For this protocol, a 10 µm section of FFPE tissue with dimensions of 1 inch x 1 inch (25.4 mm x 25.4 mm) and a mass of < 12 mg is typical.

The optimal amount of starting material will be informed by the FFPE tissue thickness, cross-sectional area, and cellularity. If a 10 µm section yields <1 µg of RNA with traditional kits (bead- or column-based), increasing the input FFPE tissue amount for RNA purification on the Ionic Purification System is acceptable. In such cases, this protocol can accommodate FFPE tissue sections totaling 20 µm in thickness (e.g., 2 x 10 µm or 4 x 5 µm). Care must be taken to avoid overloading since adding too much input FFPE tissue can lead to lower than expected RNA recovery (see Figure 1). A small pilot study is helpful in defining optimal input conditions as some FFPE tissue samples present challenges to extraction and purification requiring optimization beyond the standard protocol. Contact support@bionano.com for guidance on processing samples that do not meet the above requirements.

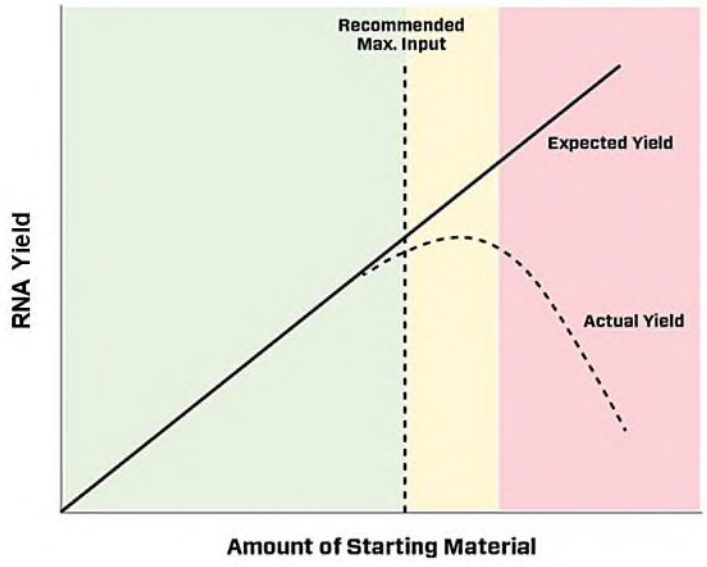

Figure 1. Impact of overloading

Lysis Procedure

Preparation of Lysates

1. Transfer each FFPE tissue section into a 1.5 mL LoBind Eppendorf tube.
2. Centrifuge tubes containing FFPE tissue sections at maximum speed (>10,000x g) for 2 minutes to move tissue to the bottom of the tube. Hold at room temperature.
3. Place one tube of Lysis Buffer 1 on the ThermoMixer and incubate at 56°C for 10 minutes at 1000 rpm. If a precipitate is present after incubation, place tube back on ThermoMixer until buffer is clear.
4. Vortex the Lysis Buffer 1 tube for 3 seconds, pulse spin and immediately proceed to the next step. Hold at room temperature.
5. Remove Proteinase K from the freezer, flick three times and pulse spin. Hold on ice.
6. To prepare Lysis Mix 1, add 135 µL of Proteinase K directly to the Lysis Buffer 1 tube. Vortex for 3 seconds and pulse spin. Hold at room temperature.
7. Add 300 µL of mineral oil to each sample tube from Step 2.
8. Using a P200 pipette, add 165 µL of prepared Lysis Mix 1 to each sample tube.

   NOTE: If a precipitate is present in Lysis Mix 1, heat at 56°C until the solution is clear before adding to tubes. Do not vortex sample tube containing mineral oil and Lysis Mix 1.
9. Place sample tubes into a ThermoMixer and incubate using a program with the following steps:
   - Incubate at 65°C for 5 minutes at 1000 rpm.
   - Incubate at 60°C for 1 hour at 500 rpm.
   - Incubate at 70°C for 1 hour at 0 rpm.
10. After the program completes, remove tubes from the ThermoMixer and hold on the benchtop for 5 minutes. Set ThermoMixer to 20°C in preparation of the DNase treatment below.

    NOTE: If upper mineral oil layer is solidified after incubation in Step 10, place at 20°C or higher until mineral oil is no longer solid.
11. Centrifuge tubes at maximum speed (>10,000x g) for 5 minutes.

    NOTE: The mixture will separate into two phases with the nucleic acid-containing lysate in the lower phase.
12. Using a P200 pipette with the tip touching the bottom of the tube, slowly aspirate and transfer 155 µL of lysate from the lower phase of each tube into new microtubes avoiding any pelleted material that may be present. Hold lysate tubes on ice.

    NOTE: A minimal amount (~5-10 µL) of mineral oil may be aspirated during transfer of the lysate and does not impact the purification process. To preserve RNA integrity, proceed immediately to the next step.

    Alternatively, store lysate tubes at -20°C for up to 7 days. Frozen lysates should be thawed on ice prior to proceeding to DNase treatment.

DNase Treatment

1. Remove DNase I from the freezer, flick three times and pulse spin. Hold on ice.
2. To prepare Lysis Mix 2, combine 630 µL of Lysis Buffer 2 and 20 µL of DNase I in a 1.5 mL tube. Invert Lysis Mix 2 ten times to mix and pulse spin. Hold on ice.

3. Add 65 µL of Lysis Mix 2 to each lysate tube from Step 12 of the Preparation of Lysates section above while on ice.
4. Invert lysate tubes ten times, pulse spin and incubate on a ThermoMixer at 20°C for 10 minutes at 300 rpm.
5. Vortex lysate tubes for 10 full seconds and pulse spin. Hold on ice for at least 5 minutes and proceed directly to Purification.

Purification

Prepare Buffer Reservoir

1. Label a 12-channel reservoir as shown in **Figure 2**, skipping every other column to prevent purification buffer cross-contamination.

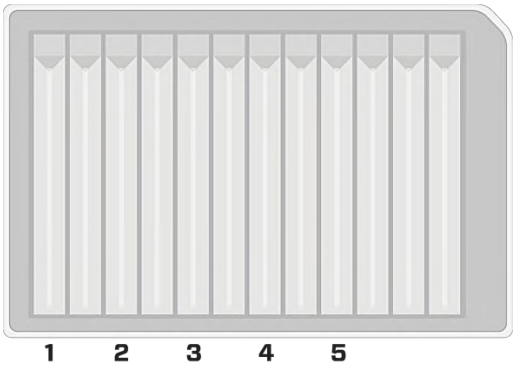

Figure 2. Reservoir column labels

2. Add purification buffers to each column according to **Table 5**.

Table 5. Reservoir buffers and volumes

Reservoir Column	Buffer from Kit	Volume
1	1 - Extraction Buffer	2.0 mL
2	2 - Anodic Buffer	2.0 mL
3	3 - Separation Buffer	3.0 mL
4	4 - Neutralization Buffer	2.0 mL
5	5 - Cathodic Buffer	2.0 mL

Setup Ionic Purification Run

1. From the **Ionic Purification System** start screen, press **Start** then select a User profile for the run.
2. Press the **G2 FFPE to RNA** button. The instrument cover will open.
3. When prompted, remove a fluidic chip from its packaging, handle only by the side skirting, and place it on the instrument stage with the barcode on the right as shown in **Figure 3**. Gently apply pressure to all four corners of the fluidic chip simultaneously to confirm that the chip is fully seated on the stage. Press the **Arrow** on the right side of the screen to continue.

   NOTE: Remove fluidic chip from its packaging and place chip directly on the instrument stage to minimize accumulation of static electricity. Avoid contact with the top and bottom surfaces of the fluidics chip.

Figure 3. Proper handling and placement orientation of an Ionic fluidic chip.

4. Enter Run Name, Chip ID, and Reagent Lot and press the **Arrow** on the right side of the screen to continue.

   NOTES:

   - Chip ID is located on the label on the top surface of the Ionic fluidic chip. Include both the P/N and L/N for the chip:

     P/N: S0223 Ionic® Fluidic Chip
     L/N: P12345560001

   - Reagent Lot number is located on the G2 FFPE to RNA Kit room temperature box label.
   - Barcodes can be scanned into the software using a handheld barcode reader connected to the instrument USB port.

5. Using a P200 multichannel pipette and the proper pipetting technique shown in **Figure 4**, add the appropriate volume of each purification buffer as shown on the screen (also see **Figure 5**), working left to right (1 to 5), to the fluidic chip**.**

   NOTE: Do *not* remove the plastic film from the sample wells at this point as the fluidic chip will not prime correctly without these wells being covered.

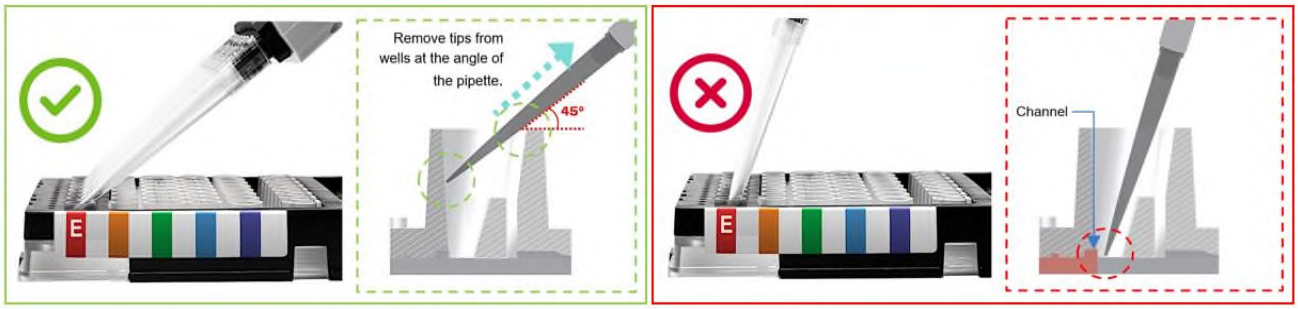

Figure 4. Proper (left) and improper (right) pipetting techniques for fluidic chip buffer wells.

   NOTE: Correct technique is important to ensure the buffers prime correctly into the fluidic chip.

   - Visually inspect tips to ensure each contains the same volume prior to adding to the fluidic chip.
   - Rest pipette tips on the top right of the wells and dispense against the left wall no more than halfway into the well with the pipette at an angle of ~45° relative to the chip surface.
   - Smoothly dispense at a steady speed to the first stop only, then remove tips from the well by dragging the tips against the top right wall of the wells.

- Always avoid contact with the bottom of the wells when dispensing.
- Change tips after each dispense.

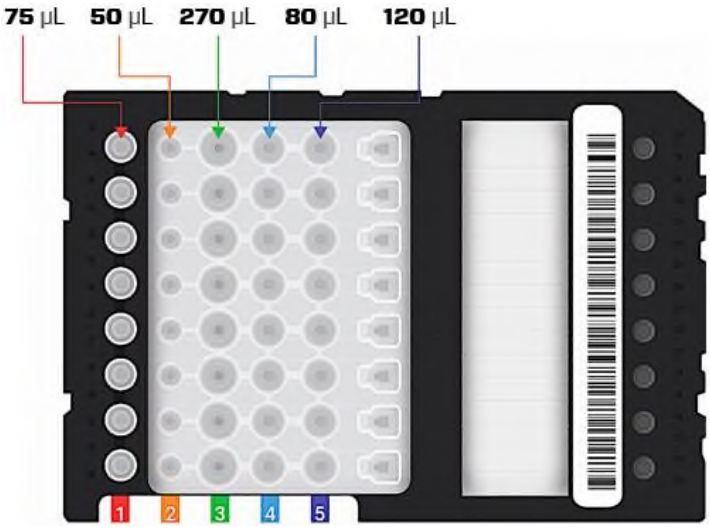

Figure 5. G2 FFPE To RNA purification buffer dispense volumes.

NOTE: The Separation Buffer should be added using two pipette transfers with fresh tips for each transfer.

6. After loading purification buffers press the **Arrow** on the right side of the screen to continue. The instrument cover will close and the fluidic chip will prime.

   NOTE: Priming takes approximately 4 minutes.

   IMPORTANT: Samples should be loaded within 10 minutes of priming completion.

7. During fluidic chip priming, vortex lysates for 5 seconds and pulse spin for 10 seconds. Hold on ice.
8. When priming is complete press the **Arrow** on the right side of the screen to continue. Press **OK** to confirm samples have been vortexed. The instrument cover will open.
9. Enter sample naming information (See Ionic User Manual for additional information on sample naming options) and press the **Arrow** on the right side of the screen to continue. Confirm run information is correct and press the **Arrow** on the right side of the screen to continue.
10. While firmly holding the chip by the side skirt, carefully remove the plastic film from the sample wells using the pull tab and press **OK** to continue (**Figure 6**).

Figure 6. Remove the plastic film from the sample wells

11. Using a P200 pipette and keeping the tip in contact with the bottom of the tube, slowly aspirate and transfer 200 µL of lysate to each sample well.

    NOTE: Insert tip no more than halfway into the well at the position noted in **Figure 7** and smoothly dispense at a steady speed to the first stop only.

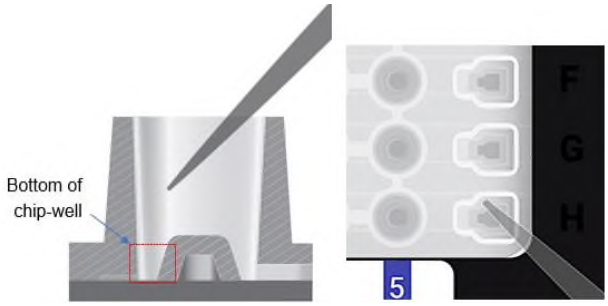

Figure 7. Proper Sample Well lysate loading positioning

12. Press **Begin Run**. The instrument cover will close, and the purification process will begin.

    NOTE: The G2 FFPE to RNA purification run will proceed for approximately 70 minutes and the instrument will indicate when the run has completed. **IMPORTANT**: extracts should be collected within one hour upon completion.

Collect RNA Extracts

1. Once the purification run has completed, click the **Arrow** on the right side of the screen to continue. The instrument cover will open.
2. Using a P200 pipette set to 60 µL, aspirate each purified RNA extract (~50 µL) by placing the tip at the *bottom* of the left wall of each Extraction Buffer well and transfer to a LoBind microcentrifuge tube or LoBind 96-well microplate.

    NOTE: As demonstrated in **Figure 7**, the Extraction Buffer well has a small ledge. Be sure to navigate the pipette tip to the left of the small ledge to reach the well bottom.

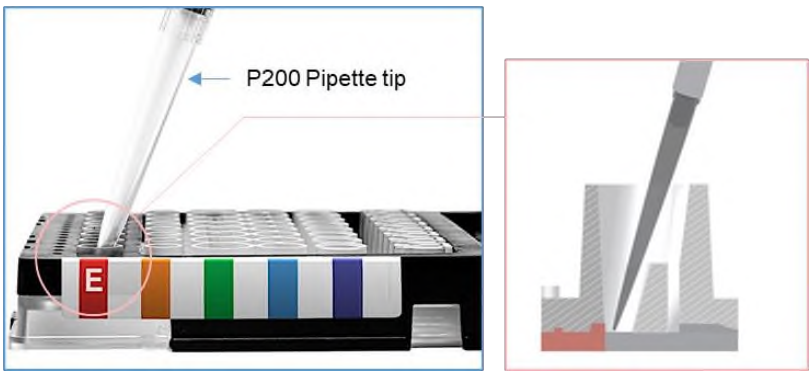

Figure 7. Aspirating the RNA extract from the Extraction Well

3. Using a P20 pipette set to 10 µL, aspirate any remaining RNA extract from the microchannel on the right side of the Extraction Buffer well and combine with the extract from Step 2. See **Figure 8**.

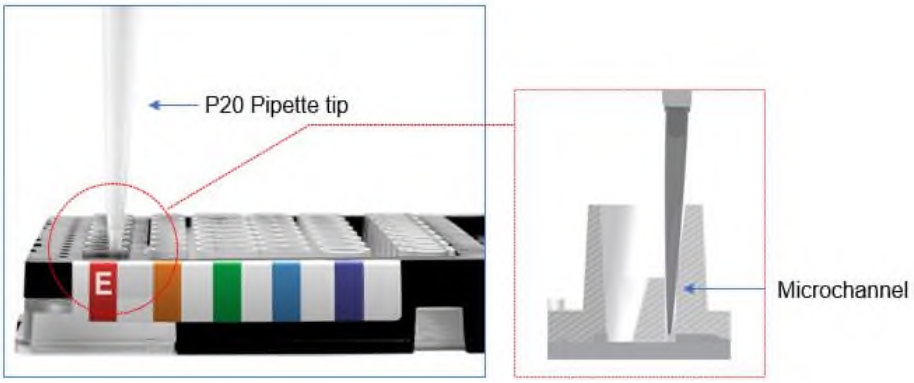

Figure 8. Recovery of any remaining RNA from the Extraction Well microchannel

4. Press the **Arrow** on the right side of the screen and confirm the extracts have been collected by clicking **OK**.
5. Remove the chip from the instrument stage and click the **Arrow** on the right side of the screen to continue. The instrument cover will close.
6. Press **Finish** to set up another purification run or return to the Home screen.
7. Vortex RNA extracts and pulse spin before using in downstream assays.

   NOTE: Store RNA extracts on ice for same-day use and -80°C for long term storage.

Troubleshooting

Ionic Purification System Feedback

The Ionic Purification System provides post-purification feedback for each sample lane as described in **Table 6**.

Table 6. Run-status icon description.

Run-status Icon	Description
	A green checkmark indicates a successful purification run for that lane. Collect samples by continuing to Step 3 below.
	A yellow warning indicates that a purification run abnormality has been detected for that lane. • Please collect the sample from the Extraction Buffer Well • If additional information is needed, please Save System Logs as described below and contact support@bionano.com
	An orange recycle icon indicates that the purification run for that lane did not initiate. • Please recover the sample from the Sample Input well as described below. • This sample is not lost and can be run on a new chip.

Recovery of Non-Initiated Lysate

If the Ionic system displays an orange recycle icon (shown above) upon completion of a purification run, follow the steps below to recover the non-initiated sample so it can be run on a new Ionic fluidic chip.

1. Before collecting non-initiated lysate(s), collect all purified extracts as described above in the Collect RNA Extracts section.
2. Remove the Ionic fluidic chip from the instrument stage and place on a level surface. Cover columns 1–5 with a plastic adhesive film leaving the Sample Well column uncovered as shown in **Figure 9**. Ensure a tight seal for each well.

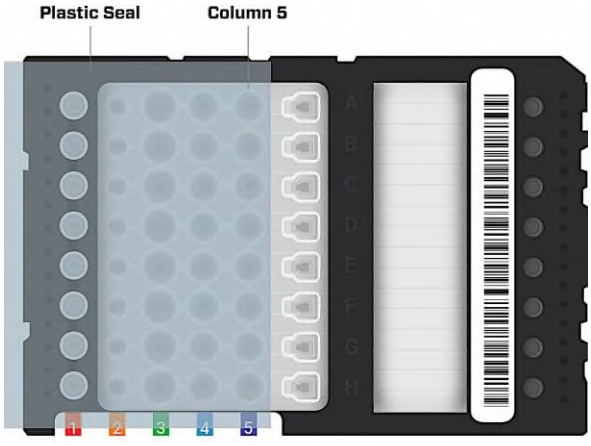

Figure 9. Proper plastic seal application for sample recovery

3. Set a 200 µL pipette to 200 µL. While using the thumb/forefinger to reinforce the seal on the well in Column 5, aspirate slowly from the bottom right side of the Sample Well of the non-initiated sample where the well and channel meet (see **Figure 10**) and transfer to a new microtube.

   NOTES: Typically, between 75–125 µL is expected to be recoverable from a non-initiated sample.

   - Aspirating from the bottom right side of the sample well allows lysate to be collected from the sample well, and potentially to the right, the sample channel.
   - Avoid Aspiration of fluid from the channel between the Cathodic Buffer (5) and the Sample Well
   - Ensure a proper seal on the Cathodic Buffer (5) well will minimize the amount of buffer that is aspirated from the channel to the left of the sample well.

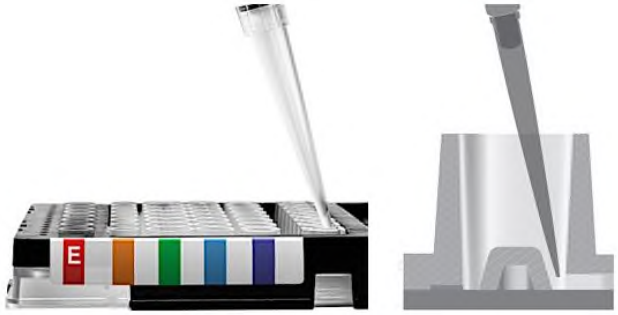

Figure 10. Sample recovery technique

4. Add a sufficient volume of FFPE Sample Buffer to the sample to bring the total volume to ~210 µL.
5. Store recovered samples at -20°C until they are ready to be re-run. Then thaw recovered samples, vortex, spin down, place samples on ice and proceed directly to purification. When re-running the sample, always use the same protocol from the original Run.

If errors are encountered while running the instrument, follow the steps in Save System Logs and then email the log file to support@bionano.com. Support will be in contact within 48 hours to follow up.

Save System Logs

The **Save System Logs** maintenance screen is used to save system log files to a USB flash drive. The system log files can be used by Bionano Support to diagnose problems with the instrument.

NOTE: The USB flash drive must be in a FAT32 format. It is recommended to have a minimum of 1 GB of available space on the flash drive**.**

Cases where this function should be used:

- If the Run results are not as expected
- If the self-test fails
- If Support personnel request a system log

1. Press the **Save System Logs** button to save system log files to a USB device, as shown in **Figure 11**.

Figure 11. Save System Logs screen - Step 1

2. Insert a USB drive into the USB slot located at the bottom-right on the front of the instrument.
3. Once a valid USB drive is detected, the next screen is displayed, shown in **Figure 12**. The definitions in **Table 7** are helpful here. Press **Write** to begin the transfer.

Figure 12. Save System Logs screen - Step 2

Table 7. Save System Logs screen.

Call Out	Screen Component	Definition
1	Write button	A button to copy the system log to the USB drive inserted in the USB slot in the font of the instrument. The system log file is copied to the root directory of the USB drive
2	Help icon	Loads the Help screen

4. A status bar is displayed on the touchscreen. The status bar may seem inactive for larger log files. Wait for the system log to be saved to the USB flash drive.
5. After the system log file is saved to the USB flash drive, press the button on the left side to return to the Maintenance & Service screen.

Technical Assistance

For technical assistance, contact Bionano Genomics Technical Support.

You can retrieve documentation on Bionano products, SDS's, certificates of analysis, frequently asked questions, and other related documents from the Support website or by request through e-mail and telephone.

TYPE	CONTACT
Email	support@bionano.com
Phone	Hours of Operation: Monday through Friday, 9:00 a.m. to 5:00 p.m., PST US: +1 (858) 888-7663
Website	www.bionano.com/support
Address	Bionano Genomics, Inc. 9540 Towne Centre Drive, Suite 100 San Diego, CA 92121

Ionic G2 FFPE Complete Kit Protocol

DOCUMENT NUMBER:
CG-00039

DOCUMENT REVISION:
B

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Revision History

REVISION	NOTES
A	Initial release

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For all procedures in this document, the use of appropriate personal protective equipment (PPE) is strongly recommended.

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- All operators should review and be familiar with the *Ionic Purification System User Guide*.
- Only Bionano qualified service engineers should service the Ionic system.

Damage to the Ionic system caused by inappropriate use, neglect to perform required maintenance, or performing inappropriate maintenance may void warranty or require services not covered by standard service contract terms.

- Do not move the instrument while it is in operation.
- Do not unplug the instrument while it is in operation.
- Do not spill liquids in any area of the instrument.
- Do not use with flammable materials or in the presence of toxic fumes.
- Do not use excessive force to open or close the system cover.
- Use only with Ionic Fluidic Chips and associated kits and protocols.

Laboratory supervisors and/or facility managers must take the necessary precautions to ensure a safe workplace and appropriate training of personnel.

- All laboratory activities should be in accordance with all national, state, and local health and safety regulations.
- Follow all applicable SDS (or MSDS) recommendations for proper handling and disposal of chemicals and reagents.
- Follow all safety guidelines for use of personal protective equipment, laboratory devices, and labware established for the laboratory where the instrument is used.

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Table 1: Contents provided in the Ionic® FFPE to Pure RNA Kit

Container	Item Name	Description	Volume	Quantity per Kit
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-20ºC Reagents Box	DNase I	DNase I Reagent	120 µL	1
-20ºC Reagents Box	RNase	RNase A Reagent	120 µL	1
-20ºC Reagents Box	Sample Buffer	Blank Sample Buffer	1.6 mL	1
RT Reagents Box	Lysis Buffer 2	Lysis Buffer 2	7.6 mL	1
RT Reagents Box	1 - Extraction Buffer	Extraction Buffer	24 mL	1
RT Reagents Box	2 - Anodic Buffer	Anodic Buffer	24 mL	1
RT Reagents Box	3 - Separation Buffer	Separation Buffer	18 mL	2
RT Reagents Box	4 - Neutralization Buffer	Neutralization Buffer	24 mL	1
RT Reagents Box	5 - Cathodic Buffer	Cathodic Buffer	24 mL	1
RT Reagents Box	Mineral Oil	Mineral Oil	15 mL	1
Fluidic Chip Set Box	Ionic Fluidic Chip	Fluidic Chips	N/A	12

Table 2: Reagents provided by the user.

User-supplied Reagents	For Lysate Preparation	For Purification
FFPE sections or scrolls	✓	

Table 3: Equipment provided by the user.

User-supplied Equipment (or equivalent)	For Lysate Preparation	For Purification
Razors/scalpels (if using slides)	✓	
12-column reservoir (Agilent 204365-100)		✓
P200 multichannel pipette		✓
P200 single channel pipette	✓	✓
P20 single channel pipette		✓
Microcentrifuge	✓	
Programmable ThermoMixer	✓	
Vortex mixer (adjustable speed)	✓	✓

Table 4: Labware provided by the user.

User-supplied Labware (or equivalent)	For Lysate Preparation	For Purification
DNA LoBind Tube, 1.5 mL (Eppendorf 22431021)	✓	✓
Optional: DNA LoBind Tube, 2.0 mL (Eppendorf 22431048)	✓	
Optional: DNA LoBind Plate, 96-well (Eppendorf 951032000)		✓

Protocol at a Glance

Lysate Preparation

	1. **Centrifuge FFPE tissue sample tubes for 2 minutes**
	2. Add 300 µL of mineral oil
	3. Prepare Lysis Mix 1 and add 325 µL to each sample
	4. Incubate samples on ThermoMixer. • 65°C for 5 mins at 1000 rpm • 60°C for 1 hour at 500 rpm • 70°C for 1 hour at 0 rpm
	5. Transfer 155 µL of lysate for RNA processing into a separate tube then proceed with RNA purification
	6. Incubate remaining lysate for DNA processing containing mineral oil on Thermomixer then proceed to DNA purification. • 70°C for 7 hours at 1000 rpm • Hold at 8°C

RNA Purification

	1. Prepare RNA Lysis Mix 2 and add 65 µL to each tube for RNA purification.
	2. Incubate on ThermoMixer • 20°C for 10 mins at 300 rpm
	3. Prepare Purification Buffer Reservoir
	3. Place Ionic Fluidic Chip onto instrument
	4. Load Purification Buffers and prime Ionic Fluidic Chip
	6. Add 200 µL of each lysate to the chip
	7. Start purification run (~70 minutes)
	8. Collect ~50 µL of each extract

DNA Purification

	1. Centrifuge tubes for DNA processing for 5 minutes and transfer lysates to new tubes
	2. Prepare DNA Lysis Mix 2 and add 65 µL to each tube
	3. Incubate on ThermoMixer • 20°C for 10 mins at 300 rpm
	4. Prepare Purification Buffer Reservoir
	5. Place Ionic Fluidic Chip onto instrument
	6. Load Purification Buffers and prime Ionic Fluidic Chip
	7. Add 200 µL of each lysate to the chip
	8. Start purification run (~70 minutes)
	9. Collect ~50 µL of each extract

Sample Requirements

This protocol is intended for the independent extraction and purification of RNA and DNA from up to eight Formalin-Fixed Paraffin-Embedded (FFPE) tissue samples in parallel and is optimized to recover the maximum amount of RNA and DNA from a minimal amount of tissue. Bionano recommends a single 10 µm section of FFPE tissue (scroll or slide-mounted) with an area of 50–300 mm^2 for most downstream applications. For this protocol, a 10 µm section of FFPE tissue with dimensions of 1 inch x 1 inch (25.4 mm x 25.4 mm) and a mass of < 12 mg is typical.

The optimal amount of starting material will be informed by the FFPE tissue thickness, cross-sectional area, and cellularity. If a 10 µm section yields <1 µg of RNA or DNA with traditional kits (bead- or column-based), increasing the input FFPE tissue amount for RNA purification on the Ionic Purification System is acceptable. In such cases, this protocol can accommodate FFPE tissue sections totaling 20 µm in thickness (e.g., 2 x 10 µm or 4 x 5 µm). Care must be taken to avoid overloading since adding too much input FFPE tissue can lead to lower than expected RNA or DNA recovery (see **Figure 1**). A small pilot study is helpful in defining optimal input conditions as some FFPE tissue samples present challenges to extraction and purification requiring optimization beyond the standard protocol. Contact support@bionano.com for guidance on processing samples that do not meet the above requirements.

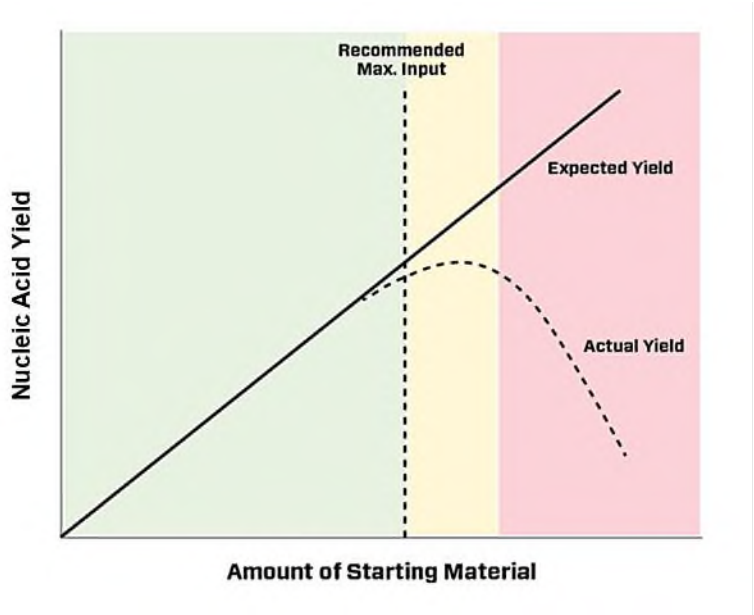

Figure 1. Impact of overloading

Lysis Procedure

Overview of the Approach

The Ionic FFPE Complete Kit provides DNA and RNA from the same starting FFPE sample by splitting the sample after lysis into two tubes, digesting the non-target nucleic acid, and then purifying the lysates on the Ionic Purification System. A summary of this procedure is shown in **Figure 2**.

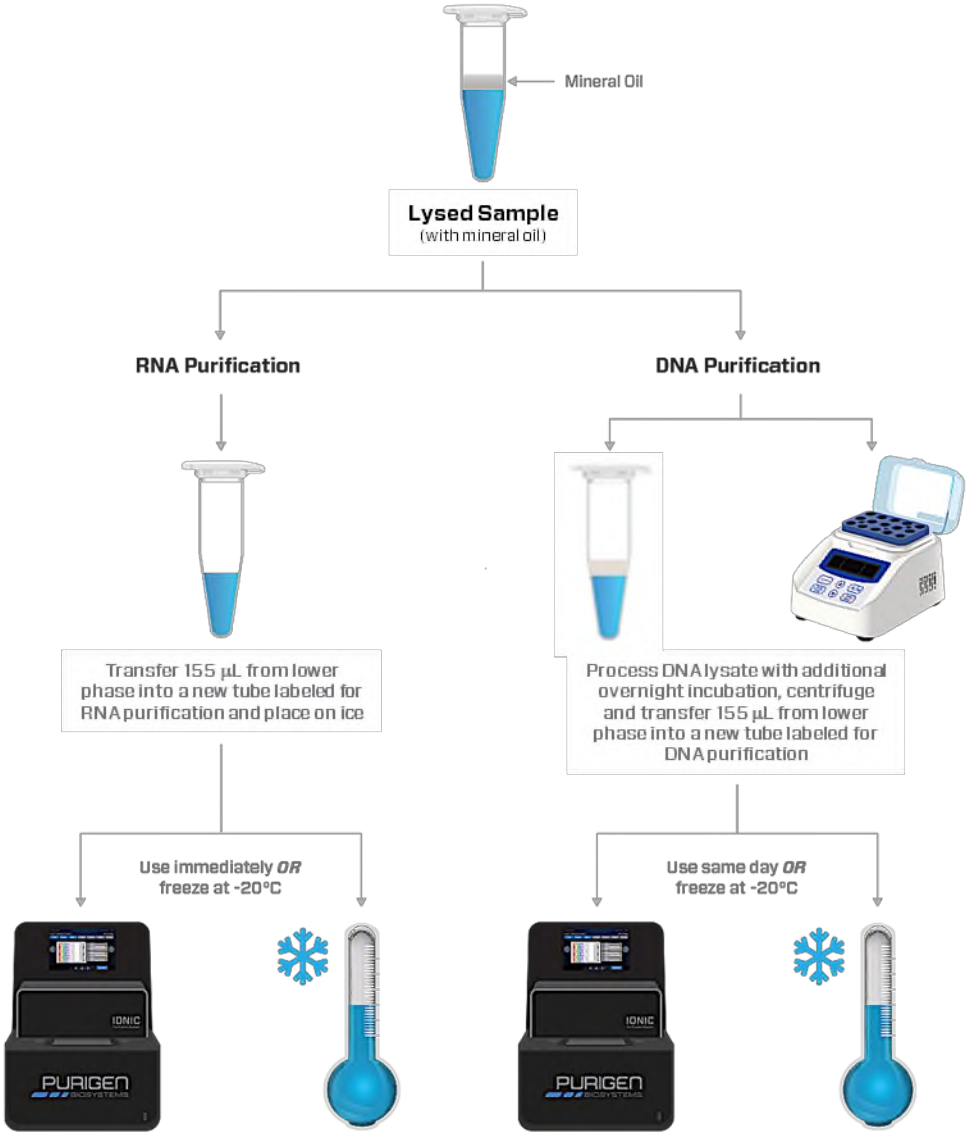

Figure 2. Overview of purification approach and pause points for the FFPE Complete Kit

Preparation of Lysates

1. Transfer each FFPE tissue section into a 1.5 mL LoBind Eppendorf tube.
2. Centrifuge tubes containing FFPE tissue sections at maximum speed (>10,000x g) for 2 minutes to move tissue to the bottom of the tube. Hold at room temperature.
3. Place two tubes of Lysis Buffer 1 on the ThermoMixer and incubate at 56°C for 10 minutes at 1000 rpm. If a precipitate is present after incubation, place tube back on ThermoMixer until buffer is clear.
4. Vortex the Lysis Buffer 1 tubes for 3 seconds, pulse spin and immediately proceed to the next step. Hold at room temperature.
5. Remove Proteinase K from the freezer, flick three times and pulse spin. Hold on ice.
6. To prepare Lysis Mix 1, add 135 µL of Proteinase K directly to each Lysis Buffer 1 tube. Vortex for 3 seconds and pulse spin. Hold at room temperature.
7. Add 300 µL of mineral oil to each sample tube from Step 2.
8. Using a P200 pipette, add 325 µL of prepared Lysis Mix 1 to each sample tube with two 162.5 µL transfers. Change tips between each dispense.

   NOTE: If a precipitate is present in Lysis Mix 1, heat at 56°C until the solution is clear before adding to tubes. Do not vortex sample tube containing mineral oil and Lysis Mix 1.
9. Place sample tubes into a ThermoMixer and incubate using a program with the following steps:
   - Incubate at 65°C for 5 minutes at 1000 rpm.
   - Incubate at 60°C for 1 hour at 500 rpm.
   - Incubate at 70°C for 1 hour at 0 rpm.
10. After the program is completed, remove tubes from the ThermoMixer, pulse spin, and hold on the benchtop for 5 minutes. Set ThermoMixer to 20°C in preparation of the DNase treatment below.

    NOTE: The mixture will separate into two phases with the nucleic acid-containing lysate in the lower phase.
11. With a P200 pipette set to 155 µL, gently pipette mix the lower nucleic acid-containing lysate 10 times.
12. Aspirate 155 µL of lysate and transfer to a new tube for RNA processing. Hold these RNA lysates on ice.

    NOTE: To preserve RNA integrity, proceed immediately to DNase treatment. Alternatively, store lysate tubes at -20°C for up to 7 days. Frozen lysates must be thawed on ice prior to proceeding to DNase treatment.
13. Hold tubes containing mineral oil and the remaining lysate from Step 11 at room temperature for DNA processing. Proceed with the Processing DNA Lysates section below immediately after the RNA lysates have completed the DNase treatment and the ThermoMixer is available.

DNase Treatment of RNA Lysates

1. Remove DNase I from the freezer, flick three times and pulse spin. Hold on ice.
2. To prepare ‘RNA Lysis Mix 2’, combine 630 µL of Lysis Buffer 2 and 20 µL of DNase I in a 1.5 mL tube. Invert ‘Lysis Mix 2’ ten times to mix and pulse spin. Hold on ice.
3. Add 65 µL of ‘RNA Lysis Mix 2’ to each lysate tube for RNA processing from Step 12 of the Preparation of Lysates section above while on ice.
4. Invert lysate tubes ten times, pulse spin and incubate on a ThermoMixer at 20°C for 10 minutes at 300 rpm.
5. Vortex lysate tubes for 10 full seconds and pulse spin. Hold on ice for at least 5 minutes and proceed directly to Purification.

Processing DNA Lysates

1. Place lysates for DNA processing from Step 13 of the Preparation of Lysates section above into a ThermoMixer and incubate using a program with the following steps:
   - Incubate at 70°C for 7 hours at 1000 rpm.
   - Hold at 8°C.
2. After the program is completed, remove tubes from ThermoMixer and hold on the benchtop for 5 minutes. Set ThermoMixer to 20°C in preparation of the RNase treatment below.

   NOTE: If mineral oil layer is solidified, place at 20°C or higher until mineral oil is no longer solid.
3. Centrifuge tubes at maximum speed (>10,000x g) for 5 minutes.

   NOTE: The mixture will separate into two phases with the nucleic acid-containing lysate in the lower phase. If mineral oil layer is solidified, place at 20°C or higher until mineral oil is no longer solid.
4. Using a P200 pipette with the tip touching the bottom of the tube, slowly aspirate and transfer 155 µL of lysate from the lower phase of each tube into new microtubes avoiding any pelleted material that may be present. Hold lysate tubes on ice.

   NOTE: A minimal amount (~5-10 µL) of mineral oil may be aspirated during transfer of the lysate and does not impact the purification process.

   To preserve DNA integrity, proceed immediately to the next step. Alternatively, store lysate tubes at -20°C for up to 7 days. Frozen lysates should be thawed on ice prior to proceeding to RNase treatment.

RNase Treatment of DNA Lysates

1. Remove RNase from the freezer, flick three times and pulse spin. Hold on ice.
2. To prepare DNA Lysis Mix 2, combine 630 µL of Lysis Buffer 2 and 20 µL of RNase in a 1.5 mL tube. Invert Lysis Mix 2 ten times to mix and pulse spin. Hold on ice.
3. Add 65 µL of DNA Lysis Mix 2 to each lysate tube for DNA processing from Step 4 of the Processing DNA Lysates section above while on ice.
4. Invert lysate tubes ten times, pulse spin and incubate on a ThermoMixer at 20°C for 10 minutes at 300 rpm.
5. Vortex lysate tubes for 10 full seconds and pulse spin. Hold on ice for at least 5 minutes and proceed directly to Purification.

Purification

Prepare Buffer Reservoir

1. Label a 12-channel reservoir as shown in **Figure 2** skipping every other column to prevent purification buffer cross-contamination.

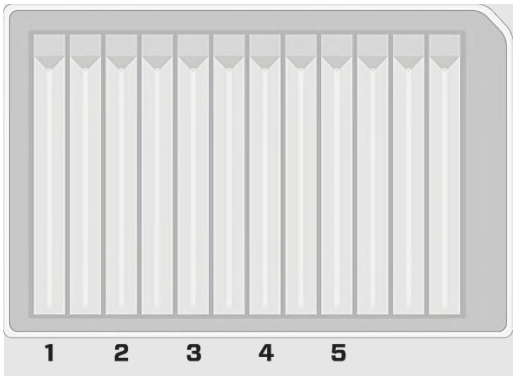

Figure 2. Reservoir column labels

2. Add purification buffers to each column according to **Table 5**.

Table 5. Reservoir buffers and volumes

Reservoir Column	Buffer from Kit	Volume
1	**1 - Extraction Buffer**	2.0 mL
2	**2 - Anodic Buffer**	2.0 mL
3	**3 - Separation Buffer**	3.0 mL
4	**4 - Neutralization Buffer**	2.0 mL
5	**5 - Cathodic Buffer**	2.0 mL

Set Up Ionic Purification Run

1. From the Ionic **Purification System** start screen, press **Start** then select a User profile for the run.
2. Press the **G2 FFPE Complete** button. The instrument cover will open.
3. When prompted, remove a fluidic chip from its packaging, handling only by the side skirting, and place it on the instrument stage with the barcode on the right as shown in **Figure 3**. Gently apply pressure to all four corners of the fluidic chip simultaneously to confirm that the chip is fully seated on the stage. Press the **Arrow** on the right side of the screen to continue.

   NOTE: Remove fluidic chip from its packaging and place chip directly on the instrument stage to minimize accumulation of static electricity. Avoid contact with the top and bottom surfaces of the fluidic chip.

Figure 3. Proper handling and placement orientation of an Ionic fluidic chip.

4. Enter Run Name, Chip ID, and Reagent Lot and press the **Arrow** on the right side of the screen to continue.

   NOTES:

   - Chip ID is located on the label on the top surface of the Ionic fluidic chip: Include both the P/N and L/N for the chip:

   - Reagent Lot number is located on the G2 FFPE to RNA Kit room temperature box label.
   - Barcodes can be scanned into the software using a handheld barcode reader connected to the instrument USB port.

5. Using a P200 multichannel pipette and the proper pipetting technique shown in **Figure 4**, add the appropriate volume of each purification buffer as shown on the screen (also see **Figure 5**), working left to right (1 to 5), to the fluidic chip**.**

   NOTE: Do not remove the plastic film from the sample wells at this point as the fluidic chip will not prime correctly without these wells covered

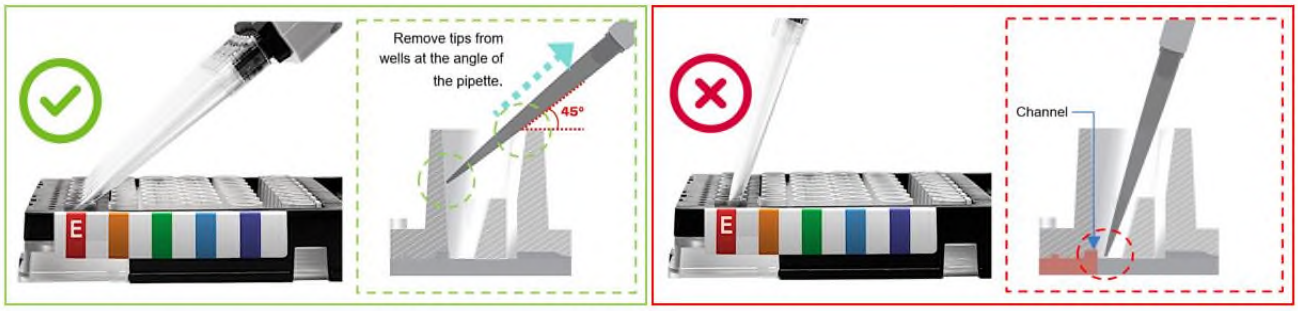

Figure 4. Proper (left) and improper (right) pipetting techniques for fluidic chip buffer wells.

   NOTE: Correct technique is important to ensure the buffers prime correctly into the fluidic chip

   - Visually inspect tips to ensure each contains the same volume prior to adding to the fluidic chip.
   - Rest pipette tips on the top right of the wells and dispense against the left wall no more than halfway into the well with the pipette at an angle of ~45° relative to the chip surface.
   - Smoothly dispense at a steady speed to the first stop only then remove tips from the well by dragging the tips against the top right wall of the wells.
   - Always avoid contact with the bottom of the wells when dispensing.

- Change tips after each dispense.

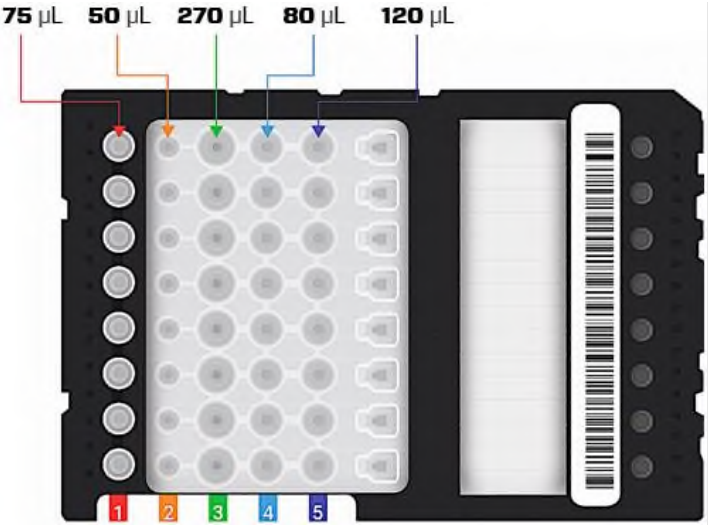

Figure 5. G2 FFPE To RNA purification buffer dispense volumes.

NOTE: The Separation Buffer should be added using two pipette transfers with fresh tips for each transfer.

6. After loading purification buffers press the **Arrow** on the right side of the screen to continue. The instrument cover will close, and the fluidic chip will prime.

   NOTE: Priming takes approximately 4 minutes.

   NOTE: Samples should be loaded within 10 minutes of priming completion.

7. During fluidic chip priming, vortex lysates for 5 seconds and pulse spin for 10 seconds. Hold on ice.
8. When priming is complete press the **Arrow** on the right side of the screen to continue. Press **OK** to confirm samples have been vortexed. The instrument cover will open.
9. Enter sample naming information (See Ionic User Manual for additional information on sample naming options) and press the **Arrow** on the right side of the screen to continue. Confirm run information is correct and press the **Arrow** on the right side of the screen to continue.
10. While firmly holding the chip by the side skirt, carefully remove the plastic film from the sample wells using the pull tab and press **OK** to continue (**Figure 6**).

Figure 6. Remove the plastic film from the sample wells.

11. Using a P200 pipette and keeping the tip in contact with the bottom of the tube, slowly aspirate and transfer 200 µL of lysate to each sample well.

    NOTE: Insert tip no more than halfway into the well at the position noted in **Figure 7** and smoothly dispense at a steady speed to the first stop only.

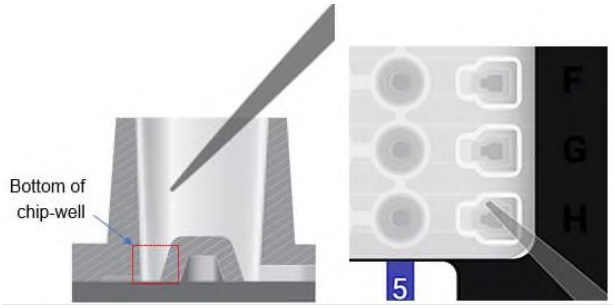

Figure 7. Proper Sample Well lysate loading positioning.

12. Press **Begin Run**. The instrument cover will close and the purification process will begin.

    NOTE: The G2 FFPE Complete purification run will proceed for approximately 70 minutes and the instrument will indicate when the run has completed.

    NOTE: Upon completion, extracts should be collected within one hour.

Collect RNA or DNA Extracts

1. Once the purification run has completed, click the **Arrow** on the right side of the screen to continue. The instrument cover will open.
2. Using a P200 pipette set to 60 µL, aspirate each purified RNA extract (~50 µL) by placing the tip at the *bottom* of the left wall of each Extraction Buffer well and transfer to a LoBind microcentrifuge tube or LoBind 96-well microplate.

    NOTE: As demonstrated in **Figure 7**, the Extraction Buffer well has a small ledge. Be sure to navigate the pipette tip to the left of the small ledge to reach the well bottom.

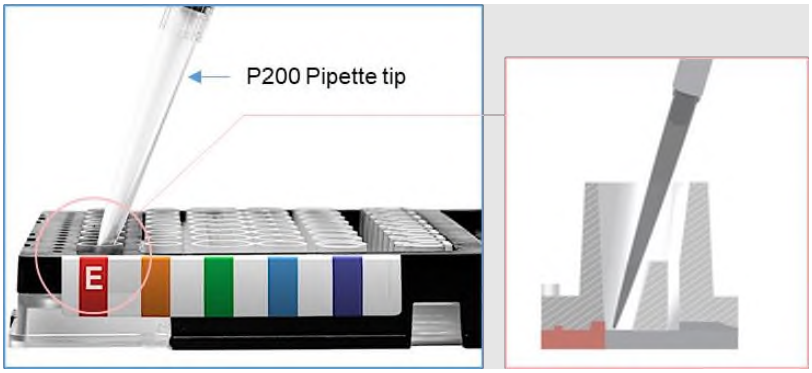

Figure 7. Aspirating the RNA extract from the Extraction Well

3. Using a P20 pipette set to 10 µL, aspirate any remaining RNA or DNA extract from the microchannel on the right side of the Extraction Buffer well and combine with the extract from Step 2. See **Figure 8**.

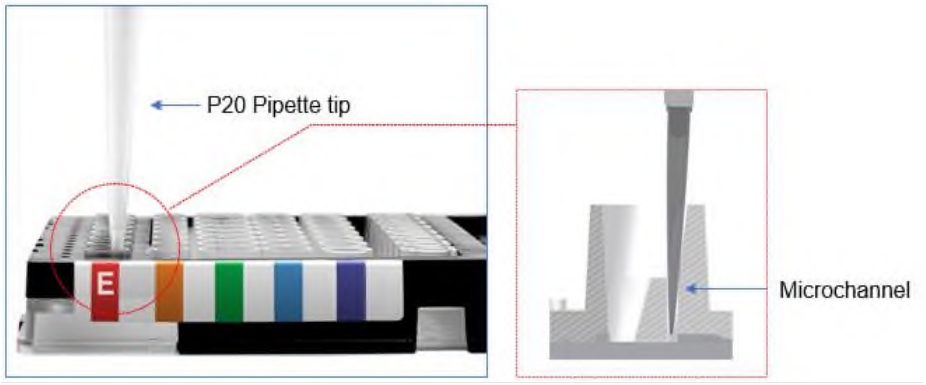

Figure 8. Recovery of any remaining RNA from the Extraction Well microchannel

4. Press the **Arrow** on the right side of the screen and confirm the extracts have been collected by clicking **OK**.
5. Remove the chip from the instrument stage and click the **Arrow** on the right side of the screen to continue. The instrument cover will close.
6. Press **Finish** to set up another purification run or return to the **Home** screen.
7. Vortex RNA or DNA extracts and pulse spin before using in downstream assays.

   NOTES: Store RNA extracts on ice for same-day use and -80°C for long term storage.

   Store DNA extracts on ice for same-day use and -20°C for long term storage.

Troubleshooting

Ionic Purification System Feedback

The Ionic Purification System provides post-purification feedback for each sample lane as described in **Table 6**.

Table 6. Run-Status icon description.

Run-status Icon	Description
	A green checkmark indicates a successful purification run for that lane. Collect samples by continuing to Step 3 below.
	A yellow warning indicates that a purification run abnormality has been detected for that lane. • Please collect the sample from the Extraction Buffer Well • If additional information is needed, please Save System Logs as described below and contact support@bionano.com
	An orange recycle icon indicates that the purification run for that lane did not initiate. • Please recover the sample from the Sample Input well as described below. • This sample is not lost and can be run on a new chip.

Recovery of Non-Initiated Lysate

If the Ionic system displays an orange recycle icon upon completion of a purification run, follow the steps below to recover the non-initiated sample so it can be run on a new Ionic fluidic chip.

1. Before collecting non-initiated lysate(s), collect all purified extracts as described above in the Collect RNA or DNA Extracts section.
2. Remove the Ionic fluidic chip from the instrument stage and place on a level surface. Cover columns 1–5 with a plastic adhesive film leaving the Sample Well column uncovered as shown in **Figure 9**.
3. Ensure a tight seal for each well.

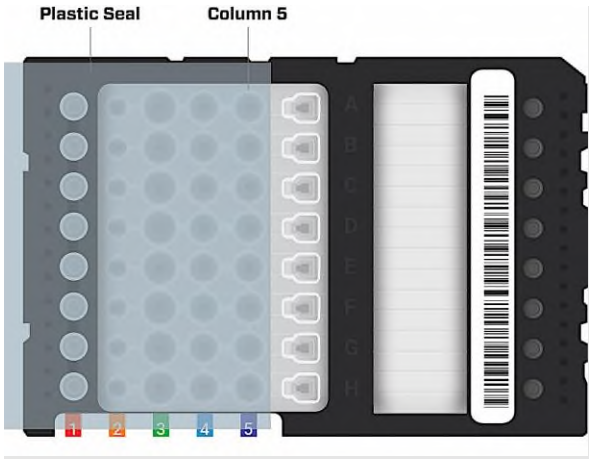

Figure 9. Proper plastic seal application for sample recovery

4. Set a 200 µL pipette to 200 µL. While using the thumb/forefinger to reinforce the seal on the well in Column 5, aspirate slowly from the bottom right side of the Sample Well of the non-initiated sample where the well and channel meet (see **Figure 10**) and transfer to a new microtube.

   NOTES: Typically, between 75–125 µL is expected to be recoverable from a non-initiated sample.

   - Aspirating from the bottom right side of the sample well allows lysate to be collected from the sample well, and potentially to the right, the sample channel.
   - Avoid Aspiration of fluid from the channel between the Cathodic Buffer (5) and the Sample Well.
   - Ensuring a proper seal on the Cathodic Buffer (5) well will minimize the amount of buffer that is aspirated from the channel to the left of the sample well.

Figure 10. Sample recovery technique

5. Add a sufficient volume of FFPE Sample Buffer to the sample to bring the total volume to ~210 µL.
6. Store recovered samples at -20°C until they are ready to be re-run. Then, thaw recovered samples, vortex, spin down, place samples on ice and proceed directly to purification. When re-running the sample, always use the same protocol from the original Run.

If errors are encountered while running the instrument, follow the steps in Save System Logs and then email the log file to support@bionano.com. Support will be in contact within 48 hours to follow up.

Save System Logs

The **Save System Logs** maintenance screen is used to save system log files to a USB flash drive. Bionano Support can use the system log files to diagnose problems with the instrument.

NOTE: The USB flash drive must be in a FAT32 format. It is recommended to have a minimum of 1 GB of available space on the flash drive.

Cases where this function should be used:

- If the Run results are not as expected
- If the self-test fails
- If Support personnel request a system log

1. Press the **Save System Logs** button to save system log files to a USB device, as shown in **Figure 11**.

Figure 11. Save System Logs screen - Step 1

2. Insert a USB drive into the USB slot located at the bottom-right on the front of the instrument.
3. Once a valid USB drive is detected, the next screen is displayed, shown in **Figure 12**. The definitions in **Table 7** are helpful here. Press **Write** to begin the transfer.

Figure 12. Save System Logs screen - Step 2

Table 7. Save System Logs screen.

Call Out	Screen Component	Definition
1	Write button	A button to copy the system log to the USB drive inserted in the USB slot in the font of the instrument. The system log file is copied to the root directory of the USB drive
2	**Help** icon	Loads the **Help** screen

4. A status bar is displayed on the touchscreen. The status bar may seem inactive for larger log files. Wait for the system log to be saved to the USB flash drive.
5. After the system log file is saved to the USB flash drive, press the button on the left side to return to the **Maintenance & Service** screen.

Technical Assistance

For technical assistance, contact Bionano Genomics Technical Support.

You can retrieve documentation on Bionano products, SDS's, certificates of analysis, frequently asked questions, and other related documents from the Support website or by request through e-mail and telephone.

TYPE	CONTACT
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Address	Bionano Genomics, Inc. 9540 Towne Centre Drive, Suite 100 San Diego, CA 92121