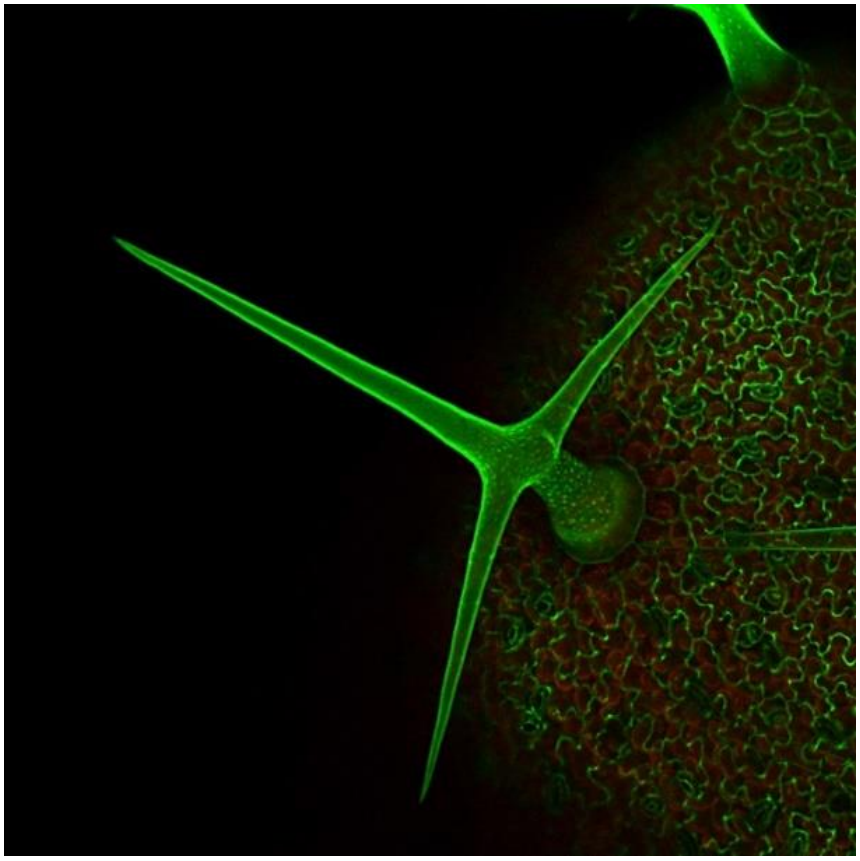
Plante-ører: Undersøgelse af trichomers rolle i planters lydperception



Projekt Forskerspirer 2024	
Titel	Plante-ører: Undersøgelse af trichomers rolle i planters lydperception
Identifikationskode	VN210
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1 Indholdsfortegnelse

2 Indledning

Det er længe blevet afvist, at planter kan høre og reagere på lyd, fordi de ikke har et oplagt organ specialiseret til hørelse.[1] Nu eksisterer der en tiltagende mængde evidens for, at planter kan 'høre', både naturlige lyde som bibrummen og unaturlige monotone lyde, og reagere på det med f.eks. produktion af sødere nektar, styrkning af deres forsvarssystemer, øget vækst og hurtigere spiring af frø.[2,3,4] Alligevel vides det stadig ikke, hvordan planters lydperception virker. Mennesker har ører, myrer har følehorn, slanger har et lydfølsomt kæbeben. Men hvad har planter?

Jeg synes, at planters lydperception er et vildt spændende emne, fordi det udfordrer den udbredte forståelse af dem som passive væsner. De er ikke passive, da de kan percipere og endda skelne mellem forskellige lyde fra deres omgivelser.[5] Vi har bare ikke fundet ud af hvordan.

[1] (Demey, Mishra, & Van Der Straeten, 2023)
[2] (Veits, et al., 2019)
[3] (Ghosh, et al., 2016)
[4] (Hassanien, Tian-zhen, Yu-feng, & Bao-Ming, 2014)
[5] (Veits, et al., 2019)

3 Problemformulering og afgrænsning

Det er allerede lykkedes forskere at identificere flere nøgleaktører i vejen fra lydbølger i luften til reaktion i planten.[6] Det vides dog endnu ikke med sikkerhed, hvordan lydbølger opfanges af planterne. Jeg vil i mit forskerspireprojekt undersøge om trichomer, en hår-agtig udvækst på planters overflade, er de første modtagere af lydbølger i planters lydperception: Strukturerne, der omdanner lydbølgerne til et signal, der kan føres videre gennem planten. Dette vil jeg gøre med afsæt i følgende problemformulering:

Hvordan påvirker manglen på eller misdannelsen af trichomer *Arabidopsis thalianas* lydperception?

Planten *Arabidopsis thaliana*, eller almindelig gåsemad på dansk, anvendes i projektet som modelorganisme, da det er den oftest anvendte modelorganisme inden for plantebiologi.[7] Derfor kan jeg bedre bygge videre på eksisterende forskning, og praktiske dele af mit projekt, såsom anskaffelse af specifikke mutanter, bliver også lettere.

Af hensyn til projektets omfang vil jeg kun tage udgangspunkt i *A.thaliana*'s reaktion på en enkelt type lyd. Det er naturligvis ikke sikkert, at trichomer spiller en lige stor rolle i perceptionen af alle lyde, men resultaterne vil stadig forbedre vores forståelse af planters lydperception.

[6] (Demey, Mishra, & Van Der Straeten, 2023)
[7] (Krämer, 2015)

4 Teori

I de følgende underafsnit gennemgås teori, der er relevant for at kunne forstå mit forsøgsdesign og metodevalg.

4.1 Forskingsfeltet og hypotesen om trichomers rolle i lydperception

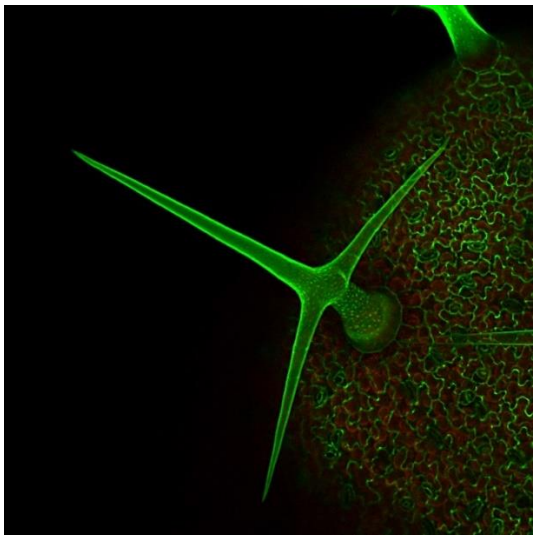
Flere potentielle aktører i vejen fra lydbølge i luften til reaktion i planten er blevet identificeret. Disse inkluderer K^+, der ved lydeksponering strømmer ud af planteceller, og sekundære budbringere som Ca^{2+}-ioner og reactive oxygen species (ROS), hvis koncentrationer i plantecellen øges ved lydeksponering.[8,9] Derudover er diverse familier af membrankanaler og overfladereceptorer blevet foreslået som mulige aktører, men disses involvering i lydopfattelsesprocessen er endnu ikke blevet påvist.[10] Ligeledes vides det heller ikke med sikkerhed, hvordan lyd kan aktivere disse overfladereceptorer.[11]

Trichomer er håragtige en- eller flercellede vedhæng, der findes på de fleste planters overflade.[12] Deres form og størrelse varierer meget mellem plantearter,[13] men ser hos *A.thaliana* ud som vist på figur 1. Et tidligere studie har vist, at trichomer fungerer som mekanoreceptorer, der åbner for membrankanaler ved fysisk berøring.[14] Da lydbølger, ligesom fysisk berøring, også er en mekanisk stimulus, tyder dette på, at trichomer også spiller en rolle i lydperception. Derudover resonerer *A.thaliana*-trichomer med tyggelyde fra *Pieris rapae*-larver.[15] Da vibrationer fra tyggende *P.rapae*-larver fører til en respons i *A.thaliana*,[16] tyder dette ligeledes på, at trichomer spiller en rolle i lydperception.

[8] (Ye, et al., 2023)
[9] (Mishra, Ghosh, & Bae, 2016)
[10] (Demey, Mishra, & Van Der Straeten, 2023)
[11] (Demey, Mishra, & Van Der Straeten, 2023)
[12] (Johnson, 1975)
[13] (Johnson, 1975)
[14] (Zhou, et al., 2016)
[15] (Liu, et al., 2017)
[16] (Appel & Cocroft, 2014)

Fordi antallet af trichomer varierer blandt plantearter og planteorganer, er det blevet foreslået, at trichomer forstærker reaktionen på lyd uden at være nødvendige for lydperception.[17] Lyde med høj intensitet vil i højere grad kunne vibrere hele planteorganer som f.eks. blade. Det er derfor muligt, at trichomer fungerer som 'antenner', der grundet deres lave masse og naturlige frekvens kan forstærke signalet fra lavintensitetslyde, der ellers kun ville have en meget lille effekt.

Hadj-Amor et al. har i et preprint fra maj 2024, der endnu ikke er gennemgået peer review, vist, at mangel på trichomer ikke påvirker planters evne til at percipere lyd.[18] Denne undersøgelse blev dog udført med 100 dB monoton lyd, hvilket er meget højt sammenlignet med de fleste naturlige lyde. Studiet modbeviser derfor ikke førnævnte hypotese, da resultatet kan forklares af den høje lydintensitet, der muligvis kan fremkalde en respons i planter uanset trichomintegritet. Derudover adskiller mit forsøg sig også metodemæssigt fra det udført af Hadj-Amor et al., da de anvendte svampeinfektion som stresskilde, mens jeg vil anvende herbivorbehandling (mere om dette i afsnit 5). Mit projekt er derfor stadig relevant.



Figur 1: Billede af et A.thaliana*-trichom taget med 600x zoom på et konfokalmikroskop.*[19]

[17] (Demey, Mishra, & Van Der Straeten, 2023)
[18] (Hadj-Amor, et al., 2024)
[19] (Runions, 2003)

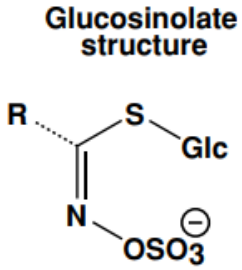
4.2 Priming

I nogle tilfælde kommer påvirkningen af lyd og vibrationer kun til udtryk som en forstærket reaktion på en anden stimulus; et fænomen kaldet priming. Et eksempel på dette er når *A.thaliana* udsættes for tyggevibrationer fra *P.rapae*. Vibrationerne alene fører ikke til nogen stigning i koncetrationen af forsvarsstoffer i plantens blade.[20] Udsættes *A.thaliana* derimod for et herbivorangreb umiddelbart efter vibrationerne, ses der en større stigning i koncentrationen af forsvarsstoffer i bladene, end hvis planten ikke var blevet udsat for vibrationer.[21]

Det er stadig ikke helt forstået hvordan priming virker på et molekylært niveau. Der er dog rimelig stor enighed om, at det involverer epigenetiske ændringer såsom DNA methylering og modifikation af histoner, hvilket påvirker udtrykkelse af gener.[22,23]

4.3 Glucosinolater

Glucosinolater er en familie af metabolitter produceret af blandt andet *A.thaliana*.[24] De spiller en rolle i planters forsvar, da de ved herbivorangreb hydroliseres til stoffer, der er giftige for mange herbivorer.[25] Glucosinolaters molekylære struktur er karakteriseret ved en negativ sulfatgruppe (figur 2), hvilket kan udnyttes når glucosinolater skal isoleres fra andre stoffer (afsnit 4.2).

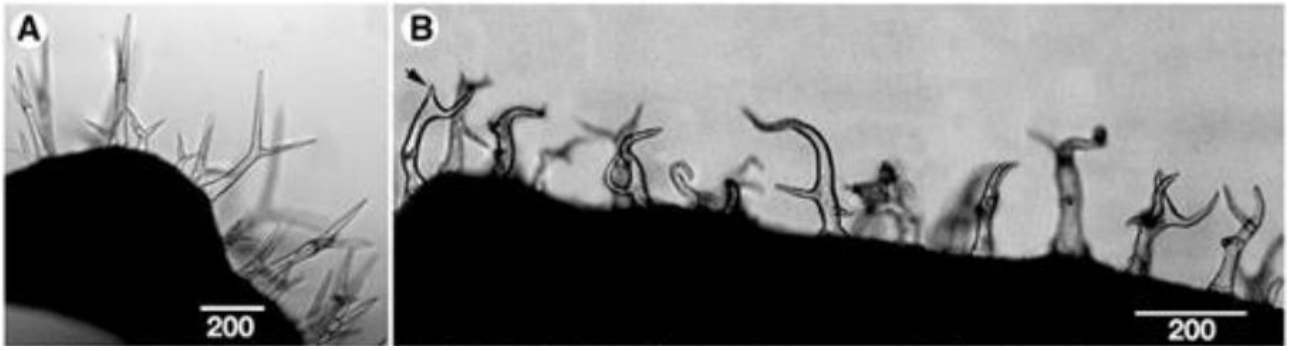


Figur 2: Den genrelle strukturformel for glucosinolat-molekyler, hvor 'R' er en variabel gruppe.[26]

[20] (Appel & Cocroft, 2014)
[21] (Appel & Cocroft, 2014)
[22] (Hilker & Schmülling, 2019)
[23] (Biswas, Seal, Majumder, & Biswas, 2023)
[24] (Halkier & Gershenzon, 2006)
[25] (Halkier & Gershenzon, 2006)
[26] (Halkier & Gershenzon, 2006)

5 Forsøgsdesign og metode

I mit projekt anvendes hypotetisk deduktiv metode. Jeg vil eksperimentielt sammenligne vildtype *A.thalina*'s reaktion på lyd med to slags trichommutanters. Den ene trichommutant kaldes GLABROUS1 (GL1) og har næsten ingen trichomer.[27] Den anden kaldes CROOKED (CRK) og har misformede trichomer (figur 3).[28] Det er nødvendigt at anvende mere end en slags mutant, da det muterede gen i hver af *A.thaliana*-mutanterne kan have andre funktioner, der ikke er trichom-relaterede. En anderledes lydperception i disse mutanter, skyldes derfor ikke nødvendigvis trichomfænotypen, men kan også være forbundet med tabet af det muterede gens andre funktioner.



Figur 3: Vildtype A.thaliana-*trichomer (A) og trichomer på en CROOKED-mutant (B). Værdierne ved målestokkene er i µm.*[29]

Forsøget er opbygget således: En gruppe *A.thaliana*-planter bestående af de 3 førnævnte trichomfænotyper udsættes for lydbehandling, og primingen måles på indholdet af forsvarsrelateret RNA i planterne. Derefter udsættes de for herbivorbehandling, hvilket bør aktivere primingen, og glucosinolatindholdet i planterne måles. Det samme gøres med en kontrolgruppe, der ikke udsættes for lydbehandling. På denne måde kan reaktionen i de lydbehandlede planter relativt til de ikke-lydbehandlede af samme fænotype sammenliges på tværs af trichomfænotyperne.

[27] (Marks & Feldmann, 1989)
[28] (Mathur, et al., 2003)
[29] (Mathur, et al., 2003)

De to metoder, som lydreaktionen måles på, fungerer som bekræftelse af hinanden. Indholdet af RNA for forsvarsrelaterede gener bør afspejles i glucosinolatindholdet og vice versa. Er dette ikke tilfældet, tyder det på fejlkilder eller mangler i den anvendte teori.

Metoderne, der anvendes i projektet, beskrives i de følgende underafsnit.

5.1 Dyrkning af *A.thaliana* og kontrol af ens vækstforhold i lyskamre

I hvert af to lyskamre plantes 10 af hver af de tre førnævnte trichom-fænotyper. Såningen foregår ved, at to frø af samme fænotype placeres i en potte på 5 x 5 cm. Når et af frøene spirer, fjernes det andet frø. Med denne metode undgås potter uden spirede frø. De tre fænotyper placeres tilfældigt iblandt hinanden i lyskammerene for at undgå placering i kammeret som en variabel. Forsøgsplanterne gros i fire uger. Efter fire uger bør planternes bladrosetter være dannet og store nok til at udføre forsøg på.[30] Ved at starte forsøget allerede efter fire uger frem for at vente længere tid, undgås det, at der er for stor forskel i planternes størrelse grundet uens vækstrater.

Da planterne i de to lyskamre senere skal sammenlignes, skal det sikres, at planterne gror ens i de to lyskamre. Dette gøres ved brug af billedbaseret plante-fænotyping. I hvert kammer tages et billede med et kamera rettet lodret ned mod planterne. Ved brug af softwaren PlantCV sammenlignes mængden af grøn farve på de to billeder. Da alle planterne efter de 4 ugers vækst stadig forventes at være små og kun bestå af en bladroset, bør der ikke være mange blade, der overlapper hinanden. Derfor er denne teknik præcis nok til formålet.

5.2 Opdrætning af *P.rapae*-larver

Der skal bruges 60 *P.rapae*-larver til herbivorbehandlingen, men 65 opdrættes i tilfælde af, at nogle dør. Larverne opdrættes på plantemateriale indtil de har gennemført 3. hamskifte. I dette udviklingsstadie er de større end yngre larver, og dermed lettere at arbejde med. *P.rapae* larver gennemgår i alt 4 hamskifte inden metamorphose.[31] Ved at anvende larverne før 4. hamskifte,

[30] (Krämer, 2015)
[31] (Hauze, n.d.)

risikeres det ikke, at larverne begynder metamorphose før forsøget er gennemført. Opdrætningen af *P.rapae* kan forventes at tage under 15 dage.[32]

5.3 Lydbehandling

Til lydbehandlingen ville det være oplagt at tage udgangspunkt i intensiteten og frekvensen af *P.rapae*-tyggelyde. Det er dog besværligt, at udsætte mange planter for så svag en lydintensitet samtidig. Appel & Cocroft forsøgte at replikere tyggelydene ved at vibrere blade direkte med piezoelektriske aktuatorer.[33] Denne metode er dog nærmere en simulering af fysisk berøring end af lydbølger i luften. I stedet anvendes lufttransmiterede lydbølger med en frekvens på 3000 Hz, da det før at blevet vist, at disse primer *A.thaliana* ligesom *P.rapae*-tyggevibrationerne, og da *P.rapae*-tyggelyde inkluderer denne frekvens.[34,35]

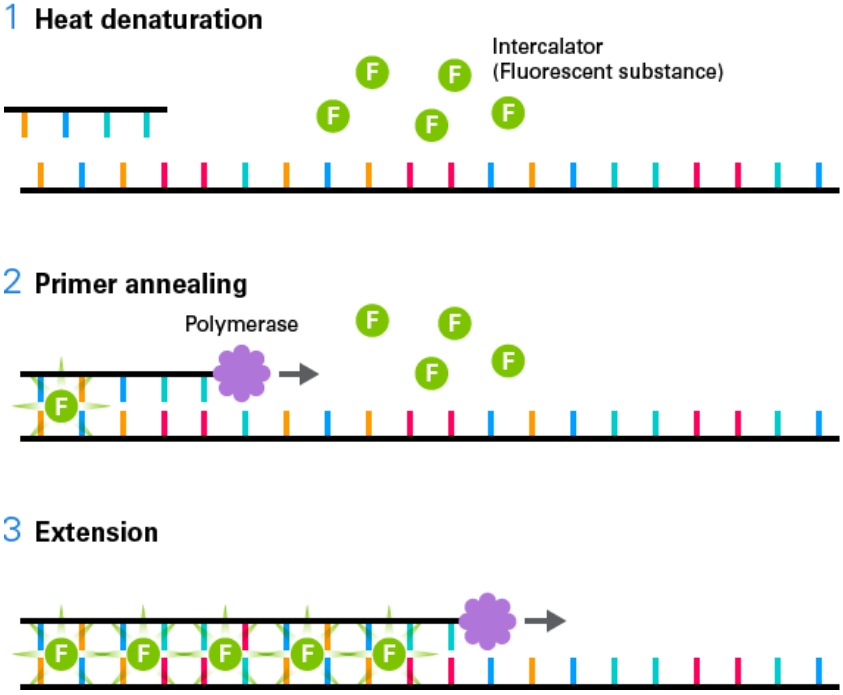
En højtaler placeret i midten af det ene lyskammer, ca. 1 meter over planterne, kalibreres, således at planterne udsættes for en lydintensitet på 40 dB, når en 3000 Hz lydfil afspilles. Planterne længst fra højtaleren forventes at udsættes for en lydintensitet inden for 1 dB af dem nærmest højtaleren (se bilag 1). Afstanden til højtaleren bør derfor ikke være en væsentlig variabel, men noteres alligevel som fejlled og indrages, når resultaterne analyseres. Lydbehandlingen sker, ved at lydfilen afspilles i 2 timer, da dette før har været nok til have en målbar effekt.[36]

5.4 RT-qPCR

Da priming påvirker udtrykkelsen af gener, kan det måles direkte gennem måling af mængden af RNA fra relevante gener i *A.thaliana*-blade. Til dette anvendes metoden RT-qPCR (reverse transcriptase quantitative polymerase chain reaction). Et af de yderste rosetblade plukkes fra alle planterne. RNA isoleres fra bladmassen og reverse-transkripteres til DNA med henholdsvis et RNA purification kit og et cDNA reverse transcription kit. Selve qPCR udføres på en real time PCR-maskine, hvor cDNA'et af 5 forsvarsrelaterede gener opformeres. De anvendte primere, genernes

[32] (Hauze, n.d.)
[33] (Appel & Cocroft, 2014)
[34] (Ghosh, et al., 2016)
[35] (Liu, et al., 2017)
[36] (Appel & Cocroft, 2014)

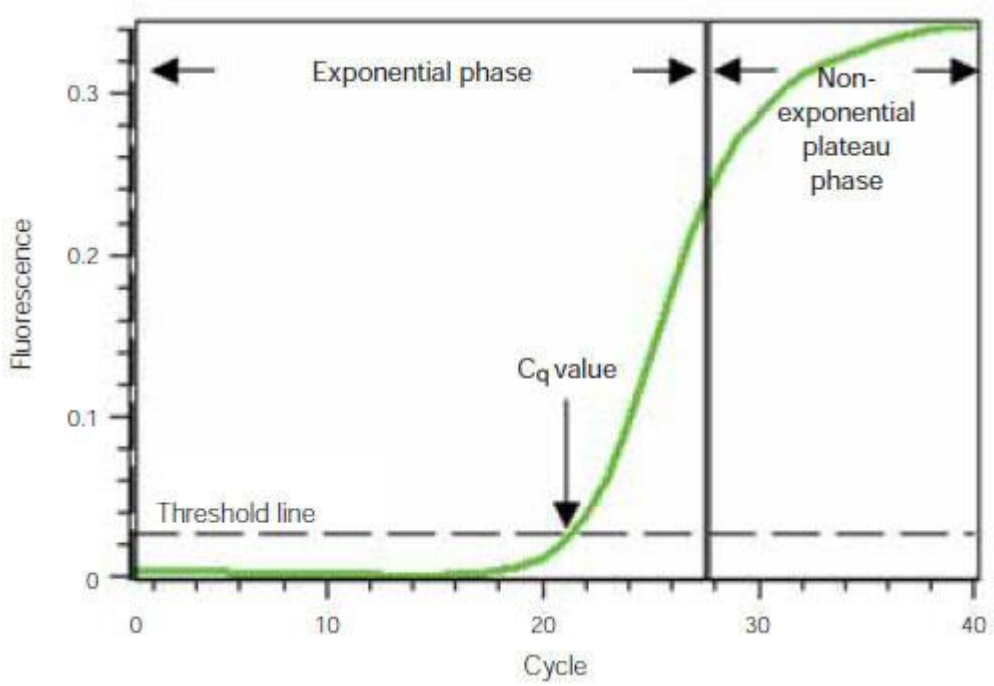
navne samt generernes konkrete funktioner vises i bilag 2. Under qPCR anvendes SYBR Green I som markør, der virker som vist på figur 4.



Figur 4: Virkemåden af SYBR Green. Under qPCR spaltes DNA-strengene under opvarmning, mens SYBR Green er frit i opløsningen og kun udsender et svagt fluorescent signal (1). DNA'et køles lidt ned igen, hvilket gør at primers kan binde sig til DNA-strengene, og DNA-polymerase kan forlænge primersne (2). SYBR Green kan binde sig til det nu dobbeltstrengede DNA, hvorved det fluorescente signal kraftigt forøges (3).[37]

RT-qPCR vil resultere i, at der for hvert analyseret gen i hvert blad dannes en graf som den på figur 5, hvor C_q-værdien er en indikator for genets udtrykkelse.[38]

[37] (Takara Bio Europe, n.d.)
[38] (Bio-Rad Laboratories Inc., u.d.)



Figur 5: Outputtet af RT-qPCR for et gen. Jo mere RNA/cDNA, der er til stede til at starte med, jo færre PCR-cyklusser skal der til, før det fluorescente signal bliver detekterbart. Dette antal cyklysser kaldes C_q-værdien.[39]

5.5 Herbivorbehandling

Metoden, som jeg vil anvende til herbivorbehandling, er baseret på den beskrevet af Appel & Cocroft.[40] Ca. 2 timer inden herbivorbehandlingen igangsættes, fjernes *P.rapae*-larverne fra deres foderkilde for at gøre dem sultne. For at herbivorbehandle en *A.thaliana* plante, påsættes en *P.rapae*-larve på et af de yderste rosetblade med en 'clip cage' (figur 3).

[39] (Bio-Rad Laboratories Inc., n.d.)
[40] (Appel & Cocroft, 2014)

Figur 6: Billede af en 'clip cage' på en plante.[41]

Da Appel & Cocroft anvendte metoden, begyndte de fleste larver at tygge på bladet med det samme.[42] Modsat Appel & Cocroft, der fjernede larverne når omkring 30% af det respektive blad var spist, fjernes alle larver efter 2 timer. Afvigelsen fra Appel & Cocroft's metode gør, at udførelsen er simplere, og at mindre afgøres på øjemål. En svaghed ved min metode er, at nogle larver vil nå at spise mere end andre. Det kan vurderes kvalitativt om dette er tilfældet. Er forskellen systematisk, f.eks. mellem lyd- og ikke-lydbehandlede planter, skal denne data også analyseres.

5.6 Måling af glucosinolatkoncentration

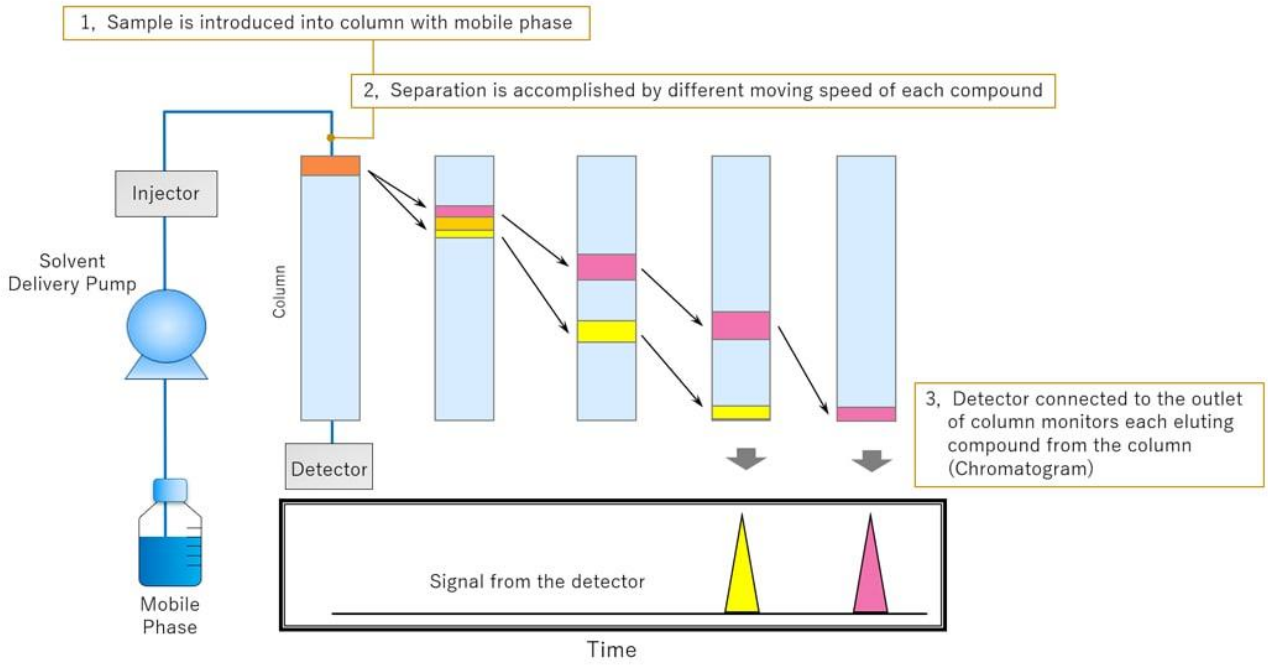
Målingen af glucosinolatkoncentrationen i *A.thalianas* blade består af to processer: ekstraktion og UHPLC. Homogeniseret bladmasse fra hver plante tilføres en kendt mængde p-hydroxybenzyl glucosinolat, og glucosinolater ekstraheres med methanol som beskrevet af Aller et al.[43] P-hydroxybenzyl glucosinolat findes ikke i forvejen i *A.thaliana,* og fungerer derfor som en intern standard, der viser, om glucosinolat er gået tabt under ekstrationen. For at fjerne urenheder tilføres glucosinolatet til Sephadex A-25, en gel der binder anioner, og derfor kan binde glucosinolater.[44] Sephadex-gelen skyldes for at fjerne ubundne molekyler, hvorefter sulfatase

[41] (Bugdorm, n.d.)
[42] (Appel & Cocroft, 2014)
[43] (Aller, Jagd, Kliebenstein, & Burow, 2018)
[44] (Cytiva, n.d.)

tilføres. Sulfatasen vil fjerne sulfatgrupperne fra glucosinolaten (figur 2 i afsnit 4.3), hvilket resulterer i desulfoglucosinolat, der ikke binder til Sephadex A-25. Derefter kan Sephadex-gelen skylles igen, hvilket resulterer i en opløsning af desulfoglucosinolat.

Desulfoglucosinolaterne kvantificeres med UHPLC/TQ-MS, hvilket står for ultra high performance liquid chromatography forbundet med triple quadrupole mass spectrometry, der udføres på en UHPLC/TQ-MS-maskine. UHPLC minder om almindelig HPLC, men er hurtigere og mere præcist.[45] En ulempe ved ved UHPLC er, at det er mere sensitivt over for urenheder[46], hvilket er årsagen til at jeg anvender Sephadex A-25 og TQ-MS, da begge disse metoder frasorterer urenheder.

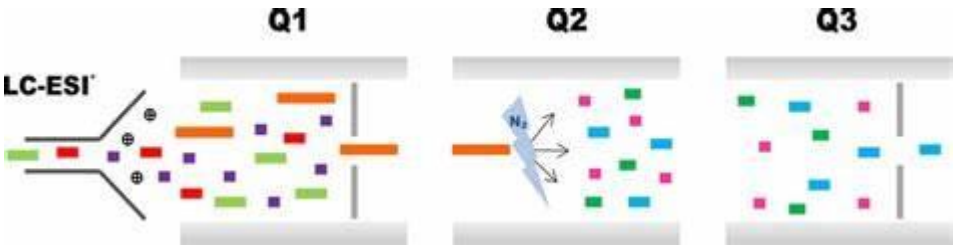
I (U)HPLC sepereres stofferne i opløsningen, ved at de føres gennem en lang kolonne, som de bevæger sig gennem med forskellige hastigheder (figur 4).[47]



Figur 7: Princippet bag UHPLC og HPLC. En opløsning føres gennem en lang kolonne af en mobil fase (1). Kolonnen indeholder et fast stof, som stofferne i opløsningen binder sig til med forskellige effektivitet, hvilket resulterer i at de bevæger sig med forskellig hastighed (2). Når stofferne kommer ud af kolonnen adskildt fra hinanden, kan koncentrationerne måles med diverse detektorer.[48]

[45] (uHPLCs, 2023)
[46] (uHPLCs, 2023)
[47] (Shimadzu Corporation, n.d.)
[48] (Shimadzu Corporation, n.d.)

Når stofferne kommer ud af kolonnen, kan koncentrationerne måles med en detektor. Jeg vælger at anvende en TQ-MS-detektor. Massespektrometri har den fordel, at de analyserede stoffer ikke behøver at have specielle egenskaber ligesom med UV-Vis-detektion, som kræver kromofore grupper, eller elektrokemisk detektion, som kræver, at stofferne udviser elektrokemisk aktivitet.[49] TQ-MS adskiller sig fra normal MS, ved at TQ-MS består af to gange MS udført efter hinanden, hvilket resulterer i højere præcision (figur 7).[50] I bilag 3 vises masse/ladnings-forholdene og spaltningsenergierne, som kræves for at isolere ti bestemte desulfoglukosinolater inklusivt den interne standard. På baggrund af disse værdier bestemmes koncentrationerne af de ti stoffer.



Figur 8: Princippet bag TQ-MS. På denne figur er indgangsopløsningen udgangsopløsningen fra liquid chromatography (LC), der er blevet ioniseret med electrospray ionisation (ESI), men det er samme princip, hvis der i stedet anvendes UHPLC. I Q1 frasorteres et bestemt stof ud fra m/z-forholdet, i Q2 fragmentes dette stof ved et energisk sammenstød med en ureaktiv gas. I Q3 frasorteres et af fragmenter, hvis koncentration til sidst kan måles ud fra ladningsdensiteten.[51]

[49] (Thermo Fischer Scientific Inc., n.d.)
[50] (Thermo Fischer Scientific Inc., n.d.)
[51] (Caballero, et al., 2020)

6 Udførelse og tidsramme

Forsøget udføres på Institut for Plante og Miljøvidenskab ved KU. For at reducere rollen af måleusikkerheder, udføres forsøget tre gange. En tidsplan for udførelsen af forsøget vises i tabel 1.

Tabel 1: Tidsplan for projektet

Tid	Forsøgsfase og relevant metodeafsnit
4 uger (udføres simultant)	Dyrkning af *A.thaliana*-planter (6.1)
	Opdrætning *P.rapae*-larver (6.2)
2 timer	Lydbehandling (6.3)
Få minutter	Indsamling af blade til qPCR (6.4)
3 timer	Herbivorbehandling (6.5)
2 dage	qPCR (6.4)
	Måling af glucosinolatkoncentration (6.6)
Tid per udførelse:	Ca. 4½ uge
Tid for 3 udførelser:	Ca. 14 uger

7 Budget

Budgettet for projektet, dvs. for tre udførelser af forsøget, er beskrevet i tabel 2. Nogle af materialerne antages det, at Institut for Plante og Miljøvidenskab stiller til rådighed. Der er efterladt et stort restbeløb i budgettet til uforudsete udgifter, f.eks. hvis KU opkræver et mindre beløb for lån af faciliteter.

Tabel 2: Budget for udførelse af forsøget tre gange.

Materiale	Antal	Pris i DKK
Vildtype-, Gl1- og CRK-A.thaliana-frø Købes fra Arabidopsis Biological Resource Center	90 af hver (sælges i pakker af 100)	549 kr
Clip cages Købes fra Bugdorm.com	60	1445 kr

cDNA reverse transcription kit **(kan anvendes til 200 reaktioner)** Købes fra Thermofisher.com	1	3755 kr
RNA isolation kit **(kan anvendes til 96 reaktioner)** Købes fra Thermofisher.com	2	4980 kr
Højtaler (prisen er for en Genelec 8010A - Active Studio Monitor) Købes fra Nordicproaudio.com	1	1982 kr
Støjmåler (præcision på ±1,5 dB) Købes fra Biltema.dk	1	249 kr
Kamera til sammenligning af vækst (prisen er for et Trust Teza 4K UHD webkamera) Købes fra Elgiganten.dk	1	879 kr
Pieris rapae-æg Købes fra Insektitaet.de	200 (sælges i pakker af 50)	1072 kr
Primers (se bilag 2 for primersekvenserne) Købes fra Eurofins Genomics	180 primer-opløsninger for hver af de 5 gener	558 kr
qPCR-maskine, UHPLC/TQ-MS-maskine og homogenizer Lånes af KU	-	0 kr
Lån af lyskamre Lånes af KU	-	0 kr
Øvrige kemikalier og laboratorieudstyr Lånes/gives af KU	-	0 kr
Pris i alt:	15469 kr	
Restbeløb:	4531 kr	

8 Konklusion

Min hypotese er, at trichommutanterne har en svagere reaktion på lyd end vildtype *A.thaliana*. Bekræftes denne hypotese, indikerer det, at trichomer spiller vigtig rolle i planters lydperception. Den nye viden vil give et udgangspunkt for yderligere forskning inden for planters lydperception. F.eks. ville det være oplagt, at undersøge hvilke membrankanaler, trichomerne er koblet til, eller om virkningen af lydbehandling er mere markant, når der anvendes lyd, der resonerer med trichomer.

Hvis forsøgsresultaterne ikke viser nogen betydelig sammenhæng mellem trichomintegritet og lydperception, vil det stadig gavne forskningsfeltet. Som forklares i afsnit 3.2, er trichomer mange gange blevet foreslået som mulige aktører i lydperception. Resultatet vil derfor være en indikator til resten af forskningsfeltet, om at de måske har ledt det forkerte sted.

I begge tilfælde vil vores viden om planters lydperception forøges, hvilket blandt andet hjælper os med at vurdere fremtidige anvendelsesmuligheder for lydbehandling af planter. Det kunne f.eks. være inden for landbrug, hvor lydbehandling potentielt kan fungere som alternativ til sprøjtegifte.

9 Tak

Tusind tak til Emma Aller, akademisk medarbejder ved sektionen for molekylær plantebiologi på Institut for Plante- og Miljøvidenskab, for vejledning og sparring, specielt om normer inden for plantevidenskab.

Derudover tak til Dorte Lind Damkjær og Peter Ruby Schmidt, mine forskerspirekoordinatorer og lærer, for sparring om akademiske overvejelser.

10 Litteraturliste

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Bilag 1: Varians i lydintensitet

Formlerne for lydintensitet og lydstyrke, der anvendes i dette bilag, er fået fra artiklen Lyd effekt, intensitet og lydstyrke af Ole Witt Hansen.[52]

Formlen for lydintensitet i et fast punkt er

$$I = \frac{P}{A}$$

Hvor P er effekten, hvormed lydbølger bevæger sig gennem et areal A, der er vinkelret på udbredelsesretningen fra lydkilden. Approximeres arealet som værende en halvkugle, og kaldes afstanden til lydkilden r, fås:

$$I = \frac{P}{2 \cdot \pi \cdot r}$$

For at omregne til dB-skalaen anvendes følgeden formel, hvor I_0 er en reference-lydintesitet på $10^{-12}\,\frac{W}{m^2}$, omtrentlig svarende til den laveste lydintensitet, som mennesker kan opfatte.

$$L = 10 \cdot \log\left(\frac{I}{I_0}\right) = 10 \cdot \log\left(\frac{P}{2 \cdot \pi \cdot r \cdot I_0}\right)$$

For at kunne udføre beregningerne, er det nødvendigt at antage, at de 60 potter på 5x5 cm er organiseret i en kvadratisk form, der skal kunne rumme alle potterne. Sidelægden af dette kvadrat må mindst være

$$s = \sqrt{0{,}05\ m \cdot 0{,}05\ m \cdot 60} = 0{,}387\ m$$

For planterne direkte under højtaleren er $r_{min} = 0{,}5\ m$. Da højtaleren er placeret over midten af kvadratet, kan Pythagoras' læresætning anvendes til at beregne afstanden til planterne længst fra højtaleren:

[52] (Hansen, 2019)

$$r_{maks} = \sqrt{(r_{min})^2 + \left(\frac{s}{2}\right)^2} = 0{,}536\ m$$

P kan justeres ved at skruge op eller ned for højtalerens lydstyrke. Indstilles lydstyrken således, at $P = 1{,}6 \cdot 10^{-6}\ W$, hvilket skal gøres ved brug af en støjmåler, er lydniveauet for planterne tættest og længst fra højtaleren følgende:

$$L_{maks} = 10 \cdot \log\left(\frac{P}{2 \cdot \pi \cdot r_{min}^2 \cdot I_0}\right) = 40{,}08\ dB$$

$$L_{min} = 10 \cdot \log\left(\frac{P}{2 \cdot \pi \cdot r_{maks}^2 \cdot I_0}\right) = 39{,}473\ dB$$

Heraf ses det, at forskellen mellem den maksimale lydstyrke, som de midterste planter udsættes for, og den minimale lydstyrke, som planterne længst fra højtaleren udsættes for, er relativt tæt på hinanden. Dette er naturligvis ikke et formelt bevis, og afstandene mellem højtaler og planter vil sandsynligvis være anderledes i virkeligheden. Dette er blot en overslagsregning, for at vise at forskellene i afstand mellem planter og højtaler er så små, at det ikke bør skabe væsentlige forskelle i lydniveau.

Bilag 2: Primere til qPCR

Tabellen herunder viser de valgte gener, hvis udtrykkelse måles ved qPCR, samt de primers, der anvendes til opformeringen. All I tabellen vises også hvilken af genets funktioner, der har fået mig til at udvælge genet. Information om generne funktioner er anskaffet fra en artikel af Gosh et al., der viste, at alle udtrykkelsen af alle disse gener bliver opreguleret ved lydbehandling med 3000 Hz.[53] Udover genets symbol, er genets TAIR-ID også angivet. TAIR-ID'et er brugbart når genet skal slåes op i TAIR-databasen, for at finde genets sekvens.

Primerne er designet med NCBI Primer-BLAST med følgende parametre:

- Smeltetemperatur af primers: $57°C$ - $63°C$.
- Maksimal forskel mellem primernes smeltetemperaturer: $3°C$.
- Primersne sammenlignet med resten af *A.thaliana*-genomet, for at sikre at de ikke binder til andre gener.

TAIR-ID	Gensymbol	Funktion/årsag til valg	Primer-sekvenser (5´→ 3´)
At4g37370	ATAPR1	Glucosinolatsyntese	Forward: GTCTGAAACGTTGCGCCTTT Reverse: CCAGTCCAGAACCAGGACAC
At4g21990	ATAPR3	Glucosinolatsyntese	Forward: AAGGTGAGACCTTTGAGGCG Reverse: CCGTTGACATTAGCGGTTGC
At2g34600	*JAZ7*	Jasmonatsyntese (jasmonat er også et forsvarsstof)	Forward: CAACGGGCACATGTGTGTTT Reverse: TTGTTGGAGGATCCGAACCG
At5g66070	At5g66070	Kitinsyntese (kitin styrker planters immunsystem)	Forward: CAAAGGGTCTCACTGGGGAC Reverse: ACCTGAAAGTCCTGAAGGCAG
At5g06320	*NHL3*	Opregulerer plantens forsvar	Forward: CGGTGATCAGCGATTCGGTA Reverse: TAGTTCCAACCACCGTCGTG

[53] (Ghosh, et al., 2016)

Bilag 3: Stoffer kvantificeret med TQ-MS og de anvendte værdier i Q1, Q2 og Q3.

Listen herunder viser de desulfoglucosinolater, hvis koncentrationer måles, forkortelser for dem, m/z-forholdet i Q1, m/z-forholdet i Q3 og spændingen, stofferne spaltes ved i Q2. Alle værdierne er fået fra et studie af Aller et al., hvori glucosinolatindholdet i *A.thaliana*-blade blev undersøgt.[54]

3-methylthiopropyl (3mtp), (+)328 > 166 [5V];

3-methylsulfinyl (3msp), (+)344 > 182 [10V];

4-methylthiobutyl (4mtb), (+)342 > 132 [15V];

4-methylsulfinylbutyl (4msb), (+)358 > 196 [5V];

5-methylsulfinylpentyl (5msp), (+)372 > 210 [5V];

7-methylthioheptyl (7mth), (+)384 > 222 [5V];

7-methylsulfinylheptyl (7msh), (+)400 > 238 [7V];

8-methylthiooctyl (8mto), (+)398 > 236 [5V];

8-methylsulfinyloctyl (8mso), (+)414 > 252 [5V];

Intern standard: p-hydroxybenzyl (pOHB), (+)346 > 184 [10V]

[54] (Aller, Jagd, Kliebenstein, & Burow, 2018)

Bilag 4: PDF-version af websider, der anvendes som kilder

Herunder ses en liste af alle websider, der anvendes som kilder. På de efterfølgende sider er alle websiderne indsat som PDF i samme rækkefølge som angivet i listen herunder.

Bio-Rad Laboratories Inc. (n.d.). What is Real-Time PCR (qPCR)? Retrieved 10 23, 2024, from Bio-Rad: https://www.bio-rad.com/en-dk/applications-technologies/what-real-time-pcr-qpcr?ID=LUSO4W8UU

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What is Real-Time PCR (qPCR)?

Nucleic acid amplification and detection techniques are among the most valuable tools in biological research today. Scientists in all areas of life science — basic research, biotechnology, medicine, forensics, diagnostics, and more — utilize these methods in a wide range of applications. For some applications, qualitative nucleic acid detection is sufficient. Other applications, however, demand a quantitative analysis. Real-time PCR can be used for both qualitative and quantitative analysis; choosing the best method for your application requires a broad knowledge of this technology. This section provides an overview of real-time PCR, reverse-transcription quantitative PCR techniques, and the choice of instruments that Bio-Rad offers for these techniques. It also provides tips for steps in RNA isolation such as sample collection, RNA extraction, and analyzing the quality and quantity of RNA..

DID YOU KNOW?

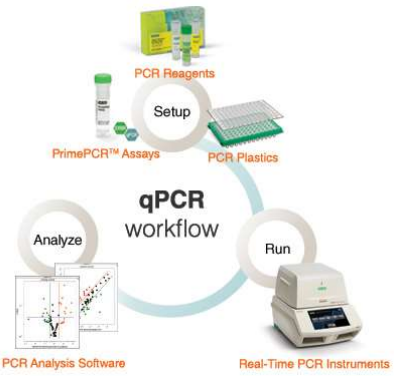
Bio-Rad offers custom and pre-designed real-time PCR Primers.
Available in the U.S., Canada, E.U., China, Australia, New Zealand, Singapore, Taiwan, Hong Kong, India, Israel, South Africa, Thailand, Macau, Korea, Japan and Brazil.

Page Contents

What Is Real-Time PCR?

In conventional PCR, the amplified DNA product, or amplicon, is detected in an end-point analysis. In real-time PCR, the accumulation of amplification product is measured as the reaction progresses, in real time, with product quantification after each cycle.

The qPCR workflow below delineates the steps in real-time PCR. First, amplification reactions are set up with PCR reagents and unique or custom primers. Reactions are then run in real-time PCR instruments and the collected data is analyzed by proprietary instrument software.



Real-time detection of PCR products is enabled by the inclusion of a fluorescent reporter molecule in each reaction well that yields increased fluorescence with an increasing amount of product DNA. The fluorescence chemistries employed for this purpose include DNA-binding dyes and

occurs. The measured fluorescence is proportional to the total amount of amplicon; the change in fluorescence over time is used to calculate the amount of amplicon produced in each cycle.

The main advantage of real-time PCR over PCR is that real-time PCR allows you to determine the initial number of copies of template DNA (the amplification target sequence) with accuracy and high sensitivity over a wide dynamic range. Real-time PCR results can either be qualitative (the presence or absence of a sequence) or quantitative (copy number). Quantitative real-time PCR is thus also known as qPCR analysis. In contrast, PCR is at best semiquantitative. Additionally, real-time qPCR data can be evaluated without gel electrophoresis, resulting in reduced bench time and increased throughput. Finally, because real-time qPCR reactions are run and data are evaluated in a unified, closed-tube qPCR system, opportunities for contamination are reduced and the need for postamplification manipulation is eliminated in qPCR analysis.

Applications of Real-Time PCR/qPCR Assays

Real-time PCR/qPCR assays have become the tool of choice for the rapid and sensitive determination and quantitation of nucleic acid in various biological samples, with diverse applications such as gene expression analysis, the detection of genetically modified organisms in food, and cancer phenotyping.

In research laboratories, qPCR assays are widely used for the quantitative measurement of gene copy number (gene dosage) in transformed cell lines or the presence of mutant genes. In combination with reverse-transcription PCR (RT-PCR), qPCR assays can be used to precisely quantitate changes in gene expression, for example, an increase or decrease in expression in response to different environmental conditions or drug treatment, by measuring changes in cellular mRNA levels.

How Does Real-Time PCR Work?

To understand how real-time PCR works, we illustrate a qPCR analysis using a typical amplification plot (Figure 1). In this plot, the number of PCR cycles is shown on the x-axis, and the fluorescence from the amplification reaction, which is proportional to the amount of amplified product in the tube, is shown on the y-axis.

The amplification plot shows two phases, an exponential phase followed by a non-exponential plateau phase. During the exponential phase, the amount of PCR product approximately doubles in each cycle. As the reaction proceeds, however, reaction components are consumed, and ultimately one or more of the components becomes limiting. At this point, the reaction slows and enters the plateau phase (cycles 28–40 in Figure 1).

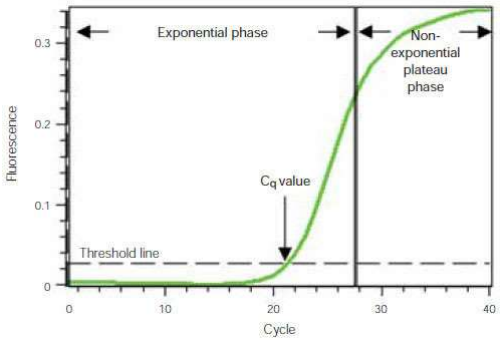


Figure 1. Amplification plot. Baseline-subtracted fluorescence versus number of PCR cycles.

Initially, fluorescence remains at background levels, and increases in fluorescence are not detectable (cycles 1–18, Figure 1) even though product accumulates exponentially. Eventually, enough amplified product accumulates to yield a detectable fluorescence signal. The cycle number at which this occurs is called the quantification cycle, or C_q. Because the C_q value is measured in the exponential phase when reagents are not limited, real-time qPCR can be used to reliably and accurately calculate the initial amount of template present in the reaction based on the known exponential function describing the reaction progress.

The C_q of a reaction is determined mainly by the amount of template present at the start of the amplification reaction. If a large amount of template is present at the start of the reaction, relatively few amplification cycles will be required to accumulate enough product to give a fluorescence signal above background. Thus, the reaction will have a low, or early, C_q. In contrast, if a small amount of template is present at the start of the reaction, more amplification cycles will be required for the fluorescence signal to rise above background. Thus, the reaction will have a high, or late, C_q. This relationship forms the basis for the quantitative aspect of real-time PCR.

RNA Isolation

Sample Collection

For RNA isolation and the quantification of gene expression, sample material should be as homogeneous as possible. If your tissue sample consists of many different cell types, pinpointing the expression pattern of your target gene may be difficult. If you have a heterogeneous sample, use one of the many methods that are available for separating and isolating specific cell types, for example, tissue dissection, needle biopsies, and laser capture microdissection. The collected cells can then be used to obtain the RNA samples.

RNA Extraction

Either total or poly(A+) RNA can be used for most real-time RT-qPCR applications. One critical consideration in working with RNA is to eliminate RNases in your solutions, consumables, and labware. Ready-to-use RNase-free solutions can be purchased, or your solutions can be treated with diethyl pyrocarbonate (DEPC) and then autoclaved. RNases on labware can also be inactivated by DEPC treatment or by baking at 250°C for 3 hr.

Prepared RNA samples may need DNase treatment to prevent potential amplification of any contaminating genomic DNA, which could lead to overestimation of the copy number of an mRNA. When starting material is limited, however, DNase treatment may be inadvisable, because the additional manipulation could result in loss of RNA. The amplification of potentially contaminating genomic DNA can be precluded by designing transcript-specific primers, for example, primers that span or amplify across splice junctions.

Analyzing Nucleic Acid Quantity and Quality

Accurate nucleic acid quantification is essential for gene expression analysis, especially when total RNA amounts are used to normalize target gene expression. RNA concentration and purity are commonly determined by measuring the ratio of UV absorbance at 260 nm and 280 nm.

Tired of RNA extractions?

Perform cell lysis and RT-qPCR assays directly from cell cultures with SingleShot™ Cell Lysis RT-qPCR Kits — no RNA purification required.

Learn more »

Reverse Transcription Quantitative PCR (RT-qPCR)

Two methods are available for quantification of gene expression by RT-qPCR: two-step RT-qPCR and one-step RT-qPCR. In both cases, RNA is reverse transcribed into cDNA, and the cDNA is then used as the template for qPCR amplification. *One-step* and *two-step* refer to whether the RT and real-time PCR amplification are performed in the same or separate tubes. In the two-step method, RNA is first transcribed into cDNA in a reaction using reverse transcriptase. An aliquot of the resulting cDNA is then used as a template for multiple qPCR reactions. In the one-step method, RT and qPCR are performed in the same tube.

qPCR/Real-Time PCR Instrumentation

A real-time PCR detection system consists of a thermal cycler equipped with an optical detection module to measure the fluorescence signal generated during each amplification cycle as the fluorophore binds to the target sequence. Bio-Rad real-time PCR detection systems feature thermal cyclers with interchangeable modules for singleplex and multiplex detection of fluorophores as well as fixed real-time PCR units. All qPCR systems feature thermal gradient functionality.

Bio-Rad real-time PCR detection systems.

Videos | Documents

1000-Series System Tour
This animated video gives you a quick overview of the 1000-series Touch thermal cyclers.

Quantitative PCR (qPCR): Validating a SYBR® Green I Assay

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Clip Cage (Small, Ø25/Ø40mm, lot of 12)

Model: BDC1540-12

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Discount	-0%	-5%	-10%
Price per Unit	$46.80 USD	$44.46 USD	$42.12 USD

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Main Material: Expanded Polyethylene (EPE) Foam
Mesh Size: 104 x 94 | 300 µm Aperture

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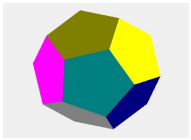
Lyd effekt, intensitet og lydstyrke

Dette er en artikel fra min hjemmeside: www.olewitthansen.dk



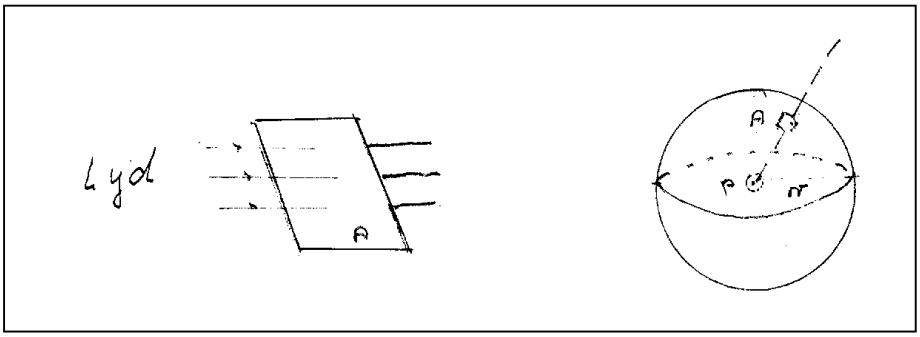
Ole Witt-Hansen 2019

Indhold

1. Effekt, intensitet og lydstyrke i dB

Lyd er et bølgefænomen, hvor udbredelsen er en følge af periodiske svingninger i et stof.
Lyd transporterer energi og impuls, mens partikelsvingningerne er stationære.
Den energi, som en lydgiver udsender per sekund kaldes for effekten og betegnes *P*.
Ifølge definitionen af *effekt* er:

$$P = \frac{\Delta E}{\Delta t}$$



Lyd er bølger, og en lydgiver har en bestemt effekt. For eksempel har en mindre stuehøjtaler en effekt på 20 *W*, mens en meget kraftig højtaler har en effekt på 100 *W*.

Den påførte effekt på f.eks. en højtaler, er imidlertid ikke nødvendigvis det samme som den udsendte effekt. Den påførte effekt er apparatets effektforbrug i Watt, mens der er et energitab i ledninger og højtalerens mekaniske dele.

Lyd intensiteten i et bestemt punkt i rummet er defineret som den effekt, som passerer et areal *A*, anbragt vinkelret på udbredelsesretningen, divideret med dette areal.

$$I = \frac{P}{A}$$ Intensiteten måles i W/m^2.

Ifølge denne definition er intensiteten (for mindre arealer, hvor effekten er konstant) uafhængig af arealets størrelse, idet den passerede effekt er proportional med arealet.

Intensiteten er det fysiske mål for hvor kraftig lyden er. Det menneskelig øres opfattelse af lyd er imidlertid meget anderledes, idet opfattelsen ikke følger en absolut skala, men snarere en logaritmisk skala. Groft sagt betyder dette, at en tidobling af intensiteten opfattes som om lydstyrken blot er forøget med 10.

Af den grund angives lydstyrken ikke som intensiteten målt i W/m^2, men den måles derimod i decibel (dB).
1 dB = 1/10 B (Bell) er opkaldt efter telefonens opfinder Alexander Graham Bell.
Decibel skalaen er imidlertid en logaritmisk skala, som har et referencepunkt ved 0 dB, som er intensiteten $I_0 = 10^{-12}$ W/m^2. Dette er høregrænsen for et normalt øre hos personer under 40 år.

Når lyd angives i dB, taler man om lydstyrke (eng, volume) og ikke om intensitet.

Hvis L betegner lydstyrken målt i dB og I betegner intensiteten, så er de to størrelser forbundet ved ligningen:

$$L = 10 \log \frac{I}{I_0}$$

Det ses, at når $I = I_0$ er $L = 0$.

Hvis intensiteten er 0,2 W/m^2 vil det svare til en lydstyrke: $L = 10 \log \frac{0{,}2}{10^{-12}} = 113 \ \ dB$,

Dette er en meget kraftig lyd, f.eks. fra en flymotor.

Hvisken svarer til en lydstyrke på omkring 30 dB, mens lyd bliver smertefuldt ved en lydstyrke over 135 dB, og denne lydstyrke betegnes derfor som smertegrænsen.

Som konsekvens af den logaritmiske skala følger det, at hvis intensiteten forøges med en faktor 10, så bliver lydstyrken målt i dB forøget med 10. Hvis intensiteten forøges med en faktor 100, så bliver lydstyrken forøget med 20 dB. Dette følger umiddelbart af definitionen på lydstyrke.

$$L_{10} = 10 \log \frac{10I}{I_0} = 10 \log 10 + 10 \log \frac{I}{I_0} = 10 \log \frac{I}{I_0} + 10$$
$$L_{100} = 10 \log \frac{100I}{I_0} = 10 \log 100 + 10 \log \frac{I}{I_0} = 10 \log \frac{I}{I_0} + 20$$

2. Afstandskvadratloven for bølger

Hvis man omslutter en lydgiver med en (matematisk) kugleflade med lydgiveren i centrum, og hvis man antager, at lyden udbreder sig isotropt (det samme i alle retninger), så er intensiteten på kuglefladen $I(r)$ overalt den samme. Hvis man endvidere antager, at der ikke er noget tab, så vil den effekt, som udsendes fra lydgiveren være den samme effekt, som passerer kuglefladen i afstanden r.
Sammenhængen mellem den effekt, som passerer et areal i afstanden r og intensiteten på arealet er:

$$I(r) = \frac{P_A}{A} \quad \Leftrightarrow \quad P_A = I(r)A$$

Vælger vi arealet A som overfladen af en kugle, så har vi $A = 4\pi r^2$, og vi får derfor:

$$I(r)4\pi r^2 = P \qquad \text{eller} \qquad I(r) = \frac{P}{4\pi r^2}$$

Den sidste ligning kaldes for *afstandskvadratloven*, og den udtrykker at intensiteten aftager omvendt proportionalt med kvadratet på afstanden fra lydgiveren til modtageren.
Der gælder således at, hvis afstanden fordobles, så vil intensiteten reduceres til en fjerdedel.

Hvis vi anvender denne formel, kan vi f.eks. vurdere lydstyrken for en tilskuer, der befinder sig i afstanden 10 m fra højtalerne, der har en samlet effekt på 4000 W.

Først beregner vi intensiteten ud fra ligningen: $I(r) = \frac{P}{4\pi r^2}$, som giver $I = \frac{4000W}{4\pi(10m^2)} = 3{,}18\ W/m^2$,

og dermed lydstyrken:

$$L = 10\log(\frac{3{,}18}{10^{-12}}) = 125\ dB$$

Bestemt en meget kraftig lydstyrke, men dog under smertegrænsen.

Vi skal derefter udlede en formel, der beskriver hvor meget lydstyrken formindskes, når afstanden øges fra r_1 til r_2. Det viser sig, at svaret er uafhængigt af intensiteten.

$$L_1 - L_2 = 10\log(\frac{I_1}{I_0}) - 10\log(\frac{I_2}{I_0}) = 10\log(\frac{I_1}{I_2}) =$$

$$10\log\left(\frac{\frac{P}{4\pi r_1^2}}{\frac{P}{4\pi r_2^2}}\right) = 10\log(\frac{r_2^2}{r_1^2}) = 20\log(\frac{r_2}{r_1})$$

Ud fra regnereglerne for logaritmer, finder vi derfor:

$$L_1 - L_2 = 20\log(\frac{r_2}{r_1})$$

Hvis vi bruger denne formel, kan man beregne, hvor meget lydstyrken er svækket, fra afstanden r_1 til r_2. For eksempel vil lydstyrken svækkes med 20 dB, hvis man bevæger sig fra afstanden 1,0 m fra lydgiveren til afstanden 10 m fra lydgiveren. Dette følger umiddelbart af formlen ovenfor.

$$L_1 - L_2 = 20\log(\frac{r_2}{r_1}) = 20\log(\frac{10}{1}) = 20\ dB$$

I et klasselokale kan læreren spekulere på, hvorvidt han kan høre, hvis to elever, som sidder på den sidste række, hvisker sammen.
Hvis de hvisker sammen med en styrke på 25 cm fra øret, og den sidste række er 7 meter fra læreren finder man:

$$L_1 - L_2 = 20\log(\frac{r_2}{r_1}) = 20\log(\frac{7}{0{,}25}) = 29\ dB$$

Idet 30 dB - 29 dB er meget tæt på høregrænsen, vil det teoretisk ikke være tilfældet.

Pieris rapae

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By Deena Hauze

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Classification

Kingdom
Animalia
animals

Class
Insecta
insects

Order
Lepidoptera

Family
Pieridae

Genus
Pieris

Species
Pieris rapae

Geographic Range

Cabbage butterflies (*Pieris rapae*), known as cabbage worms in their caterpillar stage, are found all around the world in temperate climates. They were introduced to Montreal in the 1860s and have spread throughout North America. (Barlett, 2004; Capinera, 2014)

Biogeographic Regions: nearctic ; palearctic ; oriental ; ethiopian ; neotropical ; australian

Habitat

Cabbage butterflies are found in a variety of habitats. They can be found in almost any type of open space, including meadows, bogs, forests, fields, and open spaces. (Barlett, 2004)

Habitat Regions: temperate

Terrestrial Biomes: savanna or grassland ; forest

Wetlands: marsh ; bog

Physical Description

Cabbage worms, the larval form of cabbage butterflies, are up to 35 mm in length. These caterpillars have a green, velvety appearance. The four final instars have yellow stripes running along the centers of their backs. Adult butterflies have a wingspan that ranges from 4.5 cm to 6.5 cm. Cabbage butterflies have white wings tipped in black. They have one black spot on the upper side of the hindwing. Females have two black dots in the middle of their wings and dense, white hair on their bodies. Males have a single black dot in the middle of their wings and dense, yellowish hair on their bodies. (Barlett, 2004; Capinera, 2014)

Other Physical Features: ectothermic ; bilateral symmetry

Sexual Dimorphism: sexes colored or patterned differently

Range wingspan
4.5 to 6.5 cm
1.77 to 2.56 in

Development

Cabbage worms mature for around 15 days before undergoing metamorphosis to become cabbage butterflies. During this period of development, the larvae undergo five instars and four molts. Pupation occurs in chrysalises built on food plants or nearby debris. Grey, green, yellow, or brown in color, the chrysalises are 19-20 mm in length. Metamorphosis can last from 11 days up to a few weeks. Cabbage worms that pupate late in the year may overwinter in their chrysalises before emerging. (Capinera, 2014; Richards, 1940)

Development - Life Cycle: metamorphosis

Reproduction

Female cabbage butterflies mate once as early adults. (Kingsolver, 2000)

Mating System: polygynous

Female cabbage butterflies lay between 300-400 eggs in their lifetimes. They lay one egg at a time on the undersides of leaves. The eggs are white and become more yellow as they age. A single plant can have up to 57 eggs and 48 larvae on it. (Capinera, 2014; Kingsolver, 2000)

Key Reproductive Features: semelparous ; seasonal breeding ; sexual ; fertilization (internal) ; oviparous

Breeding interval
Cabbage butterflies breed once in their lifetimes.

Breeding season
Cabbage butterflies breed from early spring to early fall.

Range eggs per season
300 to 400

Range time to independence
0 (low) minutes

Range age at sexual or reproductive maturity (female)
21 (low) days

Range age at sexual or reproductive maturity (male)
21 (low) days

Parental Investment: no parental involvement

Lifespan/Longevity

Cabbage butterflies live from 3 to 6 weeks, depending on the weather. About 3 weeks of their lifespans are spent as adults. There are 2-3 generations per year in Colorado, 3 in New England, 3-5 in California, and 6-8 near the southernmost part of the range of cabbage butterflies. (Capinera, 2014)

Range lifespan
Status: wild
21 to 42 days

Typical lifespan
Status: wild
21 to 42 hours

Behavior

Cabbage butterflies are active during the day. They fly from spring until September, but they have shorter active seasons farther north and longer active seasons in the south. (Barlett, 2004; Capinera, 2014)

Key Behaviors: flies ; diurnal ; motile ; sedentary ; solitary

Communication and Perception

Like other butterflies, cabbage butterflies have compound eyes. They are able to see ultraviolet light. (Berger, 2001)

Communication Channels: visual
Other Communication Modes: pheromones
Perception Channels: visual ; chemical

Food Habits

Adult cabbage butterflies feed on nectar, while larval cabbage worms feed on the leafy foliage. Cabbage butterflies prefer feeding on nectar from plants that contain mustard oil. They have been seen feeding from the flowers of *mustard plants*, *dandelions*, *broccolis*, *cabbages*, *Brussel sprouts*, *cauliflowers*, *collards*, *horseradish*, *kale*, *red clovers*, *asters*, and mints. Larvae feed on the leafy parts of these plants, sometimes reducing the plants to stems. They prefer *cabbage plants*, hence their name. (Barlett, 2004; Capinera, 2014)

Primary Diet: herbivore (folivore , nectarivore)
Plant Foods: leaves ; nectar

Predation

Predators include shield bugs, ambush bugs, vespid wasps, European wasps, harvestmen, and hoverflies. The species known as *white butterfly parasites* attack cabbage worms. (Ashby, 1974; Capinera, 2014; Kingsolver, 2000)

Known Predators

- shield bugs (Pentatomidae)
- ambush bugs (Phymatinae)
- vespid wasps (Vespidae)
- harvestmen (Opiliones)
- hoverflies (Syrphidae)

Ecosystem Roles

Cabbage worms and cabbage butterflies, despite being different life stages of the same creature, have very different environmental roles. Cabbage worms can negatively impact their ecosystems by wounding or killing plants through their ravenous feeding. Cabbage butterflies only the nectar of plants without destroying the foliage. Additionally, cabbage butterflies are important pollinators of crop plants, such as cabbage. (Barlett, 2004)

Ecosystem Impact: pollinates

Commensal/Parasitic Species

- white butterfly parasites (*Apanteles glomeratus*)
- parasitic flies (*Phryxe vulgaris*)

Economic Importance for Humans: Positive

Cabbage butterflies are pollinators of crop plants. (Barlett, 2004)

Positive Impacts: pollinates crops

Economic Importance for Humans: Negative

Cabbage worms, the caterpillar form of cabbage butterflies, are crop pests. They may eat crop plants down to the stems. (Capinera, 2014)

Negative Impacts: crop pest

Conservation Status

IUCN Red List	US Federal List	CITES
No special status	No special status	No special status

State of Michigan List
No special status

Contributors

Deena Hauze (author), Animal Diversity Web Staff.

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1 Overview of HPLC

HPLC is an abbreviation for High Performance Liquid Chromatography. "Chromatography" is a technique for separation, "chromatogram" is the result of chromatography, and "chromatograph" is the instrument used to conduct chromatography.

Among the various technologies developed for chromatography, devices dedicated for molecular separation called columns and high-performance pumps for delivering solvent at a stable flow rate are some of the key components of chromatographs. As related technologies became more sophisticated, the system commonly referred to as High Performance Liquid Chromatography, simply became referred to as "LC". Nowadays, Ultra High Performance Liquid Chromatography (UHPLC), capable of high-speed analysis, has also become more wide-spread.

Only compounds dissolved in solvents can be analyzed with HPLC. HPLC separates compounds dissolved in a liquid sample and allows qualitative and quantitative analysis of what components and how much of each component are contained in the sample.

Fig.1 shows a basic overview of the HPLC process. The solvent used to separate components in a liquid sample for HPLC analysis is called the mobile phase. The mobile phase is delivered to a separation column, otherwise known as the stationary phase, and then to the detector at a stable flow rate controlled by the solvent delivery pump. A certain amount of sample is injected into the column and the compounds contained in the sample are separated. The compounds separated in the column are detected by a detector downstream of the column and each compound is identified and quantified.

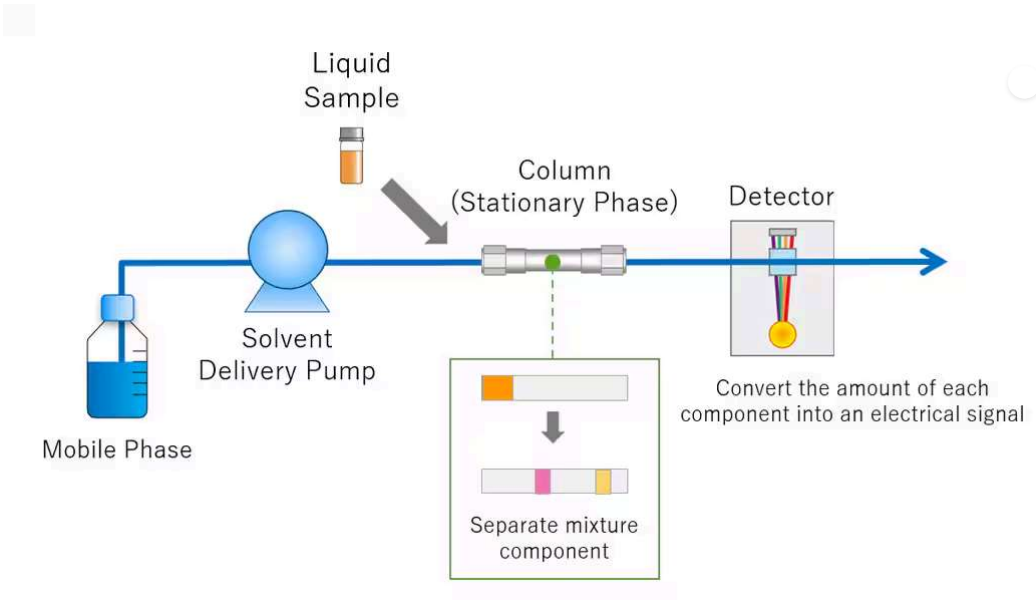


Fig.1 Overview of HPLC

2 The Apparatus of HPLC

The "Basic Overview of the HPLC process"(As shown in Fig.1) and its mechanisms have now been covered. Going into more detail, HPLC consists of a variety of components, including a solvent delivery pump, a degassing unit, a sample injector, a column oven, a detector, and a data processor. Fig.2 shows the HPLC flow diagram and the role of each component.

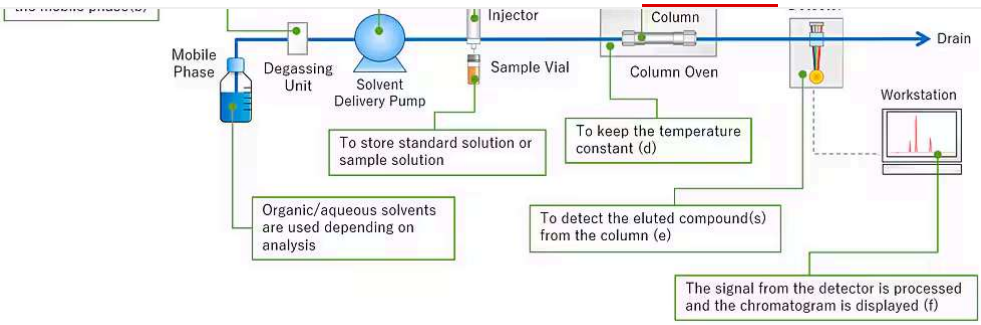


Fig.2 HPLC Flow Diagram

As for HPLC, the pump delivers the mobile phase at a controlled flow rate(a). Air can easily dissolve in the mobile phase under the standard atmospheric pressure in which we live in. If the mobile phase contains air bubbles and enters the delivery pump, troubles such as flow rate fluctuations and baseline noise/drift may occur. The degassing unit helps prevent this issue by removing air bubbles in the mobile phase(b). After the dissolved air has been removed, the mobile phase is delivered to the column. The sample injector then introduces a standard solution or sample solution into the mobile phase (c). Temperature fluctuations can affect the separation of compounds in the column. The column is placed in a column oven to keep the temperature constant(d). Compounds eluted from the column are detected by a detector which is placed downstream of the column(e). A workstation processes the signal from the detector to obtain a chromatogram to identify and quantify the compounds(f).

3 HPLC Separation

HPLC can separate and detect each compound by the difference of each compound's speed through the column. Fig.3 shows an example of HPLC separation.

There are two phases for HPLC: the mobile phase and the stationary phase. The mobile phase is the liquid that dissolves the target compound. The stationary phase is the part of a column that interacts with the target compound.

In the column, the stronger the affinity (e.g.; van der waals force) between the component and the mobile phase, the faster the component moves through the column along with the mobile phase. On the other hand, the stronger the affinity with the stationary phase, the slower it moves through the column. Fig. 3 shows an example in which the yellow component has a strong affinity with the mobile phase and moves quickly through the column, while the pink component has a strong affinity with the stationary phase and moves through slowly. The elution speed in the column depends on the affinity between the compound and the stationary phase.

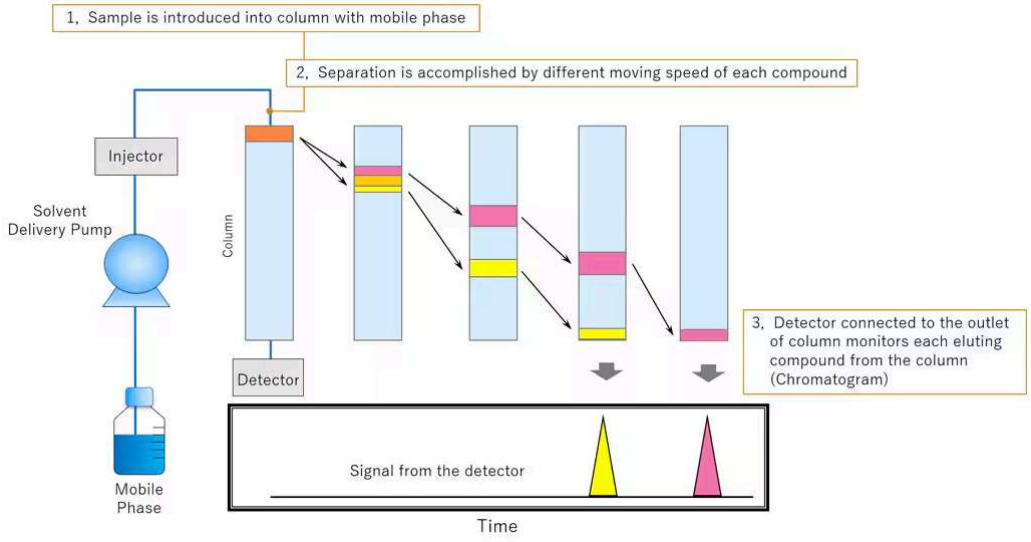


Fig.3 An Example of HPLC Separation

4 How to Read a Chromatogram

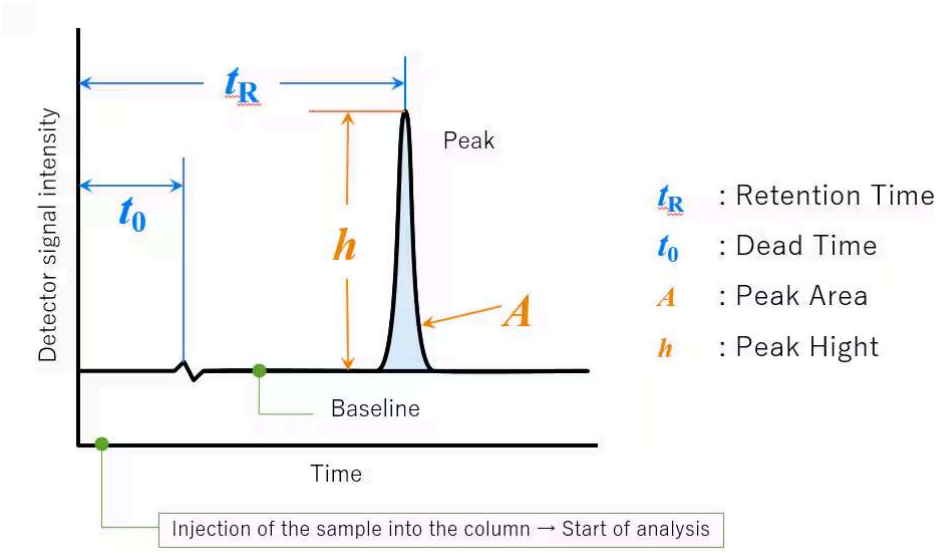
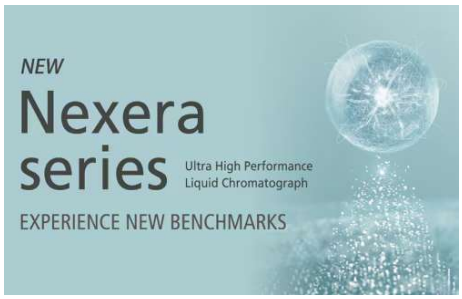


Fig.4 Chromatogram and Related Terms



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PRODUCT OVERVIEW

One-step RT-qPCR kits

At Takara Bio, great enzymes are part of our DNA. Thanks to over 90 years of experience in the pursuit of excellence in biochemistry and molecular biology technologies, we have developed an extensive portfolio of tools that have been widely published in peer-review studies. Explore below to see how we can solve your challenges with one-step reverse transcription quantitative PCR (RT-qPCR).

Advantages of one-step RT-qPCR

- Simple and rapid workflow
- Compatible with large numbers of samples (when looking at few target genes)
- Adaptable to high-throughput/automated workflows

Limitations

- Cannot optimize RT step
- Does not generate stock cDNA
- Not ideal when analyzing large numbers of target genes

Introduction

Quantitative PCR (qPCR) is a powerful technique for the accurate analysis of gene expression. When starting with RNA samples, one must first perform a reverse transcription (RT) step to generate cDNA for the subsequent qPCR reaction. One-step RT-qPCR streamlines this workflow by performing the RT step in the same tube as the qPCR reaction (Figure 1).

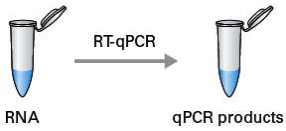


Figure 1. Schematic of a one-step RT-qPCR reaction.

In general, one-step RT-qPCR is best suited for applications where speed and throughput are required. As the single-tube protocol is easy to set up and compatible with liquid handlers and/or automated systems it allows for less hands-on time, reduces pipetting errors, and minimizes contamination. One-step RT-qPCR is best applied to workflows comprised of many samples with few target genes.

However, one-step RT-qPCR has some limitations. Since both the RT and qPCR steps take place in the same tube the reaction conditions cannot be optimized separately, which can lead to lower yields and/or efficiency in either step. Another limitation is that all the generated cDNA is used up in the subsequent qPCR step, meaning that no stocks of cDNA can be banked for further validation or experimentation.

We offer one-step RT-qPCR kits supporting both TB Green- (our proprietary green intercalating dye) and probe-based chemistries to meet your experimental needs and give you the flexibility to run a wide variety of applications.

Principle of one-step RT-qPCR

Takara Bio's One Step PrimeScript III RT-qPCR Kit allows cDNA synthesis from RNA using PrimeScript Reverse Transcriptase, followed by PCR amplification with Takara *Ex Taq* Hot Start Version in a single, uninterrupted procedure. PCR amplification products are detected and monitored in real time with either probe- or TB Green-based detection.

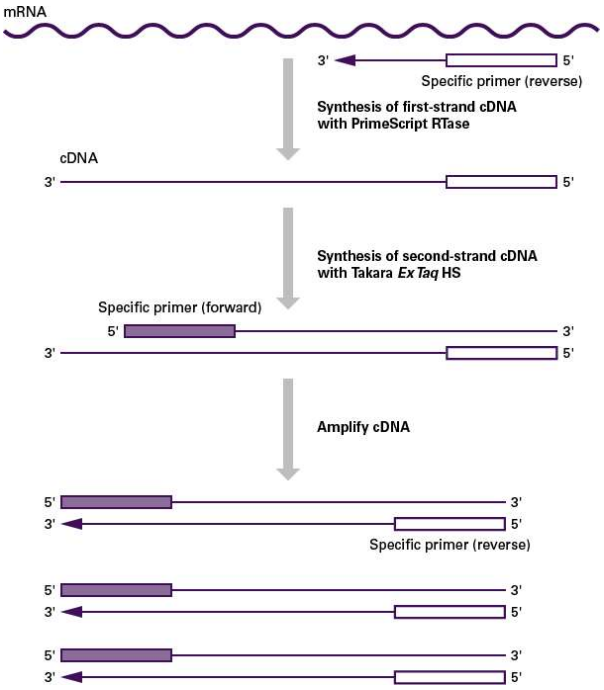


Figure 2. Workflow schematic using our PrimeScript One Step RT-PCR Kit Ver.2.

Probe-based detection

One-step RT-qPCR kits that utilize probe-based detection must be accurate, specific and reproducible. Our One Step PrimeScript RT-PCR Kit (Perfect Real Time) passes these requirements with flying colors. This kit is available in both 100 and 500 reaction sizes.

Oligonucleotides modified with a fluorophore (e.g., FAM) at the 5' end and quencher (e.g., TAMRA) at the 3' end are included in the reaction. During the annealing step, the probe specifically hybridizes to the template DNA and the fluorophore's fluorescence is suppressed by the quencher. During the extension step, the 5'→3' exonuclease activity of *Taq* DNA polymerase degrades the probe hybridized to

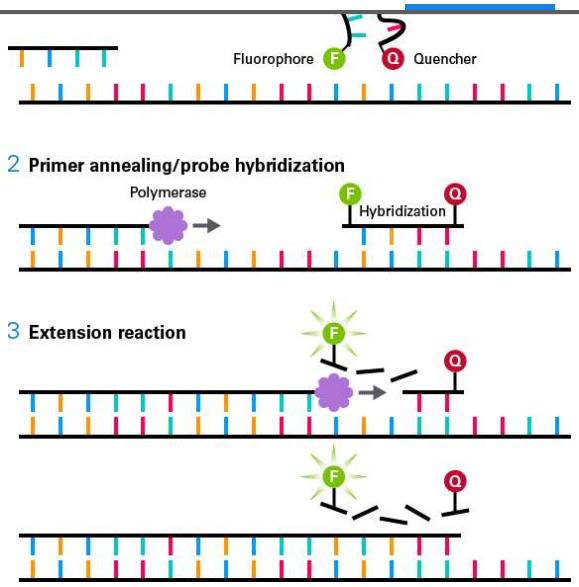


Figure 3. Schematic detailing the detection and quantitation of probe-based RT-PCR fluorescence.

TB Green-based detection

One-step RT-qPCR kits that utilize TB Green-based detection are rapid, efficient, and sensitive. When you want rapid and cost-effective results in a single-tube protocol, utilize our One-Step TB Green PrimeScript RT-PCR Kit II (Perfect Real Time). This kit is available in both 100 and 500 reaction sizes.

This method detects fluorescence produced during the amplification process by adding a DNA intercalating dye (TB Green) that fluoresces upon binding to double-stranded DNA. Following the synthesis and binding of TB Green to DNA synthesized during RT-PCR, the quantity of amplified DNA and the melting point of the resulting amplicon can be measured.

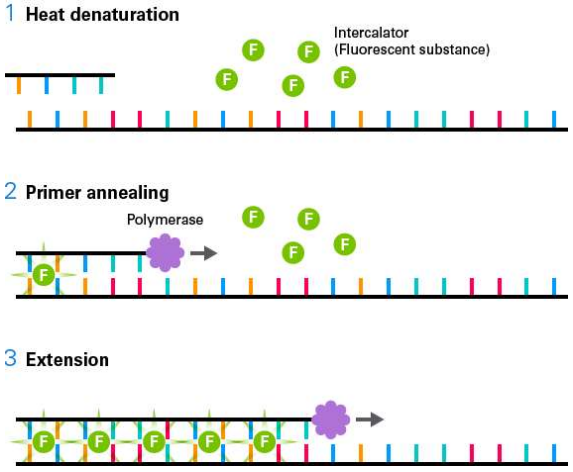


Figure 4. Schematic detailing the detection and quantitation of TB Green-based RT-PCR fluorescence.

Highlighted citations

Here are a few examples of research that's been driven by our one-step RT-qPCR kits:

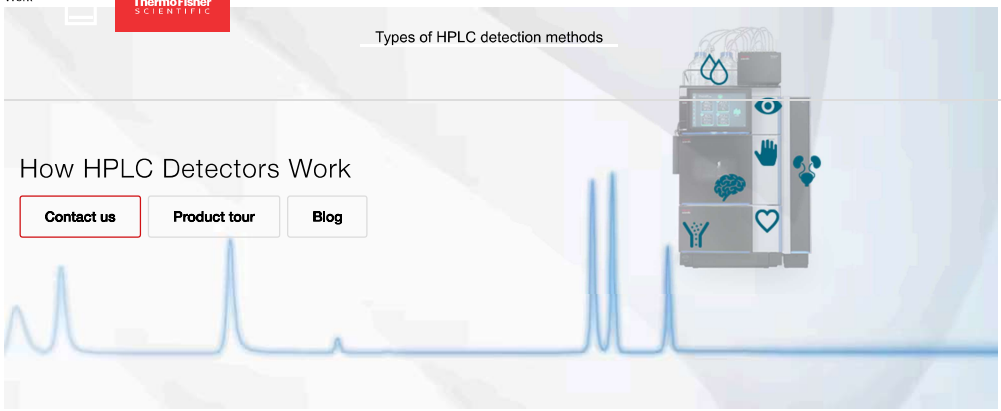
Ma, W. *et al.* Zika virus causes testis damage and leads to male infertility in mice. *Cell* **167**, 1511–1524.e10 (2016).
Cat. # RR064A was used to sensitively detect Zika viral RNA levels from multiple tissues in Zika virus-infected mice. These data were able to discern higher viral levels in testis, associated with infertility.

Xie, Y., Wang, M., Xu, D., Li, R. & Zhou, G. Simultaneous detection and identification of four sugarcane viruses by one-step RT-PCR. *J. Virol. Methods* **162**, 64–68 (2009).
Cat. # RR064A was used in the development of a one-step quadruplex RT-PCR method for detecting viruses in sugarcane. This rapid and sensitive technique greatly reduced cost and labor since multiple infections could be tested for in one sample.

Zhang, N. *et al.* Development of one-step SYBR Green real-time RT-PCR for quantifying bovine viral diarrhea virus type-1 and its comparison with conventional RT-PCR. *Virol. J.* **8**, 374 (2011).
Cat. # RR086A was used to develop a specific, sensitive, and reproducible assay for bovine viral diarrhea virus, a surrogate model for hepatitis C virus. The assay was 10-fold more sensitive than conventional assays and showed no primer dimers or nonspecific products.

Zou, Q. *et al.* Use of Praziquantel as an adjuvant enhances protection and Tc-17 responses to killed H5N1 virus vaccine in mice. *PLoS One* **7**, e34865 (2012).
Cat. # RR086A was used to measure H5N1 infection levels in mouse lung tissue. This enabled the testing of a novel adjuvant, PZQ, which was able to reduce virus loads and prolong survival.

Featured Products



Types of HPLC detection methods

After elution from the column, the mobile phase transports separated bands or analytes to the detector – the final HPLC component. While there are many HPLC detection methods, no single one can detect all possible analytes. Liquid chromatographers can utilize two or more detection methods in the same run to obtain deeper sample characterization.

HPLC system components

- **HPLC learning center**
- **HPLC system components**
- **Charged aerosol detection**
- **Vanquish LC systems**

Related resources

Vanquish HPLC & UHPLC Bibliography

Thermo Scientific AppsLab Library of Analytical Applications

HPLC & UHPLC resources

Detection method	Analyte requirements	Detection limit	Destructive detector?
UV-Vis (UVD)	Absorbs UV-Vis light between 190 – 800 nm	Nanograms	No
Fluorescence (FLD)	Has a fluorophore or labelled with a fluorescent tag	Femtograms	No
Refractive Index (RID)	No analyte restrictions	Micrograms	No
Evaporative Light Scattering (ELSD)	Non- and semi-volatile analytes	Nanograms	Yes
Electrochemical (ECD)	Undergoes a redox reaction in the presence of an electric potential	Femtograms	Yes
Charged Aerosol (CAD)	Non- and semi-volatile analytes	Picograms	Yes
Mass Spectrometry (MS)	Volatile and semi-volatile ionizable analytes	Picograms	Yes

You can learn more about the benefits of using multiple detectors in an **HPLC analysis**

How HPLC detectors work

Most HPLC detectors work by converting a physiochemical property of an analyte into an electrical signal. In other words, a detector 'sees' a sample and sends signals at consecutive time points throughout the sample run.

Signal intensity should correlate with the amount – either mass or concentration – of the detected sample at the given time point, allowing the quantification and identification of the separated analytes in a time-dependent manner.

Selecting a detector compatible with your target analytes and separation conditions is crucial when developing a method. If you use a detection method incompatible with the target analytes, you will miss the sample information.

Conversely, some mobile phase compositions or additives can produce noisy backgrounds for specific detectors, preventing proper analyte quantitation.

Ultraviolet-visible light absorption detection (UV-Vis)

Types of UV-Vis detectors

For UV-Vis detection, chromatographers can choose between three types of detectors: a variable wavelength detector (VWD), diode array detector (DAD), or multiple wavelength detector (MWD). In variable wavelength detection, a single chosen wavelength from the UV-Vis spectrum illuminates the sample. In contrast, diode array and multiple wavelength detectors exposes the sample to the entire spectrum instead of a single chosen

wavelength. The application needs or optical properties of the analyte(s) and sample matrix often determine the detector choice.

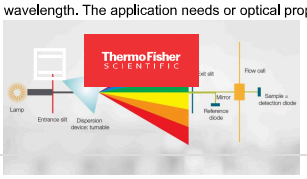


Illustration of UV-Vis detection mechanism with a VWD

Click image to enlarge

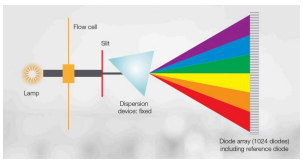
How variable wavelength detectors work

A variable wavelength detector uses a rotating grating to disperse polychromatic light into the spectrum. The light of a single wavelength is then selected and passed through the exit slit.

After the light passes through the exit slit, a beam splitter or semipermeable mirror divides the beam into two parts: one part of the light goes to a reference diode to measure the intensity without absorption. The second part passes through the flow cell, where the sample partially absorbs the light. The intensity of the remaining light is measured by the detection photodiode and translated into a quantitative signal.

By selecting a wavelength before exposing the sample, light from one wavelength is used to measure the absorption. This detection method offers high sensitivity due to the simultaneous measurement of an actual reference and reduces the total light exposure of the sample during detection.

The enhanced sensitivity of a VWD versus a DAD is essential for light-sensitive compounds.



Schematic display of UV-Vis detection mechanism with a DAD

Click image to enlarge

How diode array and multiple wavelength detectors work

Diode array and multiple wavelength detectors both use a grating to disperse the light onto a photodiode array after the light has passed through the flow cell. As a result, the absorption of all wavelengths is simultaneous, giving the analyte a full absorption spectrum.

This detection method is preferred when analyzing complex mixtures or samples of unknown composition, for example, during method development or peak purity analysis.

Types of HPLC detection methods

Fluorescence detection (FLD)

Refractive index detection (RID)

Electrochemical detection (ECD)

Electrochemical detectors are selective and extremely sensitive and often used to measure low-level, redox-active analytes in complex biological matrices. Although ECD performance often compares to the sensitivity in FLD, an advantage of electrochemical detection is a direct measurement of an analyte without using complex, time-consuming derivatization procedures.

How ECD works

An electrochemical detector measures the current produced when an electrochemically active compound undergoes oxidation or reduction at the surface of the electrode caused by an applied potential. According to Faraday's law, the resulting current is directly proportional to the concentration of the analyte experiencing the electrochemical reaction.

Charged aerosol detection (CAD)

Evaporative light scattering detection (ELSD)

Mass spectrometry (MS)

Liquid chromatography systems often pair with mass spectrometers. In combination with the retention time from the LC separation, MS detection provides an additional level of information by determining the mass-to-charge ratio of analytes contained in the sample.

Mass spectra contain information regarding the elemental and isotopic composition of analytes, which yields high detection specificity and is helpful for structural elucidation. These detectors are compatible with many analytes capable of forming gas-phase ions, from small inorganic salts to large macromolecules like proteins.

MS detection is more sensitive than other detection methods such as UV-Vis, does not require a chromophore or redox group, and enables the identification and structure elucidation of various molecules.

How mass spectrometry works

Thermo Scientific ISQ EC and ISQ EM Single Quadrupole Mass Spectrometers - How they work

Mass spectrometers have three main components: the ion source, the mass analyzer, and the detector, sometimes referred to as the electrometer. In MS, analytes are transferred into the gas phase, charged, filtered, and detected based on the mass-to-charge (*m/z*) ratio.

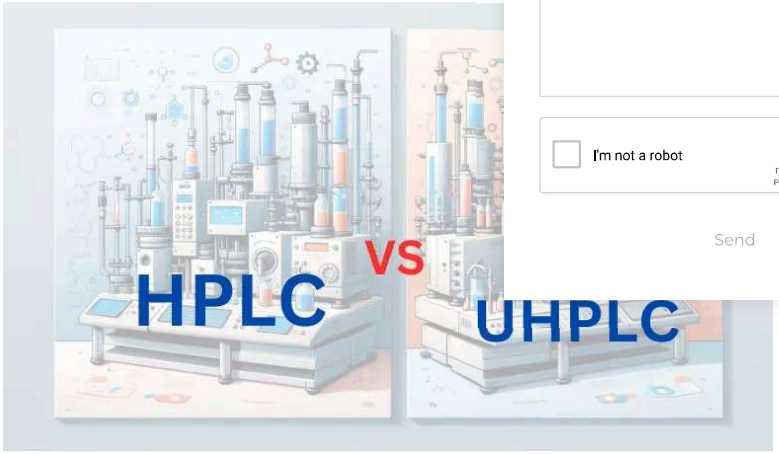
The ion source first generates gas-phase ions from the eluent stream and provides a focused ion beam to the mass analyzer. Next, the mass analyzer separates ions in time or space based on the respective *m/z*. Lastly, the detector converts the ions into time-based electrical signal and outputs a spectrum of the selected *m/z* within the scan range.

The key differentiator among mass spectrometers is the mass analyzer. Established examples include ion trap, quadrupole, time-of-flight, and orbitrap, with different analyzers providing advantages in resolution, speed, and sensitivity.

Tandem mass spectrometry (MS/MS)

Tandem mass spectrometry (MS/MS) involves using multiple stages of mass analysis to gain more structural information and/or higher specificity than single-stage MS. In the first stage, precursor ions are isolated based on the *m/z*. Next, the selected ions are fragmented, often by acceleration

What Is The Difference Between H



November 30, 2023

Table of Contents

In the realm of analytical chemistry, high-performance liquid chromatography (HPLC) and ultra-high-performance liquid chromatography (UHPLC) stand as two pillars of separation science. While both techniques excel in separating and identifying components within complex mixtures, they differ in their operating principles and capabilities, leading to distinct applications and advantages.

What is UHPLC ?

Ultra-high-performance liquid chromatography (UHPLC) is a type of liquid chromatography that uses smaller particles, higher pressures, and specialized instrumentation to achieve faster separation times, improved resolution, and enhanced sensitivity compared to traditional HPLC.

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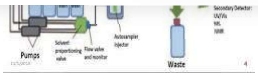

UHPLC instrument

UHPLC, the younger sibling of HPLC, emerged in the 21st cen
technology. It elevates the separation power of HPLC by utiliz
stationary phase, higher operating pressures, and specialized
combination results in significantly faster separation times, im
enhanced sensitivity.

To delve deeper into the intricacies of HPLC and UHPLC, let's

Particle Size:

HPLC typically employs particle sizes ranging from 3 to 5 micr
particles of 2 micrometers or less. This reduction in particle siz
surface area, enhancing the interaction between the sample
phase.

Operating Pressure:

HPLC systems operate at pressures around 400 bar (6,000 psi
withstand pressures up to 1,300 bar (20,000 psi). These higher
achieve faster flow rates and reduce analysis times.

Column Dimensions:

UHPLC columns generally have smaller internal diameters (2.1 mm or less) and shorter lengths (typically 100 mm) compared to HPLC columns, which typically have internal diameters of 4.6 mm and lengths of 250 mm. These smaller dimensions contribute to UHPLC's efficiency and speed.

Resolution and Sensitivity:

UHPLC's smaller particle sizes, higher pressures, and optimized column dimensions result in narrower chromatographic peaks, improved resolution, and enhanced sensitivity. This allows for the detection and quantification of even trace-level analytes.

Applications:

UHPLC is used in a variety of applications, including pharmaceutical analysis, environmental monitoring, food safety testing, and clinical diagnostics.

HPLC's versatility makes it suitable for a wide range of applications, including pharmaceutical analysis, environmental monitoring, food safety testing, and clinical diagnostics. UHPLC, with its superior resolving power and sensitivity, finds its niche in applications requiring rapid analysis, high-throughput screening, and the detection of low-abundance analytes.

In conclusion, HPLC and UHPLC, though sharing the same fundamental principles, represent distinct advancements in liquid chromatography. HPLC stands as a proven and versatile technique, while UHPLC offers unparalleled speed, resolution, and sensitivity. The choice between these techniques depends on the specific requirements of the analysis, balancing accuracy, sensitivity, sample throughput, and cost considerations.

How does UHPLC work ?

UHPLC, or Ultra-High-Performance Liquid Chromatography, is a sophisticated analytical technique that separates the components of a mixture based on their interactions with a stationary phase and a mobile phase. It's a powerful tool for identifying and quantifying various compounds in various fields, including pharmaceuticals, environmental science, food safety, and clinical diagnostics.

Here's a simplified breakdown of how UHPLC works:

1. **Sample Preparation:** The sample to be analyzed is prepared by dissolving it in a suitable solvent. This solvent will act as the mobile phase in the UHPLC system.

2. **Injection:** A precise volume of the prepared sample is injected into the UHPLC system using an autosampler. The autosampler ensures accurate and reproducible sample injection.

3. **Pumping:** A high-pressure pump forces the mobile phase through the UHPLC column. The mobile phase is typically a mixture of solvents, such as water and acetonitrile, and its composition can be varied depending on the specific separation required.

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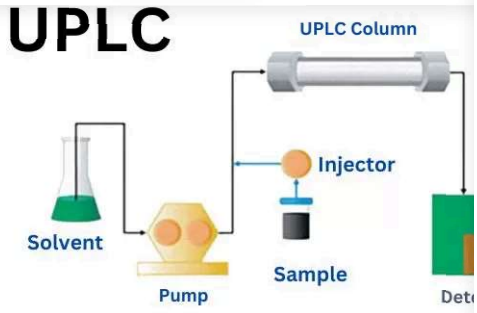

1. **Detection:** As the sample molecules elute from the column which generates a signal proportional to the concentration detectors in UHPLC include ultraviolet-visible (UV-Vis) detec (MS).

2. **Data Processing:** The detector's signal is converted into dig computer. The software converts the data into a chromatog concentration of each sample component versus time.

3. **Identification and Quantification:** The chromatogram is used to identify and quantify the sample components. Identification is based on the retention time of each peak in the chromatogram, while quantification is based on the peak area.

UHPLC offers several advantages over traditional HPLC, including:

- **Faster separation times:** UHPLC columns with smaller particle sizes and higher pressures allow for faster analysis.
- **Improved resolution:** UHPLC can separate compounds that are too close together to be resolved by HPLC.
- **Enhanced sensitivity:** UHPLC can detect and quantify compounds that are present in very low concentrations.

These advantages make UHPLC a valuable tool for a wide range of applications.

How HPLC & UHPLC Instruments Work

Can i use the same column for HPLC and UHPLC ?

The Answer No, you cannot use the same column for HPLC and UHPLC. This is because HPLC and UHPLC columns have different physical characteristics that are optimized for their respective operating conditions.

HPLC columns are typically packed with particles that are 3 to 5 micrometers in diameter and are designed to operate at pressures of up to 400 bar (6,000 psi).

UHPLC columns are packed with particles that are 2 micrometers or smaller in diameter and are designed to operate at pressures of up to 1,300 bar (20,000 psi).

Using an HPLC column in a UHPLC system would result in low efficiency and poor resolution. This is because the higher pressure in the UHPLC system would cause the mobile phase to flow too quickly through the column, resulting in insufficient interaction between the sample molecules and the stationary phase.

Conversely, using a UHPLC column in an HPLC system would not damage the column, but it would not be able to achieve the same level of performance as an HPLC column. This is because the lower pressure in the HPLC system would not be able to force the mobile phase through the column as quickly, resulting in longer analysis times.

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What are the pros and cons of UHPL

UHPLC Pros:

- **Faster separation times:** UHPLC columns with smaller part allow for faster analysis. This is because the smaller particles interaction between the sample molecules and the stationa pressures force the mobile phase through the column more
- **Improved resolution:** UHPLC can separate compounds tha resolved by HPLC. This is because the smaller particles and systems result in narrower chromatographic peaks.
- **Enhanced sensitivity:** UHPLC can detect and quantify com low concentrations. This is because the smaller particles and systems result in more efficient separation and detection of
- **Reduced solvent consumption:** UHPLC systems use smalle than HPLC systems, which results in reduced solvent consu significant advantage, especially for laboratories that are co disposal.
- **Automated operation:** UHPLC systems are typically highly the amount of time and labor required for analysis. This can laboratories that run a high volume of samples.

UHPLC Cons:

- **Higher cost:** UHPLC systems are more expensive than HPLC systems. This is because UHPLC systems require more specialized instrumentation, such as high-pressure pumps and detectors.
- **Increased complexity:** UHPLC systems are more complex to operate than HPLC systems. This is because UHPLC systems require more precise control of pressure and flow rate.
- **Greater sensitivity to impurities:** UHPLC systems are more sensitive to impurities in the mobile phase and sample than HPLC systems. This is because the smaller particle sizes and higher pressures in UHPLC systems can cause impurities to interfere with the separation of the sample molecules.
- **Limited compatibility with older methods:** UHPLC methods are not always compatible with older HPLC methods. This is because the smaller particle sizes and higher pressures in UHPLC systems can require changes to the mobile phase composition and gradient elution profile.

Overall, UHPLC is a powerful analytical technique that offers several advantages over HPLC. However, it is important to consider the pros and cons of UHPLC carefully before deciding whether it is the right technique for your needs.

Pros of UHPLC	Cons of UHPLC
1. Increased Efficiency: UHPLC offers faster separations and higher resolution compared to traditional HPLC.	**1. Cost**: UHPLC systems and their components are generally more expensive than standard HPLC systems.
2. Enhanced Sensitivity: Due to smaller particle sizes in the columns, UHPLC provides greater sensitivity and detection limits.	**2. Operational Complexity**: UHPLC requires more precision in operation and maintenance, which can be complex.
3. Reduced Solvent Consumption: UHPLC uses smaller column sizes and reduced flow rates, leading to lower solvent usage and costs.	**3. Equipment Stress**: The high pressure used in UHPLC can lead to faster wear and tear of the equipment.
4. High Throughput: The faster analysis time enables higher sample throughput, beneficial in high-demand settings.	**4. Sample Compatibility**: Some sensitive samples may degrade under the high pressures used in UHPLC.
5. Better Separation of Complex Mixtures: UHPLC is more effective in separating complex mixtures due to its higher resolution.	**5. Skilled Operation Required**: UHPLC often requires operators with more training and expertise.

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Faster Separation Times:

UHPLC's smaller particle sizes and higher operating pressures separation times compared to HPLC. This can be attributed to the stationary phase, which enhances the interaction betwee stationary phase, leading to quicker separations.

Improved Resolution:

UHPLC's narrow chromatographic peaks translate into improv separation of closely eluting compounds that would otherwis enhanced resolving power is crucial for analyzing complex mi individual components with greater precision.

Enhanced Sensitivity:

UHPLC's superior sensitivity allows for the detection and quar that would be undetectable using HPLC. This is particularly in pharmaceutical analysis and environmental monitoring, whe compounds is essential.

Reduced Solvent Consumption:

UHPLC systems typically consume less solvent due to their smaller columns and lower flow rates compared to HPLC. This translates into reduced solvent costs, environmental impact, and waste generation, making UHPLC a more sustainable analytical technique.

Automated Operation:

UHPLC systems are highly automated, minimizing manual intervention and reducing the risk of human error. This automation streamlines the analytical process, improves reproducibility, and frees up scientists for more complex tasks.

Suitability for Complex Analyses:

UHPLC's ability to handle complex mixtures with improved resolution and sensitivity makes it particularly well-suited for analyzing samples containing a wide range of components, such as biological fluids, environmental samples, and pharmaceutical formulations.

Applications:

UHPLC finds applications in diverse fields, including:

- **Pharmaceutical analysis:** Identification and quantification of pharmaceuticals in biological fluids and dosage forms
- **Environmental monitoring:** Detection of pollutants and contaminants in water, air, and soil
- **Food safety testing:** Identification and quantification of food contaminants, such as pesticides and antibiotics
- **Clinical diagnostics:** Diagnosis of diseases, such as cancer and diabetes
- **Biopharmaceutical research:** Characterization and purification of proteins and peptides

In conclusion, UHPLC offers a significant advantage over HPLC in terms of speed, resolution, sensitivity, solvent consumption, and automation. Its suitability for complex analyses makes it a valuable tool for a wide range of applications, particularly in fields where high-throughput analysis and accurate detection of trace-level analytes are crucial.

How to take care of UHPLC ms ?

Maintaining a UHPLC-MS system in optimal condition is crucial for ensuring its long-term performance and reliability. Here's a comprehensive guide to proper UHPLC-MS care and maintenance:

Daily Maintenance:

1. **Solvent Management:**

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